UNIVERSIDADE FEDERAL DE PELOTAS
Centro de Ciências Químicas, Farmacêuticas e de Alimentos
Programa de Pós-Graduação em Bioquímica e Bioprospecção

Tese

Implicações do envelhecimento na neuropatia induzida por oxaliplatina: 7-cloro-4-(fenilseleno)quinolina como estratégia terapêutica

Angélica Schiavom dos Reis

Pelotas, 2021

Angélica Schiavom dos Reis

Implicações do envelhecimento na neuropatia induzida por oxaliplatina: 7-cloro-4-(fenilseleno)quinolina como estratégia terapêutica

Tese apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, como requisito parcial para a obtenção do grau de Doutor em Bioquímica e Bioprospecção.

Orientadora: Prof.ª Drª. Ethel Antunes Wilhelm

Coorientadora: Prof.ª Drª. Cristiane Luchese

Pelotas, 2021

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

R375i Reis, Angélica Schiavom dos

Implicações do envelhecimento na neuropatia induzida por oxaliplatina: 7- cloro-4-(fenilseleno) quinolina como estratégia terapêutica / Angélica Schiavom dos Reis ; Ethel Antunes Wilhelm, orientadora ; Cristiane Luchese, coorientadora. — Pelotas, 2021.

300 f.: il.

Tese (Doutorado) — Programa de Pós-Graduação Bioquímica e Bioprospecção, Centro de Ciências Químicas Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 2021.

1. Oxaliplatina. 2. Envelhecimento. 3. Platina. 4. Neurotoxicidade. 5. Dor. I. Wilhelm, Ethel Antunes, orient. II. Luchese, Cristiane, coorient. III. Título.

CDD: 574.192

Elaborada por Gabriela Machado Lopes CRB: 10/1842

Angélica Schiavom dos Reis

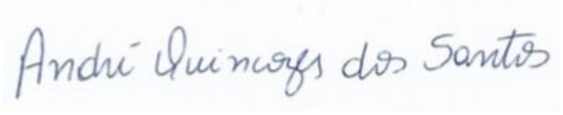
Implicações do envelhecimento na neuropatia induzida por oxaliplatina: 7-cloro-4-(fenilseleno)quinolina como estratégia terapêutica.

Tese aprovada, como requisito parcial, para obtenção do grau de Doutor em Bioquímica e Bioprospecção, Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas.

Data da Defesa: 03/09/2021

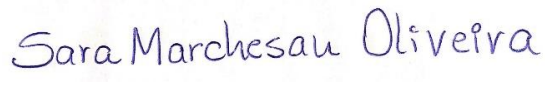
Banca examinadora:

.....
Profª. Drª. Ethel Antunes Wilhelm (Orientadora)
Doutora em Ciências Biológicas (Bioquímica Toxicológica) pela Universidade Federal de Santa Maria, UFSM, Brasil.



.....
Prof. Dr. André Quincozes dos Santos
Doutor em Ciências Biológicas (Bioquímica) pela Universidade Federal do Rio Grande do Sul, UFRGS, Brasil.

.....
Profª. Drª. Fabiana Kömmling Seixas
Doutora em Biotecnologia pela Universidade Federal de Pelotas, UFPEL, Brasil.



.....
Profª. Drª. Sara Marchesan de Oliveira
Doutora em Ciências Biológicas (Bioquímica Toxicológica) pela Universidade Federal de Santa Maria, UFSM, Brasil.

"Tu te tornas eternamente responsável pelo que cativas...Cada um que passa em nossa vida passa sozinho, pois cada pessoa é única, e nenhuma substitui outra. Cada um que passa em nossa vida passa sozinho, mas não vai só, nem nos deixa sós. Leva um pouco de nós mesmos, deixa um pouco de si mesmo. Há os que levam muito; mas não há os que não levam nada. Há os que deixam muito; mas não há os que não deixam nada. Esta é a maior responsabilidade de nossa vida e a prova evidente que duas almas não se encontram ao acaso."

(Antoine de Saint-Exupéry)

Agradecimentos

Agradeço à minha família, pelo amor e carinho, dedicados a mim, por estarem sempre ao meu lado e terem me dado todo o suporte para eu conquistar meus objetivos. Obrigada pelo incentivo ao estudo e por terem me proporcionado uma educação.

Agradeço a Universidade Federal de Pelotas, ao Programa de Pós-Graduação em Bioquímica e Bioprospecção e, a todos os professores que contribuíram para minha formação e desenvolvimento deste estudo.

Agradeço à minha orientadora. Prof.ª Drª Ethel, sou muito grata por todas as oportunidades, pelo apoio e amizade. E, também, pelo enorme crescimento profissional e pessoal que me proporcionou.

Agradeço a CAPES, pelo apoio financeiro.

Agradeço à minha coorientadora Prof.ª Drª Cristiane Luchese e à Prof.ª Drª Márcia Foster Mesko, pelas significativas contribuições com o estudo.

Agradeço ao Laboratório de Pesquisa em Farmacologia e Bioquímica (LaFarBio), meus colegas, sem a contribuição de vocês não seria possível desenvolver esse estudo. Também, agradeço a parceria com o Laboratório de Controle de Contaminates em Biomateriais (LCCBio), o Laboratório de Síntese Orgânica Limpa (Lasol), o Laboratório Genômica Estrutural e, o Grupo de Pesquisa em Oncologia Celular e Molecular (GPO). Este trabalho concretizou-se apenas com o esforço conjunto desses laboratótios da Universidade Federal de Pelotas.

Agradeço à toda equipe do Biotério Central da Universidade Federal de Pelotas.

Agradeço o apoio financeiro do Projeto Institucional de Internacionalização (CAPES/PrInt/UFPel).

Agradeço a Deus, por abençoar nossos esforços e permitir a concretização de um sonho.

RESUMO

REIS, Angélica Schiavom. **Implicações envelhecimento na neuropatia induzida por oxaliplatina: 7-cloro-4-(fenilseleno)quinolina como estratégia terapêutica.** Orientadora: Ethel Antunes Wilhelm. 2021. 300 f. Tese (Doutorado em Ciências com ênfase em Bioquímica e Bioprospecção) - Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2021.

Considerando a complexidade dos efeitos induzidos pela oxaliplatina e a falta de tratamento eficaz, principalmente para os idosos, tornam-se cada vez mais relevantes estudos que contribuam para a compreensão dos mecanismos envolvidos na neuropatia periférica induzida pela oxaliplatina (NPIO) e, que identifiquem novos alvos farmacológicos para o desenvolvimento de terapias eficazes e seguras para todas as idades. Neste contexto, destaca-se o 7-cloro-4-(fenilselanil) quinolina (4-PSQ), um derivado de quinolina contendo selênio que tem sido amplamente estudado, dado que este apresenta promissores efeitos antioxidante e neuroprotetor. Portanto, o conjunto dos fatores citados nos conduziram a investigar a ação do 4-PSQ em um modelo de NPIO em diferentes idades. Nesse sentido, a 1ª etapa deste estudo demonstrou que, apesar das defesas eficientes do sistema nervoso central (SNC), após a exposição à oxaliplatina ocorre um acúmulo de platina na medula espinhal de camundongos, o que amplia o conhecimento sobre a sua toxicidade. Ainda, foi proposto que o acúmulo de platina na medula espinhal pode causar danos oxidativos aos neurônios e prejudicar a função mitocondrial. Além disso, o 4-PSQ reduziu a dor neuropática subjacente a NPIO. Na 2ª etapa do estudo, foi demonstrada uma correlação significativa entre o comportamento ansioso e o prejuízo cognitivo, comorbidades associadas à NPIO. A administração de oxaliplatina aumentou os níveis de corticosterona plasmática e inibiu a atividade da enzima Na^+, K^+ - ATPase. O 4-PSQ reverteu as comorbidades e os seus efeitos farmacológicos parecem ser, principalmente, devido à modulação da enzima Na^+, K^+ - ATPase. Na 3ª etapa do estudo, demonstro-se pela primeira vez que a NPIO aguda foi exacerbada pelo envelhecimento por meio do aumento do dano oxidativo e modulação da Na^+, K^+ - ATPase. O 4-PSQ reverteu os danos causados pela oxaliplatina, com destaque, para os potencializados pelo envelhecimento. Na 4ª etapa do estudo, foi evidenciado pela primeira vez que a NPIO crônica foi exacerbada pelo envelhecimento. Alterações na concentração de bioelementos, no estado redox e nas vias de neuroproteção e neuroplasticidade induzidas pelo envelhecimento parecem contribuir para a NPIO. A deposição de platina foi identificada pela primeira vez no cérebro de camundongos expostos à oxaliplatina, independentemente da idade. Na 5ª etapa do estudo, foi demonstrado que o 4-PSQ reverteu a NPIO crônica e suas comorbidades. A ação farmacológica do 4-PSQ neste modelo experimental parece envolver a modulação das enzimas Na^+, K^+ - ATPase e acetilcolinesterase e, principalmente, a promoção do equilíbrio redox. Na 6ª etapa do estudo, o potencial farmacológico e antioxidante do 4-PSQ destacaram-se em um modelo de dor oncológica. Este estudo é inovador, provendo uma perspectiva sobre o impacto do envelhecimento na NPIO e, evidências sobre os efeitos do 4-PSQ.

Palavras-chave: Oxaliplatina, envelhecimento, platina, neurotoxicidade, dor.

ABSTRACT

REIS, Angélica Schiavom. **Implications of aging in oxaliplatin-induced neuropathy: 7-Chloro-4-(phenylselanyl) quinoline as a therapeutic strategy.** Adviser: Ethel Antunes Wilhelm. 2021. 300 f. Thesis (Doctorate in Science with an emphasis on Biochemistry and Bioprospecting) - Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2021.

Given the complexity of the effects induced by oxaliplatin and the lack of effective treatment, especially for the elderly, studies that contribute to the understanding of the mechanisms involved in oxaliplatin-induced peripheral neuropathy (NPIO) and that identify new pharmacological targets are becoming increasingly relevant for the development of effective and safe therapies for all ages. In this context, 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) stands out, a quinoline derivative containing selenium, that has been widely studied, as it has promising antioxidant and neuroprotective effects. Therefore, the set of factors mentioned led us to investigate the action of 4-PSQ in an NPIO model at different ages. In this sense, the 1st stage of this study showed that, despite the efficient defenses of the central nervous system (CNS), after exposure to oxaliplatin, there is an accumulation of platinum in the spinal cord of mice, which increases the knowledge about its toxicity. Furthermore, it has been proposed that the accumulation of platinum in the spinal cord can cause oxidative damage to neurons and impair mitochondrial function. In addition, 4-PSQ reduced the neuropathic pain underlying NPIO. In the 2nd stage of the study, a significant correlation was shown between anxious behavior and cognitive impairment, comorbidities associated with NPIO. Also, the administration of oxaliplatin caused an increase in plasma corticosterone levels and inhibition of the enzyme Na^+, K^+ - ATPase. 4-PSQ reversed comorbidities and its pharmacological effects seem to be mainly due to the modulation of the enzyme Na^+, K^+ - ATPase. In the 3rd stage of the study, it was demonstrated for the first time that acute NPIO was exacerbated by aging through increased oxidative damage and modulation of Na^+, K^+ - ATPase. The 4-PSQ reversed the damage caused by oxaliplatin, especially for those aggravated by aging. In the 4th stage of the study, it was shown for the first time that chronic NPIO was exacerbated by aging. Changes in bioelement concentration, oxidative status, and neuroprotection and neuroplasticity pathways seem to contribute to NPIO. Platinum deposition was first identified in the brain of mice exposed to oxaliplatin, regardless of age. In the 5th stage of the study, it was demonstrated that 4-PSQ reversed chronic NPIO and its comorbidities. The pharmacological action of 4-PSQ in this experimental model seems to involve the modulation of the enzymes Na+, K+ - ATPase and acetylcholinesterase and, mainly, the promotion of redox balance. In the 6th stage of the study, the pharmacological and antioxidant potential of 4-PSQ stood out in a cancer pain model. This study is innovative, providing a perspective on the impact of aging on NPIO and evidence on the effects of 4-PSQ.

Keywords: Oxaliplatin, aging, platinum, neurotoxicity, pain.

LISTA DE FIGURAS

Referencial Teórico

LISTA DE FIGURAS

Artigo Científico 1

LISTA DE FIGURAS

Artigo Científico 2

LISTA DE FIGURAS

Manuscrito 1

LISTA DE FIGURAS

Manuscrito 2

LISTA DE FIGURAS

Manuscrito 3

LISTA DE FIGURAS

Resultados preliminares

LISTA DE TABELAS

Artigo Científico 1

Artigo Científico 2

Manuscrito 2

LISTA DE ABREVIATURAS

AChE	Acetilcolinesterase
ATF4	Fator de ativação da transcrição 4
BDNF	Fator neurotrófico derivado do cérebro (do inglês *Brain-derived neurotrophic fator*)
CAT	Catalase
DNA	Ácido desoxirribonucleico (do inglês *deoxyribonucleic acid*)
GAPDH	Gliceraldeído 3-fosfato desidrogenase
GPx	Glutationa peroxidase
GSH	Glutationa reduzida
GST	Glutationa S-transferase
GR	Glutationa redutase
ICP-MS	Espectrometria de massa por plasma acoplado indutivamente (do inglês *inductively coupled plasma-tandem mass spectrometry*)
MDA	Malondialdeído
NADPH	Fosfato de dinucleotídeo de adenina e nicotinamida
NPIO	Neuropatia periférica induzida por oxaliplatina
NF-κB	Factor nuclear kappa B
Nrf2	Fator nuclear eritroide 2 relacionado ao fator 2 (do inglês *Nuclear factor-erythroid-2 related factor*)
NPSH	Tióis não proteicos (do inglês *non-protein thiols*)
OMS	Organização Mundial da Saúde
OXA	Oxaliplatina
PI3K	Fosfatidilinositol 3-quinases
RNA	Ácido ribonucleico (do inglês *ribonucleic acid*)
SOD	Superóxido dismutase
SNC	Sistema nervoso central
SNP	Sistema nervoso periférico
TRP	Receptor de potencial transitório (*transient receptor potential*)

SUMÁRIO

1 INTRODUÇÃO

A organização mundial da saúde (OMS) estima que 20% da população mundial terá mais de 60 anos em 2050 (FANE e WEERARATNA, 2020). O envelhecimento é um processo complexo caracterizado pelo declínio das funções fisiológicas, com degradação progressiva e redução da capacidade de reparo dos tecidos e sistemas orgânicos, levando a um risco aumentado de mortalidade. No entanto, com os crescentes avanços na saúde pública, a população mundial está vivendo mais e alcançando idades mais avançadas, aumentando a incidência de muitas doenças crônicas relacionadas à idade, incluindo doenças neurodegenerativas e cânceres (BHADRA et al., 2020).

De fato, a maior incidência de câncer ocorre em pacientes com mais de 60 anos (FANE e WEERARATNA, 2020). Em 2018, 13% dos casos novos de câncer no mundo ocorreram em indivíduos com 80 anos ou mais (PILLERON et al., 2020). De acordo com o banco de dados GLOBOCAN, o câncer colorretal é uma das doenças malignas mais comuns, uma das principais causas de morte relacionada ao câncer no mundo (FERLAY et al., 2019) e, é o terceiro tipo de câncer com maior incidência em idosos de ambos os sexos (PILLERON et al., 2020). Atualmente, apesar do progresso nos regimes quimioterápicos, que resultaram em melhorias substanciais para os pacientes, a quimioterapia leva a inúmeras alterações na estrutura e função celular, as quais estão associadas a toxicidade grave, duradoura ou irreversível.

A oxaliplatina, um fármaco quimioterápico à base de platina, é um componente importante no tratamento do câncer colorretal que aumenta significativamente a taxa de sobrevida dos pacientes (NICHETTI et al., 2019). No entanto, a neurotoxicidade induzida pela administração da oxaliplatina é um efeito adverso grave e potencialmente permanente, inicialmente caracterizado por sintomas clínicos agudos que são exacerbados com a exposição contínua a oxaliplatina, resultando no estabelecimento de neurotoxicidade crônica e dor neuropática (STAROBOVA e VETTER, 2017; MCQUADE et al., 2018; CAVALETTI e MARMIROLI, 2020). Apesar dos avanços significativos na compreensão da neurotoxicidade induzida por oxaliplatina, diversas dúvidas ainda persistem, tanto em relação aos sintomas clínicos quanto à base celular e molecular. Na neuropatia periférica aguda induzida por oxaliplatina (NPAIO), a interferência reversível da oxaliplatina e seus metabólitos, como o oxalato,

nos canais iônicos foi descrita como mecanismo principal, mas o conhecimento sobre outros mecanismos envolvidos ainda é limitado. Em relação à neuropatia periférica crônica induzida por oxaliplatina (NPCIO), a hipótese mais aceita envolve a toxicidade mitocondrial. Este mecanismo tem sido extensivamente investigado por meio de alterações na morfologia mitocondrial, bioenergética e geração de espécies reativas (CAVALETTI e MARMIROLI, 2020).

A neuropatia periférica induzida pela administração de oxaliplatina (NPIO) é a principal toxicidade limitante da dose, afetando negativamente a qualidade de vida dos pacientes e levando à descontinuação do tratamento (NICHETI et al., 2019). Pacientes com NPIO apresentam dor neuropática como um dos sintomas clínicos (FLATTERS et al., 2017) e comorbidades como o declínio cognitivo e o comportamento ansioso. Entretanto, a natureza e o impacto do conjunto dos mecanismos neurobiológicos envolvidos na NPIO não estão completamente caracterizados. De fato, os mecanismos patológicos subjacentes à neurotoxicidade induzida pela oxaliplatina constituem um problema clínico desafiador (MATSOS e JOHNSTON, 2019).

Além disso, outros fatores biológicos podem ter um impacto negativo na fisiopatologia da NPIO e agravar a dor neuropática, como o processo degenerativo causado por doença ou envelhecimento. A experiência da dor resulta de um conjunto complexo de ações e interações entre o sistema nervoso central (SNC) e o sistema nervoso periférico (SNP). Em pacientes idosos, é necessário considerar o declínio da neurogênese com a idade (NGUYEN e EHRLICH, 2020), bem como a perda progressiva das funções orgânicas, principalmente, devido ao acúmulo de dano oxidativo às macromoléculas (LIGUORI et al., 2018). Assim, uma hipótese é que pacientes idosos em tratamento com a oxaliplatina são mais suscetíveis aos efeitos adversos, principalmente à dor. Nessa linha, novas abordagens terapêuticas devem considerar as peculiaridades do envelhecimento em busca de terapia segura e eficaz para o tratamento da NPIO.

Nesse contexto, destaca-se o 7-cloro-4-(fenilselanil) quinolina (4-PSQ) (Figura 1), um composto amplamente estudado por nosso grupo de pesquisa. Primeiramente, foi demonstrada a ação antioxidante *in vitro* do 4-PSQ (SAVEGNAGO et al., 2013). A partir disso, evidenciamos um importante potencial do 4-PSQ em modelos de nocicepção e inflamação em camundongos

(PINZ et al., 2016, VOSS et al., 2018). Além disso, o composto reduziu a inflamação aguda induzida por carragenina e apresentou ações neuroprotetora e ansiolítica em camundongos (REIS et al., 2017; SILVA et al., 2017; PINZ et al., 2018; VOGT et al., 2018). Nossos resultados sugerem que os efeitos farmacológicos do 4-PSQ estão associados à sua ação antioxidante e à modulação dos sistemas serotoninérgico, nitrérgico e glutamatérgico, ou seja, o 4-PSQ é uma molécula multialvo (PINZ et al., 2016; REIS et al., 2017; SILVA et al., 2017; LUCHESE et al., 2020). Além disso, nosso grupo de pesquisa demonstrou que o tratamento com o 4-PSQ restaurou o comprometimento cognitivo causado pelo envelhecimento em ratos, uma vez que reestabeleceu os níveis de expressão das moléculas de adesão celular neural, que desempenham um papel fundamental na plasticidade sináptica e processos cognitivos (BARTH et al., 2019). E, recentemente, evidenciou-se que o 4-PSQ reverteu os danos renais e hepáticos induzidos por oxaliplatina (LEMOS et al., 2020; da MOTTA et al., 2021).

Assim, diante da necessidade de novas abordagens para a NPIO que considerem as especificidades de diferentes faixas etárias, este estudo teve como objetivo responder as seguintes questões/hipóteses: *i)* o envelhecimento altera a suceptibilidade à NPIO e suas comorbidades? *ii)* a platina se deposita no SNC e contribui para o dano oxidativo que desencadeia a NPIO? *iii)* alterações em vias oxidativas, neuroprotetoras e de neuroplasticidade; e na concentração de biolementos agravam a NPIO em idosos? *iv)* o 4-PSQ reduz a neuropatia e as comorbidades independente da idade devido ao seu potencial antioxidante? *v)* o 4-PSQ reduz a dor oncológica? Esta tese aborda um tema extremamente relevante para a saúde pública mundial, e poderá impactar significativamente na compreensão da neurotoxicidade subjacente a oxaliplatina.

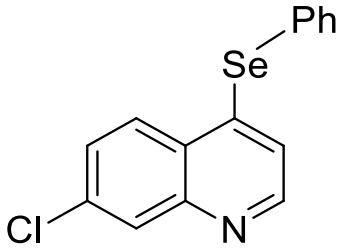


Figura 1. Estrutura química do 7-cloro-4-(fenilselanil) quinolina (4-PSQ)

OBJETIVOS

2.1 Objetivo geral

Investigar o papel do envelhecimento na neuropatia periférica induzida por oxaliplatina (NPIO) e elucidar o potencial farmacológico do composto sintético 7-cloro-4-(fenilseleno)quinolina (4-PSQ), a fim de evidenciar os mecanismos fisiopatológicos envolvidos na NPIO e buscar uma estratégia farmacológica segura e eficaz para o tratamento.

2.2 Objetivos específicos

- Avaliar se o envelhecimento altera a fisiopatologia, os sintomas, e as comorbidades emocionais e cognitivas da NPIO;
- Investigar se a platina, após o tratamento com a oxaliplatina, é capaz de permear a barreira hematoencefálica (BHE) e se acumular no SNC;
- Correlacionar as concentrações dos elementos selênio, cobre, potássio, fósforo, sódio, enxofre, magnésio, cálcio, manganês, zinco, ferro, cobalto e platina com o envelhecimento e, também, com a exposição à oxaliplatina em diferentes idades;
- Determinar o impacto causado pela associação entre o envelhecimento e a exposição à oxaliplatina no equilíbrio redox do SNC e nervo ciático.
- Comparar a expressão gênica e/ou atividade de proteínas envolvidas no equilíbrio oxidativo e na plasticidade sináptica em camundongos jovens e velhos expostos à oxaliplatina;
- Investigar as vias do Nrf2 e do BDNF como potenciais alvos farmacológicos para o tratamento da NPIO considerando as peculiaridades da idade;
- Investigar novos alvos farmacológicos, incluindo a modulação das enzimas $Na^{+}K^{+}$ - ATPase, Mg^{2+} - ATPase e acetilcolinesterase (AChE), para o tratamento da neurotoxicidade induzida pela oxaliplatina e suas comorbidades em diferentes idades;
- Elucidar o potencial farmacológico do 4-PSQ na dor associada a NPIO e suas comorbidades em camundongos/ratos velhos e jovens.
- Avaliar o potencial farmacológico do 4-PSQ na dor oncológica.

3 REFERENCIAL TEÓRICO

3.1 Envelhecimento

O envelhecimento é um processo complexo caracterizado pelo declínio da função fisiológica, com degradação progressiva e redução da capacidade de reparo dos tecidos e sistemas orgânicos, levando a um risco aumentado de doeças e mortalidade. A senescência é um processo metabólico ativo de envelhecimento celular associado ao processo de envelhecimento (BHADRA et al., 2020). As células senescentes exibem algumas propriedades do envelhecimento.

De fato, diversas características do envelhecimento incluindo a disfunção mitocondrial, detecção desregulada de nutrientes, alterações em proteínas, e instabilidade genômica induzem células normais a se tornarem senescentes, o que por sua vez pode levar à senescência de células normais próximas. A indução de células normais à senescência, associado ao declínio na atividade do sistema imunológico em idosos, causa acúmulo de células senescentes no organismo. E, em indivíduos idosos, esse acúmulo contribui para a disfunção dos tecidos e aumenta o risco de desenvolvimento de doenças associadas à idade (BORGHESAN et al., 2020).

Lesões ao ácido desoxirribonucleico (DNA), como as induzidas por estresse oxidativo, radiação, e agentes genotóxicos incluindo os quimioterápicos, ativam mecanismos de reparo celular, entretanto, a continuidade dos danos causa liberação de quimiocinas, citocinas, e proteases gerando inflamação celular e disfunção mitocondrial. Por sua vez, mitocôndrias disfuncionais induzem à senescência. A senescência associada à disfunção mitocondrial leva à hiperprodução de espécies reativas de oxigênio. A teoria do envelhecimento pela ação de espécies reativas é baseada na hipótese de que as perdas funcionais associadas à idade são devido ao acúmulo de danos oxidativos às macromoléculas (lipídios, DNA e proteínas). O mecanismo exato do envelhecimento induzido pelo estresse oxidativo ainda não está claro, mas provavelmente os níveis aumentados de espécies reativas levam à senescência celular, um mecanismo fisiológico que interrompe a proliferação celular em resposta aos danos que ocorrem durante a replicação (BORGHESAN et al., 2020).

O estresse oxidativo e a senescência celular estão envolvidos em vários processos patológicos, dada a estreita relação entre o estresse oxidativo, a inflamação e o envelhecimento. O envelhecimento é uma perda da homeostase devido a um estresse oxidativo crônico que afeta principalmente os sistemas reguladores, como os sistemas nervoso, endócrino e imunológico. A consequente ativação do sistema imunológico induz um estado inflamatório que cria um círculo vicioso em que o estresse oxidativo crônico e a inflamação se alimentam e, consequentemente, aumenta a morbimortalidade relacionada à idade (LIGUORI et al., 2018).

Atualmente, os avanços na saúde pública aumentaram e a população mundial está envelhecendo, aumentando a incidência de muitas doenças relacionadas à idade, incluindo doenças neurodegenerativas e cânceres (BHADRA et al., 2020). Nesse contexto, torna-se mais emergente a necessidade de considerar o impacto que o envelhecimento causa na fisiopatologia das doenças, bem como considerar as peculiaridades do envelhecimento no desenvolvimento de novas terapias.

3.2 A experiência da dor em idosos

No Brasil, com as mudanças nas taxas de natalidade e mortalidade nas últimas décadas, estima-se que o percentual de pessoas acima de 60 anos em 2050 corresponderá a cerca de 30% da população do país (IBGE, 2011; OMS, 2015). Assim, o tema relacionado ao envelhecimento populacional tem chamado a atenção da comunidade científica e dos órgãos públicos, sendo a qualidade de vida da população idosa um desafio constante para a ciência e a sociedade em geral. Dentre as maiores adversidades para esse tema, encontram-se o aumento dos casos de doenças crônicas e de seus impactos psicológicos, sociais e ambientais (BEARD et al., 2016).

A dor é uma experiência recorrente para muitos idosos e, atualmente, esforços significativos estão sendo empreendidos com o objetivo de melhorar a avaliação e o tratamento da dor em idosos (MORRISSEY et al., 2014; HORGAS, 2017; GLEASON et al., 2018). Essa iniciativa aumentou a conscientização sobre o problema, mas ainda há evidências de falhas no diagnóstico e gerenciamento da dor quando relacionados ao envelhecimento (HORGAS, 2017).

O manejo eficaz da dor nesta faixa etária baseia-se em uma avaliação abrangente e completa seguida por reavaliações frequentes. O diagnóstico da dor é composto por três componentes essenciais o autorrelato, avaliado através do uso de escalas, a compreensão e o estabelecimento de metas para o atendimento e tomada de decisões. Neste contexto, o autorrelato destaca-se diante da ausência de marcadores biológicos confiáveis e específicos para o diagnóstico da dor (HOGAN et al., 2016; GLEASON et al., 2018). No entanto, o autorrelato quando realizado por pacientes com idade avançada propende a ser minimizado quando relacionado a fatores como multimorbidade, polifarmácia e comprometimento cognitivo. De fato, estudos têm demonstrado que os idosos realizam um menor número de notificações relacionadas à dor, apesar desta condição apresentar-se frequentemente relacionada ao comprometimento funcional e psicológico nesta faixa etária (MORRISSEY et al., 2014; GLEASON et al., 2018).

A dor tem início a partir de um estímulo nociceptivo, podendo apresentar tipos (aguda e neuropática) e características (oncológica e não-oncológica) diferentes. Os idosos, por serem mais susceptíveis, podem ser acometidos por diferentes tipos de dor, sendo assim estão sujeitos à sobreposição destas o que pode influenciar na recuperação e na reabilitação. Nesse sentido, as doenças crônicas oncológicas vêm atingindo de maneira expressiva a população idosa e isso ocorre especialmente em virtude do envelhecimento celular e da falta de proteção hormonal, que são características da idade avançada. Nesse contexto, o gerenciamento da dor, especialmente em pacientes idosos com câncer, pode ser de difícil execução.

3.3 Dor

A dor é uma experiência sensorial e emocional desagradável associada a, ou semelhante àquela associada a, dano tecidual real ou potencial (IASP, 2020). De fato, é uma experiência complexa que representa uma das causas mais expressivas de sofrimento e deficiência no mundo. Entretanto, a dor também é um importante sintoma clínico para a detecção de diferentes patologias, visto que, age como um mecanismo de defesa do organismo.

O componente sensorial da dor é denominado nocicepção, e refere-se somente à percepção do sinal no SNC, evocado pela ativação de nociceptores,

que são receptores sensoriais especializados, provenientes de um tecido danificado. A nocicepção é o componente fisiológico da dor e compreende os processos de transdução, transmissão e modulação do estímulo nociceptivo. Uma vez instalado o estímulo nociceptivo, diversas alterações neuroendócrinas acontecem, promovendo um estado de hiperexcitabilidade do SNC e SNP. Diante disso, entre a exposição inicial ao estímulo nocivo até a percepção da dor há uma série de mecanismos complexos envolvidos. Sendo assim, o estímulo nociceptivo está diretamente relacionado a ação de mediadores e, alguns destes, são comuns ao processo inflamatório, especialmente, em casos de dor crônica. Após a lesão tecidual os nociceptores são ativados através da liberação de vários neurotransmisssores e neuromoduladores, tais como, glutamato, bradicinina, prostaglandinas, histamina, serotonina e citocinas. Como resultado, ocorre a sensibilização de fibras aferentes primárias que, por sua vez, são responsáveis por transmitir a informação da dor para os neurônios do corno-dorsal e, posteriormente, para o centro superior do cérebro, resultando no estabelecimento da dor. Por fim, após a percepção da dor nas regiões superiores do encéfalo, o sistema de antinocicepção é ativado pelo organismo através de vias descendentes inibitórias. Essas influenciam a transmissão do estímulo nociceptivo dos neurônios aferentes primários para SNC. Logo, tal mecanismo é responsável pela restauração da homeostasia (COLLOCA et al., 2017).

A dor tem início a partir de um estímulo nociceptivo, podendo ser classificada em aguda, crônica ou neuropática. A dor aguda é caracterizada por lesão tecidual provocada frequentemente por trauma, intervenção cirúrgica ou doença e pode durar de alguns dias a semanas, desaparecendo com a resolução da lesão. Por sua vez, a dor crônica permanece mesmo após a resolução do dano e pode durar de meses até anos, causando sofrimento e incapacidade (KING e FRASER, 2013; TAYLOR et al., 2016).

A dor neuropática, uma condição debilitante que acomete em torno de 10% da população mundial, é causada por meio de uma lesão ao sistema somatossenrorial, incluindo as fibras periféricas e/ou os neurônios centrais, de modo que o dano ocasionado, geralmente, excede a capacidade de reparo do organismo (SISIGNANO et al., 2014; FINNERUP et al., 2016; GILRON et al., 2015). A dor neuropática prejudica a qualidade de vida dos pacientes, sendo

assim transtornos de ansiedade, depressão e distúrbios do sono são comorbidades frequentemente associadas que acometem, com maior incidência, as mulheres e os idosos (FINNERUP et al., 2016; COLLOCA et al, 2017).

Diversas patologias prejudicam o sistema somatossensorial, mais especificamente a medula espinhal e/ou cérebro, causando dor neuropática central. Em contrapartida, a dor neuropática de origem periférica é comumente causada por patologias que acometem, principalmente, as fibras C não mielinizadas e as fibras A mielinizadas (COLLOCA et al., 2017). O manejo da dor neuropática é realizado com terapia medicamentosa e intervenções não medicamentosas, entretanto, na maioria das vezes, esses procedimentos são insuficientes para aliviar os sintomas. Uma vez que esse tipo de dor é frequentemente mais severo, as terapias clínicas disponíveis são muitas vezes inadequadas e sua eficácia é imprevisível, além de, na maioria dos casos apresentar efeitos adversos severos (KAMERMAN et al., 2015). Nesse sentido, o tratamento adequado e eficaz da dor neuropática é de difícil execução.

3.4 Neuropatia periférica

A neuropatia periférica é uma síndrome caracterizada por degeneração ou disfunção dos nervos periféricos (LUCZKOWSKA et al., 2018). A ampla variabilidade e elevada prevalência da neuropatia periférica estão relacionadas às suas diferentes causas (CALLAGHAN et al., 2015; MERKIES et al., 2015). Sabe-se que os diferentes padrões de neuropatias estão relacionados a mecanismos distintos, e que numerosos processos fisiopatológicos podem prejudicar a funcionalidade dos nervos periféricos. Processos metabólicos, imunológicos, traumáticos, genéticos, infecciosos, e tóxicos podem comprometer os nervos periféricos em múltiplos níveis e por meio de várias vias moleculares (CALLAGHAN et al., 2015; COLLOCA et al., 2017). O diabetes e o uso de agentes quimioterápicos estão entre as principais causas de polineuropatia sensorial distal (PAPANAS et al., 2011). De fato, com o aumento na incidência de câncer, a neuropatia periférica induzida por quimioterapia torna-se um problema cada vez mais relevante (FITZMAURICE et al., 2017; BARRELL e SMITH, 2019).

As patologias do sistema nervoso periférico são diversas e envolvem uma pluralidade de vias e mecanismos. Resumidamente, a neuropatia periférica altera as propriedades elétricas dos nervos sensoriais, o que conduz a um desequilíbrio entre a sinalização central excitatória e inibitória, de modo que neurônios inibitórios e sistemas de controle descendente são prejudicados. Por sua vez, a transmissão de sinais sensoriais e os seus mecanismos de desinibição são alterados (COLLOCA et al., 2017). Sob este aspecto, o diagnóstico da neuropatia periférica é baseado no reconhecimento de padrões, refletindo a localização neuroanatômica. As fibras de diâmetro menor, mielinizadas ou não mielinizadas, transmitem informações de dor térmica e mecânica. Nesse sentido uma lesão nestas fibras causa sensações de queimaduras, formigamentos, choques elétricos, hiperalgesia, ou seja, sensibilidade aumentada a estímulos dolorosos, e/ou alodinia, que é a sensação dolorosa causado por um estímulo inócuo. Por sua vez, as fibras mielinizadas com diâmetro médio ou grande transmitem sensações de vibração e posição articular, consequentemente um dano a estas fibras implica em dormência e desequilíbrio (STINO, 2017; BARRELL e SMITH, 2019).

Considerando, as dificuldades impostas pela neuropatia periférica, devido à (i) elevada prevalência, principalmente em idosos; (ii) dificuldade em realizar um diagnóstico decisivo, apesar das escalas, testes e análises disponíveis; e (iii) falta de tratamento adequado; evidencia-se que há a necessidade de mais estudos para suprir as falhas na compreensão e manejo desta patologia. Uma possibilidade de suprir essas falhas é avaliar de forma separada os mecanismos envolvidos na origem de cada tipo de neuropatia periférica.

3.5 Neuropatia periférica induzida por quimioterapia

As drogas quimioterápicas, atualmente, são a base do tratamento sistêmico do câncer. Entretanto, apesar da eficácia destes fármacos em limitar ou inibir o crescimento tumoral, a quimioterapia é frequentemente acompanhada de efeitos adversos. A neuropatia periférica induzida por quimioterapia torna-se um problema cada vez mais proeminente e oneroso, à medida que a incidência e a sobrevivência ao câncer aumentam. Este efeito adverso causado pelo tratamento com fármacos quimioterápicos é comum e

dependente da dose (DOUGHTY e BOWLEY, 2019; IBRAHIM e EHRLICH, 2020). A neuropatia periférica pode comprometer a terapia, uma vez que leva a redução da dose ou descontinuação do tratamento, além de diminuir severamente a qualidade de vida dos pacientes, constituindo a principal causa de dor persistente em sobreviventes do câncer (IBRAHIM e EHRLICH, 2020). De fato, apesar de alguns pacientes apresentarem melhora nos sintomas da dor neuropática induzida por quimioterápicos, após a interrupção do tratamento, a reversão absoluta é infrequente.

Os mecanismos envolvidos na ação antitumoral dos agentes antineoplásicos são relativamente bem compreendidos. Entretanto, os mecanismos subjacentes à neuropatia periférica induzida por quimioterapia, ainda não foram inteiramente elucidados (STAROBOVA e VETTER, 2017; IBRAHIM e EHRLICH, 2020). Inúmeros fatores são determinantes para a ocorrência da neuropatia periférica induzida pelos quimioterápicos, entre esses, a faixa etária dos pacientes e condições pré-existentes, como diabetes e obesidade, ou o estilo de vida, como tabagismo e uso crônico de álcool, são condições que podem aumentar o risco.

Do mesmo modo, o fármaco quimioterápico utilizado é considerado um preditivo de neuropatia periférica, sendo os principais a cisplatina, a oxaliplatina, a vincristina, o paclitaxel e o bortezomibe (BOYETTE-DAVIS et al., 2018). Os agentes antineoplásicos podem causar sintomas específicos que diferem em relação às características clínicas, a gravidade e a recuperação. Fármacos quimioterápicos, como os agentes à base de platina, taxanos, e alcalóides da vinca, que interferem no ciclo levando à morte celular e degradação do tumor, causam neuropatias periféricas graves (GRISOLD et al., 2012; STAROBOVA e VETTER, 2017; BOYETTE-DAVIS et al., 2018).

As diferenças na sintomatologia da neuropatia periférica, induzida por cada agente quimioterápico, não podem ser elucidadas exclusivamente através dos potenciais farmacológicos, metabólitos formados, e propriedades farmacocinéticas dos medicamentos. Nesse sentido, é fundamental que se compreenda especificamente os mecanismos envolvidos na neuropatia periférica induzida por cada um destes quimioterápicos. De modo geral, os processos fisiopatológicos subjacentes à neuropatia periférica induzida por quimioterapia são multifatoriais e envolvem disfunção mitocondrial,

mecanismos apoptóticos, alterações na homeostase do cálcio, degeneração do axônio e remodelação da membrana, além de processos imunes e neuroinflamatórios (ARETI et al., 2014; CALLAGHAN et al., 2015; STAROBOVA e VETTER, 2017).

Como discutido anteriormente, a intervenção clínica em pacientes com neuropatia periférica ainda representa um desafio (BARRELL e SMITH, 2019), e este fato se estende a neuropatia periférica induzida por quimioterapia. Além disso, outro fator a ser considerado para a intervenção farmacológica é a idade, uma vez que o envelhecimento é um problema para o gerenciamento de pacientes com neuropatia periférica induzida por quimioterapia. Pacientes idosos utilizam maior número de medicamentos, e a polifarmácia associada ao protocolo quimioterápico representa uma limitação para a prescrição de medicamentos para o tratamento dos sintomas clínicos da neuropatia, principalmente devido a preocupação com interações medicamentosas. Além disso, a Associação Americana de Geriatria publicou uma lista de medicamentos que devem ser evitados por idosos, o que limita ainda mais o número de medicamentos a disposição dos prescritores para o tratamento dos sintomas da neuropatia periférica induzida por quimioterapia (GUERARD et al, 2017; STAROBOVA e VETTER, 2017; IBRAHIM e EHRLICH, 2020).

De fato, inúmeros estudos têm sido realizados com o objetivo de encontrar um tratamento e/ou prevenção da neuropatia periférica induzida pela quimioterapia, entretanto até a presente data não houve transferências bem-sucedidas de resultados pré-clínicos para a prática clínica em termos de abordagens preventivas ou terapêuticas (IBRAHIM e EHRLICH, 2020). Contudo, avanços importantes na compreensão dos mecanismos implícitos, no manejo, e abordagem terapêutica foram alcançados (BOYETTE-DAVIS et al., 2018; CALLS et al., 2020; IBRAHIM e EHRLICH, 2020).

É consenso que uma boa comunicação entre o médico e o paciente é decisiva para o gerenciamento desta patologia, principalmente quando os pacientes são idosos. Inicialmente, o gerenciamento é baseado na modificação do regime quimioterápico, incluindo alteração na dose, ciclos de tratamento, tempo, forma de dosagem e duração (BOYETTE-DAVIS et al., 2018; IBRAHIM e EHRLICH, 2020). No entanto, estes procedimentos prejudicam o efeito farmacológico do agente antineoplásico. Intervenções não farmacológicas se

baseiam no uso de produtos naturais e mudanças no estilo de vida. Outro procedimento disponível é a intervenção farmacológica, que consiste principalmente na prescrição de inibidores da recaptação de serotonina-noradrenalina, fármacos indicados para o tratamento do transtorno bipolar, anticonvulsivantes e analgésicos. Com base no que foi exposto, diversas abordagens são utilizadas, entretanto, não fornecem um nível satisfatório de alívio nos sintomas para muitos pacientes, principalmente os idosos (STAROBOVA e VETTER, 2017; BOYETTE-DAVIS et al., 2018; IBRAHIM e EHRLICH, 2020).

A neuropatia periférica induzida por quimioterápicos é uma condição recursiva, caracterizada por dor neuropática e prejuízo das funções, sensitiva e motora. Avaliando o número crescente de sobreviventes do câncer e reconhecendo as limitações das terapias disponíveis, medicamentosas ou não, para o manejo da neuropatia periférica, uma análise crítica da literatura bem como estudos inovadores são essenciais para compreensão e gerenciamento apropriado desta síndrome dolorosa. Nesse sentido, buscar as especificidades envolvidas em cada padrão de neuropatia, esclarecer os mecanismos envolvidos em cada tipo de indução, e elucidar as particularidades de cada agente indutor podem ser definitivos na busca da prevenção ou tratamento eficaz para as neuropatias periféricas, incluindo a causada por agentes quimioterápicos.

3.6 Neurotoxicidade induzida pela oxaliplatina

A oxaliplatina é considerada um agente alquilante que inibe a síntese e a replicação do DNA por meio de ligações cruzadas estabelecidas pelos complexos de platina com células tumorais (VANDAMME et al., 2014). No entanto, o tratamento com a oxaliplatina pode provocar anomalias na membrana neuronal bem como na atividade dos canais iônicos e, de tal modo, causar transtornos neuronais graves. Além disso, uma característica única da oxaliplatina é a geração do metabólito oxalato, uma das poucas características que a diferenciam da cisplatina, e este tem sido proposto como o mecanismo responsável pelas diferenças farmacológicas e, também, pela severa sensibilidade ao frio induzida pela oxaliplatina (MIYAKE et al., 2016; STAROBOVA e VETTER, 2017).

A oxaliplatina ao ser administrada sofre, primeiramente, uma reação de deslocamento do grupo oxalato provocada por nucleófilos fracos (CVITKOVIC, 1998). A partir disso, reações não enzimáticas subsequentes originam intermediários instáveis, os quais tem o grupo 1,2-DACH-platina deslocado por hidrólise. Por fim, os metabólitos ativos formados são o monocloro-DACH platina, dicloro-DACH platina e monoaqua-1,2-DACH platina, moléculas que reagem com o DNA. Resumidamente, a oxaliplatina é convertida em oxalato e derivados de platina. Nos compostos derivados da platina, o grupo platina do metabólito ativo forma um complexo que se liga irreversivelmente às proteínas plasmáticas. Após a administração da oxaliplatina, 15% da platina é encontrada na circulação sistêmica, enquanto os 85% restantes estão distribuídos nos tecidos. Os complexos de platina, eliminados principalmente por via renal, apresentam um período de meia vida de 5 dias, entretanto, complexos de platina já foram identificados em pacientes décadas após a infusão (CALLS et al., 2020).

Os pacientes submetidos ao tratamento com a oxaliplatina frequentemente apresentam sintomas neurológicos agudos, os quais iniciam-se durante a infusão do fármaco e atingem o pico nas primeiras 24 a 48 horas que transcorrem a administração. Os sintomas podem desaparecer dentro de aproximadamente uma semana, contudo, infusões subsequentes podem torná-los crônicos (LEHKY et al., 2004). De fato, embora demonstrando uma eficiente atividade antitumoral, a oxaliplatina está associada com a neuropatia periférica grave. Diante disso, evidencia-se dois padrões de neurotoxicidade, a neurotoxicidade aguda que é compreendida como uma neuropatia sensorial caracterizada por sensibilidade ao frio, e a neurotoxicidade cumulativa tardia ou crônica que na maioria das vezes manifesta-se como parestesias dolorosas e dormência.

3.6.1 Neuropatia periférica aguda induzida pela oxaliplatina (NPAIO)

A NPAIO é uma síndrome neurotóxica aguda, transitória e, independe da dose administrada do fármaco, causa dor em aproximadamente 90% dos pacientes em tratamento com a oxaliplatina (STAROBOVA e VETTER, 2017). Os sintomas clínicos da NPAIO, que são caracterizados por parestesia grave, geralmente acometem a região oral, laringofaringe, mãos e pés. Além de

manifestarem-se logo nas primeiras horas de infusão do fármaco, os sintomas clínicos são intensificados pelo frio, e causam alterações na amplitude dos potenciais de ação dos nervos sensoriais.

Além disso, os testes de vibração e limiar de percepção sensorial térmica, também costumam ser prejudicados, bem como os parâmetros de refratariedade e superexcitabilidade (CALLS et al., 2020). Disfunções sensitivas e motoras são relatadas, tais como fraqueza generalizada e outros sinais de hiperexcitabilidade de nervos periféricos (LEONARD et al., 2005). Estes sintomas clínicos representam algumas das principais queixas dos pacientes em tratamento com a oxaliplatina.

As manifestações neurológicas agudas ocasionadas pela oxaliplatina podem apresentar resolução espontânea, o que é incomum, ou tornarem-se crônicas (CALLS et al., 2020). Estudos neurofisiológicos demonstraram que ocorre um aumento nos potenciais de ação motora na síndrome dolorosa da NPAIO, após um único estímulo elétrico, bem como descargas neuronais até 48 horas após a infusão da oxaliplatina (LEHKY et al., 2004). A neurotoxicidade periférica induzida por esse fármaco pode se desenvolver rapidamente, logo no início do tratamento, mas também de forma tardia. O fenômeno é conhecido como "efeito de inércia" e ocorre quando os sintomas clínicos surgem ou agravam-se até 6 meses após o término ou a interrupção do tratamento (BRIANI et al., 2014; VELASCO e BRUNA, 2014).

Evidências crescentes indicam que a hiperexcitabilidade dos neurônios sensoriais está relacionada a NPAIO, uma vez que, o tratamento com a oxaliplatina modula a atividade dos principais efetores da atividade elétrica neuronal. De fato, diversos estudos demonstraram que a oxaliplatina altera as propriedades eletrofisiológicas dos canais de sódio, potássio e cálcio dependentes de voltagem (GROLLEAU et al., 2001; SITTL et al., 2010; THIBAULT et al., 2012; CHIORAZZI et al., 2015; SCHMITT et al., 2018).

Além disso, outro mecanismo envolvido na suscetibilidade ao frio causado pela oxaliplatina é decorrente do aumento da sensibilidade dos canais iônicos potencial receptor transiente (TRP) à ação das espécies reativas de oxigênio, aumentadas pelo oxalato. O aumento da produção de espécies reativas foi observado em neurônios do gânglio da raiz dorsal durante a dor neuropática induzida pelo tratamento com oxaliplatina, possivelmente devido a

disfunção mitocondrial (ZHENG et al., 2011; ARETI et al., 2014; MIYAKE et al., 2016). De fato, inúmeras alterações são induzidas pelo tratamento com a oxaliplatina, entretanto a totalidade dos mecanismos moleculares envolvidos na NPAIO ainda não estão completamente elucidados.

3.6.2 Neuropatia periférica crônia induzida pela oxaliplatina (NPCIO)

A NPCIO é caracterizada por neurotoxicidade cumulativa ou crônica, que acomete em torno de 70% dos pacientes em tratamento com a oxaliplatina. Esta forma é dependende da dose da infusão de oxaliplatina, geralmente manifesta-se com doses acima de 540 mg/m^2, e com as repetições dos ciclos de tratamento. Os sintomas clínicos assemelham-se com os da NPAIO e incluem sensibilidade à temperatura, parestesias e disestesias das mãos e pés. Além disso, as atividades que exigem coordenação motora fina podem ser alteradas, com doses cumulativas superiores a 780 mg/m^2 (CERSOSIMO, 2005). A NPCIO é a principal causa para o ajuste de dose ou interrupção do tratamento com oxaliplatina (NICHETI et al., 2019).

Os compostos de platina apresentam elevada afinidade pelo sistema nervoso periférico, devido à dificuldade de permear a barreira hematoencefálica, o que faz com que os seus principais locais de depósito sejam os gânglios da raiz dorsal. Estudos têm demonstrado que o desenvolvimento da NPCIO pode ser em decorrência de exposições repetidas a oxaliplatina e, por episódios frequentes de NPAIO (STAROBOVA e VETTER, 2017; WEICKHARDT et al., 2011).

Nesse sentido, tem sido proposto que com as repetições no ciclo de tratamento, o conjunto de mecanismos que contribuem para o desenvolvimento da NPCIO incluem alterações na excitabilidade axonal e na homeostase do cálcio, assim como na sensibilização de canais TRP e dano oxidativo (ARETI et al., 2014; MILTENBURG e BOOGERD, 2014; STAROBOVA e VETTER, 2017; CALLS et al., 2020).

3.6.3 Mecanismos subjacentes a NPIO

A sensibilização dos canais de cátions TRPA$_1$ é um dos principais mecanismos envolvidos na NPAIO. Diversos estudos sugerem que as proteínas da família TRP desempenham um papel decisivo no desenvolvimento

de neuropatias periféricas, principalmente o canal $TRPA_1$ na NPIO (STAROBOVA e VETTER, 2017; CHUKYO et al., 2018; CALLS et al., 2020) (Figura 2). O $TRPA_1$ é um nociceptor polimodal ativado por ERO que desempenha um papel importante na geração da dor, uma vez que é um mediador da alodínia causada pelo frio (MIYAKE et al., 2016; NAZIROGLU e BRAIDY, 2017). O frio excessivo estimula a produção mitocondrial de ERO, contudo é necessária uma sensibilização prévia do canal, uma vez que somente as ERO não são capazes de ativar o $TRPA_1$. Após a infusão da oxaliplatina, o metabólito oxalato causa inibição da atividade da proteína prolil hidroxilase, o que impede a hidroxilação do resíduo de prolina do $TRPA_1$ e conduz a um estado potencializado da sensibilidade deste canal iônico. Assim, a indução da sensibilidade ao frio observada na NPAIO é causada, principalmente, por meio da associação entre dois fatores, o aumento da sensibilização do $TRPA_1$, causado pelo oxalato, e o aumento da produção mitocondrial de ERO, provocado pela oxaliplatina (MIYAKE et al., 2016).

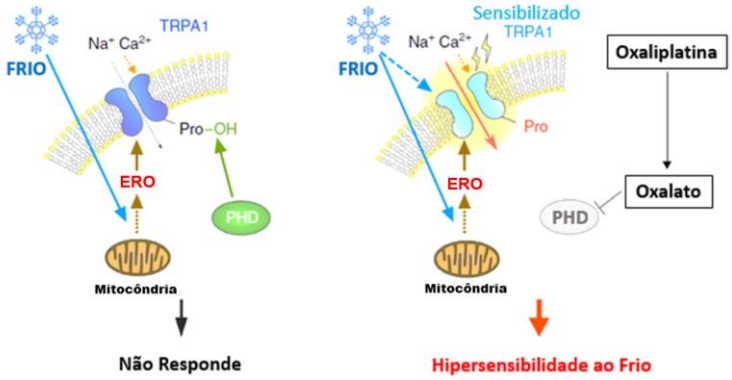


Figura 2. Representação esquemática da hipersensibilidade aguda ao frio desencadeada pela oxaliplatina e mediada por $TRPA_1$ (Adaptado a partir de MIYAKE et al., 2016). Espécies reativas de oxigênio (ERO); Prolil hidroxilase (PHD).

Está bem estabelecido que a ativação do canal iônico $TRPA_1$ está relacionada à sensibilidade térmica e dor neuropática induzida pelo frio na NPIO. Alem disso, foi demonstrado por CHUKYO e colaboradores (2018) que a infusão de oxaliplatina, além de potencializar a sensibilização do canal $TRPA_1$, como descrito acima, também causa um aumento nos níveis de expressão deste canal catiônico em neurônios sensoriais presentes nos gânglios da raiz dorsal. Ainda, para corroborar a relação entre o $TRPA_1$ e a dor neuropática observada na NPIO, os autores demonstraram que o aumento nos níveis de

expressão do canal iônico $TRPA_1$ está diretamente relacionado à hiperalgesia causada pela NPIO, tanto mecânica quanto térmica (CHUKYO et al., 2018).

Outra hipótese amplamente aceita, subjacente aos mecanismos envolvidos na NPIO, está relacionada à hiperexcitabilidade dos neurônios sensoriais, uma vez que, a oxaliplatina altera a cinética dos principais efetores da atividade neuronal elétrica (CALLS et al., 2020). Inúmeros estudos têm buscado elucidar as alterações que este fármaco causa nos canais de sódio, potássio e cálcio dependentes de voltagem. Nesse sentido, foi demonstrado que a oxaliplatina aumentou as correntes de sódio e alterou a dependência da voltagem do canal de sódio, conduzindo a potenciais mais hiperpolarizados no gânglio da raiz dorsal (Figura 3A) (CALLS et al., 2020).

De fato, a NPIO está diretamente relacionada a ação do oxalato nos canais de sódio axonais (XIAO et al., 2012). O oxalato é capaz de quelar o cálcio extracelular, manter a abertura prolongada dos canais de sódio dependentes de voltagem, e, assim, interferir na despolarização dos neurônios sensoriais causando hiperexcitabilidade da membrana (GROLLEAU et al., 2001). Os canais de sódio dependente de voltagem estão sendo investigados como alvo promissor na busca por um tratamento para dor neuropática causada por oxaliplatina. Neste contexto, foi demonstrado que o tratamento com antagonistas dos canais de sódio reverte temporariamente a hiperalgesia mecânica e fria induzida pela oxaliplatina (EGASHIRA et al., 2010; KAWASHIRI et al., 2012).

Os canais de potássio dependente de voltagem, assim como os canais de sódio, são alvos importantes nos estudos envolvendo a NPIO. Estudos sugerem que a oxaliplatina altera a cinética dos canais de potássio dependentes de voltagem (Figura 3B) (KAGIAVA et al., 2008; SITTL et al., 2010; THIBAULT et al., 2012). Nesse sentido, KAGIAVA e colaboradores (2008) sugeriram que os canais de potássio dependentes de voltagem são inibidos após a infusão da oxaliplatina, e, com isso, ocorre uma redução na corrente de potássio axonal o que favorece a hiperexcitabilidade neuronal. (STILL et al., 2010; CALLS, 2020).

O trabalho desenvolvido por THIBAULT e colaboradores (2012) reitera esses resultados. Os autores demonstraram que, embora os mecanismos que levam a hiperexcitabilidade nos neurônios corticais observada na NPIO ainda

não tenham sido completamente elucidados, de fato a inibição dos canais de potássio voltagem dependente é um fator relevante para o desenvolvimento da dor neuropática causada pela NPCIO (THIBAULT et al., 2012).

Ainda, a fim de elucidar particularmente o envolvimento dos canais de cálcio voltagem dependente na NPIO, foi demonstrado que o tratamento com a oxaliplatina aumentou as correntes de cálcio e os níveis de expressão dos canais de cálcio dependente de voltagem, nos neurônios do gânglio da raiz dorsal (Figura 3C) (SCHMITT et al., 2018). Além disso, uma relação positiva entre o aumento dos canais de cálcio dependes de voltagem e vias de apoptose mediada pela caspase-3 foi observada. O oxalato é um agente quelante de cálcio, e por isso na NPIO ocorre um aumento na condutância de sódio, redução do potencial limiar e da resistência da membrana. Como consequência desses fenômenos, a hiperexcitabilidade conduz à dor neuropática. O uso de cálcio e magnésio antes da infusão de oxaliplatina foi avaliado como uma estratégia de prevenção para a dor neuropática na NPIO, entretanto nenhum benefício clínico consistente foi observado (JORDAN et al., 2016, STAROBOVA e VETTER, 2017).

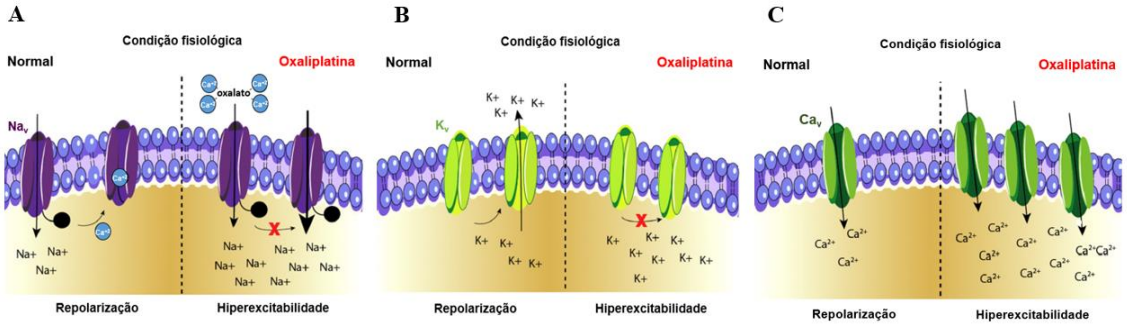


Figura 3. Representação esquemática das alterações causadas pela oxaliplatina nos canais de sódio (A), potássio (B) e, cálcio (C) dependentes de voltagem (Adaptado a partir de Calls et al., 2020).

De fato, o distúrbio na homeostase do cálcio tem sido proposto como um dos mecanismos envolvidos na gênese da dor neuropática proveniente da NPIO. A manutenção da concentração do cálcio intracelular é de suma importância, uma vez que, os íons cálcio desempenham um papel fundamental na regulação de inúmeros processos celulares, entre esses, a liberação de neurotransmissores, excitabilidade da membrana, plasticidade sináptica, bem como, a ativação de vias de apoptose e morte celular (ARETI et al., 2014). Nesse sentido, é importante salientar que SCHULZE e colaboradores (2011)

demonstraram que exclusivamente a exposição crônica à oxaliplatina alterou a sinalização intracelular de cálcio.

Recentemente foi sugerido que a apoptose é o principal mecanismo de morte celular envolvido na perda de neurônios sensoriais periféricos, posterior ao tratamento com a oxaliplatina. Diante disso, cabe salientar que a cascata apoptótica é iniciada, principalmente, a partir do dano estrutural não reparado ao DNA, devido aos adutos formados entre a platina e o DNA, bem como, em função de danos oxidativos ao DNA, causado por ERO que aumentam significativamente com a infusão de oxaliplatina (CALLS et al., 2020).

De fato, estudos têm demonstrado que o aumento dos adutos entre o DNA e a platina se correlaciona com o aumento dos fenômenos apoptóticos e com a atrofia neuronal (TA et al., 2006; YAN et al., 2015). O reparo por excisão de nucleotídeo (NER) é o mecanismo responsável por restaurar os adutos formados entre a platina e o DNA, enquanto o reparo por excisão de base (BER) representa o principal mecanismo responsável pela correção dos danos oxidativos causados ao DNA. Entretanto, no caso do tratamento com fármacos derivados da platina a eficácia dos mecanismos de reparo NER e BER é deficiente. Neste contexto, a ineficiência dos mecanismos de reparo tem sido avaliada como um mecanismo subjacente a NPIO, visto que a quantidade de adutos formados nos neurônios do gânglio da raiz dorsal, ou seja, danos ao DNA devido a ineficiência dos mecanismos de reparo, foi diretamente relacionada à gravidade da neuropatia periférica (DZAGNIDZE et al., 2007; CALLS et al., 2020).

De fato, o estresse oxidativo induzido pela disfunção mitocondrial destaca-se como um dos principais mecanismos envolvidos na NPIO. As mitocôndrias são organelas celulares responsáveis por diversos processos fisiológicos, incluindo a sinalização e armazenamento de cálcio, apoptose, regulação do potencial da membrana e produção de energia. A produção mitocondrial de adenosina trifosfato (ATP), via respiração aeróbica, é imprescindível para a manutenção das funções biológicas. Em condições normais, as mitocôndrias produzem ERO em quantidade necessária, uma vez que, essas moléculas/radicais desempenham funções importantes nas vias de sinalização celular. Entretanto, em condições patológicas, as mitocôndrias são um alvo para lesão induzida por ERO. De fato, uma correlação direta entre o

dano oxidativo e o comprometimento mitocondrial após o tratamento com a oxaliplatina e demais agentes de platina é amplamente aceita (Figura 4) (STAROBOVA e VETTER, 2017; CALLS et al., 2020).

A mitocôndria é particularmente susceptível a ação dos derivados de platina, porque os mecanismos fisiológicos de reparo do DNA, incluindo NER e BER como discutido acima, não protegem o DNA mitocondrial de formar adutos com a platina (CALLS et al., 2020). Assim, muitos estudos têm buscado elucidar a relação entre o dano mitocondrial e a neuropatia periférica induzida pelos agentes de platina (PARK et al., 2000; DI CESARE MANNELLI et al., 2013; KRUKOWSKI et al., 2017; STAROBOVA e VETTER, 2017; CALLS et al., 2020).

A oxaliplatina prejudica a replicação e a transcrição do DNA mitocondrial, levando à síntese proteica alterada e prejuízos funcionais na cadeia respiratória, causando aumento patológico na produção mitocondrial de ERO. Adicionalmente, o estresse oxidativo causa oxidação de biomoléculas intracelulares (enzimas, proteínas e lipídios). De fato, moléculas lipídicas, como as que compõe a bainha de mielina, são altamente susceptíveis ao dano oxidativo, por isso o tratamento com a oxaliplatina resulta em desmielinização dos nervos periféricos e crescente sensibilização dos processos de transdução de sinal (STAROBOVA e VETTER, 2017; CALLS et al., 2020).

Os danos ao DNA mitocondrial causado por derivados de platina, além de causar aumento na produção mitocondrial de ERO, conduzem a ativação de vias de apoptose. Inúmeros estudos buscam elucidar os mecanismos envolvidos na ativação destas vias, entretanto, a grande maioria utiliza a cisplatina com fármaco de escolha para investigação (CALLS et al., 2020). Sendo que, a apoptose é, principalmente, iniciada através do dano ao DNA mitocondrial, causado pela formação dos adutos entre a platina e o DNA, considera-se que ambos os fármacos, oxaliplatina e cisplatina, provocam o mesmo tipo de comportamento. Contudo, nesse contexto observa-se uma limitação na literatura.

ZANARDELLI e colaboradores (2015) demonstraram que o dano mitocondrial motivado pelo tratamento com a oxaliplatina, em células nervosas não tumorais, causou liberação citosólica do citocromo C, aumentou os níveis de radical ânion superóxido, e reduziu os níveis de expressão da proteína

antiapoptótica Bcl-2. Contudo, a oxaliplatina não alterou os níveis de expressão da capase-8, um dos principais iniciadores de apoptose. Neste estudo, os autores observaram que a oxaliplatina produz efeitos diferentes em células normais e tumorais. Ao se investigar o efeito da oxaliplatina sobre as células tumorais, foi observado um aumento na atividade da caspase-8, ativando a apoptose extrínseca, e um aumento na expressão de Bcl-2, o que bloqueou o dano mitocondrial nas células tumorais. É importante ressaltar, que os resultados obtidos no estudo definiram que há diferença entre os mecanismos envolvidos no efeito farmacológico em relação à neurotoxicidade induzida pela oxaliplatina.

Os dados acima citados sugerem que a ativação da via de apoptose mitocondrial prevalece nas células nervosas normais, após o dano induzido pela oxaliplatina, enquanto a via de apoptose extrínseca é ativada nas células tumorais. Esse fato pode indicar uma possível estratégia para o planejamento de novas terapias para o tratamento da NPIO sem alterar negativamente o efeito farmacológico da oxaliplatina (ZANARDELLI et al., 2015).

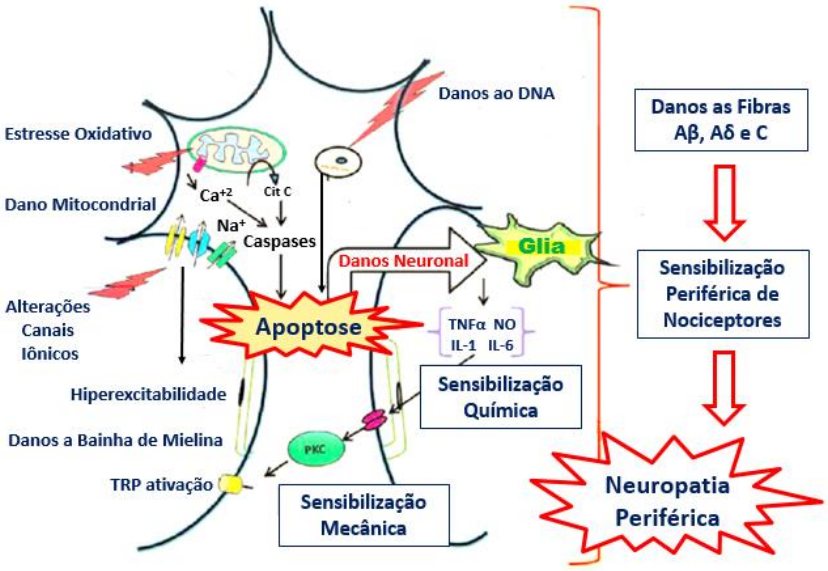


Figura 4. Representaçao esquemática dos mecanismos subjacentes a neuropatia periférica induzida por oxaliplatina (Adaptado a partir de ARETI et al., 2016). Òxido nítrico (NO); Interleucina (IL); Fator de necrose tumoral α (TNFα); Citocromo C (Cit C); Canal iônico potencial receptor transiente (TRP).

3.7 4-PSQ como estratégia terapêutica para a NPIO e suas comorbidades

Avanços significativos foram observados na compreensão dos mecanismos envolvidos na neurotoxicidade induzida pela oxaliplatina,

entretanto, a complexidade dos eventos contribui para a falta de uma terapia eficaz e segura para o tratamento. O tratamento para NPIO não deve interferir com os efeitos antitumorais da oxaliplatina, além de fornecer neuroproteção e alívio dos sintomas. Nesse sentido, as estratégias de tratamento são predominantemente baseadas na modificação do regime de quimioterapia e no manejo sintomático usando abordagens farmacológicas. Os medicamentos indicados incluem, principalmente, anticonvulsivantes (gabapentina, carbamazepina, oxcarbazepina, lamotrigina, topiramato, pregabalina) e antidepressivos (venlafaxina). No entanto, esses fármacos além de induzirem inúmeros efeitos adversos incluindo náusea e tontura, também, precisam de ajuste da dose em casos de insuficiência hepática e/ou renal ou são contraindicados para pacientes idosos. Além disso, não fornecem um nível satisfatório de alívio para muitos pacientes com NPIO, tornando necessárias pesquisas adicionais para identificar novas abordagens terapêuticas. A dor neuropática, o prejuízo cognitivo e emocional, a falta de alvos seletivos e fármacos específicos para o tratamento representam as principais limitações para o manejo dos pacientes. Em particular, o tratamento da NPIO nos idosos é ainda mais complexo, além da suscetibilidade destes pacientes às comorbidades associadas ao tratamento com a oxaliplatina (BANACH et al., 2017).

Sob este aspecto, o estresse oxidativo é identificado como um dos responsáveis pelos danos neuronais causados pela oxaliplatina. A neurodegeneração mediada pelo estresse oxidativo pode ser executada por diferentes mecanismos, tais como, depleção de defesas antioxidantes, dano a biomoléculas, desmielinização, neuroinflamação e morte neuronal por apoptose (ARETI et al., 2014). Diante disto, antioxidantes podem representar uma alternativa promissora para o tratamento da NPIO, em especial em pacientes idosos.

Neste sentido, destacam-se os compostos orgânicos de selênio que têm recebido a atenção dos pesquisadores por apresentarem síntese simples e atividades farmacológicas relevantes, visto que, alguns compostos possuem ação mimética à enzima glutationa peroxidase (BORTOLATTO et al., 2013; KUDVA et al., 2015; SARAIVA et al., 2016) e ações antioxidante, antinociceptiva e anti-inflamatória (NOGUEIRA e ROCHA, 2011; WILHELM et

al., 2014). De fato, diversos estudos revelaram compostos orgânicos de selênio com potencial antioxidante e ações efetivas sobre a dor e processos inflamatórios em modelos experimentais (WILHELM et al., 2009, 2014; BRUNING et al., 2010; NOGUEIRA e ROCHA, 2011; PINZ et al., 2016; SILVA et al., 2017).

Paralelamente aos compostos de selênio, a versatilidade da quinolina e seus derivados têm atraído grande atenção no campo do desenvolvimento de medicamentos. Uma variedade de derivados de quinolina tem sido utilizada como antiviral, anticâncer, antibacteriano, antifúngico, antiobesidade e agentes anti-inflamatórios (JUNG et al., 2012; MANTOVANI et al., 2014; MANERA et al., 2015). Especialmente, os derivados da 7-cloroquinolina são unidades biológicas ativas e exibem uma ampla gama de atividades farmacológicas (DE SOUZA et al., 2009; MACEDO et al., 2010; SINGH et al., 2012). Devido à sua importância como subestrutura em uma ampla variedade de produtos sintéticos e naturais, consideráveis esforços foram direcionados ao desenvolvimento de novas estruturas baseadas em 7-cloroquinolina.

Com base nas principais propriedades farmacológicas dos derivados de quinolina e selênio, nosso grupo de pesquisa juntamente com o Laboratório de Síntese Orgânica Limpa (LASOL), planejou a síntese do 4-PSQ (Figura 4), uma molécula com o núcleo quinolínico e um substituinte organosselênio. Desde então, nosso grupo de pesquisa (LaFarBio) tem buscado elucidar as propriedades farmacológicas do 4-PSQ em diversos modelos experimentais (PINZ et al., 2016; REIS et al., 2017; SILVA et al., 2017; DUARTE et al., 2017; PINZ et al., 2018; VOGT et al., 2018; VOSS et al., 2018; BARTH et al., 2019; LEMOS et al., 2020; LUCHESE et al., 2020; PALTIAN et al., 2020; DA MOTTA et al., 2021; RODRIGUES et al., 2021). Além disso, outros pesquisadores também têm dedicado esforços ao estudo desta molécula (SAVEGNAGO et al., 2013; SALGUEIRO et al., 2017; DE FREITAS COUTO et al., 2019; DE AQUINO SILVA et al., 2021). Inicialmente, o 4-PSQ apresentou efeito antioxidante *in vitro* (SAVEGNAGO et al., 2013). A partir disso, foi ampliada a investigação do potencial farmacológico do composto. O 4-PSQ demonstrou efeitos antinociceptivo, ansiolítico e, neuroprotetor em modelos animais e, as suas ações farmacológicas parecem estar relacionadas às propriedades antioxidante e anti-iflamatória e, também, à sua habilidade de modular os

sistemas serotoninérgico, nitrérgico, glutamatérgico e colinérgico (PINZ et al., 2016; SILVA et al., 2017; REIS et al., 2017; VOGT et al., 2018; BARTH et al., 2019; PALTIAN et al., 2020). Pinz e colaboradores (2016) evidenciaram a potente ação antinociceptiva e anti-inflamatória do 4-PSQ. Os resultados revelaram que o composto reduziu a nocicepção e a inflamação, em modelos animais agudos, com doses a partir de 0,1 mg Kg^{-1}. REIS e colaboradores (2017) evidenciaram que o 4-PSQ reduziu a captação de glutamato no córtex cerebral e protegeu contra o comportamento relacionado à ansiedade. Importamtemente, estudos prévios demonstraram que a atividade antioxidante contribui significativamente para o efeito neuroprotetor do 4-PSQ (VOGT et al., 2018; PINZ et al., 2018) e, para o seu efeito cicatrizante no tratamento de lesões cutâneas semelhantes a dermatite atópica (VOSS et al., 2018). O 4-PSQ exerce ações de aprimoramento de memória em ratos velhos por meio da modulação do sistema colinérgico e, por meio da modulação da plasticidade sináptica, aumentando a molécula de adesão de células neurais (NCAM) e os níveis de polissialiltransferase (BARTH et al., 2019). O potencial antioxidante do 4-PSQ se destacou também contra o dano oxidativo relacionado à senescência (LUCHESE et al., 2020). Corroborando estudos anteriors, Paltian e colaboradores (2020) demosntraram que o 4-PSQ elicita ação ansiolítica modulando os sistemas serotoninérgico, gabaérgico, além de, vias de neuroproteção e plasticidade neuronal associadas ao fator neurotrófico derivado do cérebro (BDNF). Recentemente, foi demonstrado que o 4-PSQ reverteu o dano hepático e renal induzido pela oxaliplatina, por meio da modulação do sistema antioxidante (LEMOS et al., 2020; DA MOTTA et al., 2021).

De fato, evidências crescentes demonstram que o 4-PSQ é um composto multialvo, com um significativo potencial antioxidante, sendo promissor no campo de desenvolvimento de medicamentos, principalmente porque exerce ações farmacológicas relevantes sem causar distúrbios locomotores e toxicidade nos rins, fígado e cérebro (PINZ et al., 2016; REIS et al., 2017). Neste contexto, o 4-PSQ pode ser uma promissora estratégia na busca de novos compostos com propriedades proeminentes para o tratamento da dor neuropática e das comorbidades causadas pela NPIO.

4 CAPÍTULOS

Os resultados que fazem parte desta tese estão divididos em capítulos e apresentados sob a forma de artigo e manuscrito. As seções materiais e métodos, resultados, discussão e referências encontram-se nos artigos e nos manuscritos, representando a íntegra deste estudo. Os estudos estão estruturados de acordo com as regras das revistas científicas.

Capítulo 1

Elucidação de mecanismos envolvidos na fisiopatologia da NPIO e do potencial farmacológico do 4-PSQ

Inicialmente, a dor neuropática causada pela NPIO, bem como o potencial farmacológico do 4-PSQ foram investigados em camundongos (etapa 1). Essa etapa do estudo foi fundamental para ampliar a compreensão sobre os mecanismos subjacentes a NPIO. Neste contexto, foi dada uma atenção especial ao impacto que a oxaliplatina causa nas defesas antioxidantes no SNC, uma vez que, o dano oxidativo causado pela oxaliplatina no SNP, principalmente no gânglio da raiz dorsal, tem sido amplamente estudado. Ainda, a modulação da via colinérgica na NPIO foi investigada por meio da enzima AChE. Destaca-se que o objetivo principal desta etapa foi definir se a oxaliplatina permeia a barreira hematoencefálica e, para isso a concentração de platina na medula espinhal dos camundongos foi determinada. Ainda, uma das principais metas desta etapa foi determinar o potencial farmacológico do 4-PSQ em reduzir a dor neuropática associada à NPIO.

Resultados relevantes obtidos pelo presente estudo ampliaram a compreensão sobre os mecanismos envolvidos na fisiopatologia NPIO, uma vez que, pela primeira vez, foi detectada platina na medula espinhal de camundongos tratados com a oxaliplatina. Portanto, aqui foi definido que a platina, proveniente da oxaliplatina, permeia o SNC através da barreira hematoencefálica (Figura 5). Nesta etapa, observou-se que o 4-PSQ reduziu a hipersensibilidade mecânica e térmica induzida pela administração da oxaliplatina. O 4-PSQ reverteu o aumento na produção de espécies reativas no SNC induzido pelo quimioterápico, principalmente por meio da modulação da atividade e expressão das enzimas antioxidantes glutationa peroxidase (GPx), superóxido dismutase (SOD) e, catalase (CAT). Foi observado que o tratamento com a oxaliplatina alterou a atividade e a expressão da AChE, sugerindo que a via colinernérgica pode estar implicada na fisiopatologia da neuropatia periférica induzida pela OXA. O 4-PSQ normalizou ambas atividade e expressão da AChE. De fato, o 4-PSQ apresentou resultados promissores frente ao dano induzido pela oxaliplatina. Portanto, o 4-PSQ pode ser um bom protótipo para o estudo de uma terapia mais eficaz para o tratamento da NPIO.

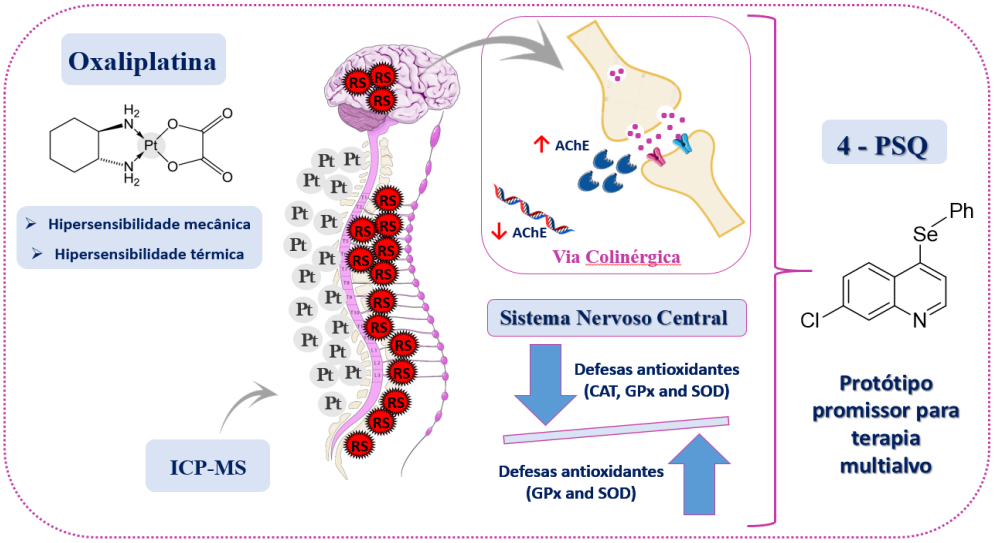


Figura 5. Representação esquemática dos principais resultados obtidos na etapa 1.

Artigo 1

Os resultados deste capítulo da tese estão apresentados sob a forma de artigo científico, o qual se encontra assim organizado. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se no próprio artigo.

O artigo científico encontra-se publicado na revista *Molecular Neurobiology.*

Molecular Neurobiology
https://doi.org/10.1007/s12035-020-02048-4

ORIGINAL ARTICLE

Advances in the Understanding of Oxaliplatin-Induced Peripheral Neuropathy in Mice: 7-Chloro-4-(Phenylselanyl) Quinoline as a Promising Therapeutic Agent

Angélica S. Reis[1] · Jaini J. Paltian[1] · William B. Domingues[2] · Diogo L. R. Novo[3] · Gabriel P. Costa[4] · Diego Alves[4] · Vinicius F. Campos[2] · Marcia F. Mesko[3] · Cristiane Luchese[1] · Ethel A. Wilhelm[1]

Received: 17 May 2020 / Accepted: 28 July 2020

Abstract

In this study, the deposition of platinum in oxaliplatin (OXA)-exposed mice and the effects of the oxidative damage on the central nervous system were investigated. The relationship between the reactive species (RS) levels as well as the expression and activity of enzymes, such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and acetylcholinesterase (AChE), in the development of peripheral neuropathy after OXA exposure, was evidenced. The effects of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on OXA-induced peripheral neuropathy was also investigated. *Swiss* mice received OXA (10 mg kg^{-1}) or vehicle by intraperitoneal route (days 0 and 2). Oral administration of 4-PSQ (1 mg kg^{-1}) or vehicle was performed on days 2 to 14. Behavioural tasks started on day 9, after the first OXA administration. It was observed that 4-PSQ reduced the mechanical and thermal hypersensitivity induced by OXA. 4-PSQ and OXA did not affect locomotor and exploratory activities. The results revealed, for the first time, a high concentration of platinum in the spinal cord of mice exposed to OXA. 4-PSQ reversed the increased levels of RS in the spinal cord, cerebral cortex and hippocampus of mice exposed to OXA. The alterations in the activity and expression of the GPx, SOD, CAT and AChE induced by OXA exposure were normalized by 4-PSQ. Therefore, the 4-PSQ might be a good prototype for the development of a more effective drug for the treatment of OXA-induced peripheral neuropathy. The results obtained by the present study expanded the knowledge about the mechanisms involved in the physiopathology of peripheral neuropathy.

Highlights
- A high platinum concentration in the spinal cord of mice exposed to OXA was revealed.
- Platinum deposition influenced oxidative damage on central nervous system of mice.
- 4-PSQ was a promising agent to reduce the hypersensitivity OXA-induced.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12035-020-02048-4) contains supplementary material, which is available to authorized users.

✉ Cristiane Luchese
cristiane_luchese@yahoo.com.br

✉ Ethel A. Wilhelm
ethelwilhelm@yahoo.com.br

[1] Programa de Pós-graduação em Bioquímica e Bioprospecção, Laboratório de Pesquisa em Farmacologia Bioquímica, CCQFA, Universidade Federal de Pelotas, UFPel, Pelotas, RS CEP 96010-900, Brazil

[2] Programa de Pós-graduação em Biotecnologia, Laboratório de Genômica Estrutural, Biotecnologia, Universidade Federal de Pelotas, UFPel, Pelotas, RS CEP 96010-900, Brazil

[3] Programa de Pósgraduação em Química, Laboratório de Controle de Contaminantes em Biomateriais, CCQFA, Universidade Federal de Pelotas, UFPel, Pelotas CEP 96010-900, Brazil

[4] Programa de Pós-graduação em Química, Laboratório de Síntese Orgânica Limpa, CCQFA, Universidade Federal de Pelotas, UFPel, Pelotas, RS CEP 96010-900, Brazil

Keywords Neuropathy · Selenium · Pain · Platinum · Acetylcholinesterase · Oxaliplatin

Abbreviations

4-PSQ	7-Chloro-4-(phenylselanyl) quinoline
OXA	Oxaliplatin
NMDA	N-methyl-D-aspartate
RS	Reactive species
CAT	Catalase
GPx	Glutathione peroxidase
SOD	Superoxide dismutase
AChE	Acetylcholinesterase
CNS	Central nervous system
NADPH	β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
ICP-MS	Inductively coupled plasma mass spectrometry

Introduction

Peripheral neuropathy is a common adverse effect caused by several chemotherapeutic drugs and is the main cause of ongoing pain in cancer survivors [1]. Currently, cancer is the second leading cause of death worldwide [2]. Despite the advancements in chemotherapy, there were 17.5 million cancer cases worldwide and over 8.8 million deaths due to cancer. Colorectal cancer is among the 3rd most common incident cancer in both sexes and the main cause of death from cancer for women [2]. Oxaliplatin (OXA) is a platinum-based chemotherapeutic agent widely used in colorectal cancer treatment [1].

Treatment with OXA induces various side effects, including peripheral neuropathy [3]. Peripheral neuropathy induced by OXA administration is the main dose-limiting toxicity, negatively affecting patients' quality of life and leading to treatment discontinuation [1]. The neurotoxic effects induced by OXA initially include the development of an acute neuropathy that occurs in approximately 85–96% of patients, and continued exposure to OXA can lead to the development of chronic peripheral neuropathy that occurs in around 40–93% of patients in treatment [3–5]. Many factors contribute to the variability in the incidence of peripheral neuropathy induced by OXA, such as the dose per cycle, the number of cycles and the duration of treatments [6, 7].

Despite the heterogeneity of the published results, several studies indicate that the incidence of peripheral neuropathy is higher in the treatment with OXA than with other chemotherapeutic drugs; besides, it shows a more complex symptom profile [1, 5–8]. Neuropathic pain induced by OXA is mainly caused by injury to the somatosensory nervous system, and various pathophysiological mechanisms, such as mitochondrial dysfunction [9], sensitization of the transient receptor potential (TRP) channels [10], oxidative damage [11], apoptosis through activation of mitogen-activated protein kinase (MAPK) pathway [12] and up-regulation of N-methyl D-aspartate (NMDA) receptors [13], are involved. All these actions of OXA alter the sensory neurons and lead to neuropathic pain [3, 4].

The mitochondrial dysfunction contributes to the accumulation of reactive species (RS) in the neurons that leads to the development and progression of peripheral neuropathy induced by chemotherapeutic [9, 14]. Besides that, it has been widely observed that platinum compounds cause depletion in enzymatic antioxidant defences [15]. Therefore, the increase of the RS associated with loss in the enzymatic antioxidant defences occurs with the accumulation of oxidant-damaged proteins and organelles, which might be responsible for neurodegeneration and pain observed in the peripheral neuropathy [4, 9, 15].

The management of neuropathic pain is generally focused on treating symptoms because the cause of the pain can be rarely treated, once a large number of known and unknown mechanisms are involved in the pathophysiology of neuropathic pain induced by OXA [3, 4]. The early approach to the management of a patient with neuropathic pain is to initiate pharmacological treatment; however, patients generally do not respond to the used therapy [16, 17]. Therefore, the development of new therapies, which can minimize the peripheral neuropathy caused by OXA treatment, is urgently needed [18]. In this context, 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) has been extensively studied by our research group [19–25] and some of us [26–29]. Among these studies, it was showed that this compound exerts memory enhancer actions in ageing rats through cholinergic system modulation [25]. 4-PSQ also elicits acute anti-inflammatory and antinociceptive effects that are correlated with its antioxidant property [19] and to the modulation of serotonergic, nitrergic and glutamatergic systems [21]. Reis et al. [20] evidenced that 4-PSQ reduced glutamate uptake in cerebral cortices and protected against kainate-induced anxiety-related behaviour in mice. Indeed, a growing body of evidences demonstrates that 4-PSQ is a promising multi-target compound in the field of drug development.

Thus, the present study was motivated by the following factors: (i) increased cancer survival rates and, consequently, sequelae caused by chemotherapy; (ii) pharmacological potential of 4-PSQ; and (iii) the need for studies that seek a better understanding of the factors that determine the development of chemotherapy-induced neuropathy. Considering these motivations, in this research, the deposition of platinum in the spinal cord of the mice and the influence on central nervous system (CNS) oxidative damage after OXA exposure were investigated. In addition, the relationship between the RS levels as well as the expression and activity of enzymes, such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and acetylcholinesterase (AChE), with the development of peripheral neuropathy after exposure to OXA/4-PSQ was studied.

Materials and Methods

Animals

All experiments were performed in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA 4506-2017), and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) and International Guiding Principles for Biomedical Research Involving Animals. The tests were carried out using male adult *Swiss* mice (25–30 g). This species has been used in several neuropathic pain studies [16, 30]. Animals were maintained at 22 ± 2 °C with free access to water and food, under a 12-:12-h light/dark cycle (with lights on at 6:00 a.m.); 7 animals were housed in each cage with the following dimensions: 34-cm width, 40-cm length and 16-cm height. Mice were acclimatized to the behaviour room for at least 1 h. All efforts were made to minimize the number of animals used and their discomfort.

The group size of animals used for each experiment was based on studies that used protocols such as those proposed in this research [16, 30]. The number of animals and intensities of noxious stimuli used were the minimum needed to demonstrate consistent effects of the treatments. Allocation concealment was performed using a randomization procedure (http://www.randomizer.org/). Behavioural evaluations were performed blindly on drug administration. All experiments were carried out between 08:00 and 17:00 h.

Drugs

4-PSQ was prepared and characterized in our laboratory. Nuclear magnetic resonance analysis (^{1}H and ^{13}C) showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 4-PSQ (99.9%) was determined by gas chromatography coupled with mass spectrometry (GC-MS) [26, 27] (see supplementary material).

4-PSQ and OXA were dissolved in canola oil and in 5% glucose solution, respectively. OXA was obtained from Eurofarma pharmaceutical company. All other chemicals used in this study were of analytical grade and obtained from Sigma-Aldrich, (St. Louis, MO, USA). Mice received treatments by oral (p.o.—intragastric gavage) or intraperitoneal (i.p.) routes at a constant volume of 10 mL kg^{-1} of body weight.

Experimental Design

Firstly, mice were randomly divided into four groups (8 animals/group): (i) control, (ii) OXA, (iii) 4-PSQ and (iv) 4-PSQ + OXA. On days 0 and 2, mice of the control and 4-PSQ groups received a 5% glucose solution (10 mL kg^{-1}, i.p.), whereas mice of the OXA and 4-PSQ + OXA groups received OXA (10 mg kg^{-1}, i.p.) at a dose of 10 mg kg^{-1}. From day 2 to day 14 of the experimental protocol, mice of the control and OXA groups received canola oil (10 mL kg^{-1}, p.o.), whereas mice of the 4-PSQ and 4-PSQ + OXA groups received 4-PSQ (1 mg kg^{-1}, p.o.), once a day. Twenty-four hours after the last treatment, the animals were euthanized by inhalation of isoflurane anaesthetic. The spinal cord, cerebral cortex and hippocampus samples were rapidly dissected, weighed and placed on ice then used for ex vivo assays (Fig. 1). OXA exposure caused a mortality around 8%. Given that the damage caused by OXA in regions such as the dorsal root ganglion and peripheral nerves is known, in this study, we search enhancing knowledge about other regions, mainly involved in pain pathways. The operators in the behavioural tests and data analysis were blinded. The dose and the protocol of 4-PSQ treatment were based on a previous study [23]. Since other studies evaluating the pharmacological actions of the 4-PSQ have been carried out [19–29], to minimize the number of animals used, a dose-response curve was not performed in the present study.

Behavioural Tests

Measurement of Mechanical Sensitivity

Mechanical sensitivity was carried out in mice according to the method previously described by Alamri et al. [31], with some modifications. For this test, mice were placed individually inside acrylic cages with wire grid floors 30 min before the start of testing performed in a quiet room. Before paw stimulation, the animals were quieted, without exploratory movements and not resting on their paws. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer (digital aesthesiometer, Insight, São Paulo, Brazil) adapted with a polypropylene tip. The paw withdrawal threshold was measured by applying the polypropylene tip perpendicular to the middle of the plantar surface of the hind paw at a constant progressive pressure until paw withdrawal, and the pressure value was automatically recorded.

Measurement of Thermal Sensitivity

Thermal sensitivity was tested in mice as reported by Woolfe and MacDonald [32], with some modifications. The hot plate test has been used for several years as a model of acute non-inflammatory nociception to investigate the central effect of analgesic drugs [32]. It is a behavioural model of nociception in which behaviours such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. For this, animals were placed in a glass box on a heated metal plate maintained at 52 ± 1 °C. The latency of nociceptive responses such as licking or shaking one of the paws or jumping was recorded

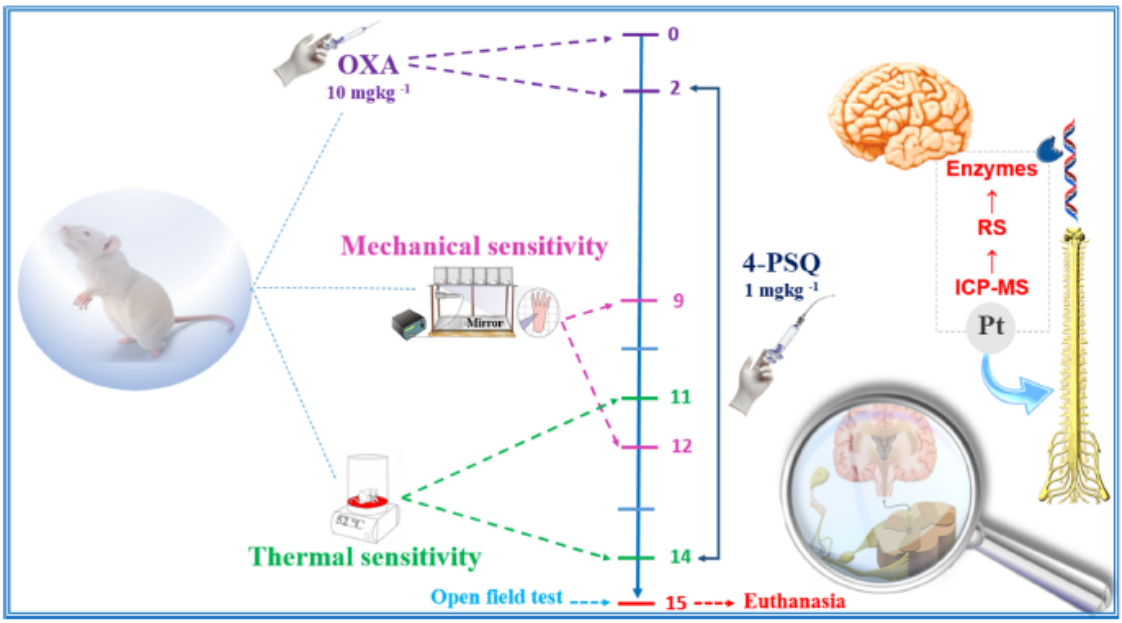


Fig. 1 Schedule of experimental protocol

as the reaction. To avoid damage to the paws of animals, time standing on the plate was limited to 45 s.

Assessment of Locomotor and Exploratory Domains

The open field test was performed on the fifteenth day of the experimental protocol to evaluate the general locomotor and exploratory behaviour of mice. The open field was made of plywood and surrounded by 30-cm-high walls. The floor of the open field, 45 cm long and 45 cm wide, was divided by masking tape markers into 9 squares (3 rows of 3). In this test, each animal was placed at the centre of the open field and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory (number of rearing on the hind limbs) activities [33].

Ex Vivo Assays

Motivated by behavioural test results, ex vivo assays were performed to extend the knowledge about the factors that determine the development of OXA-induced peripheral neuropathy and that contribute to therapeutic effect of 4-PSQ. Twenty-four hours after the last treatment, animals were killed by inhalation of isoflurane anaesthetic. Spinal cord samples were collected to determine platinum concentration. Additionally, samples of spinal cord, cerebral cortex and hippocampus were collected to determine oxidative stress markers, such as CAT, GPx and SOD activities, as well as RS levels. Furthermore, given that a decrease on cholinergic neurotransmission in neuropathic pain condition has been reported [34], the involvement of the AChE enzyme in the cerebral cortex and hippocampus was evaluated. For these analyses, samples were homogenized in 50 mmol L^{-1} TrisHCl pH 7.4 or, specifically for AChE activity, in 0.25 mol L^{-1} sucrose buffer (1:10 w v^{-1}) and centrifuged at 900 ×*g* for 10 min to yield a supernatant (S_1). Moreover, samples of cerebral cortex and hippocampus were collected to determine of CAT, GPx, SOD and AChE expressions using the qRT-PCR technique.

Neuropathic pain is a debilitating condition, whose brain circuit remains poorly understood. There is experimental evidence that demonstrated altered functioning of the spinal cord and supraspinal regions, including the hippocampus and cerebral cortex, associated with this condition. For example, neuropathic pain causes chronic stress that induces a neuroinflammatory response in the hippocampus [35, 36]. Also, it has been largely suggested that disturbance in the cerebral cortex areas pain-modulation circuits may be crucial for the maintenance of neuropathic pain [37–39]. Indeed, the development of neuropathic pain includes several mechanisms that extend from the periphery to the CNS through connections of the spinal cord [40, 41].

Determination of Platinum in Spinal Cord

Nitric acid (Merck, Germany) and hydrochloric acid used during sample preparation and determination steps were purified using a sub-boiling system (Duopur, Milestone, Italy). Ultrapure water (18 MΩ cm) was obtained from a purification system (Mega Up, Megapurity, South Korea). The reference standard solution of platinum (1000 μg mL^{-1}, in 10% HCl, SPEX CertiPrep, USA) was used to prepare the external calibration curve (0.01 to 10 μg L^{-1}) for the determination using inductively coupled plasma mass spectrometry (ICP-MS) and

also to perform the recovery tests for accuracy evaluation. High-purity argon (99.998%, White Martins, Brazil) was used for plasma generation, nebulization and as auxiliary gas in the determination step.

Mice spinal cord masses were measured on an analytical balance (AUY220, Shimadzu, Philippines), with a maximum load of 220 g and a resolution of 0.0001 g. The digestion of samples was performed in a water bath (Thermomix BM - B, Braun Biotech International, Germany). The determination of platinum was performed using an inductively coupled plasma mass spectrometer (NexION 300X, Perkin-Elmer, Canada), equipped with a concentric nebulizer (Meinhard Associates, USA), a cyclonic spray chamber (Glass Expansion Inc., Australia) and a quartz torch with a quartz injector tube (2 mm i.d.). Instrumental performance was optimized following previous work published in the literature [42, 43].

The digestion procedure was performed using around 90 mg of mice spinal cord, 250 μL of HNO_3 and 750 μL of HCl in 2 mL microtubes. The microtubes were heated in a water bath (± 95 °C) for 2 h. The final digests were diluted with water up to 2 mL, and the platinum concentration was determined by ICP-MS using isotope 194 [42, 43]. Six independent experiments for each group were performed. Results were expressed considering the spinal cord weight (nanograms of platinum per gramme of spinal cord weighted). Limit of detection (LOD) was calculated from the mean of the blank values plus three times the standard deviation obtained for ten replicates of the blank. The sample mass (90 mg) and the final volume of the digests (2 mL) were also taken into account during LOD calculation.

Involvement of Oxidative Stress

RS Levels

The RS levels were determined using a spectrofluorimetric method, using 2′,7′-dichlorofluorescein diacetate (DCHF-DA) assay according to Loetchutinat et al. [44]. S_1 (50 μL) was incubated with 20 μL of DCHF-DA (1 mmol L^{-1}) and 2430 μL of Tris HCl (10 mmol L^{-1}) in pH 7.4. The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 525 nm (with 488-nm excitation) 60 min after the addition of DCHF-DA to the medium (Shimadzu RF-5301PC fluorometer). RS levels were expressed as arbitrary units of fluorescence.

Antioxidant Enzymes

CAT Activity

CAT activity was measured spectrophotometrically using the method of Aebi [45], which involves monitoring the consumption of hydrogen peroxide (H_2O_2) in the presence of S_1 at 240 nm. The enzymatic reaction was initiated by adding an aliquot of S_1 (100 μL) and the substrate (H_2O_2, 105 μL) to a concentration of 0.3 mmol L^{-1} in a medium containing 50 mmol L^{-1} potassium phosphate buffer and pH 7.0. The enzymatic activity was expressed in units per milligramme of protein (1 U decomposes 1 mmol of H_2O_2 per minute at pH 7 at 25 °C).

GPx Activity

GPx activity was assayed spectrophotometrically by the method of Wendel [46], which involves monitoring of the dismutation of H_2O_2 in the presence of S_1 at 340 nm. S_1 (50 μL) was added in a system composed by reduced glutathione (GSH)/NADPH/glutathione reductase (GR), and the enzymatic reaction was initiated by the addition of H_2O_2 (100 μL). In this assay, the enzymatic activity is indirectly measured by NADPH decay. H_2O_2 is reduced and generates oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by the GR present in the analysis medium at the expense of NADPH. The enzymatic activity was expressed as nanomoles per minute per milligramme of protein.

SOD Activity

This method is based on the capacity of SOD to inhibit autoxidation of epinephrine. SOD activity was measured spectrophotometrically according to Misra and Fridovich's method [47]. S_1 (6, 12 or 18 μL) was added to a 0.05 mol L^{-1} Na_2CO_3 buffer, and the enzymatic reaction was started by adding epinephrine (30 μL). The colour reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26 °C. The enzymatic activity was expressed as units per milligramme of protein.

Involvement of AChE Enzyme

The AChE activity was assayed following a modified method of Ellman [48], using acetylthiocholine as the substrate. The reaction mixture (2 mL final volume) contained S_1 (100 μL), 100 mM K^+-phosphate buffer, pH 7.5 and 1 mM 5,5′-dithio-bis-nitrobenzoic acid (DTNB). The method is based on the formation of the yellow anion, 5,5′-dithio-bis-nitrobenzoate, measured spectrophotometrically at 412 nm during 2 min. The enzyme was pre-incubated for 2 min at 25 °C. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide. The enzymatic activity was expressed as micromolars per hour per milligramme of protein.

RNA Extraction, cDNA Synthesis and Quantitative Real-time Polymerase Chain Reaction

Total mRNA was extracted from thawed samples of hippocampus and cerebral cortex weighing between 50 and 70 mg using TRIzol reagent (Invitrogen™, Carlsbad, USA), followed by DNase treatment with DNase I Amplification Grade (Invitrogen™, Carlsbad, USA) to ensure minimum DNA contamination of the samples. The total RNA isolated was quantified, and its purity (260/280 and 260/230 ratios) was examined using a NanoVue spectrophotometer (GE, Fairfield, CT, USA).

The cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription (AppliedBiosystems™, UK) according to the manufacturer's protocol. For reverse transcription, 2 μg of total RNA was used in a reaction volume of 20 μL. The amplification was made with GoTaq® qPCR Master Mix (Promega, Madison, WI) using the Agilent Mx3005P qPCR System (Agilent Technologies Inc., Santa Clara, CA). The sequence of primers used is indicated in Table 1. The qPCR conditions were as follows: 10 min at 95 °C to activate the hot-start Taq polymerase, followed by 35 cycles of denaturation for 15 s at 95 °C, primer annealing for 60 s at 60 °C and extension for 30 s at 72 °C (fluorescence signals were detected at the end of every cycle). Baseline and threshold values were automatically set by the Stratagene MxPro software.

The number of PCR cycles required to reach the fluorescence threshold in each sample was defined as the Ct value, and each sample was analysed in duplicate to obtain an average Ct for each sample. The $2^{-\Delta\Delta CT}$ method was used to normalize the fold change in gene expressions [53], using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene.

Protein Determination

The protein concentration was measured spectrophotometrically at 595 nm by the method of Bradford [54], using bovine serum albumin as the standard. It is a rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. The reaction mixture contained S1 (50 μL) and Coomassie brilliant blue (2.5 mL). The reaction mixture was incubated for 10 min. The protein level was expressed as milligrammes of protein per millilitre.

Data and Statistical Analysis

The normality of the data was evaluated using the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed using GraphPad Prism 6.0 software (San Diego, CA, USA). Data were analysed by one-way analysis of variance (ANOVA) followed by the Tukey or Newman–Keuls test when appropriated for parametric data. Data were expressed as mean ± standard error of the mean (S.E.M.). Post hoc tests were performed only when the F value achieved the necessary level of statistical significance ($P < 0.05$) and when there was no significant variance in homogeneity. All data were evaluated for outliers (ROUT ($Q = 1.0\%$)). The outliers were excluded from the results. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology [55].

Results

Reproducibility of OXA-Induced Mechanical and Thermal Sensitivities

OXA administration (10 mg kg^{-1}, i.p.) produced robust and reliable mechanical and thermal sensitivities in *Swiss* mice (Fig. 2). These effects lasted for at least 12 ($F_{(3,\ 28)} = 69.96$, $P < 0.0001$) or 14 ($F_{(3,\ 28)} = 4.589$, $P < 0.01$) days post initiation of administration. On the evaluated days, the peak of the allodynic effect was observed on day 9 ($F_{(3,\ 28)} = 77.22$, $P < 0.0001$) for the mechanical sensitivity, and on day 11 ($F_{(3,\ 28)} = 6.707$, $P < 0.01$) for the thermal sensitivity. A reduction of 58% in the paw withdrawal threshold in the mice

Table 1 Primers used for quantitative real-time polymerase chain reaction. Listed are the forward and reverse primer sequences used to amplify each target gene as well as the GAPDH endogenous control

Primer Name	Sequence	Reference
CAT forward	5′AGAGAGCGGATTCCTGAGAGA3′	[49]
CAT reverse	5′ACCTTTCCCTTGGAGTATCTG3′	
SOD forward	5′GGACCTCATTTTAATCCTCAC3′	
SOD reverse	5′TGCCCAGGTCTCCAACATG3′	
AChE forward	5′TTAGGGCTGGGATATAATACGAC3′	[50]
AChE reverse	5′GCCCCTAGTGGGAGGAAGT3′	
GPx forward	5′TGTGGAAATGGATGAAAGTCCAG3′	[51]
GPx reverse	5′CATGGGACCATAGCGCTTCAC3′	
GAPDH forward	5′TGCGACTTCAACAGCAACTC3′	[52]
GAPDH reverse	5′ATGTAGGCAATGAGGTCCAC3′	

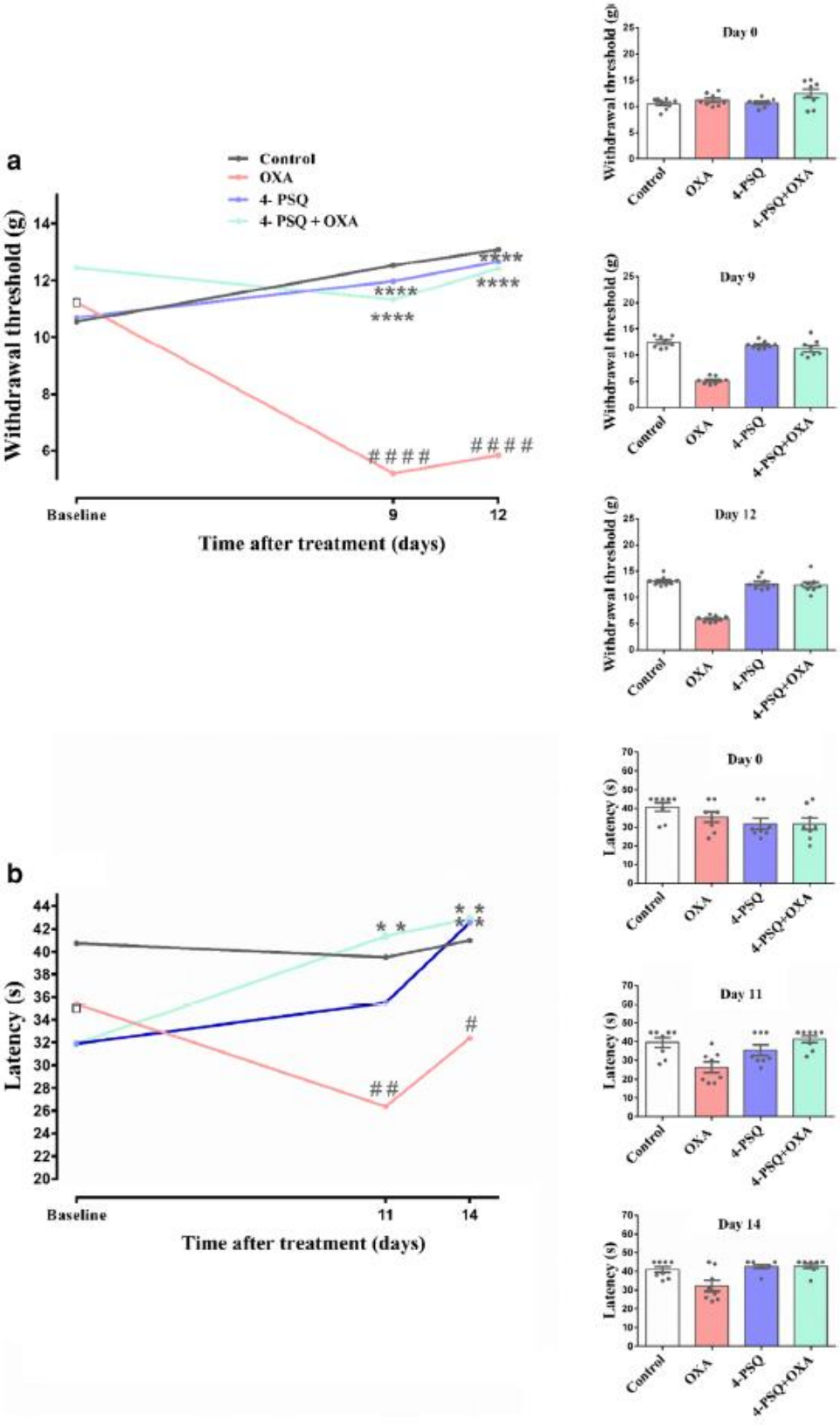


Fig. 2 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the **a** paw withdrawal threshold to mechanical stimulus in the von Frey test and **b** on the latency to thermal stimulus in the hot plate test. Each point represents the mean of 8 mice in each group. (*) $P < 0.05$, (**) $P < 0.01$ and (****) $P < 0.0001$ denotes significance levels when compared with the OXA group; (##) $P < 0.01$ and (####) $P < 0.0001$ denote significance levels when compared with the control group (one-way ANOVA followed by Tukey's test)

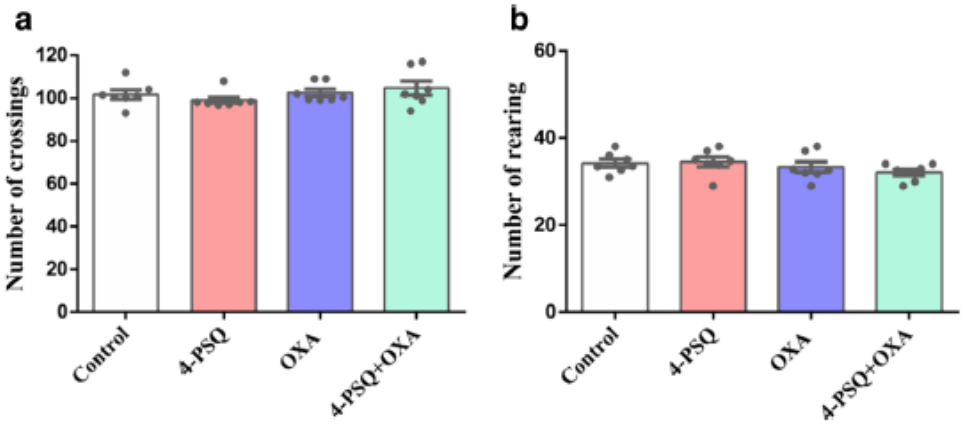


Fig. 3 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the **a** number of crossings and **b** number of rearings in the open field test. Each column represents the mean ± S.E.M. of 7 mice in each group (one-way ANOVA followed by Tukey's test)

exposed to OXA was verified, characterizing a mechanical hypersensitivity. In turn, OXA administration (10 mg kg^{-1}, i.p.) produced a reduction of the latency on the hot plate test by 33%, characterizing the thermal hypersensitivity in the mice, corroborating with the results of the mechanical sensitivity.

Effect of 4-PSQ on OXA-Induced Mechanical and Thermal Sensitivities

The animals that were treated with 4-PSQ (1 mg kg^{-1}, p.o.) demonstrated a reduction in the mechanical hypersensitivity induced by OXA, with maximum inhibition of 117% and 112% on days 9 ($F_{(3,\ 28)} = 77.22$, $P < 0.0001$) and 12 ($F_{(3,\ 28)} = 69.96$, $P < 0.0001$), respectively. The mechanical sensitivity of animals in the 4-PSQ + OXA group was similar to that of the control group (Fig. 2a). No significant difference was observed in the 4-PSQ group when compared with the control group (Fig. 2a).

The 4-PSQ treatment effect (1 mg kg^{-1}, p.o.) on the hot plate test is demonstrated in Fig. 2b. These results revealed that the treatment with 4-PSQ protected against OXA-induced thermal hypersensitivity. Analysis of the results from days 11 ($F_{(3,\ 28)} = 6.707$, $P < 0.01$) and 14 ($F_{(3,\ 27)} = 6.681$, $P < 0.01$) indicated that 4-PSQ increased the latency time by 56% and 32%, respectively, when compared with the OXA group.

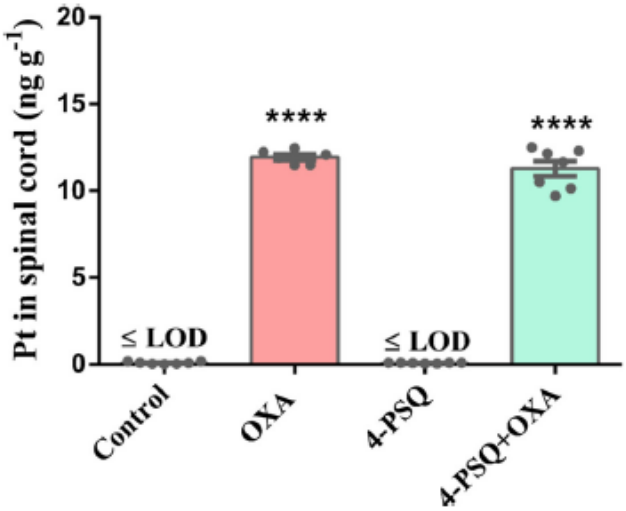


Fig. 4 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the deposition of platinum in the spinal cord of mice. Each column represents the mean ± S.E.M. of 6–7 mice in each group. (****) $P < 0.0001$ denotes significance levels when compared with the control group and ≥LOD denotes below detection limit (one-way ANOVA followed by Tukey's test). Limit of detection: 0.1 ng g^{-1}.

Effect of 4-PSQ and OXA on Locomotor and Exploratory Behaviour

The number of crossings and rearings in the open field is presented in Fig 3 a and b, respectively. The data analysis revealed that the treatment of mice with 4-PSQ and/or OXA did not cause any significant change in the number of crossings (ANOVA $F_{(3,24)} = 1.054$, $P > 0.05$) or rearing (ANOVA $F_{(3,24)} = 1.188$, $P > 0.05$) (Fig. 3a, b, respectively).

Deposition of Platinum in the Spinal Cord of the Mice after OXA Exposure and Effect of 4-PSQ

The results of the present study revealed, for the first time, high concentration of platinum in the spinal cord of mice exposed to OXA (10 mg kg^{-1}, i.p.) (Fig. 4) ($F_{(3,\ 23)} = 792.4$, $P < 0.0001$). An increase in the platinum levels of 175 times in the spinal cord was observed in animals exposed to OXA when compared with the control group. The results presented in Fig. 4 show that treatment with 4-PSQ (1 mg kg^{-1}, p.o.) did not reduce the concentration of platinum in the spinal cord of the mice when compared with the OXA group.

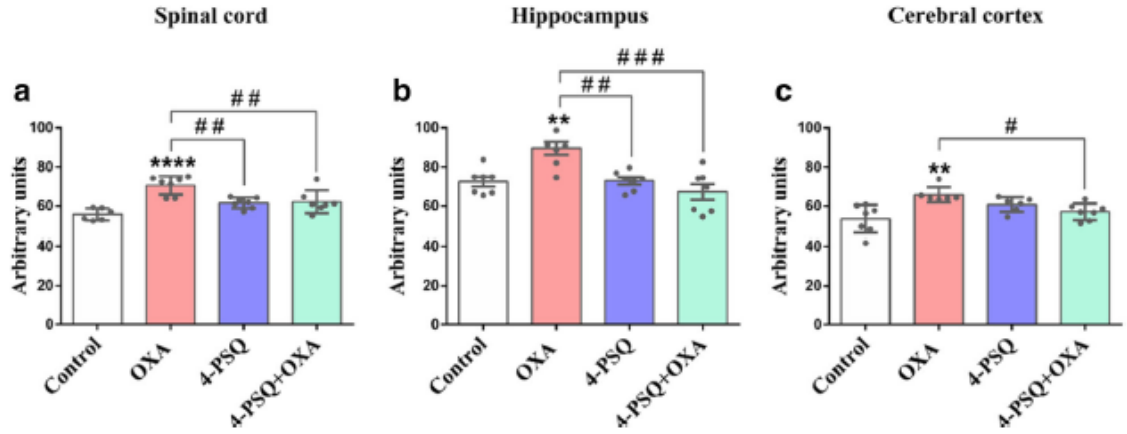


Fig. 5 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the RS levels spinal cord (**a**), hippocampus (**b**) and cerebral cortex (**c**) of mice. Each column represents the mean ± S.E.M. of 6–7 mice in each group. (**) $P<0.01$ and (****) $P<0.0001$ denote significance levels when compared with the control group; (##) $P<0.01$ and (###) $P<0.001$ denote significance levels when compared with OXA group (one-way ANOVA followed by Tukey's test)

Involvement of Oxidative Stress on OXA-Induced Peripheral Neuropathy in Mice and Antioxidant Effect of 4-PSQ

To confirm the contribution of oxidative stress to OXA-induced peripheral neuropathy, RS levels, activity and/or expression of antioxidant enzymes (CAT, GPx and SOD) were evaluated in the spinal cord, cerebral cortex and hippocampus of mice (Fig. 5). An increase in the levels of RS was detected in the spinal cord (27%) (Fig. 5a) ($F_{(3,\ 23)}=13.11$, $P<0.0001$), hippocampus (24%) (Fig. 5b) ($F_{(3,\ 24)}=10.06$, $P<0.001$) and cerebral cortex (23%) (Fig. 5c) ($F_{(3,\ 22)}=7.128$, $P<0.01$) of mice exposed to OXA (10 mg kg^{-1}, i.p.) on the day 15 of the experimental protocol, compared with mice of the control group. The results showed that treatment with 4-PSQ (1 mg kg^{-1}, p.o.) reversed the increase in the RS

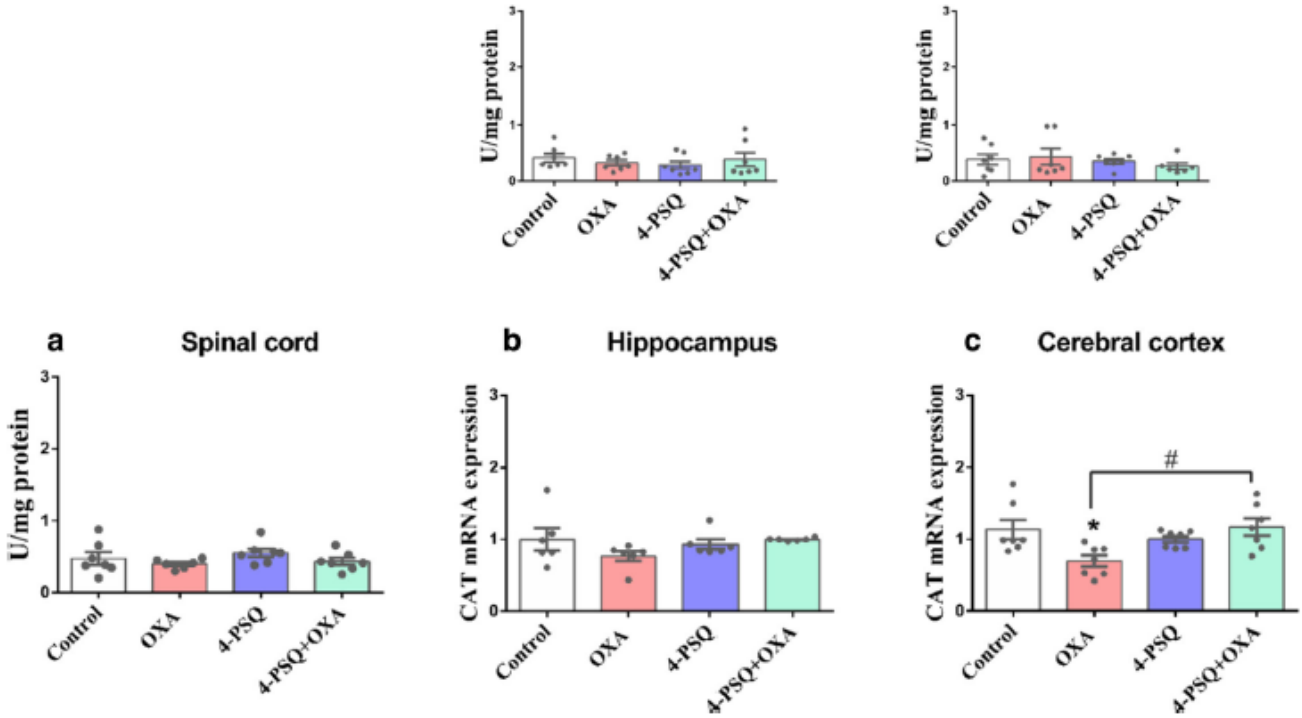


Fig. 6 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the catalase activity in the spinal cord (**a**), and expression levels in the hippocampus (**b**) and cerebral cortex(**c**) of mice. Inserts show the effect of 4-PSQ (1 mg kg^{-1}, p.o.) and OXA (10 mg kg^{-1}, i.p.) on the catalase activity in the cerebral cortex and hippocampus of mice. Each column represents the mean ± S.E.M. of 6–7 mice in each group. (*) $P<0.05$ denotes significance levels when compared with the control group; (#) $P<0.05$ denotes significance levels when compared with OXA group (one-way ANOVA followed by Tukey's test)

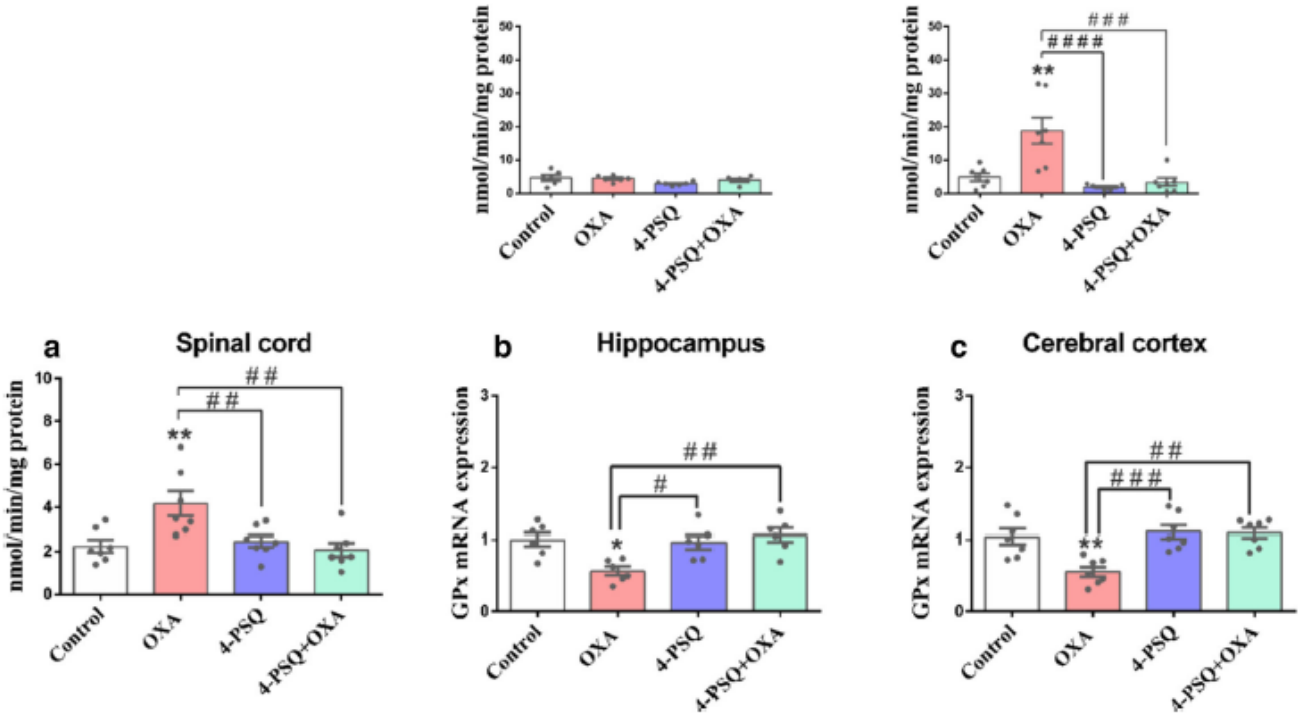


Fig. 7 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on glutathione peroxidase activity in the spinal cord (**a**), and hippocampus (**b**) and cerebral cortex (**c**). Inserts show the effect of 4-PSQ (1 mg kg^{-1}, p.o.) and OXA (10 mg kg^{-1}, i.p.) on the glutathione peroxidase activity in the cerebral cortex and hippocampus of mice. Each column represents the mean ± S.E.M. of 6–7 mice in each group. (*) $P < 0.05$ and (**) $P < 0.01$ denote significance levels when compared with the control group; (#) $P <$ and (##) $P < 0.01$ 0.05, (###) $P < 0.001$, (####) and $P < 0.0001$ denote significance levels when compared with OXA group (one-way ANOVA followed by Tukey's test)

levels induced by OXA in all tissues evaluated, when compared with the OXA group.

Figure 6 summarizes the results obtained regarding the activity and expression of the enzyme CAT analysed after the administration of OXA (10 mg kg^{-1}, i.p.) and/or treatment with 4-PSQ (1 mg kg^{-1}, p.o.). CAT activity remained unchanged in the spinal cord (Fig. 6a) ($F_{(3,\ 23)} = 1.217$, $P > 0.05$), cerebral cortex (insert Fig. 6c) ($F_{(3,\ 23)} = 0.4957$, $P > 0.05$) and hippocampus (insert Fig. 6b) ($F_{(3,\ 24)} = 0.4968$, $P > 0.05$) of the mice after the treatments. A reduction of 39% in the CAT expression (Fig. 6c) in the cerebral cortex of mice exposed to OXA was observed ($F_{(3,\ 24)} = 4.577$, $P < 0.05$). The treatment with 4-PSQ (1 mg kg^{-1}, p.o.) was effective in protecting against this alteration. No alteration in the CAT expression in the hippocampus (Fig. 6b) ($F_{(3,\ 20)} = 1.45$, $P > 0.05$) of mice exposed to OXA and/or 4-PSQ was observed.

OXA administration significantly increased GPx activity in the spinal cord (Fig. 7a) ($F_{(3,\ 24)} = 6.550$, $P < 0.01$) and cerebral cortex of mice (insert Fig. 7c) ($F_{(3,\ 24)} = 13.17$, $P < 0.0001$), to 87% and 291%, respectively. In the mice hippocampus, no alteration in the GPx activity was observed after the treatments, when compared with the control group (insert Fig. 7b) ($F_{(3,\ 20)} = 1.987$, $P > 0.05$). On the other hand, the analysis of the results revealed that the GPx expression of mice exposed to OXA decreased by 46% and 44% in the cerebral cortex (Fig. 7c) ($F_{(3,\ 24)} = 9.209$, $P < 0.001$) and hippocampus (Fig. 7b) ($F_{(3,\ 20)} = 6.509$, $P < 0.01$), respectively. OXA-induced alterations in the activity and expression of the GPx, in all tissues evaluated, were normalized to control levels by 4-PSQ treatment (1 mg kg^{-1}, p.o.) (Fig. 7a–c).

As shown in Fig. 8, OXA administration increased the SOD activity to 24% and 63% in the spinal cord (Fig. 8a) ($F_{(3,\ 24)} = 10.52$, $P < 0.0001$) and hippocampus (insert Fig. 8b) ($F_{(3,\ 24)} = 13.11$, $P < 0.0001$), respectively, of mice. SOD activity in the cerebral cortex of mice was not changed by treatments (insert Fig. 8c) ($F_{(3,\ 23)} = 1.864$, $P > 0.05$). 4-PSQ (1 mg kg^{-1}, p.o.) restored the spinal cord (Fig. 8a) and hippocampus (insert Fig. 8b) SOD activity of mice exposed to OXA to control values. The data presented in Fig. 8 b and c display the 4-PSQ effects on the SOD expression after OXA exposure. OXA significantly decreased the hippocampus (43%) ($F_{(3,\ 20)} = 9.737$, $P < 0.001$) and cerebral cortex (58%) ($F_{(3,\ 24)} = 12.00$, $P < 0.0001$) SOD expression when compared with the control group (Fig. 8b, c). Statistical analysis revealed that 4-PSQ (1 mg kg^{-1}, p.o.) restored hippocampus and cerebral cortex SOD expression to control levels (Fig. 8b, c).

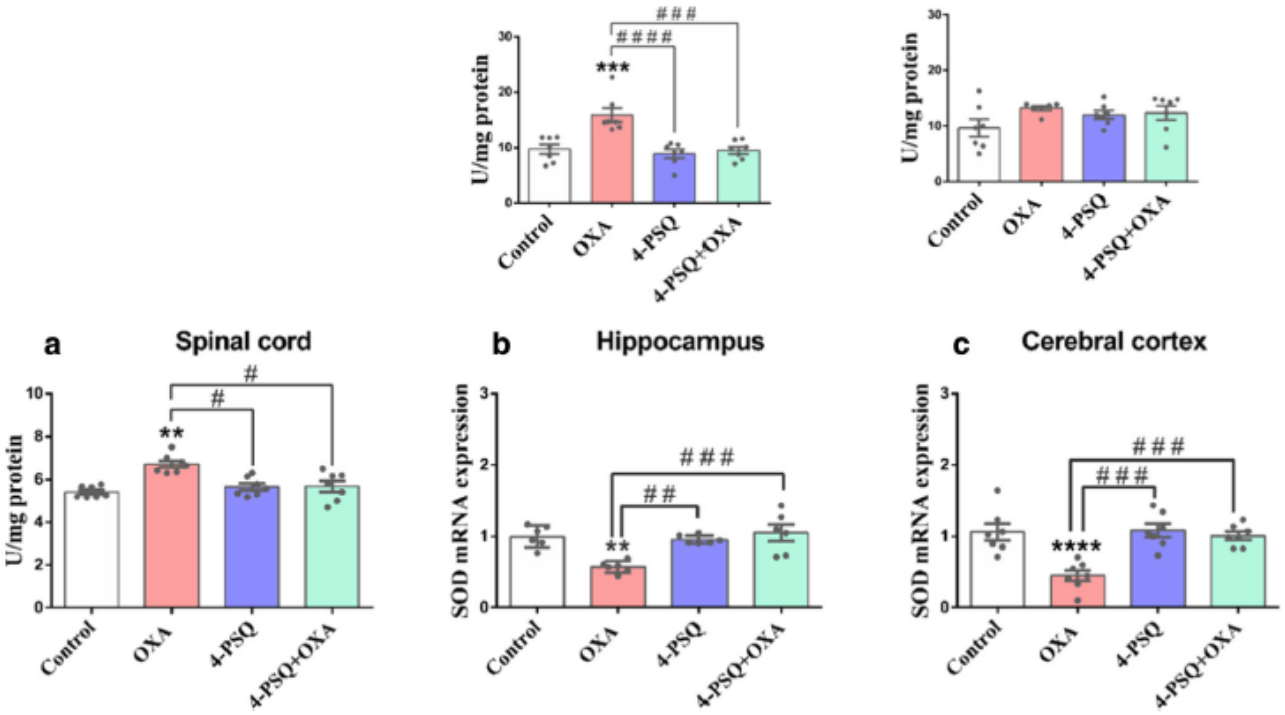


Fig. 8 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the superoxide dismutase activity in the spinal cord (**a**), and expression levels hippocampus (**b**) and cerebral cortex (**c**). Inserts show the effect of 4-PSQ (1 mg kg^{-1}, p.o.) and OXA (10 mg kg^{-1}, i.p.) on the superoxide dismutase activity in the cerebral cortex and hippocampus of mice. Each column represents the mean ± S.E.M. of 6–7 mice in each group. (**) $P<0.01$, (***) $P<0.001$ and (****) $P<0.0001$ denote significance levels when compared with the control group; ($^{\#}$) $P<0.05$, ($^{\#\#}$) $P<0.01$, ($^{\#\#\#}$) $P<0.001$ and ($^{\#\#\#\#}$) $P<0.0001$ denote significance levels when compared with OXA group (One-way ANOVA followed by Tukey's test)

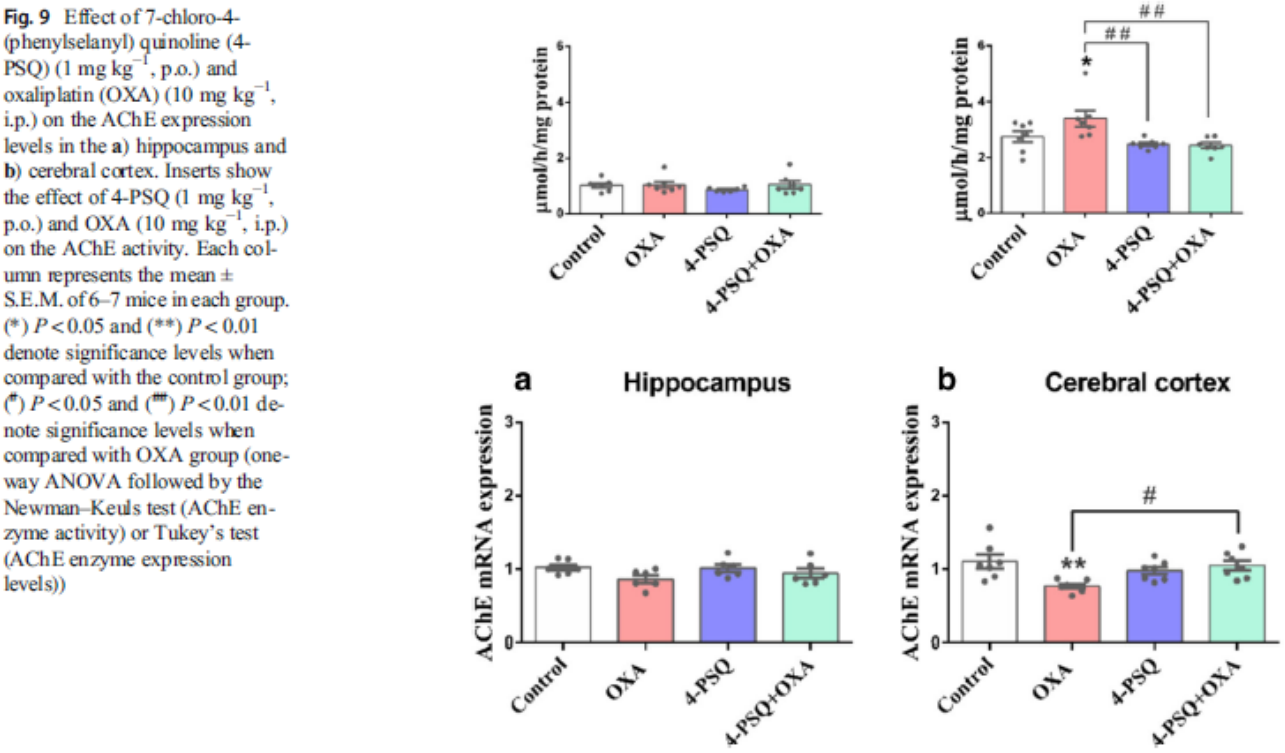


Fig. 9 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the AChE expression levels in the **a**) hippocampus and **b**) cerebral cortex. Inserts show the effect of 4-PSQ (1 mg kg^{-1}, p.o.) and OXA (10 mg kg^{-1}, i.p.) on the AChE activity. Each column represents the mean ± S.E.M. of 6–7 mice in each group. (*) $P<0.05$ and (**) $P<0.01$ denote significance levels when compared with the control group; ($^{\#}$) $P<0.05$ and ($^{\#\#}$) $P<0.01$ denote significance levels when compared with OXA group (one-way ANOVA followed by the Newman–Keuls test (AChE enzyme activity) or Tukey's test (AChE enzyme expression levels))

Involvement of Cerebral AChE on OXA-Induced Peripheral Neuropathy in Mice and the Effect of 4-PSQ

An increase of 23% in the cerebral cortex AChE activity in the mice exposed to OXA was observed when compared with the control group (insert Fig. 9b) ($F_{(3,\ 24)} = 5.733$, $P < 0.01$). No alteration was evidenced on hippocampus AChE activity after treatments (insert Fig. 9a) ($F_{(3,\ 24)} = 0.6741$, $P > 0.05$). Animals exposed to OXA exhibited a reduction of 31% in the expression of AChE in the cerebral cortex (Fig. 9b) ($F_{(3,\ 24)} = 5.231$, $P < 0.01$), whereas no alteration was observed in the hippocampus (Fig. 9a) ($F_{(3,\ 20)} = 1.998$, $P > 0.05$). OXA-induced alterations in the activity and expression of the cerebral cortex AChE were normalized to control levels by the 4-PSQ treatment (1 mg kg^{-1}, p.o.) (Fig. 9b).

Discussion

The physiopathology process of the OXA-induced peripheral neuropathy is multi-factorial [56, 57]. Importantly, it has been reported that oxidative stress contributes to development of the OXA-induced peripheral neuropathy [3, 17]. However, not all the physiopathology processes of the OXA-induced peripheral neuropathy remain completely understood and it is a challenging clinical problem.

For a better understanding of the mechanisms involved in the OXA-induced peripheral neuropathy, the concentration of platinum in the spinal cord of the mice was quantified in this study. Jain in 2001 reported an increase of platinum compounds and of their metabolites on the dorsal root ganglion [58]. In this case, the susceptibility of the peripheral nervous system (PNS) is mainly due to the absence of an efficient barrier, like the blood brain-barrier (BBB) in the CNS [58]. On the other hand, an increase in platinum compounds and their metabolites in the CNS is poorly reported. Recently, Branca et al. [59] suggested the OXA exposure opening of the BBB starting from RS formation; however, more studies is still necessary for better understand.

Despite the efficient defences of the CNS, in the present study, it was observed for the first time that the concentration of platinum in the spinal cord of mice treated with OXA was higher than in the control mice, which expands the knowledge about its toxicity. Considering these results, a hypothesis is that platinum accumulation in the spinal cord could cause oxidative damage in the neurons and impairment of mitochondrial function, similarly to what occurs in the PNS [58]. As expected, the analysis of the data revealed an increase in the RS levels in the spinal cord of mice treated with OXA.

Understanding that OXA causes damage directly to both the CNS and PNS, the direct relationship between the activity and expression of the AChE with OXA treatment was investigated. AChE, an enzyme that degrades acetylcholine in the synaptic cleft, was selected because it has an unquestionable importance to the functioning of cholinergic synapses present in the CNS and PNS. It was observed that OXA increased the AChE activity in the cerebral cortex, suggesting that alterations in the AChE activity might be implicated in the physiopathology of the OXA-induced peripheral neuropathy. These results are in agreement with those obtained by Ferrier et al. [34], which demonstrated that OXA reduced levels of acetylcholine and increased the choline levels in the cerebral cortex of rats. In addition, the choline concentration was correlated with mechanical hypersensitivity, suggesting a causal link between both. In this sense, based on the obtained results, it was possible to evidence a reduction in the AChE expression in the cerebral cortex of mice exposed to OXA. A hypothesis is that the downregulation of OXA-induced the AChE gene expression could be understood as a compensatory mechanism for the increase in the activity. In this regard, different expression and activity patterns were also explored by Wieczorek et al. [60].

To explore other mechanisms involved in the OXA-induced peripheral neuropathy, possible changes in markers of oxidative damage in the CNS were evaluated. It is well known that increased oxidative stress is a common mechanism of neurotoxicity induced by several chemotherapeutic agents such as OXA [4, 61]. Neurodegeneration caused by oxidative stress can occur through bioenergetic failure, biomolecular damage or depletion of antioxidant defences [4, 62]. However, the underlying mechanism by which oxidative damage generates pain signals in the OXA-induced peripheral neuropathy is poorly understood. Here, taking into account the obtained results, it was possible to observe that the administration of OXA produced a significant increase in the levels of RS in the spinal cord, cerebral cortex and hippocampus of mice.

Based on the evidence from the present study, it is believed that the corresponding increase in the RS levels generation is mediated by platinum deposits in the CNS and contributes to higher mechanical and thermal sensitivity observed in animals exposed to OXA. The balance between RS production and antioxidant defences determines the degree of oxidative stress. Indeed, the burden of RS production is largely counteracted by an intricate antioxidant system. SOD speeds the conversion of superoxide to H_2O_2, whereas CAT and GPx convert H_2O_2 to water. Considering the obtained results, it was observed that OXA induced a reduction in the expression of CAT, GPx and SOD in the cerebral tissues of mice. In this line, it is important to highlight that many studies have shown that excessive oxidative stress causes damage to enzymes, resulting in the loss of antioxidant defences [63, 64]. On the other hand, these data are in contrast with results presented by Wang et al. [65] that demonstrated that OXA is an effective activator of the nuclear factor E2–related factor-2 (Nrf2)/antioxidant response elements (AREs) signalling pathway in vitro and in vivo. Nrf2 is considered the key regulator of the body's antioxidant

response and is responsible for inducing the expression of genes encoding antioxidant proteins and enzymes. Hence, a hypothesis to explain the reduction in the expression of CAT, GPx and SOD in the cerebral tissues of mice after OXA exposure is that the burden of RS production induces the DNA damage and 13 days after exposure to this platinum agent there is accumulation of oxidant-damaged nuclear DNA, resulting in lower protein synthesis.

As a compensatory mechanism, an increase in the activity of GPx (cerebral cortex and spinal cord) and SOD (hippocampus and spinal cord) was observed in animals exposed to OXA. Indeed, when the stress is severe, survival is dependent on the ability of the cell to adapt and resist the stress, as well as to repair or replace the damaged molecules [66]. Several stress response mechanisms have evolved to help the cell and organism adapt to acute stress [67].

All harmful OXA-induced effects can cause damage the sensory neurons, which leads to neuropathic pain characterized by an increase in the mechanical and thermal sensitivity [3]. Indeed, OXA produced a significant increase in the mechanical and thermal sensitivity in mice. The management of the OXA-induced neuropathic pain generally focuses on treating symptoms, since the cause of the pain still cannot be treated. In view of this, 4-PSQ was investigated for the first time as a therapeutic strategy for the treatment of the OXA-induced neuropathic pain. The findings indicated that 4-PSQ, which has already been characterized as a promising compound with several pharmacological effects [19, 20, 25], reversed the mechanical and thermal hypersensitivity induced by OXA exposure, without impairing the locomotor and exploratory capacity of the animals. Of particular importance in this study, the OXA-induced alterations in the activity and expression of the AChE were normalized by 4-PSQ. Moreover, 4-PSQ reversed the oxidative imbalance caused by OXA because it reduced levels of RS and normalized the activity and expression of the GPx, CAT and SOD antioxidant enzymes in all tissues evaluated. It is important to emphasize that these results can partially explain the beneficial effect of 4-PSQ in the treatment of the OXA-induced neuropathic pain, especially because the effects here demonstrated were taken during daily 4-PSQ dosing. The same protocol is followed in the pharmacological treatment with nonsteroidal anti-inflammatory drugs or opioids in the management of neuropathic pain [16, 17]. However, more studies are required to define any long-lasting effects of the 4-PSQ on neuropathy-causing mechanisms.

Flatters et al. [62] reported that currently a combination of therapies is required for the most effective management of chemotherapeutic-induced peripheral neuropathy. In this sense, based on the obtained results, it is possible to reaffirm that 4-PSQ is a promising molecule as a therapeutic strategy for the OXA-induced peripheral neuropathy, because 4-PSQ acts on multiple targets that are altered in this pathology. 4-PSQ elicits antinociceptive, antioxidant and neuroprotective effects in animal models [19, 22]. The pharmacological actions of 4-PSQ seem to be related to its antioxidant and anti-inflammatory activities, as well as its ability to modulate the serotonergic, nitrergic, glutamatergic and cholinergic systems [19–21, 23, 25]. Importantly, our previous research related to 4-PSQ showed that this compound reduced glutamate uptake in the brain of mice [20]. In addition, it modulated the synaptic plasticity by enhancing the neural cell adhesion molecule (NCAM) and polysialyltransferase levels in the cerebral cortex and hippocampus of aged rats [25]. Considering that complex disorders, such as OXA-induced peripheral neuropathy, are more likely to be healed or alleviated though simultaneous modulation of multiple targets, the versatility of 4-PSQ deserves great attention.

Although consistent results have been obtained, it should be noted that some limitations of this study are the lack of data about the effects in female mice, since sex is an important biological and experimental variable. Also, the hypothesis that 4-PSQ could interact with OXA, interfering in the antineoplastic effect, cannot be completely ruled out. Investigating this point is the main objective of our research group for the next studies.

To summarize the current knowledge, the present research helped to expand understanding about the mechanisms involved in the physiopathology of the OXA-induced peripheral neuropathy. Here, the increase in the concentration of platinum in the spinal cord of mice treated with OXA and its relationship with the increase in the RS levels and modulation of the enzymes SOD, CAT, GPx and AChE in the CNS was reported for the first time. In addition, the multi-target 4-PSQ-based therapy reduced the OXA-induced peripheral neuropathy in mice. These results suggest that 4-PSQ might be a good prototype for the development of a more effective agent for OXA-induced peripheral neuropathy treatment.

Authors' Contributions A.S.R., J.J.P., C.L and E.A.W. conceived and designed the study. W.B.D. and V.F.C. were responsible for perform qRT-PCR. D.N and M.F.M were responsible for quantify the concentration of platinum by ICP-MS. G.P.C and D.A. performed the 4-PSQ synthesis. A.S.R. and E.A.W. wrote the manuscript. E.A.W supervised the study. All authors approved the final version of the manuscript.

Funding Information This study received financial support and scholarships from the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (429,859/2018-0) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) (PqG 17/2551-0001013-2, PRONEM 16/2551-0000240-1). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível superior–Brasil (CAPES)-Finance Code 001. C.L.; E.A.W.; D.A.; M.F.M. and V.F.C. are recipients of CNPq fellowship. This study also received financial assistance from L'ORÉAL-UNESCO-ABC for Women in Science.

Compliance with Ethical Standards

Animal care and all experimental procedures were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) and in accordance with

the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 4506-2017). All efforts were made to minimize the number of animals used and their suffering.

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Nichetti F, Falvella FS, Miceli R, Cheli S, Gaetano R, Fucà G, Infante G, Martinetti A et al (2019) Is a pharmacogenomic panel useful to estimate the risk of oxaliplatin-related neurotoxicity in colorectal cancer patients? Pharmacogenomics J 19:465–472. https://doi.org/10.1038/s41397-019-0078-0
2. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, Dicker DJ, Chimed-Orchir O et al (2017) Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol 3:524–548. https://doi.org/10.1001/jamaoncol.2016.5688
3. Starobova H, Vetter I (2017) Pathophysiology of chemotherapy-induced peripheral neuropathy. Front Mol Neurosci 10:174. https://doi.org/10.3389/fnmol.2017.00174
4. Areti A, Yerra VG, Naidu VGM, Kumar A (2014) Oxidative stress and nerve damage: Role in chemotherapy induced peripheral neuropathy. Redox Biol 2:289–295. https://doi.org/10.1016/j.redox.2014.01.006
5. Colvin LA (2019) Chemotherapy-induced peripheral neuropathy: where are we now? Pain 160:S1–S10. https://doi.org/10.1097/j.pain.0000000000001540
6. Gebremedhn EG, Shortland PJ, Mahns DA (2018) The incidence of acute oxaliplatin-induced neuropathy and its impact on treatment in the first cycle: a systematic review. BMC Cancer 18:410. https://doi.org/10.1186/s12885-018-4185-0
7. Quintão NLM, Santin JR, Stoeberl LC, Corrêa TP, Melato J, Costa R (2019) Pharmacological treatment of chemotherapy-induced neuropathic pain: PPARγ agonists as a promising tool. Front Neurosci 13:907. https://doi.org/10.3389/fnins.2019.00907
8. Molassiotis A, Cheng HL, Lopez V, Au JS, Chan A, Bandla A, Leung KT, Li YC et al (2019) Are we mis-estimating chemotherapy-induced peripheral neuropathy? Analysis of assessment methodologies from a prospective, multinational, longitudinal cohort study of patients receiving neurotoxic chemotherapy. BMC Cancer 19:132. https://doi.org/10.1186/s12885-019-5302-4
9. Zheng H, Xiao WH, Bennett GJ (2011) Functional deficits in peripheral nerve mitochondria in rats with paclitaxel-and oxaliplatin-evoked painful peripheral neuropathy. Exp Neurol 232:154–161. https://doi.org/10.1016/j.expneurol.2011.08.016
10. Nassini R, Gees M, Harrison S, De Siena G, Materazzi S, Moretto N, Failli P, Preti D et al (2011) Oxaliplatin elicits mechanical and cold allodynia in rodents via TRPA1 receptor stimulation. Pain 152:1621–1631. https://doi.org/10.1016/j.pain.2011.02.051
11. Joseph EK, Chen X, Bogen O, Levine JD (2008) Oxaliplatin acts on IB4-positive nociceptors to induce an oxidative stress-dependent acute painful peripheral neuropathy. J Pain 9:463–472. https://doi.org/10.1016/j.jpain.2008.01.335
12. Scuteri A, Galimberti A, Ravasi M, Pasini S, Donzelli E, Cavaletti G, Tredici G (2010) NGF protects dorsal root ganglion neurons from oxaliplatin by modulating JNK/Sapk and ERK1/2. Neurosci Lett 486:141–145. https://doi.org/10.1016/j.neulet.2010.09.028
13. Mihara Y, Egashira N, Sada H, Kawashiri T, Ushio S, Yano T, Ikesue H, Oishi R (2011) Involvement of spinal NR2B-containing NMDA receptors in oxaliplatin-induced mechanical allodynia in rats. Mol Pain 7:1744–8069. https://doi.org/10.1186/1744-8069-7-8
14. Kanat O, Ertas H, Caner B (2017) Platinum-induced neurotoxicity: a review of possible mechanisms. World J Clin Oncol 8:329–335. https://doi.org/10.5306/wjco.v8.i4.329
15. Zanardelli M, Micheli L, Cinci L, Failli P, Ghelardini C, Di Cesare ML (2014) Oxaliplatin neurotoxicity involves peroxisome alterations. PPARγ agonism as preventive pharmacological approach. PLoS One 9:e102758. https://doi.org/10.1371/journal.pone.0102758
16. Brusco I, Camponogara C, Carvalho FB, Schetinger MRC, Oliveira MS, Trevisan G, Ferreira J, Oliveira SM (2017) α-Spinasterol: a COX inhibitor and a transient receptor potential vanilloid 1 antagonist presents an antinociceptive effect in clinically relevant models of pain in mice. Br J Pharmacol 174:4247–4262. https://doi.org/10.1111/bph.13992
17. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A et al (2017) Neuropathic pain. Nat Rev Dis Primers 3:17002. https://doi.org/10.1038/nrdp.2017.2
18. Urru M, Muzzi M, Coppi E, Ranieri G, Buonvicino D, Camaioni E, Coppini R, Pugliese AM et al (2020) Dexpramipexole blocks Nav1.8 sodium channels and provides analgesia in multiple nociceptive and neuropathic pain models. Pain 161:831–841. https://doi.org/10.1097/j.pain.0000000000001774
19. Pinz M, Reis AS, Duarte V, da Rocha MJ, Goldani BS, Alves D, Savegnago L, Luchese C et al (2016) 4-Phenylselenyl-7-chloroquinoline, a new quinoline derivative containing selenium, has potential antinociceptive and anti-inflammatory actions. Eur J Pharmacol 780:122–128. https://doi.org/10.1016/j.ejphar.2016.03.039
20. Reis AS, Pinz M, Duarte LFB, Roehrs JA, Alves D, Luchese C, Wilhelm EA (2017) 4-phenylselenyl-7-chloroquinoline, a novel multitarget compound with anxiolytic activity: Contribution of the glutamatergic system. J Psychiatr Res 84:191–199. https://doi.org/10.1016/j.jpsychires.2016.10.007
21. Silva VD, Reis AS, Pinz MP, da Fonseca CA, Duarte LFB, Roehrs JA, Alves D, Luchese C et al (2017) Further analysis of acute antinociceptive and anti-inflammatory actions of 4-phenylselenyl-7-chloroquinoline in mice. Fundam Clin Pharmacol 31:513–525. https://doi.org/10.1111/fcp.12295
22. Vogt AG, Voss GT, de Oliveira RL, Paltian JJ, Duarte LF, Alves D, Jesse CR, Roman SS et al (2018) Organoselenium group is critical for antioxidant activity of 7-chloro-4-phenylselenyl-quinoline. Chem Biol Interact 282:7–12. https://doi.org/10.1016/j.cbi.2018.01.003
23. Pinz MP, Reis AS, Vogt AG, Krüger R, Alves D, Jesse CR, Roman SS, Soares MP et al (2018) Current advances of pharmacological properties of 7-chloro-4-(phenylselanyl) quinoline: prevention of cognitive deficit and anxiety in Alzheimer's disease model. Biomed Pharmacother 105:1006–1014. https://doi.org/10.1016/j.biopha.2018.06.049
24. Voss GT, Oliveira RL, de Souza JF, Duarte LF, Fajardo AR, Alves D, Luchese C, Wilhelm EA (2018) Therapeutic and technological potential of 7-chloro-4-phenylselanyl quinoline for the treatment of atopic dermatitis-like skin lesions in mice. Mater Sci Eng C 84:90–98. https://doi.org/10.1016/j.msec.2017.11.026
25. Barth A, Vogt AG, Reis AS, Pinz MP, Krüger R, Domingues WB, Alves D, Campos VF et al (2019) 7-Chloro-4-(Phenylselanyl) quinoline with memory enhancer action in aging rats: modulation of neuroplasticity, acetylcholinesterase activity, and cholesterol levels. Mol Neurobiol 56:6398–6408. https://doi.org/10.1007/s12035-019-1530-5
26. Savegnago L, Vieira AI, Seus N, Goldani BS, Castro MR, Lenardão EJ, Alves D (2013) Synthesis and antioxidant properties

of novel quinoline–chalcogenium compounds. Tetrahedron Lett 54:40–44. https://doi.org/10.1016/j.tetlet.2012.10.067

27. Duarte LFB, Barbosa ES, Oliveira RL, Pinz MP, Godoi B, Schumacher RF, Luchese C, Wilhelm EA et al (2017) A simple method for the synthesis of 4-arylselanyl-7-chloroquinolines used as in vitro acetylcholinesterase inhibitors and in vivo memory improvement. Tetrahedron Lett 58:3319–3322. https://doi.org/10.1016/j.tetlet.2017.07.039
28. Salgueiro WG, Goldani BS, Peres TV, Miranda-Vizuete A, Aschner M, da Rocha JBT (2017) Insights into the differential toxicological and antioxidant effects of 4-phenylchalcogenil-7-chloroquinolines in Caenorhabditis elegans. Free Radic Biol Med 110:133–141. https://doi.org/10.1016/j.freeradbiomed.2017.05.020
29. de Freitas CS, Araujo SM, Bortolotto VC, Poetini MR, Pinheiro FC, Musachio EAS, Meichtry LB, Sacramento M et al (2019) 7-chloro-4-(phenylselanyl) quinoline prevents dopamine depletion in a Drosophila melanogaster model of Parkinson's-like disease. J Trace Elem Med Biol 54:232–243. https://doi.org/10.1016/j.jtemb.2018.10.015
30. Furgała A, Sałat R, Sałat K (2018) Acute cold allodynia induced by oxaliplatin is attenuated by amitriptyline. Acta Neurobiol Exp 78:315–321. https://doi.org/10.21307/ane-2018-030
31. Alamri FF, Shoyaib A, Biggers A, Jayaraman S, Guindon J, Karamyan VT (2018) Applicability of the grip strength and automated von Frey tactile sensitivity tests in the mouse photothrombotic model of stroke. Behav Brain Res 336:250–255. https://doi.org/10.1016/j.bbr.2017.09.008
32. Woolfe G, MacDonald AD (1944) The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther 80:300–307
33. Walsh RN, Cummins RA (1976) The open-field test: a critical review. Psychol Bull 83:482–504. https://doi.org/10.1037/0033-2909.83.3.482
34. Ferrier J, Bayet-Robert M, Dalmann R, Guerrab A, Aissouni Y, Graveron-Demilly D, Chalus M, Pinguet J et al (2015) Cholinergic neurotransmission in the posterior insular cortex is altered in preclinical models of neuropathic pain: key role of muscarinic M2 receptors in donepezil-induced antinociception. J Neurosci 35:16418–16430. https://doi.org/10.1523/JNEUROSCI.1537-15.2015
35. Fiore NT, Austin PJ (2018) Glial-cytokine-neuronal adaptations in the ventral hippocampus of rats with affective behavioral changes following peripheral nerve injury. Neuroscience 390:119–140. https://doi.org/10.1016/j.neuroscience.2018.08.010
36. Li Q, Liu S, Zhu X, Mi W, Maoying Q, Wang J, Yu J, Wang Y (2019) Hippocampal PKR/NLRP1 inflammasome pathway is required for the depression-like behaviors in rats with neuropathic pain. Neuroscience 412:16–28. https://doi.org/10.1016/j.neuroscience.2019.05.025
37. Chen SD, Gu YX, Xu DB, Dai QX, Wang JL (2019) Involvement of miR-34a and p53 protein of cerebral cortex in electroacpuncture analgesia in mice with neuropathic pain. Zhen ci yan jiu =. Acupuncture Res 44:632–636. https://doi.org/10.13702/j.1000-0607.190149
38. Huang J, Gadotti VM, Chen L, Souza IA, Huang S, Wang D, Ramakrishnan C, Deisseroth K et al (2019) A neuronal circuit for activating descending modulation of neuropathic pain. Nat Neurosci 22:1659–1668. https://doi.org/10.1038/s41593-019-0481-5
39. Liu Y, Latremoliere A, Li X, Zhang Z, Chen M, Wang X, Fang C, Zhu J et al (2018) Touch and tactile neuropathic pain sensitivity are set by corticospinal projections. Nature 561:547–550. https://doi.org/10.1038/s41586-018-0515-2
40. Widerström-Noga E (2017) Neuropathic pain and spinal cord injury: phenotypes and pharmacological management. Drugs 77:967–984. https://doi.org/10.1007/s40265-017-0747-8
41. Deng M, Chen SR, Pan HL (2019) Presynaptic NMDA receptors control nociceptive transmission at the spinal cord level in neuropathic pain. Cell Mol Life Sci 76:1889–1899. https://doi.org/10.1007/s00018-019-03047-y
42. Muller AL, Oliveira JS, Mello PA, Muller EI, Flores EM (2015) Study and determination of elemental impurities by ICP-MS in active pharmaceutical ingredients using single reaction chamber digestion in compliance with USP requirements. Talanta 136:161–169. https://doi.org/10.1016/j.talanta.2014.12.023
43. Hartwig CA, Pereira RM, Novo DLR, Oliveira DTT, Mesko MF (2017) Green and efficient sample preparation method for the determination of catalyst residues in margarine by ICP-MS. Talanta 174:394–400. https://doi.org/10.1016/j.talanta.2017.06.037
44. Loetchutinat C, Kothan S, Dechsupa S, Meesungnoen J, Jay-Gerin JP, Mankhetkorn S (2005) Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′, 7′-dichlorofluorescein diacetate assay. Radiat Phys Chem 72:323–331. https://doi.org/10.1016/j.radphyschem.2004.06.011
45. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126. https://doi.org/10.1016/S0076-6879(84)05016-3
46. Wendel A (1981) Glutathione peroxidase. Methods in enzymol 77:325–333. https://doi.org/10.1016/S0076-6879(81)77046-0
47. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175 http://www.jbc.org/content/247/10/3170
48. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem 82:70–77. https://doi.org/10.1016/0003-9861(59)90090-6
49. d'Avila JC, Siqueira LD, Mazeraud A, Azevedo EP, Foguel D, Castro-Faria-Neto HC, Sharshar T, Chrétien F et al (2018) Age-related cognitive impairment is associated with long-term neuroinflammation and oxidative stress in a mouse model of episodic systemic inflammation. J Neuroinflammation 15:15–28. https://doi.org/10.1186/s12974-018-1059-y
50. Silverman HA, Dancho M, Regnier-Golanov A, Nasim M, Ochani M, Olofsson OS, Ahmed M, Miller EJ et al (2014) Brain region-specific alterations in the gene expression of cytokines, immune cell markers and cholinergic system components during peripheral endotoxin-induced inflammation. Mol Med 20:601–611. https://doi.org/10.2119/molmed.2014.00147
51. Zhang R, Miao QW, Zhu CX, Zhao Y, Liu L, Yang J, An L (2015) Sulforaphane ameliorates neurobehavioral deficits and protects the brain from amyloid β deposits and peroxidation in mice with Alzheimer-like lesions. Am J Alzheimers Dis Other Dement 30:183–191. https://doi.org/10.1177/1533317514542645
52. Turabelidze A, Guo S, DiPietro LA (2010) Importance of housekeeping gene selection for accurate reverse transcription-quantitative polymerase chain reaction in a wound healing model. Wound Repair Regen 18:460–466. https://doi.org/10.1111/j.1524-475X.2010.00611.x
53. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
54. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3
55. Curtis MJ, Alexander S, Cirino G, Docherty JR, George CH, Giembycz MA, Hoyer D, Insel PA et al (2018) Experimental design and analysis and their reporting II: updated and simplified

guidance for authors and peer reviewers. Br J Pharmacol 175:987–993. https://doi.org/10.1111/bph.14153
56. Potenzieri A, Riva B, Rigolio R, Chiorazzi A, Pozzi E, Ballarini E, Cavaletti G, Genazzani AA (2020) Oxaliplatin-induced neuropathy occurs through impairment of haemoglobin proton buffering and is reversed by carbonic anhydrase inhibitors. Pain 161:405–415. https://doi.org/10.1097/j.pain.0000000000001722
57. Illias AM, Gist AC, Zhang H, Kosturakis AK, Dougherty PM (2018) Chemokine CCL2 and its receptor CCR2 in the dorsal root ganglion contribute to oxaliplatin-induced mechanical hypersensitivity. Pain 159:1308–1316. https://doi.org/10.1097/j.pain.0000000000001212
58. Jain KK (2001) Drug-induced neurological disorders. Hogrefe & Huber, Seattle
59. Branca JJV, Maresca M, Morucci G, Becatti M, Paternostro F, Gulisano M, Ghelardini C, Salvemini D et al (2018) Oxaliplatin-induced blood brain barrier loosening: A new point of view on chemotherapy-induced neurotoxicity. Oncotarget 9:23426. https://doi.org/10.18632/oncotarget.25193
60. Wieczorek E, Jablonowski Z, Tomasik B, Gromadzinska J, Jablonska E, Konecki T, Fendler W, Sosnowski M et al (2017) Different gene expression and activity pattern of antioxidant enzymes in bladder cancer. Anticancer Res 37:841–848. https://doi.org/10.21873/anticanres.11387
61. Conklin KA (2004) Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integr Cancer Ther 3:294–300. https://doi.org/10.1177/1534735404270335
62. Flatters SJL, Dougherty PM, Colvin LA (2017) Clinical and preclinical perspectives on chemotherapy-induced peripheral neuropathy (CIPN): a narrative review. Br J Anaesth 119:737–749. https://doi.org/10.1093/bja/aex229
63. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5:9–19. https://doi.org/10.1097/WOX.0b013e3182439613
64. Griess B, Tom E, Domann F, Teoh-Fitzgerald M (2017) Extracellular superoxide dismutase and its role in cancer. Free Radic Biol Med 112:464–479. https://doi.org/10.21307/ane-2018-030
65. Wang XJ, Li Y, Luo L, Wang H, Chi Z, Xin A, Wu J, Tang X (2014) Oxaliplatin activates the Keap1/Nrf2 antioxidant system conferring protection against the cytotoxicity of anticancer drugs. Free Radic Biol Med 70:68–77. https://doi.org/10.1016/j.freeradbiomed.2014.02.010
66. Allen RG, Tresini M (2000) Oxidative stress and gene regulation. Free Radic Biol Med 28:463–499. https://doi.org/10.1016/S0891-5849(99)00242-7
67. Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408:239–247. https://doi.org/10.1038/35041687

Capítulo 2

Modulação farmacológica da Na^+, K^+ - ATPase para o tratamento das comorbidades associadas à NPIO: 4-PSQ como estratégia terapêutica

Na etapa 2 desta tese, o objetivo principal foi investigar o envolvimento da enzima Na^+, K^+ - ATPase na neurotoxicidade induzida pela administração da oxaliplatina e propor a modulação desta enzima como alvo farmacológico para o tratamento das comorbidades associadas à NPIO. Esta proposta baseou-se no fato desta enzima, fundamental para a manutenção das funções cerebrais, ser particularmente vulnerável à ação de medicamentos. Ainda, considerando que a atividade disfuncional da enzima Na^+, K^+ - ATPase pode contribuir para a patogênese de diversas doenças do SNC, foi determinado o efeito da oxaliplatina sobre o comportamento ansiogênico e o déficit cognitivo em camundongos adultos jovens, como comorbidades relacionadas à NPIO. Além disso, considerando que: *i)* o 4-PSQ tem ação ansiolítica em camundongos jovens; *ii)* o 4-PSQ restaurou o declínio de memória causado pelo envelhecimento em ratos velhos e, *iii)* os promissores resultados observados na etapa 1; nesta etapa da tese foi determinado o efeito desta selenoquinolina sobre o comportamento ansiogênico e o déficit cognitivo relacionadas à NPIO. Neste contexto, a Na^+, K^+ - ATPase foi investigada como um novo alvo farmacológico para o tratamento das comorbidades associadas a NPIO.

A hipótese desta etapa foi comprovada pelos resultados obtidos. De fato, a administração da oxaliplatina modulou o funcionamento da enzima Na^+, K^+ ATPase no SNC. A oxaliplatina inibiu a atividade e reduziu a expressão da enzima Na^+, K^+ - ATPase. Também, nessa etapa, ratificou-se a correlação positiva significativa entre o comportamento ansioso e o prejuízo cognitivo induzidos pelo tratamento com oxaliplatina. Corroborando a correlação demonstrada, a administração de oxaliplatina aumentou os níveis de corticosterona plasmática. Ainda, os resultados obtidos aqui confirmaram os promissores efeitos do 4-PSQ em reverter a neurotoxicidade causada pela administração da oxaliplatina. De fato, o tratamento com 4-PSQ reverteu o comportamento ansioso e o comprometimento cognitivo induzido pela exposição à oxaliplatina, por meio da modulação da enzima Na^+, K^+ - ATPase, bem como, pela normalização dos níveis de corticosterona (Figura 6). É importante reiterar que o 4-PSQ é uma molécula multialvo, o que certamente contribui para os efeitos relevantes demonstrados neste estudo. Nesta etapa

do estudo, os resultados novamente ajudaram a ampliar os conhecimentos sobre os mecanismos envolvidos na fisiopatologia da NPIO e suas comorbidades.

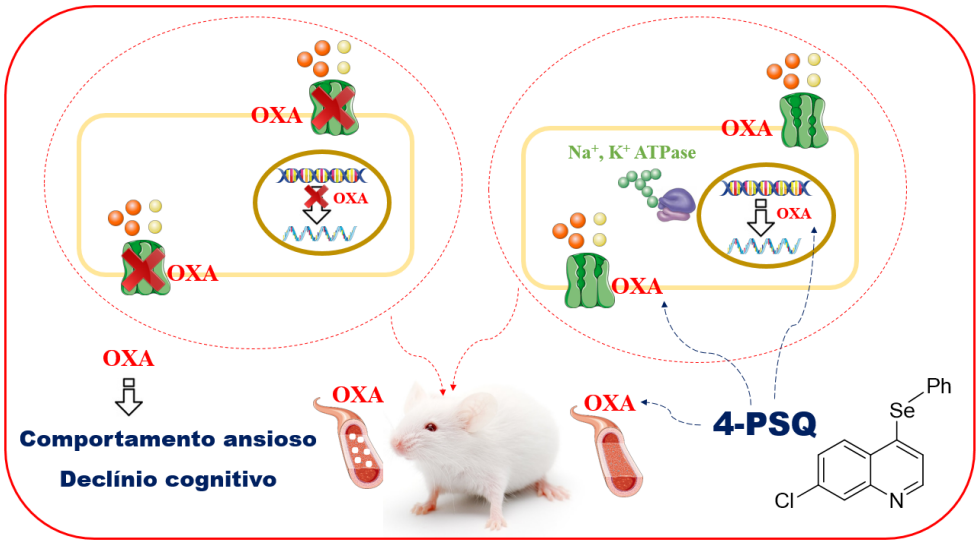


Figura 6. Representação esquemática dos principais resultados obtidos na etapa 2.

Artigo 2

Os resultados deste capítulo da tese estão apresentados sob a forma de artigo científico, o qual se encontra assim organizado. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados, Conclusões e Referências Bibliográficas encontram-se no próprio artigo.

O artigo científico encontra-se publicado na revista *Brain Research Bulletin.*

Brain Research Bulletin 162 (2020) 282–290

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Pharmacological modulation of Na^+, K^+-ATPase as a potential target for OXA-induced neurotoxicity: Correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline

Angélica S. Reis[a], Jaini J. Paltian[a], William B. Domingues[b], Gabriel P. Costa[c], Diego Alves[c], Janice L. Giongo[d], Vinicius F. Campos[b], Cristiane Luchese[a,*], Ethel A. Wilhelm[a,*]

[a] Programa de Pós-graduação em Bioquímica e Bioprospecção, Laboratório de Pesquisa em Farmacologia Bioquímica, CCQFA - Universidade Federal de Pelotas, UFPel - CEP, 96010-900, Pelotas, RS, Brazil
[b] Programa de Pós-graduação em Biotecnologia, Laboratório de Genômica Estrutural, Biotecnologia - Universidade Federal de Pelotas, UFPel – CEP, 96010-900, Pelotas, RS, Brazil
[c] Programa de Pós-graduação em Química, Laboratório de Síntese Orgânica Limpa, CCQFA - Universidade Federal de Pelotas, UFPel – CEP, 96010-900, Pelotas, RS, Brazil
[d] Pharmacy Department, Faculdade Anhanguera – CEP – 96055000, Pelotas, RS, Brazil

ARTICLE INFO

Keywords:
Oxaliplatin
Neurotoxicity
Behavior
Corticosterone
Chemotherapy
4-PSQ

ABSTRACT

Growing evidence demonstrates that Oxaliplatin (OXA) is commonly associated with neurotoxicity that leads to emotional and cognitive impairments. The aim of the present study was to evaluate the OXA and Na^+, K^+-ATPase interaction and to correlate anxious behavior and cognitive impairment induced by this chemotherapeutic in Swiss mice. Also, considering the pharmacological modulation of Na^+, K^+-ATPase as a potential target for OXA-induced neurotoxicity, the therapeutic potential of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) was evaluated. Mice received OXA (10 mg kg^{-1}) or vehicle by intraperitoneal route (days 0 and 2). Oral administration of 4-PSQ (1 mg kg^{-1}) or vehicle was performed from days 2–14. Behavioral tasks started from day 12 onwards. On day 15, the animals were sacrificed, and the tissues collected. The effects of OXA and 4-PSQ on activity and expression level of Na^+, K^+-ATPase in the hippocampus and cerebral cortex, and the plasmatic corticosterone levels were determined. The findings demonstrated a significant positive correlation between anxious behavior and cognitive impairment induced by OXA. OXA caused an increase on the plasmatic corticosterone levels and reduced activity and expression level of Na^+, K^+-ATPase. 4-PSQ reduced both anxious behavior and cognitive impairment induced by OXA. 4-PSQ effect seems to be due to the modulation of Na^+, K^+-ATPase and reduction of corticosterone levels. Our results helped to expand knowledge about the mechanisms involved in the physiopathology of the OXA-induced neurotoxicity and strongly indicated that 4-PSQ may be a good prototype for the treatment of anxious behavior and cognitive impairment induced by OXA exposure.

1. Introduction

Chemotherapy is a widely used anti-cancer treatment. However, despite the advancement in chemotherapy, several chemotherapeutic drugs promote emotional and cognitive impairments in cancer survivors (Tofthagen et al., 2013; Cruzado et al., 2014; Dubois et al., 2014; Sales et al., 2019; Matsos and Johnston., 2019; Witlox et al., 2019). Nowadays, cancer is the second leading cause of death worldwide. Colorectal cancer is among the 3 most common types of cancers in both sexes, and it is the main cause of deaths from cancer in women (Fitzmaurice et al., 2017).

Oxaliplatin (OXA) is an important component in colorectal cancer treatment (Nichetti et al., 2019), having significantly increased patient survival rates, although it induces various side effects including myelotoxicity, peripheral neuropathy and neurotoxicity (Sales et al., 2019; Starobova and Vetter., 2017). Several factors are associated with the side effects induced by treatment with OXA, such as cognitive decline and anxious behavior, but the nature and impact of the neurobiological mechanisms involved are not clearly characterized. Indeed, pathological mechanisms underlying neurotoxicity induced by OXA are a challenging clinical problem (Matsos and Johnston., 2019).

The neurotoxicity induced by treatment with OXA is a dose-limiting

* Corresponding authors.
E-mail addresses: cristiane_luchese@yahoo.com.br (C. Luchese), ethelwilhelm@yahoo.com.br (E.A. Wilhelm).

https://doi.org/10.1016/j.brainresbull.2020.06.021
Received 23 March 2020; Received in revised form 21 June 2020; Accepted 29 June 2020
Available online 03 July 2020

toxicity, which negatively affects patient's quality of life and leads to patients dropping out of the treatment discontinuations, in addition to causing disorders of an emotional and cognitive nature (Tofthagen et al., 2013; Matsos and Johnston., 2019; Witlox et al., 2019). There are studies on the anxious behavior, cognitive impairment and neurotoxicity experienced by patients after treatment with OXA, but until now little is known about the mechanisms underlying these effects (Tofthagen et al., 2013; Cruzado et al., 2014; Dubois et al., 2014). Thus, there is a need to search for a better understanding of the mechanisms involved in the neurotoxicity induced by OXA and the development of therapeutic alternatives more effective and safer.

In this context, Na^+, K^+-ATPase is particularly vulnerable to drug actions (Wang and O'Doherty, 2012). Increasing evidence suggests that platinum-based chemotherapeutic agents can inhibit Na^+, K^+-ATPase activity (Huličiak et al., 2012; Kubala et al., 2014). Tummala et al. (2009) demonstrated that reduced expression of β1 subunit in the Na^+, K^+-ATPase protein is associated with OXA resistance in cancer cells. Besides that, a reduction on the Na^+, K^+-ATPase activity directly affects neurotransmitter signaling and neural activity. A deficiency in Na^+, K^+-ATPase genes causes memory deficits and anxiety-related behavior in mice (Moseley et al., 2007). Thus, Na^+, K^+-ATPase modulators can be promising in the development therapy alternatives for the emotional and cognitive disorders induced by OXA exposure.

In this sense, here we highlight 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (Fig. 1), a quinoline derivative containing selenium, which has several pharmacological properties already described in the literature (Pinz et al., 2016; Reis et al., 2017; Silva et al., 2017; Vogt et al., 2018; Barth et al., 2019). Our research group (Barth et al., 2019) showed that this compound modulated the cholinergic system in a study about memory and aging in rats. Besides, it underscored the fact that the glutamatergic system is strongly involved in the anxiolytic and antinociceptive effects exerted by 4-PSQ (Reis et al., 2017; Silva et al., 2017). Indeed, the 4-PSQ is a promising compound in the field of drug development.

According to the facts mentioned, the present study explored the role of the OXA exposure on emotional and cognitive domains. Posteriorly, the underlying mechanisms to OXA-induced neurotoxicity and, the modulation of Na^+, K^+-ATPase enzyme as a pharmacological target were investigated. In addition, the therapeutic potential of 4-PSQ was evaluated.

2. Materials and methods

2.1. Animals

Experiments were performed following the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA 4506 – 2017) and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996). The tests were carried using male adult Swiss mice (25 – 30 g). Animals were maintained at 22 ± 2 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 6:00 a.m.). Mice were acclimatized to the behavior room for at least 1 h before the test. All efforts were made to minimize the number of animals used and their discomfort.

The group size used for each experiment was based on studies that used protocols like those proposed here. The number of animals and discomfort used was the minimum needed to demonstrate the consistent effects of the treatments. For the present study, twenty-eight animals were divided into four groups, with seven animals per group. Allocation concealment was performed using a randomization procedure (http://www.randomizer.org/). Behavioral evaluations were performed blindly on drug administration. The experiments were carried out between 08:00 am and 05:00 pm.

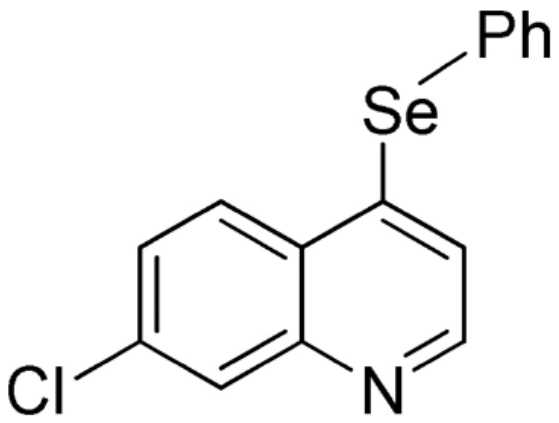


Fig. 1. Chemical structure of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ).

2.2. Drugs

4-PSQ was prepared and characterized (Duarte et al., 2017) in our laboratory and analysis of the ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 4-PSQ (99.9 %) was determined by GC/MS.

4-PSQ and OXA were dissolved in canola oil and 5% glucose solution, respectively. Mice received 4-PSQ or its vehicle by per oral route with intragastric (i.g.) gavage, and OXA or 5% glucose solution by intraperitoneal (i.p.) route. OXA was purchased from commercial sources. All other chemicals used in this study were of analytical grade and obtained from standard commercial suppliers.

2.3. Experimental design

Firstly, mice were randomly divided into groups: Control, OXA, 4-PSQ and 4-PSQ + OXA, totalizing four experimental groups (n = 7 animals/group). As shown in Fig. 2, the mice in the control and 4-PSQ groups received a 5 % glucose solution (10 mL kg^{-1}, i.p. route), whereas the mice in the OXA and 4-PSQ + OXA groups received OXA (10 mg kg^{-1}, i.p. route), at days 0 and 2. In the second day, after 30 min of the treatment with OXA, the mice in the control and OXA groups received canola oil (10 mL kg^{-1}, i.g. route), whereas the mice in the 4-PSQ and 4-PSQ + OXA groups received 4-PSQ (1 mg kg^{-1}, i.g. route), up to the fourteenth day. After 24 h of last treatment, the mice were anesthetized and euthanized by isoflurane inhalation. A cardiac puncture was performed, and the blood was collected in tubes containing heparin to further determine corticosterone levels. Additionally, cerebral cortex and hippocampus samples were rapidly dissected, weighed, and placed on ice. Tissue samples were used to determine the involvement of Na^+, K^+-ATPase activity and expression level by the qRT-PCR technique. Operators were blinded in the behavioral tests and data analyses.

3. Behavioral tests

3.1. Assessment of locomotor and exploratory domains

The open field test evaluated the general locomotor and exploratory behaviors of mice. The open field was made of plywood and surrounded by 30 cm-high walls. The floor of the open field, 45 cm long and 45 cm wide, was divided by masking tape markers into 9 squares (3 rows of 3). Mice were evaluated on the twelfth day, before the object recognition task. In this test, each animal was placed at the center of the open field and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory (number of rearings on the

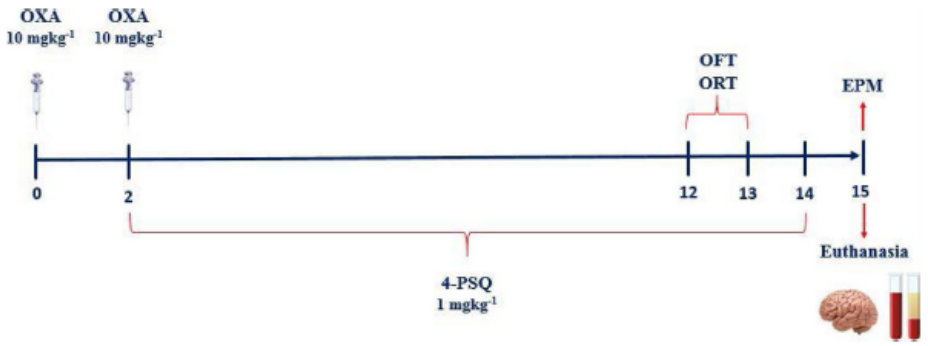


Fig. 2. The experimental protocol. Mice received oxaliplatin (OXA) (10 mg kg^{-1}) or vehicle by intraperitoneal (i.p.) route (days 0 and 2) and were intragastrically (i.g.) treated for 12 days with 4-PSQ (1 mg kg^{-1}) or canola oil (1 mL kg^{-1}). Open field test (OFT) was performed on the 12th day. Object recognition test (ORT) was performed on the 12th and 13th days. On the 15th day, the elevated plus-maze test (EPM) was performed, and posteriorly the animals were euthanized by inhalation of isoflurane for *ex vivo* assays.

hind limbs) activities (Walsh and Cummins, 1976). Mice were tested only once.

3.2. Assessment of cognitive domain

The object recognition task was carried out according to the method previously described by Stangherlin et al. (2009). This task has been widely used to evaluate short-term (STM) and long-term (LTM) memories. The task was performed in an open field apparatus on the twelfth and thirteenth days of the experimental protocol. On the day of the task (the twelfth day of the experimental protocol) each animal was submitted to a habituation session in the absence of objects for 5 min. Posteriorly, four objects were used: A1, A2, B and C. The A1 and A2 objects were two identical balls, the B object was a cube and the C object was a square. The objects used were made of plastic material, measuring 10 × 10 cm (length x height) and had the following color pattern: blue, red, and yellow. During the training, the animals were placed for 5 min in the arena containing two identical objects (objects A1 and A2) to explore. Exploration was accounted when the animal directed its nose around 2 cm of the object while sniffing, touching or looking at it. In the presence of a familiar object (A1) and a new object (B), 1.5 h after training, the STM of mice was evaluated. The time to explore was defined in 5 min, enough to measure learning and recognition memory. In turn, LTM was assessed 24 h after training. For this, the mice were placed to explore a familiar object (A1) and a new object (C) for 5 min. Time spent exploring each object was reported. Data were expressed as a percentage of the exploratory preference and calculated as follows: Training = (A2/(A1 + A2))×100; STM = (B/(A1 + B))×100; LTM = (C/(A1 + C))×100.

3.3. Assessment of emotional domain

The elevated plus-maze apparatus consists of two opposed open arms (16cm × 5cm) and two opposed closed arms (16cm × 5cm x 10 cm) mounted at an angle of 90°, all facing a central platform (5cm × 5cm) elevated 50 cm from the floor. This test is widely validated to measure anxiety in rodents (Pellow et al., 1985). On the fifteenth day, all animals were evaluated in the elevated plus-maze test. Each animal was placed individually at the center of the apparatus facing one of the open arms. The frequency of entries into either open or closed arms and the time spent in each type of arm were measured for 5 min. The anxiolytic effects of a drug are illustrated by a significant statistical increase of parameters in open arms.

3.4. Ex vivo assays

Based on the results obtained in the behavioral models, the mechanisms that could be involved in the OXA and 4-PSQ actions were investigated. Our aim was extending the knowledge about OXA-induced emotional and cognitive impairment and the pharmacological properties of 4-PSQ.

After the elevated plus-maze test, animals were anesthetized and euthanized. Blood samples were collected, and the plasma was obtained by centrifugation at 4.000 rotation per minute (rpm) for 10 min at 4 °C (hemolyzed plasma was discarded) and used to determine corticosterone levels. Samples of the cerebral cortex and hippocampus were collected to determine Na^+, K^+-ATPase activity. For this, samples were homogenized in 50 mM Tris HCl pH 7.5 (1:10 w/v) and centrifuged at 2500 rpm for 10 min to yield a supernatant (S_1). Moreover, samples of the cerebral cortex and hippocampus were also collected to determine Na^+, K^+-ATPase expression by the qRT-PCR technique.

3.5. Investigation of possible modulation of the enzyme Na^+, K^+-ATPase as a physiopathological mechanism involved in OXA-induced neurotoxicity

For the Na^+, K^+-ATPase activity assay, a reaction mixture was used containing S_1, 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samplings were performed under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at 37 °C for 30 min and the incubation was stopped by adding a 10 % trichloroacetic acid solution (TCA) with 10 mM $HgCl_2$. Na^+, K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured according Fiske and Subbarow (Fiske and Subbarow., 1925). Enzyme activity was expressed as nmol of Pi/mg of Protein/min.

For the expression of Na^+, K^+-ATPase the total mRNA was extracted from thawed samples of hippocampus and cerebral cortex, weighing between 50-70 mg, using TRIzol reagent (Invitrogen™, Carlsbad, USA) followed by DNase treatment with DNase I Amplification Grade (Invitrogen™, Carlsbad, USA) in order to ensure minimum DNA contamination of the samples. The total isolated RNA was quantified and its purity (260/280 and 260/230 ratios) was examined by spectrophotometer NanoVue (GE, Fairfield, CT, USA).

The cDNA synthesis was performed using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems™, UK) according to the manufacturer's protocol. For reverse transcription, 2 µg of total RNA was used in a reaction volume of 20 µl. The amplification was made with GoTaq® qPCR Master Mix (Promega, Madison, WI) using the Agilent Mx3005 P qPCR System (Agilent Technologies Inc., Santa Clara, CA) and the sequence of primer used is indicated in Table 1. The qPCR conditions were as follows: 10 min at 95 °C to activate the hot-start Taq polymerase, followed by 35 cycles of denaturation for 15 s at 95 °C, primer annealing for 60 s at 60 °C, and extension for 30 s at 72 °C (fluorescence signals were detected at the end of every cycle). Baseline and threshold values were automatically set by the Stratagene MxPro software.

The number of PCR cycles required to reach the fluorescence threshold in each sample was defined as the Ct value, and each sample was analyzed in duplicate to obtain an average Ct for each sample. The $2^{-\Delta\Delta CT}$ method was used to normalize the fold change in gene expressions (Livak and Schmittgen., 2001), using glyceraldehyde-3-

Table 1
Primers used for a quantitative real-time polymerase chain reaction. Listed are the forward and reverse primer sequences used to amplify Na^+, K^+ATPase target gene as well as the GAPDH endogenous control.

Primer Name	Sequence	Reference
Na^+, K^+-ATPase Forward	5′TTTCAGAACGCCTACCTAGAGC3′	(Wang et al., 2015)
Na^+, K^+-ATPase Reverse	5′TGGAGATAAGACCCACGAAGC3′	
GAPDH Forward	5′TGCGACTTCAACAGCAACTC3′	(Turabelidze et al., 2010)
GAPDH Reverse	5′ATGTAGGCAATGAGGTCCAC3′	

phosphate dehydrogenase (GAPDH) as a housekeeping gene.

3.6. Determination of plasmatic corticosterone levels in OXA-induced neurotoxicity

The determination of plasma corticosterone levels was performed according to Zenker and Bernstein (Zenker and Bernstein., 1958) with modifications. After blood centrifugation, aliquots of plasma were incubated with chloroform and centrifuged for 5 min at 2500 rpm, followed by addition of 0.1 M NaOH and another round of centrifugation. After the addition of the fluorescence reagent (H_2SO_4 and 50 % ethanol), samples were again centrifuged (5 min at 2500 rpm) and incubated at room temperature for 2 h. Fluorescence intensity emission, corresponding to plasma corticosterone levels, was recorded at Ex: 247, EM: 540 and corticosterone levels were expressed as ng/mL.

3.7. Protein assessment

The protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as the standard.

3.8. Data and statistical analysis

The normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed using GraphPad Prism 6.0 software (San Diego, CA, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's or Newman–Keuls test when appropriate. Pearson's correlation analysis was used to analyze the correlation between variables. Data were expressed as mean ± standard error of the mean (S.E.M.). Post hoc tests were performed only when the F-value achieved the necessary level of statistical significance ($P < 0.05$) and when there was no significant variation in homogeneity (Curtis et al., 2018).

4. Results

4.1. Behavioral tests

4.1.1. Assessment of locomotor and exploratory domains

The possible effects of the treatments on locomotor and exploratory activities of mice were evaluated in the open field test. The data analysis revealed that treatment of mice with 4-PSQ or OXA did not cause any significant change in the number of crossings (ANOVA: $F_{(3,24)} = 0.5152$, $P > 0.05$) or rearings (ANOVA: $F_{(3,24)} = 0.3019$, $P > 0.05$) (Fig. 3A and B, respectively).

4.1.2. Effect of 4-PSQ on OXA-induced cognitive impairment

In the object recognition task, during the habituation phase, there was no significant difference among groups in the percentage of exploratory preference (data not shown). The One-way ANOVA of STM revealed a significant effect on the percentage of exploratory preference for a novel object ($F_{(3,24)} = 13.72$, $P < 0.0001$). OXA administration (10 mg kg^{-1}, i.p.) reduced (around 51 %) the exploratory preference for the new object when compared with the control group (Fig. 4A). At the same time, the results demonstrate that the treatment with 4-PSQ (1 mg kg^{-1}, p.o.) reversed (around 173 %) the reduction in this OXA-induced memory parameter. A similar result was observed for LTM, the one-way ANOVA revealed a significant effect on the exploratory preference percentage for a novel object ($F_{(3,24)} = 10.07$, $P < 0.001$). OXA administration caused exploratory preference to be reduced by around 26 % when compared with the control group (Fig. 4B). 4-PSQ treatment normalized (around 52 %) the percentage of preference for a novel object when compared to the control group.

4.1.3. Effect of 4-PSQ in OXA-induced anxious behavior

One-way ANOVA revealed that OXA (10 mg kg^{-1}, i.p.) induced a reduction in the percentage of open arm entries ($F_{(3,24)} = 11.38$, $P < 0.0001$) (Fig. 5A), percentage of time spent in the open arms ($F_{(3,24)} = 19.93$, $P < 0.0001$) (Fig. 5B) and number of dives ($F_{(3,24)} = 13.38$, $P < 0.0001$) (Fig. 5C) by 61 %, 77 % and 74 %, respectively, when compared OXA group to the control group. Treatment with 4-PSQ (1 mg kg^{-1}, i.p.) reversed the decrease of the percentage of open arm entries (around 312 %), percentage of time spent in the open arms (around 777 %) and number of dives (around 688 %) altered by the treatment with OXA in the elevated plus-maze test when compared to control group.

4.1.4. Correlation between anxious behavior and cognitive impairment after treatment with OXA

Pearson's correlation test was carried out to test the correlations between anxious behaviors and cognitive impairment after treatment with OXA. The results illustrated in Fig. 6A showed the correlation coefficients between the percentage of time spent on the open arm and the exploratory preference percentage in the short-term memory assessment ($r = 0.8855$, $P < 0.0001$). Fig. 6B showed the correlation coefficients between the percentage of time spent on the open arm and the exploratory preference percentage in the long-term memory assessment ($r = 0.7082$, $P < 0.01$). Indeed, all evaluated parameters were positively correlated.

4.2. Ex vivo assays

4.2.1. Effect of 4-PSQ on OXA-induced Na^+, K^+-ATPase impairment

One-way ANOVA revealed that OXA (10 mg kg^{-1}, i.p.) significantly decreased the levels of mRNA expression of the enzyme Na^+, K^+-ATPase in cerebral cortex (57 %) (ANOVA: $F_{(3,24)} = 24.07$, $P < 0.0001$) (Fig. 7A) and hippocampus (40 %) (ANOVA: $F_{(3,24)} = 13.46$, $P < 0.0001$) (Fig. 7B) of mice, when compared OXA group with the control group. Despite this, we highlight that the treatment with the compound 4-PSQ (1 mg kg^{-1}, p.o.) normalized the expression level of the enzyme in the cerebral cortex (around 168 %) and hippocampus (around 73 %) when compared OXA + 4-PSQ group to the control group. Also, Fig. 7 showed that the OXA reduced Na^+, K^+-ATPase activity in the cerebral cortex (53 %) (ANOVA: $F_{(3,24)} = 5.619$, $P < 0.05$) (Fig. 7C) and hippocampus (77 %) (ANOVA: $F_{(3,24)} = 10.29$, $P < 0.001$) (Fig. 7D) of mice. 4-PSQ (1 mg kg^{-1}, p.o.) treatment normalized enzyme activity in the cerebral cortex (around 167 %) and hippocampus (around 287 %) when compared OXA + 4-PSQ group to the control group, since there was no significant difference between the control group and the 4-PSQ + OXA group.

4.2.2. Effect of 4-PSQ on OXA-induced disorders in the hypothalamic-pituitary-adrenal (HPA) axis

The corticosterone levels in the plasma of the mice were elevated by OXA exposure (ANOVA: $F_{(3, 22)} = 3.529$, $P < 0.05$) (Fig. 8). Besides that, one-way ANOVA revealed that the administration of 4-PSQ reversed the increase on the plasmatic corticosterone levels induced by OXA.

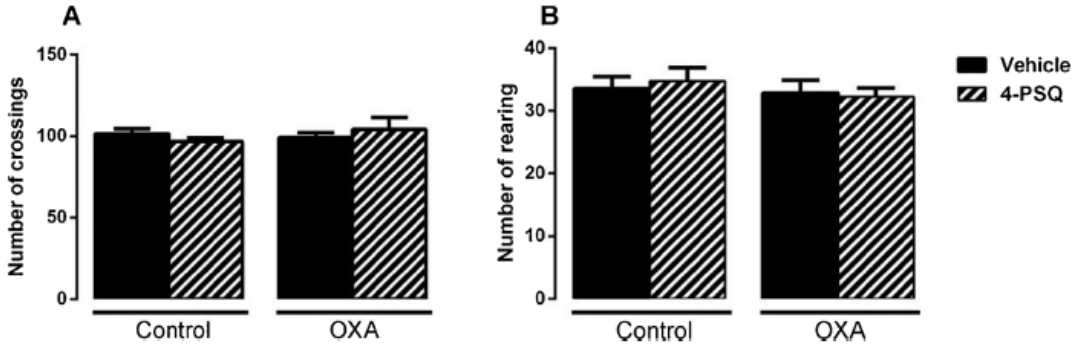


Fig. 3. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the (A) number of crossings and (B) number of rearings. Each column represents the mean ± S.E.M. of 7 mice in each group (One-way ANOVA followed by the Tukey's test).

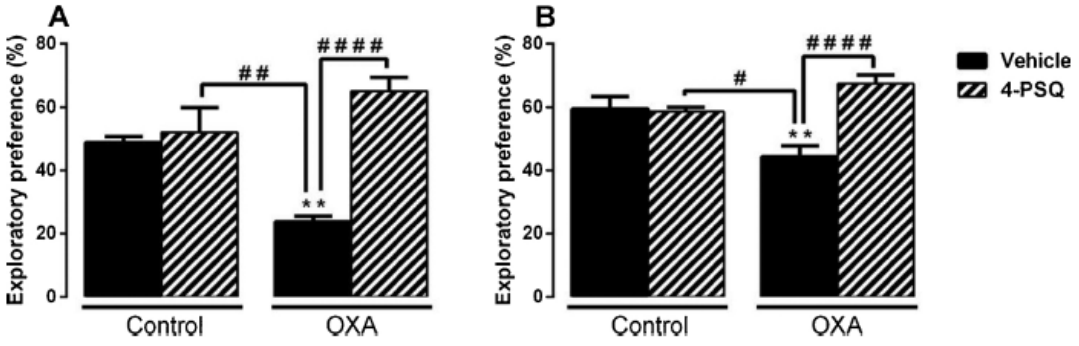


Fig. 4. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on (A) short-term and (B) long-term memories in the object recognition task. Each column represents the mean ± S.E.M. of 7 mice in each group. (**) $P < 0.01$ denotes significance levels when compared with the control group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with OXA group (One-way ANOVA followed by the Tukey's test).

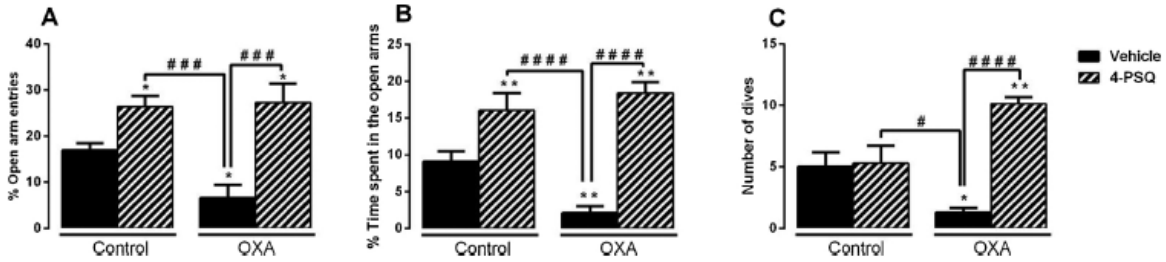


Fig. 5. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on behavioral parameters in the elevated plus-maze test in mice. (A) Percentage of entries in the open arms, (B) percentage of time spent in the open arms and (C) number of dives. Each column represents the mean ± S.E.M. of 7 mice in each group. (*) $P < 0.05$ and (**) $P < 0.01$ denote significance levels when compared with the control group; ($^{\#}$) $P < 0.05$, ($^{\#\#\#}$) $P < 0.001$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with OXA group (One-way ANOVA followed by the Newman - Keuls' test).

5. Discussion

Cancer survivors treated with chemotherapeutic agents show emotional and cognitive impairments (Tofthagen et al., 2013; Dubois et al., 2014; Sales et al., 2019). In the present study, it was demonstrated for the first time the correlation between anxious behavior and cognitive impairment induced by OXA exposure in mice. In addition, our findings indicated the pharmacological modulation of Na^+, K^+-ATPase as a potential target for OXA-induced neurotoxicity. Besides, OXA damage can be related with the increase on plasmatic corticosterone levels. Moreover, the present study also provided evidence for the potential effect of 4-PSQ on the OXA-related emotional and cognitive impairment in mice by modulating Na^+, K^+-ATPase and corticosterone levels. Indeed, 4-PSQ treatment has shown promising results, mainly in experimental models of anxiety and cognition (Reis et al., 2017; Barth et al., 2019; Pinz et al., 2018).

Here, OXA treatment caused anxious behavior in mice, as evidenced by reduction of the entries and time spent in the open arms in the elevated plus-maze. The elevated plus-maze test has been widely validated to measure anxiety in rodents (Quines et al., 2015; Socała and Wláz., 2016; Sousa et al., 2018). This test is based on the natural aversion of rodents for open and elevated areas as well as on their natural spontaneous exploratory behavior in novel environments. It is widely reported that the anxiety causes activation of the HPA axis, which leads to glucocorticoid release, such as corticosterone, from the adrenal cortex into the circulation (McGill et al., 2006). As expected, OXA treatment altered the HPA axis, as evidenced by the increase in corticosterone levels in plasma, which supports the results obtained in the behavioral test.

On the other hand, treatment with OXA also is associated with a decline in cognitive function, including learning and memory (Sales et al., 2019; Matsos and Johnston., 2019). In the current study, we

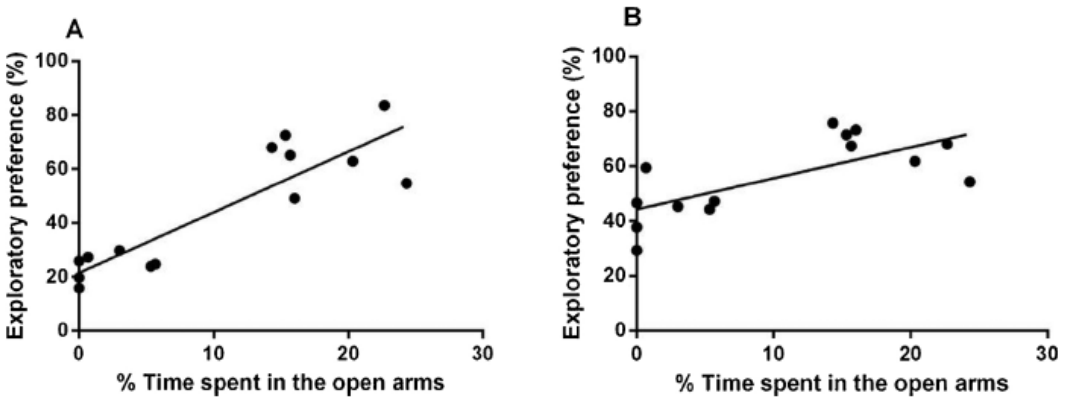


Fig. 6. Correlation between anxious behavior and cognitive impairment induced for treatment with OXA. Correlation coefficients between the percentage of time spent on the open arm and exploratory preference percentage in the short-term (A) and long-term (B) memory. Pearson correlation (*r*) and P-value were used to verify the correlation between data.

analyzed recognition memory with the object recognition task, a non-spatial memory test based on rodents' natural tendency to explore novel objects, which mainly relies on the function of the perirhinal cortex and hippocampus (Barker and Warburton., 2011). Our results demonstrated that mice treated with OXA had a deficit in STM and LTM, as evidenced by decreasing in the exploratory preference for the new object in the object recognition task. Corroborating our results, Matsos and Johnston (Matsos and Johnston., 2019) showed that OXA reduced preference for the novel object in the object recognition task.

Among the most important findings of this study, the significant positive correlation between anxious behavior and cognitive impairment induced by exposure to OXA stands out. The neurotoxicity remains the more common adverse effect of OXA, limiting its clinical use. Despite the efforts of several researchers engaged in the search for a better understanding of the physiopathological mechanisms involved in OXA-induced neurotoxicity, not all processes are completely understood. Thus, a common mechanism causing emotional and cognitive impairments after treatment with OXA was hypothesized in the present study.

In this sense, to increase the knowledge about the physiopathological mechanisms involved in OXA-induced neurotoxicity, the relationship between the Na^+, K^+-ATPase enzyme and the disorders

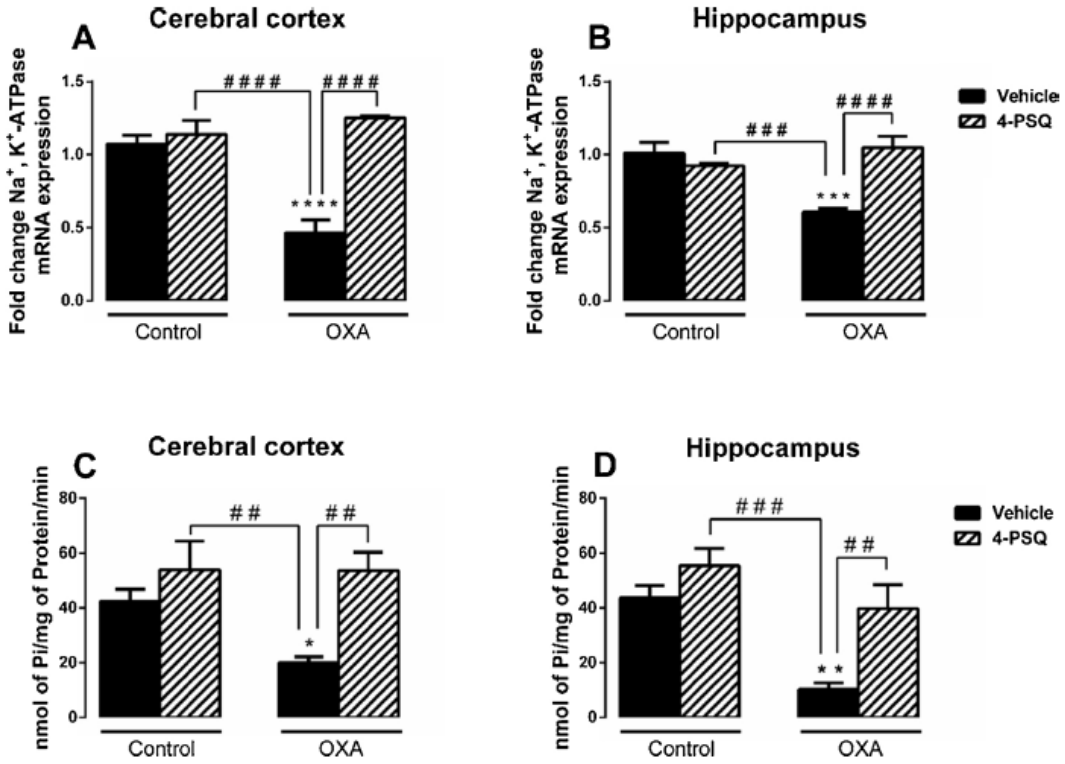


Fig. 7. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the mRNA Na^+, K^+-ATPase expression levels in the (A) cerebral cortex, (B) hippocampus, and on the Na^+, K^+-ATPase activity in the (C) cerebral cortex and (D) hippocampus. Each column represents the mean ± S.E.M. of 6 - 7 mice in each group. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ and (****) $P < 0.0001$ denote significance levels when compared with the control group; ($^{\#\#}$) $P < 0.01$, ($^{\#\#\#}$) $P < 0.001$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with OXA group (One-way ANOVA followed by the Newman - Keuls' test or Tukey's test).

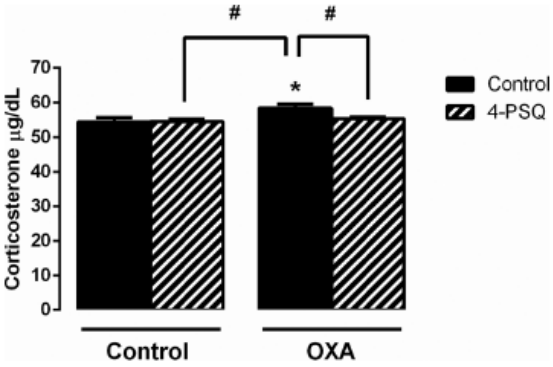


Fig. 8. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the corticosterone levels in plasma. Each column represents the mean ± S.E.M. of 6 - 7 mice in each group. (*) $P < 0.05$ denotes significance levels when compared with the control group and (#) $P < 0.05$ denotes significance levels when compared with the OXA group (One-way ANOVA followed by the Newman - Keuls' test).

induced by the treatment with OXA was investigated. Na^+, K^+-ATPase, which is the most important membrane protein expressed in almost all types of cells in the brain, has a crucial importance because neuronal activity widely depends on this enzyme that maintains the ion gradients and sets the foundation of excitability. Any impairment of its function can inevitably influence a variety of cellular processes (Baldissera et al., 2017). The Na^+, K^+-ATPase, in addition to the transport of sodium and potassium ions across the cell membrane, to perform many functions including maintaining and re-establishing the electrochemical gradient necessary for neuronal excitability. Also, it is responsible for the regulation of neuronal cellular volume and plays an important role in neuronal and synaptic plasticity. In this sense, decreased Na^+, K^+-ATPase activity has been associated with directly impaired signal transduction, which is important for cellular physiology as well as for the disease's progression. Besides that, decreased Na^+, K^+-ATPase activity was linked to oxidative damage (Barker and Warburton., 2011; Baldissera et al., 2017; Della-Pace et al., 2013). Indeed, deficiency on Na^+, K^+-ATPase has been pertinent in several disorders, such as increased anxiety-related behavior, depression, memory deficits, and Alzheimer's disease (Tofthagen et al., 2013; Cruzado et al., 2014; Dubois et al., 2014; Sales et al., 2019; Moseley et al., 2007; Goldstein et al., 2006; Kurauchi et al., 2019).

Moseley and contributors (Moseley et al., 2007) showed that Na^+, K^+-ATPase modulate cognitive deficit. A reduction on the activity of the Na^+, K^+-ATPase in the hippocampal cells may become the limiting factor in maintaining function, thereby compromising sensitive networks involved in processes such as learning and memory. Although models of hippocampal function emphasize its well-known role in cognitive functions, it has also been viewed as a neural mediator of emotion. Besides, it has been well described that brain Na^+, K^+-ATPase activity, including important regions such as the hippocampus and prefrontal cortex, may be involved in the etiology of neuropsychiatric disorders (Goldstein et al., 2006; Kurauchi et al., 2019).

Following our hypothesis about the relationship between enzyme Na^+, K^+-ATPase and the side effects of the chemotherapy drug OXA, data analysis revealed that OXA exposure reduced both the activity and levels of expression of the Na^+, K^+-ATPase in the hippocampus and cerebral cortex of mice. Still, our results also evidenced that Na^+, K^+-ATPase activity inhibition induced by OXA in the hippocampus was greater than in the cerebral cortex, despite the lower reduction in the levels of expression of the Na^+, K^+-ATPase in the hippocampus. In this sense, these data lead us to understand that the OXA could directly inhibit the activity of the enzyme in the brain structures studied, mainly in the hippocampus, not only indirectly through the reduction of expression levels. The reduction of the Na^+, K^+-ATPase activity and/or expression causes an imbalance in sodium and potassium ions, which are necessary to maintain the electric potential of brain cells, as well as the ionic gradient to neuronal excitability. Growing evidence suggests that inhibitors Na^+, K^+-ATPase cause memory deficits and behavioral disorders, including hyperactivity and impulsivity, which are common symptoms in several psychiatric disorders (Baldissera et al., 2017; Kurauchi et al., 2019).

According to (Tummala et al. (2009)), an increase in the expression of Na^+, K^+-ATPase enzyme leads to improved uptake of OXA by carcinoma cells. Thus, the authors suggest a potentially important role played by this enzyme in both prognosis and therapy of OXA-resistant malignancies. These results are in agreement with ours. In our study, the treatment with OXA reduced both activity and expression of the Na^+, K^+-ATPase enzyme. Therefore, the mechanism involved in the anxious behavior and cognitive impairment observed in this study can also be related to cells resistant to OXA and the consequent reduction of the pharmacological effect.

Indeed, there is a need for improving the quality of life of patients undergoing a treatment with OXA, mainly because the management of the harmful effects induced by this drug generally focuses on treating symptoms, since the cause still can not be treated. In this sense, the therapeutic potential of 4-PSQ on OXA-induced emotional and cognitive impairments was investigated. As expected, 4-PSQ restored emotional and cognitive impairment induced by treatment with OXA. Of particular importance in this study, 4-PSQ reestablished the activity and expression of the Na^+, K^+-ATPase, which was reduced by the OXA. Also, 4-PSQ reduced corticosterone levels in plasma, regulating the HPA axis.

4-PSQ elicits antinociceptive, antioxidant and neuroprotective effects in animal models (Pinz et al., 2016; Vogt et al., 2018). Reis and contributors (Reis et al., 2017) showed that 4-PSQ elicited anxiolytic-like behavior in mice. Indeed, 4-PSQ reduced glutamate uptake in cerebral cortices and protected against kainate-induced anxiety-related behavior. Thus, the current findings suggest that the glutamatergic pathway is implicated in the anxiolytic-like effect of 4-PSQ. In addition, it is believed that the decrease of glutamate uptake is a compensatory mechanism for another action exerted by 4-PSQ. Hence, a hypothesis to explain the anxiolytic-like action induced by 4-PSQ could be the inhibition of the glutamate action on its receptors, acting, for example, as an antagonist. Of particular importance, our previous study also demonstrated that the 4-PSQ effect started at 0.5 h and remained significant up to 72 h after administration, revealing a rapid and long-lasting action.

In addition, it was underscored the fact that the glutamatergic system, in particular the NMDA receptor, is strongly involved in the pharmacological actions of 4-PSQ (Silva et al., 2017). GLAST and GLT-1 are the most important transporters of glutamate in brain tissue transporting glutamate from the extracellular space into the cell protected against glutamate toxicity. In this context, GLAST and GLT-1 are dependent on sodium and potassium gradients which are generated principally by Na^+, K^+-ATPase to produce ion gradients that drive glutamate uptake (Zhang et al., 2009; Rose and Chatton., 2016). Thus, Na^+, K^+-ATPase is necessary for maintaining the glutamatergic pathway.

Kurauchi et al. (2019) showed that memantine, a blocker of the NMDA receptor, prevents the inhibition of the Na^+, K^+-ATPase activity. Besides, cognitive and emotional processes, mediated through the hippocampus and cerebral cortex, were affected by inhibition of the Na^+, K^+-ATPase (Baldissera et al., 2017; Kurauchi et al. (2019)). Consistent with these results, 4-PSQ protected against the decrease in the activity and expression of the Na^+, K^+-ATPase caused by treatment with OXA, in the hippocampus and cerebral cortex of mice. Consequently, the treatment with 4-PSQ reestablished the electric potential of brain cells and the ionic gradient to neuronal excitability, required for maintenance of the brain cellular processes.

Recently, our research group demonstrated that the 4-PSQ restored

the cognitive impairment caused by aging through synaptic plasticity modulation in rats (Barth et al., 2019). In addition, 4-PSQ was effective in protecting against anxiety and also against learning and memory impairment in a mouse model of Alzheimer's disease induced by amyloid β-peptide (Pinz et al., 2018). Anxiety is a neuropsychiatric symptom frequently observed in patients with Alzheimer's disease, a progressive neurodegenerative disorder. In our previous study, the treatment with 4-PSQ significantly attenuated the anxiogenic behavior induced by amyloid β-peptide, and the anticholinesterase and antioxidant actions contributed for the pharmacological effect of the compound (Pinz et al., 2018). Here, 4-PSQ restored the cognitive impairment and anxious behavioral caused by OXA, as evidenced by the object recognition task and in the elevated plus-maze test. It is important to mention that 4-PSQ is a multi-target molecule, and this certainly contributes to the relevant effects demonstrated in this study.

6. Conclusion

To summarize, our findings demonstrated that 4-PSQ-based therapy restored the emotional and cognitive impairment caused by OXA. Besides, the effects of 4-PSQ seem to be due to regulating the HPA axis through reducing corticosterone levels in plasma and mainly its ability to reestablish activity and expression of the Na^+, K^+-ATPase enzyme. Indeed, based on previous studies and according to the results obtained here, it was possible to infer that the modulation of the Na^+, K^+-ATPase had a fundamental role for the 4-PSQ effects on OXA-induced emotional and cognitive impairment. In addition, our results helped to expand knowledge about the mechanisms involved in the pathophysiology of the harmful effects OXA-induced, and suggest that the pharmacological modulation of Na^+, K^+-ATPase as a potential target for OXA-induced neurotoxicity. Thus, these results strongly contribute to the research of a novel therapeutic agent for emotional and cognitive impairment induced by OXA.

Authors statement

The manuscript and the data reported here have not been published previously and they are not under consideration for publication elsewhere. All listed authors have contributed significantly to the research and manuscript preparation. They consent to their names on the manuscript, approving thus the final article. All other authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the *Brain Research Bulletin*. There is no conflict of interest in the conduct and reporting of research (e.g., financial interests in a test or procedure, funding by pharmaceutical companies for drug research).

Author contributions

A.S.R., J.J.P., J.L.G., C.L and E.A.W. conceived and designed the study. W.B.D. and V.F.C. were responsible for perform qRT-PCR. G.P.C and D.A. were responsible for the synthesis of the compound. A.S.R., J.J.P, C.L. and E.A.W. wrote the manuscript. E.A.W and C.L. supervised the study. All authors approved the final version of the manuscript.

Declaration of transparency and scientific rigor

This declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigor of preclinical research recommended by funding agencies, publishers and other organizations engaged with supporting research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to UFPel, CNPq (00490362036, 429859/2018-0), L'ORÉAL-UNESCO-ABC "Para Mulheres na Ciência", and FAPERGS (PqG 17/2551-0001013-2) for financial support. C.L. and E.A.W. are recipients of CNPq fellowship. The publication of this paper was partially supported by PRPPGI/UFPel and CAPES.

References

Baldissera, M.D., Souza, C.F., Grando, T.H., et al., 2017. Nerolidol-loaded nanospheres prevent behavioral impairment via ameliorating Na^+, K^+-ATPase and AChE activities as well as reducing oxidative stress in the brain of Trypanosoma evansi-infected mice. Naunyn-Schmiedeberg's Arc. Pharmacol. 390, 139–148.

Barker, G.R., Warburton, E.C., 2011. When is the hippocampus involved in recognition memory? J. Neurosci. 31, 10721–10731.

Barth, A., Vogt, A.G., dos Reis, A.S., et al., 2019. 7-chloro-4-(Phenylselanyl) quinoline with memory enhancer action in aging rats: modulation of neuroplasticity. Acetylcholinesterase Activity, and Cholesterol Levels Mol Neurobiol. 1–11.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Cruzado, J.A., López-Santiago, S., Martínez-Marín, V., et al., 2014. Longitudinal study of cognitive dysfunctions induced by adjuvant chemotherapy in colon cancer patients. Support. Care Cancer 22, 1815–1823.

Curtis, M.J., Alexander, S., Cirino, G., et al., 2018. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br. J. Pharmacol. 175, 987–993.

Della-Pace, I.D., Rambo, L.M., Ribeiro, L.R., et al., 2013. Triterpene 3β, 6β, 16β trihidroxilup-20 (29)-ene protects against excitability and oxidative damage induced by pentylenetetrazol: the role of Na^+, K^+-ATPase activity. Neuropharmacology 67, 455–464.

Duarte, L.F.B., Oliveira, R.L., Rodrigues, K.C., et al., 2017. Organoselenium compounds from purines: synthesis of 6- arylselanylpurines with antioxidant and anticholinesterase activities and memory improvement effect. Bioorg. Med. Chem. 25, 6718–6723.

Dubois, M., Lapinte, N., Villier, V., et al., 2014. Chemotherapy-induced long-term alteration of executive functions and hippocampal cell proliferation: role of glucose as adjuvant. Neuropharmacology 79, 234–248.

Fiske, C.H., Subbarow, Y., 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66, 375–400.

Fitzmaurice, C., Allen, C., Barber, R.M., et al., 2017. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol. 3, 524–548.

Goldstein, I., Levy, T., Galili, D., et al., 2006. Involvement of Na+, K+-ATPase and endogenous digitalis-like compounds in depressive disorders. Biol. Psychiatry 60, 491–499.

Huličiak, M., Vacek, J., Šebela, M., et al., 2012. Covalent binding of cisplatin impairs the function of Na+/K+-ATPase by binding to its cytoplasmic part. Biochem. Pharmacol. 83, 1507–1513.

Kubala, M., Geleticova, J., Huliciak, M., et al., 2014. Na+/K+-ATPase inhibition by cisplatin and consequences for cisplatin nephrotoxicity. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. 158, 194–200.

Kurauchi, Y., Yoshimaru, Y., Kajiwara, Y., et al., 2019. Na+, K+-ATPase inhibition causes hyperactivity and impulsivity in mice via dopamine D2 receptor-mediated mechanism. Neurosci. Res. 146, 54–64.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression datausing real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.

Matsos, A., Johnston, I.N., 2019. Chemotherapy-induced cognitive impairments: a systematic review of the animal literature. Neurosci. Biobehav. Rev. 102, 382–399.

McGill, B.E., Bundle, S.F., Yaylaoglu, M.B., et al., 2006. Enhanced anxiety and stress-induced corticosterone release are associated with increased Crh expression in a mouse model of Rett syndrome. Proc Natl Acad Sci U S A. 103, 18267–18272.

Moseley, A.E., Williams, M.T., Schaefer, T.L., et al., 2007. Deficiency in Na, K-ATPase α isoform genes alters spatial learning, motor activity, and anxiety in mice. J. Neurosci. 27, 616–626.

Nichetti, F., Falvella, F.S., Miceli, R., et al., 2019. Is a pharmacogenomic panel useful to estimate the risk of oxaliplatin-related neurotoxicity in colorectal cancer patients? Pharmacogenomics J. https://doi.org/10.1038/s41397-019-0078-0.

Pellow, S., Chopin, P., File, S.E., et al., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.

Pinz, M., Reis, A.S., Duarte, V., et al., 2016. 4-Phenylselenyl-7-chloroquinoline, a new quinoline derivative containing selenium, has potential antinociceptive and anti-inflammatory actions. Eur. J. Pharmacol. 780, 122–128.

Pinz, M.P., dos Reis, A.S., Vogt, A.G., et al., 2018. Current advances of pharmacological properties of 7-chloro-4-(phenylselanyl) quinoline: prevention of cognitive deficit and anxiety in Alzheimer's disease model. Biomed. Pharmacother. 105, 1006–1014.

Quines, C.B., Da Rocha, J.T., Sampaio, T.B., et al., 2015. Involvement of the serotonergic system in the anxiolyticlike effect of 2-phenylethynyl butyltellurium in mice. Behav. Brain Res. 277, 221–227.
Reis, A.S., Pinz, M., Duarte, L.F.B., Roehrs, J.A., et al., 2017. 4-phenylselenyl-7-chloroquinoline, a novel multitarget compound with anxiolytic activity: contribution of the glutamatergic system. J. Psychiatr. Res. 84, 191–199.
Rose, C.R., Chatton, J.Y., 2016. Astrocyte sodium signaling and neuro-metabolic coupling in the brain. Neuroscience 323, 121–134.
Sales, M.V.C., Suemoto, C.K., Apolinario, D., et al., 2019. Effects of adjuvant chemotherapy on cognitive function of patients with early-stage colorectal cancer. Clin Colorectal Canc. 18, 19–27.
Silva, V.D., Reis, A.S., Pinz, M., et al., 2017. Further analysis of acute antinociceptive and anti-inflammatory actions of 4-phenylselenyl-7-chloroquinoline in mice. Fundam. Clin. Pharmacol. 31, 513–525.
Socała, K., Wlάz, P., 2016. Evaluation of the antidepressant- and anxiolytic-like activity of spinasterol, a plant derivative with TRPV1 antagonistic effects, in mice. Behav. Brain Res. 303, 19–25.
Sousa, F.S.S., Birmann, P.T., Balaguez, R., et al., 2018. A-(phenylselanyl) acetophenone abolishes acute restraint stress induced-comorbid pain, depression and anxiety-related behaviors in mice. Neurochem. Int. 120, 112–120.
Stangherlin, E.C., Rocha, J.B.T., Nogueira, C.W., 2009. Diphenyl ditelluride impairs short-term memory and alters neurochemical parameters in young rats. Pharmacol Biochem Be. 91, 430–435.
Starobova, H., Vetter, I., 2017. Pathophysiology of chemotherapy-induced peripheral neuropathy. Front. Mol. Neurosci. 10, 174.
Tofthagen, C., Donovan, K.A., Morgan, M.A., et al., 2013. Oxaliplatin-induced peripheral neuropathy's effects on health-related quality of life of colorectal cancer survivors. Support. Care Cancer 21, 3307–3313.
Tummala, R., Wolle, D., Barwe, S.P., et al., 2009. Expression of Na, K-ATPase-β 1 subunit increases uptake and sensitizes carcinoma cells to oxaliplatin. Cancer Chemother. Pharmacol. 64, 1187–1194.
Turabelidze, A., Guo, S., DiPietro, L.A., 2010. Importance of housekeeping gene selection for accurate reverse transcription-quantitative polymerase chain reaction in a wound healing model. Wound Repair Regen. 18, 460–466.
Vogt, A.G., Voss, G.T., de Oliveira, R.L., et al., 2018. Organoselenium group is critical for antioxidant activity of 7-chloro-4-phenylselenyl-quinoline. Chem-biol interact. 282, 7–12.
Walsh, R.N., Cummins, R.A., 1976. The open-field test: a critical review. Psychol. Bull. 83, 482–504.
Wang, H.Y.L., O'Doherty, G.A., 2012. Modulators of Na/K-ATPase: a patent review. Expert Opin. Ther. Pat. 22, 587–605.
Wang, F., Cai, B., Li, K.C., et al., 2015. FXYD2, a γ subunit of Na +, K +-ATPase, maintains persistent mechanical allodynia induced by inflammation. Cell Res. 25, 318.
Witlox, L., Schagen, S.B., de Ruiter, M.B., et al., 2019. Effect of physical exercise on cognitive function and brain measures after chemotherapy in patients with breast cancer (PAM study): protocol of a randomised controlled trial. BMJ Open 9, e028117.
Zenker, N., Bernstein, D.E., 1958. The estimation of small amounts of corticosterone in rat plasma. J. Biol. Chem. 231, 695–701.
Zhang, D., Hou, Q., Wang, M., et al., 2009. Na, K-ATPase activity regulates AMPA receptor turnover through proteasome-mediated proteolysis. J. Neurosci. 29, 4498–4511.

Capítulo 3

A influência da idade sobre a NPAIO: Novas perspectivas sobre a modulação da Na $^{+}$, K $^{+}$ - ATPase, estresse oxidativo e terapia com 4-PSQ

Considerando que em torno de 90% dos pacientes em tratamento com oxaliplatina desenvolvem neuropatia periférica aguda, na etapa 3 desta tese, o objetivo central consistiu em investigar o impacto causado pelo envelhecimento sobre os sintomas clínicos e mecanismos envolvidos na NPAIO e o potencial farmacológico do 4-PSQ. A fim de compreender essa relação e considerando os resultados obtidos anteriormente, aqui foi investigada a modulação da Na $^{+}$, K $^{+}$ - ATPase, enzima susceptível a processos neurodegenerativos induzidos por ambos os fatores estudados, bem como, o efeito da associação entre envelhecimento e o tratamento com a oxaliplatina sobre a produção de espécies reativas e defesas antioxidantes no SNC.

Os resultados obtidos demonstraram pela primeira vez que o envelhecimento exacerbou os sintomas clínicos observados na NPAIO, principalmente, por meio dos danos oxidativos progressivos causados pelo desequilíbrio redox, como evidenciado pela crescente produção de espécies reativas associada à redução das defesas antioxidantes e, também, por meio da modulação das enzimas Na^{+}, K^{+} - ATPase e Mg^{+2} - ATPase. De fato, os resultados sugerem que os danos causados aos neurônios sensoriais no SNC pelo desequilíbrio redox e, também, pela falta de energia em decorrência da inibição da Na^{+}, K^{+} - ATPase, podem estar relacionados ao aumento da hiperalgesia quando associado o tratamento com a oxaliplatina e a idade avançada.

Novamente, nesta etapa do estudo, o tratamento com o 4-PSQ apresentou resultados promitentes diante dos danos induzidos pela administração da oxaliplatina e, neste protocolo, exacerbados pelo envelhecimento. O 4-PSQ reverteu a hipersensibilidade induzida pela administração da oxaliplatina e agravada pelo envelhecimento por meio da redução das espécies reativas e modulação das enzimas antioxidantes GPx e SOD. Ainda, o 4-PSQ restabeleceu parcialmente a atividade da Na^{+}, K^{+} ATPase. Este estudo foi o primeiro desse tipo, aprovisionando uma nova visão sobre o impacto do envelhecimento nos mecanismos envolvidos na NPAIO (Figura 7). Além disso, este estudo corroborou as evidências sobre os efeitos

promissores do 4-PSQ na neurotoxicidade induzida pela oxaliplatina, agora, também considerando os agravantes induzidos pelo envelhecimento.

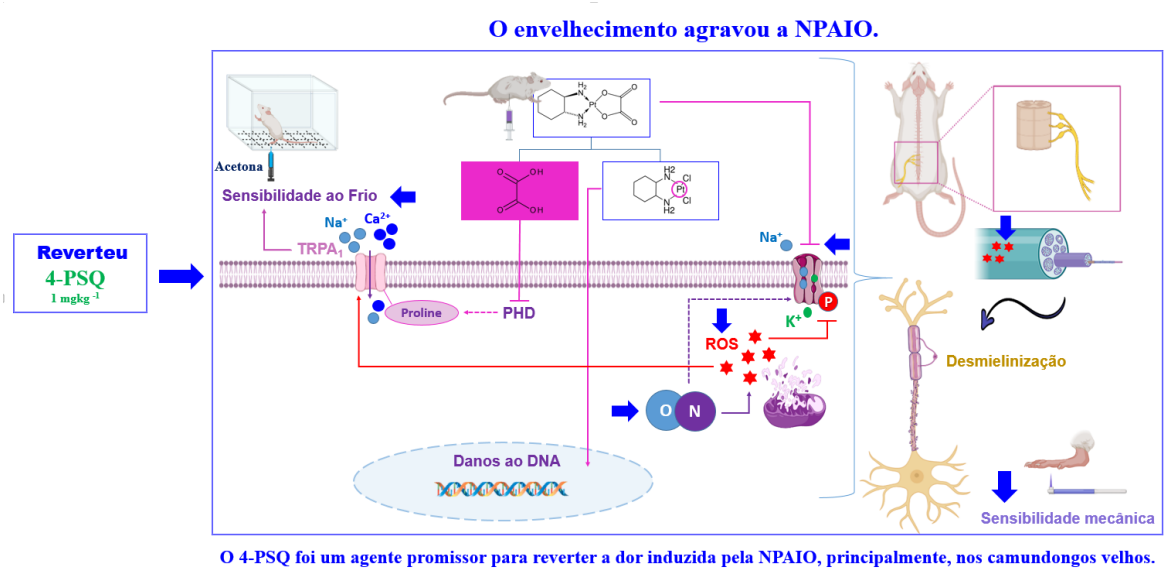


Figura 7. Representação esquemática dos principais resultados obtidos na etapa 3.

Manuscrito 1

Os resultados deste capítulo da tese estão apresentados sob a forma de manuscrito científico, o qual se encontra assim organizado. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados, Conclusões e Referências Bibliográficas encontram-se no próprio manuscrito.

O manuscrito 1 encontra-se aceito para publicação na revista *Molecular Neurobiology*.

Interface of aging and acute peripheral neuropathy induced by Oxaliplatin in mice: target-directed approaches for Na^+, K^+ - ATPase, oxidative stress and 7-chloro-4-(phenylselanyl) quinoline therapy

Angélica S. Reis[a], Carolina C. Martins[a], Ketlyn P. da Motta[a], Jaini J. Paltian[a], Gabriel P. Costa[b], Diego Alves[b], Cristiane Luchese[a*], Ethel A. Wilhelm[a*]

[a]*Programa de Pós-graduação em Bioquímica e Bioprospecção, Laboratório de Pesquisa em Farmacologia Bioquímica, CCQFA - Universidade Federal de Pelotas, UFPel - CEP - 96010-900, Pelotas, RS, Brasil.* [b]*Programa de Pós-graduação em Química, Laboratório de Síntese Orgânica Limpa, CCQFA - Universidade Federal de Pelotas, UFPel - CEP - 96010-900, Pelotas, RS, Brasil.*

*Address for correspondence:

Ethel Antunes Wilhelm; e-mail: ethelwilhelm@yahoo.com.br / Phone: +55-53-32757360

Cristiane Luchese; e-mail: cristiane_luchese@yahoo.com.br / Phone: +55-53-32757233

Programa de Pós-graduação em Bioquímica e Bioprospecção (PPGBBio), Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas (UFPel), Campus Capão do Leão, Pelotas, CEP 96010-900, RS, Brasil

Abstract

Almost 90% of patients develop pain immediately after oxaliplatin (OXA)-treatment. Here, the impact of aging on OXA-induced acute peripheral neuropathy and the potential of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) as a new therapeutic strategy were evaluated. In *Swiss* mice, the oxidative damage, and its influence on Mg^{2+} - ATPase and Na^{+}, K^{+} - ATPase activities were investigated. The relationship between the reactive species (RS) and nitrate and nitrite (NOx) levels, the activity of glutathione peroxidase (GPx), and superoxide dismutase (SOD) with the development of OXA-induced acute peripheral neuropathy was also studied. In this study, it was evidenced that OXA-induced acute peripheral neuropathy was exacerbated by aging through increased oxidative damage as well as Na^{+}, K^{+} - ATPase, and Mg^{+2} - ATPase inhibition. 4-PSQ reversed hypersensitivity induced by OXA and aging-aggravated by reducing RS and NOx levels, through modulation of GPx and SOD activities. 4-PSQ partially reestablish Na^{+}, K^{+} - ATPase activity, but not Mg^{2+} - ATPase activity. Locomotor and exploratory activities were not affected. This study is the first of its kind, providing new insight into the aging impact on mechanisms involved in OXA-induced acute peripheral neuropathy. Also, it provides evidence on promising 4-PSQ effects on this condition, mainly on aging.

Keywords: Aging; neuropathy; oxaliplatin; selenium; Na^{+}, K^{+} - ATPase; pain.

Abbreviations

4-PSQ, 7-chloro-4-(phenylselanyl) quinoline; OXA, Oxaliplatin; NMDA, N-methyl-D-aspartate; RS, reactive species; GPx, glutathione peroxidase; SOD, superoxide dismutase; NOx, nitrate and nitrite; CNS, central nervous system; NADPH, β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

Introduction

Oxaliplatin (OXA) is an important component in the treatment of colorectal cancer, which has significantly increased patient survival rates [1]. However, toxicity induced by OXA is associated with severe, frequently lasting, or permanent adverse effects. OXA-induced neurotoxicity is the major dose-limiting adverse effect leading to treatment discontinuation [1]. Of note, the OXA-induced neurotoxicity has been extensively investigated; however, significant challenges persist for the understanding of symptoms clinical and its cellular–molecular basis [1–3]. It is well-established that neurotoxicity induced by OXA causes initially the development of acute peripheral neuropathy and, posteriorly, continued exposure to OXA can lead to the development of chronic peripheral neuropathy.

At present, the mechanisms underlying peripheral neuropathy induced by OXA also remain unclear. It has been proposed that the accumulation of platinum lead to increase of oxidative stress mainly caused by superoxide and nitrite species [4, 5]. In fact, in OXA-induced acute peripheral neuropathy the more accepted hypothesis is due to impaired mitochondrial function, principally for failure bioenergetics, and reactive species (RS) generation [6]. Also, it was postulated as mechanism-basic the reversible interference with ion channels, and alterations on neuronal lipid synthesis and metabolism, but knowledge about other involved mechanisms is still limited [2].

In addition, many biological factors could have a negative impact on physiopathology of OXA-induced acute peripheral neuropathy and aggravate neuropathic pain, as degenerative process caused by disease or aging. Nowadays, the advances in public health, enhance the number of people that live into old age. There is estimates that in 2050 one-fifth of the world population will be over the age of 60 [7]. Within this age bracket, occurs the greater incidence of cancers [7], with a highlight to

colorectal cancer, principal cause of cancer-related death worldwide [8]. Indeed, aging is considered a risk factor for several chronic diseases, including neurodegenerative disorders and cancers. Aging-process occurs dynamically and progressively, and it is defined by a decline on repair capacity associated to decrease in physiological function and gradual deterioration of tissues and organ systems [9].

The experience of pain results from a complex set of actions and interactions between the central nervous system (CNS) and peripheral nervous system (PNS). In old age patients, is necessary to consider the neurogenesis declines with age [10], as well as progressive loss of tissue and organ function, particularly, due to the accumulation of oxidative damage to macromolecules [11]. Thus, old patients in OXA-treatment can be more susceptible to side effects, mainly pain. In this line, new therapeutic approaches should consider the aging-peculiarities in search for safe and effective therapy for OXA-induced acute peripheral neuropathy treatment.

Growing evidence has shown the importance of Na^+, K^+ - ATPase in basic cellular functions and, it has been suggested that dysfunctional activity of this enzyme can contribute to the pathogenesis of several CNS diseases. Indeed, previous studies suggested that dysfunctional Na^+, K^+ - ATPase activity throughout the aging-process renders neurons more susceptible to degeneration. Inhibited Na^+, K^+ - ATPase activity in neurons causes energy deficiency, increases intracellular Na^+, which leads to hyper-excitability cellular, thereby affecting neuronal functions. In turn, malfunction of this enzyme in astrocytes causes an increase in extracellular glutamate and rendering the CNS more vulnerable to the neurodegenerative processes [12, 13]. Moreover, Na^+, K^+ - ATPase enzyme activity is particularly vulnerable to drugs' action including the OXA. Recently, our research group demonstrated that the treatment with OXA caused neurotoxicity mainly by inhibiting Na^+, K^+ - ATPase activity, and reducing its

expression [14]. In this context, Na^+, K^+ - ATPase modulators can be promising in the development of alternative therapy for the acute peripheral neuropathy induced by OXA exposure, mainly in the elderly.

7-Chloro-4-(phenylselanyl) quinoline (4-PSQ) is an organoselenium compound and a quinoline derivative, which has been extensively studied by our research group [5, 14–26] and others [27–31]. Our research group showed that 4-PSQ exerts a memory enhancer action by modulating the neuroplasticity and the acetylcholinesterase activity in aging rats [18]. Also, it was demonstrated that 4-PSQ reduced chronic pain and reversed oxidative damage in a model of OXA-induced chronic neuropathy peripheral [5]. 4-PSQ reduced OXA-induced anxious behaviour and cognitive impairment, and the effects seem to be due to the modulation of Na^+, K^+ - ATPase [14]. Furthermore, 4-PSQ reversed kidney and liver damage induced by OXA [19, 20].

Given the promising effects of 4-PSQ against OXA-induced long-term toxicity, here we look for investigate whether this compound also mitigates OXA-induced acute peripheral neuropathy in mice. The aim of the present study also included to evaluate the

impact of aging, highlighting the reduction of oxidative stress and the modulation of Mg^{2+} - ATPase and Na^+, K^+ - ATPase activities as a pharmacological target to reduce the OXA-induced acute peripheral neuropathy in mice.

Materials and Methods

Animals

All experiments were performed following the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA 4506-2017) and in accordance with the National Institute of Health

Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) [32] and International Guiding Principles for Biomedical Research Involving Animals. The tests were carried out using adult (2 months) and old (20 months) *Swiss* male mice. This species has been used in several neuropathic pain studies [33, 34]. The adult mice were housed five per cage in a colony, whereas old mice were housed one per cage. All animals were maintained at 22 ± 2 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 6:00 a.m.). Mice were acclimatized to the behavioural room for at least 1 h. All efforts were made to minimize the number of animals used and their discomfort. The number of animals and intensities of noxious stimuli used was the minimum needed to demonstrate consistent effects of the treatment. Allocation concealment was performed using a randomization procedure (http://www.randomizer.org/). Behavioural evaluations were performed blindly on drug administration. All experiments were carried out between 08:00 and 17:00 h.

Drugs

4-PSQ was prepared and characterized in our laboratory. Nuclear magnetic resonance analysis (^{1}H and ^{13}C) showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 4-PSQ (99.9%) was determined by gas chromatography coupled with mass spectrometry (GC-MS) [27, 28].

4-PSQ and OXA were dissolved in canola oil and 5% glucose solution, respectively. OXA was obtained from Eurofarma pharmaceutical company. All other chemicals used in this study were of analytical grade and obtained from Sigma-Aldrich, (St. Louis, MO, USA). Mice received treatments by oral (p.o. - intragastric gavage) or intraperitoneal (i.p.) routes, at a constant volume of 10 mL kg^{-1} of body weight.

Experimental Design

Firstly, mice were randomly divided into six groups (7 animals/group): *i)* Young, *ii)* Young+OXA, *iii)* Young+OXA+4-PSQ, *iv)* Old, *v)* Old+OXA, and *vi)* Old+OXA+4-PSQ. On days 0 and 2, mice of the Young and Old groups received a 5% glucose solution (10 mL kg^{-1}, i.p.), whereas mice of the Young+OXA, Young+OXA+4-PSQ, Old+OXA, and Old+OXA+4-PSQ groups received OXA (10 mg kg^{-1}, i.p.) at a dose of 10 mg kg^{-1}. On day 2 of the experimental protocol, 0.5 h after OXA-administration, mice of the Young, Young+OXA, Old, and Old+OXA groups received canola oil (10 mL kg^{-1}, p.o.), whereas mice of the Young+OXA+4-PSQ and Old+OXA+4-PSQ groups received a single administration of 4-PSQ (1 mg kg^{-1}, p.o.). The behavioural tests were measured before (baseline values) and at 0.5 h after the 4-PSQ treatment to evaluate the acute OXA-induced thermal and mechanical sensitivities in *Swiss* mice.

Posteriorly, the animals were euthanized by inhalation of isoflurane anesthetic. The sciatic nerve, spinal cord, and cerebral cortex samples were rapidly dissected, weighed, and placed on ice then used for *ex vivo* assays (Fig. 1). The operators in the behavioural tests and data analysis were blinded. The dose and the protocol of treatment with 4-PSQ were based on previous studies [5, 14]. Given that other studies evaluating the pharmacological actions of the 4-PSQ on the OXA-induced neurotoxicity have been carried out [5, 14], a dose-response curve was not performed in the present study to minimize the number of animals used.

Behavioural tests

Measurement of thermal sensitivity

Thermal sensitivity was tested in mice as reported by Brusco et al. [33], with some modifications. Cold sensitivity was assessed by the acetone drop method. In this sense, a drop (20 μL) of acetone was applied three times in each right hind paw and cumulative scores were then generated following a 4-point scale: 0 = no response; 1 = quick withdrawal, flick or stamp of the paw; 2 = prolonged withdrawal or repeated flicking, and 3 = repeated flicking of the paw with licking directed at the ventral side of the paw.

Measurement of mechanical sensitivity

Mechanical sensitivity was carried out in mice according to the method previously described by Alamri et al. [35], with some modifications. For this test, mice were placed individually inside acrylic cages with wire grid floors 30 min before the start of testing performed in a quiet room. Before paw stimulation, the animals were quieted, without exploratory movements and not resting on their paws. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer (digital aesthesiometer, Insight, São Paulo, Brazil) adapted with a polypropylene tip. The paw withdrawal threshold was measured by applying the polypropylene tip perpendicular to the middle of the plantar surface of the hind paw at constant progressive pressure until paw withdrawal, and the pressure value was automatically recorded.

Assessment of locomotor and exploratory domains

The open field test was performed to evaluate the general locomotor and exploratory behaviours of mice. The open field was made of plywood and surrounded by 30 cm-high walls. The floor of the open field, 45 cm long and 45 cm wide, was divided by masking tape markers into 9 squares (3 rows of 3). In this test, each animal

was placed at the center of the open field and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory (number of rearing on the hind limbs) activities [36].

***Ex vivo* assays**

Based on the results of the behavioural tests, it was possible to elucidate the aging as well as 4-PSQ effects on pain caused by OXA-induced acute peripheral neuropathy. Posteriorly, *ex vivo* assays were performed to elucidate the aging targets that impact on OXA-induced acute peripheral neuropathy and, also, to the therapeutic effect of 4-PSQ. Sciatic nerve samples were collected to determine RS levels. Additionally, spinal cord and cerebral cortex samples were collected to determine oxidative stress markers, such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, as well as RS levels. Also, given that nitrate and nitrite (NOx) levels is an indicator of NO production, and it modulates Na^+, K^+ - ATPase activity, NOx levels were quantified in the spinal cord.

Furthermore, given that a decrease in activity and expression level of Na^+, K^+-ATPase in treatment with OXA has been reported [14], the involvement of the Na^+, K^+-ATPase, and Mg^{2+} - ATPase on OXA-induced acute peripheral neuropathy was evaluated in the spinal cord and cerebral cortex of mice. For these analyses, samples were homogenized in 50 mmol L^{-1} TrisHCl pH 7.4 centrifuged at 900 x g for 10 min to yield a supernatant (S_1). Specifically for NOx levels, samples were homogenized in 200 mM $ZnSO_4$ and acetonitrile (96%), centrifuged at 13,000 x g at 4 °C for 30 min, and the S_1 was collected.

Involvement of oxidative stress

RS levels

The RS levels were determined by a spectrofluorimetric method, using 2',7'-dichlorofluorescein diacetate (DCHF-DA) assay according to Loetchutinat et al. [37]. S_1 (50 μL) was incubated with 20 μL of DCHF-DA (1 mmol L^{-1}) and 2430 μL of Tris HCl (10 mmol L^{-1}) in pH 7.4. The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 525 nm (with 488 nm excitation) 60 min after the addition of DCHF-DA to the medium (Shimadzu RF-5301PC fluorometer). RS levels were expressed as arbitrary units of fluorescence.

NOx levels

NOx levels were assayed spectrophotometrically according to a previously published study by Miranda et al. [39]. Spinal cord samples were weighed and homogenized, the S_1 was used to determine the nitrate and nitrite content, an indicator of nitric oxide (NO) production. NOx content was estimated in a medium containing 2 % VCl_3 (in 5 % HCl), 0.1 % N-(1-naphthyl) ethylene-diamine dihydrochloride and 2 % sulfanilamide (in 5 % HCl). After incubating at 37 °C for 60 min, nitrite levels were determined spectrophotometrically at 540 nm, based on the reduction of nitrate to nitrite by VCl_3. Tissue nitrate/nitrite levels were expressed as nmol of NOx/mg of protein.

Antioxidant enzymes

GPx activity

GPx activity was assayed spectrophotometrically by the method of Wendel [40], which involves monitoring of the dismutation of H_2O_2 in the presence of S_1 at 340 nm. S_1 (50 μL) was added in a system composed of reduced glutathione

(GSH)/NADPH/glutathione reductase (GR), and the enzymatic reaction was initiated by the addition of H_2O_2 (100 µL). In this assay, the enzymatic activity is indirectly measured by NADPH decay. H_2O_2 is reduced and generates oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by the GR present in the analysis medium at the expense of NADPH. The enzymatic activity was expressed as nmol/min/mg protein.

SOD activity

This method is based on the capacity of SOD to inhibit the autoxidation of epinephrine. SOD activity was measured spectrophotometrically according to Misra and Fridovich' s method [41]. S_1 (6, 12, or 18 µL) was added to a 0.05 mol L^{-1} Na_2CO_3 buffer, and the enzymatic reaction was started by adding epinephrine (30 µL). The color reaction was measured at 480 nm. One unit of the enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26 °C. The enzymatic activity was expressed as U/mg protein.

Involvement of Na^+, K^+-ATPase and Mg^{2+} - ATPase activity

For the Na^+, K^+-ATPase activity assay, a reaction mixture was used containing S_1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samplings were performed under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at 37 °C for 30 min and the incubation was stopped by adding a 10 % trichloroacetic acid solution (TCA) with 10 mM $HgCl_2$. Na^+, K^+-ATPase activity was calculated by the difference between the two assays. Released

inorganic phosphate (Pi) was measured according to Fiske and Subbarow [42]. Enzyme activity was expressed as nmol of Pi/mg of protein/min.

For the Mg^{2+}- ATPase activity assay, a reaction mixture was used containing S_1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4 [43]. Controls to correct for non-enzymatic substrate hydrolysis were prepared by adding sample preparations after the reactions were stopped with TCA. To determine the Mg^{2+}-ATPase activity, ouabain (1 mM) was added to the reaction medium. The reaction was initiated by adding ATP and was stopped after 30 min of incubation by the addition of 10% TCA. Enzymatic activity was expressed as nmol of Pi/mg of protein/min.

Protein determination

The protein concentration was measured spectrophotometrically at 595 nm by the method of Bradford [44], using bovine serum albumin as the standard. It is a rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. The reaction mixture contained S_1 (50 µL) and coomassie brilliant blue (2.5 mL). The reaction mixture was incubated for 10 min. The protein level was expressed as mg protein/mL.

Data and statistical analysis

The normality of the data was evaluated using the D' Agostino and Pearson omnibus normality test. Statistical analysis was performed using GraphPad Prism 6.0 software (San Diego, CA, USA). Data were analyzed by two-way analysis of variance (ANOVA) followed by the Tukey test when appropriated for parametric data. Data were expressed as mean ± standard error of the mean (S.E.M.). Post hoc tests were performed only when the F-value achieved the necessary level of statistical significance ($P < 0.05$)

and when there was no significant variance in the homogeneity. All data were evaluated for outliers (ROUT (Q = 1.0 %)). The outliers were excluded from the results. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology [45].

Results

Sensitivity aging-caused exacerbate cold and mechanical hypersensitivities induced by OXA

To establish the impact of aging on OXA-induced acute peripheral neuropathy and identify the molecular mechanisms underlying, it is necessary to consider cold and mechanical sensitivities. In this sense, we employed well-established tests of the acetone drop method and von Frey test, in Young and Old *Swiss* mice. Interestingly, we observed that older animals are more sensitive to cold stimulus (Fig. 2), as evidenced by the increase in the cumulative scores of the test scale; and to mechanical stimulus (Fig. 3), as evidenced by the decrease in the paw withdrawal threshold. In this sense, old mice presented higher cold (around 500%) and mechanical (17%) sensitivity than young mice.

Here, it was administrated a well-defined dose of OXA (10 mg kg^{-1}, i.p.) to induced pain in mice. In fact, a stable and robust thermal (Fig.2) and mechanical sensitivities (Fig. 3) were observed. These acute effects were observed 0.5 h after OXA-administration on day 2 of the experimental protocol. Old mice that received OXA showed an increase of 66% in the cumulative scores of cold sensitivity, whereas it was observed a reduction of 40% in the paw withdrawal threshold in the mechanical sensitivity test. In turn, after administration of OXA, young mice presented an increase of 560% in the cumulative scores of cold sensitivity, whereas the paw withdrawal

threshold decreased 35% in the mechanical sensitivity test. In addition, it was observed that the old mice that received OXA presented higher cold (54%) and mechanical (25%) hypersensitivities than young mice, also treated with OXA. Indeed, it was observed that older animals are more sensitive to a mechanical and thermal stimulus. Concomitantly, aging also increased the animals' vulnerability to effects OXA-induced acute peripheral neuropathy. Overall, these results show that the OXA-administration (10 mg kg^{-1}, i.p.) produced cold and mechanical hypersensitivities in Young and Old mice, however, Old mice were more susceptible to the toxic effects of OXA and presented higher sensitivity.

4-PSQ reduces acute cold and mechanical sensitivities induced by OXA in aged and young mice

Given the increase in the cold and mechanical sensitivities induced by aging and/or OXA, we subsequently aimed to investigate whether the treatment with 4-PSQ could restore pain sensitization. The old and young animals exposed to OXA (10 mg kg^{-1}, i.p.) that received 4-PSQ (1 mg kg^{-1}, p.o.) demonstrated normalized sensitivity, the cold (Fig. 2) and mechanical (Fig. 3A) sensitivities of Old+OXA+4-PSQ and Young+OXA+4-PSQ groups were similar to the Young group (vehicle). These results revealed that the treatment with 4-PSQ protected against hypersensitivity induced by both OXA and aging.

Locomotor and exploratory behaviours of young and old mice are not altered by 4-PSQ and OXA

The results of number of rearings and crossings in the open field test are presented in Fig. 3A and B, respectively. The data analysis revealed that treatment of

mice with 4-PSQ and/or OXA did not cause any significant change in the number of crossings or rearings in young and old animals.

Oxidative stress contributes to acute peripheral neuropathy induced by OXA in mice and 4-PSQ exerts antioxidant effect

To reinforce the contribution of oxidative stress to OXA-induced acute peripheral neuropathy, RS levels (Fig. 4), NOx levels (Fig.5), and activity of antioxidant enzymes (GPx and SOD) (Fig. 6) were evaluated in the sciatic nerve, spinal cord and/or cerebral cortex of mice. Aging led to increased levels of RS in the spinal cord (51%) (Fig. 5B) and cerebral cortex (38%) (Fig. 5C) of mice. Besides, it was observed increased levels of RS in the sciatic nerve, spinal cord, and cerebral cortex of the old and young mice that received OXA. In particular, old mice that received OXA showed higher values of RS, in the spinal cord and cerebral cortex, than Young+OXA mice.

In turn, the results showed that treatment with 4-PSQ (1 mg kg^{-1}, p.o.) reversed the increase in the RS levels induced by OXA in the sciatic nerve of old and young mice (Fig. 5A). Also, treatment with 4-PSQ reversed increased levels of RS induced by OXA in the spinal cord of young mice (Fig. 5B). However, 4-PSQ-treatment partially reversed the increase in the RS levels induced by OXA in the spinal cord of the old mice. In the cerebral cortex, the treatment with 4-PSQ reduced the RS levels induced by OXA in old and young mice (Fig. 5C).

To determine whether especially RS of the nitrogen plays a key role in OXA-induced acute peripheral neuropathy, NOx levels were evaluated. As shown in Fig. 6, aging increased the NOx levels in the spinal cord of mice. Also, OXA administration significantly increased NOx levels in the spinal cord of young and old mice. On the other hand, the analysis of the results revealed that the treatment with 4-PSQ reduced

the levels of NOx in the spinal cord of young and old mice exposed to OXA. However, we highlight that the old mice that received OXA showed higher values of the NOx in the spinal cord than young mice OXA-administrated.

The results suggest that increased RS and NOx levels contribute to OXA-induced acute peripheral neuropathy and, to elderly susceptibility for OXA-toxicity. Also, results indicate that the antioxidant effect of 4-PSQ can be involved in its analgesic action. Based on this, next, we examined whether antioxidants enzymes GPx and SOD contribute to OXA and 4-PSQ effects, and to the vulnerability of old to OXA.

Fig. 7 summarizes the results obtained regarding the GPx and SOD activities analyzed after the administration of OXA (10 mg kg^{-1}, i.p.) and/or treatment with 4-PSQ (1 mg kg^{-1}, p.o.) in old and young mice. OXA administration significantly increased GPx activity in the spinal cord (Fig. 7A) and cerebral cortex (Fig. 7B) of young mice, to 161% and 205%, respectively. In the spinal cord (Fig. 7A) and cerebral cortex (Fig. 7B) of Old, Old+OXA, and Old+OXA+4-PSQ mice groups no alteration in the GPx activity was observed after the treatments. On the other hand, the analysis of the results revealed that the increase in the GPx activity induced by OXA in young mice was normalized in the cerebral cortex (Fig. 7B) and reversed partially in the spinal cord (Fig. 7A) after 4-PSQ treatment.

As shown in Fig. 7, OXA (10 mg kg^{-1}, i.p.) administration increased the SOD activity to 126% in the cerebral cortex (Fig. 7D) of young mice. However, old mice that received OXA did not modify SOD activity in the spinal cord and the cerebral cortex. An increase in the spinal cord (Fig. 7C) and cerebral cortex (Fig. 7D) SOD activity of young and old animals exposed to OXA and treated with 4-PSQ was observed.

Aging and/or OXA exposure modify Na^+, K^+ - ATPase, and Mg^{2+} - ATPase activities: target approaches of 4-PSQ

Aging significantly decreased the Na^+, K^+- ATPase activity in the spinal cord of mice (Fig. 8A). Also, in the spinal cord and cerebral cortex of mice, Mg^{+2} - ATPase activity was reduced by aging (Fig. 8C and 8D). In turn, a decreased in the spinal cord Na^+, K^+- ATPase activity of the young mice exposed to OXA was observed (Fig. 8A). OXA administration also reduced Na^+, K^+- ATPase activity in the cerebral cortex of young and old mice (Fig. 8B). Concerning the Mg^{+2} - ATPase activity, no alteration was evidenced on the spinal cord and cerebral cortex of old and young mice after treatment with OXA respectively (Fig. 8C and 8D).

Despite this, we highlight that the treatment with the compound 4-PSQ partially reversed the effects caused by aging and OXA on the Na^+, K^+- ATPase activity in the spinal cord of young mice (Fig. 8A). Besides, the 4-PSQ-treatment reversed the effects caused by aging and OXA on the Na^+, K^+- ATPase activity in the spinal cord of old mice (Fig. 8A). 4-PSQ also reversed the effects caused by aging and OXA on the Na^+, K^+- ATPase activity in the cerebral cortex of young and old mice (Fig. 8B). No alteration was evidenced on the Mg^{+2} - ATPase activity in the spinal cord and cerebral cortex of old and young mice after treatment with OXA and 4-PSQ (Fig. 8C and 8D).

Discussion

Acute neuropathy is specifically caused by OXA, affects most patients undergoing treatment, and is mainly characterized by cold hypersensitivity and dysesthesia of the peripheral regions. Furthermore, there is no defined specific pathophysiology for the disease or appropriate diagnosis and treatment to date [6]. In the present study, it was demonstrated that aging exacerbates acute peripheral

neuropathy induced by treatment with OXA. Here, we evidenced that aging exacerbated cold and mechanical hypersensitivity OXA-induced, mainly through oxidative damage induced to PNS and CNS, and by inhibiting Na^+, K^+-ATPase activity. Importantly, also it was observed that treatment with 4-PSQ reversed cold and mechanical hypersensitivity OXA-induced in both ages evaluated. In this way, findings lead us to believe that the 4-PSQ modulated the pain and suppress the signaling pathways of OXA-induced acute peripheral neuropathy in the aged and young, mainly, through its antioxidant activity. Consequently, the 4-PSQ reduced the cold and mechanical hypersensitivity as well as normalized Na^+, K^+-ATPase enzyme activity.

Indeed, it has been reported that oxidative stress contributes to the development of OXA-induced neurotoxicity [46, 47]. In this sense, recently our research group demonstrated, for the first time, that the treatment with OXA causes deposition of platinum in the spinal cord. On well-established model of OXA-induced chronic peripheral neuropathy in mice, we showed that the platinum crosses the blood-brain-barrier and starting RS formation [5]. Besides, the key mechanism involved in OXA-induced acute peripheral neuropathy is due to oxalate metabolites, which can alter the functional properties of voltage-gated Na^+ channels, lead to hyper-excitability of dorsal root ganglion sensory neurons [48]. However, despite continued efforts for a better understanding of the mechanisms involved in the OXA-induced acute peripheral neuropathy, not all the physiopathology processes were completely understood. For a better understanding of the mechanisms involved in this condition, in the present study the impact aging-caused in OXA-induced acute peripheral neuropathy was investigated.

Firstly, we showed that aging caused hypersensitivity (pain in response to innocuous stimuli) in the animals. Indeed, previous studies suggest that the impact caused by aging, mainly on the brain, contributes to the development of pain [49, 50]. In

addition, as previously reported aging can alter many aspects involved in the physiopathology of the pain, including increased oxidative damage, and decline of antioxidant defense [51]. Here, aging led to oxidative damage, as evidenced by the increase in the RS and NOx levels on CNS and spinal cord, respectively. Corroborating with the results of this study, previously published data demonstrated that the process of aging affects the production of RS and thus, the cellular redox state. Aging reduces the ability of the cell to maintain its proteome [52]. Here, the relationship between aging and inhibition of Mg^{2+} - ATPase enzyme activity in the spinal cord and cerebral cortex was observed. In this line, we can suggest that alterations in intracellular Mg^{2+} concentration on CNS also contribute to disorders associated with aging such as pain. The Mg^{2+} - ATPase enzyme is one of the Mg^{2+} transporting systems essential, which is localized mainly in the plasma membrane. It is known that Mg^{2+} plays a crucial role in a living organism's functioning including protein synthesis and enzymatic cofactor involved in carbohydrate metabolism and DNA synthesis [53]. In turn, in the present study Na^{+}, K^{+} - ATPase, SOD, and GPx activities were not changed by aging.

Posteriorly, we highlight that the aging exacerbated OXA-induced mechanical and cold hypersensitivity. Indeed, cold hypersensitivity is one of the major characteristics of OXA-induced acute peripheral neuropathy, which is manifested clinically in patients as severe pain in response to a normal cold stimulation [48]. Here, we also demonstrated that OXA-administration caused oxidative damage in young mice, and enhancement oxidative damage in old mice, as evidenced by the increase in the RS levels, on PNS and CNS, as well as by an increase in the spinal cord NOx levels.

In the present study, the response of the antioxidant system to oxidative damage caused by OXA was different between aged and young mice. Indeed, young animals exposed to OXA presented an increase in the SOD and GPx activities on the CNS.

Accordingly, Wang et al. [54] demonstrated that OXA is an activator of the Nuclear factor-erythroid-2 related factor 2 (Nrf2) signaling pathway, which is considered the key regulator of the body's antioxidant response and is responsible for inducing the expression of genes encoding antioxidant proteins and enzymes such as SOD and GPx. However, results obtained in the present study highlight old mice, which also received OXA, did not present an increase in the activity of enzymatic antioxidant defenses evaluated. It is established that cellular functionality is modulated by balance redox, mainly because RS can seriously damage cellular macromolecule, including proteins as antioxidant enzymes. In this sense, considering our results, it was established that the deficiencies in the enzymatic antioxidant system caused by aging exacerbated the OXA-induced acute peripheral neuropathy and could be related to aggravating hypersensitivity to pain observed in the old mice that received OXA. Corroborating with our results, recently the use of antioxidant molecules has been indicated as a potential strategy to manage pain [55, 56]. Antioxidants reduced mitochondria damage and pain induced by chemotherapeutic drugs supporting a pivotal role for oxidative stress in the pain [56].

To explore other mechanisms involved in the OXA-induce acute neuropathic pain, possible changes in Mg^{2+} - ATPase and Na^{+}, K^{+} - ATPase activities on CNS were evaluated. Although aging has inhibited Mg^{2+} - ATPase activity on CNS, treatment with OXA did not modify the activity of this enzyme. However, results obtained indicate that the treatment with OXA inhibited Na^{+}, K^{+} - ATPase activity. In accordance with our results, increasing evidence suggests that occurs a negative interaction between Na^{+}, K^{+} - ATPase enzyme and platinum-based chemotherapeutic agents [14, 57–59]. A reduction in the expression of β1 subunit in the Na^{+}, K^{+} - ATPase protein is associated with OXA resistance in cancer cells [59]. Recently, our research group demonstrated

that treatment with OXA inhibited the Na^+, K^+ - ATPase activity in young mice [14]. Further, we also showed that the treatment with 4-PSQ reduced both anxious behaviour and cognitive impairment induced by OXA in young mice, mainly through the modulation of Na^+, K^+ - ATPase enzyme.

Neuronal Na^+, K^+ - ATPase in both pre- and post-synaptic sites, and on astrocytes membrane is responsible for the maintenance of ionic gradient within the cells that form the CNS. Around 50% amount of ATP of a healthy brain is expensed to maintain Na^+, K^+ - ATPase activity. On the other hand, the inhibition of Na^+, K^+ - ATPase plays crucial role in several diseases on CNS, including neurological, neuropsychiatric, and neurodegenerative disorders [13]. OXA-induced acute peripheral neuropathy is a neurological disorder that causes mitochondrial dysfunction leading to accumulation of RS and oxidative damage [60, 61]. In this line, our findings are according to previously published data. Our data evidenced that the growing oxidative damage, as well as inhibition of Na^+, K^+ - ATPase, are mechanisms involved in OXA-induced acute peripheral neuropathy, mainly in the aged. Importantly, it has been reported that oxidative stress indirectly affects Na^+, K^+ - ATPase activity. In several neurological diseases, the increased oxidative stress due to reduced mitochondrial activity, as OXA-induced, leads to reduced ATP – synthesis on the CNS, impairing the function of Na^+, K^+ - ATPase enzyme. Also, a reduction in Na^+, K^+ - ATPase activity was correlated with hyper-excitability due to increases intracellular Na^+, increased glutamate-induced calcium influx, and neurodegeneration on CNS [13].

On the other hand, Munhoz and contributors [62] demonstrate that stimulation of NMDA receptor-NOS by glutamate leads to increased Na^+, K^+ - ATPase activity. Through Ca^{2+} influx induced by glutamate occurs the activation of neuronal NO synthase and, successively, increased production of NO. In turn, NO increases

concentrations of the second messenger cGMP, lead to up-regulating cGMP-dependent protein kinase (PKG). PKG modulates Na^+, K^+ - ATPase activity, both centrally and peripherally. Concerning OXA, this result can be explained by a pharmacological inhibition due to a negative interaction between OXA and Na^+, K^+ - ATPase. Considering aging, degenerative processes associated with aging could be responsible for a decrease in the Na^+, K^+ - ATPase activity. This hypothesis is supported by previous studies that demonstrated also a decline in Na^+, K^+ - ATPase activity aging-induced, through of modulate in the glutamate-cGMP-PKG pathway [12].

The damage of sensory neurons caused by OXA leads to neuropathic pain, characterized by an increase in mechanical and thermal sensitivity [46]. Here, OXA produced a significant increase in the cold and mechanical sensitivity in young and mainly old mice, through growing oxidative stress that led to inhibiting of the Na^+, K^+ - ATPase. In turn, the management of OXA-induced acute neuropathic pain generally focuses on treating symptoms, since the cause of the OXA-induced acute neuropathic pain still cannot be treated. In view of promising effects of 4-PSQ on neurotoxicity induced by treatment with OXA, including reduced chronic pain [5], and, also, anxious behaviour and cognitive impairment induced by OXA [14], this molecule was investigated for the first time as a possible therapeutic strategy for the treatment of OXA-induced acute peripheral neuropathy. 4-PSQ has been characterized as a promising compound with several pharmacological effects including antinociceptive [23] and anxiolytic [22, 25], also reduced aging-induced cognitive impairment [18]. 4-PSQ elicits effects through antioxidant [16, 20, 27, 31], anti-inflammatory [26], and neuroprotective [14, 15] properties. Additionally, 4-PSQ modulates serotonergic, nitrergic, glutamatergic, and cholinergic systems [24, 25, 26,]. In this study, 4-PSQ

reversed the cold and mechanical hypersensitivity induced by OXA acute exposure, without impairing the locomotor and exploratory capacity of the animals.

It is important to emphasize that 4-PSQ-effects on cold and mechanical hypersensitivity were no different in both old and young mice. Of particular importance in this study, 4-PSQ reversed the oxidative imbalance caused by OXA also in both ages analyzed, because it reduced levels of RS and NOx in all tissues evaluated. Beyond these processes, it is also known that OXA modulates the enzymatic antioxidant defense system. Indeed, we demonstrate that when administered to young mice, OXA causes an increase in the GPx and SOD activities on CNS to balance the increase of pro-oxidant molecules. In this case, the treatment with 4-PSQ partly or completely reversed GPx activity, as well as increased or did not change SOD activity in the spinal cord and cerebral cortex, respectively. This result can be explained by the antioxidant effect of 4-PSQ because reducing pro-oxidant molecules lead to normalization of antioxidant defenses. Nevertheless, when aging mice received OXA did not any significant change in the activity of the antioxidant enzymes on CNS, even in front of an increase of pro-oxidant molecules. In this case, the treatment with 4-PSQ induced an increase on SOD activity in the spinal cord and cerebral cortex but did not change the GPx activity was observed. Indeed, we reiterated that treatment with 4-PSQ reversed the increase of RS and NOx levels of aged that received OXA in all tissues analyzed.

Consistent with these results, 4-PSQ protected against the inhibition in the activity of the Na^+, K^+ - ATPase caused by treatment with OXA on the CNS, in both aged and young. Consequently, the treatment with 4-PSQ reestablished the electric potential of brain cells and the ionic gradient to neuronal excitability, required for maintenance of the brain cellular processes. Although 4-PSQ did not reverse the inhibition of Mg^{2+} - ATPase induced by aging, this enzyme is seen not to be involved

with the mechanisms of OXA-induced acute peripheral neuropathy. Based on the obtained results, it is possible to suggest that 4-PSQ is a promising molecule in search of a new therapeutic strategy for OXA-induced acute peripheral neuropathy because it acts on multiple targets that are altered in this pathology, regardless of age.

Conclusions

To summarize the current knowledge, this study helped to expand understanding about the mechanisms involved in the physiopathology of the OXA-induced acute peripheral neuropathy and advanced in the search for a new therapy. Here, the relationship between OXA-administration, aging, and the increase in the RS and NOx levels, and modulation of the enzymes SOD, GPx, Na^+, K^+ - ATPase, and Mg^{2+} - ATPase in the CNS was reported. In this sense, our findings demonstrated that 4-PSQ-based therapy reduced cold and mechanical hypersensitivity caused by OXA. Besides, the effects of 4-PSQ seem to be due to antioxidant and neuroprotective effects, mainly for its ability to reestablish activity of the Na^+, K^+ - ATPase enzyme. These results suggest that 4-PSQ might be a good prototype for OXA-induced acute peripheral neuropathy treatment.

Declarations

Funding Information

This study received financial support and scholarships from the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (429859/2018-0, 312747/2020-9), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) (PqG 17/2551-0001013-2). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível superior – Brasil

(CAPES) - Finance Code 001. C.L.; E.A.W.; D.A. are recipients of CNPq fellowship. This study also received financial assistance from L'ORÉAL-UNESCO-ABC for Women in Science.

Author contributions

A.S.R., C.C.M., K.P.M, J.J.P., C.L. and E.A.W. conceived and designed the study. G.P.C. and D.A. performed the 4-PSQ synthesis. A.S.R. and E.A.W. wrote the manuscript. E.A.W. supervised the study. All authors approved the final version of the manuscript.

Availability of Data and Material

All data generated or analyzed during this study are included in this published article.

Code Availability

Not applicable.

Compliance with Ethical Standards

Animal care and all experimental procedures were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996). Also, this study was performed in line with the principles of the Declaration of Helsinki, and in accordance with the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 4506-2017). All efforts were made to minimize the number of animals used and their suffering.

Conflict of interest

The authors declare that they have no conflicts of interest.

Consent for Publication

Not applicable.

Consent to participate

Not applicable.

Acknowledgements

Not applicable.

References

1. Nichetti F, Falvella FS, Miceli R, et al (2019) Is a pharmacogenomic panel useful to estimate the risk of oxaliplatin-related neurotoxicity in colorectal cancer patients? Pharmacogenomics J. https://doi.org/10.1038/s41397-019-0078-0
2. Rimola V, Hahnefeld L, Zhao J, et al (2020) Lysophospholipids contribute to oxaliplatin-induced acute peripheral pain. J Neurosci 40:9519–9532. https://doi.org/10.1523/JNEUROSCI.1223-20.2020
3. Cavaletti G, Marmiroli P cancers Management of Oxaliplatin-Induced Peripheral Sensory Neuropathy. https://doi.org/10.3390/cancers12061370
4. GJ B, T D, D S (2014) Mitotoxicity in distal symmetrical sensory peripheral neuropathies. Nat Rev Neurol 10:326–336. https://doi.org/10.1038/NRNEUROL.2014.77
5. Reis AS, Paltian JJ, Domingues WB, et al (2020) Advances in the Understanding of Oxaliplatin-Induced Peripheral Neuropathy in Mice: 7-Chloro-4-

(Phenylselanyl) Quinoline as a Promising Therapeutic Agent. Mol Neurobiol 57:5219–5234. https://doi.org/10.1007/s12035-020-02048-4

6. Cavaletti G, Marmiroli P (2020) Management of oxaliplatin-induced peripheral sensory neuropathy. Cancers (Basel). 12
7. Fane M, Weeraratna AT (2020) How the ageing microenvironment influences tumour progression. Nat. Rev. Cancer 20:89–106
8. Ferlay J, Colombet M, Soerjomataram I, et al (2019) Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int. J. Cancer 144:1941–1953
9. Bhadra M, Howell P, Dutta S, et al (2020) Alternative splicing in aging and longevity. Hum Genet 139:357–369. https://doi.org/10.1007/s00439-019-02094-6
10. Nguyen LD, Ehrlich BE Cellular mechanisms and treatments for chemobrain: insight from aging and neurodegenerative diseases. https://doi.org/10.15252/emmm.202012075
11. Liguori I, Russo G, Curcio F, et al (2018) Oxidative stress, aging, and diseases. Clin Interv Aging 13:757–772. https://doi.org/10.2147/CIA.S158513
12. Kinoshita PF, Leite JA, Orellana AMM, et al (2016) The influence of Na+, K+-ATPase on glutamate signaling in neurodegenerative diseases and senescence. Front Physiol 7:1–19. https://doi.org/10.3389/fphys.2016.00195
13. Shrivastava AN, Triller A, Melki R (2020) Cell biology and dynamics of Neuronal Na+/K+-ATPase in health and diseases. Neuropharmacology 169:. https://doi.org/10.1016/j.neuropharm.2018.12.008
14. Reis AS, Paltian JJ, Domingues WB, et al (2020) Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity:

Correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline. Brain Res Bull 162:282–290. https://doi.org/10.1016/j.brainresbull.2020.06.021

15. Rodrigues KC, Bortolatto CF, da Motta KP, et al (2021) The neurotherapeutic role of a selenium-functionalized quinoline in hypothalamic obese rats. Psychopharmacology (Berl). https://doi.org/10.1007/s00213-021-05821-y

16. Vogt AG, Voss GT, de Oliveira RL, et al (2018) Organoselenium group is critical for antioxidant activity of 7-chloro-4-phenylselenyl-quinoline. Chem Biol Interact 282:7–12. https://doi.org/10.1016/j.cbi.2018.01.003

17. Voss GT, Oliveira RL, de Souza JF, et al (2018) Therapeutic and technological potential of 7-chloro-4-phenylselanyl quinoline for the treatment of atopic dermatitis-like skin lesions in mice. Mater Sci Eng C 84:90–98. https://doi.org/10.1016/j.msec.2017.11.026

18. Barth A, Vogt AG, dos Reis AS, et al (2019) 7-Chloro-4-(Phenylselanyl) Quinoline with Memory Enhancer Action in Aging Rats: Modulation of Neuroplasticity, Acetylcholinesterase Activity, and Cholesterol Levels. Mol Neurobiol. https://doi.org/10.1007/s12035-019-1530-5

19. KP da M, BB L, JJ P, et al (2021) 7-Chloro-4-(phenylselanyl) quinoline reduces renal oxidative stress induced by oxaliplatin in mice. Can J Physiol Pharmacol. https://doi.org/10.1139/CJPP-2021-0090

20. Lemos BB, Motta KP da, Paltian JJ, et al (2020) Role of 7-chloro-4-(phenylselanyl) quinoline in the treatment of oxaliplatin-induced hepatic toxicity in mice. Can J Physiol Pharmacol 1–11. https://doi.org/10.1139/cjpp-2020-0134

21. Luchese C, Vogt AG, Pinz MP, et al (2020) Amnesia-ameliorative effect of a quinoline derivative through regulation of oxidative/cholinergic systems and

Na+/K+-ATPase activity in mice. Metab Brain Dis 35:589–600. https://doi.org/10.1007/s11011-020-00535-0

22. Paltian JJ, dos Reis AS, de Oliveira RL, et al (2020) The anxiolytic effect of a promising quinoline containing selenium with the contribution of the serotonergic and GABAergic pathways: Modulation of parameters associated with anxiety in mice. Behav Brain Res 393:. https://doi.org/10.1016/j.bbr.2020.112797

23. Pinz M, Reis AS, Duarte V, et al (2016) 4-Phenylselenyl-7-chloroquinoline, a new quinoline derivative containing selenium, has potential antinociceptive and anti-inflammatory actions. Eur J Pharmacol 780:122–128. https://doi.org/10.1016/j.ejphar.2016.03.039

24. Pinz MP, dos Reis AS, Vogt AG, et al (2018) Current advances of pharmacological properties of 7-chloro-4-(phenylselanyl) quinoline: Prevention of cognitive deficit and anxiety in Alzheimer's disease model. Biomed Pharmacother 105:1006–1014. https://doi.org/10.1016/j.biopha.2018.06.049

25. Reis AS, Pinz M, Duarte LFB, et al (2017) 4-phenylselenyl-7-chloroquinoline, a novel multitarget compound with anxiolytic activity: Contribution of the glutamatergic system. J Psychiatr Res 84:191–199. https://doi.org/10.1016/j.jpsychires.2016.10.007

26. Silva VDG, Reis AS, Pinz MP, et al (2017) Further analysis of acute antinociceptive and anti-inflammatory actions of 4-phenylselenyl-7-chloroquinoline in mice. Fundam Clin Pharmacol 31:513–525. https://doi.org/10.1111/fcp.12295

27. Savegnago L, Vieira AI, Seus N, et al (2013) Synthesis and antioxidant properties of novel quinoline-chalcogenium compounds. Tetrahedron Lett 54:40–

44. https://doi.org/10.1016/j.tetlet.2012.10.067

28. Duarte LFB, Barbosa ES, Oliveira RL, et al (2017) A simple method for the synthesis of 4-arylselanyl-7-chloroquinolines used as in vitro acetylcholinesterase inhibitors and in vivo memory improvement. Tetrahedron Lett 58:3319–3322. https://doi.org/10.1016/j.tetlet.2017.07.039

29. Salgueiro WG, Goldani BS, Peres T V., et al (2017) Insights into the differential toxicological and antioxidant effects of 4-phenylchalcogenil-7-chloroquinolines in Caenorhabditis elegans. Free Radic Biol Med 110:133–141. https://doi.org/10.1016/j.freeradbiomed.2017.05.020

30. de Freitas Couto S, Araujo SM, Bortolotto VC, et al (2019) 7-chloro-4-(phenylselanyl) quinoline prevents dopamine depletion in a Drosophila melanogaster model of Parkinson's-like disease. J Trace Elem Med Biol 54:232–243. https://doi.org/10.1016/j.jtemb.2018.10.015

31. de Aquino Silva D, Silva MRP, Guerra GP, et al (2021) 7-chloro-4-(phenylselanyl) quinoline co-treatment prevent oxidative stress in diabetic-like phenotype induced by hyperglycidic diet in Drosophila melanogaster. Comp Biochem Physiol Part - C Toxicol Pharmacol 239:. https://doi.org/10.1016/j.cbpc.2020.108892

32. Book GETT (1996) Guide for the Care and Use of Laboratory Animals

33. Brusco I, Justino AB, Silva CR, et al (2019) Kinins and their B1 and B2 receptors are involved in fibromyalgia-like pain symptoms in mice. Biochem Pharmacol 168:119–132. https://doi.org/10.1016/j.bcp.2019.06.023

34. Bobinski F, Teixeira JM, Sluka KA, et al (2019) HHS Public Access. 159:437–450. https://doi.org/10.1097/j.pain.0000000000001109.IL-4

35. Alamri FF, Shoyaib A Al, Biggers A, et al (2018) Applicability of the grip

strength and automated von Frey tactile sensitivity tests in the mouse photothrombotic model of stroke. Behav Brain Res 336:250–255. https://doi.org/10.1016/j.bbr.2017.09.008

36. Walsh RN, Cummins RA (1976) The Open-Field Test : A Critical Review. 83:482–504

37. Loetchutinat C, Kothan S, Dechsupa S, et al (2005) Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′,7′-dichlorofluorescein diacetate assay. Radiat Phys Chem 72:323–331. https://doi.org/10.1016/j.radphyschem.2004.06.011

38. Brüning CA, Prigol M, Luchese C, et al (2012) Protective effect of diphenyl diselenide on ischemia and reperfusion-induced cerebral injury: Involvement of oxidative stress and pro-inflammatory cytokines. Neurochem Res 37:2249–2258. https://doi.org/10.1007/s11064-012-0853-7

39. KM M, MG E, K Y, et al (2001) Unique oxidative mechanisms for the reactive nitrogen oxide species, nitroxyl anion. J Biol Chem 276:1720–1727. https://doi.org/10.1074/JBC.M006174200

40. Wendel A (1981) [44] Glutathione Peroxidase By. 77:

41. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

42. Fiske, 1925

43. FC T, JM G, MSP S, et al (2020) Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions. Psychopharmacology (Berl) 237:811–823.

https://doi.org/10.1007/S00213-019-05419-5

44. MM B (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1006/ABIO.1976.9999

45. Curtis MJ, Alexander S, Cirino G, et al (2018) Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br J Pharmacol 175:987–993. https://doi.org/10.1111/bph.14153

46. Starobova H, Vetter I (2017) Pathophysiology of chemotherapy-induced peripheral neuropathy. Front Mol Neurosci 10:1–21. https://doi.org/10.3389/fnmol.2017.00174

47. Colloca L, Ludman T, Bouhassira D, et al (2017) Neuropathic pain [Review]. Nat Rev Dis Prim 3:17002. https://doi.org/10.1038/nrdp.2017.2

48. Kang L, Tian Y, Xu S, Chen H (2020) Oxaliplatin-induced peripheral neuropathy: clinical features, mechanisms, prevention and treatment. J Neurol. https://doi.org/10.1007/s00415-020-09942-w

49. Cruz-Almeida Y, Fillingim RB, Riley JL, et al (2019) Chronic pain is associated with a brain aging biomarker in community-dwelling older adults. Pain 160:1119–1130. https://doi.org/10.1097/j.pain.0000000000001491

50. Cruz-Almeida Y, Cole J (2020) Pain, aging, and the brain: new pieces to a complex puzzle. Pain 161:461–463

51. Zhang H, Davies KJA, Forman HJ (2015) Oxidative stress response and Nrf2 signaling in aging. Free Radic. Biol. Med. 88:314–336

52. Korovila I, Hugo M, Castro JP, et al (2017) Redox Biology Proteostasis , oxidative stress and aging. 13:550–567

53. Nozadze E, Arutinova N, Tsakadze L, et al (2015) Molecular Mechanism of Mg-

ATPase Activity. J Membr Biol 248:295–300. https://doi.org/10.1007/s00232-014-9769-2

54. Wang XJ, Li Y, Luo L, et al (2014) Oxaliplatin activates the Keap1/Nrf2 antioxidant system conferring protection against the cytotoxicity of anticancer drugs. Free Radic Biol Med 70:68–77. https://doi.org/10.1016/j.freeradbiomed.2014.02.010

55. Ge Y, Jiao Y, Li P, et al (2018) Coregulation of endoplasmic reticulum stress and oxidative stress in neuropathic pain and disinhibition of the spinal nociceptive circuitry

56. Khasabova IA, Khasabov SG, Olson JK, et al (2019) Pioglitazone, a PPARγ agonist, reduces cisplatin-evoked neuropathic pain by protecting against oxidative stress. Pain 160:688–701. https://doi.org/10.1097/j.pain.0000000000001448

57. Huličiak M, Vacek J, Šebela M, et al (2012) Covalent binding of cisplatin impairs the function of Na +/K +-ATPase by binding to its cytoplasmic part. Biochem Pharmacol 83:1507–1513. https://doi.org/10.1016/j.bcp.2012.02.015

58. Kubala M, Geleticova J, Huliciak M, et al (2014) Na+/K+-ATPase inhibition by cisplatin and consequences for cisplatin nephrotoxicity. Biomed Pap 158:194–200. https://doi.org/10.5507/bp.2014.018

59. Tummala R, Wolle D, Barwe SP, et al (2009) Expression of Na,K-ATPase-β1 subunit increases uptake and sensitizes carcinoma cells to oxaliplatin. Cancer Chemother Pharmacol 64:1187–1194. https://doi.org/10.1007/s00280-009-0985-x

60. Areti A, Yerra VG, Naidu VGM, Kumar A (2014) Oxidative stress and nerve damage: Role in chemotherapy induced peripheral neuropathy. Redox Biol

2:289–295. https://doi.org/10.1016/j.redox.2014.01.006

61. Kanat O, Ertas H, Caner B (2017) Platinum-induced neurotoxicity: A review of possible mechanisms. World J Clin Oncol 8:329–333. https://doi.org/10.5306/wjco.v8.i4.329

62. Munhoz CD, Kawamoto EM, De Sá Lima L, et al (2005) Glutamate modulates sodium-potassium-ATPase through cyclic GMP and cyclic GMP-dependent protein kinase in rat striatum. Cell Biochem Funct 23:115–123. https://doi.org/10.1002/cbf.1217

Captions of figures

Fig. 1 Experimental Protocol

Fig. 2. Effect of aging (Old), 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the cold sensitivity in the acetone test. Each point represents the mean of 7 mice in each group. (****) $P < 0.0001$ denotes significance levels when compared with the Young group; ($^{\#\#}$) $P < 0.01$ denotes significance levels when compared with the Old group; ($^{+++}$) $P < 0.001$ denotes significance levels when compared with the Young+OXA+4-PSQ group; ($^{\&\&\&\&}$) $P < 0.0001$ denotes significance levels when compared with the Old+OXA+4-PSQ group. (Two-way ANOVA followed by the Tukey's test)

Fig. 3 Effect of aging (Old), 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the paw withdrawal threshold to mechanical stimulus in the von Frey test. Each point represents the mean of 7 mice in each group. (****) $P < 0.001$ and (****) $P < 0.0001$ denote significance levels when compared with the Young group; ($^{\#\#\#\#}$) $P < 0.001$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote

significance levels when compared with the Old group; ($^{++++}$) P < 0.0001 denotes significance levels when compared with the Young+OXA+4-PSQ group; ($^{\&\&\&\&}$) P < 0.0001 denotes significance levels when compared with the Old+OXA+4-PSQ group. (Two-way ANOVA followed by the Tukey's test)

Fig. 4 Effect of aging (Old), 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the (A) number of rearings and the (B) number of crossings in the open field test. Each point represents the mean of 7 mice in each group. (Two-way ANOVA followed by the Tukey's test)

Fig. 5 Effect of aging (Old), 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the RS levels in the (A) sciatic nerve, (B) spinal cord and (C) cerebral cortex of mice. Each point represents the mean of 7 mice in each group. (*) P < 0.05, (***) P < 0.001 and (****) P < 0.0001 denote significance levels when compared with the Young group; ($^{\#}$) P < 0.05 and ($^{\#\#}$) P < 0.01 denote significance levels when compared with the Old group; ($^{+++}$) P < 0.001 denotes significance levels when compared with the Young+OXA+4-PSQ group; ($^{\&\&}$) P < 0.01, ($^{\&\&\&}$) P < 0.001 and ($^{\&\&\&\&}$) P < 0.0001 denote significance levels when compared with the Old+OXA+4-PSQ group. (Two-way ANOVA followed by the Tukey's test)

Fig. 6 Effect of aging (Old), 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the nitrate and nitrite (NOx) levels in the spinal cord of mice. Each point represents the mean of 7 mice in each group. (*) P < 0.05 and (***) P < 0.001 denote significance levels when compared with the Young group; ($^{\#}$) P < 0.05 denotes significance levels when compared with the Old group; ($^{+}$) P < 0.05 and ($^{+++}$) P < 0.001 denote significance levels when compared with the

Young+OXA+4-PSQ group; ($^{\&}$) P < 0.05 and ($^{\&\&\&}$) P < 0.001 denote significance levels when compared with the Old+OXA+4-PSQ group. (Two-way ANOVA followed by the Tukey's test)

Fig. 7 Effect of aging (Old), 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on glutathione peroxidase activity in the (A) spinal cord and (B) cerebral cortex, and on superoxide dismutase in the (C) spinal cord and (D) cerebral cortex of mice. Each point represents the mean of 7 mice in each group. (*) P < 0.05, (**) P < 0.01, (***) P < 0.001 and (****) P < 0.0001 denote significance levels when compared with the Young group; ($^{\#}$) P < 0.05 and ($^{\#\#}$) P < 0.01 denote significance levels when compared with the Old group; ($^{++++}$) P < 0.0001 denotes significance levels when compared with the Young+OXA+4-PSQ group; ($^{\&}$) P < 0.05 and ($^{\&\&\&}$) P < 0.001 denote significance levels when compared with the Old+OXA+4-PSQ group. (Two-way ANOVA followed by the Tukey's test)

Fig. 8 Effect of aging (Old), 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (1 mg kg^{-1}, p.o.) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on Na^{+}, K^{+} - ATPase activity in the (A) spinal cord and (B) cerebral cortex, and on and Mg^{2+} - ATPase activity in the (C) spinal cord and (D) cerebral cortex of mice. Each point represents the mean of 7 mice in each group. (*) P < 0.05, (**) P < 0.01, (***) P < 0.001 and (****) P < 0.0001 denote significance levels when compared with the Young group; ($^{\#}$) P < 0.05 and ($^{\#\#\#\#}$) P < 0.0001 denote significance levels when compared with the Old group; ($^{++}$) P < 0.01 denotes significance levels when compared with the Young+OXA+4-PSQ group; ($^{\&}$) P < 0.05 and ($^{\&\&}$) P < 0.01 denote significance levels when compared with the Old+OXA+4-PSQ group. (Two-way ANOVA followed by the Tukey's test)

Fig. 1

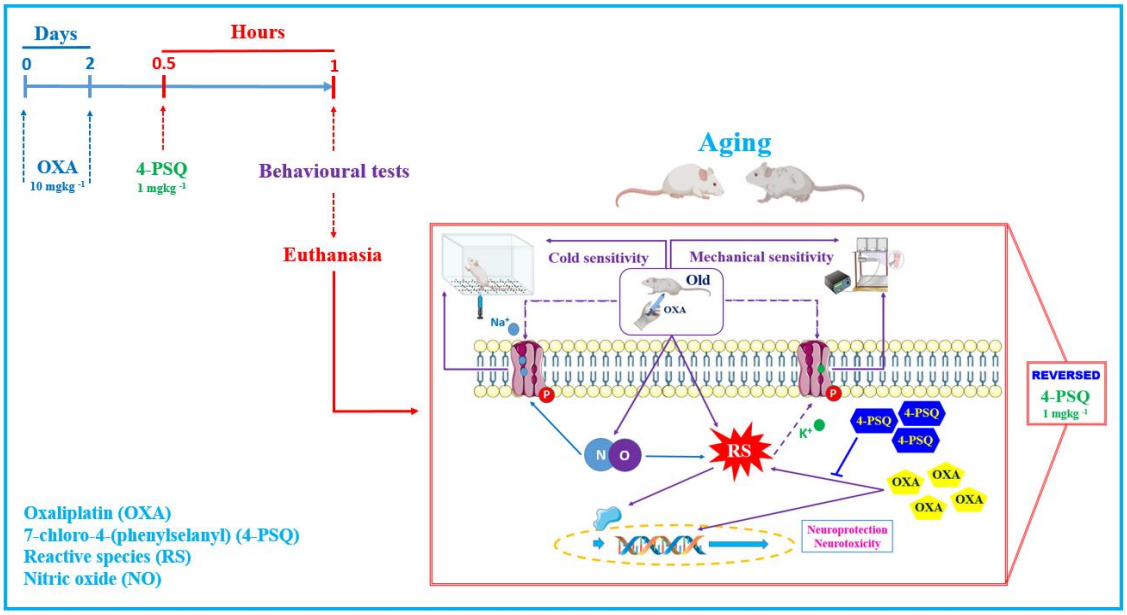

Days
Hours
0
2
0.5
1
OXA
10 mgkg -1
4-PSQ
1 mgkg -1
Behavioural tests
Euthanasia
Aging
Cold sensitivity
Mechanical sensitivity
Old
OXA
Na+
K+
RS
N O
4-PSQ
OXA
Neuroprotection
Neurotoxicity
REVERSED
4-PSQ
1 mgkg -1
Oxaliplatin (OXA)
7-chloro-4-(phenylselanyl) (4-PSQ)
Reactive species (RS)
Nitric oxide (NO)

Fig. 2

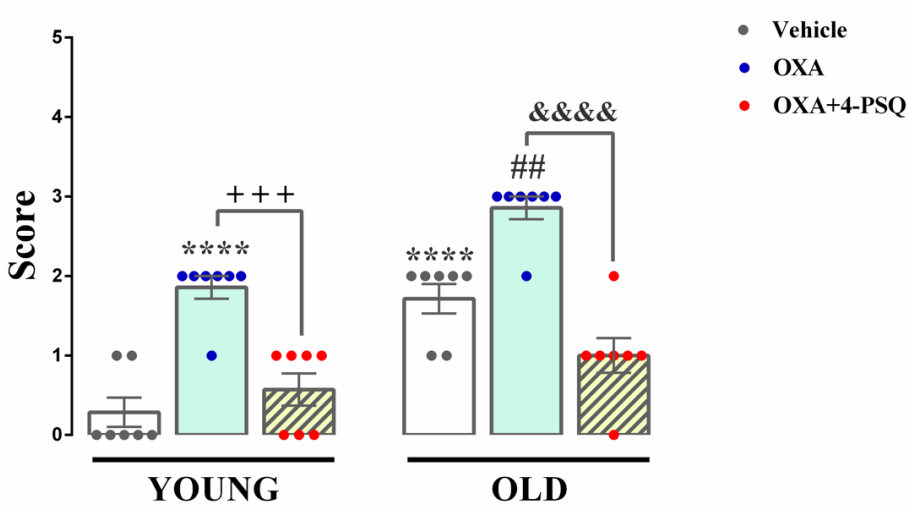

Vehicle
OXA
OXA+4-PSQ
Score
0
1
2
3
4
5
****
+++
##
&&&&
YOUNG
OLD

Fig. 3

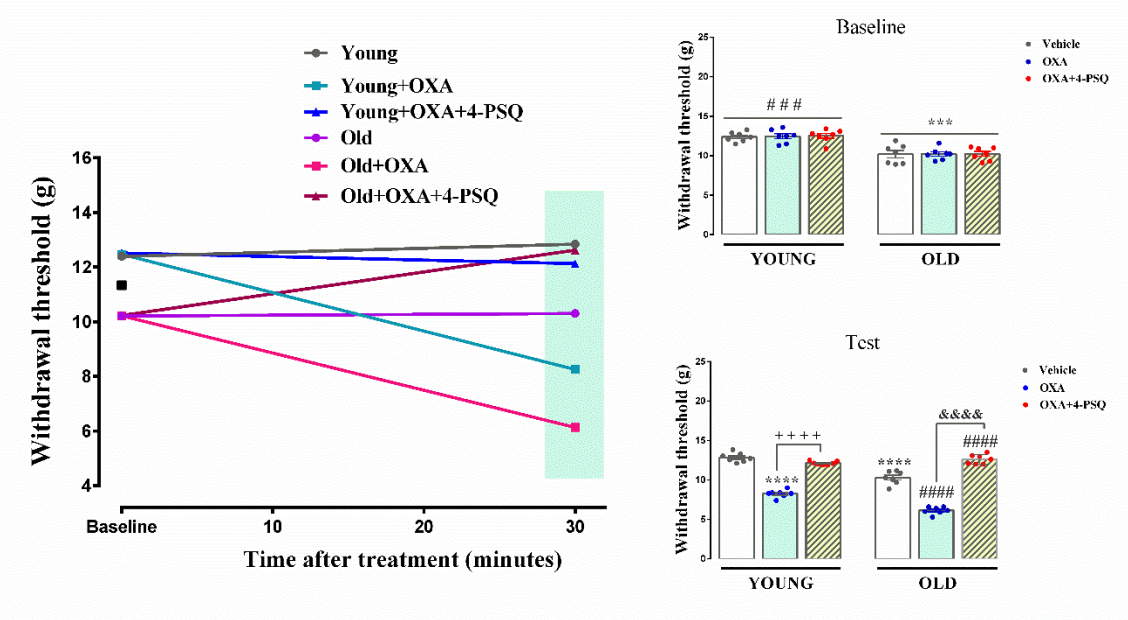


Fig. 4

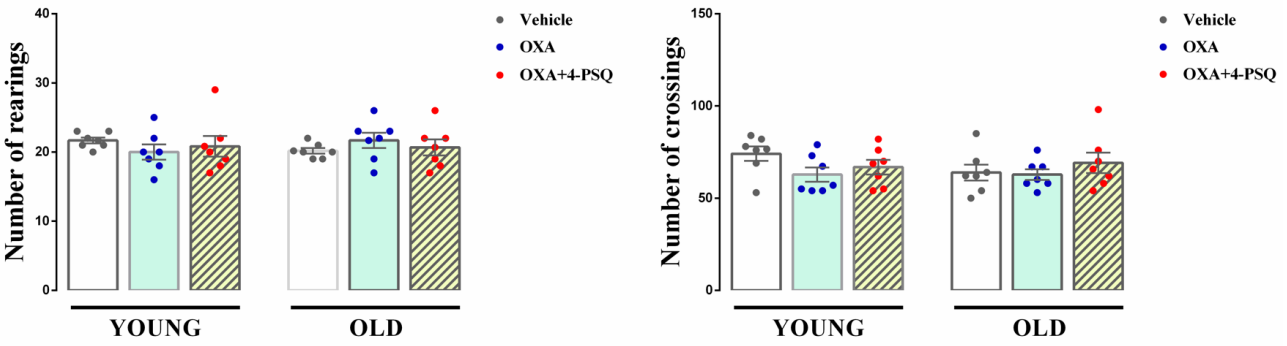


Fig. 5

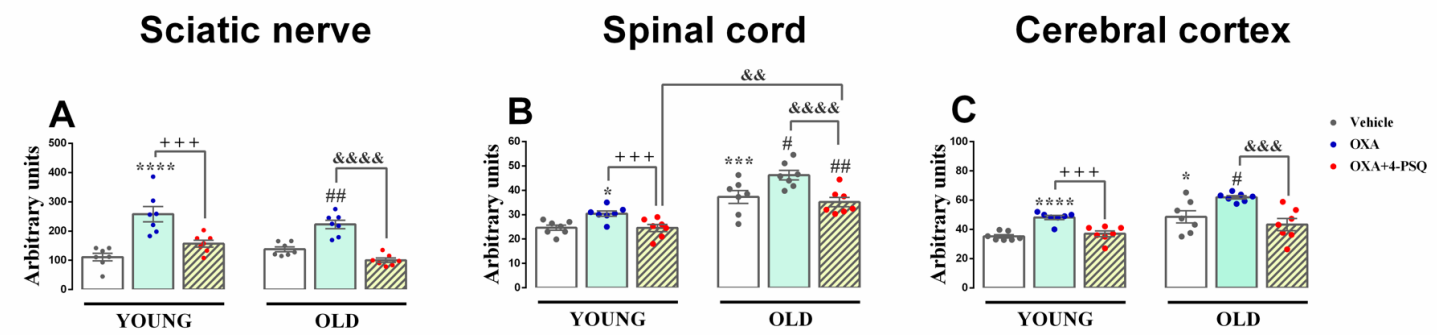


Fig. 6

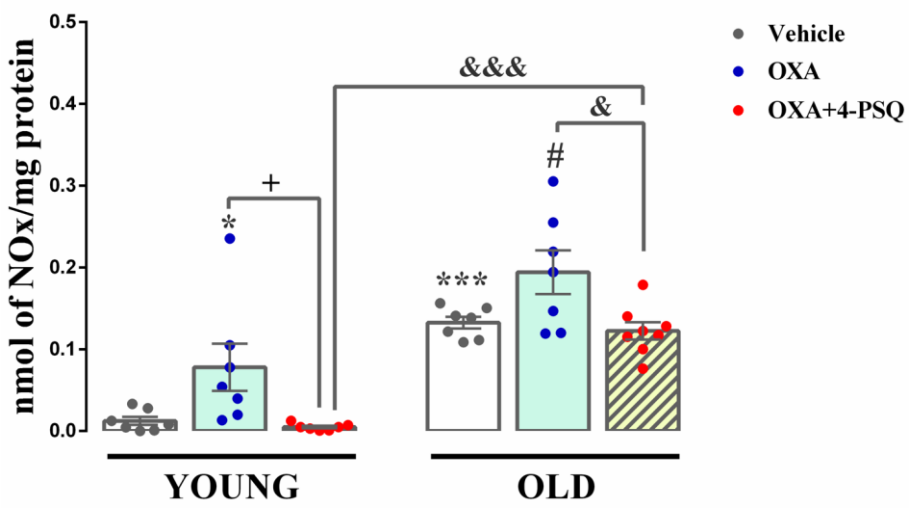

nmol of NOx/mg protein
0.5
0.4
0.3
0.2
0.1
0.0
&&&
&
+
*
#
***
Vehicle
OXA
OXA+4-PSQ
YOUNG
OLD

Fig. 7

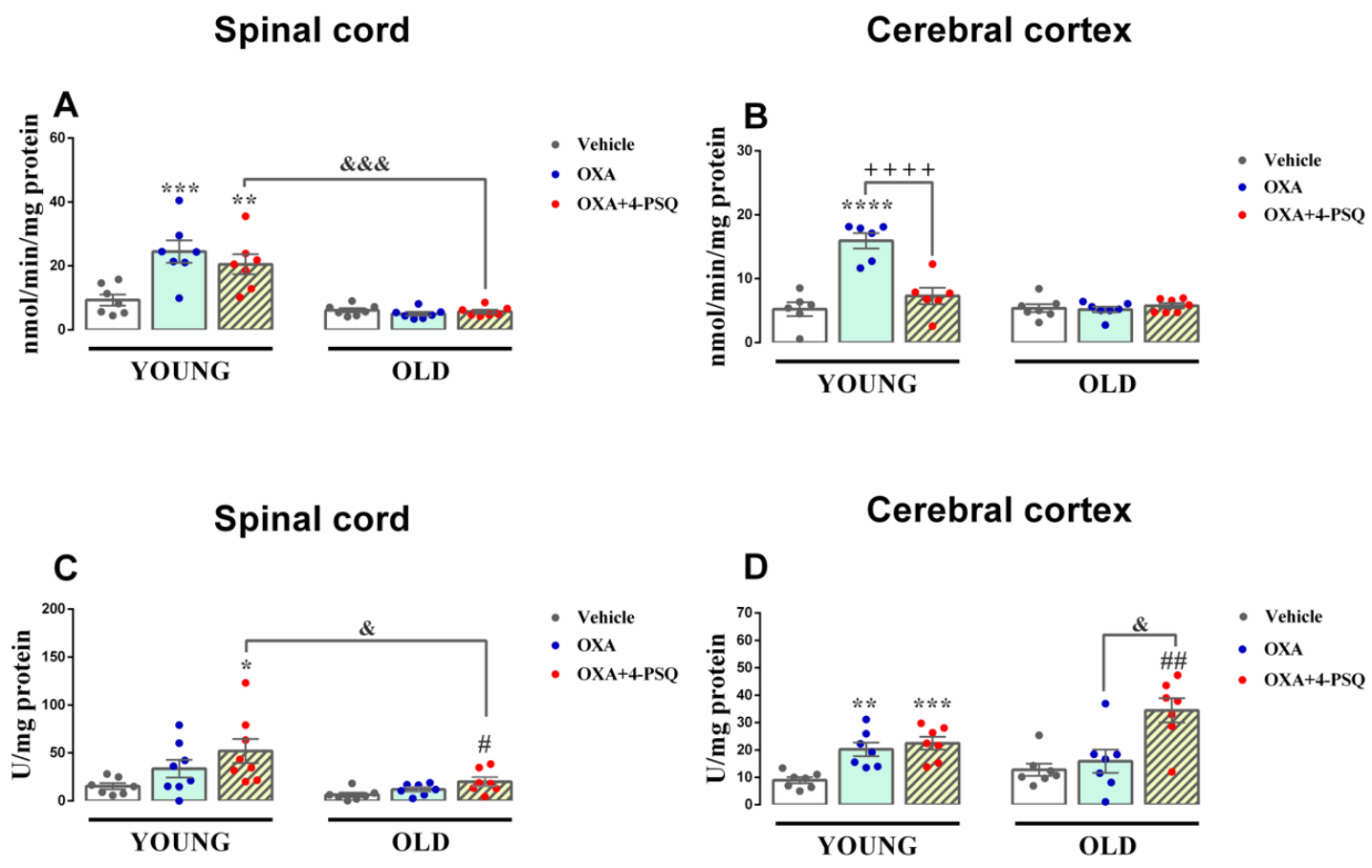
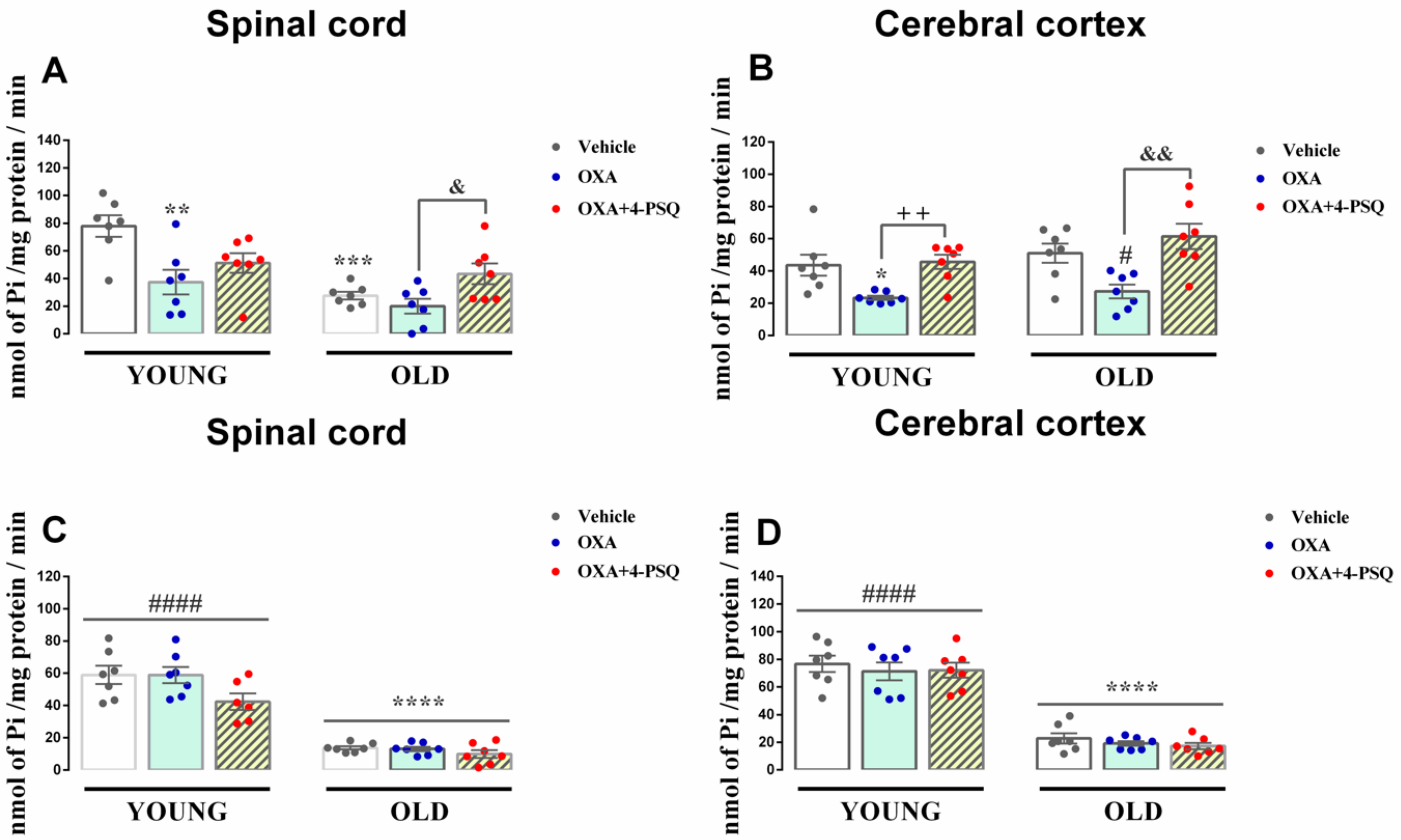
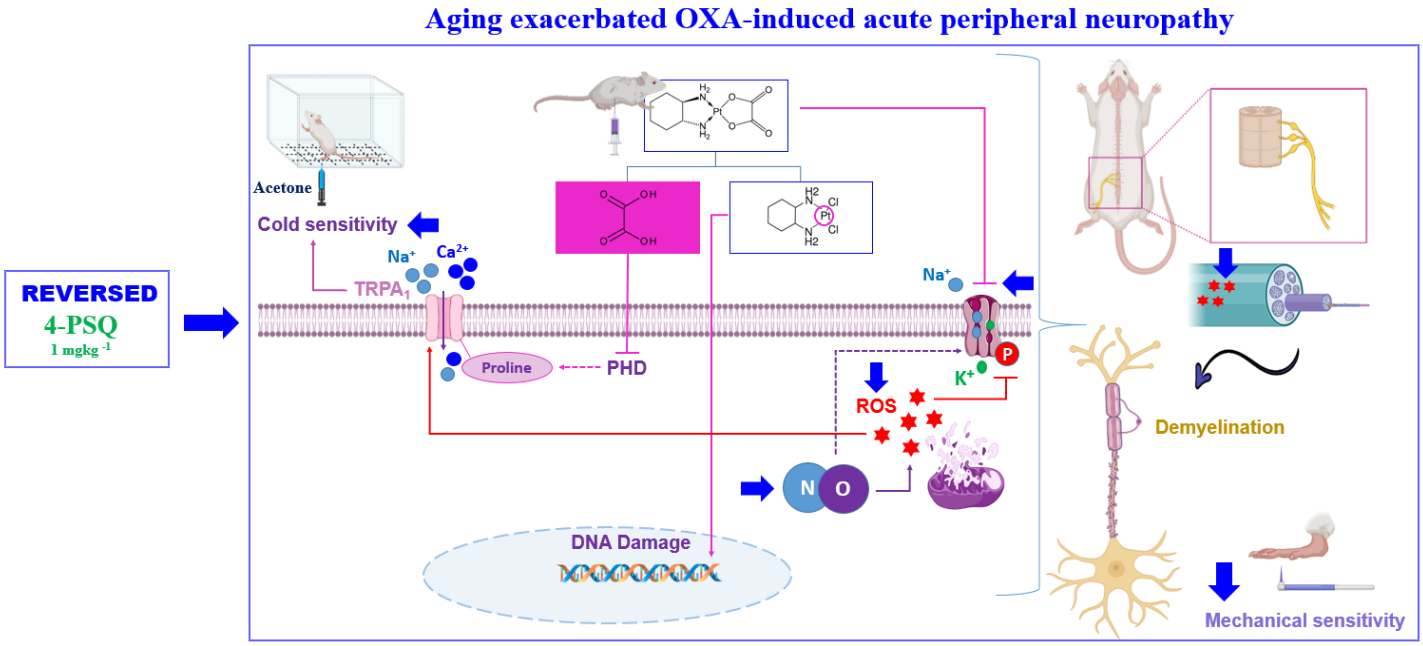

Spinal cord
Cerebral cortex
A
B
C
D
nmol/min/mg protein
U/mg protein
***
**
&&&
++++
****
*
&
#
##
Vehicle
OXA
OXA+4-PSQ
YOUNG
OLD

Fig. 8


Spinal cord
A
nmol of Pi /mg protein / min
140
120
100
80
60
40
20
0
**
***
&
YOUNG
OLD
Vehicle
OXA
OXA+4-PSQ
Cerebral cortex
B
120
100
80
60
40
20
0
*
++
#
&&
YOUNG
OLD
Vehicle
OXA
OXA+4-PSQ
Spinal cord
C
120
100
80
60
40
20
0
####
****
YOUNG
OLD
Vehicle
OXA
OXA+4-PSQ
Cerebral cortex
D
140
120
100
80
60
40
20
0
####
****
YOUNG
OLD
Vehicle
OXA
OXA+4-PSQ

Graphical Abstract



4-PSQ was a promising agent to reverse OXA-induced acute pain, mainly in the aging

Capítulo 4

Elucidação do papel do envelhecimento nos mecanismos subjacentes à NPCIO

Está bem estabelecido que muitos fatores contribuem para a incidência ou exacerbação da NPIO. Nesta tese, foi primeiramente demonstrado que, apesar das defesas eficientes do SNC, após a exposição ao medicamento oxaliplatina ocorre um acúmulo de platina na medula espinhal de camundongos, o que ampliou o conhecimento sobre sua toxicidade. O acúmulo de platina na medula espinhal pode causar danos oxidativos aos neurônios e prejudicar a função mitocondrial. De fato, foi demonstrado complementarmente que a administração da oxaliplatina causa estresse oxidativo devido ao aumento na produção de espécies reativas e, colapso das defesas antioxidantes. Corroborando os achados citados e, igualmente relevante, foi demonstrado que a enzima Na^+, K^+ - ATPase é um alvo da oxaliplatina e, sua inibição está envolvida na gênese da NPIO, de fato, o aumento na produção de espécies reativas, também, pode levar a inibição da atividade desta enzima fundamental para o funcionamento do SNC. Concomitantemente, foi evidenciado pela primeira vez nesse estudo que o avanço da idade agrava os mecanismos e sintomas clínicos da NPAIO.

Considerando os resultados relevantes obtidos nos capítulos 1, 2 e 3, buscou-se elucidar a influência dos mecanismos subjacentes ao envelhecimento sobre a NPCIO. A etapa 4 desse estudo foi decisiva para contemplar um dos objetivos principais desta tese, que envolve a compreensão dos mecanismos e alvos alterados pelo envelhecimento que aumentam a suscetibilidade à neurotoxicidade induzida pela oxaliplatina. Esta etapa foi desenvolvida a fim de identificar alvos farmacológicos promissores, além de, buscar uma terapia eficaz e segura para o tratamento da NPIO, considerando as particularidades dos idosos. Para investigar os mecanismos envolvidos na NPCIO e a influência causada pelo envelhecimento, a concentração de bioelementos e platina e sua influência no dano oxidativo, neuroproteção e vias de neuroplasticidade foram avaliadas.

Os resultados obtidos na etapa 4 desta tese, demonstraram que o tratamento com a oxaliplatina exacerbou a dor, o comportamento ansioso, mas não o comprometimento cognitivo causado pelo envelhecimento. A deposição de platina foi identificada na medula espinhal e, pela primeira vez, no cérebro

de camundongos expostos a oxaliplatina, independentemente da idade. Alterações na concentração de bioelementos, dano oxidativo e nas vias de neuroproteção e neuroplasticidade induzidas pelo envelhecimento parecem contribuir para a NPCIO (Figura 8). Os resultados buscaram prover uma base para intervenções terapêuticas na NPCIO, considerando as especificidades da idade.

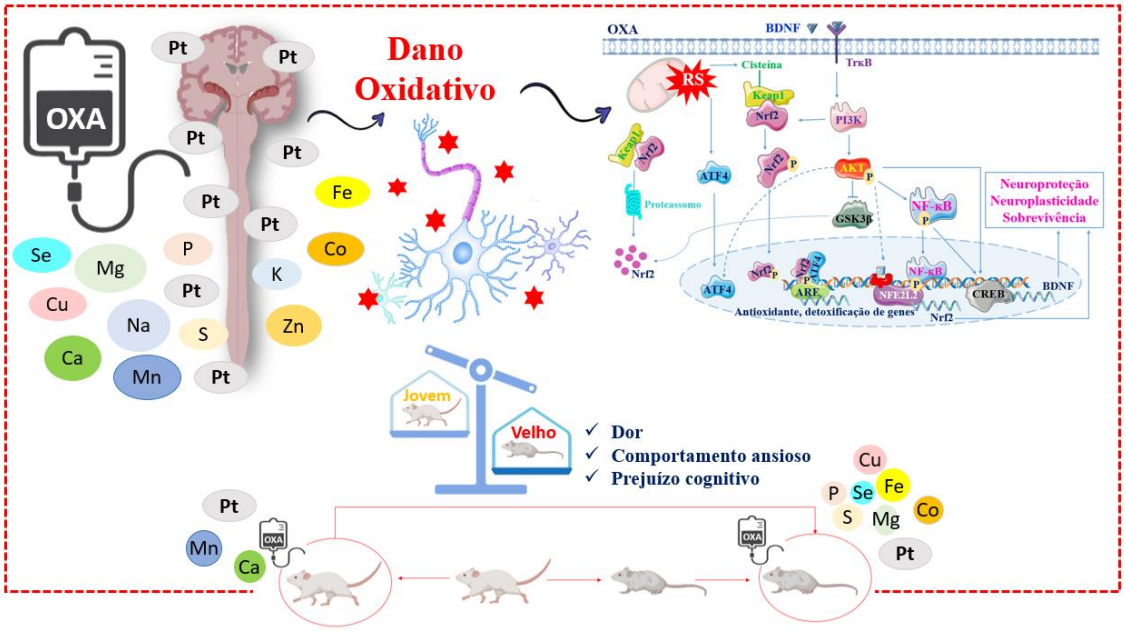


Figura 8. Representação esquemática dos principais resultados obtidos na etapa 4.

Manuscrito 2

Os resultados deste capítulo da tese estão apresentados sob a forma de manuscrito científico, o qual se encontra assim organizado. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se no próprio manuscrito.

O manuscrito 2 será submetido à revista *Progress in Neurobiology.*

Does age influence oxaliplatin-induced peripheral neuropathy in mice?

Angélica S. Reis[a], Jaini J. Paltian[a], William B. Domingues[b], Diogo L. R. Novo[c], Eduardo Bolea-Fernandez[d], Thibaut V. Acker[d], Vinicius F. Campos[b], Cristiane Luchese[a], Frank Vanhaecke[d], Marcia F. Mesko[c*], Ethel A. Wilhelm[a*]

[a]*Programa de Pós-graduação em Bioquímica e Bioprospecção, Laboratório de Pesquisa em Farmacologia Bioquímica, CCQFA - Universidade Federal de Pelotas, UFPel - CEP - 96010-900, Pelotas, RS, Brasil.*

[b]*Programa de Pós-graduação em Biotecnologia, Laboratório de Genômica Estrutural, Biotecnologia - Universidade Federal de Pelotas, UFPel - CEP - 96010-900, Pelotas, RS, Brasil.*

[c]*Programa de Pós-graduação em Química, Laboratório de Controle de Contaminantes em Biomateriais, CCQFA - Universidade Federal de Pelotas, UFPel - CEP - 96010-900, Pelotas, RS, Brasil.*

[d]*Department of Chemistry, Atomic & Mass Spectrometry, A&MS research unit, Ghent University, 9000 Ghent, Belgium.*

The authors declare that they have no conflict of interest.

*Address for correspondence:

Ethel Antunes Wilhelm; e-mail: ethelwilhelm@yahoo.com.br/ Marcia Foster Mesko; e-mail: marciamesko@yahoo.com.br / Phone: +55-53-32757360

Programa de Pós-graduação em Bioquímica e Bioprospecção (PPGBBio), Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas (UFPel), Campus Capão do Leão, Pelotas, CEP 96010-900, RS, Brasil

Abstract

Many factors can contribute to the incidence or exacerbation of peripheral neuropathy induced by oxaliplatin (OXA). Recently, the accumulation of platinum in spinal cord of mice after exposure to OXA, despite the efficient defenses of the central nervous system, has been demonstrated by our research group, which expands the knowledge about its toxicity. One hypothesis is platinum accumulation in spinal cord causing oxidative damage to neurons and impairing mitochondrial function. Thus, the main aim of this study was to investigate the relationship between aging and OXA-induced neuropathic pain and its comorbidities including anxious behavior and cognitive impairment. Using *Swiss* mice OXA-induced peripheral neuropathy model, platinum and bioelements concentration and their influence on oxidative damage, neuroprotection and neuroplasticity pathways were evaluated. As results, OXA-treatment exacerbated pain and anxious behavior, but not cognitive impairment aging-induced. Platinum deposition in spinal cord and, for the first time, in brain of mice OXA-exposed, age-independently, was identified. Alterations in bioelements concentration, oxidative damage and neuroprotection and neuroplasticity pathways induced by aging contribute to OXA-induced peripheral neuropathy. Our results strive to provide a basis for therapeutic interventions to peripheral neuropathy induced by OXA considering age specificities.

Keywords: Neuropathy; aging; oxaliplatin; platinum; Nrf2; BDNF

Abbreviations

OXA, Oxaliplatin; RS, reactive species; CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase; AChE, acetylcholinesterase; CNS, central nervous system; NADPH, β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; ICP-MS/MS, inductively coupled plasma-tandem mass spectrometry.

1. Introduction

Aging is a complex process characterized by the physiological function decline with progressive degradation stage and by the reduction of repair capacity of tissues and organ systems, leading to an increased risk of mortality. Around 20% of the world's population will be over 60 age by 2050 (Fane and Weeraratna, 2020). Advances in public health have increased the life expectancy and the world population will reach older ages in the next years. These facts will be accompanied by the increase of many chronic age-related diseases incidence, such as those neurodegenerative and cancers (Bhadra et al., 2020). The highest incidence of cancers occurs in patients over 60 age (Fane and Weeraratna, 2020). In 2018, around 2.3 million new cancer cases worldwide were in 80 age or older individuals (13% of all cancer cases) (Pilleron et al., 2020).

According to the GLOBOCAN database, colorectal is one of the most common malignancies cancer worldwide (Ferlay et al., 2019). Colorectal cancer is among the 3rd most common cancer in oldest females and males (Pilleron et al., 2020). Currently, despite progress in chemotherapeutic regimens resulting in substantial improvements in the long-term outcome, chemotherapy treatments have led numerous cellular structure/function changes associated with severe, sometimes long-lasting, or irreversible, side effects. Oxaliplatin (OXA), a platinum-based chemotherapeutic agent widely used in colorectal cancer treatment, induces various side effects, including peripheral neuropathy (Cavaletti and Marmiroli, 2020).

Neurotoxicity induced by OXA-administration is a severe and potentially permanent side effect, initially characterized by acute clinical symptoms that are exacerbated resulting in the establishment of chronic neurotoxicity and neuropathic pain. Despite significant advances in the understanding of OXA-induced neurotoxicity,

several challenges persist in both clinical level and cellular–molecular basis. The reversible interference with ion channels was postulated as mechanism-basic to the acute neuropathy, but the knowledge about chronic neuropathy mechanisms is still limited. Hypothesis more accepted is due to the mitochondrial toxicity. This mechanism has been extensively investigated through the changes in mitochondrial morphology, bioenergetics, and reactive species (RS) generation (Cavaletti and Marmiroli, 2020).

In the case of elderly patients, associated to the OXA-induced neurotoxicity side effects, neurogenesis declines with age (Nguyen and Ehrlich, 2020) and with progressive loss of tissue and organ function, particularly due to the oxidative damage accumulation to the macromolecules (Liguori et al., 2018). In this context, some new approaches can be helpful to elucidate molecular-mechanisms innovative investigations involving OXA-induced peripheral neuropathy, considering mainly the aging peculiarities.

Nuclear factor-erythroid-2 related factor 2 (Nrf2) is a master regulator of the oxidative stress response, highlighting its effect in the mitochondrial quality control in oxidative stress conditions (Kasai et al., 2019). In some studies considering Nrf2 effects have been pointed its modulation as an interesting strategy to treatment of OXA-induced peripheral neuropathy (Yang et al., 2018) and losses of cellular protection response during aging (Silva-Palacios et al., 2018). However, more studies are necessary to evaluate both effects in the same patient.

In the same context, the identification of biomarkers to enhance cell survival can be a strategy against establishing of OXA-induced peripheral neuropathy in the elderly (Nguyen and Ehrlich, 2020) considering the restoring neurons lost due to daily wear and tear by neurogenesis and the reduction of neurogenesis as a common factor in aging and neurodegenerative diseases. Thus, the brain-derived neurotrophic factor (BDNF) is a

neurotrophin secreted into the extracellular environment to promote neurogenesis. Understand BDNF-TrkB pathway changes is an important tool to outline OXA-induced peripheral neuropathy impact in elderly patients.

Some studies have been concentrated on effects of chemotherapeutic agent OXA on central (CNS) and peripheral (CNP) nervous systems in adult animals (Areti et al., 2014; Cavaletti and Marmiroli, 2020; Reis et al., 2020b). Major advances have been made in better understand the determinant factors for the development of OXA-induced neuropathy. Nevertheless, there are few studies exanimating the interaction between OXA-induced neuropathy and aging. Thus, the hypothesis of the present study seeks to answer the following questions: i*)* Does age influence OXA-induced peripheral neuropathy in mice? *ii)* Are OXA-induced peripheral neuropathy comorbidities altered by the aging? *iii)* Can changes in the concentration of different bioelements, the deposition of platinum, and its influence on oxidative damage and neuroprotection and neuroplasticity pathways contribute to the aggravation of symptoms of OXA-induced neuropathy?

2. Materials and Methods

2.1 Animals

All experiments were performed in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA 4506-2017) and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) (Book, 1996) and International Guiding Principles for Biomedical Research Involving Animals. The tests were carried out using adult (2 months) and old (20 months) *Swiss* male mice. This species has been used in

neuropathic pain studies (Bobinski et al., 2019; Brusco et al., 2019). All animals were maintained at 22 ± 2 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 6:00 a.m.). Mice were acclimatized to the behavior room for at least 1 h. All efforts were made to minimize the number of animals used and their discomfort. The number of animals and intensities of noxious stimuli were the minimum needed to demonstrate consistent effects of the treatment. Allocation concealment was performed using a randomization procedure (http://www.randomizer.org/). Behavioral evaluations were performed blindly on drug administration. All experiments were carried out between 08:00 and 17:00 h.

2.2 Drugs

OXA was obtained from Eurofarma pharmaceutical company (São Paulo, Brazil), and this drug was dissolved in 5% glucose solution. All other chemicals used in this study were of analytical grade from Sigma-Aldrich (St. Louis, MO, USA). Mice received OXA by intraperitoneal (i.p.) route at a constant volume of 10 mL kg^{-1} of body weight.

2.3 Treatment scheme

Mice were randomly divided into the following four groups (7 animals/group): *i)* Young, *ii)* Young + OXA, *iii)* Old and *iv)* Old + OXA. On days 0 and 2, mice of the Young and Old groups received a 5% glucose solution (10 mL kg^{-1}, i.p.), whereas mice of the Young + OXA and Old + OXA groups received OXA (10 mg kg^{-1}, i.p.). From day 5 to day 13 of the experimental protocol (Fig. 1), mice were subjected to behavioral tests. On day 14, the animals were euthanized by inhalation of isoflurane anesthetic and, spinal cord, cerebral cortex and hippocampus samples were rapidly dissected, weighed,

and placed on ice, then used for *ex vivo* assays. OXA exposure caused a mortality around 8% and 40% in young and old mice, respectively. Given that the damage caused by OXA in regions such as dorsal root ganglion and peripheral nerves is known, other regions mainly involved in pain pathways were investigated in the present study. In the behavioral tests and data analysis the operators were blinded.

2.4 Behavioral tests

2.4.1 Measurement of mechanical sensitivity

Mechanical sensitivity was carried out in mice according to the method previously described in the literature with some modifications (Alamri et al., 2018). Mechanical sensitivity was evaluated on the fifth, eighth, and twelfth days of the experimental protocol. Around 30 min before starting the test in a quiet room, mice were individually placed inside acrylic cages with wire grid floors. Before paw stimulation, the animals were quieted without exploratory movements and not resting their paws. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer (digital aesthesiometer, Insight, São Paulo, Brazil) adapted with a polypropylene tip. The paw withdrawal threshold was measured by applying the polypropylene tip perpendicular to the middle of the plantar surface of the hind paw at a constant progressive pressure until paw withdrawal, and the pressure value was automatically recorded. Data of the withdrawal threshold were expressed in gram (g).

2.4.2 Measurement of thermal sensitivity

Thermal sensitivity was tested in mice as reported in the literature with some modifications (Woolfe and MacDonald, 1944). Hot-plate test is a model of acute non-inflammatory nociception to investigate the central effect of analgesic drugs. Thermal

sensitivity was evaluated on the sixth, ninth, and thirteenth days of the experimental protocol. Animals were placed in a glass box on a heated metal plate maintained at 52 ± 1 °C. The latency of nociceptive responses such as licking or shaking one of the paws or jumping was recorded as the reaction to a noxious thermal stimulus. To avoid animals paw damage, time standing on the plate was limited to 45 s. Data of the latency were expressed in the seconds (s).

2.4.3 Assessment of locomotor and exploratory domains

The general locomotor and exploratory behaviors of mice was evaluated by open field test. The open field was made of plywood and surrounded by 30 cm-high walls. The floor of the open field, 45 cm long and 45 cm wide, was divided by masking tape markers into 9 squares (3 rows of 3). Mice were observed in the open field test on the seventh day of the experimental protocol. Each animal was placed at the center of the open field and observed for 4 min to record the locomotor and exploratory activities (Walsh and Cummins, 1976). The arena was cleaned with 40% ethanol after each session and each mouse was tested only once. Data of the locomotor and exploratory activities were expressed as number of segments crossed with the four paws and, number of rearings on the hind limbs, respectively.

2.4.4 Assessment of cognitive domain

The object recognition task was carried out according to the method previously described in the literature (Stangherlin et al., 2009). This task has been widely used to evaluate short-term (STM) and long-term (LTM) memories. The task was performed in an open field apparatus on the tenth and eleventh days of the experimental protocol. On the day of the task each animal was submitted to a habituation session in the absence of

objects for 5 min. Posteriorly, four objects named as A1 and A2 (balls), B (cube) and C (square) were used. The objects were made of plastic material, measuring 10 x 10 cm (length x height) as following color pattern: blue, red, and yellow. During the training, the animals were placed for 5 min in the arena containing two identical objects (A1 and A2). Exploration was accounted when the animal directed its nose - sniffing, touching, or looking - around 2 cm of the object. In the presence of a familiar object (A1) and a new object (B), 1.5 h after training, the STM of mice was evaluated. The time to explore was defined in 5 min, enough to measure learning and recognition memory. In turn, LTM was assessed in 24 h after training. The mice were placed to explore a familiar object (A1) and a new object (C) for 5 min. Time spent exploring each object was reported. Data were expressed as a percentage of the exploratory preference and calculated as follows: Training = (A2/(A1+A2))×100; STM = (B/(A1+B))×100; LTM = (C/(A1+C))×100.

2.4.5 Assessment of anxiety-like behavior

The elevated plus-maze apparatus consists of two opposed open arms (16 cm x 5 cm) and two opposed closed arms (16 cm x 5 cm x 10 cm) mounted at an angle of 90º, all facing a central platform (5 cm x 5 cm) elevated 50 cm from the floor. This test is widely validated to measure anxiety in rodents (Pellow et al., 1985). On the fourteenth day, all animals were evaluated in the elevated plus-maze test. Each animal was individually placed at the center of the apparatus facing one of the open arms. The frequency of entries into either open or closed arms and the time spent in each type of arm were measured for 5 min. The anxiolytic effect of a drug is illustrated by a significant statistical increase of parameters in open arms. Data were expressed as a percentage of the number of entries and of the time spent in the open arms.

2.5 *Ex vivo* assays

Ex vivo assays were performed to extend the knowledge about the influence of aging in the development of OXA-induced peripheral neuropathy. Neuropathic pain induced by OXA is a debilitating condition, whose brain circuit remains poorly understood. There is some experimental evidence about the functioning alteration of spinal cord and supraspinal regions (including the hippocampus and cerebral cortex) caused by neuropathic pain induced by OXA . The development of neuropathic pain includes several mechanisms extend from the periphery to the CNS through connections of spinal cord (Deng et al., 2019; Widerström-Noga, 2017). Disturbance in cerebral cortex areas pain-modulation circuits may be crucial for the maintenance of neuropathic pain (Chen et al., 2019; Huang et al., 2019), as well as neuroinflammatory responses in hippocampus (Fiore and Austin, 2018; Li et al., 2019). Thus, spinal cord, hippocampus and/or cerebral cortex tissues were selected for these investigations.

On the fourteenth day, after plus-maze test, animals were killed by isoflurane anesthetic inhalation. Spinal cord and brain were collected aiming to determine platinum and biolements concentration. Additionally, cerebral cortex was collected aiming to determine oxidative stress markers, such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities, and RS levels. Furthermore, given that a decrease on cholinergic neurotransmission in neuropathic pain condition has been reported (Ferrier et al., 2015), the activity and/or expression of acetylcholinesterase (AChE) in cerebral cortex and hippocampus were evaluated. Samples were homogenized in 50 mmol L^{-1} TrisHCl, pH 7.4, or specifically for AChE activity in 0.25 mol L^{-1} sucrose buffer (1:10 w v^{-1}) and centrifuged at 900 x g for 10 min - supernatant (S_1). Spinal cord, hippocampus, and/or cerebral cortex were also used

aiming to determine CAT, GPx, SOD, Nrf2, phosphoinositide 3-kinase (PI3K), activating transcription factor 4 (ATF4), factor nuclear kappa B (NF-κB), BDNF and cAMP response element binding protein (CREB) expressions using the qRT-PCR technique.

2.5.1 Determination of platinum and bioelements in the spinal cord and brain

Only high-purity reagents were used for the determination of platinum derived from oxaliplatin and of other bioelements in spinal cord and brain. Ultra-pure water (resistivity > 18.2 MΩ cm) was obtained from a Milli-Q Element water purification system (Millipore, France). Pro-analysis purity level 14.4 mol L^{-1} HNO_3 (ChemLab, Belgium) further purified by sub-boiling distillation and ultra-pure 9.8 mol L-1 H_2O_2 (Sigma Aldrich, Belgium) were used for sample digestion. Appropriate dilutions of 1 g L^{-1} single element standard solutions of Ca, Co, Cu, Fe, Ga, Mg, Mn, Na, P, Pt, S, Se, Y, and Zn (Instrument solutions, The Netherlands) were used for method development, optimization, and calibration purposes. For quantitative element determination, external calibration was relied on as calibration approach (0, 0.5, 1.0, 5.0, 10, 25, 50 and100 µg L-1 for Ca, Co, Cu, Fe, Mg, Mn, Na, Pt and Zn; 0, 1.0, 5.0, 10, 25 and 50 µg L-1 for K; 0, 0.05, 0.1, 0.5 and 1 µg L-1 for Se; and 0, 5.0, 10, 25 and 50 µg L-1 for P and S; all standard solutions were prepared in 0.35 mol L-1 HNO3) with Ga and Y (5 µg L-1) as internal standards (IS) to correct for instrument instability, signal drift and matrix effects.

(Ultra-)trace element determination was carried out using an Agilent 8800 ICP-MS/MS instrument (ICP-QQQ, Agilent Technologies, Japan). The sample introduction system is equipped with a concentric nebulizer (MicroMist, 400 µL min-1) mounted onto a Peltier-cooled (2 °C) Scott-type spray chamber. This instrument is equipped with

a tandem mass spectrometry configuration (MS/MS mode) consisting of two quadrupole units (Q1 and Q2) and a collision/reaction cell (CRC) located in-between both quadrupole mass filters (the configuration is Q1-CRC-Q2). The double mass selection offered by ICP-MS/MS instrumentation provides a better control over the ion-molecule processes occurring within the CRC, thus resulting in an improvement over the in-cell chemistry (chemical resolution). ICP-MS/MS provides additional means to deal with spectral overlap in a more straightforward way compared to traditional single-quadrupole ICP-MS instrumentation (Balcaen et al., 2015; Bolea-Fernandez et al., 2017). In this work, the CRC was pressurized with NH3/He (10% NH3 in He) and O2 as reaction gases to promote the formation of reaction product ions. This approach, called mass-shift, has been used to remove spectral interferences efficiently and selectively in the context of the determination of challenging elements at (ultra-)trace concentration levels via ICP-MS/MS (the instrument settings are shown in Table 1) (Resano et al., 2020). The product ion scanning (PIS) tool available for ICP-MS/MS instrumentation was used for the identification of the best suited reaction product ions for each of the target analytes. To maximize the sample throughput, two multi-element methods were developed for the simultaneous determination of (i) Ca, Co, Cu, Fe, Mg, Mn, Na, Pt and Zn and (ii) P, S and Se, while K was determined in a monoelement method (see Table 1).

Prior to ICP-MS/MS analysis, the samples were acid-digested in Teflon Savillex beakers, which had been pre-cleaned with HNO3 and HCl and subsequently rinsed with Milli-Q water. The digestion procedure was performed using approx. 300 mg of brain and 100 mg of spinal cord (the sample mass was accurately weighed for appropriate quantification), 4 mL of 14.4 mol L-1 HNO3 and 1 mL of 9.8 mol L-1 H2O2. The mixture was heated at 110 °C on a hot plate for approx. 24 h. The digested samples

were appropriately diluted with Milli-Q water or HNO3 until a final volume of 20 mL and an acid concentration of 0.35 M. Six independent experiments were performed for each group. The results are expressed considering wet spinal cord/brain weight (nanograms of element per gram of spinal cord/brain weighted). The limits of detection (LODs) were calculated as 3 times the standard deviation on 10 consecutive measurements of a blank solution divided by the slope of the calibration curve. A certified reference material NIST CRM 8414 (Bovine Muscle Powder) was also analyzed for quality assurance and quality control (QA/QC) of the analytical method.

2.5.2 Involvement of oxidative stress

2.5.2.1 RS levels

RS levels were determined using a spectrofluorimetric method, 2',7'-dichlorofluorescein diacetate (DCHF-DA) assay (Loetchutinat et al., 2005). S_1 (50 µL) was incubated with 20 µL of DCHF-DA (1 mmol L^{-1}) and 2430 µL of Tris HCl (10 mmol L^{-1}) in pH 7.4. The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured using a quartz cuvette for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 525 nm (with 488 nm excitation) 60 min after the addition of DCHF-DA to the medium (Shimadzu RF-5301PC fluorometer). RS levels were expressed as arbitrary units of fluorescence.

2.5.2.2 CAT activity

CAT activity was spectrophotometrically measured monitoring the consumption of H_2O_2 in the presence of S_1 at 240 nm (Aebi, 1984). The enzymatic reaction was initiated by adding an aliquot of S_1 (100 µL) and the substrate (H_2O_2, 105 µL) until 0.3 mmol L^{-1} in a medium containing 50 mmol L^{-1} potassium phosphate buffer, pH 7.0. The

enzymatic activity was expressed in U/mg protein (1 U decomposes 1 mmol L^{-1} of H_2O_2 per minute at pH 7 at 25 °C).

2.5.2.3 GPx activity

GPx activity was spectrophotometrically assayed monitoring the dismutation of H_2O_2 in the presence of S_1 at 340 nm (Wendel, 1981). S_1 (50 µL) was added in a system composed by reduced glutathione (GSH)/NADPH/glutathione reductase (GR), and the enzymatic reaction was initiated by H_2O_2 (100 µL). The enzymatic activity is indirectly measured by NADPH decay. H_2O_2 is reduced, generating oxidized glutathione (GSSG) from GSH. GSSG is back regenerated to GSH by GR present in the analysis medium at the expense of NADPH. The enzymatic activity was expressed as nmol/min/mg protein.

2.5.2.4 SOD activity

SOD activity was spectrophotometrically measured based on the capacity of SOD to inhibit autoxidation of epinephrine (Misra and Fridovich, 1972). S_1 (6, 12, or 18 µL) was added to 0.05 mol L^{-1} Na_2CO_3 buffer, and the enzymatic reaction was started by adding epinephrine (30 µL). The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26 °C. The enzymatic activity was expressed as U/mg protein.

2.5.3 Involvement of AChE enzyme

AChE activity was assayed based on the formation of the 5,5′-dithio-bis-acid nitrobenzoic, yellow anion, measured spectrophotometrically at 412 nm during 2 min. This method was based in the literature with some modifications using acetylthiocholine as substrate (Ellman, 1959). The reaction mixture (2 mL final volume) contained as

following: S_1 (100 µL), 100 mM K^+-phosphate buffer, pH 7.5 and 1 mM 5,5′-dithio-bis-nitrobenzoic acid (DTNB). The enzyme was pre-incubated for 2 min at 25 °C. The reaction was initiated by the addition of 0.8 mmol L^{-1} acetylthiocholine iodide. The enzymatic activity was expressed as µmol/h/mg protein.

2.5.4 RNA extraction, cDNA synthesis and quantitative real-time polymerase chain reaction (PCR)

Total mRNA was extracted from 50 and 70 mg thawed samples of spinal cord, hippocampus and/or cerebral cortex using TRIzol reagent (Invitrogen™, Carlsbad, USA), followed by DNase treatment with DNase I Amplification Grade (Invitrogen™, Carlsbad, USA) to ensure minimum DNA contamination. Total RNA isolated was quantified, and its purity (260/280 and 260/230 ratios) examined using a NanoVue spectrophotometer (GE, Fairfield, CT, USA).

cDNA synthesis was performed using High-Capacity cDNA Reverse Transcription (AppliedBiosystems™, UK) according to the manufacturer's protocol. 2 µg of total RNA was used in a reaction volume of 20 µL. The amplification was made using GoTaq® qPCR Master Mix (Promega, Madison, WI) in Agilent Mx3005P qPCR System (Agilent Technologies Inc., Santa Clara, California). The sequence of primers is indicated in Table 2. The qPCR conditions were as following: 10 min at 95 °C to activate the hot-start Taq polymerase, followed by 35 cycles of denaturation for 15 s at 95 °C, primer annealing for 60 s at 60 °C, and extension for 30 s at 72 °C (fluorescence signals were detected at the end of every cycle). Baseline and threshold values were automatically set by Stratagene MxPro software.

The number of PCR cycles to reach the fluorescence threshold in each sample was defined as Ct value, and each sample was analyzed in duplicate to obtain an

average Ct. The $2^{-\Delta\Delta CT}$ method was used to normalize the fold change in gene expressions (Livak and Schmittgen, 2001) using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as housekeeping gene.

2.5.5 Protein determination

The protein concentration was spectrophotometrically measured at 595 nm using bovine serum albumin as standard (Bradford, 1976). This is a rapid and sensitive strategy for the microgram quantities quantitation of protein using protein-dye binding principle. The reaction mixture containing S1 (50 µL) and Coomassie brilliant blue (2.5 mL) was incubated for 10 min. Several biochemical analyses rely on accurate protein concentration quantitation. The protein level was expressed as mg protein/mL.

2.5.6 Data and statistical analysis

The normality of the data was evaluated using D' Agostino and Pearson omnibus normality test. Statistical analysis was performed using GraphPad Prism 6.0 software (San Diego, CA, USA). Data were analyzed by two-way analysis of variance (ANOVA) followed by the Tukey's test when appropriated for parametric data. Data were expressed as mean ± standard error of the mean (S.E.M.). Post hoc tests were performed only when F-value achieved necessary level of statistical significance ($P < 0.05$) and when there was no significant variance in homogeneity. All data were evaluated for outliers (ROUT - Q = 1.0 %) and outliers were excluded. Data and statistical analysis comply with the recommendations on experimental design and pharmacology analysis (Curtis et al., 2018).

3. Results

3.1 Aging induces neuropathy and exacerbates mechanical and thermal hypersensitivities induced by OXA exposure

Initially, to establish the aging impact in OXA-induced peripheral neuropathy and to identify the molecular mechanisms underlying, the behavior of animals through mechanical and thermal sensitivities should be considered. Well-established tests (von Frey and hot plate) in young and old *Swiss* mice were employed. Interestingly, OXA exposure produced robust growth in mechanical and thermal sensitivities in both ages (Fig. 2). These effects started in 5 or 6 days post OXA administration during mechanical and thermal sensitivities, respectively, and lasted in least 12 or 13 days, respectively.

Moreover, mechanical (Fig. 2A) and thermal (Fig. 2B) sensitivities were compared between young and old mice controls. Old animals were more sensitive to mechanical and thermal stimulus than young animals. Concomitantly, aging also increased the animal's vulnerability to the several effects caused by OXA-induced peripheral neuropathy. A reduction in the paw withdrawal threshold (Fig. 2A) and in the latency time (Fig. 2B) was observed in older animals exposed to OXA on days 12 and 13 of the experimental protocol, respectively. Overall, these results indicate OXA-administration as producing mechanical and thermal hypersensitivity in young and old mice. However, the toxic effects of OXA are more pronounced in old mice and these animals are more sensible than young mice.

3.2 The magnitude comorbidities of OXA-induced peripheral neuropathy is modified by aging

Beyond the seriously debilitating effects of neuropathic pain caused by OXA-treatment, the presence of multiple comorbidities including anxiety and cognitive

impairment (Reis et al., 2020a) can complicate several challenges during oncologic treatment. Thus, OXA-exposure caused anxious behavior in young and old mice (Fig. 3). Aging-induced anxious behavior in mice was established by reduction of the entries (Fig. 3A) and time spent (Fig. 3B) in the open arms in the elevated plus-maze. Interestingly, anxious behavior in old mice was exacerbated during the treatment with OXA. A reduction in both entries (Fig. 3A) and time spent (Fig. 3B) in the open arms in the elevated plus-maze test was observed in old mice exposed to OXA comparing with young mice exposed to the chemotherapy.

OXA-exposure also induced a cognitive impairment in young mice (Fig. 3C and D). Nevertheless, aging caused a decline in cognitive function, and old mice presented a deficit in STM (Fig. 3C) and LTM (Fig. 3D) evidenced by decreasing in the exploratory preference for the new object in the object recognition task. Cognitive damage aging-induced was not aggravated by OXA-treatment. The effect of OXA and/or aging on locomotor and exploratory behaviors of mice was determined. The number of crossings and rearings in the open field is presented in Figures 3E and 3F, respectively. There was no significant change in the evaluated parameters considering the treatment and the aging.

3.3 Platinum deposition occurs in both the spinal cord and the brain after exposure to OXA

OXA-exposure can cause an opening of blood-brain-barrier (BBB) starting from RS formation (Branca et al., 2018). Recently, our research group (Reis et al., 2020b) has demonstrated, for the first time, the platinum deposition in spinal cord of young mice submitted to OXA treatment. These results establishes the platinum from OXA-treatment crossing the BBB. In order to complement the existing knowledge, the present

study revealed, for the first time, high platinum concentration in brain of mice exposed to OXA (10 mg kg^{-1}, i.p.), regardless of age (Fig. 4). Furthermore, platinum concentration in spinal cord of young mice exposed to OXA was higher than old mice exposed to the chemotherapy (Fig. 4).

3.4 Aging, OXA treatment and, its interaction impact the bioelements concentration in the CNS

Appropriate levels of bioelements are crucial for the physiological condition maintenance in CNS. Bioelements imbalance caused by neurotoxic activities are linked with decreased enzymatic activities, increased proteins aggregation, and oxidative stress. These processes can result in neurodegeneration and cell death (Kabir et al., 2021). The concentration of some bioelements in CNS was determined to investigate the role of aging in the OXA-induced peripheral neuropathy. The concentration of calcium, cobalt, copper, iron, magnesium, manganese, potassium, phosphorus, selenium, sodium, sulfur, and zinc was measured in spinal cord and brain in young and old mice (Table 3).

According to the data, the concentration of sodium and cobalt in spinal cord was increased by aging while the concentration of manganese in spinal cord was decreased by aging. In brain, the concentration of manganese was reduced while the concentration of cobalt and copper was increased. Any changes in manganese and copper levels can lead to redox imbalance considering their cofactors function in antioxidant enzymes SOD (MnSOD and CuZnSOD) and CAT (Leyssens et al., 2017; Żwierełło et al., 2020). An increase sodium concentration can change the body fluid and electrolyte homeostasis (Begg, 2017). High systemic cobalt levels are related with complex clinical

syndrome characterized by neurological, cardiovascular, and endocrine deficits (Leyssens et al., 2017).

Imbalance in some bioelements concentration was observed considering OXA-treatment. Calcium concentration in spinal cord of young mice exposed to OXA was higher than in young. Phosphorus, sulfur, selenium, magnesium, manganese and cooper levels in spinal cord of old OXA-treatment mice was higher than in old. An increase on phosphorus, sulfur, selenium, magnesium, iron, cobalt, and copper in spinal cord of old OXA-treatment mice was observed comparing with young OXA-treatment mice. Elemental concentration in brain revealed a manganese concentration reduction in young mice exposed to OXA and a potassium, phosphorus and copper reduction in old mice exposed to OXA, comparing both with same age without OXA. Cobalt and copper concentration of old OXA-treatment mice was higher than in young OXA-treatment mice.

Many bioelements are necessary cofactors for properly metabolism function. These elements play an important key in both survival and longevity. Magnesium presents crucial roles in several cellular mechanisms, such as synaptic plasticity and energy metabolism (Kabir et al., 2021). Copper, iron, manganese, selenium and zinc are cofactors enzymes required for important organism functions, such as antioxidant system performance and redox balance maintenance (Mehta and Gowder, 2015). Vitamins and minerals deficiencies are associated with chronic disease and premature aging (Ames, 2018; Kabier et al., 2021). Phosphorus deficiencies can lead to muscle weakness, and selenium deficiency to muscular diseases. Calcium, magnesium, and selenium are important to prevent and/or treat sarcopenia, age-related muscle mass losses, muscle strength, and physical performance, besides support healthy aging

without pain (van Dronkelaar et al., 2018). Considering aging and chronic diseases possibility, pain is constantly experienced (Watkins, 2018).

In summary, old OXA-treatment mice presented cobalt, copper, iron, magnesium, phosphorus, selenium, and sulfur levels in CNS higher than in young OXA-treatment mice. These results demonstrate for the first time bioelements imbalance concentration in CNS caused by OXA treatment. This mechanism can be involved in the high vulnerability of the old mice to OXA-induced peripheral neuropathy. These results indicate the need for caution about nutritional supplementation of old during OXA treatment to avoidance toxicity caused by high bioelements concentration.

3.5 Oxidative stress plays pivotal role on the increased pain sensitization on the OXA-induced peripheral neuropathy of elderly mice

Bioelemental concentration evaluation infers aging and/or OXA exposure as influencers in platinum and bioelements concentration imbalance in CNS. Moreover, alteration in antioxidant enzymes and bioelements levels have been frequently related to oxidative stress (Koekkoek and Van Zanten, 2016). The physiopathology process of the OXA-induced peripheral neuropathy is multi-factorial, and oxidative stress contributes to their development (Areti et al., 2014). Oxidative stress-mediated cell damage has been recognized as a fundamental mechanism to the OXA-induced peripheral neuropathy pathophysiology (Colloca et al., 2017). However, despite continued efforts for better understanding OXA-induced peripheral neuropathy mechanisms, the physiopathology processes are still not fully understood. This issue remains a big challenge and an important question to be resolved.

To expand the mechanisms underlying OXA-induced peripheral neuropathy knowledge, the different possible ways considering the changes associated with this pathology and aging should be investigated. For these reasons, in the present study, RS levels and activity of antioxidant enzymes (CAT, GPx, and SOD) were firstly determined and, these results in cerebral cortex of mice are summarized in Figure 5. As results, an increase in RS levels was observed in cerebral cortex of old mice comparing with young (Fig. 5A) and an increase in RS levels was observed in young and old mice both treated with OXA, comparing same age without chemotherapy (Fig. 5A).

RS levels in cerebral cortex of old OXA-treatment mice were higher than in young OXA-treatment mice. RS increase levels in aging-induced was aggravated by OXA treatment (Fig. 5A). However, RS levels in young OXA-treatment and old without OXA-treatment were quite similar. Despite RS levels increase, the aging decreases CAT enzyme activity (Fig. 5C). Nevertheless, the highest SOD (Fig. 5B) and GPx (Fig. 5D) activities, different of CAT activity, was observed in young OXA-treatment mice. In turn, no alteration was observed in the activity of antioxidant defenses analyzed in the cerebral cortex of old OXA-treatment mice compared with same age without OXA treatment.

Oxidant production is increased during the aging while enzymatic antioxidant defenses are decreased (Zhang et al., 2015). Several factors may influence cellular redox status. The balance between oxidant and antioxidant is constantly maintained through the regulation of the antioxidant levels in response to oxidative stress, mainly through of signaling pathway of Nrf2. Recently, a reduction in the expression of SOD, CAT, and GPx in cerebral tissues of mice caused by OXA-treatment has been reported by our research group (Reis et al., 2020b). Other studies have shown the damage in some enzymes caused by the excessive oxidative stress, resulting in antioxidant

defenses losses (Birben et al., 2012; Griess et al., 2017). However, the aging was still not considered. An increase in RS levels and a modification in antioxidant enzymes activity (SOD, CAT, and GPx) in cerebral cortex of mice in both aging and/or OXA-treatment induced support the hypothesis that expression levels of antioxidant defenses can be modified. To confirm this hypothesis, expression levels of antioxidant enzymes (SOD, CAT, and GPx) were evaluated in spinal cord, cerebral cortex, and hippocampus of mice (Fig. 6).

A reduction in SOD expression levels in hippocampus (Fig. 6B) and cerebral cortex (6C) of old OXA-treatment and young OXA-treatment mice was observed comparing the same age without chemotherapy. CAT expression levels were reduced considering only the aging in spinal cord (Fig. 6D) and in cerebral cortex (Fig. 6F). On the other hand, CAT expression levels in spinal cord (Fig. 6D) of old OXA-treatment and young OXA-treatment mice were lower than those in same age mice without treatment. A decrease in the CAT enzyme expression was detected in hippocampus (Fig. 6E) and cerebral cortex (Fig. 6F) of old and young mice both OXA-treatment comparing with young mice. Still, young mice OXA-treatment presented similar CAT expression levels in cerebral cortex while old OXA-treatment mice presented a reduction CAT expression levels in cerebral cortex, both compared with old mice without OXA-treatment (Fig. 6F). A reduction in GPx expression levels in spinal cord (Fig. 6G), hippocampus (Fig. 6H), and cerebral cortex (Fig. 6I) was observed in mice with chemotherapy in both ages.

Thus, considering the antioxidant enzymes expression (SOD, CAT, and GPx) in CNS tissues, aging and OXA treatment cooperatively decreased the expression levels of CAT in spinal cord (Fig. 6D) and in cerebral cortex (Fig. 6F). Indeed, these results demonstrate aging and/or OXA-treatment augmenting the oxidative stress, evidenced by

increase RS production and antioxidant enzymatic defenses activity (SOD and GPx), and decrease SOD, CAT, and GPx expression levels. Importantly, the oxidative stress aging-induced was aggravated by OXA-treatment. Considering Nrf2/EpRE signaling as regulator of many antioxidant enzymes expression, including SOD, CAT and GPx, as well as NRf2 and ATF4 as important role in the mitochondrial homeostasis maintenance (Zhang et al., 2015; Yang et al., 2018), the impact between aging and OXA-treatment on NRf2, PI3K and ATF4 molecules signaling was also investigated to expand the knowledge involving oxidative stress in OXA-induced peripheral neuropathy and aging.

As shown in Fig. 7, the Nrf2 expression levels in hippocampus (Fig.7B) and in cerebral cortex (Fig. 7C) was decreased by aging, as well as PI3K in hippocampus (Fig.7E) and in cerebral cortex (Fig. 7F), and ATF4 in spinal cord (Fig. 7G) and in hippocampus (Fig. 7H), all of them without chemotherapy. Nrf2 expression in spinal cord (Fig. 7A) and in hippocampus (Fig. 7B) was reduced in animals with OXA administration in same age while Nrf2 expression in cerebral cortex was increased (Fig. 7C). The treatment with OXA increased PI3K expression levels in spinal cord (Fig. 7E) in young mice comparing same age without OXA-treatment.

PI3K expression was reduced in spinal cord of old OXA-treatment mice comparing same age without chemotherapy and young mice with chemotherapy. The PI3K expression in old mice OXA-treatment was decreased in hippocampus (Fig. 7E) in relation to young mice and increased in cerebral cortex (Fig. 7F) in relation to old mice, both without chemotherapy. A reduction in ATF4 expression was observed in spinal cord (Fig. 7G) of both ages OXA-treatment mice. As can be seen in Fig. 7H, the lowest reduction in ATF4 expression levels in hippocampus was observed in young. In cerebral cortex (Fig. 7I), both ages OXA-treatment mice presented levels of ATF4 lower than

young mice while old OXA-treatment mice presented ATF4 in the cerebral cortex lower than in same age mice without OXA treatment (Fig 7I).

Based on the evidence from the present study, the alterations in the Nrf2 pathway induced by OXA can be correlated with the age and tissue analyzed. Nrf2/ARE signaling system is regulated in several levels (Kasai et al., 2019). Indeed, findings of this study indicate the neuroprotective effects associated with Nrf2 pathway as an interesting target for ameliorating the OXA-induced neuropathic pain during aging.

3.6 BDNF signaling pathway is involved in OXA-induced peripheral neuropathy and in the changes aging-mediated

Neuropathic pain is usually associated with several comorbidities such as anxiety, depression, and disability. Neuropathic pain represents a unique challenge to the primary provider caring for the patient (Wang and Mullally, 2020). OXA-induced peripheral neuropathy causes neuropathic pain in young and exacerbated pain in old. Associated with OXA-induced neuropathic pain, anxious behavior in young and old mice as well as cognitive impairment in young mice were observed in this study. To better understand the relationship between OXA-induced neuropathic pain and its comorbidities, mainly observed during aging, BDNF signaling pathway was also investigated. BDNF is a neurotrophin containing high expression level that regulates brain synapses. In addition to its well-established neurotrophic action, BDNF also plays other important neuroprotective effects including antioxidant (Pradhan et al., 2019). From data obtained in this study, aging has increased susceptibility of OXA toxic effects in peripheral neuropathy and other comorbidities mainly due to oxidative stress. Thus, aging and/or OXA-treatment influence in BDNF, CREB and NF-κB expression levels was studied.

BDNF (Fig. 8A) and CREB (Fig. 8C) expression levels in the hippocampus were not changed by aging while BDNF (Fig. 8B) and CREB (Fig. 8D) expression levels in cerebral cortex were reduced. The role of BDNF/TrkB pathway in pain perception has been extensively studied; however, not yet completely elucidated. At brain level, BDNF promotes the nociceptive facilitation due to increased plasticity and indirectly stimulation of descending nociceptive inhibition. Data indicate BDNF involved as far in the initial sensitization of pain as its increase. Sustained release of BDNF can lead to chronic pain (Cappoli et al., 2020; Nijs et al., 2015). CREB regulates BDNF-gene transcription.

According to the results, NF-κB expression levels in hippocampus (Fig. 8E) and cerebral cortex (Fig. 8F) of mice was increased by aging. On the other hand, OXA-treatment reduced BDNF levels in hippocampus (Fig. 8A) and cerebral cortex (Fig. 8B), and CREB levels in cerebral cortex (Fig. 8D) in both age OXA-treatment mice. Old OXA-treatment mice presented a reduction in BDNF expression levels in hippocampus (Fig. 8A) and cerebral cortex (Fig. 8B), and also CREB in cerebral cortex (Fig. 8D), comparing with old mice without OXA-treatment. A reduction in BDNF (Fig. 8B) and CREB (Fig. 8D) expression levels in cerebral cortex in old OXA-treatment mice was also observed comparing young OXA-treatment mice.

The nuclear transcription factor NF-κB is a regulator role in innate and acquire immunity, inflammatory response, and tumors progression (Wang et al., 2019). NF-κB expression in hippocampus (Fig. 8E) and cerebral cortex (Fig. 8F) was increased during OXA treatment in both ages compared with only young mice. Indeed, NF-κB signaling pathway is activated by RS excessive production (Wang et al., 2019), as caused by OXA-treatment and aging.

3.7 Cortical AChE is modulated by OXA exposure, but not by aging

Reis and collaborators (2020b) demonstrated an increased activity and a reduced expression level of AChE enzyme in cerebral cortex of mice exposed to OXA. In this sense, to understand the involvement of cerebral AChE on OXA-induced peripheral neuropathy during aging, in the present study, the activity and the AChE expression level were evaluated in young and old mice. Firstly, alteration of AChE activity or expression levels in cerebral cortex of old mice exposed to OXA is still not reported. The results obtained in this study corroborate with those previously published. Indeed, OXA-treatment induced an increase in the activity (insert Fig. 9B) and a reduction in AChE enzyme expression (Fig. 9B) in cerebral cortex of young and old mice comparing the same aging without chemotherapy. However, no difference in AChE expression levels in hippocampus was observed.

4. Discussion

The present study establishes aging exacerbating peripheral neuropathy induced by OXA. Bioelements play an important role in aging which can cause an imbalance in their concentration, as demonstrated in this study. Although bioelements role is no clear in the OXA-induced peripheral neuropathy, a link between both was evidenced in this study. Imbalances of intracellular bioelements homeostasis are linked with some factors such as oxidative damage, reduction synaptic plasticity, deficit neurological, and premature aging. Old OXA-treated and young OXA-treated mice presented different concentrations of several bioelements in CNS, including calcium, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sulfur, and zinc. However, the results are inquiring comparing the alterations in spinal cord and brain induced by OXA in young and old mice. OXA-exposure modified the levels of cobalt, copper, iron,

magnesium, phosphorus, selenium, and sulfur in old mice, while OXA-exposure modified only the levels of calcium and manganese in young mice. Considering these results, a hypothesis could be that some bioelements may not be being used by certain enzymes, and for this reason they may be migrating more easily between tissues.

Indeed, the bioelements level differences induced by OXA-treatment was still not clear. Our evidence suggests the occurrence of facts leading changes during aging. Then, a nutritional intervention in old requirement during OXA-treatment deserves attention. Bioelements accomplish decisive functions to human health, deficiency of them can lead undesirable pathological conditions. Nevertheless, supplementation should be carefully controlled, given several toxic effects ascribed to them in quantities exceeding those required for accomplishing biological functions (Fraga, 2005, Kaur et al., 2019; Miquel et al., 2018)). Considering the results obtained, the bioelemental changes observed can contribute to the peripheral neuropathy induced by OXA, predominantly during aging.

Association between aging and OXA-treatment increases the pain sensitivity and its comorbidities, mainly through oxidative damage induced in CNS due to platinum accumulation, as well as by reduced neuroprotection and neuroplasticity. OXA-treatment caused platinum accumulation in spinal cord. This data corroborate with those previously published (Bernocchi et al., 2015; Branca et al., 2018; Reis et al., 2020b). However, stand out that only in this study the results demonstrated, for the first time, OXA-treatment causing platinum accumulation in brain. Toxic platinum compounds effects to the CNS has been mainly reported considering oxidative stress mediated by RS (Bernocchi et al., 2015; Branca et al., 2018; Reis et al., 2020b). Platinum can affect mitochondrial DNA leading mitochondrial dysfunction, such as DNA mutations, decreasing mitochondrial respiratory function, and inducing neuronal apoptosis (Areti et

al., 2014; Girolimetti et al., 2017).

The evidence from the current study suggests platinum deposition in CNS undetermined by aging. Young and old mice exposed to OXA presented platinum deposition; however, oxidative stress was directly affected by aging. In this sense, RS levels are exacerbated by mitochondrial dysfunction induced by platinum, but other pathways contribute to this difference between ages. Indeed, results highlight different enzymatic antioxidant defense responses in young and old mice exposed to OXA. To maintain redox balance after RS formation induced by OXA-treatment, an increase in SOD and GPx activities in cerebral cortex of young mice exposed to chemotherapy was observed. However, this increase in the activity of antioxidant enzymes was not observed to old OXA-treatment mice, leading to a high oxidative damage caused by OXA. The redox state impacts significantly cellular functionality and RS present well-established able to damage cellular macromolecule as proteins, which are abundant in the cells.

Corroborating with the present study, the influence of aging and the RS production effects - the cellular redox state – has been previously demonstrated in published data. Aging reduces the ability of the cell to maintain its proteome (Korovila et al., 2017). Basal and inducible expression levels of antioxidant enzymes including the SOD, CAT, and GPx, are regulate by Nrf2 transcription factor. OXA exposure caused a reduction in the antioxidant enzymes expression levels in spinal cord, hippocampus, and/or cerebral cortex of young and old mice. This raises the possibility that the deficiencies in the antioxidant system caused by aging exacerbated the OXA-induced peripheral neuropathy. This consequence could be correlated to the increasing of the sensitivity to pain in the old OXA-treatment mice. Recently, inhibiting oxidative stress has been indicated as a potential strategy to manage neuropathic pain (Ge et al., 2018;

Khasabova et al., 2019). Antioxidants reduced mitochondria damage and pain induced by chemotherapeutic drugs, supporting a role for oxidative stress in the neuropathic pain (Khasabova et al., 2019).

Given redox imbalance caused by OXA-induced neurotoxicity was enhanced by aging, an immediate question was the association of both factors with the Nrf2 pathway change. Nowadays, Nrf2 role in the oxidative imbalance related to aging is still not clearly established (Silva-Palacios et al., 2018); however, some reports suggest OXA as an effective activator of Nrf2 pathway (Fang et al., 2020). Interestingly, aging *per se* reduced the Nrf2 expression in brain. On the other hand, OXA effects on Nrf2 expression levels dependent of the tissue analyzed. Downregulates Nrf2 expression levels are in accordance with SOD, CAT and GPx enzymes expression reduction, since Nrf2 controls the genes expression whose products include antioxidant enzymes. However, divergent results were observed in cerebral cortex. Findings suggest that Nrf2-mediated antioxidant response failed to protect young and old mice against OXA neurotoxicity. There is a strong indication the elucidation of this pathway signaling, as protective mechanism particularly to old patients, will help to develop novel therapeutic interventions to prevent or treat the peripheral neuropathy and its comorbidities induced by OXA.

Previous studies demonstrate have found that Nrf2 positive regulators like PI3K decrease with age (Yamamoto et al., 2018; Zhang et al., 2015). The results of this study confirmed a reduction of PI3K expression in hippocampus and cerebral cortex, but not in spinal cord, during aging. In turn, OXA-treatment regulated PI3K expression in spinal cord according with age - young over expression and old down expression. In this aspect, mitochondrial disturbances, like OXA-caused, can indirectly activate PI3K (Kasai et al., 2019) as way to defenses. Here, in the old mice, this mechanism was

activated only in cerebral cortex. In the current context, despite the associations between aging, oxidative stress, and Nrf2 pathway, the hypothesis is that Nrf2 presents an impact on OXA-induced neuropathy peripheral in old and this fact has not been sufficiently investigated. In this respect, the present study demonstrated that aging and OXA downregulate ATF4 expression.

These findings are consistent with the results published by Hussain and Ramaiah (2007). Results suggest, despite the increase in PI3K expression, the low levels of Nrf2 in spinal cord could be related to reduced ATF4 level. Supporting this notion, recent data demonstrated Nrf2 knockout aggravated OXA-induced peripheral neuropathy, mainly through the increase RS production, decrease mitochondrial membrane potential which led to abnormal intracellular calcium levels, and induce cytochrome c-related (Cyt C) apoptosis, and overexpression the transient receptor potential (TRP) protein family (Yang et al., 2018). This suggests Nrf2 pathway may play a critical role in pain management associated with OXA-induced peripheral neuropathy, particularly during aging.

BDNF levels play a crucial role as neuromodulator in pain transmission which is also widely accepted for its involvement in cellular processes underlie cognition and other complex behaviors as anxiety (Notaras and van den Buuse, 2020). After an initial increase in BDNF levels favoring neuroplasticity and leading chronic pain as defined in previous studies (Cappoli et al., 2020; Nijs et al., 2015), BDNF levels decrease in hippocampus and cerebral cortex reducing neuroplasticity and supporting chronic pain maintenance. Our results suggest the reduced BDNF levels were caused by OXA-treatment and aging related to nociceptive effects, cognitive impairment, and anxious behavior. Data also highlight the importance of BDNF on the aggravated pain in old OXA-treated mice. This hypothesis is supported by results recently published by

Cappoli and collaborators (2020). The authors reported, despite some evidence that BDNF released by neurons determines nociceptive response, the exogenous BDNF leads to an antinociceptive effect with the microglial cells involvement. Thus, low expression of BDNF could be related to pain maintenance. Reduced BDNF levels were associated with high depressive and anxiety-like behaviors and working memory impairment due to reduced neuroplasticity.

The transcription factor CREB mediates the transcription of genes essential for survival and neurons differentiation and for BDNF transcription (Lima Giacobbo et al., 2019). Our results demonstrated that aging reduced CREB expression in cerebral cortex of mice. Additionally, OXA-treatment reduced CREB expression in both ages. However, the old presented CREB levels lower than young. These findings corroborate with those refer to BDNF expression achieved in this study. BDNF can promote anti-inflammatory action of microglia and neurological recovery through TrkB signaling. BDNF-TrkB pathway determines the upregulation of different anti-inflammatory microglial markers and regulation of the NF-κB pathway (Cappoli et al., 2020; Yin et al., 2020).

Aging increased NF-κB level in both hippocampus and cerebral cortex of mice. These results corroborate with other data obtained in this study, demonstrating increased RS level and reduced BDNF expression caused by aging. Results suggest that NF-κB pathway is activated by aging, mainly through oxidative stress. In turn, OXA-treatment increased NF-κB expression in both tissues analyzed only in young mice, despite of high RS level exhibited in old OXA-treatment mice. On the other hand, NF-κB expression contributes to increase the production and release of proinflammatory cytokines (Wang et al., 2019). Consecutively, an increase of proinflammatory mediators leads to peripheral sensitization mainly through TRP vanilloid activation (Areti et al.,

2014), supporting our results about the role of aging in the increase of mechanical sensitivity.

The RS play a major role in intracellular signaling. However, the redox imbalance leading to the progression of neurodegenerative disorders. The Nrf2 increases antioxidant defenses neutralizing RS, thus reduces RS-mediated NF-κB activation. Also, the Nrf2 reduces NF-κB nuclear translocation and transcription of pro-inflammatory genes. On the other hand, NF-κB can inhibit Nrf2 activity preventing antioxidant gene transcription (Sivandzade et a., 2019). Here, our findings demonstrated that in the hippocampus occurred an increase of the NF-κB expression level and a reduction in the Nrf2 expression level. By contrast, Nrf2 and NF-κB gene transcription increased in the cerebral cortex.

Cognitive impairment aging-induced is mainly mediated by neuroinflammation and cholinergic dysfunction (Benfante et al., 2019). In this study, the involvement of NF-κB in neuroinflammation associated with aging and its relationship with cognitive decline were demonstrated. Indeed, in the proinflammatory states associated with the aging process occurs an upregulation of NF- κB (Xia et al., 2016). OXA-treatment induced neuroinflammation in young mice and cognitive decline through NF-κB expression regulation. In addition, old mice exposed to OXA presented cognitive impairment and NF-κB levels similar with those in old without chemotherapy. Thus, the cholinergic dysfunction occurrence in old with OXA-induced peripheral neuropathy should be investigated.

Our research group has demonstrated the reduction of AChE expression and the increase of AChE activity in the cerebral cortex of young mice caused by OXA (Reis et al., 2020b). The results of this study corroborate with these data, and similar results were observed in old mice treated with OXA. Cholinergic neurotransmission is altered

during aging, and this fact can lead to cognitive impairment, mainly through neuronal degeneration development and normal neural circuitry disruption (Richardson et al., 2020). These findings supporting the aging role in the cognitive impairment observed; however, considering part of cholinergic neurotransmission, in particular the activity and expression levels of AChE, OXA effects were not altered by aging.

To summarize the current knowledge, the present research helped to expand the understanding of the mechanisms underlying the physiopathology of OXA-induced peripheral neuropathy, highlighting aging role. Indeed, aging exacerbated the pain and anxious behavior caused by OXA-treatment and the deposition of platinum in brain of mice treated with OXA was reported for the first time. Taken together, the results suggest important modulated targets for OXA-induced peripheral neuropathy prevention or treatment, considering the peculiarities aging-mediated.

Funding Information

This study received financial support and scholarships from the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (429859/2018-0), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) (PqG 17/2551-0001013-2). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível superior – Brasil (CAPES) - Finance Code 001 and by Institutional Internationalization Project - CAPES/PrInt/UFPel (041/2017). C.L.; E.A.W.; D.A.; M.F.M. and V.F.C. are recipients of CNPq fellowship. EBF thanks FWO-Vlaanderen for his postdoctoral grant (12ZA320N). This study also received financial assistance from L'ORÉAL-UNESCO-ABC for Women in Science.

Compliance with Ethical Standards

Animal care and all experimental procedures were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) and in accordance with the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 4506-2017). All efforts were made to minimize the number of animals used and their suffering.

Author contributions

A.S.R., J.J.P., C.L and E.A.W. conceived and designed the study. W.B.D. and V.F.C. were responsible for perform qRT-PCR. D.L.R.N, E.B.F, T.V.A., F.V. and M.F.M were responsible for elemental quantification. A.S.R. and E.A.W. wrote the manuscript. E.A.W supervised the study. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Alamri, F.F., Shoyaib, A. Al, Biggers, A., Jayaraman, S., Guindon, J., Karamyan, V.T., 2018. Applicability of the grip strength and automated von Frey tactile sensitivity tests in the mouse photothrombotic model of stroke. Behav. Brain Res. 336, 250–255. https://doi.org/10.1016/j.bbr.2017.09.008

Ames, B.N., 2018. Prolonging healthy aging: Longevity vitamins and proteins. Proc.

Natl. Acad. Sci. U. S. A. 115, 10836–10844. https://doi.org/10.1073/pnas.1809045115

Areti, A., Yerra, V.G., Naidu, V.G.M., Kumar, A., 2014. Oxidative stress and nerve damage: Role in chemotherapy induced peripheral neuropathy. Redox Biol. 2, 289–295. https://doi.org/10.1016/j.redox.2014.01.006

Balcaen, L., Bolea-Fernandez, E., Resano, M., Vanhaecke, F., 2015. Inductively coupled plasma - Tandem mass spectrometry (ICP-MS/MS): A powerful and universal tool for the interference-free determination of (ultra)trace elements – A tutorial review. Anal Chim Acta, 24; 7-19. doi: 10.1016/j.aca.2015.08.053.

Begg, D.P., 2017. Disturbances of thirst and fluid balance associated with aging. Physiol. Behav. 178, 28–34. https://doi.org/10.1016/j.physbeh.2017.03.003

Bellaver, B., Bobermin, L.D., Souza, D.G., Rodrigues, M.D.N., de Assis, A.M., Wajner, M., Gonçalves, C.A., Souza, D.O., Quincozes-Santos, A., 2016. Signaling mechanisms underlying the glioprotective effects of resveratrol against mitochondrial dysfunction. Biochim. Biophys. Acta - Mol. Basis Dis. 1862, 1827–1838. https://doi.org/10.1016/j.bbadis.2016.06.018

Benfante, R., Di Lascio, S., Cardani, S., Fornasari, D., 2019. Acetylcholinesterase inhibitors targeting the cholinergic anti-inflammatory pathway: a new therapeutic perspective in aging-related disorders. Aging Clin. Exp. Res. 1-12. https://doi.org/10.1007/s40520-019-01359-4

Bernocchi, G., Fanizzi, F.P., De Pascali, S.A., Piccolini, V.M., Gasperini, C., Insolia, V., Bottone, M.G., 2015. Neurotoxic effects of platinum compounds: Studies in vivo on intracellular calcium homeostasis in the immature Central Nervous System. Toxics 3, 224–248. https://doi.org/10.3390/toxics3020224

Bhadra, M., Howell, P., Dutta, S., Heintz, C., Mair, W.B., 2019. Alternative splicing in

aging and longevity 139, 357–369. https://doi.org/10.1007/s00439-019-02094-6

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defence. World Allergy Organ. J. 5, 9-19. https://doi.org/10.1097/WOX.0b013e3182439613

Bobinski, F., Teixeira, J.M., Sluka, K.A., Roberto, A., Santos, S., Biology, F., Science, R., City, I., 2019. HHS Public Access 159, 437–450. https://doi.org/10.1097/j.pain.0000000000001109.IL-4

Bolea-Fernandez, E., Balcaen, L., Resano, M., Vanhaecke, F., 2017. Overcoming spectral overlap via inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS). A tutorial review, J. Anal. At. Spectrom. 32, 1660-1679. DOI https://doi.org/10.1039/C7JA00010C

Book, G.E.T.T., 1996. Guide for the Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals. https://doi.org/10.17226/5140

Branca, J.J.V., Maresca, M., Morucci, G., Becatti, M., Paternostro, F., Gulisano, M., Ghelardini, C., Salvemini, D., Mannelli, L.D.C., Pacini, A., 2018. Oxaliplatin-induced blood brain barrier loosening: A new point of view on chemotherapy-induced neurotoxicity. Oncotarget 9, 23426–23438. https://doi.org/10.18632/oncotarget.25193

Brusco, I., Justino, A.B., Silva, C.R., Fischer, S., Cunha, T.M., Scussel, R., Machado-de-Ávila, R.A., Ferreira, J., Oliveira, S.M., 2019. Kinins and their B1 and B2 receptors are involved in fibromyalgia-like pain symptoms in mice. Biochem. Pharmacol. 168, 119–132. https://doi.org/10.1016/j.bcp.2019.06.023

Cappoli, N., Tabolacci, E., Aceto, P., Dello Russo, C., 2020. The emerging role of the BDNF-TrkB signaling pathway in the modulation of pain perception. J. Neuroimmunol. 349, 577406. https://doi.org/110.1016/j.jneuroim.2020.577406

Cavaletti, G., Marmiroli, P., 2020. Management of oxaliplatin-induced peripheral sensory neuropathy. Cancers 12, 1370. https://doi.org/10.3390/cancers12061370

Chen, S.D., Gu, Y.X., Xu, D.B., Dai, Q.X., Wang, J.L., 2019. Involvement of miR-34a and p53 protein of cerebral cortex in electroacpuncture analgesia in mice with neuropathic pain. Zhen ci yan jiu = Acupunct. Res. 44, 632–636. https://doi.org/10.13702/j.1000-0607.190149

Colloca, L., Ludman, T., Bouhassira, D., Baron, R., Dickenson, A.H., Yarnitsky, D., Freeman, R., Truini, A., Attal, N., Finnerup, N.B., Eccleston, C., Kalso, E., Bennett, D.L., Dworkin, R.H., Raja, S.N., 2017. Neuropathic pain [Review]. Nat. Rev. Dis. Prim. 3, 17002. https://doi.org/10.1038/nrdp.2017.2

Costa, J., Domett Siqueira, L., Mazeraud, A., Pereira Azevedo, E., Foguel, D., Caire Castro-Faria-Neto, H., Sharshar, T., Chrétien, F., Augusto Bozza, F., 2018. Age-related cognitive impairment is associated with long-term neuroinflammation and oxidative stress in a mouse model of episodic systemic inflammation. J. Neuroinflammation 15, 28. https://doi.org/10.1186/s12974-018-1059-y

Deng, M., Chen, S.R., Pan, H.L., 2019. Presynaptic NMDA receptors control nociceptive transmission at the spinal cord level in neuropathic pain. Cell. Mol. Life Sci. 76, 1889-1899. https://doi.org/10.1007/s00018-019-03047-y

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch Biochem 82:70–77. https://doi.org/10.1016/0003-9861(59)90090-6

Fane, M., Weeraratna, A.T., 2020. How the ageing microenvironment influences tumour progression. Nat. Rev. Cancer. 20, 89-106. https://doi.org/10.1038/s41568-019-0222-9

Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D.M., Piñeros, M., Znaor, A., Bray, F., 2019. Estimating the global cancer incidence and mortality in

2018: GLOBOCAN sources and methods. Int. J. Cancer. 144, 1941-1953. https://doi.org/10.1002/ijc.31937

Ferrier, J., Bayet-Robert, M., Dalmann, R., Guerrab, A. El, Aissouni, Y., Graveron-Demilly, D., Chalus, M., Pinguet, J., Eschalier, A., Richard, D., Daulhac, L., Marchand, F., Balayssac, D., 2015. Cholinergic neurotransmission in the posterior insular cortex is altered in preclinical models of neuropathic pain: Key role of muscarinic M2 receptors in donepezil-induced antinociception. J. Neurosci. 35, 16418–16430. https://doi.org/10.1523/JNEUROSCI.1537-15.2015

Fiore, N.T., Austin, P.J., 2018. Glial-cytokine-neuronal Adaptations in the Ventral Hippocampus of Rats with Affective Behavioral Changes Following Peripheral Nerve Injury. Neuroscience 390, 119–140. https://doi.org/10.1016/j.neuroscience.2018.08.010

Fraga, C.G., 2005. Relevance, essentiality and toxicity of trace elements in human health. Mol. Asp. Med. 26, 235-244. https://doi.org/10.1016/j.mam.2005.07.013

Fukuchi, M., Izumi, H., Mori, H., Kiyama, M., Otsuka, S., Maki, S., Maehata, Y., Tabuchi, A., Tsuda, M., 2017. Visualizing changes in brain-derived neurotrophic factor (BDNF) expression using bioluminescence imaging in living mice. Sci Rep. 7, 4949. https://doi.org/10.1038/s41598-017-05297-x

Ge, Y., Jiao, Y., Li, P., Xiang, Z., Li, Z., Wang, L., Li, W., Gao, H., Shao, J., Wen, D., Yu, W., 2018. Coregulation of endoplasmic reticulum stress and oxidative stress in neuropathic pain and disinhibition of the spinal nociceptive circuitry, Pain. 159, 894-906. https://doi.org/10.1097/j.pain.0000000000001161

Girolimetti, G., Guerra, F., Iommarini, L., Kurelac, I., Vergara, D., Vidone, M., Amato, L.B., Leone, G., Dusi, S., Tiranti, V., Perrone, A.M., Bucci, C., Porcelli, A.M., Gasparre, G., 2017. Platinum-induced mitochondrial DNA mutations confer lower

sensitivity to paclitaxel by impairing tubulin cytoskeletal organization. Hum. Mol. Genet. 26, 2961-2974. https://doi.org/10.1093/hmg/ddx186

Griess, B., Tom, E., Domann, F., Teoh-Fitzgerald, M., 2017. Extracellular superoxide dismutase and its role in cancer. Free Radic. Biol. Med. 112, 464-479. https://doi.org/10.1016/j.freeradbiomed.2017.08.013

Huang, J., Gadotti, V.M., Chen, L., Souza, I.A., Huang, S., Wang, D., Ramakrishnan, C., Deisseroth, K., Zhang, Z., Zamponi, G.W., 2019. A neuronal circuit for activating descending modulation of neuropathic pain. Nat. Neurosci. 22, 1659–1668. https://doi.org/10.1038/s41593-019-0481-5

Kabir, M.T., Uddin, M.S., Zaman, S., Begum, Y., Ashraf, G.M., Bin-Jumah, M.N., Bungau, S.G., Mousa, S.A., Abdel-Daim, M.M., 2020. Molecular Mechanisms of Metal Toxicity in the Pathogenesis of Alzheimer's Disease. Mol. Neurobiol. 58, 1-20. https://doi.org/10.1007/s12035-020-02096-w

Kasai, S., Yamazaki, H., Tanji, K., Engler, M.J., Matsumiya, T., Itoh, K., 2019. Role of the ISRATF4 pathway and its cross talk with Nrf2 in mitochondrial quality control. J. Clin. Biochem. Nutr. 64, 1–12. https://doi.org/10.3164/jcbn.1837

Kaur, D., Rasane, P., Singh, J., Kaur, S., Kumar, V., Mahato, D.K., Dey, A., Dhawan, K., Kumar, S., 2029. Nutritional interventions for elderly and considerations for the development of geriatric foods. Curr. Aging Sci. 12, 15-27. https://doi.org/10.2174/1874609812666190521110548

Khasabova, I.A., Khasabov, S.G., Olson, J.K., Uhelski, M.L., Kim, A.H., Albino-Ramírez, A.M., Wagner, C.L., Seybold, V.S., Simone, D.A., 2019. Pioglitazone, a PPARγ agonist, reduces cisplatin-evoked neuropathic pain by protecting against oxidative stress. Pain 160, 688–701. https://doi.org/10.1097/j.pain.0000000000001448

Koekkoek, W.A.C., Van Zanten, A.R.H., 2016. Antioxidant Vitamins and Trace Elements in Critical Illness. Nutr. Clin. Pract. 31, 457–474. https://doi.org/10.1177/0884533616653832

Korovila, I., Hugo, M., Castro, J.P., Weber, D., Höhn, A., 2017. Proteostasis , oxidative stress and aging. Redox Biol. 13, 550–567. https://doi.org/10.1016/j.redox.2017.07.008

Leyssens, L., Vinck, B., Van Der Straeten, C., Wuyts, F., Maes, L., 2017. Cobalt toxicity in humans—A review of the potential sources and systemic health effects. Toxicology 387, 43–56. https://doi.org/10.1016/j.tox.2017.05.015

Li, N., Alam, J., Venkatesan, M.I., Eiguren-Fernandez, A., Schmitz, D., Di Stefano, E., Slaughter, N., Killeen, E., Wang, X., Huang, A., Wang, M., Miguel, A.H., Cho, A., Sioutas, C., Nel, A.E., 2004. Nrf2 Is a Key Transcription Factor That Regulates Antioxidant Defense in Macrophages and Epithelial Cells: Protecting against the Proinflammatory and Oxidizing Effects of Diesel Exhaust Chemicals. J. Immunol. 173, 3467–3481. https://doi.org/10.4049/jimmunol.173.5.3467

Li, Q., Liu, S., Zhu, X., Mi, W., Maoying, Q., Wang, J., Yu, J., Wang, Y., 2019. Hippocampal PKR/NLRP1 Inflammasome Pathway Is Required for the Depression-Like Behaviors in Rats with Neuropathic Pain. Neuroscience 412, 16–28. https://doi.org/10.1016/j.neuroscience.2019.05.025

Li, X., Su, L., Zhang, X., Zhang, C., Wang, L., Li, Y., Zhang, Y., He, T., Zhu, X., Cui, L., 2017. Ulinastatin downregulates TLR4 and NF-kB expression and protects mouse brains against ischemia/reperfusion injury. Neurol. Res. 39, 367–373. https://doi.org/10.1080/01616412.2017.1286541

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging,

and diseases. Clin. Interv. Aging 13, 757–772. https://doi.org/10.2147/CIA.S158513

Lima Giacobbo, B., Doorduin, J., Klein, H.C., Dierckx, R.A.J.O., Bromberg, E., de Vries, E.F.J., 2019. Brain-Derived Neurotrophic Factor in Brain Disorders: Focus on Neuroinflammation. Mol. Neurobiol. 56, 3295–3312. https://doi.org/10.1007/s12035-018-1283-6

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262

Loetchutinat, C., Kothan, S., Dechsupa, S., Meesungnoen, J., Jay-Gerin, J.P., Mankhetkorn, S., 2005. Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′,7′-dichlorofluorescein diacetate assay. Radiat. Phys. Chem. 72, 323–331. https://doi.org/10.1016/j.radphyschem.2004.06.011

Mehta, S.K., Gowder, S.J.T., 2015. Members of Antioxidant Machinery and Their Functions. Basic Princ. Clin. Significance Oxidative Stress 59-85. https://doi.org/10.5772/61884

Mesclon, F., Lambert-Langlais, S., Carraro, V., Parry, L., Hainault, I., Jousse, C., Maurin, A.C., Bruhat, A., Fafournoux, P., Averous, J., 2017. Decreased ATF4 expression as a mechanism of acquired resistance to long-term amino acid limitation in cancer cells. Oncotarget 8, 27440–27453. https://doi.org/10.18632/oncotarget.15828

Miquel, S., Champ, C., Day, J., Aarts, E., Bahr, B. A., Bakker, M., Bánáti, D., Calabrese, V., Cederholm, T., Cryan, J., Dye, L., Farrimond, J.A., Korosi, A., Layé, S., Maudsley, S., Milenkovic, D., Mohajeri, M.H., Sijben, J., Solomon, A.,

Spencer, J.P.E., Thuret, S., Berghe, W.V., Vauzour, D., Vellas, B., Wesnes, K., Willatts, P., Wittenberg, R., Geurts, L., 2018. Poor cognitive ageing: Vulnerabilities, mechanisms and the impact of nutritional interventions. Ageing Res. Rev. https://doi.org/42, 40-55. 10.1016/j.arr.2017.12.004

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3175. http://www.jbc.org/content/247/10/3170

Muller, A.L.H., Oliveira, J.S.S., Mello, P.A., Muller, E.I., Flores, E.M.M., 2015. Study and determination of elemental impurities by ICP-MS in active pharmaceutical ingredients using single reaction chamber digestion in compliance with USP requirements. Talanta 136, 161–169. https://doi.org/10.1016/j.talanta.2014.12.023

Nguyen, L.D., Ehrlich, B.E., 2020. Cellular mechanisms and treatments for chemobrain: insight from aging and neurodegenerative diseases. 12, EMBO Mol. Med e12075 https://doi.org/10.15252/emmm.202012075

Nijs, J., Meeus, M., Versijpt, J., Moens, M., Bos, I., Knaepen, K., Meeusen, R., 2015. Brain-derived neurotrophic factor as a driving force behind neuroplasticity in neuropathic and central sensitization pain: A new therapeutic target? Expert Opin. Ther. Targets 19, 565-576. https://doi.org/10.1517/14728222.2014.994506

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167. https://doi.org/10.1016/0165-0270(85)90031-7

Pilleron, S., Soto-Perez-de-Celis, E., Vignat, J., Ferlay, J., Soerjomataram, I., Bray, F., Sarfati, D., 2020. Estimated global cancer incidence in the oldest adults in 2018 and projections to 2050. Int. J. Cancer ijc.33232. https://doi.org/10.1002/ijc.33232

Pradhan, J., Noakes, P.G., Bellingham, M.C., 2019. The Role of Altered BDNF/TrkB

Signaling in Amyotrophic Lateral Sclerosis. Front. Cell. Neurosci. 13, 1–16. https://doi.org/10.3389/fncel.2019.00368

Reis, A.S., Paltian, J.J., Domingues, W.B., Costa, G.P., Alves, D., Giongo, J.L., Campos, V.F., Luchese, C., Wilhelm, E.A., 2020a. Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity: Correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline. Brain Res. Bull. 162, 282–290. https://doi.org/10.1016/j.brainresbull.2020.06.021

Reis, A.S., Paltian, J.J., Domingues, W.B., Novo, D.L.R., Costa, G.P., Alves, D., Campos, V.F., Mesko, M.F., Luchese, C., Wilhelm, E.A., 2020b. Advances in the Understanding of Oxaliplatin-Induced Peripheral Neuropathy in Mice: 7-Chloro-4-(Phenylselanyl) Quinoline as a Promising Therapeutic Agent. Mol. Neurobiol. 57, 5219–5234. https://doi.org/10.1007/s12035-020-02048-4

Resano, M., Aramendía, M., Nakadi, F.V., García-Ruiz, E., Alvarez-Llamas, C., Bordel, N., Pisonero, J., Bolea-Fernández, E., Liu, T., Vanhaeckee, F., 2020. Breaking the boundaries in spectrometry. Molecular analysis with atomic spectrometric techniques. Trends Anal. Chem, 129, 115955. https://doi.org/10.1016/j.trac.2020.115955

Richardson, B.D., Sottile, S.Y., Caspary, D.M., 2020. Mechanisms of GABAergic and cholinergic neurotransmission in auditory thalamus: Impact of aging. Hear. Res. 108003. https://doi.org/10.1016/j.heares.2020.108003

Shankar, D.B., Cheng, J.C., Kinjo, K., Federman, N., Moore, T.B., Gill, A., Rao, N.P., Landaw, E.M., Sakamoto, K.M., 2005. The role of CREB as a proto-oncogene in hematopoiesis and in acute myeloid leukemia. Cancer Cell 7, 351–362. https://doi.org/10.1016/j.ccr.2005.02.018

Silva-Palacios, A., Ostolga-Chavarría, M., Zazueta, C., Königsberg, M., 2018. Nrf2: Molecular and epigenetic regulation during aging. Ageing Res. Rev. 47, 31–40. https://doi.org/10.1016/j.arr.2018.06.003

Silverman, H.A., Dancho, M., Regnier-Golanov, A., Nasim, M., Ochani, M., Olofsson, P.S., Ahmed, M., Miller, E.J., Chavan, S.S., Golanov, E., Metz, C.N., Tracey, K.J., Pavlov, V.A., 2014. Brain region-specific alterations in the gene expression of cytokines, immune cell markers and cholinergic system components during peripheral endotoxin-induced inflammation. Mol. Med. 20, 601–611. https://doi.org/10.2119/molmed.2014.00147

Sivandzade, F., Prasad, S., Bhalerao, A., Cucullo, L., 2019. NRF2 and NF-κB interplay in cerebrovascular and neurodegenerative disorders: molecular mechanisms and possible therapeutic approaches. Redox biol. 21, 101059. https://doi.org/10.1016/j.redox.2018.11.017

Stangherlin, E.C., Rocha, J.B.T., Nogueira, C.W., 2009. Diphenyl ditelluride impairs short-term memory and alters neurochemical parameters in young rats. Pharmacol. Biochem. Behav. 91, 430–435. https://doi.org/10.1016/j.pbb.2008.08.020

Turabelidze, A., Guo, S., Dipietro, L.A., 2010. Importance of housekeeping gene selection for accurate reverse transcription-quantitative polymerase chain reaction in a wound healing model. Wound Repair Regen. 18, 460–466. https://doi.org/10.1111/j.1524-475X.2010.00611.x

van Dronkelaar, C., van Velzen, A., Abdelrazek, M., van der Steen, A., Weijs, P.J.M., Tieland, M., 2018. Minerals and Sarcopenia; The Role of Calcium, Iron, Magnesium, Phosphorus, Potassium, Selenium, Sodium, and Zinc on Muscle Mass, Muscle Strength, and Physical Performance in Older Adults: A Systematic Review. J. Am. Med. Dir. Assoc. 19, 6-11.e3.

https://doi.org/10.1016/j.jamda.2017.05.026

Xia, S., Zhang, X., Zheng, S., Khanabdali, R., Kalionis, B., Wu, J., Wan, W., Tai, X., 2016. An update on inflamm-aging: mechanisms, prevention, and treatment. J Immunol Res. 2016. https://doi.org/10.1155/2016/8426874

Walsh, R.N., Cummins, R.A., 1976. The Open-Field Test : A Critical Review 83, 482–504. https://doi.org/10.1037/0033-2909.83.3.482

Wang, V.C., Mullally, W.J., 2020. Pain Neurology. Am. J. Med. 133, 273-280. https://doi.org/10.1016/j.amjmed.2019.07.029

Wang, Y., Wang, L., Wen, X., Hao, D., Zhang, N., He, G., Jiang, X., 2019. NF-κB signaling in skin aging. Mech. Ageing Dev. 184, 111160. https://doi.org/10.1016/j.mad.2019.111160

Watkins, B.A., 2018. Endocannabinoids, exercise, pain, and a path to health with aging. Mol. Aspects Med. 64, 68–78. https://doi.org/10.1016/j.mam.2018.10.001

Wendel, A., 1981. Glutathione peroxidase. Methods in enzymol 77, 325–333. https://doi.org/10.1016/S0076-6879(81)77046-0

Widerström-Noga, E., 2017. Neuropathic Pain and Spinal Cord Injury: Phenotypes and Pharmacological Management. Drugs 77, 967-984. https://doi.org/10.1007/s40265-017-0747-8

Yang, Y., Luo, L., Cai, X., Fang, Y., Wang, J., Chen, G., Yang, J., Zhou, Q., Sun, X., Cheng, X., Yan, H., Lu, W., Hu, C., Cao, P., 2018. Nrf2 inhibits oxaliplatin-induced peripheral neuropathy via protection of mitochondrial function. Free Radic. Biol. Med. 120, 13–24. https://doi.org/10.1016/j.freeradbiomed.2018.03.007

Yin, R., Zhao, S., Qiu, C., 2020. Brain-derived neurotrophic factor fused with a collagen-binding domain inhibits neuroinflammation and promotes neurological

recovery of traumatic brain injury mice via TrkB signalling. J. Pharm. Pharmacol. 72, 539–550. https://doi.org/10.1111/jphp.13233

Zhang, H., Davies, K.J.A., Forman, H.J., 2015. Oxidative stress response and Nrf2 signaling in aging. Free Radic. Biol. Med 88, 314-336. https://doi.org/10.1016/j.freeradbiomed.2015.05.036

Zhang, R., Miao, Q.-W., Zhu, C.-X., Zhao, Y., Liu, L., Yang, J., An, L., 2015. Sulforaphane Ameliorates Neurobehavioral Deficits and Protects the Brain From Amyloid b Deposits and Peroxidation in Mice With Alzheimer-Like Lesions. Am. J. Alzheimer's Dis. 30, 183-191. https://doi.org/10.1177/1533317514542645

Żwierełło, W., Styburski, D., Maruszewska, A., Piorun, K., Skórka-Majewicz, M., Czerwińska, M., Maciejewska, D., Baranowska-Bosiacka, I., Krajewski, A., Gutowska, I., 2020. Bioelements in the treatment of burn injuries – The complex review of metabolism and supplementation (copper, selenium, zinc, iron, manganese, chromium and magnesium). J. Trace Elem. Med. Biol. 62. https://doi.org/10.1016/j.jtemb.2020.126616

Captions of figures

Fig. 1 Schedule of experimental protocol.

Fig. 2 Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the (A) paw withdrawal threshold to mechanical stimulus in the von Frey test and (B) on the latency

to thermal stimulus in the hot plate test. Each point represents the mean of 7 mice in each group. (*) P < 0.05, (***) P < 0.001, and (****) P < 0.0001 denote significance levels when compared with the Young group; ($^{\#}$) P < 0.05, ($^{\#\#\#}$) P < 0.001, and ($^{\#\#\#\#}$) P < 0.0001 denote significance levels when compared with the Old group; ($^{+}$) P < 0.05, and ($^{++++}$) P < 0.0001 denote significance levels when compared with the Old + OXA group (Two-way ANOVA followed by the Tukey's test).

Fig. 3 Effect of aging (Old) and oxaliplatin (OXA) (10 mg/kg, i.p.) on the (A) percentage of entries in the open arms and (B) percentage of time spent in the open arms in the elevated plus-maze; (C) percentage of exploratory preference - short-term and (D) percentage of exploratory preference - long-term memories in the object recognition task; (E) number of crossings and (F) number of rearings in the open field test. Each column represents the mean ± S.E.M. of 7 mice in each group. (*) P < 0.05, (**) P < 0.01, (***) P < 0.001, and (****) P < 0.0001 denote significance levels when compared with the Young group; ($^{\#}$) P < 0.05, ($^{\#\#\#}$) P < 0.001, and ($^{\#\#\#\#}$) P < 0.0001 denote significance levels when compared with the Old group; ($^{+}$) P < 0.05 denotes significance levels when compared with the Old + OXA group (Two-way ANOVA followed by the Tukey's test).

Fig. 4 Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the deposition of platinum in the spinal cord and brain of mice. Each column represents the mean ± S.E.M. of 5 mice in each group. (****) P < 0.0001 denotes significance levels when compared with the Young group; ($^{\#\#\#\#}$) P < 0.0001 denotes significance levels when compared with the Old group; ($^{+}$) P < 0.05 denotes significance levels when compared

with the Old + OXA group and (≤ LOD) denotes below detection limit (Two-way ANOVA followed by the Tukey's test). Limit of detection: 0.1 ng g^{-1}.

Fig. 5 Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the (A) reactive species levels, (B) superoxide dismutase activity, (C) catalase activity, and (D) glutathione peroxidase activity in the cerebral cortex of mice. Each column represents the mean ± S.E.M. of 7 mice in each group. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, and (****) $P < 0.0001$ denotes significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$, and ($^{\#\#\#\#}$) $P < 0.0001$ denotes significance levels when compared with the Old group; ($^{+}$) $P < 0.05$, ($^{++}$) $P < 0.01$, and ($^{++++}$) $P < 0.0001$ denotes significance levels when compared with the Old + OXA group (Two-way ANOVA followed by the Tukey's test).

Fig. 6 Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the superoxide dismutase expression levels in the spinal cord (A), hippocampus (B) and cerebral cortex (C); catalase expression levels in the spinal cord (D), hippocampus (E) and cerebral cortex (F); and glutathione peroxidase expression levels in the spinal cord (G), hippocampus (H) and cerebral cortex (I) of mice. Each column represents the mean ± S.E.M. of 6 - 7 mice in each group. (*) $P < 0.05$, (**) $P < 0.01$, and (****) $P < 0.0001$ denotes significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$, ($^{\#\#\#}$) $P < 0.001$, and ($^{\#\#\#\#}$) $P < 0.0001$ denotes significance levels when compared with the Old group; ($^{+}$) $P < 0.05$, and ($^{++}$) $P < 0.01$ denotes significance levels when compared with the Old + OXA group (Two-way ANOVA followed by the Tukey's test).

Fig. 7 Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the Nrf2 expression levels in the spinal cord (A), hippocampus (B) and cerebral cortex (C); PI3K expression levels in the spinal cord (D), hippocampus (E) and cerebral cortex (F); and ATF4 expression levels in the spinal cord (G), hippocampus (H) and cerebral cortex (I) of mice. Each column represents the mean ± S.E.M. of 6 mice in each group. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, and (****) $P < 0.0001$ denotes significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$, ($^{\#\#\#}$) $P < 0.001$, and ($^{\#\#\#\#}$) $P < 0.0001$ denotes significance levels when compared with the Old group (Two-way ANOVA followed by the Tukey's test).

Fig. 8 Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the NF-κB expression levels in the hippocampus (A) and cerebral cortex (B); BDNF expression levels in the hippocampus (C) and cerebral cortex (D); and CREB expression levels in the hippocampus (E) and cerebral cortex (F) of mice. Each column represents the mean ± S.E.M. of 6 - 7 mice in each group. (*) $P < 0.05$, (**) $P < 0.01$, and (****) $P < 0.0001$ denotes significance levels when compared with the Young group; ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#}$) $P < 0.001$ denotes significance levels when compared with the Old group; ($^{++}$) $P < 0.01$ denotes significance levels when compared with the Old + OXA group (Two-way ANOVA followed by the Tukey's test).

Fig. 9 Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on the AChE expression levels in the (A) hippocampus and (B) cerebral cortex of mice. Inserts show the effect of aging (Old) and OXA (10 mg kg^{-1}, i.p.) on the AChE activity. Each column represents the mean ± S.E.M. of 7 mice in each group. (***) $P < 0.01$ and (****) $P < 0.0001$ denotes significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$

and ($^{\#\#\#\#}$) P < 0.0001 denotes significance levels when compared with the Old group (Two-way ANOVA followed by the Tukey's test).

Fig. 10 Mechanisms involved in the exacerbation of symptoms OXA-induced peripheral neuropathy aging-caused.

Tables

Table 1. Instrumental settings for the Agilent 8800 ICP-QQQ instrument.

Conditions in ICP-MS/MS			
Reaction gas	NH_3	NH_3	O_2
Scan type	MS/MS	MS/MS	MS/MS
Plasma mode	Low matrix	Cold plasma	Low matrix
RF power (W)	1550	600	1550
Extract 1 (V)			
Q1 bias (V)	-1 V	-1 V	-1 V
Reaction gas flow rate setting (mL min^{-1})			0.30
Q1→Q2	Na: 23→40	K: 39→39	S: 31→47
	Mg: 24→75	Ga (IS): 71→71	S: 32→48
	Mg: 25→76	Y (IS): 89→89	S: 34→50
	Ca: 40→57		Se: 80→96
	Mn: 55→89		Ga (IS): 71→71
	Fe: 56→90		Y (IS): 89→89
	Fe: 57→91		
	Co: 59→93		
	Cu: 63→97		
	Zn: 64→98		
	Cu: 65→99		
	Zn: 66→100		
	Pt: 194→245		
	Pt: 195→246		
	Ga (IS): 71→71		
	Y (IS): 89→89		
Octopole bias (V)	−5	−5	−5
Energy discrimination (V)	−8	−8	−8
Extract 2 (V)			
Q2 QP bias (V)	−13	−13	−13
Wait time offset (ms)	2	2	2
Sweeps / replicate	100	100	100
Integration time / mass (s)	1	1	1
Replicates	10	10	10
Total analysis time/sample (s)			

Table 2. Primers used for quantitative real-time polymerase chain reaction. Listed are the forward and reverse primer sequences used to amplify each target gene as well as the GAPDH endogenous control.

Primer Name	Sequence	Reference
CAT Forward	5'AGAGAGCGGATTCCTGAGAGA3'	
CAT Reverse	5'ACCTTTCCCTTGGAGTATCTG3'	d'Avila et al., 2018
SOD Forward	5'GGACCTCATTTTAATCCTCAC3'	
SOD Reverse	5'TGCCCAGGTCTCCAACATG3'	
GPx Forward	5'TGTGGAAATGGATGAAAGTCCAG3'	Zhang et al., 2015
GPx Reverse	5'CATGGGACCATAGCGCTTCAC3'	
Nrf2 Forward	5'CTCGCTGGAAAAAGAAGTG3'	Li et al., 2004
Nrf2 Reverse	5' CCGTCCAGGAGTTCAGAGG3'	
PI3K Forward	5' CTCTCCTGTGCTGGCTACTGT3'	Bellaver et al., 2016
PI3K Reverse	5'GCTCTCGGTTGATTCCAAACT3'	
ATF4 Forward	5' TCGATGCTCTGTTTCGAATG3'	Mesclon et al., 2017
ATF4 Reverse	5' AGAATGTAAAGGGGGCAACC3'	
BDNF Forward	5' AAGGACGCGGACTTGTACAC3'	Fukuchi et al., 2017
BDNF Reverse	5' CGCTAATACTGTCACACACGC3'	
CREB Forward	5'AAGCTGAAAGTCAACAAATGACAGTT3'	Shankar et al., 2005
CREB Reverse	5' TGGACTGTCTGCCCATTGG3'	
NF-κB Forward	5' AGAGAAGCACAGATACCACTAAG3'	Li et al., 2017
NF-κB Reverse	5' CAGCCTCATAGAAGCCATCC3'	
AChE Forward	5'TTAGGGCTGGGATATAATACGAC3'	Silverman et al., 2014
AChE Reverse	5'GCCCCTAGTGGGAGGAAGT3'	
GAPDH Forward	5'TGCGACTTCAACAGCAACTC3'	Turabelidze et al., 2010
GAPDH Reverse	5'ATGTAGGCAATGAGGTCCAC3'	

Table 3. Effect of aging (Old) and oxaliplatin (OXA) (10 mg kg^{-1}, i.p.) on elements concentration.

ELEMENT	CONCENTRATION (NG G^{-1})							
	Spinal cord				Brain			
	Young	*Young + OXA*	*Old*	*Old + OXA*	*Young*	*Young + OXA*	*Old*	*Old + OXA*
POTASSIUM	3062.0±39.7	3883.0±344.5	3586.0±91.3	4426.0±577.7	4049.0±150.5	4112.0±166.0	4585.0±245.7	3471.0±271.4$^{\#}$
PHOSPHORUS	613.0±56.1	556.6±29.98^{+++}	694.8±28.1	969.7±70.9$^{***\#\#}$	689.6±24.1	649.0±18.8$^{\#\#}$	694.2±11.6	531.6±18.3$^{***\ \#\#\#\#}$
SULFUR	291.6±26.6	248.4±5.6^{+++}	321.9±7.3	424.5±37.4$^{**\#}$	421.0±16.2	397.5±13.5	406.3±10.1	368.0±20.9
SELENIUM	94.7±1.8	97.0±2.3^{++}	98.8±1.9	130.4±11.5$^{**\#\#}$	135.8±4.6	128.5±4.7	142.9±2.3	131.2±1.9
SODIUM	1452.0±32.5	1583.0±34.2	2036.0±118.8*	1930.0±204.4	1423.0±68.9	1301.0±48.7	1337.0±40.9	1483.0±156.3
MAGNESIUM	145.8±2.0	154.4±5.2^{++}	154.8±1.4	200.1±15.7$^{**\#\#}$	151.2±6	132.2±5.3	142.2±3.3	140.2±8.8
CALCIUM	50.6±0.9	125.0±29.2*	79.5±6.9	80.3±2.8	59.1±4.4	50.5±2.1	57.5±2.4	61.8±4.7
MANGANESE	419.5±9.7	394.2±10.7$^{\#}$	299.5±10.8**	392.8±32.0$^{\#}$	368.0±8.0	314.8±15.6*	289.1±7.1***	289.9±11.2***
IRON	11.7±0.1	11.8±0.3^{+}	13.2±0.7	14.9±1.1*	18.1±0.8	15.7±0.3	19.0±3.3	20.1±0.6
COBALT	4.7±0.1	3.8±0.3$^{\#\#\#\ ++++}$	8.5±0.2**	10.4±1.1****	4.8±0.4	4.3±0.1$^{\#\#\#\#\ ++++}$	11.8±1.3****	11.3±0.7***
COPPER	1.8±0.1	1.9±0.1^{+}	2.3±0.1	2.9±0.2$^{***\#\#\#}$	2.6±0.1	2.3±0.1$^{\#\#\#\#\ ++++}$	4.2±0.1****	3.5±0.1$^{****\#\#\#}$
ZINC	4.5±0.1	5.2±0.3	5.3±0.5	7.14±0.7**	7.1±0.2	6.6±0.2	7.1±0.2	8.0±0.7

Each column represents the mean ± S.E.M. of 5 mice in each group. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, and (****) $P < 0.0001$ denotes significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$, ($^{\#\#\#}$) $P < 0.001$, and ($^{\#\#\#\#}$) $P < 0.0001$ denotes significance levels when compared with the Old group; ($^{+}$) $P < 0.05$, ($^{++}$) $P < 0.01$, ($^{+++}$) $P < 0.001$, and ($^{++++}$) $P < 0.0001$ denotes significance levels when compared with the Old + OXA group (Two-way ANOVA followed by the Tukey's test).

Figures

Fig. 1. Schedule of experimental protocol

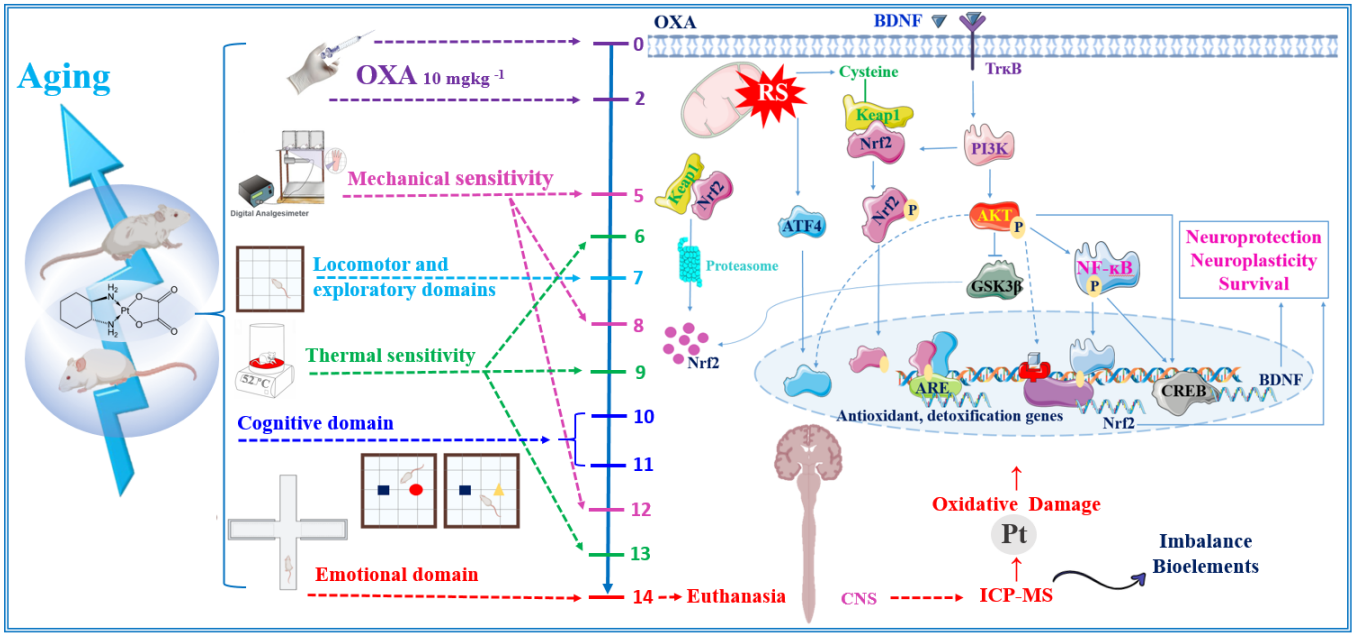
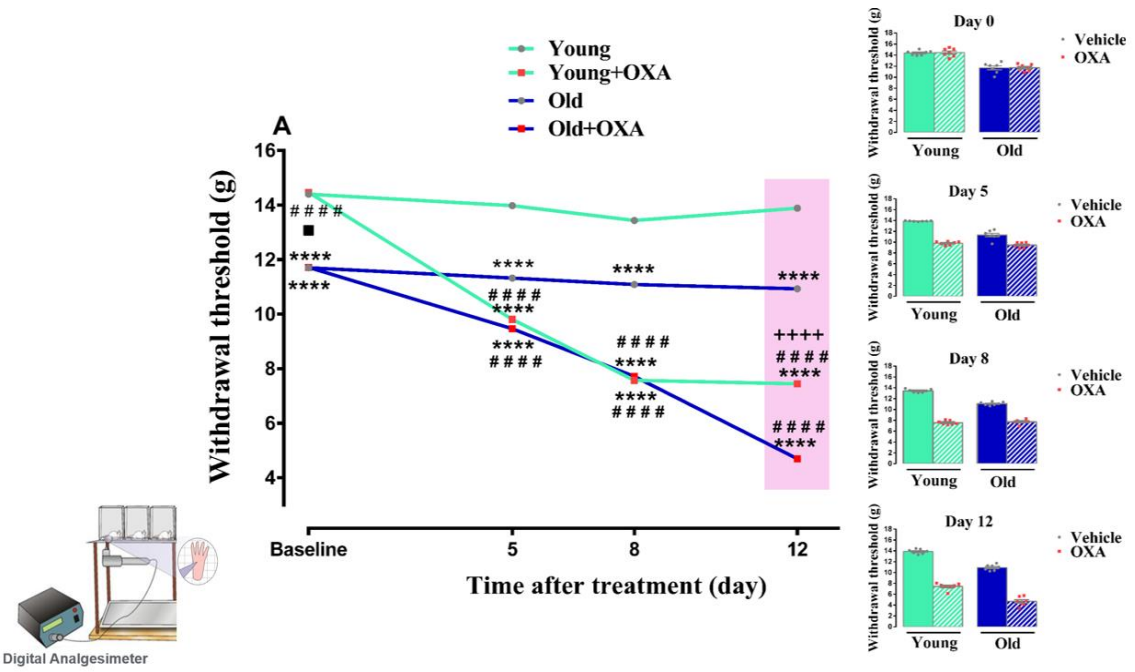


Fig. 2.


A
Young
Young+OXA
Old
Old+OXA
Withdrawal threshold (g)
Baseline
5
8
12
Time after treatment (day)
Digital Analgesimeter
Day 0
Day 5
Day 8
Day 12
Vehicle
OXA
Young
Old

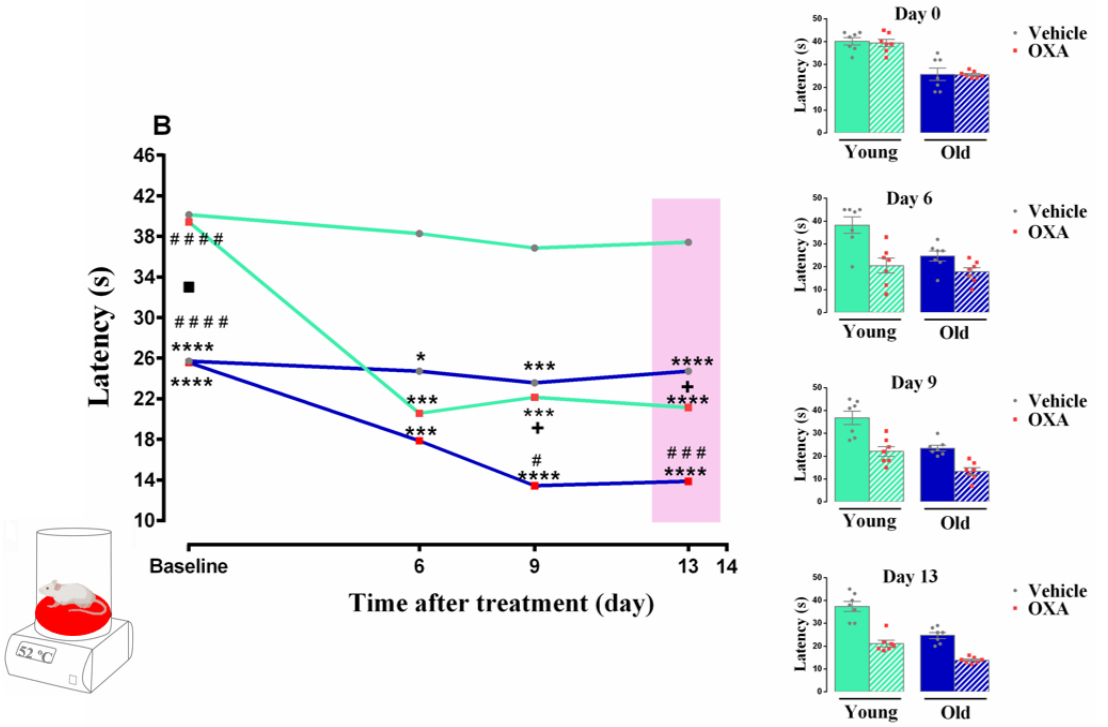

B
Latency (s)
Baseline
6
9
13
14
Time after treatment (day)
52 °C
Day 0
Day 6
Day 9
Day 13
Vehicle
OXA
Young
Old

Fig. 3.

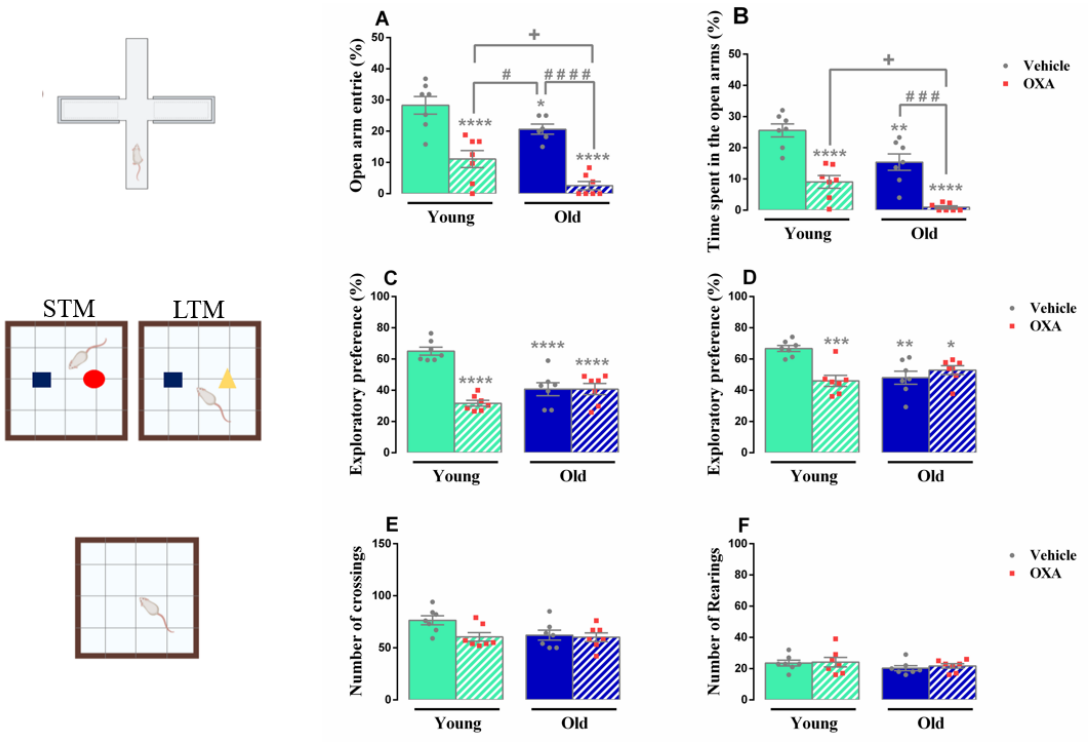

STM
LTM
A
Open arm entrie (%)
Young
Old
B
Time spent in the open arms (%)
Vehicle
OXA
C
Exploratory preference (%)
D
E
Number of crossings
F
Number of Rearings

Fig. 4.

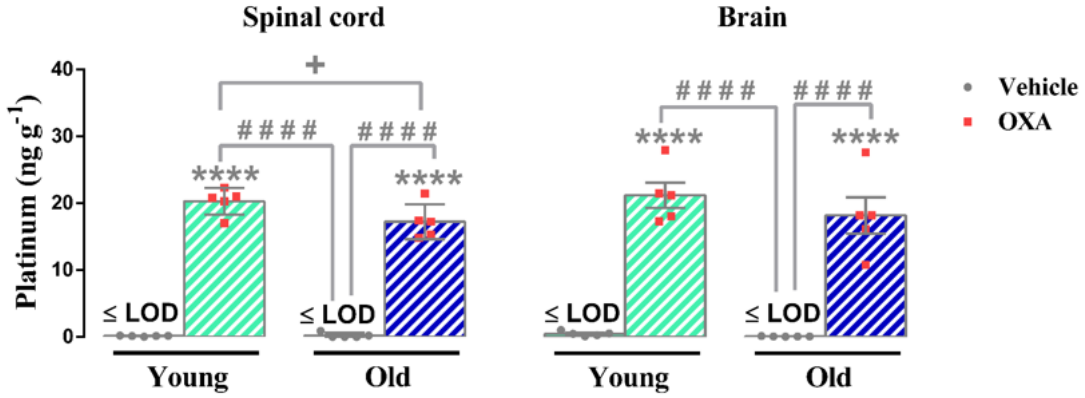

Spinal cord
Brain
Platinum (ng g-1)
≤ LOD
Young
Old
Vehicle
OXA

Fig. 5.

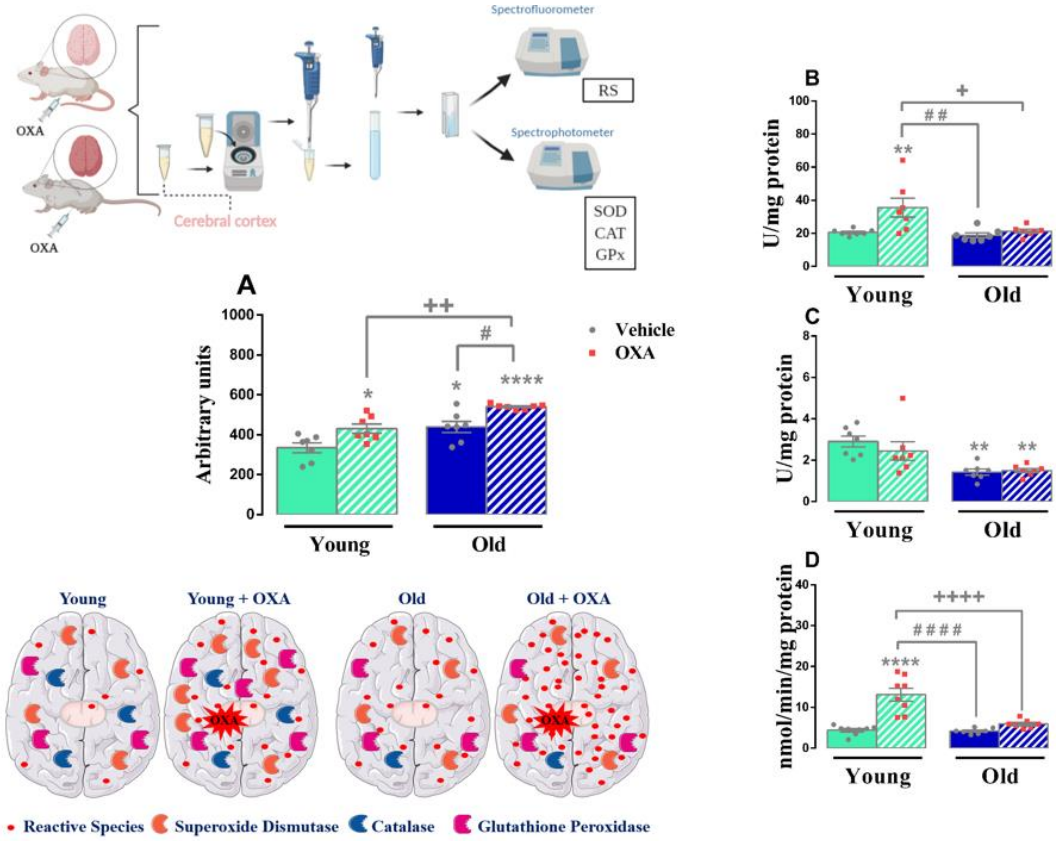

OXA
OXA
Cerebral cortex
Spectrofluorometer
RS
Spectrophotometer
SOD
CAT
GPx
A
Arbitrary units
Vehicle
OXA
Young
Old
B
U/mg protein
C
U/mg protein
D
nmol/min/mg protein
Young
Young + OXA
Old
Old + OXA
Reactive Species
Superoxide Dismutase
Catalase
Glutathione Peroxidase

Fig. 6.

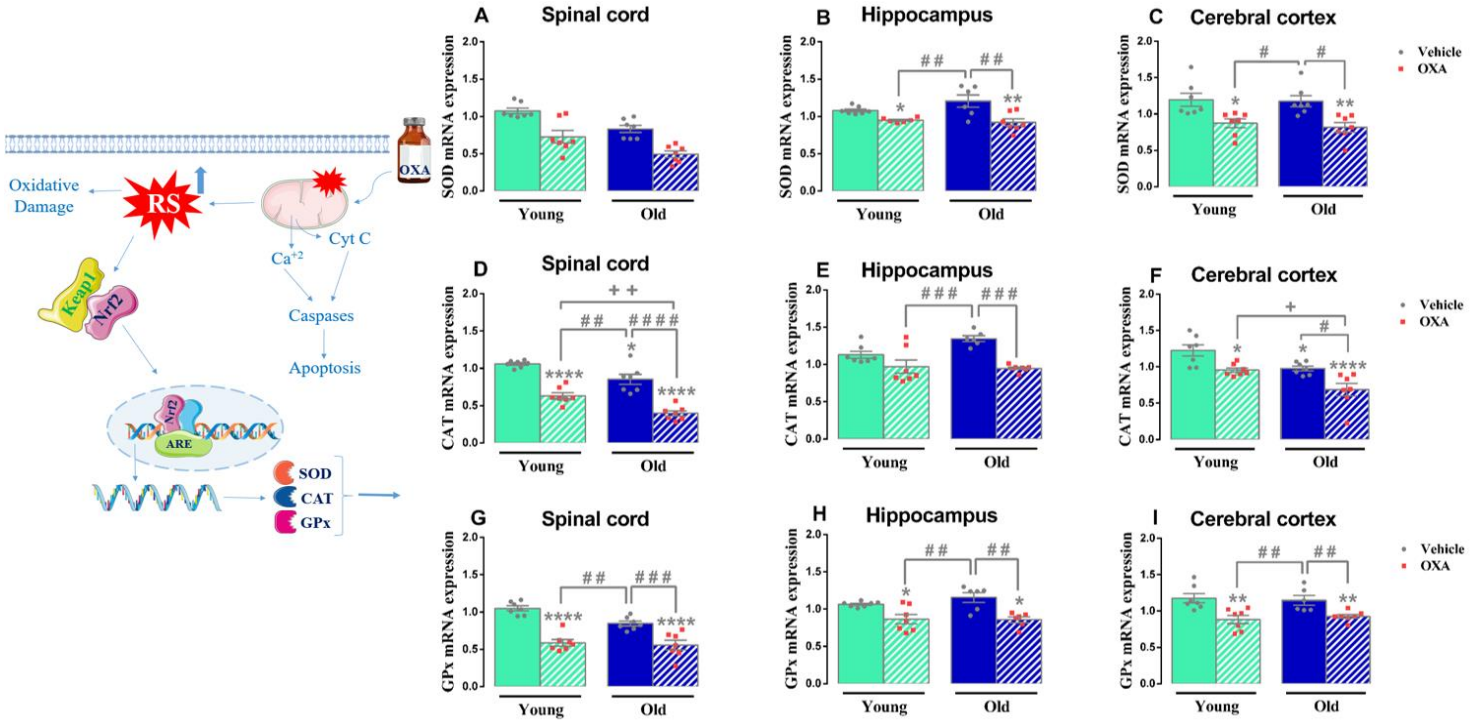

Oxidative Damage
RS
OXA
Cyt C
Ca+2
Keap1
Nrf2
Caspases
Apoptosis
ARE
SOD
CAT
GPx
A Spinal cord
B Hippocampus
C Cerebral cortex
D Spinal cord
E Hippocampus
F Cerebral cortex
G Spinal cord
H Hippocampus
I Cerebral cortex
SOD mRNA expression
CAT mRNA expression
GPx mRNA expression
Young
Old
Vehicle
OXA

Fig. 7

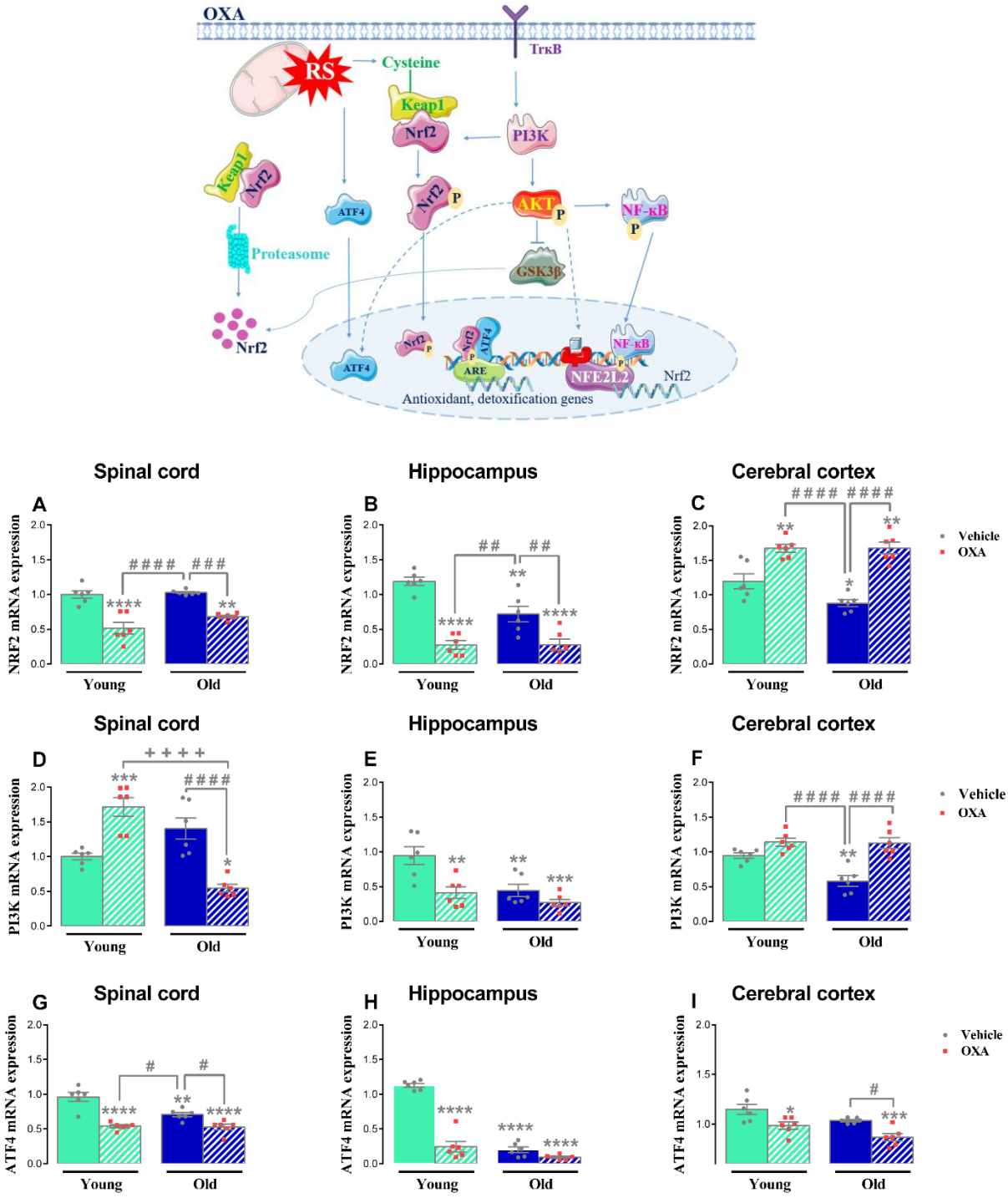

OXA
TrκB
RS
Cysteine
Keap1
Nrf2
PI3K
ATF4
AKT
P
NF-κB
Proteasome
GSK3β
Nrf2
ARE
NFE2L2
Antioxidant, detoxification genes
Spinal cord
Hippocampus
Cerebral cortex
NRF2 mRNA expression
PI3K mRNA expression
ATF4 mRNA expression
Young
Old
Vehicle
OXA

Fig. 8

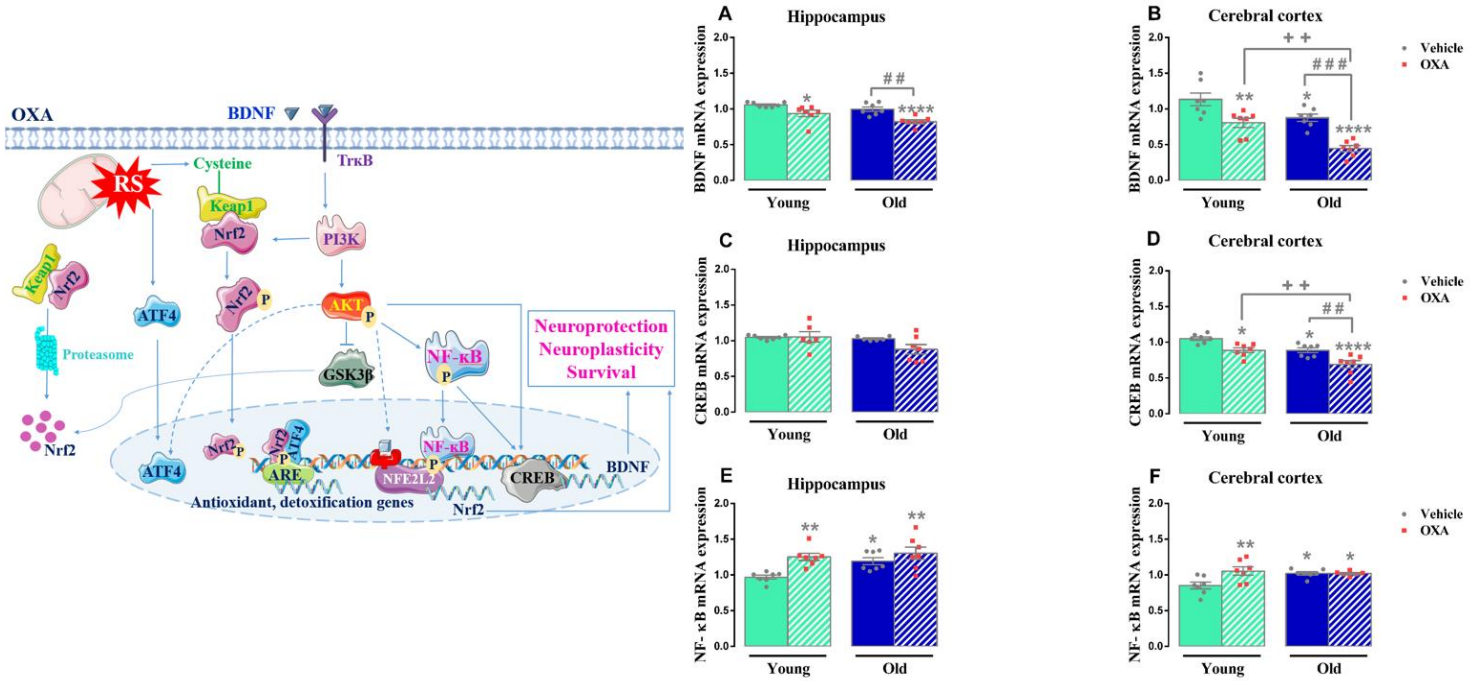

OXA
BDNF
TrκB
RS
Cysteine
Keap1
Nrf2
PI3K
ATF4
AKT
P
Proteasome
GSK3β
NF-κB
Neuroprotection
Neuroplasticity
Survival
ARE
NFE2L2
CREB
BDNF
Antioxidant, detoxification genes
A
Hippocampus
BDNF mRNA expression
B
Cerebral cortex
C
CREB mRNA expression
D
E
NF- κB mRNA expression
F
Young
Old
Vehicle
OXA

Fig. 9

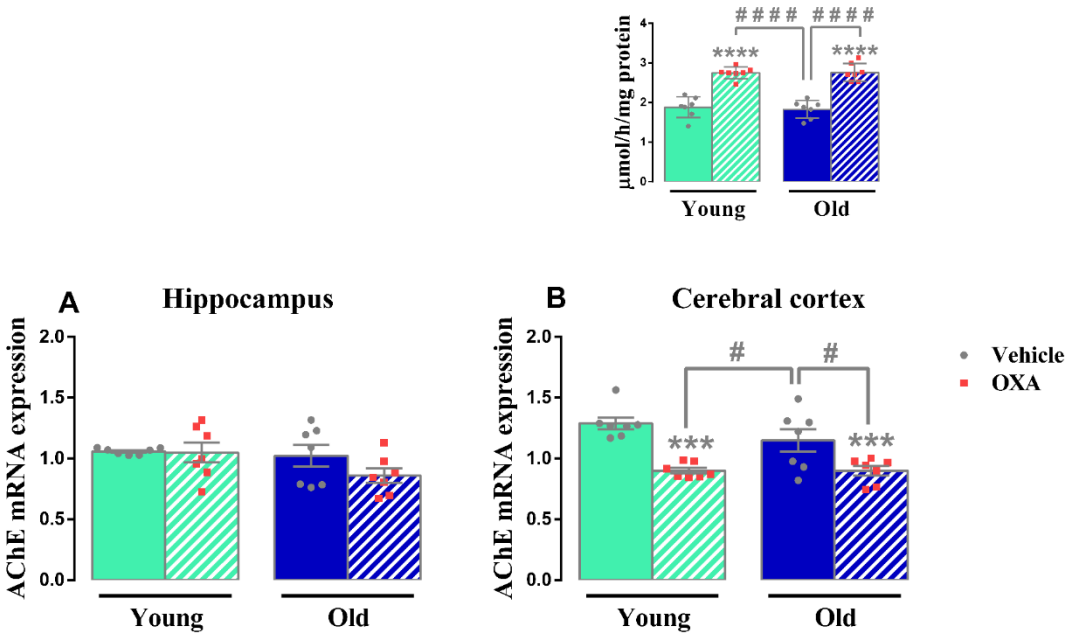
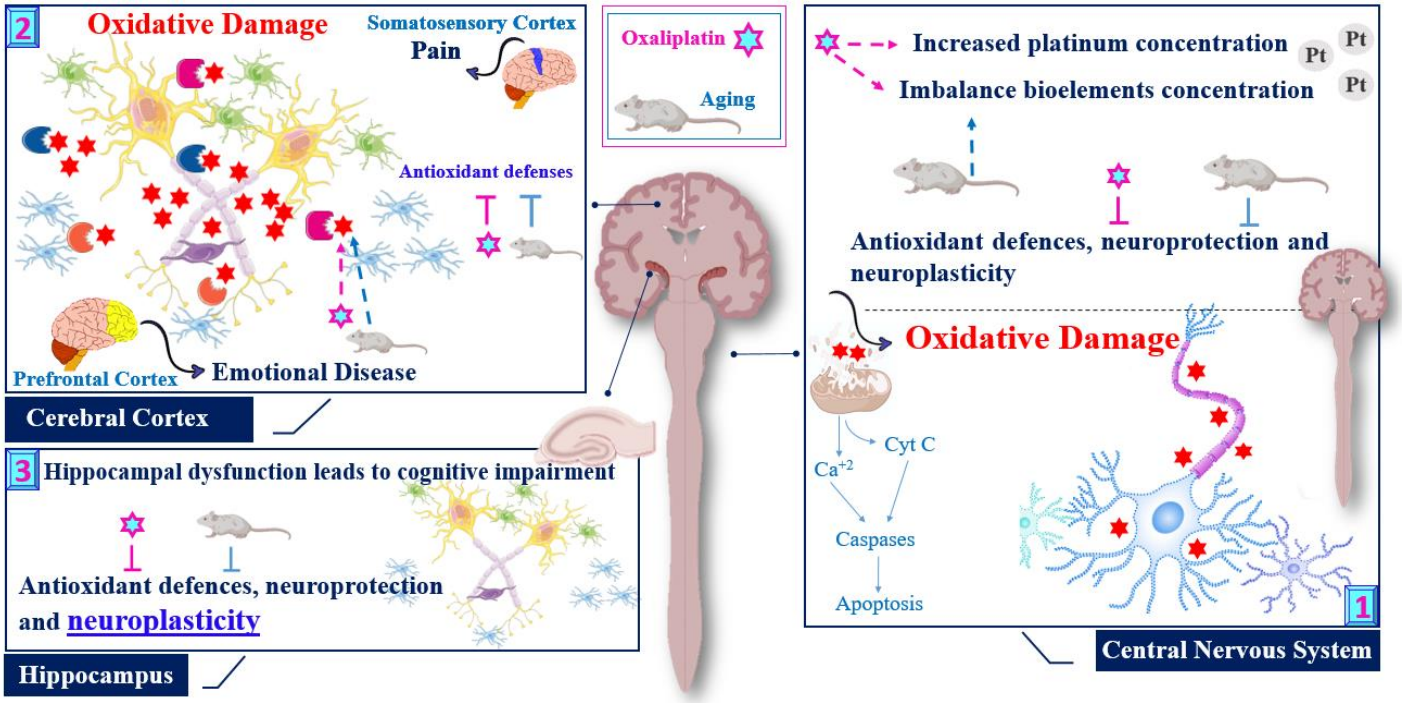

µmol/h/mg protein
Young
Old
A
Hippocampus
AChE mRNA expression
B
Cerebral cortex
Vehicle
OXA

Fig. 10


2
Oxidative Damage
Somatosensory Cortex
Pain
Antioxidant defenses
Prefrontal Cortex
Emotional Disease
Cerebral Cortex
3
Hippocampal dysfunction leads to cognitive impairment
Antioxidant defences, neuroprotection and neuroplasticity
Hippocampus
Oxaliplatin
Aging
Increased platinum concentration
Pt
Pt
Pt
Imbalance bioelements concentration
Antioxidant defences, neuroprotection and neuroplasticity
Oxidative Damage
Cyt C
Ca+2
Caspases
Apoptosis
1
Central Nervous System

Highlights

- Aging exacerbates the OXA-induced peripheral neuropathy.
- Association between aging and OXA enhances anxiety and oxidative damage.
- Spinal cord and brain Pt deposition after OXA-exposed occurs age-independently.
- OXA-induced imbalance in CNS bioelements levels is exacerbated by aging.
- OXA impairs neuroprotection and neuroplasticity pathways in aged mice.

Capítulo 5

4-PSQ reduz sintomas clínicos da NPIO e suas comorbidades independentemente da idade

O processo fisiopatológico da NPIO é multifatorial. Por meio dos resultados obtidos, foi evidenciado que o estresse oxidativo contribui para o desenvolvimento da NPIO, no entanto, nem todos os processos fisiopatológicos envolvidos na sua gênese foram completamente compreendidos e, isso é um problema clínico desafiador.

É importante ressaltar que, a pesquisa desenvolvida nesse estudo, contribuiu de forma significativa para ampliar a compreensão dos mecanismos envolvidos na NPIO. A partir das evidências obtidas aqui, foi definido que a platina, proveniente do metabolismo da oxaliplatina, permeia a barreira hematoencefálica, se acumula no SNC e, causa extenso dano oxidativo nesses tecidos. Anteriormente, os estudos indicavam que os metabólitos da oxaliplatina se acumulavam a causavam danos diretos apenas ao SNP. Além disso, ressaltamos que a oxaliplatina, também, altera a funcionalidade do SNC por meio da inibição da enzima Na^+, K^+ - ATPase. Resumidamente, foi identificado que o aumento do dano oxidativo prejudicou as funções do SNC, como evidenciado pela inibição da Na^+, K^+ - ATPase. Desta forma, uma hipótese é que a administração da oxaliplatina conduziu à uma redução da produção de energia no SNC.

Mesmo diante das modificações biológicas causadas pelo envelhecimento aos tecidos e sistemas orgânicos, este fator tem sido negligenciado nas pesquisas que envolvem a toxicidade induzida pelo tratamento com a oxaliplatina. Nas etapas anteriores deste estudo, foi definido que somente o envelhecimento aumentou a sucetibilidade à dor, através do estresse oxidativo, evidenciado pelo aumento na produção de espécies reativas. Além disso, os processos de neurodegeneração causados pelo envelhecimento foram observados por meio da redução na atividade de enzimas cerebrais.

De fato, quando associado os dois fatores, envelhecimento e tratamento com a oxaliplatina, foi observado um agravamento significativo dos sintomas clínicos da NPIO e de suas comorbidades. Principalmente, o dano oxidativo

excessivo ao SNC foi destacado por meio do aumento das espécies reativas, colapso das defesas antioxidantes, alteração das funções do SNC e, modulação das vias relacionadas à neuproteção (Fator nuclear eritroide 2 relacionado ao fator 2 (Nrf2)) e neuroplasticidade (Fator neurotrófico derivado do cérebro (BDNF)). A concentração de bioelementos, necessários para a manutenção adequada das funções biológicas, foi alterada como observado na etapa 4 do estudo.

Nas etapas anteriores, o potencial farmacológico do 4-PSQ foi investigado. De fato, os promissores efeitos do composto se destacaram ao longo do estudo. O 4-PSQ reverteu a sensibilidade induzida pela oxaliplatina e, também, reduziu as comorbidades associadas. O 4-PSQ é uma molécula multialvo com um significativo potencial antioxidante e neuroprotetor. Embora o 4-PSQ não tenha impedido ou reduzido o acúmulo de platina no SNC, a sua ação antioxidante ficou em evidência nesse estudo e, este composto modulou diferentes mecanismos envolvidos na NPIO, tanto aguda quanto a crônica. O tratamento com o 4-PSQ reduziu os níveis de espécies reativas, modulou positivamente as defesas antioxidantes diante dos danos induzidos pela oxaliplatina e, reestabeceu o funcionamento adequado da enzima Na^+, K^+ - ATPase. De fato, os dados sugerem que o tratamento com o 4-PSQ pode contribuir para o reestabelimento das funções cerebrais, prejudicadas pela administração da oxaliplatina.

Baseado nos fatos descritos, o objetivo da etapa 5 foi avaliar os efeitos do 4-PSQ na NPCIO, considerando as especificidades do envelhecimento. Os resultados obtidos demonstraram que o tratamento com a oxaliplatina exacerbou a dor, o comportamento ansioso, e, também, o comprometimento cognitivo causado pelo envelhecimento em ratos. Corroborando os resultados anteriormente obtidos, o desequilíbrio redox causado pelo envelhecimento também foi elevado pela administração de oxaliplatina. Além disso, a associação dos fatores, alterou o funcionamento adequado do SNC, como demonstrado através da atividade das enzimas Na^+, K^+ - ATPase e AChE.

O tratamento com o 4-PSQ, por sua vez, reverteu o desequibrio oxidativo. De fato, a redução dos danos oxidativos ao SNC pode explicar o

efeito analgésico evidenciado pelo 4-PSQ diante dos danos causados pela oxaliplatina (Figura 9). Considerando o conjunto de evidências coletadas nesse estudo a respeito do potencial farmacológico do 4-PSQ, pode-se sugerir que esta molécula é promissora para o desenvolvimento de uma nova terapia para o tratamento da NPIO e suas comorbidades.

É importante ressaltar que, algumas análises previstas para serem realizadas nessa etapa 5, principalmente, por meio de parceria com outros laboratórios da UFPel e outras universidades brasileiras e do exterior, foram atrasadas devido à pandemia mundial do coronavirus (Covid 19) que acomete o Brasil de forma devastadora. As análises incluem uma busca por microRNA, moléculas que podem ser utilizadas como marcadores genéticos para os danos induzidos pela associação dos fatores; a expressão de marcadores como Nrf2 e BDNF, pela técnica de qRT-PCR; bem como, a quantificação de bioelementos, necessários para a manutenção adequada do organismo. Essas análises serão realizadas, o que representa uma conquista diante da situação que enfrentamos, mas, infelizmente não farão parte deste documento, mesmo tendo sido pensadas como parte deste estudo.

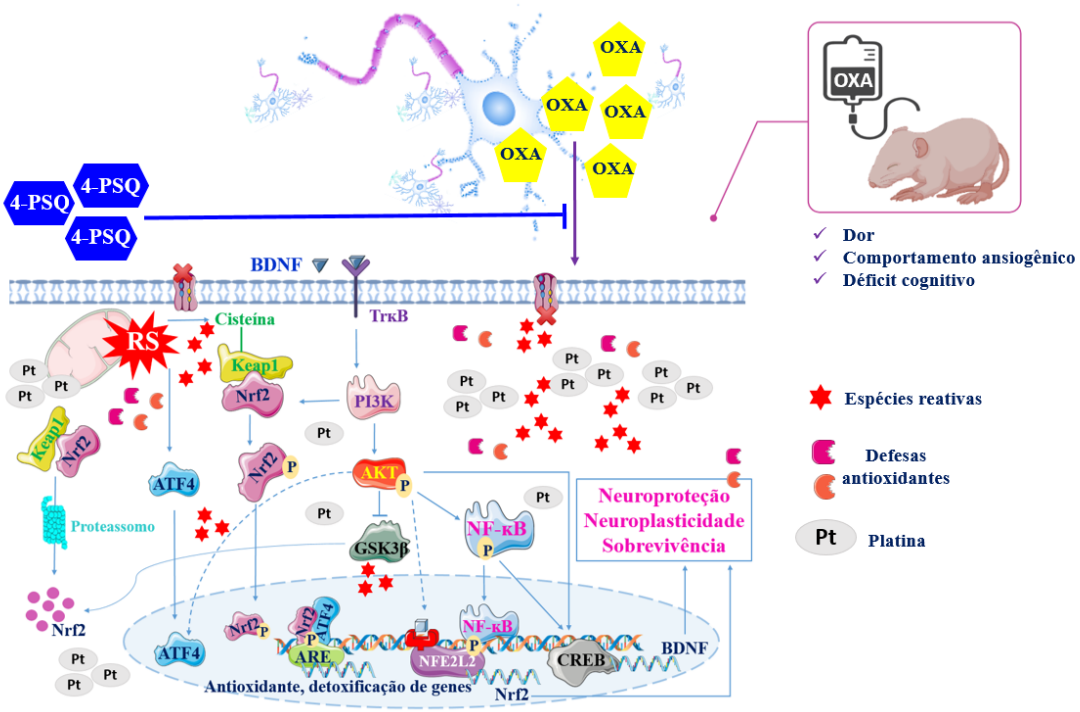


Figura 9. Representação esquemática dos principais resultados obtidos na etapa 5.

Manuscrito 3

Os principais resultados deste capítulo da tese estão apresentados sob a forma de manuscrito, o qual se encontra assim organizado. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se no próprio manuscrito.

O manuscrito 3 será submetido à revista *Biogerontology*.

Behavioral and biochemical insight of 7-chloro-4-(phenylselanyl) quinoline role on peripheral neuropathy, emotional and cognitive impairments induced by Oxaliplatin in aged rats

Angélica S. Reis[a], Carolina C. Martins[a], Gabriel P. Costa[b], Diego Alves[b], Cristiane Luchese[a*], Ethel A. Wilhelm[a*]

[a]*Programa de Pós-graduação em Bioquímica e Bioprospecção, Laboratório de Pesquisa em Farmacologia Bioquímica, CCQFA - Universidade Federal de Pelotas, UFPel - CEP - 96010-900, Pelotas, RS, Brasil.* [b]*Programa de Pós-graduação em Química, Laboratório de Síntese Orgânica Limpa, CCQFA - Universidade Federal de Pelotas, UFPel - CEP - 96010-900, Pelotas, RS, Brasil.*

*Address for correspondence:

Ethel Antunes Wilhelm; e-mail: ethelwilhelm@yahoo.com.br / Phone: +55-53-32757360

Cristiane Luchese; e-mail: cristiane_luchese@yahoo.com.br / Phone: +55-53-32757233

Programa de Pós-graduação em Bioquímica e Bioprospecção (PPGBBio), Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas (UFPel), Campus Capão do Leão, Pelotas, CEP 96010-900, RS, Brasil

Abstract

The underlying mechanisms of oxaliplatin (OXA)-induced peripheral neuropathy remain uncertain, however, oxidative damage significantly contributes to its development. On the other hand, aging is characterized by a progressive deterioration of tissues and organ systems, concurrently followed by an increase in oxidative stress. Thus, in the current study, the association between these factors and the potential of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) as a new therapeutic strategy were investigated. The administration of OXA to old Wistar rats aggravated pain sensibility, anxious behavior, and cognitive impairment through increased oxidative damage in the nervous system central (CNS) and peripheral (PNS), as well as by Na^+, K^+ - ATPase and acetylcholinesterase (AChE) activities inhibition. Locomotor and exploratory activities were not affected. 4-PSQ-treatment reversed pain sensibility, emotional and cognitive impairment, mainly through reducing oxidative damage. 4-PSQ reduced reactive species (RS) and lipid peroxidation, also increased non-protein thiols (NPSH) levels, and modulated the activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the CNS and/or PNS. 4-PSQ reestablished the activity of Na^+, K^+ - ATPase, but not of AChE enzyme. Mg^{2+} - ATPase activity was not changed. This study is the first to provide insight into the antioxidant potential of 4-PSQ in the oxidative damage to PNS in an OXA-induced peripheral neuropathy model in aged rats. 4-PSQ highlights as a good prototype for the development of a more effective drug for the treatment of OXA-induced peripheral neuropathy, regardless the age.

Keywords: Neuropathy; aging; oxaliplatin; oxidative damage; 4-PSQ

Abbreviations

OXA, Oxaliplatin; RS, reactive species; GPx, glutathione peroxidase; SOD, superoxide dismutase; AChE, acetylcholinesterase; CNS, central nervous system; PNS, peripheral nervous system; NADPH, β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; BBB, blood brain-barrier

1. Introduction

Oxaliplatin (OXA)-induced peripheral neuropathy is a severe and potentially permanent adverse effect, initially characterized by acute clinical symptoms which are exacerbated resulting in the establishment of chronic peripheral neuropathy. Several factors and mechanisms contribute to the incidence or aggravation of peripheral neuropathy induced by OXA, however, at present many of these yet remain unclear (Cavaletti and Marmiroli 2020). In this context, considering the highest incidence of cancers in patients over the age of 60 (Fane and Weeraratna, 2020), age is an important factor to be considered in the exacerbation of OXA-induced peripheral neuropathy. Indeed, was estimated that by 2050 one-fifth of the world population will be over the age of 60 (Fane and Weeraratna, 2020).

Besides, the management of OXA-induced peripheral neuropathy clinical is generally focused on treating symptoms because the causes can be rarely treated. In aged patients, is necessary to consider also many particular features, including the neurogenesis declines, progressive loss of tissue and organ function, and imbalance redox (Nguyen and Ehrlich, 2020; Liguori et al. 2018). Indeed, one hypothesis is that elderly people could be more susceptible to the side effects of OXA. Thus, new therapeutic approaches for OXA-induced peripheral neuropathy treatment should consider the aging-peculiarities.

Recently, several studies of the our research group investigated the physiopathology of adverse effects of OXA and the pharmacological effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (Figure 1) for its treatment (Reis et al. 2020a, b; Lemos et al. 2020; da Motta et al. 2021). It is well established the susceptibility of the peripheral nervous system (PNS) to oxidative damage induced by metabolites of the

OXA, mainly platinum derivatives (Cavaletti and Marmiroli; Areti et al. 2014; Starobova and Vetter 2017). However, recently our data established that platinum from OXA crosses the blood-brain-barrier (BBB), accumulating in the CNS, and causing oxidative damage (Reis et al. 2020a). This study demonstrated the involvement of the cholinergic pathway in OXA-induced neurotoxicity. Besides, our research group showed for the first time the potential antioxidant of 4-PSQ to reverse the redox imbalance induced by treatment with OXA in the CNS and thus reduce the pain sensitivity.

Based on the aforementioned facts, and given that the neurotoxicity OXA-induced is mediated mainly by oxidative damage in the CNS, we investigated the pharmacological modulation of Na^+, K^+-ATPase as a potential target for the development of new therapies (Reis et al. 2020b). Indeed, the Na^+, K^+ - ATPase enzyme is particularly vulnerable to reactive species (RS) (Shrivastava et al. 2020). In this sense, it was initially demonstrated a correlation between anxiety and cognitive decline, both caused by the administration of the OXA. As hypothesized, the treatment with OXA inhibited Na^+, K^+ - ATPase activity and, also, reduced the expression levels of this enzyme in the CNS. Corroborating with the results of the first study, the treatment with 4-PSQ restored the emotional and cognitive impairment caused by the administration of the OXA. 4-PSQ effects seem to be due the regulation of the hypothalamic-pituitary-adrenal axis, reduction of plasmatic corticosterone and mainly due to it is ability to reestablish activity and expression of the Na^+, K^+-ATPase enzyme. These results contribute to establishing Na^+, K^+-ATPase enzyme as a promising target for the treatment of the OXA-induced neurotoxicity and, in addition, to demonstrate that the

modulation of the Na^+, K^+- ATPase had a fundamental role in the effects of the 4-PSQ against neurotoxicity caused by OXA administration (Reis et al. 2020b).

OXA is a chemotherapy drug effective in cancer therapy. However, their clinical benefit is constantly challenged by side effects on the many organ systems including the brain, kidney, and liver. The imbalance redox induced by metabolites of OXA on the liver (Lemos et al. 2020) and kidney (da Motta et al. 2021) causes injury on the organs and loss functions. Against the OXA-induced hepatic injury, the treatment with the 4-PSQ normalized the plasmatic activity of the aspartate and alanine aminotransferase, both markers of damage hepatic. In addition, 4-PSQ reduced OXA-induced hepatic injury, due to a decrease in the oxidation of lipids and proteins. This study corroborated the antioxidant action of 4-PSQ in OXA-induced toxicity (Lemos et al. 2020). Experimental evidences support the involvement of RS in OXA-induced renal injury (da Motta et al. 2021). Indeed, the OXA-treatment also inhibited Na^+, K^+ -ATPase enzyme in the kidney. Moreover, 4-PSQ acted on the oxidative balance, reestablishing Na^+, K^+-ATPase activity in mice exposed to OXA. Thus, 4-PSQ treatment reestablished renal cell functionality.

Considering the important effects of 4-PSQ against neurotoxicity, hepatic injury, and damage renal induced by OXA, in the current study the 4-PSQ effects against OXA-induced chronic peripheral neuropathy and its comorbidities were investigated in aging rats.

2. Materials and Methods

2.1 Animals

All experiments were performed in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA 4506-2017) and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) (Book 1996) and International Guiding Principles for Biomedical Research Involving Animals. The tests were carried out using adult (2 months) and old (20 months) male Wistar rats. All animals were maintained at 22 ± 2 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 6:00 a.m.). Rats were acclimatized to the behavior room for at least 1 h. All efforts were made to minimize the number of animals used and their discomfort. The number of animals and intensities of noxious stimuli used were the minimum needed to demonstrate consistent effects of the treatment. Allocation concealment was performed using a randomization procedure (http://www.randomizer.org/). Behavioral evaluations were performed blindly on drug administration. All experiments were carried out between 08:00 and 17:00 h.

2.3 Drugs

OXA was obtained from Eurofarma pharmaceutical company, and it was dissolved in 5% glucose solution. All other chemicals used in this study were of analytical grade and obtained from Sigma-Aldrich, (St. Louis, MO, USA). Rats received OXA by intraperitoneal (i.p.) route at a constant volume of 1 mL kg^{-1} of body weight.

4-PSQ was prepared and characterized according to previous studies (Savegnago et al. 2013; Duarte et al. 2017) in our laboratory and analysis of the ^{1}H NMR and ^{13}C

NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 4-PSQ (99.9 %) was determined by GC/MS. 4-PSQ was dissolved in canola oil. Rats received 4-PSQ or its vehicle by per oral route with intragastric (i.g.) gavage at a constant volume of 1 mL kg^{-1} of body weight.

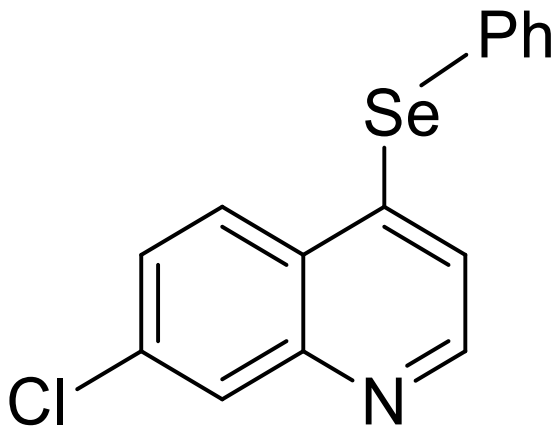


Figure 1. Chemical structure of 4-PSQ.

2.3 Treatment scheme

In this study, rats were randomly divided into four experimental groups (n = 10 animals/group): i) Young, ii) Old, iii) Old+OXA, and iv) Old+OXA+4-PSQ. From day 1 up to day 5, rats of the Young and Old groups were treated with 5% glucose solution (10 mL kg^{-1}, i.p.), whereas OXA (2 mg kg^{-1}) was administered by i.p. route to rats of the Old+OXA and Old+OXA+4-PSQ groups. On day 5, 30 min after the treatment with OXA, up to day 14, rats of the Young, Old, and Old+OXA groups received canola oil (10 mL kg^{-1}, i.g. route), whereas the rats of the Old+OXA+4-PSQ group were treated with 4-PSQ (5 mg kg^{-1}) by i.g. route. From day 5, rats were subjected to behavioral tests. On day 15, the animals were euthanized by inhalation of isoflurane anaesthetic

and, tissue samples of the CNS and PNS were rapidly dissected and stored to be used for *ex vivo* assays. In the behavioral tests and data analysis, the operators were blinded.

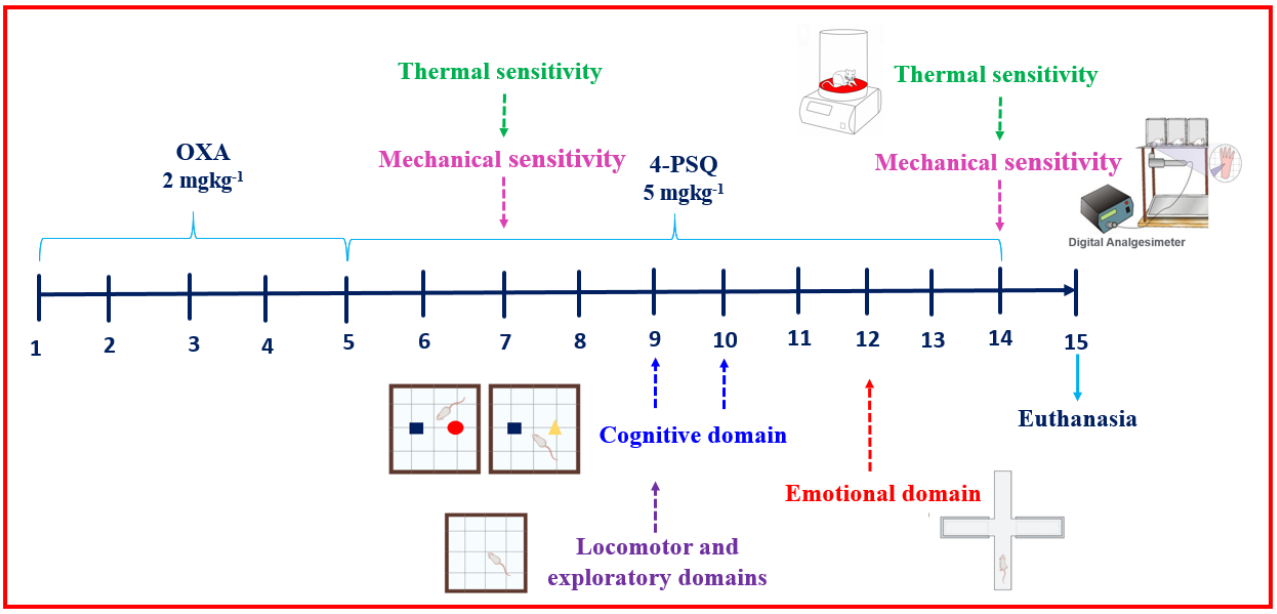


Figure 2. Schedule of experimental protocol

2.4 Behavioral tests

The tests were performed on the days indicated in the experimental protocol schedule (Figure 2).

2.4.1 Measurement of mechanical sensitivity

Mechanical sensitivity was carried as reported by Alamri et al. (2018). The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer (digital aesthesiometer, Insight, São Paulo, Brazil) adapted with a polypropylene tip. The paw withdrawal threshold was measured by applying the polypropylene tip perpendicular to the middle of the plantar surface of the hind paw at a constant progressive pressure until

paw withdrawal, and the pressure value was automatically recorded. Data of the withdrawal threshold were expressed in gram (g).

2.4.2 Measurement of thermal sensitivity

Thermal sensitivity was tested according to Woolfe and Macdonald (1944). In the hot-plate test, animals were placed in a glass box on a heated metal plate maintained at 52 ± 1 °C. The latency of nociceptive responses such as licking or shaking one of the paws or jumping was recorded as the reaction to a noxious thermal stimulus. To avoid damage to the paws of animals, time standing on the plate was limited to 45 s. Data of the latency were expressed in the seconds (s).

2.4.3 Assessment of locomotor and exploratory domains

The open field test evaluated the general locomotor and exploratory behaviors of rats. The open field was made of plywood and surrounded by 30 cm-high walls. The floor of the open field, 45 cm long and 45 cm wide, was divided by masking tape markers into 9 squares (3 rows of 3). Rats were evaluated in the open field test on the ninth day of the experimental protocol. In this test, each animal was placed at the center of the open field and observed for 4 min to record the locomotor and exploratory activities (Walsh and Cummins 1976). The arena was cleaned with 40% ethanol after each session and each rat was tested only once. Data of the locomotor and exploratory activities were expressed as number of segments crossed with the four paws and, number of rearings on the hind limbs, respectively.

2.4.4 Assessment of cognitive domain

The object recognition task was performed according to Stangherlin et al. (2009). It is widely used to evaluate short-term (STM) and long-term (LTM) memories. The task was performed in an open field apparatus on the ninth and tenth day of the experimental protocol. On the day of the task each animal was submitted to a habituation session in the absence of objects for 5 min. Posteriorly, four objects were used: A1, A2, B and C. The A1 and A2 objects were two identical balls, the B object was a cube, and the C object was a square. The objects used were made of plastic material, measuring 10 x 10 cm (length x height) and had the following color pattern: blue, red, and yellow. During the training, the animals were placed for 5 min in the arena containing two identical objects (objects A1 and A2) to explore. Exploration was accounted when the animal directed its nose around 2 cm of the object while sniffing, touching, or looking at it. In the presence of a familiar object (A1) and a new object (B), 1.5 h after training, the STM of rats was evaluated. The time to explore was defined in 5 min, enough to measure learning and recognition memory. In turn, LTM was assessed 24 h after training. For this, the rats were placed to explore a familiar object (A1) and a new object (C) for 5 min. Time spent exploring each object was reported. Data were expressed as a percentage of the exploratory preference and calculated as follows: Training = (A2/(A1+A2))×100; STM = (B/(A1+B))×100; LTM = (C/(A1+C))×100.

2.4.5 Assessment of anxiety-like behavior

The elevated plus-maze apparatus consists of two opposed open arms (50 cm x 10 cm) and two opposed closed arms (50 cm x 10 cm x 40 cm) mounted at an angle of 90º, all facing a central platform (10 cm x 10 cm) elevated 50 cm from the floor. This test is widely validated to measure anxiety in rodents (Pellow et al. 1985). On the

twelfth day, all animals were evaluated in the elevated plus-maze test. Each animal was placed individually at the center of the apparatus facing one of the open arms. The frequency of entries into either open or closed arms and the time spent in each type of arm were measured for 5 min. The anxiolytic effect of a drug is illustrated by a significant statistical increase of parameters in open arms. Data were expressed as a percentage of the number of entries and of the time spent in the open arms.

2.5 *Ex vivo* assays

Considering behavioral tests results, *ex vivo* assays were performed aiming to understand the 4-PSQ effects and their role in the treatment of the OXA-induced peripheral neuropathy in aged rats. On the 15th day of the experimental protocol, animals were killed by inhalation of isoflurane anaesthetic. Sciatic nerve, spinal cord, cerebellum, cerebral cortex, and hippocampus samples were collected to determine oxidative stress markers, such as RS, lipid peroxidation through thiobarbituric acid reactive species (TBARS), and non-protein thiols (NPSH) levels, also, antioxidant enzymes activities as superoxide dismutase (SOD) and glutathione peroxidase (GPx). Moreover, given that a decrease on cholinergic neurotransmission in neuropathic pain conditions has been reported (Ferrier et al., 2015; Reis et al., 2020), the activity of AChE in the spinal cord, cerebellum, and cerebral cortex of rats was evaluated. Also, considering that Na^+, K^+-ATPase enzyme is a target for OXA toxicity (Reis et al. 2020b), here Na^+, K^+-ATPase and Mg^{2+} - ATPase activities were evaluated. For these analyses, samples were homogenized in 50 mmol L^{-1} TrisHCl pH 7.4 or, specifically for AChE activity, in 0.25 mol L^{-1} sucrose buffer (1:10 w v^{-1}) and centrifuged at 900 *x* g for 10 min to yield a supernatant (S_1).

2.5.1 Involvement of oxidative stress

2.5.1.1 RS levels

The RS levels were determined using a spectrofluorimetric method, using 2',7'-dichlorofluorescein diacetate (DCHF-DA) assay according to Loetchutinat et al. (2005). S_1 (50 μL) was incubated with 20 μL of DCHF-DA (1 mmol L^{-1}) and 2430 μL of Tris HCl (10 mmol L^{-1}) in pH 7.4. The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 525 nm (with 488 nm excitation) 60 min after the addition of DCHF-DA to the medium (Shimadzu RF-5301PC fluorometer). RS levels were expressed as arbitrary units of fluorescence.

2.5.1.2 Lipid peroxidation assay (TBARS method)

TBARS assay was performed to indirectly determine the malondialdehyde (MDA) levels, an important lipid peroxidation marker. As previously described by Ohkawa et al. (1979), MDA reacts with 2-thiobarbituric acid (TBA) under acidic conditions and high temperatures to yield the chromogen. The S_1 aliquots were incubated with 0.8% TBA, acetic acid buffer (pH 3.4) and 8.1% sodium dodecyl sulfate (SDS) for 2 h at 95ºC. The color reaction was measured at 532 nm and the results were expressed as nmol of MDA/mg of tissue.

2.5.1.3 NPSH assay

NPSH levels were determined as described of Ellman (1959). An aliquot of S_1 was mixed (1:1) with 10 % trichloroacetic acid (TCA) and centrifuged at 900 *x* g for 10

min. After the centrifugation, the protein pellet was discarded and free –SH groups were determined in the clear supernatant. An aliquot of S_1 was added in 1 M potassium phosphate buffer, pH 7.4, and 10 mM 5,5-dithiobis (2-nitrobenzoic acid) (DTNB). The color reaction was measured at 412 nm. NPSH levels were expressed as µmol NPSH/g tissue.

2.5.1.4 GPx activity

GPx activity was assayed spectrophotometrically by the method of Wendel (1981), which involves monitoring of the dismutation of H_2O_2 in the presence of S_1 at 340 nm. S_1 (50 µL) was added in a system composed by reduced glutathione (GSH)/NADPH/glutathione reductase (GR), and the enzymatic reaction was initiated by the addition of H_2O_2 (100 µL). In this assay, the enzymatic activity is indirectly measured by NADPH decay. H_2O_2 is reduced and generates oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by the GR present in the analysis medium at the expense of NADPH. The enzymatic activity was expressed as nmol/min/mg protein.

2.5.1.5 SOD activity

SOD activity was measured spectrophotometrically according to Misra and Fridovich (1972) method. This method is based on the capacity of SOD to inhibit autoxidation of epinephrine. S_1 (6, 12 or 18 µL) was added to a 0.05 mol L^{-1} Na_2CO_3 buffer, and the enzymatic reaction was started by adding epinephrine (30 µL). The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of

enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26 °C. The enzymatic activity was expressed as U/mg protein.

2.5.2 Involvement of AChE enzyme

The AChE activity was assayed following a modified method of Ellman (1959), using acetylthiocholine as the substrate. The reaction mixture (2 mL final volume) contained S_1 (100 µL), 100 mM K^+-phosphate buffer, pH 7.5 and 1 mM 5,5′-dithio-bis-nitrobenzoic acid (DTNB). The method is based on the formation of the yellow anion, 5,5′-dithio-bis-acid nitrobenzoic, measured spectrophotometrically at 412 nm during 2 min. The enzyme was pre-incubated for 2 min at 25 °C. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide. The enzymatic activity was expressed as µmol/h/mg protein.

2.5.3 Involvement of Na^+, K^+-ATPase and Mg^{2+} - ATPase activities

For the Na^+, K^+-ATPase activity assay, a reaction mixture was used containing S_1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samplings were performed under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at 37 °C for 30 min and the incubation was stopped by adding a 10 % trichloroacetic acid solution (TCA) with 10 mM $HgCl_2$. Na^+, K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured according to Fiske and Subbarow (1925). Enzyme activity was expressed as nmol of Pi/mg of protein/min.

For the Mg^{2+}- ATPase activity assay, a reaction mixture was used containing S_1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4 (Teixeira et al., 2020). Controls to correct for non-enzymatic substrate hydrolysis were prepared by adding sample preparations after the reactions were stopped with TCA. To determine the Mg^{2+}-ATPase activity, ouabain (1 mM) was added to the reaction medium. The reaction was initiated by adding ATP and was stopped after 30 min of incubation by the addition of 10% TCA. Enzymatic activity was expressed as nmol of Pi/mg of protein/min.

2.5.5 Protein determination

The protein concentration was measured spectrophotometrically at 595 nm by the method of Bradford (1976), using bovine serum albumin as the standard. It is a rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. The reaction mixture contained S_1 (50 µL) and Coomassie brilliant blue (2.5 mL). The reaction mixture was incubated for 10 min. Several biochemical analyses rely on accurate quantitation of protein concentration. The protein level was expressed as mg protein/mL.

2.5.6 Data and statistical analysis

The normality of the data was evaluated using the D' Agostino and Pearson omnibus normality test. Statistical analysis was performed using GraphPad Prism 6.0 software (San Diego, CA, USA). Data were analyzed by two-way analysis of variance (ANOVA) followed by the Tukey's test when appropriated for parametric data. Data were expressed as mean ± standard error of the mean (S.E.M.). Post hoc tests were

performed only when the F-value achieved the necessary level of statistical significance ($P < 0.05$) and when there was no significant variance in homogeneity. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al. 2018).

3. Results

3.1 Mechanical and thermal hypersensitivities induced by aging are are aggravated by OXA-induced chronic peripheral neuropathy

Aging decreased the mechanical (Figure 3A) and thermal (Figure 3B) sensitivities in response to force transducer and hot plate tests, respectively, featuring allodynia. Mechanical and thermal allodynia induced by aging, were observed on the first day before of the OXA-administration. Aging promoted a reduction of 23% of the mechanical threshold and, decrease 25% of the latency time (Figure 3).

Interestingly, when OXA (2 mg kg^{-1}, i.p.) was administered to aging rats, an aggravating of the allodynia was observed, followed by robust growth in the mechanical (43%) and thermal (23%) sensitivities (Figure 3). These effects started from day 7th and worsened for until last day 14^{th} of the experimental protocol. Of note, OXA increased the animals' vulnerability to effects of aging.

3.2 4-PSQ reverses allodynia caused by OXA-induced chronic peripheral neuropathy in aging rats

The effects of 4-PSQ against OXA-induced chronic peripheral neuropathy in rats was evaluated in the present study considering the aging specificities. Here, 4-PSQ administration reversed the mechanical hypersensitivity, with a maximum inhibition of

39% and 143% on days 7 and 14, respectively, evidencing, a growing and powerful analgesic effect on mechanical hypersensitivity caused by chronic peripheral neuropathy induced by aging and aggravated by OXA treatment (Figure 3A).

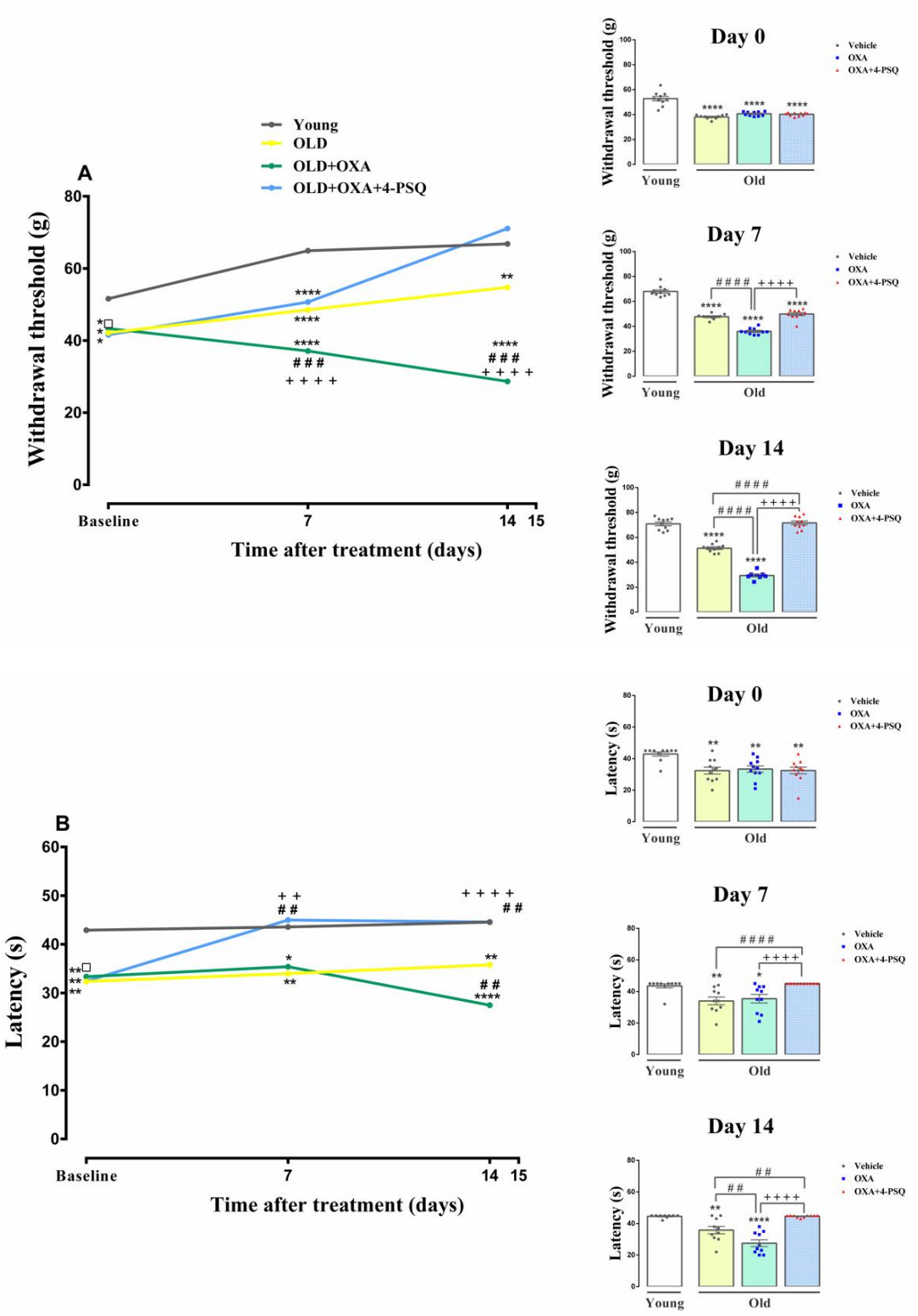


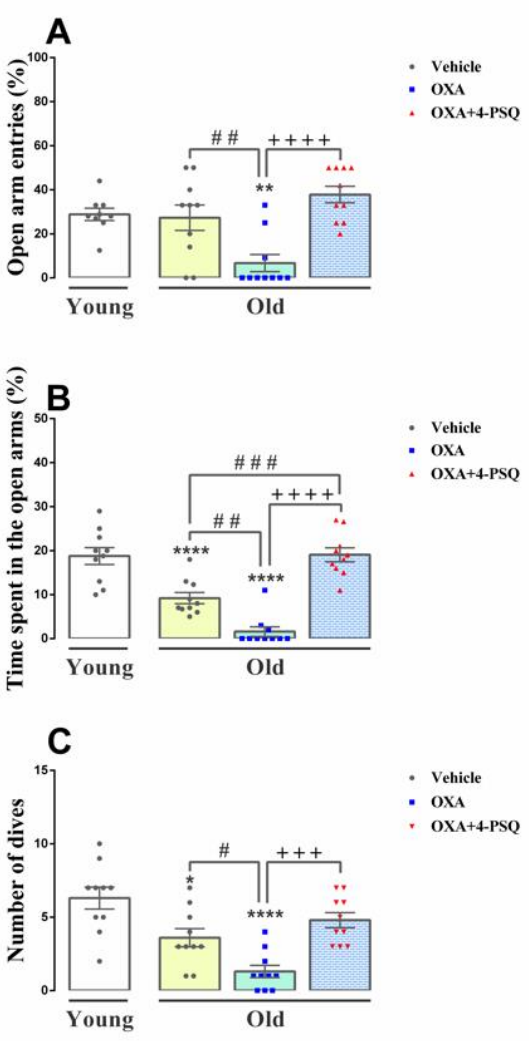
Figure 3. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (5 mg kg^{-1}, p.o.), aging (Old) and oxaliplatin (OXA) (2 mg kg^{-1}, i.p.) on the (A) paw withdrawal threshold to mechanical stimulus in the von Frey test and (B) on the latency to thermal stimulus in the hot plate test. Each point represents the mean of 10 rats in each group. (*) $P < 0.05$, (**) $P < 0.01$, and (****) $P < 0.0001$ denote significance levels when compared with the Young group; ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with the Old group; ($^{++++}$) $P < 0.0001$ denotes significance levels when compared with the Old+OXA group (Two-way ANOVA followed by the Tukey's test).

Also, treatment with 4-PSQ reversed the thermal sensitivity caused by aging and maximized by OXA. The treatment with the compound increased the latency time by 28% and 62%, on days 7 and 14, respectively (Figure 3B). Based on these results (Figure 3A and 3B), the subsequent experiments were all performed aiming to elucidate 4-PSQ effects on comorbidities of the OXA-induced chronic peripheral neuropathy in aged rats.

3.3 4-PSQ reduces comorbidities of chronic peripheral neuropathy induced by OXA-administration in aging rats

Recently, our research group demonstrated comorbidities including emotional and cognitive impairment induced by OXA neuropathy, and the beneficial effects of 4-PSQ treatment (Reis et al. 2020b). Complementing this data, here it was demonstrated that aging provoked anxious behavior in rats and we highlighted that when OXA was administered to old rats, the anxious behavior was exacerbated, as demonstrated by the

reduction in the percentage of entries in the open arms (Figure 4A), percentage of time spent in the open arms (Figure 4B), and the number of dives (Figure 4C), all parameters evaluated in the elevated plus-maze, a test widely used to determinate anxious behavior. Moreover, when 4-PSQ effects were investigated on cognitive impairment in old rats that received OXA, data revealed that the 4-PSQ reversed the reduction in the percentage of exploratory preference in the short-term (Figure 4D) and long-term (Figure 4E) memories in the object recognition task. According to the results, the evaluated parameters on the open field test, number of rearings (Figure 4F), and crossings (Figure 4G) were not significantly changed by 4-PSQ, OXA, and/or aging.



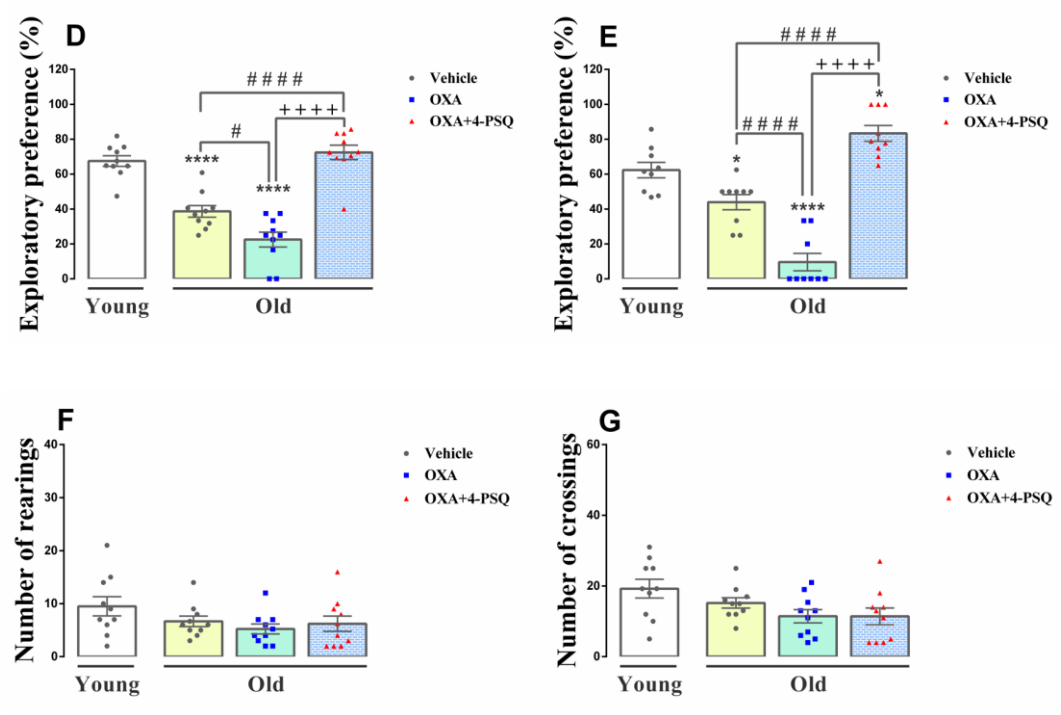


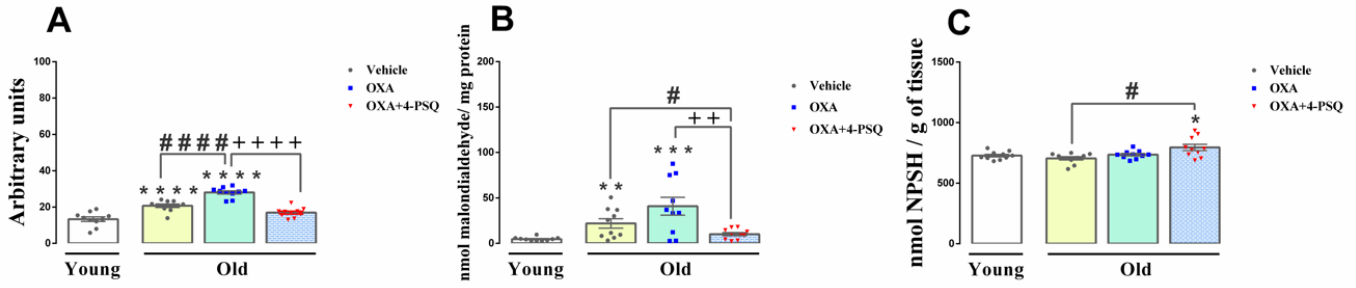
Figure 4. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (5 mg kg^{-1}, p.o.), aging (Old) and/or oxaliplatin (OXA) (2 mg kg^{-1}, i.p.) on the (A) percentage of entries in the open arms, (B) percentage of time spent in the open arms, and (C) number of dives in the elevated plus-maze; (D) percentage of exploratory preference - short-term and (E) percentage of exploratory preference - long-term memories in the object recognition task; (F) number of crossings and (G) number of rearings in the open field test. Each column represents the mean ± S.E.M. of 10 rats in each group. (*) $P < 0.05$, (**) $P < 0.01$, and (****) $P < 0.0001$ denote significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$, ($^{\#\#\#}$) $P < 0.001$, and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with the Old group; ($^{+++}$) $P < 0.001$ and ($^{++++}$) $P < 0.0001$ denote significance levels when compared with the Old+OXA group (Two-way ANOVA followed by the Tukey's test).

3.4 Oxidative stress plays a central role in the development and advancement of OXA-induced chronic peripheral neuropathy, mainly in the aged rats: highlighting the potential antioxidant of the 4-PSQ

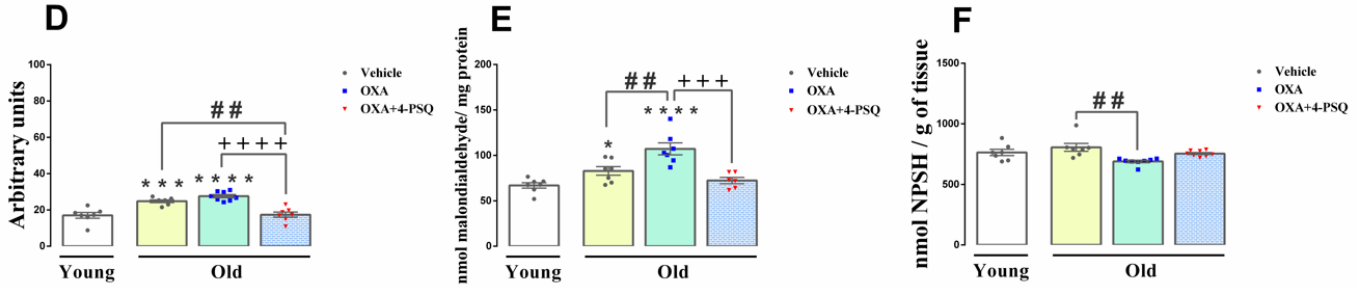
Despite the efficient defenses of the CNS, it was proposed that exposure to OXA lead to an opening of the BBB starting RS formation (Branca et al. 2018) in the CNS. In this sense, recently our research group corroborated this hypothesis, given that we demonstrated that the OXA-administration causes an accumulation of platinum in the spinal cord of mice (Reis et al. 2020a).

Based on the above considerations, here we investigated 4-PSQ antioxidant effects on oxidative damage caused by the association between exposure to OXA and aging. At first, we observed that in all tissues of the CNS investigated, it was evidenced an increase in the RS levels caused by aging, and we highlight that this increase was exacerbated by administration of OXA in the spinal cord (Figure 5A), cerebral cortex (Figure 5G), and hippocampus (Figure 5J). Thus, the elderly exposed to OXA showed the highest RS levels. Also, corroborated the increase in the RS levels, an increase in the lipid peroxidation levels on the spinal cord (Figure 5B), cerebellum (Figure 5E), and cerebral cortex (Figure 5H) induced by aging was observed. In the cerebellum and cerebral cortex, the lipid peroxidation was aggravated when old rats received OXA. Here, aging did not change the NPSH levels in the tissues analyzed (Figure 5), but in the cerebellum (Figure 5F) and hippocampus (Figure 5L) of the old rats treated with OXA a reduction in the NPSH levels was observed. GSH is the most abundant thiol-containing molecule in the cytosolic proteome of human cells and the major molecule measured in the NPSH test.

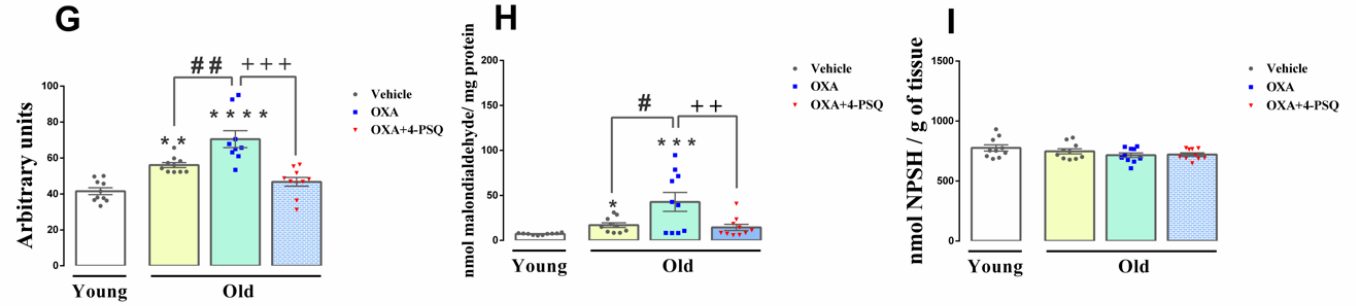
Spinal cord



Cerebellum



Cerebral cortex



Hippocampus

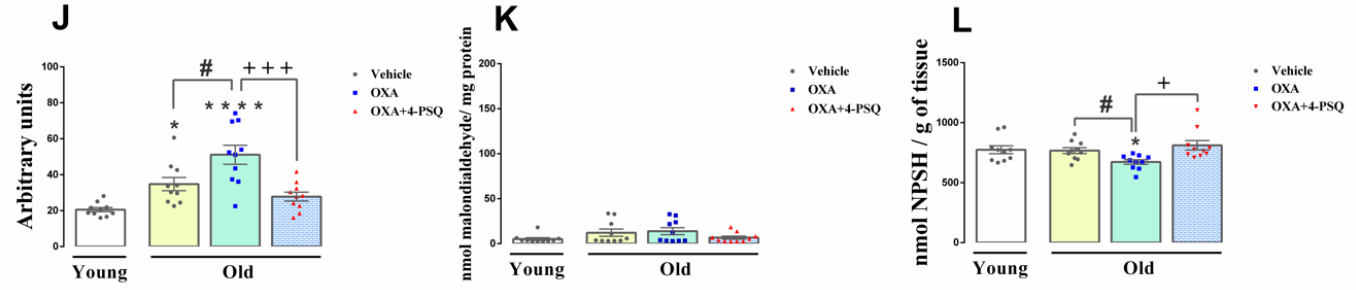


Figure 5. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (5 mg kg^{-1}, p.o.), aging (Old) and/or oxaliplatin (OXA) (2 mg kg^{-1}, i.p.) on the levels of (A) RS, (B) TBARS, and (C) NPSH in the spinal cord of the rats; (D) RS, (E) TBARS, and (F) NPSH in the cerebellum of the rats; (G) RS, (H) TBARS, and (I) NPSH in the cerebral cortex of the rats; (J) RS, (K) TBARS, and (L) NPSH in the hippocampus of the rats. Each column represents the mean ± S.E.M. of 10 rats in each group. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, and (****) $P < 0.0001$ denote significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$, and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with the Old group; ($^{+}$) $P < 0.05$, ($^{++}$) $P < 0.01$, ($^{+++}$) $P < 0.001$, and ($^{++++}$) $P < 0.0001$ denote significance levels when compared with the Old+OXA group (Two-way ANOVA followed by the Tukey's test).

In addition, here the 4-PSQ antioxidant effect was the highlight of the results set. In the spinal cord of old rats treated with OXA, the administration of the 4-PSQ reversed the oxidative damage, as evidenced by a reduction in the RS (Figure 5A) and lipid peroxidation (Figure 5B) levels and increase in the NPSH (Figure 5C) levels. In the cerebellum and cerebral cortex the 4-PSQ treatment reduced to control levels the increase in the RS and lipid peroxidation induced by OXA in the old rats. In the cerebral cortex, the 4-PSQ also reversed the oxidative stress since reduced RS (Figure 5G) and lipid peroxidation (Figure 5H) levels in the old rats that received OXA. In the hippocampus of old rats OXA-administrated, the RS (Figure 5J) and NPSH (Figure 5L) levels were normalized by treatment with 4-PSQ. Indeed, 4-PSQ reduced the oxidative damage induced by aging and exacerbated by OXA-treatment, in an OXA-induced chronic peripheral neuropathy model in rats.

3.4 4-PSQ, aging and OXA modulate enzymatic antioxidant defenses in the CNS

The degree of oxidative stress is determined by the imbalance between RS production and antioxidant defenses. In the CNS, appropriate levels of RS are crucial for the maintenance of physiological conditions. Indeed, redox imbalance can result in neurodegeneration and cell death (Korovila et al. 2017). Here, it was demonstrated that OXA administration to old rats induced extensive oxidative damage, and concurrently, 4-PSQ exerted the antioxidant effect. Indeed, the enzymatic antioxidant defenses play an important role in the maintenance of redox balance in this model.

Old rats exposed to OXA exhibited an inhibition in the GPx activity in the cerebellum (Figure 6D) and hippocampus (Figure 6H). However, despite SOD be already known as the most important line of the antioxidant enzyme defense system in a cell the interaction of the factors changed not SOD activity, in neither tissue analyzed. Here, one more time, the treatment with 4-PSQ reversed the changes induced by aging and OXA given that 4-PSQ normalized GPx activity in both tissues, cerebellum, and hippocampus.

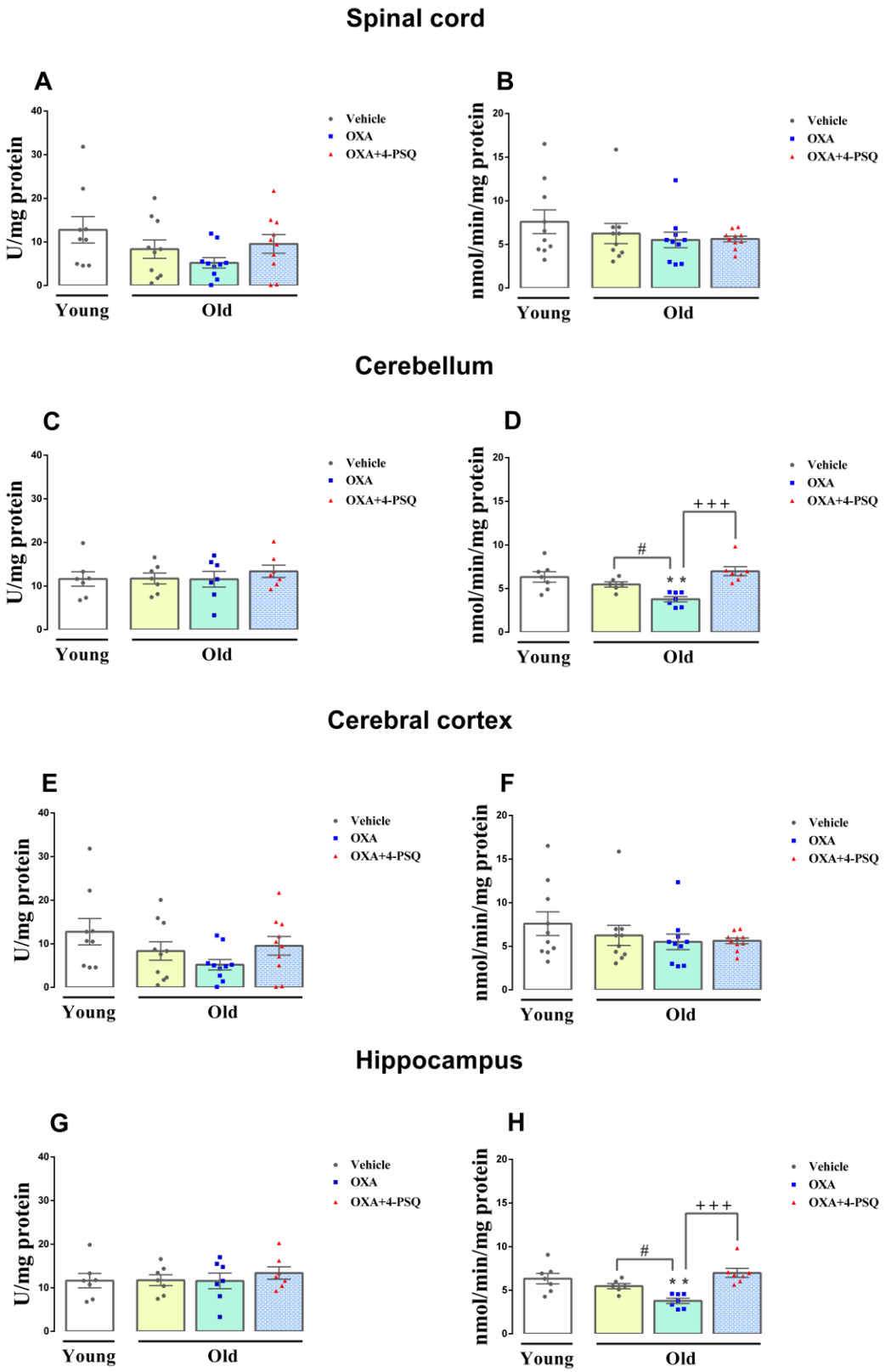

Spinal cord
A
U/mg protein
Young
Old
Vehicle
OXA
OXA+4-PSQ
B
nmol/min/mg protein
Cerebellum
C
D
#
**
+++
Cerebral cortex
E
F
Hippocampus
G
H

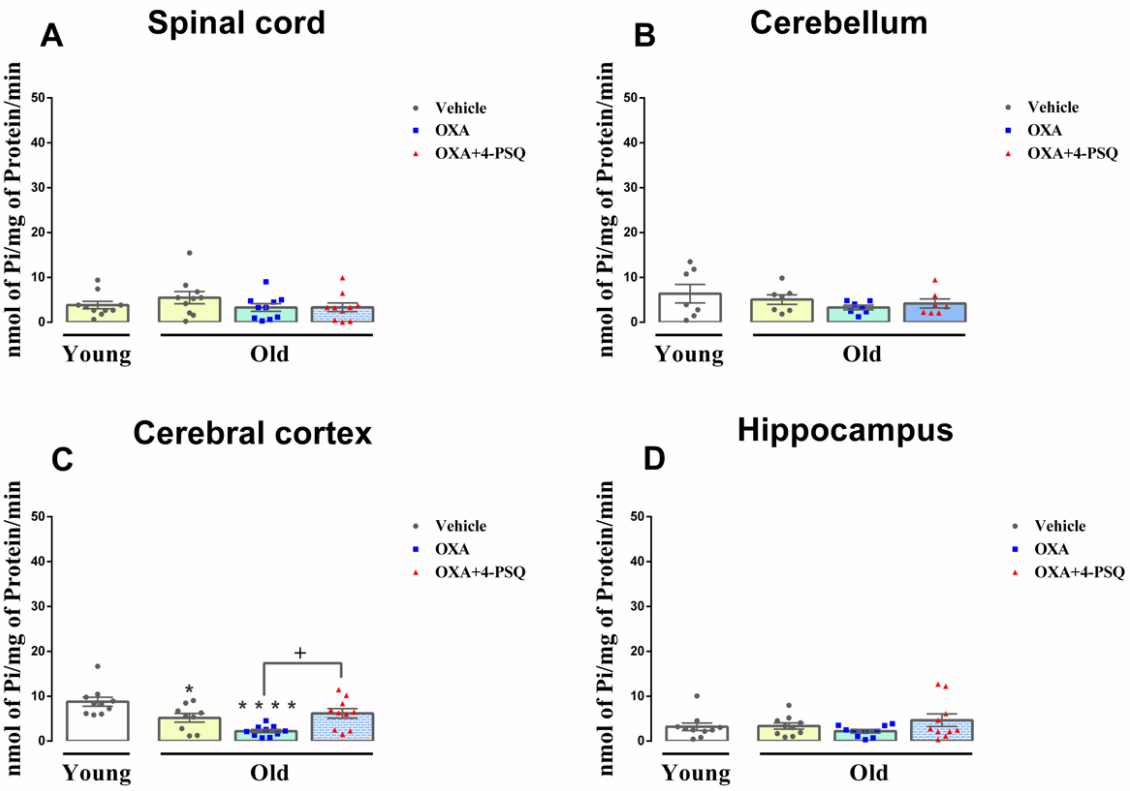
Figure 6. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (5 mg kg^{-1}, p.o.), aging (Old) and/or oxaliplatin (OXA) (2 mg kg^{-1}, i.p.) on the activity of the enzymes (A) SOD and (B) GPx in the spinal cord of the rats; (C) SOD and (D) GPx in the cerebellum of the rats (E) SOD and (F) GPx in the cerebral cortex of the rats; (G) SOD and (H) GPx in the hippocampus of the rats. Each column represents the mean ± S.E.M. of 10 rats in each group. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, and (****) $P < 0.0001$ denote significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$, and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with the Old group; ($^{+}$) $P < 0.05$, ($^{++}$) $P < 0.01$, ($^{+++}$) $P < 0.001$, and ($^{++++}$) $P < 0.0001$ denote significance levels when compared with the Old+OXA group (Two-way ANOVA followed by the Tukey's test).

3.5 Na^{+}, K^{+} - ATPase is a target of the 4-PSQ in the OXA-induced chronic peripheral neuropathy treatment in old rats

Recently, our research group demonstrated that modulation of Na^{+}, K^{+}-ATPase enzyme is a promising target for OXA-induced neurotoxicity treatment (Reis et al. 2020b). However, this previous study did not consider the aging factor. Moreover, imbalance redox also can modulate the activity of the Na^{+}, K^{+} - ATPase (Shrivastava et al. 2020), leading to impairment mitochondrial activity and reduced ATP synthesis.

In this view, here the activity of the Na^{+}, K^{+} - ATPase was investigated. In the present study, Old rats that received OXA showed an inhibition of the Na^{+}, K^{+}-ATPase activity in the cerebral cortex (Figure 7C), corroborating the results previously demonstrated (Reis et al. 2020b). Also, 4-PSQ normalized the Na^{+}, K^{+}-ATPase activity

in the cerebral cortex. As demonstrated in Figures 7E-H, 4-PSQ, aging, OXA-treatment, and the interaction between the factors did not cause any significant change in the activity of the Mg^{2+} - ATPase in all tissues analyzed.



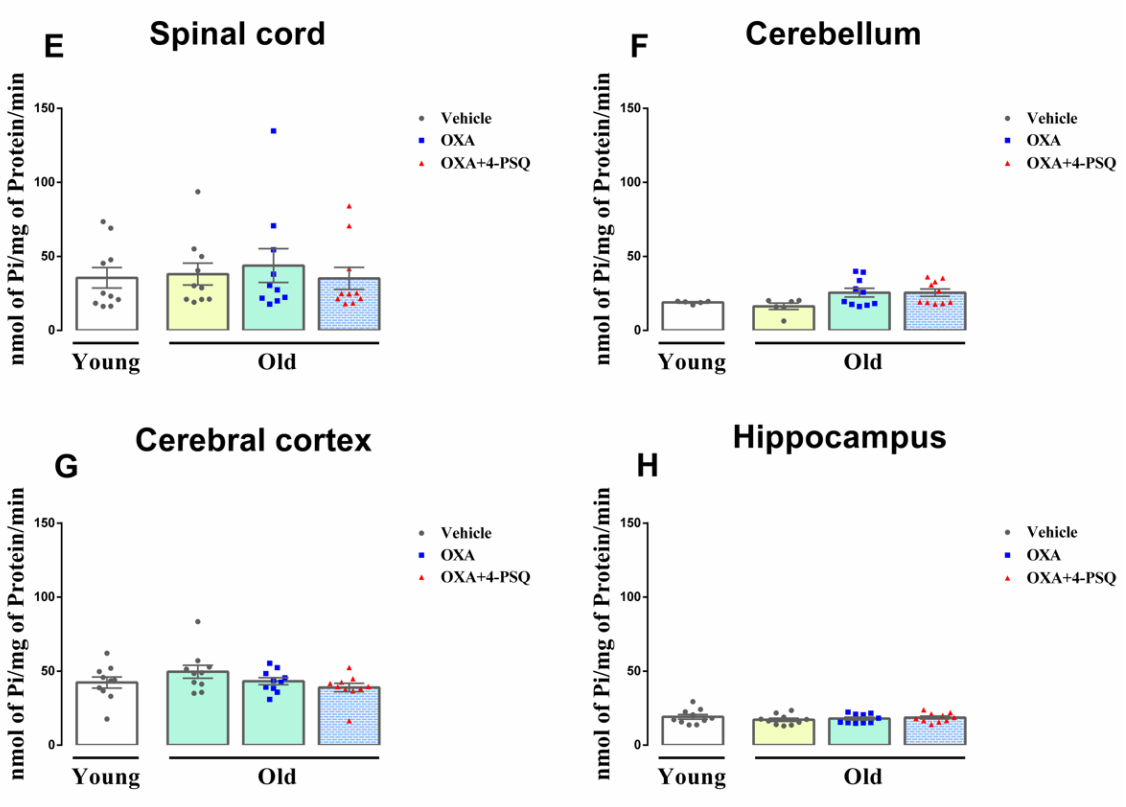


Figure 7. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (5 mg kg^{-1}, p.o.), aging (Old) and/or oxaliplatin (OXA) (2 mg kg^{-1}, i.p.) on the activity of the Na^+, K^+ - ATPase in the (A) spinal cord, (B) cerebellum, (C) cerebral cortex, and (D) hippocampus; and of the Mg^{2+} - ATPase in the (E) spinal cord, (F) cerebellum, (G) cerebral cortex, and (H) hippocampus of the rats. Each column represents the mean ± S.E.M. of 10 rats in each group. (*) $P < 0.05$, and (****) $P < 0.0001$ denote significance levels when compared with the Young group; ($^+$) $P < 0.05$ denotes significance levels when compared with the Old+OXA group (Two-way ANOVA followed by the Tukey's test).

3.6 Aging, but not 4-PSQ and/or OXA exposure, impacts the cerebellum AChE activity in rats

Recently, it was demonstrated that OXA exposure increases the activity and reduces the expression levels of the AChE enzyme in the cerebral cortex of adult mice (Reis et al. 2020a), which was reversed by 4-PSQ treatment. Because of that, here it was studied the aging impact on OXA and 4-PSQ effects. As demonstrated in Figure 8B, aging inhibited the AChE activity in the cerebellum. Treatments with OXA and/or 4-PSQ did not alter the activity of the AChE enzyme (Figure 8) in the tissues of rats studied.

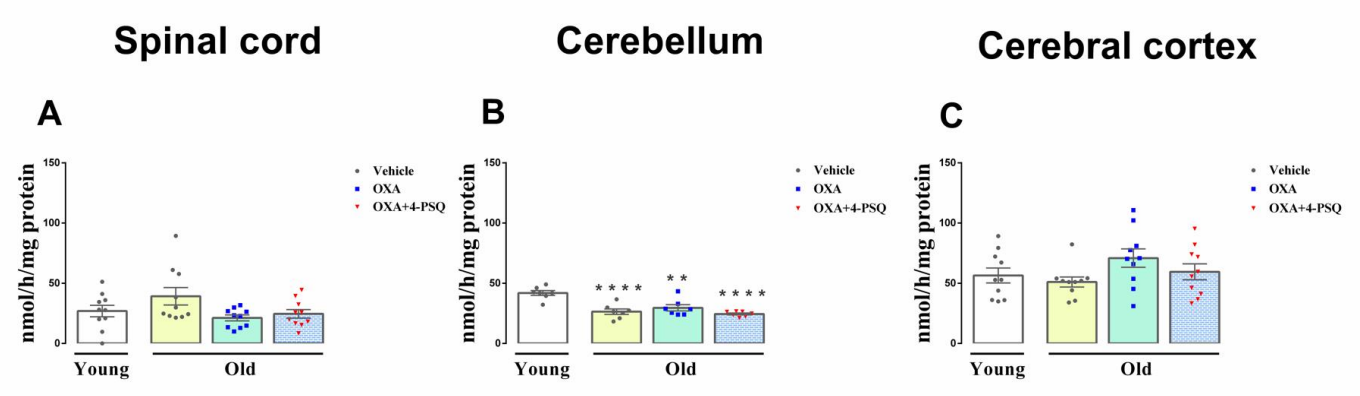
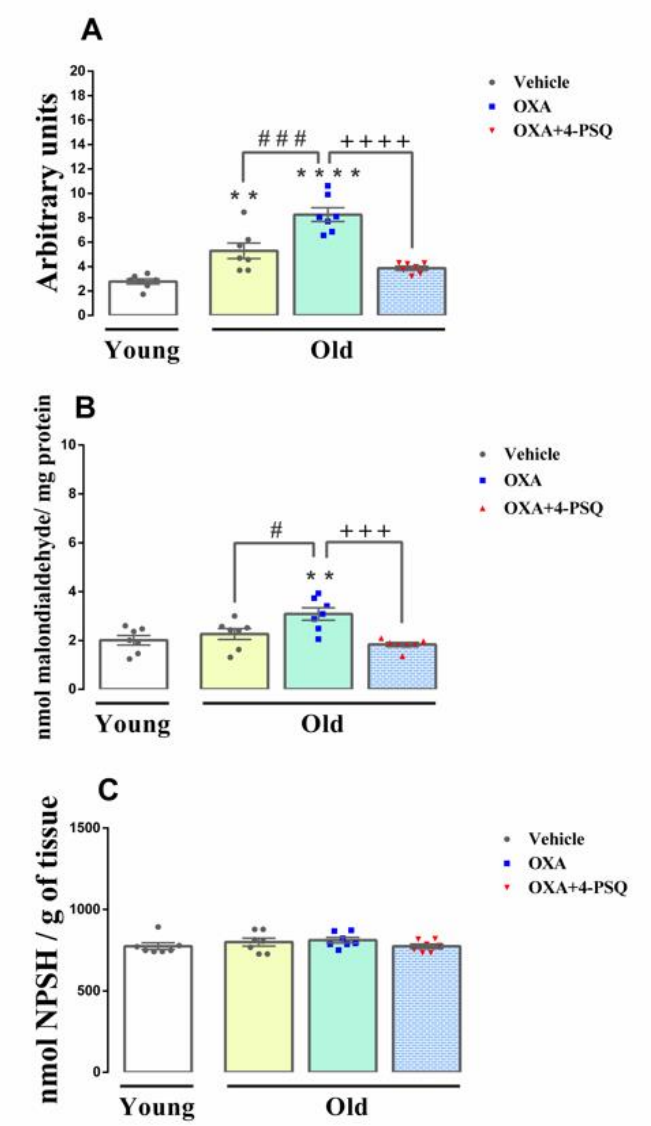


Figure 8. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (5 mg kg^{-1}, p.o.), aging (Old) and/or oxaliplatin (OXA) (2 mg kg^{-1}, i.p.) on the activity of the AChE in the (A) spinal cord, (B) cerebellum, and (C) cerebral cortex of the rats. Each column represents the mean ± S.E.M. of 10 rats in each group. (**) $P < 0.01$, and (****) $P < 0.0001$ denote significance levels when compared with the Young group (Two-way ANOVA followed by the Tukey's test).

3.7 4-PSQ reverses the oxidative damage in the sciatic nerve of old rats induced by aging and exacerbated by OXA exposure

Promising 4-PSQ effects on the OXA-induced neurotoxicity were recently demonstrated (Reis et al. 2020a, b). However, 4-PSQ effects in the PNS are not

elucidated yet. In this context, we investigate 4-PSQ-contributions to the treatment of the OXA-induced chronic peripheral neuropathy in the sciatic nerve of the old rats. As showed in Figure 9, 4-PSQ reversed also oxidative damage induced by OXA exposure in the sciatic nerve. We highlight that aging caused an increase on the RS levels in the sciatic nerve. In addition, treatment with OXA exacerbated the increase on the RS levels induced by aging and lead to an increase on the lipid peroxidation levels in the sciatic nerve of old rats. The treatment with 4-PSQ normalized all changes that OXA and aging caused.



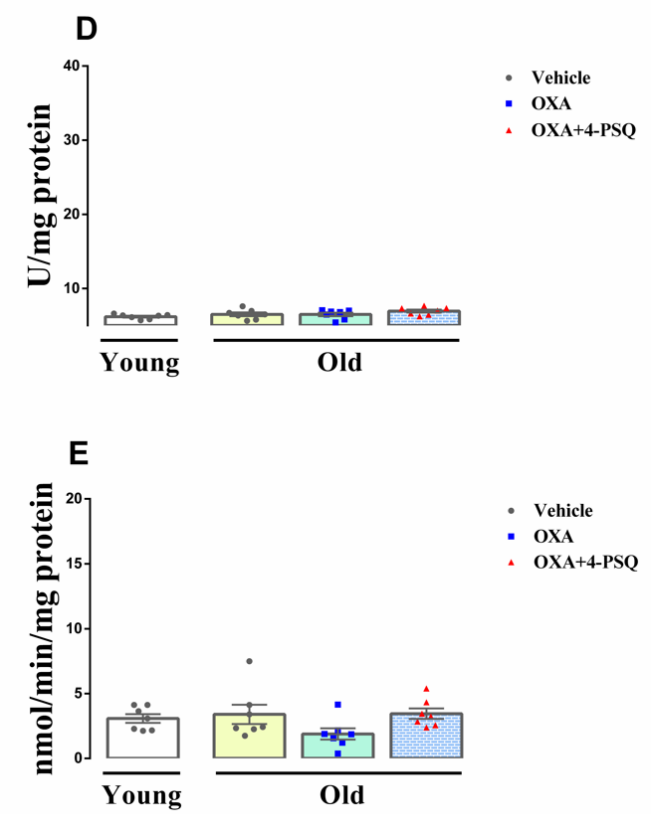


Figure 9. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) (5 mg kg^{-1}, p.o.), aging (Old) and/or oxaliplatin (OXA) (2 mg kg^{-1}, i.p.) on the (A) RS levels, (B) lipid peroxidation levels (TBARS), (C) NPSH levels, (D) SOD activity, and (E) GPx activity in the sciatic nerve of rats. Each column represents the mean ± S.E.M. of 10 rats in each group. (**) $P < 0.01$ and (****) $P < 0.0001$ denote significance levels when compared with the Young group; ($^{\#}$) $P < 0.05$ and ($^{\#\#\#}$) $P < 0.001$ denote significance levels when compared with the Old group; ($^{+++}$) $P < 0.001$ and ($^{++++}$) $P < 0.0001$ denote significance levels when compared with the Old+OXA group (Two-way ANOVA followed by the Tukey's test).

4. Discussion

Importantly, in the present study, we provided evidence for the effect of 4-PSQ

on the OXA-induced chronic peripheral neuropathy in aged rats. Here, we evidenced that OXA administration intensified the pain sensitivity, as well as increase anxious behavior and cognitive impairment induced by aging, mainly due to oxidative damage induced to CNS and PNS, and through inhibition of the Na^+, K^+-ATPase and AChE activities. Furthermore, one of the most important finding in this study is that the 4-PSQ treatment restored the oxidative balance, and thus reestablished the activity of Na^+, K^+-ATPase enzyme. In addition, the treatment with 4-PSQ reversed the pain sensitivity, anxious behavior, and cognitive impairment induced by aging and exacerbate by OXA exposure. For the first time, it was demonstrated that 4-PSQ also reversed the aging-induced oxidative damage, aggravated by OXA, in the sciatic nerve of rats.

In this sense, it is important to highlight some advantages of 4-PSQ. The design of this compound was carried out with the objective of structural improvement. Our strategy was based on compounds successfully in the treatment of nociception. In fact, in the initial study 4-PSQ revealed a potential antioxidant action *in vitro*, without toxicological effects (Savegnago et al. 2013). Posteriorly, it was demonstrated the antinociceptive and anti-inflammatory potential of the 4-PSQ and it is pharmacologic actions were mainly correlated to antioxidant property, and also, by modulating the serotonergic, nitrergic, and glutamatergic systems (Pinz et al. 2016; Silva et al. 2017; Luchese et al. 2020). In this line, our data demonstrated that the treatment with 4-PSQ reversed the OXA-induced imbalance redox and, thus, reduced neuropathic pain (Reis et al., 2020a). Here, the treatment with 4-PSQ reduced the RS levels induced by aging and exacerbated by administration of OXA in rats. In addition, corroborating the results obtained, it was observed that 4-PSQ also reduced the lipid peroxidation in analyzed tissues. Based in these evidence and in previously studies (Areti et al. 2014; Cavaletti

and Marmiroli 2020) we believed that the imbalance redox contributes to higher mechanical and thermal sensitivity observed in aged animals exposed to OXA. In this sense, antioxidant action of 4-PSQ can be associated with the reduction of pain sensitivity.

It is a recognized fact that oxidative imbalance plays a key role in the development and progress of OXA-induced peripheral neuropathy. Oxidative stress generated by OXA treatment causes mitochondrial dysfunction, increase of RS, and demyelination of the neurons (Areti et al. 2014; Cavaletti and Marmiroli 2020). In this study, the increase in the RS level induced by aging was aggravated by treatment with OXA in the CNS and PNS. In accordance with this result, an increase in the lipid peroxidation levels in both systems, peripheral and central, was observed. The PNS is vulnerable to RS generated during treatment with OXA due to the lack of an adequate barrier, as BBB in the CNS, and of efficient lymph drainage, and because of that, nerves as sciatic are highly susceptible to OXA toxicity (Areti et al. 2014; Starobova and Vetter 2017). Moreover, recently our research group showed that despite the efficient defenses of the CNS, OXA metabolism products cross the BBB, causes platinum accumulation in the CNS leading to imbalance redox (Reis et al. 2020a).

Process of aging besides enhanced the production of RS, also, reduces the ability of the cell to maintain its proteome, leading to failure in the antioxidant defenses (Zhang et al. 2015; Korovila et al. 2017). Here, despite increase RS levels caused by aging, the antioxidant enzymes SOD and GPx did not show alterations in the activity. On the other hand, considering that mitochondrial dysfunction induced by treatment with OXA can lead to increase in the production of free radicals, antioxidant depletion, and damage to cellular protein and lipid (Wei et al. 2021), yet again the results obtained in the present

study are according to published data, given that the administration of OXA to old rats caused a reduction in the GPx activity in two tissues of the CNS, cerebellum and hippocampus. Preclinical studies have suggested that antioxidant compounds like 4-PSQ can inhibit the production of free radicals and reduced OXA-induced peripheral neuropathy (Wei et al. 2021). Corroborating, here the treatment with the 4-PSQ besides of the reduce RS levels and lipid peroxidation, also normalized GPx activity and reduced the pain sensitivity in old rats exposed to OXA, reenforcing the pharmacological potential of this compound regardless of age.

Transient receptor potential ankyrin 1 (TRPA1) a polymodal nociceptor that plays a key role in pain establishing, is activated by RS (Miyake et al. 2016) and inactivated by GSH (Lee et al., 2017). GSH is a potent antioxidant that protects from imbalance redox. In old rats that received OXA, we observed a decrease in the GSH amount, as well as an increase in RS levels, which also contributes to the highest pain sensitivity in old rats exposed to OXA. Indeed, GSH can prevent OXA-induced peripheral neuropathy (Kang et al. 2020; Lee et al. 2017), however, it can also reducing chemotherapy-treatment response with OXA. In this study, the treatment with the 4-PSQ compound improved the GSH levels only in the CNS. From the data obtained in this study, one hypothesis is that the 4-PSQ will not alter chemotherapy-treatment response with OXA modulating the GSH in the PNS. The administration of 4-PSQ could be a key approach to reduce the RS levels in the CNS, through of modulation of GSH levels, and the symptoms as the pain in the elderly.

Under normal physiological conditions, a stable RS level is maintained, because the RS are signal mediators involved in several pathways with functions such as growth, differentiation, progression, and death cellular. The redox state impacts cellular

functionality significantly, mainly in the CNS, where an increase in RS generates leads to a decrease in the Na^+, K^+-ATPase activity (Bejˇcek et al. 2021). There are exogenous and endogenous modulators of Na^+, K^+-ATPase activity including, epinephrine, insulin, and RS. Recently, it was demonstrated that the OXA inhibits the Na^+, K^+-ATPase activity in the CNS, leading to anxious behavior and cognitive impairment (Reis et al. 2020b). In this line, here we observed firstly that OXA exposure did not modify the inhibition of Na^+, K^+-ATPase activity caused by aging.

The inhibition of the Na^+, K^+-ATPase activity reduces the Na^+ gradient that is needed for glutamate transport and clearance from the synapse cleft, thus it can lead the overactivation of glutamatergic neurons and excitotoxicity induced by glutamate. In addition, Na^+, K^+-ATPase activity inhibition contributes to synaptic transmission failure, mediated by downregulates the synaptic AMPA receptor. For these reasons, impairment Na^+, K^+-ATPase activity play an important role in cognitive decline, as well as neurodegenerative and emotional disease age-related (Kinoshita et al. 2016; Kurauchi et al. 2019). Indeed, here we demonstrated that the administration of the OXA exacerbates the anxious behavior and cognitive impairment induce by aging. Corroborating our results, both factors evaluated in this study, aging and OXA-treatment, caused oxidative damage and impairment Na^+, K^+-ATPase activity. Importantly, 4-PSQ normalized the balance redox and the Na^+, K^+-ATPase activity. Consequently, the hypothesis is that powerful 4-PSQ antioxidant action contributes to its anxiolytic effects and enhancer of cognitive function in aged rats exposed to OXA.

4-PSQ elicited anxiolytic-like behavior and neuroprotective effects in animals models (Reis et al. 2017; Vogt et al. 2018; Pinz et al. 2018), mainly by modulating the glutamatergic pathway and oxidative stress. Indeed, 4-PSQ reduced glutamate uptake in

the brain of the mice and protected against kainate-induced anxiety behavior. We believe that 4-PSQ could be an inhibitor of the glutamate action on its receptors, acting as a glutamatergic antagonist. Because of that, 4-PSQ reduces glutamate uptake as a compensatory mechanism (Reis et al. 2017). This hypothesis corroborates our data since in the current study the treatment with 4-PSQ restored Na^+, K^+-ATPase activity. Besides, in an Alzheimer's disease model induced by amyloid β-peptide, the 4-PSQ protected against cognitive decline and anxiety behavior, mainly modulating cholinergic pathway and reducing lipid peroxidation (Pinz et al. 2018). Also, our group study establishes that the 4-PSQ presented antioxidant action in the CNS and could be a candidate for the therapy of the diseases associated to cerebral oxidative stress (Vogt et al. 2018). Importantly, our previous research related to 4-PSQ showed that this compound reduced cognitive impairment age-related. 4-PSQ modulated the synaptic plasticity by enhancing the neural cell adhesion molecule (NCAM) and polysialyltransferase levels in the brain of aged rats (Barth et al. 2019).

Modulation of distributed networks in the cerebellum is associated with several key functions, and cerebellar injuries can cause motor disorders of ataxia and cognitive-affective (Schmahmann 2019). In this study, we observed a reduction in the AChE activity in the cerebellum of the aged rats that received OXA. A previous study of our research group evidenced that the AChE enzyme plays an important role in age-related cholinergic neurotransmission dysfunction (Barth et al., 2019). Aging led to inhibition of the AChE activity in the cerebral cortex and hippocampus of rats, causing cognitive impairment. Indeed, the current study also evidenced a decrease in the AChE activity in the CNS and cognitive deficit. On the other hand, we observed that the treatment with 4-PSQ reversed the AChE activity inhibition age-related (Barth et al., 2019). Also, our

research group demonstrated that the administration of OXA induced an increase in the AChE activity and reduction in the expression levels in young adult mice exposed to OXA. In addition, it was evidenced that the treatment with 4-PSQ reversed the dysfunction on the AChE induced by OXA (Reis et al. 2020a). However, here the treatment with 4-PSQ did no reverse the inhibition of the AChE activity in aged rats exposed to OXA. Here, it is necessary to consider that neurogenesis declines with age (Nguyen and Ehrlich), as well as progressive loss of tissue and organ function, particularly, due to the accumulation of oxidative damage to macromolecules (Liguori et al. 2018). Therefore, in this case, we also can observe the impact of OXA exposure on damage induced by aging in rats.

In summary, the present study highlighted that the effects of OXA on aging rats are complex and engage a range of pathways. OXA exacerbated the pain sensitivity, cognitive decline, and anxious behavior caused by aging mainly through oxidative damage and inhibition of Na^+, K^+-ATPase and AChE activities. Also, the results suggest that therapy with 4-PSQ reversed the peripheral neuropathy and emotional and cognitive impairments aging-related and exacerbated by OXA-treatment. Powerful antioxidant 4-PSQ action reversed the oxidative damage in the CNS and, for the first time, in the PNS, reversing the Na^+, K^+-ATPase activity inhibition. In addition, our results helped to expand knowledge about the mechanisms involved in the pathophysiology of OXA-induced chronic peripheral neuropathy, considering aging specificities. Thus, the results strongly contribute to the research of a novel therapeutic agent for the treatment of OXA-induced chronic peripheral neuropathy, mainly for the aged.

Declarations

Funding Information

This study received financial support and scholarships from the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (429859/2018-0, 312747/2020-9), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) (PqG 17/2551-0001013-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível superior – Brasil (CAPES) - Finance Code 001. C.L.; E.A.W.; D.A. are recipients of CNPq fellowship. This study also received financial assistance from L'ORÉAL-UNESCO-ABC for Women in Science.

Author contributions

A.S.R., C.C.M., K.P.M, J.J.P., C.L. and E.A.W. conceived and designed the study. G.P.C. and D.A. performed the 4-PSQ synthesis. A.S.R. and E.A.W. wrote the manuscript. E.A.W. supervised the study. All authors approved the final version of the manuscript.

Availability of Data and Material

All data generated or analyzed during this study are included in this published article.

Code Availability

Not applicable.

Compliance with Ethical Standards

Animal care and all experimental procedures were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996). Also, this study was performed in line with the principles of the Declaration of Helsinki, and in accordance with the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 4506-2017). All efforts were made to minimize the number of animals used and their suffering.

Conflict of interest

The authors declare that they have no conflicts of interest.

Consent for Publication

Not applicable.

Consent to participate

Not applicable.

Acknowledgements

Not applicable.

References

Alamri FF, Shoyaib A Al, Biggers A, et al (2018) Applicability of the grip strength and automated von Frey tactile sensitivity tests in the mouse photothrombotic model of

stroke. Behav Brain Res 336:250–255. https://doi.org/10.1016/j.bbr.2017.09.008

Areti A, Yerra VG, Naidu VGM, Kumar A (2014) Oxidative stress and nerve damage: Role in chemotherapy induced peripheral neuropathy. Redox Biol 2:289–295. https://doi.org/10.1016/j.redox.2014.01.006

Barth A, Vogt AG, dos Reis AS, et al (2019) 7-Chloro-4-(Phenylselanyl) Quinoline with Memory Enhancer Action in Aging Rats: Modulation of Neuroplasticity, Acetylcholinesterase Activity, and Cholesterol Levels. Mol Neurobiol 56:6398–6408. https://doi.org/10.1007/s12035-019-1530-5

Book GETT (1996) Guide for the Care and Use of Laboratory Animals

Branca JJV, Maresca M, Morucci G, et al (2018) Oxaliplatin-induced blood brain barrier loosening: A new point of view on chemotherapy-induced neurotoxicity. Oncotarget 9:23426–23438. https://doi.org/10.18632/oncotarget.25193

Cavaletti G, Marmiroli P (2020) Management of oxaliplatin-induced peripheral sensory neuropathy. Cancers (Basel). 12

Cavaletti G, Marmiroli P cancers Management of Oxaliplatin-Induced Peripheral Sensory Neuropathy. https://doi.org/10.3390/cancers12061370

Curtis MJ, Alexander S, Cirino G, et al (2018) Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br J Pharmacol 175:987–993. https://doi.org/10.1111/bph.14153

Duarte LFB, Barbosa ES, Oliveira RL, et al (2017) A simple method for the synthesis of 4-arylselanyl-7-chloroquinolines used as in vitro acetylcholinesterase inhibitors and in vivo memory improvement. Tetrahedron Lett 58:3319–3322. https://doi.org/10.1016/j.tetlet.2017.07.039

Ellman GL (1959) Tissue Su ~ yd ~ l Groups. 70–77

Fane M, Weeraratna AT How the ageing microenvironment influences tumour progression HHS Public Access. https://doi.org/10.1038/s41568-019-0222-9

Fiske CH, Subbarow Y, (1925). The colorimetric determination of phosphorus.

J. Biol. Chem. 66, 375e400.

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Bejˇcek J, Spiwok V, Kmoníˇcková E, Rimpelová S (2021) Na +/K +-ATPase Revisited: On Its Mechanism of Action, Role in Cancer, and Activity Modulation. Molecules 26:. https://doi.org/10.3390/MOLECULES26071905

Schmahmann JD (2019) The cerebellum and cognition. Neurosci Lett 688:62–75. https://doi.org/10.1016/J.NEULET.2018.07.005

Kang L, Tian Y, Xu S, Chen H (2020) Oxaliplatin-induced peripheral neuropathy: clinical features, mechanisms, prevention and treatment. J Neurol. https://doi.org/10.1007/s00415-020-09942-w

Kinoshita PF, Leite JA, Orellana AMM, et al (2016) The influence of Na+, K+-ATPase on glutamate signaling in neurodegenerative diseases and senescence. Front Physiol 7:1–19. https://doi.org/10.3389/fphys.2016.00195

Korovila I, Hugo M, Castro JP, et al (2017) Redox Biology Proteostasis , oxidative stress and aging. 13:550–567

da Motta KP, Lemos BB, Paltian JJ, et al (2021) 7-Chloro-4-(phenylselanyl) quinoline reduces renal oxidative stress induced by oxaliplatin in mice. Can J Physiol Pharmacol. https://doi.org/10.1139/CJPP-2021-0090

Kurauchi Y, Yamada T, Hisatsune A, et al (2019) Chronic memantine administration

prevents ouabain-induced hyperactivity in mice via maintenance of Na+, K+-ATPase activity in the hippocampus. J Pharmacol Sci 140:295–299. https://doi.org/10.1016/j.jphs.2019.06.007

Lee M, Cho S, Roh K, Chae J, et al (2017). Glutathione alleviated peripheral neuropathy in oxaliplatin-treated mice by removing aluminum from dorsal root ganglia. Am J Transl Res 9: 926-939.

Lemos BB, Motta KP da, Paltian JJ, et al (2020) Role of 7-chloro-4-(phenylselanyl) quinoline in the treatment of oxaliplatin-induced hepatic toxicity in mice. Can J Physiol Pharmacol 1–11. https://doi.org/10.1139/cjpp-2020-0134

Liguori I, Russo G, Curcio F, et al (2018) Oxidative stress, aging, and diseases. Clin Interv Aging 13:757–772. https://doi.org/10.2147/CIA.S158513

Loetchutinat C, Kothan S, Dechsupa S, et al (2005) Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′,7′-dichlorofluorescein diacetate assay. Radiat Phys Chem 72:323–331. https://doi.org/10.1016/j.radphyschem.2004.06.011

Luchese C, Barth A, da Costa GP, et al (2020) Role of 7-chloro-4-(phenylselanyl) quinoline as an anti-aging drug fighting oxidative damage in different tissues of aged rats. Exp Gerontol 130:110804. https://doi.org/10.1016/j.exger.2019.110804

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1006/ABIO.1976.9999

Nguyen LD, Ehrlich BE Cellular mechanisms and treatments for chemobrain: insight from aging and neurodegenerative diseases. https://doi.org/10.15252/emmm.202012075

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167. https://doi.org/10.1016/0165-0270(85)90031-7

Pinz M, Reis AS, Duarte V, et al (2016) 4-Phenylselenyl-7-chloroquinoline, a new quinoline derivative containing selenium, has potential antinociceptive and anti-inflammatory actions. Eur J Pharmacol 780:122–128. https://doi.org/10.1016/j.ejphar.2016.03.039

Pinz MP, dos Reis AS, Vogt AG, et al (2018) Current advances of pharmacological properties of 7-chloro-4-(phenylselanyl) quinoline: Prevention of cognitive deficit and anxiety in Alzheimer's disease model. Biomed Pharmacother 105:1006–1014. https://doi.org/10.1016/j.biopha.2018.06.049

Reis AS, Paltian JJ, Domingues WB, et al (2020a) Advances in the Understanding of Oxaliplatin-Induced Peripheral Neuropathy in Mice: 7-Chloro-4-(Phenylselanyl) Quinoline as a Promising Therapeutic Agent. Mol Neurobiol 57:5219–5234. https://doi.org/10.1007/s12035-020-02048-4

Reis AS, Paltian JJ, Domingues WB, et al (2020b) Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity: Correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline. Brain Res Bull 162:282–290. https://doi.org/10.1016/j.brainresbull.2020.06.021

Reis AS, Pinz M, Duarte LFB, et al (2017) 4-phenylselenyl-7-chloroquinoline, a novel

multitarget compound with anxiolytic activity: Contribution of the glutamatergic system. J Psychiatr Res 84:191–199. https://doi.org/10.1016/j.jpsychires.2016.10.007

Savegnago L, Vieira AI, Seus N, et al (2013) Synthesis and antioxidant properties of novel quinoline-chalcogenium compounds. Tetrahedron Lett 54:40–44. https://doi.org/10.1016/j.tetlet.2012.10.067

Shrivastava AN, Triller A, Melki R (2020) Cell biology and dynamics of Neuronal Na+/K+-ATPase in health and diseases. Neuropharmacology 169:. https://doi.org/10.1016/j.neuropharm.2018.12.008

Silva VDG, Reis AS, Pinz MP, et al (2017) Further analysis of acute antinociceptive and anti-inflammatory actions of 4-phenylselenyl-7-chloroquinoline in mice. Fundam Clin Pharmacol 31:513–525. https://doi.org/10.1111/fcp.12295

Stangherlin EC, Rocha JBT, Nogueira CW (2009) Diphenyl ditelluride impairs short-term memory and alters neurochemical parameters in young rats. Pharmacol Biochem Behav 91:430–435. https://doi.org/10.1016/j.pbb.2008.08.020

Starobova H, Vetter I (2017) Pathophysiology of chemotherapy-induced peripheral neuropathy. Front Mol Neurosci 10:1–21. https://doi.org/10.3389/fnmol.2017.00174

Miyake T, Nakamura S, Zhao M, So K, et al (2016) Cold sensitivity of TRPA1 is unveiled by the prolyl hydroxylation blockade-induced sensitization to ROS. Nat Commun 7:. https://doi.org/10.1038/NCOMMS12840

Vogt AG, Voss GT, de Oliveira RL, et al (2018) Organoselenium group is critical for antioxidant activity of 7-chloro-4-phenylselenyl-quinoline. Chem Biol Interact 282:7–12. https://doi.org/10.1016/j.cbi.2018.01.003

Walsh RN, Cummins RA (1976) The Open-Field Test : A Critical Review. 83:482–504

Wei G, Gu Z, Gu J, et al (2021) Platinum accumulation in oxaliplatin-induced peripheral neuropathy. https://doi.org/10.1111/jns.12432

Wendel A (1981) [44] Glutathione Peroxidase By. 77:

Woolfe G, Macdonald AD (1944) THE EVALUATION OF THE ANALGESIC ACTION OF PETHIDINE HYDROCHLORIDE (DEMEROL). J Pharmacol Exp Ther 80:

Zhang H, Davies KJA, Forman HJ (2015) Oxidative stress response and Nrf2 signaling in aging. Free Radic. Biol. Med. 88:314–336

Capítulo 6

4-PSQ reduz a dor oncológica

Baseado nos promissores resultados obtidos nesta tese, como uma etapa complementar, foi realizado um estudo com o objetivo de investigar os efeitos do 4-PSQ em um modelo de dor oncológica (causada por associação do câncer ao tratamento com a oxaliplatina). Estudos têm demonstrado que a dor é o sintoma mais comum, temido e oneroso presente em pacientes oncológicos, e, geralmente, aumenta com a progressão do câncer, afetando de 75 a 90 % dos pacientes com câncer metastático ou em estágio avançado. Infelizmente, esta etapa da tese não foi concluída devido à pandemia do coronavirus (Covid 19).

Para os experimentos, foram utilizados camundongos machos da raça Swiss, provenientes do biotério da Universidade Federal de Pelotas (UFPel), e todos os experimentos foram conduzidos de acordo com as normas preconizadas pela Comissão de Ética em Experimentação Animal da Universidade Federal de Pelotas (CEEA 24440-2019). Os animais foram randomicamente divididos em cinco grupos: i) Controle, ii) S180, iii) S180 + OXA, iv) S180 + 4-PSQ e, v) S180 + OXA + 4-PSQ. No primeiro dia do protocolo experimental após os testes basais, foram inoculados por via intraplantar (i.pl.) 25 µl de uma suspensão contendo a concentração de 10^6 células tumorais de sarcoma 180 (S180), na pata posterior esquerada dos camundongos dos grupos experimentais S180, S180 + OXA, S180 + 4-PSQ e, S180 + OXA + 4-PSQ. No mesmo dia, os animais do grupo Controle receberam por via i.pl. 25 µl de solução ringer com lactato (veículo) na pata posterior esquerda. Nos dias 2 e 4 do protocolo experimental foi administrado a oxaliplatina (10 mg kg^{-1}, i.p.) nos animais dos grupos S180 + OXA e, S180 + OXA + 4-PSQ. No mesmo período, os animais dos grupos Controle, S180 e, S180 + 4-PSQ receberam por via i.p. solução de glicose 5% (veículo). A partir do quarto dia do protocolo experimental, 30 minutos após a indução, por onze dias consecutivos foi administrado por via i.g. o composto 4-PSQ, na dose de 1 mg kg^{-1}, aos animais dos grupos S180 + 4-PSQ e S180 + OXA + 4-PSQ,

enquanto, para os animais dos grupos Controle, S180 e, S180 + OXA foi administrado, também por via i.g., óleo de canola (veículo) 10 ml kg^{-1}.

Nos dias 1 (basal), 9 e 13 os animais foram submetidos ao teste de alodínia mecânica (von Frey) (ALAMRI et al., 2018), e analgesia térmica (placa quente) (WOOLFE AND MACDONALD, 1944). Nos dias 11 e 12 foram avaliadas as possíveis alterações cognitivas nas memórias de curto e longo prazo, no teste do reconhecimento do objeto (STANGHERLIN et al., 2009), bem como a atividade locomotora e exploratória, no teste do campo aberto (WALSH AND CUMMINS, 1976). Por fim, no décimo quarto dia do protocolo experimental, foi avaliado o comportamento ansiogênico dos animais por meio do teste do labirinto em cruz elevado (PELLOW et al., 1985). Vinte e quatro horas após o último tratamento, os animais foram anestesiados por via inalatória com o isoflurano, um anestésico de ação central, e foram submetidos à eutanásia. Amostras de tecidos foram coletadas, pesadas e armazenadas para, posteriormente, serem utilizadas nos ensaios *ex vivo*. O edema da pata foi determinado, por meio da diferença de peso entre a pata posterior esquerda e direita do mesmo animal. Amostras dos tecidos da pata, medula espinhal e/ou córtex cerebral foram utilizadas para quantificar os níveis de espécies reativas (LOETCHUTINAT et al., 2005), de tióis não proteico (ELLMAN, 1959), e de peroxidação lipídica (OHKAWA et al., 1979). Além disso, a atividade das enzimas SOD (MISRA e FRIDOVICH, 1972), GPx (WENDEL, 1981), AChE (ELLMAN, 1961), Mg^{2+} - ATPase (TEIXEIRA et al., 2020) e Na^{+} K^{+} - ATPase (FISKE e SUBBAROW, 1925), foram determinadas. A análise estatística dos dados foi realizada por meio do programa GraphPad Prism 6.0 software (San Diego, CA, USA). Os dados foram analisados pela ANOVA de duas vias seguido pelo teste de Tukey, $P < 0,05$ foi considerado significativo.

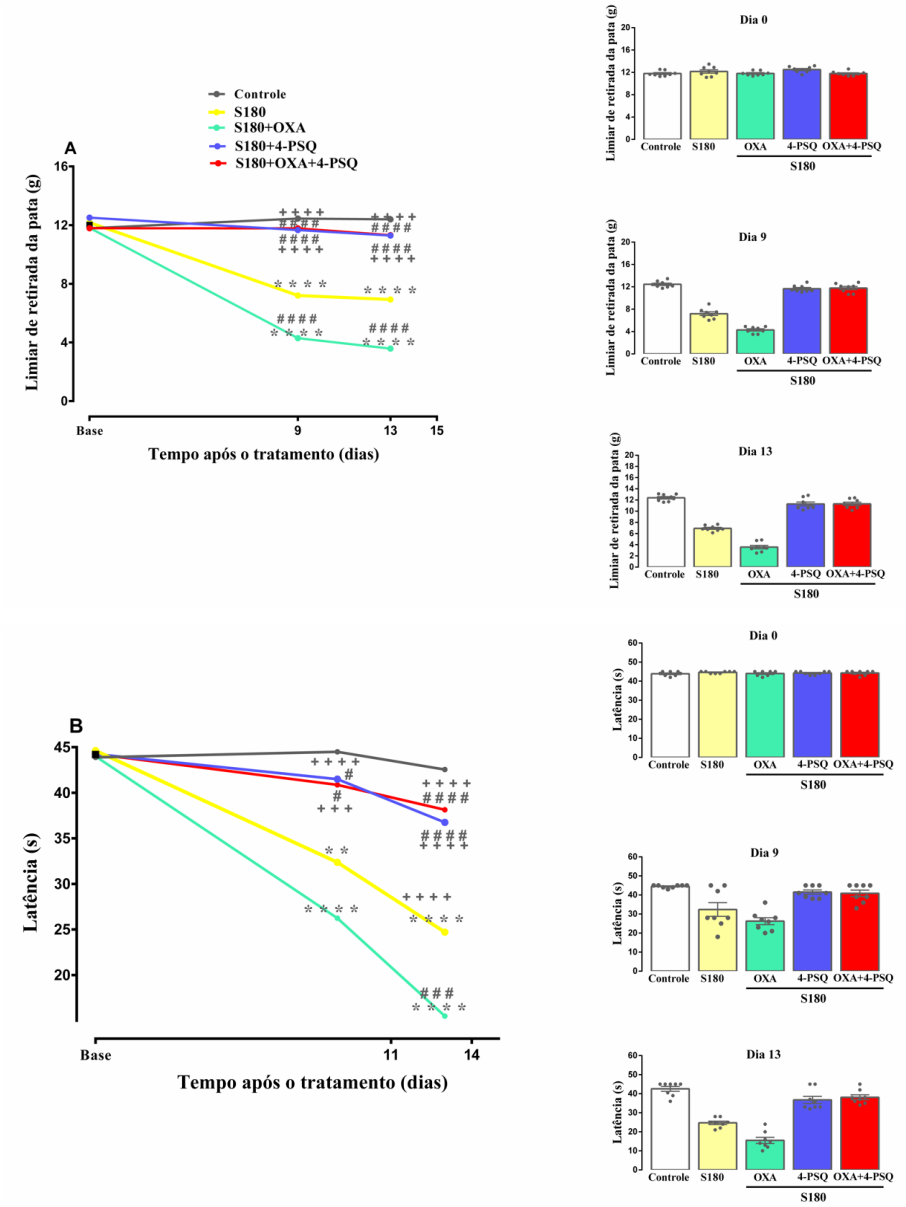


Figura 1. Efeito do 4-PSQ nas sensibilidades (A) mecânica e (B) térmica nos testes de Von Frey e placa quente, respectivamente, em um modelo de dor oncológica. (**) P < 0,01, e (****) P < 0,0001 indicam níveis de significância quando comparado ao grupo controle, (#) P < 0,05, (###) P < 0,001, e (####) P < 0,0001 comparado ao grupo S180 e (+++) P < 0,001, (++++) P < 0,0001 comparado ao grupo S180+OXA. (ANOVA de duas vias, seguido pelo teste de Tukey).

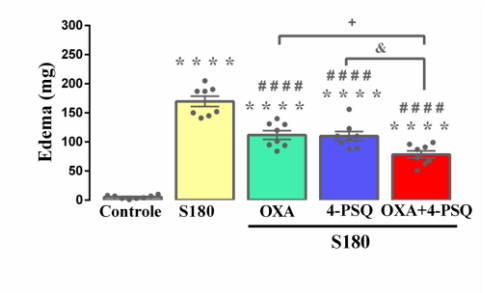


Figura 2. Efeito do 4-PSQ no edema de pata em um modelo de dor oncológica. (****) P < 0,0001 indica nível de significância quando comparado ao grupo controle, (####) P < 0,0001 comparado ao grupo S180, (+) P < 0,05 comparado ao grupo S180+OXA, e (&) P < 0,05 comparado ao grupo S180+4-PSQ. (ANOVA de duas vias, seguido pelo teste de Tukey).

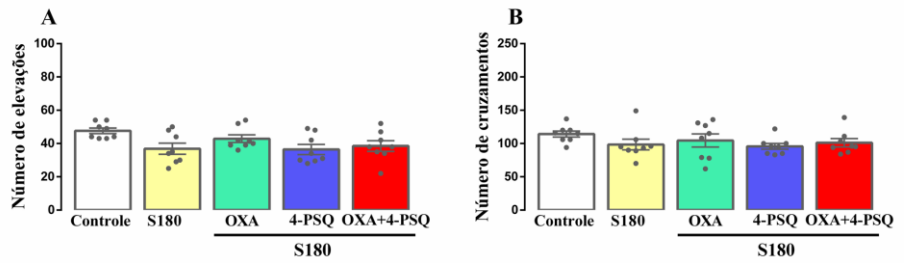


Figura 3. Efeito do 4-PSQ no (A) número de elevações, e (B) no número de cruzamentos, no teste do campo aberto em um modelo de dor oncológica. (ANOVA de duas vias, seguido pelo teste de Tukey).

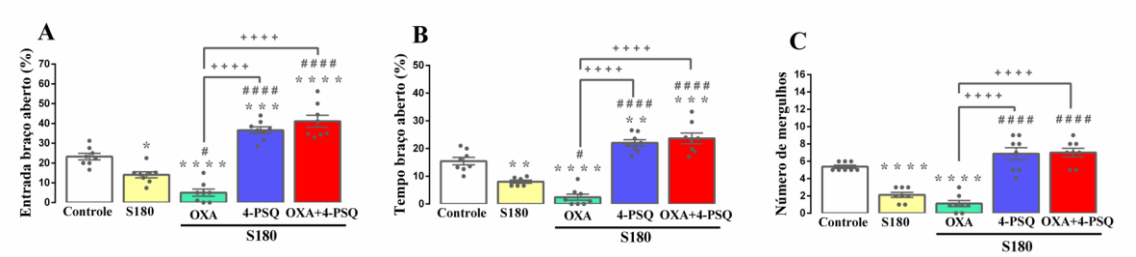


Figura 4. Efeito do 4-PSQ no (A) percentual de entrada nos braços abertos, (B) percentual de tempo gasto nos braços abertos, e no (C) número de mergulho, no teste do labirinto em cruz elevada em um modelo de dor oncológica. (*) P < 0,05, (**) P < 0,01, (***) P < 0,001, e (****) P < 0,0001 indicam níveis de significância quando comparado ao grupo controle, (#) P < 0,05, (####) P < 0,0001 comparado ao grupo S180, e (++++) P < 0,0001

comparado ao grupo S180+OXA. (ANOVA de duas vias, seguido pelo teste de Tukey).

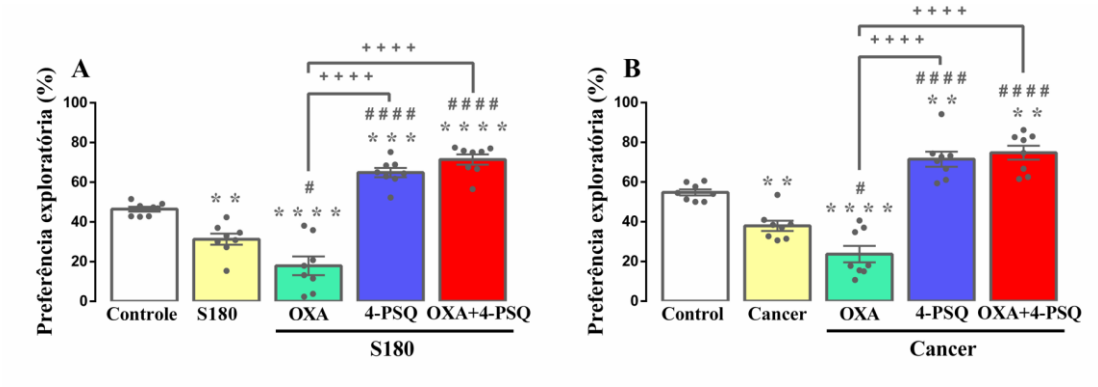
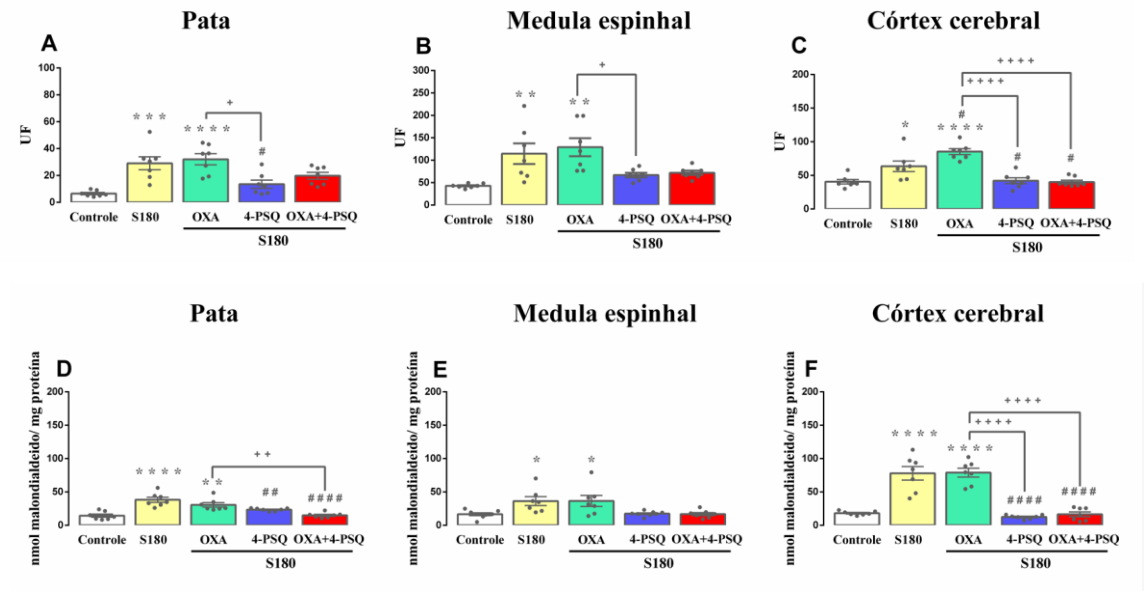


Figura 5. Efeito do 4-PSQ no percentual de preferência exploratória na memória de (A) curto, e (B) longo prazo, no teste do reconhecimento do objeto em um modelo de dor oncológica. (**) P < 0,01, (***) P < 0,001, e (****) P < 0,0001 indicam níveis de significância quando comparado ao grupo controle, (#) P < 0,05 e (####) P < 0,0001 comparado ao grupo S180, e (++++) P < 0,0001 comparado ao grupo S180+OXA. (ANOVA de duas vias, seguido pelo teste de Tukey).



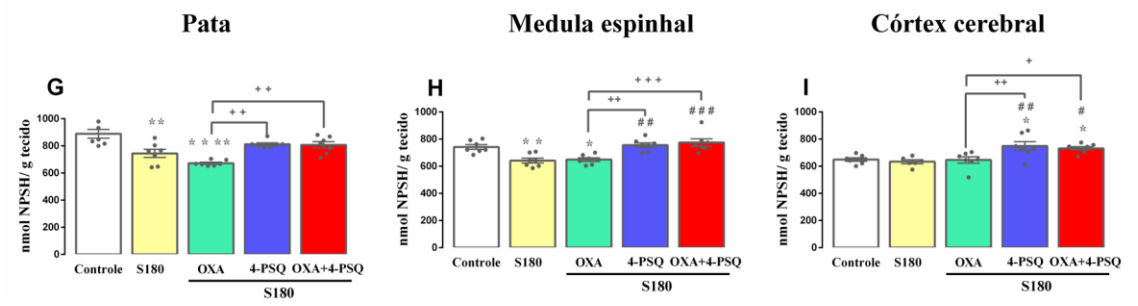


Figura 6. Efeito do 4-PSQ nos níveis de espécies reativas na (A) pata, (B) medula espinhal, e (C) córtex cerebral; nos níveis de espécies reativas ao ácido tiobarbitúrico na (D) pata, (E) medula espinhal, e (F) córtex cerebral; e nos níveis de tióis não proteico na (G) pata, (H) medula espinha, e (I) córtex cerebral de camundongos em um modelo de dor oncológica. (*) P < 0,05, (**) P < 0,01, (***) P < 0,001, e (****) P < 0,0001 indicam níveis de significância quando comparado ao grupo controle, ($^{\#}$) P < 0,05, ($^{\#\#}$) P < 0,01, ($^{\#\#\#}$) P < 0,001, ($^{\#\#\#\#}$) P < 0,0001 comparado ao grupo S180, e ($^{+}$) P < 0,05, ($^{++}$) P < 0,01, ($^{+++}$) P < 0,001 e ($^{++++}$) P < 0,0001 comparado ao grupo S180+OXA (ANOVA de duas vias, seguido pelo teste de Tukey).

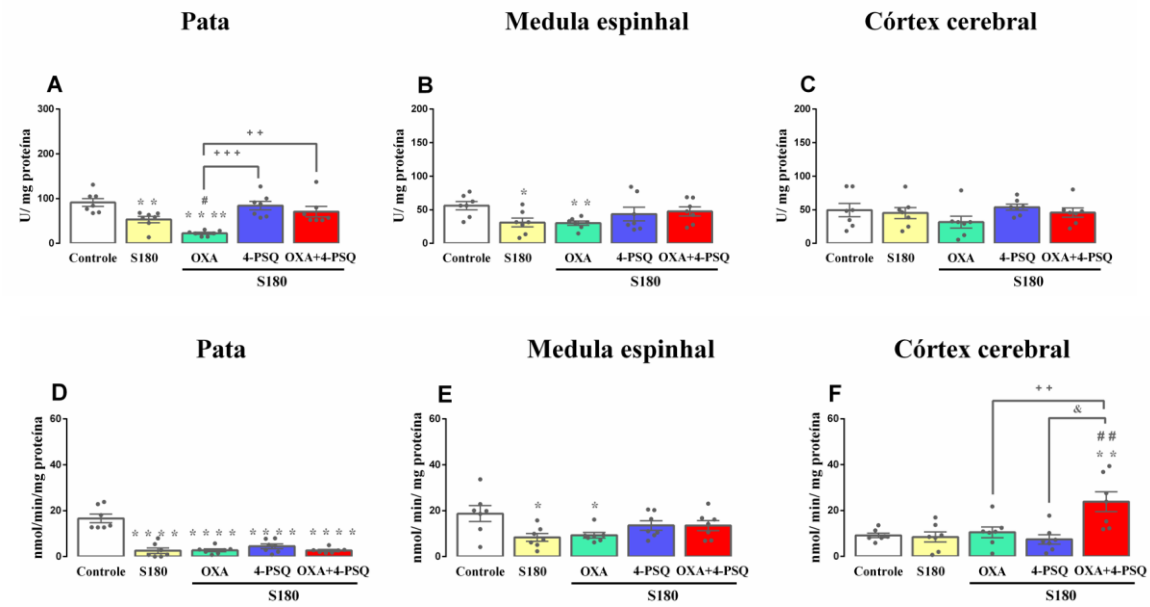


Figura 7. Efeito do 4-PSQ na atividade da enzima superóxido dismutase na (A) pata, (B) medula espinhal, e (C) córtex cerebral; e na atividade da enzima glutationa peroxidase na (D) pata, (E) medula espinhal, e (F) córtex cerebral de camundongos em um modelo de dor oncológica. (*) P < 0,05, (**) P

< 0,01, e (****) P < 0,0001 indicam níveis de significância quando comparado ao grupo controle, (#) P < 0,05 e (##) P < 0,01 comparado ao grupo S180, (++) P < 0,01 (+++) e P < 0,001 comparado ao grupo S180+OXA, e (&) P < 0,05 comparado ao grupo S180+4-PSQ. (ANOVA de duas vias, seguido pelo teste de Tukey).

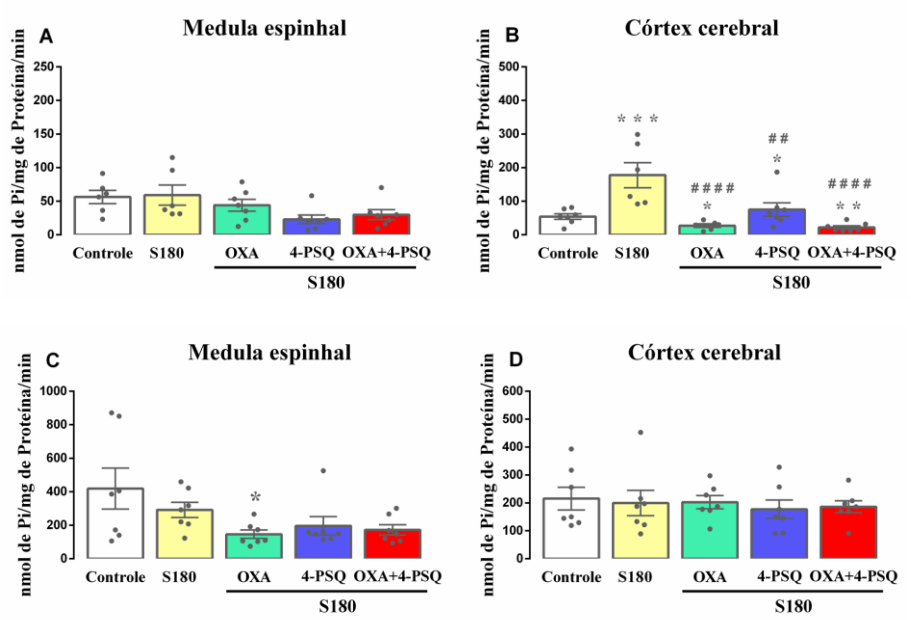


Figura 8. Efeito do 4-PSQ na atividade da enzima Na^+ k^+ - ATPase na (A) medula espinhal, e no (B) córtex cerebral; e atividade da enzima Mg^{2+} - ATPase na (C) medula espinhal e no (D) córtex cerebral de camundongos em um modelo de dor oncológica. (*) P < 0,05, (**) P < 0,01, e (***) P < 0,001 indicam níveis de significância quando comparado ao grupo controle, e (##) P < 0,01 e (####) P < 0,0001 comparado ao grupo S180. (ANOVA de duas vias, seguido pelo teste de Tukey).

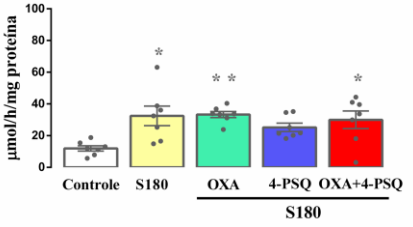


Figura 9. Efeito do 4-PSQ na atividade da enzima acetilcolinesterase no córtex cerebral de camundongos em um modelo de dor oncológica. (*) P < 0,05

indica nível de significância quando comparado ao grupo controle. (ANOVA de duas vias, seguido pelo teste de Tukey).

Os resultados preliminares indicaram que no modelo de dor oncológica, a hipersensibilidade mecânica e térmica (Figura 1A e 1B, respectivamente), o comportamento ansiogênico (Figura 4), e o declínio cognitivo (Figura 5) causado pelo câncer (S180), foram agravados pela administração concomitante de oxaliplatina. Este agravamento deu-se principalmente por meio de dano oxidativo, evidenciado através do aumento das espécies reativas e peroxidação lipídica, além da redução nos níveis de tióis não proteico (Figura 6), e modulação das enzimas antioxidantes SOD e GPx (Figura 7). Além disso, a atividade das enzimas Na^+, K^+ - ATPase (Figura 8) e AChE (Figura 9) foram aleradas no SNC. O edema da pata, induzido pelas células S180, foi reduzido por meio da administração da oxaliplatina. Nenhuma alteração na atividade locomotora e exploratória dos animais foi observada (Figura 3). De fato, o modelo de dor oncológica desenvolvido para esse estudo, apresentou-se promissor.

Ainda, podemos destacar os efeitos do 4-PSQ, uma vez que, o tratamento com o composto reduziu a dor oncológica e suas comorbidades. Nesse modelo, o 4-PSQ evidenciou-se como um potente antioxidante. Apoiando essa afirmativa, o 4-PSQ restaurou a atividade da enzima Na^+, K^+ - ATPase, reiterando sua ação neuroprotetora. Importamtemente, o 4-PSQ reduziu o edema da pata dos animais, em relação ao grupo S180+OXA, assim sendo, pode-se sugerir que o composto reduz a dor oncológica e suas comorbidades sem interferir na ação farmacológica da oxaliplatina. Entretanto, demais análises estão sendo realizadas com o objetivo de elucidar a interação entre o 4-PSQ e a oxaliplatina nas células tumorais.

5 DISCUSSÃO

O presente estudo estabeleceu que o envelhecimento exacerbou a NPIO, incluindo os sintomas agudos e crônicos. De fato, foi demonstrado pela primeira vez, que apesar das defesas eficientes do SNC, a platina, proveniente do tratamento com a oxaliplatina, permeia a barreira hematoencefálica, se acumula na medula espinhal e no cérebro, aumenta a produção de espécies reativas e, leva a um desequilíbrio redox no SNC. Em relação às defesas antioxidantes, diante dos danos oxidativos causados pela administração da oxaliplatina, evidenciou-se que organismos jovens apresentam aumento na atividade e redução na expressão gênica de algumas enzimas antioxidantes. Nesse sentido, os dados sugerem que, apesar de uma tentativa inicial de combater o estresse oxidativo, a exposição continuada à oxaliplatina conduz ao esgotamento das defesas antioxidantes. Por outo lado, os organismos envelhecidos apresentaram um sistema antioxidante deficitário, ou seja, com o aumento na produção de espécies reativas causado pela administração de oxaliplatina, o sistema antioxidante enzimático não foi capaz de aumentar a atividade das enzimas, investigadas aqui, a fim de evitar o desequilíbrio redox.

O dano oxidativo causado pela administração da oxaliplatina ao SNC foi extenso, mas, quando associado ao envelhecimento, foi consideravelmente maior. O resultado dos danos oxidativos ao SNC foi constatado através do aumento dos níveis de espécies reativas totais e, especificamente, do NOx, bem como, por meio do aumento da peroxidação lipídica dos tecidos. Ainda, a intensidade dos danos foi observada, indiretamente, por meio do agravamento dos sintomas clínicos analisados. Está bem estabelecido que a administração de oxaliplatina causa dor neuropática e comorbidades (TOFTHAGEN et al., 2013; ARETI et al., 2014; STAROBOVA e VETTER, 2017; SALES et al., 2019). No presente estudo foi demonstrado que a administração de oxaliplatina causou hiperalgesia, comportamento ansioso e déficit cognitivo. Cabe destacar, que o quadro clínico foi consideravelmente agravado pelo envelhecimento. Corroborando com os resultados deste estudo, dados publicados anteriormente demonstraram que o processo de envelhecimento aumenta a produção de espécies reativas e, consequentemente, causa desequilíbrio do estado redox

celular. Com isso, o envelhecimento reduz a capacidade das células de manter seu proteoma (KOROVILA et al., 2017).

Neste contexto, há evidências que o estresse oxidativo modula a atividade da enzima Na^+, K^+ - ATPase. Em diversas doenças neurológicas, o aumento do estresse oxidativo devido à redução da atividade mitocondrial, como causado pela oxaliplatina, leva à redução da síntese de adenosina trifosfato (ATP) no SNC, prejudicando as funções da enzima Na^+, K^+ - ATPase. Além disso, sabe-se que o envelhecimento também causa declínio na atividade da Na^+, K^+ - ATPase, por meio da modulação na via glutamatérgica (KINOSHITA et al., 2016). Os resultados obtidos nesta tese estão de acordo com os publicados, uma vez que a oxaliplatina, assim como o envelhecimento, inibiram a enzima Na^+, K^+ - ATPase. Ainda, a partir da análise dos dados, podemos sugerir que o desequilíbrio redox, induzido por ambos os fatores, contribuiu para as alterações observadas na atividade e expressão dessa enzima. Baseado nessas evidências, acreditamos que a modulação da enzima Na^+, K^+ - ATPase é um mecanismo relevante na gênese da NPIO, e pode ser um alvo farmacológico para o tratamento da dor e das comorbidades relacionadas à neurotoxicidade induzida pela oxaliplatina, principalmente, quando o fator envelhecimento é considerado.

No presente estudo foi demonstrada pela primeira vez a correlação entre o comportamento ansioso e o prejuízo cognitivo induzidos pela exposição à oxaliplatina. Além disso, salientamos que essas comorbidades foram agravadas pelo envelhecimento. MOSELEY e colaboradores (2007) relataram que uma redução na atividade da enzima Na^+, K^+ - ATPase no SNC compromete significativamente redes neuronais envolvidas nos processos cognitivos. Além disso, diversos estudos têm proposto que a atividade disfuncional da Na^+, K^+ - ATPase no SNC está envolvida na fisiopatologia de distúrbios neuropsiquiátricos, como a ansiedade (GOLDSTEIN et al., 2006; KURAUCHI et al., 2019). Diante disso, podemos sugerir que a inibição da enzima Na^+, K^+ - ATPase está relacionada com o comportamento ansioso e o prejuízo cognitivo induzidos pela administração da oxaliplatina. Esses dados corroboram nossa hipótese sobre a modulação da enzima Na^+, K^+ - ATPase

como um alvo promissor para o tratamento da neurotoxicidade induzida pela oxaliplatina.

Compreendendo que a fisiopatologia da NPIO é um processo multifatorial (ARETI et al., 2014), nesse estudo buscamos explorar novos mecanismos envolvidos nesse efeito adverso do medicamento, a fim de, prover uma nova perspectiva sobre a patologia, sua interação com os processos fisiológicos inerentes do envelhecimento, e elucidar novos alvos farmacológicos para o desenvolvimento de uma terapia segura e eficaz. Tendo em vista que os danos induzidos pela oxaliplatina ao SNP têm sido amplamente investigados, aqui nós buscamos novos mecanismos direcionando as análises para as alterações causadas ao SNC. Diante disso, está definido que níveis apropriados de bioelementos são cruciais para a manutenção das condições fisiológicas do SNC (KABIR et al., 2021). E, paralelamente, está bem estabelecido que os bioelementos desempenham um papel importante nos processos do envelhecimento, assim como, que o envelhecimento pode desequilibrar suas concentrações. Aqui, nós investigamos a contribuição dos bioelementos para a NPIO.

Nossos resultados revelaram que há uma relação entre NPIO e alterações na concentração de bioelementos no SNC. Entretanto, os resultados obtidos até o momento ainda não nos permitem definir o papel definitivo dos bioelementos na fisiopatologia da NPIO. De fato, a administração da oxaliplatina alterou a concentração de diversos bioelementos no SNC, incluindo cálcio, potássio, fósforo, enxofre, selênio, magnésio, manganês, ferro, cobalto, cobre e zinco. A exposição à oxaliplatina modificou os níveis de fósforo, enxofre, selênio, magnésio, manganês, cobre, e potássio quando associada ao envelhecimento, enquanto, na ausência desse fator, só alterou as concentrações de cálcio e manganês. Ainda não temos respostas para essa diferença induzida pelo tratamento com oxaliplatina em diferentes idades. Nossas evidências sugerem que algo está levando a mudanças maiores em idosos. Assim, uma intervenção nutricional em idosos em tratamento com oxaliplatina requer atenção.

Os bioelementos cumprem funções decisivas para a manutenção da saúde humana, e suas deficiências ou acúmulo levam a condições patológicas indesejáveis. De fato, os bioelementos são cofatores necessários para o funcionamento adequado do metabolismo e desempenham um papel fundamental na sobrevivência e na longevidade. O magnésio desempenha papel crucial em vários mecanismos celulares, como na plasticidade sináptica e no metabolismo energético (KABIR et al., 2021). Além disso, para o bom desempenho do sistema antioxidante e manutenção do equilíbrio redox, as enzimas requerem cofatores, como selênio, ferro, cobre, zinco e manganês (MEHTA e GOWDER, 2015). Há evidências de que as deficiências de nutrientes, incluindo vitaminas e minerais, estão associadas às doenças crônicas e ao envelhecimento prematuro (AMES, 2018; KABIR et al., 2021). Por exemplo, deficiências de fósforo podem levar à fraqueza muscular, enquanto a falta de selênio está associada às doenças musculares. Além disso, magnésio, selênio e cálcio são minerais promissores para prevenir e/ou tratar a sarcopenia, que é a perda de massa muscular, força muscular e desempenho físico relacionada à idade, além de apoiar um envelhecimento saudável sem dor (VAN DRONKELAAR et al., 2018).

No entanto, a suplementação deve ser controlada com cautela, uma vez que, efeitos tóxicos são atribuídos a alguns bioelementos quando presentes em quantidades superiores às necessárias para o cumprimento de suas funções biológicas (FRAGA, 2005, KAUR et al., 2019; MIQUEL et al., 2018). Considerando os resultados observados nesse estudo, podemos sugerir que as mudanças observadas na concentração dos elementos contribuem para o desenvolvimento da NPIO, predominantemente em idosos. Está bem definido, que desequilíbrios na homeostase dos bioelementos intracelulares estão ligados a dano oxidativo, redução da plasticidade sináptica, déficit neurológico e envelhecimento prematuro (KABIR et al., 2021).

Curiosamente, os dados indicam que ocorre uma convergência dos mecanismos envolvidos na NPIO, investigados nesse estudo e em outros (ARETI 2014; CAVALETTI e MARMIROLI, 2020), para um ponto em comum, o estresse oxidativo. Nesse contexto, podemos destacar a importância da

compreensão do alcance de suas implicações na NPIO. Considerando que o desequilíbrio redox causado pela neurotoxicidade induzida pela administração de oxaliplatina foi potencializado pelo envelhecimento, uma questão imediata que surgiu no desenvolvimento deste estudo foi se a associação de ambos os fatores poderia alterar a via neuroprotetora mediada pelo Nrf2. O Nrf2 é um regulador da resposta ao estresse oxidativo, destacando seu efeito no controle de qualidade mitocondrial em condições de desequilíbrio redox (KASAI et al., 2019). Estudos têm sugerido que, devido aos efeitos do Nrf2, sua modulação parece ser uma estratégia interessante de abordagem para o tratamento da NPIO (YANG et al., 2018) e, também, para prevenir a perda da resposta de proteção celular durante o envelhecimento (SILVA- PALACIOS et al., 2018). Aqui, a análise dos resultados revelou que o envelhecimento por si só reduziu a expressão de Nrf2 no SNC. Por outro lado, quando associado à administração da oxaliplatina, os níveis de expressão do Nrf2 foram alterados de forma diferente, dependente do tecido analisado no SNC. Ou seja, em algumas regiões houve aumento, enquanto, outras apresentaram redução da expressão do Nrf2. A regulação negativa do Nrf2 está de acordo com outros resultados obtidos no estudo, como a redução na expressão das enzimas SOD, CAT e GPx, uma vez que o Nrf2 controla a expressão de genes cujos produtos incluem enzimas antioxidantes. No entanto, resultados divergentes foram observados no córtex cerebral, onde, a associação entre o envelhecimento e o tratamento com a oxaliplatina levou à regulação positiva do Nrf2. Diante disso, os resultados sugerem que neste contexto, a resposta antioxidante mediada pelo Nrf2 falhou.

O Nrf2 forma um complexo com Keap1 no citosol celular, mas para a ativação do Nrf2, a separação do complexo é um pré-requisito essencial. Essa dissociação pode ocorrer por meio da ativação de quinases como fosfatidilinositol 3-quinases (PI3K), que fosforilam o Nrf2 ou pode ser mediada por estresse oxidativo. O Nrf2 dissociado transloca o núcleo da célula, onde se liga ao elemento de resposta antioxidante (ARE) após formar um heterodímero com outras proteínas, como o fator de ativação da transcrição 4 (ATF4). Por sua vez, a proteína ATF4, um gene protetor que regula a adaptação de células

a fatores de estresse, como estresse de retículo endoplasmático e estresse oxidativo, pode funcionar tanto como um ativador transcricional como repressor, incluindo da via do Nrf2 (KASAI et al., 2019). De fato, os resultados obtidos aqui confirmaram que o envelhecimento reduziu também a expressão de PI3K no cérebro, mas não na medula espinhal. Por sua vez, na medula espinhal, o tratamento com a oxaliplatina regulou a expressão de PI3K de acordo com a idade, enquanto os jovens apresentaram superexpressão, os mais velhos apresentaram uma regulação negativa da expressão do PI3K. Nesse sentido, foi previamente estabelecido que distúrbios mitocondriais, como os causados pela oxaliplatina, podem ativar indiretamente o PI3K (KASAI et al., 2019) como forma de defesa. Aqui, nós observamos que, quando houve a associação entre o envelhecimento e o tratamento com a oxaliplatina, esse mecanismo foi ativado apenas no córtex cerebral. Além disso, foi demonstrado que a associação dos fatores regulou negativamente a expressão de ATF4. Esses achados são consistentes com os resultados publicados por HUSSAIN e RAMAIAH (2007). YANG e colaboradores (2018) demonstraram, que a regulação negativa do Nrf2 agravou a NPIO, principalmente, por meio do aumento da produção de espécies reativas, diminuição do potencial de membrana mitocondrial, que levou a níveis anormais de cálcio intracelular e induziu apoptose relacionada ao citocromo C (Cyt C) e, superexpressão da família de proteínas do receptor de potencial transitório (TRP) que favorece a dor neuropática. Esses fatos em conjunto sugerem que a via de neuroproteção mediada pelo Nrf2 pode desempenhar um papel crítico, mas ainda pouco esclarecido, na NPIO, particularmente em idosos, que são mais suscetíveis aos danos oxidativos.

Um dos sintomas clínicos da NPIO é a dor neuropática, além disso, geralmente a NPIO está associada à comorbidades como a ansiedade, a depressão e a incapacidade, e por isso o seu manejo e/ou tratamento representa um desafio (WANG e MULLALLY, 2020). De acordo com os dados obtidos, foi demonstrado que a administração da oxaliplatina causou dor neuropática, como evidenciado pela hipersensibilidade mecânica e térmica (frio e calor). Ainda, nós comprovamos que o envelhecimento exacerbou a dor

neuropática, assim como, as demais comorbidades (dependendo do modelo animal estudado). Nesse sentido, para compreender melhor o agravamento desse quadro clínico e as alterações causadas no SNC, a via de sinalização do BDNF foi investigada. O BDNF é uma neurotrofina secretada para promover a neurogênese. Considerando que a neurogênese fornece condições para restaurar neurônios perdidos devido ao desgaste diário e, que a neurogênese reduzida é um fator comum no envelhecimento e em doenças neurodegenerativas, a identificação de biomarcadores capazes de aumentar a sobrevivência celular também pode ser uma estratégia contra o estabelecimento da NPIO em idosos (NGUYEN e EHRLICH, 2020). Esta neurotrofina também desempenha um papel crucial como neuromodulador na transmissão da dor. Além disso, é amplamente aceito o envolvimento do BDNF em processos celulares que fundamentam a cognição e outros comportamentos complexos como a ansiedade (NOTARAS e VAN DEN BUUSE, 2020).

Os dados coletados neste estudo indicaram que o tratamento com a oxaliplatina reduziu a expressão de BDNF no SNC, independentemente da idade. Após um aumento inicial nos níveis de BDNF, que favorece a neuroplasticidade e leva à dor crônica, conforme definido por estudos anteriores (CAPPOLI et al., 2020; NIJS et al., 2015), os níveis de BDNF diminuem no SNC, como evidenciado pelos resultados obtidos nesse estudo, reduzindo a neuroplasticidade e apoiando a manutenção da dor crônica. De fato, os resultados sugerem que os níveis reduzidos de BDNF causados pela exposição à oxaliplatina e pelo envelhecimento estão relacionados à dor neuropática, prejuízo cognitivo e comportamento ansioso. Essa hipótese é corroborada por CAPPOLI e colaboradores (2020). Os autores relataram que níveis reduzidos de BDNF foram associados aos comportamentos depressivos e ansiosos elevados e memória de trabalho prejudicada devido à neuroplasticidade reduzida.

O BDNF também pode modular a regulação gênica pela ativação de fatores de transcrição, incluído o fator nuclear kappa B (NF-κB) e a proteína de ligação responsiva ao AMPc (CREB) (LIMA GIACOBBO et al., 2019). O fator

de transcrição CREB medeia a transcrição de genes essenciais para a sobrevivência e a diferenciação dos neurônios e, a transcrição do próprio BDNF (LIMA GIACOBBO et al., 2019). Nossos resultados demonstraram que o envelhecimento reduziu a expressão do CREB no SNC, enquanto a administração da oxaliplatina reduziu a expressão do CREB independentemente da idade. Porém, nós destacamos que os mais velhos apresentaram níveis de CREB mais baixos em comparação com os mais jovens. Esses achados corroboram os que se referem à expressão do BDNF alcançada neste estudo. Por sua vez, o fator de transcrição nuclear NF-κB desempenha um papel regulador na resposta inflamatória e na progressão de tumores (WANG et al., 2019).

Aqui, o envelhecimento aumentou o nível de NF-κB no SNC. Esses resultados corroboram outros dados obtidos neste estudo, que demonstraram aumento do nível de espécies reativas e redução da expressão do BDNF, induzidos pelo envelhecimento. De fato, os resultados sugerem que a via do NF-κB é ativada no envelhecimento, principalmente por meio do estresse oxidativo. A administração de oxaliplatina, por sua vez, aumentou a expressão de NF-κB apenas nos mais jovens. O aumento dos níveis de NF-κB contribui para a produção e liberação de citocinas pró-inflamatórias (WANG et al., 2019), o que leva à sensibilização periférica principalmente por meio da ativação do TRP vanilóide (ARETI et al., 2014), corroborando os resultados obtidos neste estudo sobre o papel do envelhecimento no aumento da sensibilidade mecânica.

O comprometimento cognitivo induzido pelo envelhecimento é mediado principalmente por neuroinflamação, aqui demonstrado por meio da regulação gênica do NF-κB, e disfunção colinérgica (BENFANTE et al., 2019). Portanto, outra hipótese em torno do declínio cognitivo causado pela administração da oxaliplatina envolve o sistema colinérgico. De fato, a exposição à oxaliplatina reduziu a expressão e aumentou a atividade da AChE no SNC, em ambas as idades. A neurotransmissão colinérgica é alterada com o envelhecimento, levando ao comprometimento cognitivo, principalmente por meio do desenvolvimento de degeneração neuronal e interrupção do circuito neural

normal (RICHARDSON et al., 2020). Esses achados apoiam o papel do envelhecimento no comprometimento cognitivo observado no presente estudo. Porém, considerando parte da neurotransmissão colinérgica, em particular os níveis de atividade e expressão da AChE, o envelhecimento não exacerbou os efeitos da administração da oxaliplatina.

Resumidamente, o conjunto de resultados obtidos nesse estudo demonstrou que após a exposição à oxaliplatina ocorre um acúmulo de platina no SNC de camundongos. Diante disso, sugerimos que o acúmulo de platina no SNC pode causar desequilíbrio redox celular e, prejudicar a função mitocondrial. Aqui, foi relatado que o envelhecimento exacerbou a dor neuropática induzida pela NPIO, além de suas comorbidades, incluindo o comportamento ansioso e o prejuízo cognitivo, principalmente por meio do estresse oxidativo, disfunção das enzimas Na^+, K^+ - ATPase e AChE, alteração da concentração de bioelementos, e modulação das vias de neuroproteção e de neuroplasticidade. Vale salientar, que todos os danos foram causados ao SNC. Parte do objetivo desta tese consistiu em fornecer uma base para as intervenções terapêuticas na NPIO, considerando as especificidades da idade. Enquanto a outra parte buscou por uma molécula inovadora, com efeitos promissores, que possa vir a se tornar um medicamento estratégico para o tratamento ou prevenção da NPIO com eficácia e segurança, considerando as características da idade.

Neste contexto, o 4-PSQ foi a molécula selecionada para o estudo. O 4-PSQ é um composto organosselênio e um derivado da quinolina, que têm sido extensivamente estudado por nosso grupo de pesquisa (PINZ et al., 2016, 2018; SILVA et al., 2017; VOGT et al., 2018; VOSS et al., 2018; BARTH et al., 2019;; LEMOS et al., 2020; LUCHESE et al., 2020; PALTIAN et al., 2020; REIS et al., 2020b, 2020a, 2017; da MOTTA et al., 2021; RODRIGUES et al., 2021), e outros (SAVEGNAGO et al., 2013; DUARTE et al., 2017; SALGUEIRO et al. , 2017; DE FREITAS COUTO et al., 2019; DE AQUINO SILVA et al., 2021). De fato, os efeitos apresentados pelo 4-PSQ nos estudos pré-clínicos, acima referenciados, foram proeminentes.

No presente estudo, o 4-PSQ reverteu a hipersensibilidade mecânica e térmica induzida pela exposição à oxaliplatina, sem prejudicar a capacidade locomotora e exploratória dos animais jovens e velhos. Neste estudo, o 4-PSQ reverteu também a hipersensibilidade ao frio, principal sintoma da NPAIO que acomete em torno de 90% dos pacientes e pode manifestar-se horas após a infusão do fármaco, em ambas as idades. Corroborando esses resultados, foi previamente demonstrado que o 4-PSQ apresenta efeito antinociceptivo em doses baixas, a partir de 0,1 mg kg^{-1}, em modelos de nocicepção aguda químico e térmico (PINZ et al., 2016).

Neste estudo, o 4-PSQ restaurou o comprometimento emocional e o declínio cognitivo induzidos pela administração de oxaliplatina. De fato, os prejuízos emocionais e cognitivos são amplamente aceitos como comorbidades associadas a NPIO. Além disso, corroborando o efeito ansiolítico, o 4-PSQ reduziu os níveis de corticosterona no plasma, regulando o eixo hipotálamo-hipófise-adrenal (HPA). De particular importância, o 4-PSQ restabeleceu a atividade e/ou a expressão da Na^+, K^+ - ATPase em jovens e idosos, um dos mecanismos que se destacou como um alvo farmacológico promissor, para o tratamento da NPIO e suas comorbidades. Consequentemente, o tratamento com o 4-PSQ restabeleceu o potencial elétrico das células cerebrais e o gradiente iônico para a excitabilidade neuronal, necessária para a manutenção dos processos celulares cerebrais. Além disso, as alterações induzidas pela administração da oxaliplatina na atividade e expressão da enzima AChE no SNC foram normalizadas pelo 4-PSQ, dependendo do modelo estudado. Assim, nós destacamos a importância destes resultados para o restabelecimento das funções no SNC na NPIO.

Diante do extenso desequilíbrio redox causado pela associação entre o envelhecimento e o tratamento com a oxaliplatina, nesse estudo se destacou o potencial antioxidante do 4-PSQ. De fato, a administração diária do 4-PSQ, na dose de 1 mg kg^{-1}, reverteu o desequilíbrio oxidativo causado pela oxaliplatina, nas duas idades analisadas. Aqui, de particular importância, o tratamento com o 4-PSQ reverteu o aumento das espécies reativas e a peroxidação lipídica no SNC e no nervo ciático, SNP. Está bem estabelecido que os metabólitos da

oxaliplatina se acumulam nos nervos periféricos e causam danos oxidativos. Diante disso, foi evidenciado que o 4-PSQ reverteu os danos causados pela administração da oxaliplatina e exacerbados pela idade, tanto no SNC, foco deste estudo, quanto no SNP. O 4-PSQ aumentou os níveis de glutationa, contribuindo para o equilíbrio redox celular.

De fato, nós demonstramos que quando administrada à camundongos jovens, a oxaliplatina modulou positivamente o sistema antioxidante enzimático no SNC, para equilibrar o desequilíbrio redox causado pelo excesso de moléculas pró-oxidantes. Nesse caso, o tratamento com o 4-PSQ normalizou a atividade das enzimas antioxidantes estudadas. Esse resultado pode ser explicado pelo efeito antioxidante do 4-PSQ, pois a redução das moléculas pró-oxidantes pode contribuir para a normalização das defesas antioxidantes. No entanto, quando os camundongos envelhecidos receberam a oxaliplatina, não houve aumento na atividade das enzimas antioxidantes no SNC, mesmo diante de um aumento das moléculas pró-oxidantes. Neste caso, o tratamento com 4-PSQ modulou positivamente o sistema antioxidante enzimático no SNC, para combater o excesso de espécies reativas. Neste contexto, destacamos que o composto, modulou o sistema antioxidante de acordo com a necessidade específica de cada idade, e conduziu ao equilíbrio redox tanto nos jovens quanto nos idosos. Recentemente, foi demonstrado que o 4-PSQ restaurou os níveis de selênio e contribui para a restauração dos danos causados pelo envelhecimento em ratos. Os autores sugerem que o composto poderia ser um candidato promissor para o desenvolvimento de uma nova terapia para o gerenciamento dos danos oxidativos relacionados à idade, atuando como um fármaco anti-envelhecimento (LUCHESE et al., 2020.

Consistente com os resultados observados nesse estudo, o tratamento com o 4-PSQ provoca efeitos antinociceptivos, ansiolíticos, antioxidantes e neuroprotetores em modelos animais. As ações farmacológicas do 4-PSQ parecem estar relacionadas às suas atividades antioxidantes e anti-inflamatórias, bem como sua capacidade de modular os sistemas serotonérgico, nitrérgico, glutamatérgico e colinérgico (REIS et al., 2017; SILVA et al., 2017; BARTH et al., 2019; LUCHESE et al., 2020; PALTIAN et al., 2020).

Ainda, particularmente importante dentre os efeitos do 4-PSQ, destacamos que o ele reverteu o dano hepático e renal induzidos pela administração da oxaliplatina (LEMOS et al., 2020; DA MOTTA et al., 2021). É importante mencionar que o 4-PSQ é uma molécula multialvo, o que certamente contribuiu para os efeitos relevantes demonstrados neste estudo. De fato, considerando o perfil multifatorial da NPIO, certamente, isso é uma vantagem.

Diante dos resultados apresentados pelo 4-PSQ no tratamento da NPIO, investigamos seu potencial farmacológico em um modelo de dor oncológica. De fato, os resultados desta molécula são impressionantes, entretanto, até a 5° etapa do estudo, temos somente indícios de que o 4-PSQ não interfere no efeito farmacológico da oxaliplatina. Nesse sentido, nesta última etapa, procuramos, também, avançar nesse sentido e elucidar se o 4-PSQ compromete a ação antineoplásica da oxaliplatina. O modelo de dor oncológica, desenvolvido para esse estudo, demosntrou-se promissor, uma vez que, a associação entre o câncer (S180) e o tratamento com oxaliplatina, agravou sintomas e comorbidades, relacionados à dor oncológica, pricipalmente, por meio do aumento do dano oxidativo e modulação da enzima Na^+, K^+ - ATPase. Corroborando os dados anteriores obtidos no estudo, o tratamento com o 4-PSQ reverteu a hipersensibilidade e as comorbidades associadas à dor oncológica. Novamente, o efeito antioxidante do 4-PSQ reduziu o desequibrio redox e normalizou a atividade das enzimas Mg^{2+} - ATPase e Na^+, K^+ - ATPase no SNC. Consistente com nossa hipótese, que o 4-PSQ não altera o efeito farmacológico da oxaliplatina, quando analisado o resultado do edema da pata, observamos que o tratamento com a oxaliplatina reduziu o edema causado pelo tumor, ou seja, desenvolveu uma ação antitumoral. Quando o 4-PSQ foi administrado junto com a oxaliplatina aos animais com câncer, o edema foi significativamente reduzido, sugerindo que o 4-PSQ potencializou o efeito antitumoral da oxaliplatina. Entretanto estas conclusões serão reforçadas com a realização de experimentos adicionais que não foram finalizados ainda devido à pandemia de COVID-19.

6 CONCLUSÕES

Nesse estudo, de forma inovadora, foi relatado que o envelhecimento exacerbou a NPIO e suas comorbidades. O envelhecimento agravou a dor, o comportamento ansioso, e o comprometimento cognitivo induzidos pela exposição à oxaliplatina. A partir dos resultados obtidos aqui, foi estabelecido que a administração da oxaliplatina leva à deposição de platina no SNC, independentemente da idade. Resumidamente, o presente estudo ajudou a expandir a compreensão sobre os mecanismos envolvidos na fisiopatologia NPIO. Aqui, o aumento da concentração de platina no SNC e sua relação com desequilíbrio redox, como evidenciado por meio do aumento dos níveis de espécies reativas, aumento de peroxidação lipídica, e modulação as enzimas SOD, CAT, GPx, AChE e Na^+, K^+ - ATPase e Mg^{+2} - ATPase no SNC, foi relatado pela primeira vez. Além disso, de acordo com os resultados aqui obtidos, foi possível inferir que a modulação da Na^+, K^+ - ATPase teve um papel fundamental no desenvolvimento da NPIO, particularmente, nas suas comorbidades. Além disso, alterações na concentração de bioelementos e na sinalização do Nrf2 e BDNF no SNC, vias relacionadas com neuroproteção e neuroplasticidade, respectivamente, parecem contribuir de forma significativa para a NPIO. Por sua vez, a terapia multialvo baseada na administração do 4-PSQ reduziu a NPIO e suas comorbidades, em ambas as idades, e a dor oncológica. Além disso, os efeitos do 4-PSQ parecem ser decorrentes de ações antioxidantes e neuroprotetoras, principalmente por sua capacidade de restabelecer a atividade das enzimas Na^+, K^+ - ATPase e AChE. O 4-PSQ também regulou o eixo HPA por meio da redução dos níveis de corticosterona no plasma. Em conjunto, os resultados sugerem que o 4-PSQ pode ser um bom protótipo para o desenvolvimento de um agente mais eficaz para o tratamento da NPIO, em jovens e idosos. De fato, este estudo ajudou a ampliar o entendimento sobre os mecanismos envolvidos na fisiopatologia da NPIO e avançou na busca por uma nova terapia.

PERSPECTIVAS

Como perspectiva, para dar continuidade aos estudos envolvendo os efeitos do composto 4-PSQ e o medicamento quimioterápico oxaliplatina, pretende-se avaliar se os camundongos velhos são mais susceptíveis ao desenvolvimento da dor oncológica (câncer + oxaliplatina), bem como, investigar os mecanismos subjacentes à dor oncológica, e o potencial terapêutico do 4-PSQ.

REFERÊNCIAS

AEBI, Hugo. Catalase in vitro. In: **Methods in enzymology**. Academic Press, v. 105, p. 121-126, 1984

ALAMRI, Faisal F. et al. Applicability of the grip strength and automated von Frey tactile sensitivity tests in the mouse photothrombotic model of stroke. **Behavioural brain research**, v. 336, p. 250-255, 2018.

AMES, Bruce N. Prolonging healthy aging: longevity vitamins and proteins. **Proceedings of the National Academy of Sciences**, v. 115, n. 43, p. 10836-10844, 2018.

ARETI, Aparna et al. Oxidative stress and nerve damage: role in chemotherapy induced peripheral neuropathy. **Redox biology**, v. 2, p. 289-295, 2014.

BANACH, Marta; JURANEK, Judyta K.; ZYGULSKA, Aneta L. Chemotherapy-induced neuropathies—a growing problem for patients and health care providers. **Brain and behavior**, v. 7, n. 1, p. e00558, 2017.

BARRELL, Kelsey; SMITH, Gordon. Peripheral Neuropathy. **Medical Clinics of North America**, v. 103, n. 2, p. 383-397, 2019.

BARTH, Anelise et al. 7-Chloro-4-(Phenylselanyl) Quinoline with Memory Enhancer Action in Aging Rats: Modulation of Neuroplasticity, Acetylcholinesterase Activity, and Cholesterol Levels. **Molecular neurobiology**, v. 56, n. 9, p. 6398-6408, 2019.

BAHARI, Zahra; MEFTAHI, Gholam Hossein. Spinal α2-adrenoceptors and neuropathic pain modulation; therapeutic target. **British journal of pharmacology**, v. 176, n. 14, p. 2366-2381, 2019.

BEARD, John R. et al. The World report on ageing and health: a policy framework for healthy ageing. **The lancet**, v. 387, n. 10033, p. 2145-2154, 2016.

BELLAVER, Bruna et al. Signaling mechanisms underlying the glioprotective effects of resveratrol against mitochondrial dysfunction. **Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease**, v. 1862, n. 9, p. 1827-1838, 2016.

BHADRA, Malini et al. Alternative splicing in aging and longevity. **Human genetics**, v. 139, n. 3, p. 357-369, 2020.

BORGHESAN, M et al. A senescence-centric view of aging: implications for longevity and disease. **Trends in Cell Biology**, v. 30, n. 10, p. 777-791, 2020.

BORTOLATTO, Cristiani F. et al. Organoselenium bis selenide attenuates 3-nitropropionic acid-induced neurotoxicity in rats. **Neurotoxicity research**, v. 23, n. 3, p. 214-224, 2013.

BOYETTE-DAVIS, Jessica A. et al. An updated understanding of the mechanisms involved in chemotherapy-induced neuropathy. **Pain management**, v. 8, n. 5, p. 363-375, 2018.

BRADFORD, Marion M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical biochemistry**, v. 72, n. 1-2, p. 248-254, 1976.

BRIANI, Chiara et al. Long-term course of oxaliplatin-induced polyneuropathy: a prospective 2-year follow-up study. **Journal of the Peripheral Nervous System**, v. 19, n. 4, p. 299-306, 2014.

BRUNING, César Augusto et al. Evidence for the involvement of μ-opioid and δ-opioid receptors in the antinociceptive effect caused by oral administration of m-

trifluoromethyl-diphenyl diselenide in mice. **Behavioural pharmacology**, v. 21, n. 7, p. 621-626, 2010.

BRUSCO, Indiara et al. α-Spinasterol: a COX inhibitor and a transient receptor potential vanilloid 1 antagonist presents an antinociceptive effect in clinically relevant models of pain in mice. **British journal of pharmacology**, v. 174, n. 23, p. 4247-4262, 2017.

BURDETTE-RADOUX, Susan; MUSS, Hyman B. Adjuvant chemotherapy in the elderly: whom to treat, what regimen?. **Oncologist-Miamisburg**, v. 11, n. 3, p. 234, 2006.

CALLAGHAN, Brian C. et al. Diabetic neuropathy: clinical manifestations and current treatments. **The Lancet Neurology**, v. 11, n. 6, p. 521-534, 2012.

CALLS, Aina et al. Pathogenesis of platinum-induced peripheral neurotoxicity: Insights from preclinical studies. **Experimental Neurology**, v. 325, p. 113-141, 2020.

CAPPOLI, Natalia et al. The emerging role of the BDNF-TrkB signaling pathway in the modulation of pain perception. **Journal of Neuroimmunology**, p. 577406, 2020.

CAVALETTI, Guido; MARMIROLI, Paola. Management of oxaliplatin-induced peripheral sensory neuropathy. **Cancers**, v. 12, n. 6, p. 1370, 2020.

CERLES, Olivier et al. Preventive action of benztropine on platinum-induced peripheral neuropathies and tumor growth. **Acta neuropathologica communications**, v. 7, n. 1, p. 1-23, 2019.

CERSOSIMO, Robert J. Oxaliplatin-associated neuropathy: a review. **Annals of Pharmacotherapy**, v. 39, n. 1, p. 128-135, 2005.

CUNHA, Thiago. M. et al. An electronic pressure-meter nociception paw test for mice. **Brazilian Journal of Medical and Biological Research**, v. 37, n. 3, p. 401-407, 2004.

CHIORAZZI, Alessia; SEMPERBONI, Sara; MARMIROLI, Paola. Current view in platinum drug mechanisms of peripheral neurotoxicity. **Toxics**, v. 3, n. 3, p. 304-321, 2015.

CHUKYO, Akiko et al. Oxaliplatin-induced changes in expression of transient receptor potential channels in the dorsal root ganglion as a neuropathic mechanism for cold hypersensitivity. **Neuropeptides**, v. 67, p. 95-101, 2018.

COLLOCA, Luana et al. Neuropathic pain. **Nature reviews Disease primers**, v. 3, n. 1, p. 1-19, 2017.

CRUZADO, Juan Antonio et al. Longitudinal study of cognitive dysfunctions induced by adjuvant chemotherapy in colon cancer patients. **Supportive Care in Cancer**, v. 22, n. 7, p. 1815-1823, 2014.

CVITKOVIC, Esteban. A historical perspective on oxaliplatin: rethinking the role of platinum compounds and learning from near misses. In: **Seminars in oncology**, v. 25, n. 2, p. 1-3, 1998.

D'AVILA, Joana Costa et al. Age-related cognitive impairment is associated with long-term neuroinflammation and oxidative stress in a mouse model of episodic systemic inflammation. **Journal of neuroinflammation**, v. 15, n. 1, p. 28, 2018.

DA MOTTA, Ketlyn P. et al. 7-Chloro-4-(phenylselanyl) quinoline reduces renal oxidative stress induced by oxaliplatin in mice. **Canadian Journal of Physiology and Pharmacology**, n. ja, 2021.

DE AQUINO SILVA, Daiane et al. 7-chloro-4-(phenylselanyl) quinoline co-treatment prevent oxidative stress in diabetic-like phenotype induced by hyperglycidic diet in Drosophila melanogaster. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 239, p. 108892, 2021.

DE FREITAS COUTO, Shanda et al. 7-chloro-4-(phenylselanyl) quinoline prevents dopamine depletion in a Drosophila melanogaster model of Parkinson's-like disease. **Journal of Trace Elements in Medicine and Biology**, v. 54, p. 232-243, 2019.

DE SANTANA, Marilia Trindade et al. Citronellal, a monoterpene present in Java citronella oil, attenuates mechanical nociception response in mice. **Pharmaceutical biology**, v. 51, n. 9, p. 1144-1149, 2013.

DE SOUZA, Marcus VN et al. Synthesis and in vitro antitubercular activity of a series of quinoline derivatives. **Bioorganic & medicinal chemistry**, v. 17, n. 4, p. 1474-1480, 2009.

DI CESARE MANNELLI, L. et al. Oxaliplatin-induced oxidative stress in nervous system-derived cellular models: could it correlate with in vivo neuropathy?. **Free Radical Biology and Medicine**, v. 61, p. 143-150, 2013.

DZAGNIDZE, Anna et al. Repair capacity for platinum-DNA adducts determines the severity of cisplatin-induced peripheral neuropathy. **Journal of Neuroscience**, v. 27, n. 35, p. 9451-9457, 2007.

DOUGHTY, Christopher T.; BOWLEY, Michael P. Entrapment neuropathies of the upper extremity. **Medical Clinics**, v. 103, n. 2, p. 357-370, 2019.

DUARTE, Luis Fernando B. et al. A simple method for the synthesis of 4-arylselanyl-7-chloroquinolines used as in vitro acetylcholinesterase inhibitors and in vivo memory improvement. **Tetrahedron Letters**, v. 58, n. 33, p. 3319-3322, 2017.

DUBOIS, Martine et al. Chemotherapy-induced long-term alteration of executive functions and hippocampal cell proliferation: role of glucose as adjuvant. **Neuropharmacology**, v. 79, p. 234-248, 2014.

EGASHIRA, Nobuaki et al. Mexiletine reverses oxaliplatin-induced neuropathic pain in rats. **Journal of pharmacological sciences**, v. 112, n. 4, p. 473-476, 2010.

ELLMAN. George L. Tissue sulfhydryl groups. **Arch Biochem Biophys**, v. 82, p. 70-77, 1959.

ELLMAN, George L. et al. A new and rapid colorimetric determination of acetylcholinesterase activity. **Biochemical pharmacology**, v. 7, n. 2, p. 88-95, 1961.

FANE, Mitchell; WEERARATNA, Ashani T. How the ageing microenvironment influences tumour progression. **Nature Reviews Cancer**, v. 20, n. 2, p. 89-106, 2020.

FERRIER, Jérémy et al. Cholinergic neurotransmission in the posterior insular cortex is altered in preclinical models of neuropathic pain: key role of muscarinic M2 receptors in donepezil-induced antinociception. **Journal of Neuroscience**, v. 35, n. 50, p. 16418-16430, 2015.

FLATTERS, Sarah. J. L.; DOUGHERTY, Patrick M.; COLVIN, L. A. Clinical and preclinical perspectives on chemotherapy-induced peripheral neuropathy (CIPN): a narrative review. **BJA: British Journal of Anaesthesia**, v. 119, n. 4, p. 737-749, 2017.

FINNERUP, Nanna B. et al. Neuropathic pain: an updated grading system for research and clinical practice. **Pain**, v. 157, n. 8, p. 1599, 2016.

FISKE, Cyrus H.; SUBBAROW, Yellapragada. The colorimetric determination of phosphorus. **Journal of Biological Chemistry**, v. 66, n. 2, p. 375-400, 1925.

FITZMAURICE, Christina et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. **JAMA oncology**, v. 3, n. 4, p. 524-548, 2017.

FRAGA, Cesar G. Relevance, essentiality and toxicity of trace elements in human health. **Molecular aspects of medicine**, v. 26, n. 4-5, p. 235-244, 2005.

FUKUCHI, Mamoru et al. Visualizing changes in brain-derived neurotrophic factor (BDNF) expression using bioluminescence imaging in living mice. **Scientific reports**, v. 7, n. 1, p. 1-10, 2017.

GIACOBBO, Bruno Lima et al. Brain-derived neurotrophic factor in brain disorders: focus on neuroinflammation. **Molecular neurobiology**, v. 56, n. 5, p. 3295-3312, 2019.

GILRON, Ian; BARON, Ralf; JENSEN, Troels. Neuropathic pain: principles of diagnosis and treatment. In: **Mayo Clinic Proceedings**, v. 90, n. 4, p. 532-545, 2015.

GLEASON, Lauren J.; ESCUE, Emily D.; HOGAN, Teresita M. Older Adult Emergency Department Pain Management Strategies. **Clinics in geriatric medicine**, v. 34, n. 3, p. 491-504, 2018.

GOLDSTEIN, Inbal et al. Involvement of Na+, K+-ATPase and endogenous digitalis-like compounds in depressive disorders. **Biological psychiatry**, v. 60, n. 5, p. 491-499, 2006.

GRISOLD, Wolfgang; CAVALETTI, Guido; WINDEBANK, Anthony J. Peripheral neuropathies from chemotherapeutics and targeted agents: diagnosis, treatment, and prevention. **Neuro-oncology**, v. 14, n. 4, p. 45-54, 2012.

GROLLEAU, Françoise et al. A possible explanation for a neurotoxic effect of the anticancer agent oxaliplatin on neuronal voltage-gated sodium channels. **Journal of neurophysiology**, v. 85, n. 5, p. 2293-2297, 2001.

GUERARD, Emily J.; CLEARY, James F. Managing cancer pain in older adults. **The Cancer Journal**, v. 23, n. 4, p. 242-245, 2017.

HADJISTAVROPOULOS, Thomas et al. Pain assessment in elderly adults with dementia. **The Lancet Neurology**, v. 13, n. 12, p. 1216-1227, 2014.

HOGAN, Teresita M. et al. Improving Pain Relief in Elder Patients (I-PREP): An Emergency Department Education and Quality Intervention. **Journal of the American Geriatrics Society**, v. 64, n. 12, p. 2566-2571, 2016.

HORGAS, Ann L. Pain assessment in older adults. **Nursing Clinics**, v. 52, n. 3, p. 375-385, 2017.

HUSSAIN, Syed G.; RAMAIAH, Kolluru VA. Reduced eIF2α phosphorylation and increased proapoptotic proteins in aging. **Biochemical and biophysical research communications**, v. 355, n. 2, p. 365-370, 2007.

IBRAHIM, Eiman Y.; EHRLICH, Barbara E. Prevention of Chemotherapy-Induced Peripheral Neuropathy: A Review of Recent Findings. **Critical Reviews in Oncology/Hematology**, v. 145, p. 102831, 2020.

JIANG, Yanlin et al. Implications of apurinic/apyrimidinic endonuclease in reactive oxygen signaling response after cisplatin treatment of dorsal root ganglion neurons. **Cancer research**, v. 68, n. 15, p. 6425-6434, 2008.

JIANG, Shu-Ping et al. Celecoxib reverts oxaliplatin-induced neuropathic pain through inhibiting PI3K/Akt2 pathway in the mouse dorsal root ganglion. **Experimental neurology**, v. 275, p. 11-16, 2016.

JORDAN, Berit et al. Calcium and magnesium infusions for the prevention of oxaliplatin-induced peripheral neurotoxicity: a systematic review. **Oncology**, v. 90, n. 6, p. 299-306, 2016.

JUNG, Yongwoo et al. 8-(Tosylamino) quinoline inhibits macrophage-mediated inflammation by suppressing NF-κB signaling. **Acta Pharmacologica Sinica**, v. 33, n. 8, p. 1037-1046, 2012.

KABIR, Md Tanvir et al. Molecular mechanisms of metal toxicity in the pathogenesis of Alzheimer's disease. **Molecular Neurobiology**, v. 58, n. 1, p. 1-20, 2021.

KAGIAVA, Alexia et al. The effects of oxaliplatin, an anticancer drug, on potassium channels of the peripheral myelinated nerve fibres of the adult rat. **Neurotoxicology**, v. 29, n. 6, p. 1100-1106, 2008.

KAMERMAN, Peter R. et al. World Health Organization (WHO) essential medicines lists: where are the drugs to treat neuropathic pain?. **Pain**, v. 156, n. 5, p. 793, 2015.

KASAI, Shuya et al. Role of the ISR-ATF4 pathway and its cross talk with Nrf2 in mitochondrial quality control. **Journal of clinical biochemistry and nutrition**, p. 18-37, 2018.

KAUR, Damanpreet et al. Nutritional interventions for elderly and considerations for the development of geriatric foods. **Current aging science**, v. 12, n. 1, p. 15-27, 2019.

KAWASHIRI, Takehiro et al. L type Ca2+ channel blockers prevent oxaliplatin-induced cold hyperalgesia and TRPM8 overexpression in rats. **Molecular pain**, v. 8, p. 1744-8069, 2012.

KING, Kirsten M. et al. Single and combined effects of Δ9-tetrahydrocannabinol and cannabidiol in a mouse model of chemotherapy-induced neuropathic pain. **British journal of pharmacology**, v. 174, n. 17, p. 2832-2841, 2017.

KING, Nicholas B.; FRASER, Veronique. Untreated pain, narcotics regulation, and global health ideologies. **PLoS medicine**, v. 10, n. 4, p. e1001411 2013.

KINOSHITA, Paula F. et al. The influence of Na+, K+-ATPase on glutamate signaling in neurodegenerative diseases and senescence. **Frontiers in physiology**, v. 7, p. 195, 2016.

KOROVILA, Ioanna et al. Proteostasis, oxidative stress and aging. **Redox biology**, v. 13, p. 550-567, 2017.

KRAYCHETE, Durval Campos; SAKATA, Rioko Kimiko. Neuropatias periféricas dolorosas. **Revista Brasileira de Anestesiologia**, v. 61, n. 5, p. 649-658, 2011.

KRUKOWSKI, Karen et al. CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic pain. **Journal of Neuroscience**, v. 36, n. 43, p. 11074-11083, 2016.

KUDVA, Avinash K.; SHAY, Ashley E.; PRABHU, K. Sandeep. Selenium and inflammatory bowel disease. **American Journal of Physiology-Gastrointestinal and Liver Physiology**, v. 309, n. 2, p. 71-77, 2015.

KURAUCHI, Yuki et al. Na+, K+-ATPase inhibition causes hyperactivity and impulsivity in mice via dopamine D2 receptor-mediated mechanism. **Neuroscience research**, v. 146, p. 54-64, 2019.

LEHKY, Tanya. J. et al. Oxaliplatin-induced neurotoxicity: acute hyperexcitability and chronic neuropathy. **Muscle & nerve**, v. 29, n. 3, p. 387-392, 2004.

LEMOS, Briana B. et al. Role of 7-chloro-4-(phenylselanyl) quinoline in the treatment of oxaliplatin-induced hepatic toxicity in mice. **Canadian Journal of Physiology and Pharmacology**, v. 99, n. 4, p. 378-388, 2021.

LEONARD, Gregory D. et al. Survey of oxaliplatin-associated neurotoxicity using an interview-based questionnaire in patients with metastatic colorectal cancer. **BMC cancer**, v. 5, n. 1, p. 116, 2005.

LI, Ning et al. Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. **The Journal of Immunology**, v. 173, n. 5, p. 3467-3481, 2004.

LI, Xiaofang et al. Ulinastatin downregulates TLR4 and NF-kB expression and protects mouse brains against ischemia/reperfusion injury. **Neurological research**, v. 39, n. 4, p. 367-373, 2017.

LIGUORI, Ilaria et al. Sarcopenia: assessment of disease burden and strategies to improve outcomes. **Clinical interventions in aging**, v. 13, p. 913, 2018.

LIMA-COSTA, Maria Fernanda et al. Tendências em dez anos das condições de saúde de idosos brasileiros: evidências da Pesquisa Nacional por Amostra de Domicílios (1998, 2003, 2008). **Ciência & saúde coletiva**, v. 16, n. 9, p. 3689-3696, 2011.

LIVAK, Kenneth J.; SCHMITTGEN, Thomas D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. **Methods**, v. 25, n. 4, p. 402-408, 2001.

LOETCHUTINAT, Chatchanok et al. Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′, 7′-dichlorofluorescein diacetate assay. **Radiation Physics and Chemistry**, v. 72, n. 2-3, p. 323-331, 2005.

LUCHESE, Cristiane et al. Role of 7-chloro-4-(phenylselanyl) quinoline as an anti-aging drug fighting oxidative damage in different tissues of aged rats. **Experimental gerontology**, v. 130, p. 110804, 2020.

LUCZKOWSKA, Karolina. et al. Pathophysiology of drug-induce peripheral neuropathy in patients with multiple myeloma. **Journal of Physiology and Pharmacology**, v. 69, n. 2, p. 165-172, 2018.

MACEDO, Bruno et al. Synthesis and anti-prion activity evaluation of aminoquinoline analogues. **European journal of medicinal chemistry**, v. 45, n. 11, p. 5468-5473, 2010.

MANERA, Clementina et al. New quinolone-and 1, 8-naphthyridine-3-carboxamides as selective CB2 receptor agonists with anticancer and immuno–modulatory activity. **European journal of medicinal chemistry**, v. 97, p. 10-18, 2015.

MANTOVANI, Anderson C. et al. Synthesis of pharmacologically active 1-amino-isoquinolines prepared via silver triflate-catalyzed cyclization of o-alkynylbenzaldoximes with isocyanates. **European Journal of Pharmaceutical Sciences**, v. 51, p. 196-203, 2014.

MATSOS, Antigone; JOHNSTON, Ian N. Chemotherapy-induced cognitive impairments: a systematic review of the animal literature. **Neuroscience & Biobehavioral Reviews**, v. 102, p. 382-399, 2019.

MEHTA, Shalini Kapoor; GOWDER, Sivakumar Joghi Thatha. Members of antioxidant machinery and their functions. **Basic principles and clinical significance of oxidative stress**, p. 60-85, 2015.

MERKIES, Ingemar SJ; FABER, Catharina G.; LAURIA, Giuseppe. Advances in diagnostics and outcome measures in peripheral neuropathies. **Neuroscience letters**, v. 596, p. 3-13, 2015.

MESCLON, Florent et al. Decreased ATF4 expression as a mechanism of acquired resistance to long-term amino acid limitation in cancer cells. **Oncotarget**, v. 8, n. 16, p. 27440, 2017.

MESKO, Marcia F. et al. Evaluation of metal distributions in small samples of mouse brain lesions (hematoma) by inductively coupled plasma mass spectrometry after sampling by laser microdissection (LMD). **International Journal of Mass Spectrometry**, v. 307, n. 1-3, p. 137-141, 2011.

MIQUEL, Sophie et al. Poor cognitive ageing: Vulnerabilities, mechanisms and the impact of nutritional interventions. **Ageing research reviews**, v. 42, p. 40-55, 2018.

MISRA, Hara P.; FRIDOVICH, Irwin. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. **Journal of Biological chemistry**, v. 247, n. 10, p. 3170-3175, 1972.

MIYAKE, Takahito et al. Cold sensitivity of TRPA1 is unveiled by the prolyl hydroxylation blockade-induced sensitization to ROS. **Nature communications**, v. 7, n. 1, p. 1-10, 2016.

MILTENBURG, Nienke. C.; BOOGERD, Willen. Chemotherapy-induced neuropathy: a comprehensive survey. **Cancer treatment reviews**, v. 40, n. 7, p. 872-882, 2014.

MCQUADE, Rachel M. et al. Oxaliplatin-induced enteric neuronal loss and intestinal dysfunction is prevented by co-treatment with BGP-15. **British journal of pharmacology**, v. 175, n. 4, p. 656-677, 2018.

MOSELEY, Amy E. et al. Deficiency in Na, K-ATPase α isoform genes alters spatial learning, motor activity, and anxiety in mice. **Journal of Neuroscience**, v. 27, n. 3, p. 616-626, 2007.

MORRISSEY, Marry B. et al. An interdisciplinary look at the potential of policy to improve the health of an aging America: focus on pain. **The Gerontological Society of America, Washington (DC)**, v. 55, p. 245-251, 2014.

NAVARRO-ALARCON, Miguel; LÓPEZ-MARTINEZ, María C. Essentiality of selenium in the human body: relationship with different diseases. **Science of the Total Environment**, v. 249, n. 1-3, p. 347-371, 2000.

NAZIROĞLU, Mustafa; BRAIDY, Nady. Thermo-sensitive TRP channels: novel targets for treating chemotherapy-induced peripheral pain. **Frontiers in physiology**, v. 8, p. 1040, 2017.

NGUYEN, Lien D.; EHRLICH, Barbara E. Cellular mechanisms and treatments for chemobrain: insight from aging and neurodegenerative diseases. **EMBO molecular medicine**, v. 12, n. 6, p. e12075, 2020.

NICHETTI, Federico et al. Is a pharmacogenomic panel useful to estimate the risk of oxaliplatin-related neurotoxicity in colorectal cancer patients?. **The pharmacogenomics journal**, v. 19, n. 5, p. 465-472, 2019.

NIJS, Jo et al. Brain-derived neurotrophic factor as a driving force behind neuroplasticity in neuropathic and central sensitization pain: a new therapeutic target?. **Expert opinion on therapeutic targets**, v. 19, n. 4, p. 565-576, 2015.

NOGUEIRA, Cristina W.; ROCHA, João BT. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. **Archives of Toxicology**, v. 85, n. 11, p. 1313-1359, 2011.

NOTARAS, Michael; VAN DEN BUUSE, Maarten. Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders. **Molecular psychiatry**, v. 25, n. 10, p. 2251-2274, 2020.

OHKAWA, Hiroshi; OHISHI, Nobuko; YAGI, Kunio. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Analytical biochemistry**, v. 95, n. 2, p. 351-358, 1979.

PALTIAN, Jaini J. et al. The anxiolytic effect of a promising quinoline containing selenium with the contribution of the serotonergic and GABAergic pathways: Modulation of parameters associated with anxiety in mice. **Behavioural Brain Research**, v. 393, p. 112797, 2020.

PAPANAS, Nikolaos; VINIK, Aaron I.; ZIEGLER, Dan. Neuropathy in prediabetes: does the clock start ticking early?. **Nature Reviews Endocrinology**, v. 7, n. 11, p. 682, 2011.

PARK, Sun Ah et al. Cisplatin-induced apoptotic cell death in mouse hybrid neurons is blocked by antioxidants through suppression of cisplatin-mediated accumulation of p53 but not of Fas/Fas ligand. **Journal of neurochemistry**, v. 75, n. 3, p. 946-953, 2000.

PELLOW, Sharon et al. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. **Journal of neuroscience methods**, v. 14, n. 3, p. 149-167, 1985.

PILLERON, Sophie et al. Estimated global cancer incidence in the oldest adults in 2018 and projections to 2050. **International journal of cancer**, v. 148, n. 3, p. 601-608, 2021.

PINZ, Mikaela et al. 4-Phenylselenyl-7-chloroquinoline, a new quinoline derivative containing selenium, has potential antinociceptive and anti-inflammatory actions. **European journal of pharmacology**, v. 780, p. 122-128, 2016.

PINZ, Mikaela P. et al. Current advances of pharmacological properties of 7-chloro-4-(phenylselanyl) quinoline: Prevention of cognitive deficit and anxiety in Alzheimer's disease model. **Biomedicine & Pharmacotherapy**, v. 105, p. 1006-1014, 2018.

RAJA, Srinivasa N. et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. **Pain**, v. 161, n. 9, p. 1976-1982, 2020.

REID, M. Carrington. Expanding Targets for Intervention in Later Life Pain: What Role Can Patient Beliefs, Expectations and Pleasant Activities Play?. **Clinics in geriatric medicine**, v. 32, n. 4, p. 797, 2016.

REIS, Angélica S. et al. 4-phenylselenyl-7-chloroquinoline, a novel multitarget compound with anxiolytic activity: Contribution of the glutamatergic system. **Journal of psychiatric research**, v. 84, p. 191-199, 2017.

RODRIGUES, Karline C. et al. The neurotherapeutic role of a selenium-functionalized quinoline in hypothalamic obese rats. **Psychopharmacology**, p. 1-15, 2021.

RICHARDSON, B. D.; SOTTILE, S. Y.; CASPARY, D. M. Mechanisms of GABAergic and cholinergic neurotransmission in auditory thalamus: Impact of aging. **Hearing Research**, v. 402, p. 108003, 2021.

SALES, Manuela Vasconcelos Castro et al. Effects of adjuvant chemotherapy on cognitive function of patients with early-stage colorectal cancer. **Clinical colorectal cancer**, v. 18, n. 1, p. 19-27, 2019.

SALGUEIRO, Willian G. et al. Insights into the differential toxicological and antioxidant effects of 4-phenylchalcogenil-7-chloroquinolines in Caenorhabditis elegans. **Free Radical Biology and Medicine**, v. 110, p. 133-141, 2017.

SARAIVA, Maiara T. et al. 7-Chloroquinoline-1, 2, 3-triazoyl carboxylates: organocatalytic synthesis and antioxidant properties. **Journal of the Brazilian Chemical Society**, v. 27, n. 1, p. 41-53, 2016.

SAVEGNAGO, Lucielli et al. Synthesis and antioxidant properties of novel quinoline–chalcogenium compounds. **Tetrahedron Letters**, v. 54, n. 1, p. 40-44, 2013.

SCHMITT, Linda-Isabell et al. Oxaliplatin modulates the characteristics of voltage-gated calcium channels and action potentials in small dorsal root ganglion neurons of rats. **Molecular neurobiology**, v. 55, n. 12, p. 8842-8855, 2018.

SCHULZE, Christin et al. Prolonged oxaliplatin exposure alters intracellular calcium signaling: a new mechanism to explain oxaliplatin-associated peripheral neuropathy. **Clinical colorectal cancer**, v. 10, n. 2, p. 126-133, 2011.

SHANKAR, Deepa B. et al. The role of CREB as a proto-oncogene in hematopoiesis and in acute myeloid leukemia. **Cancer cell**, v. 7, n. 4, p. 351-362, 2005.

SILVA, Vanessa DG et al. Further analysis of acute antinociceptive and anti-inflammatory actions of 4-phenylselenyl-7-chloroquinoline in mice. **Fundamental & clinical pharmacology**, v. 31, n. 5, p. 513-525, 2017.

SILVA-PALACIOS, A. et al. Nrf2: Molecular and epigenetic regulation during aging. **Ageing research reviews**, v. 47, p. 31-40, 2018.

SILVERMAN, Harold A. et al. Brain region-specific alterations in the gene expression of cytokines, immune cell markers and cholinergic system components during peripheral endotoxin-induced inflammation. **Molecular medicine**, v. 20, n. 1, p. 601-611, 2014.

SINGH, Pardeep et al. Synthesis, docking and in vitro antimalarial evaluation of bifunctional hybrids derived from β-lactams and 7-chloroquinoline using click chemistry. **Bioorganic & medicinal chemistry letters**, v. 22, n. 1, p. 57-61, 2012.

SISIGNANO, Marco et al. Mechanism-based treatment for chemotherapy-induced peripheral neuropathic pain. **Nature Reviews Neurology**, v. 10, n. 12, p. 694, 2014.

SITTL, Ruth et al. Enhancement of axonal potassium conductance reduces nerve hyperexcitability in an in vitro model of oxaliplatin-induced acute neuropathy. **Neurotoxicology**, v. 31, n. 6, p. 694-700, 2010.

SONG, Su J et al. Incidence of taxane-induced peripheral neuropathy receiving treatment and prescription patterns in patients with breast cancer. **Supportive Care in Cancer**, v. 25, n. 7, p. 2241-2248, 2017.

STANGHERLIN, Eluza C; ROCHA, João B T; NOGUEIRA, Cristina W. Diphenyl ditelluride impairs short-term memory and alters neurochemical parameters in young rats. **Pharmacology Biochemistry and Behavior**, v. 91, n. 3, p. 430-435, 2009.

STAROBOVA, Hana; VETTER, Irina. Pathophysiology of chemotherapy-induced peripheral neuropathy. **Frontiers in molecular neuroscience**, v. 10, p. 174, 2017.

STINO, Amro M.; SMITH, Albert G. Peripheral neuropathy in prediabetes and the metabolic syndrome. **Journal of diabetes investigation**, v. 8, n. 5, p. 646-655, 2017.

TA, Lauren E. et al. Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum–DNA binding. **Neurotoxicology**, v. 27, n. 6, p. 992-1002, 2006.

TAYLOR, Stephanie J.C. et al. Novel three-day, community-based, nonpharmacological group intervention for chronic musculoskeletal pain (COPERS): a randomised clinical trial. **PLoS medicine**, v. 13, n. 6, p. e1002040, 2016.

TEIXEIRA, Fernanda Cardoso et al. Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions. **Psychopharmacology**, v. 237, n. 3, p. 811-823, 2020.

THIBAULT, Karine et al. Cortical effect of oxaliplatin associated with sustained neuropathic pain: exacerbation of cortical activity and down-regulation of potassium channel expression in somatosensory cortex. **PAIN®**, v. 153, n. 8, p. 1636-1647, 2012.

TOFTHAGEN, Cindy et al. Oxaliplatin-induced peripheral neuropathy's effects on health-related quality of life of colorectal cancer survivors. **Supportive Care in Cancer**, v. 21, n. 12, p. 3307-3313, 2013.

TURABELIDZE, Anna; GUO, Shujuan; DIPIETRO, Luisa A. Importance of housekeeping gene selection for accurate reverse transcription-quantitative polymerase chain reaction in a wound healing model. **Wound repair and regeneration**, v. 18, n. 5, p. 460-466, 2010.

VANDAMME, Marian; PAUWELS, Walter; BLEECKER, Jan D. A case of delayed oxaliplatin-induced pseudo-obstruction: an atypical presentation of oxaliplatin neurotoxicity. **Acta Clinica Belgica**, v. 70, n. 3, p. 207-210, 2015.

VAN DRONKELAAR, Carliene et al. Minerals and sarcopenia; the role of calcium, iron, magnesium, phosphorus, potassium, selenium, sodium, and zinc on muscle mass, muscle strength, and physical performance in older adults: a systematic review. **Journal of the American Medical Directors Association**, v. 19, n. 1, p. 6-11. e3, 2018.

VELASCO, Roser; BRUNA, Jordi. Oxaliplatin neurotoxicity. **Current Colorectal Cancer Reports**, v. 10, n. 3, p. 303-312, 2014.

VOGT, Ane G. et al. Organoselenium group is critical for antioxidant activity of 7-chloro-4-phenylselenyl-quinoline. **Chemico-biological interactions**, v. 282, p. 7-12, 2018.

VOSS, Guilherme T. et al. Therapeutic and technological potential of 7-chloro-4-phenylselanyl quinoline for the treatment of atopic dermatitis-like skin lesions in mice. **Materials Science and Engineering: C**, v. 84, p. 90-98, 2018.

WALSH, Roger N.; CUMMINS, Robert A. The open-field test: a critical review. **Psychological bulletin**, v. 83, n. 3, p. 482, 1976.

WANG, Hua-Yu Leo; O'DOHERTY, George A. Modulators of Na/K-ATPase: a patent review. **Expert opinion on therapeutic patents**, v. 22, n. 6, p. 587-605, 2012.

WANG, Yujia et al. NF-κB signaling in skin aging. **Mechanisms of ageing and development**, v. 184, p. 111160, 2019.

WANG, Victor C.; MULLALLY, William J. Pain neurology. **The American journal of medicine**, v. 133, n. 3, p. 273-280, 2020.

WATSON, James C.; DYCK, James B. Peripheral neuropathy: a practical approach to diagnosis and symptom management. In: **Mayo Clinic Proceedings**. v. 90, n. 7, p. 940-951, 2015.

WEICKHARDT, Andrew. J. et al. Vascular endothelial growth factor D expression is a potential biomarker of bevacizumab benefit in colorectal cancer. **British journal of cancer**, v. 113, n. 1, p. 37-45, 2015.

WENDEL, Albrecht. Glutathione peroxidase. In: **Methods in enzymology**. Academic Press, v. 77, p. 325-333, 1981.

WILHELM, Ethel A. et al. Antinociceptive and anti-allodynic effects of 3-alkynyl selenophene on different models of nociception in mice. **Pharmacology Biochemistry and Behavior**, v. 93, n. 4, p. 419-425, 2009.

WILHELM, Ethel A. et al. Organocatalytic synthesis and evaluation of 7-chloroquinoline-1, 2, 3-triazoyl carboxamides as potential antinociceptive, anti-inflammatory and anticonvulsant agent. **RSC Advances**, v. 4, n. 78, p. 41437-41445, 2014.

WITLOX, Lenja et al. Effect of physical exercise on cognitive function and brain measures after chemotherapy in patients with breast cancer (PAM study): protocol of a randomised controlled trial. **BMJ open**, v. 9, n. 6, p. e028117, 2019.

WOOLFE, GAND; MACDONALD, Alan D. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). **Journal of Pharmacology and Experimental Therapeutics**, v. 80, n. 3, p. 300-307, 1944.

WORLD HEALTH ORGANIZATION. **World report on ageing and health**. World Health Organization, 2015.

XIAO, Wenhua H.; ZHENG, Huaien; BENNETT, Gary J. Characterization of oxaliplatin-induced chronic painful peripheral neuropathy in the rat and comparison with the neuropathy induced by paclitaxel. **Neuroscience**, v. 203, p. 194-206, 2012.

YAN, Fang et al. Role of platinum DNA damage-induced transcriptional inhibition in chemotherapy-induced neuronal atrophy and peripheral neurotoxicity. **Journal of neurochemistry**, v. 135, n. 6, p. 1099-1112, 2015.

YANG, Yang et al. Nrf2 inhibits oxaliplatin-induced peripheral neuropathy via protection of mitochondrial function. **Free Radical Biology and Medicine**, v. 120, p. 13-24, 2018.

ZANARDELLI, Matteo et al. Different apoptotic pathways activated by oxaliplatin in primary astrocytes vs. colo-rectal cancer cells. **International journal of molecular sciences**, v. 16, n. 3, p. 5386-5399, 2015.

ZENKER, Nicolas; BERNSTEIN, Donald E. The estimation of small amounts of corticosterone in rat plasma. **Journal of Biological Chemistry**, v. 231, n. 2, p. 695-701, 1958.

ZHANG, Dawei et al. Na, K-ATPase activity regulates AMPA receptor turnover through proteasome-mediated proteolysis. **Journal of Neuroscience**, v. 29, n. 14, p. 4498-4511, 2009.

ZHENG, Huaien; XIAO, Wen Hua; BENNETT, Gary J. Functional deficits in peripheral nerve mitochondria in rats with paclitaxel-and oxaliplatin-evoked painful peripheral neuropathy. **Experimental neurology**, v. 232, n. 2, p. 154-161, 2011.

ANEXOS

ANEXO A

Carta de aprovação do protocolo experimental pela Comissão de Ética e Experimentação Animal da Universidade Federal de Pelotas

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **142/2018/CEEA/REITORIA**
PROCESSO Nº 23110.050294/2018-14

Certificado

Certificamos que a solicitação de **adendo** (mudança de metodologia e acréscimo de animais) à proposta intitulada **"Investigação do papel do envelhecimento na neuropatia induzida por quimioterápicos: 7-cloro-4-(fenilseleno)quinolina como alternativa terapêutica"** processo número 23110.050294/2018-14 (CEEA 4506-2017), de responsabilidade de Ethel Antunes Wilhelm - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua complementação pela Comissão de Ética em Experimentação Animal, em reunião de 10/12/2018.

Finalidade	(X) Pesquisa ( ) Ensino
Espécie/linhagem/raça	*Mus musculus*/Swiss
Nº de animais	Acréscimo de 390
Idade	195 com 2 meses e 195 com 20 meses
Sexo	Machos
Origem	Biotério Central - UFPel

Código para cadastro **CEEA 4506-2017**

Documento assinado eletronicamente por **ANELIZE DE OLIVEIRA CAMPELLO FELIX**, **Médico Veterinário**, em 19/12/2018, às 09:50, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site http://sei.ufpel.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **0394984** e o código CRC **952DA388**.

Referência: Processo nº 23110.050294/2018-14 SEI nº 0394984

ANEXO B

Carta de aprovação do protocolo experimental pela Comissão de Ética e Experimentação Animal da Universidade Federal de Pelotas

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **39/2020/CEEA/REITORIA**
PROCESSO Nº 23110.054738/2019-63

Certificado

Certificamos que a solicitação de **adendo** à proposta intitulada **"Investigação do papel do envelhecimento na neuropatia induzida por quimioterápicos: 7-cloro-4-(fenilseleno) quinolina como alternativa terapêutica."**, registrada com o nº CEEA 4506-2017, sob a responsabilidade de **xxXX** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **30 de abril de 2020**.

Solicitação: acréscimo de 68 ratos wistar machos (55 com 20 meses e 13 com 2 meses).

M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Documento assinado eletronicamente por **ANELIZE DE OLIVEIRA CAMPELLO FELIX, Médico Veterinário**, em 06/05/2020, às 10:34, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site http://sei.ufpel.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **0941208** e o código CRC **78738638**.

SEI/UFPel - 0941208 - Parecer 11/05/20 10:56

ANEXO C

Carta de aprovação do protocolo experimental pela Comissão de Ética e Experimentação Animal da Universidade Federal de Pelotas

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **39/2019/CEEA/REITORIA**
PROCESSO Nº 23110.024440/2019-29

Certificado

Certificamos que a proposta intitulada **"Investigação do papel do envelhecimento na dor oncológica: Investigação do 7-cloro-4-(fenilselanil)quinolina como alternativa terapêutica"**, processo nº 23110.002440/2019-29, sob a responsabilidade de Ethel Antunes Wilhelm que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de 16/07/2019.

Finalidade	(X) Pesquisa ( ) Ensino
Vigência da autorização	18/07/2019 a 01/07/2024
Espécie/linhagem/raça	*Mus musculus*/Swiss
Nº de animais	260
Idade	60 dias e 18-20 meses
Sexo	Machos
Origem	Biotério Central - UFPel

Ccódigo para cadastro nº **CEEA 24440-2019**

M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Documento assinado eletronicamente por ANELIZE DE OLIVEIRA CAMPELLO FELIX, Médico Veterinário, em 17/07/2019, às 13:09, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site http://sei.ufpel.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador 0623983 e o código CRC 495F9AAF.

Referência: Processo nº 23110.024440/2019-29 SEI nº 0623983

ANEXO D

Permissão para rebublicação

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Aug 16, 2021

This Agreement between Mrs. Angélica Reis ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	5131121068007
License date	Aug 16, 2021
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Molecular Neurobiology
Licensed Content Title	Advances in the Understanding of Oxaliplatin-Induced Peripheral Neuropathy in Mice: 7-Chloro-4-(Phenylselanyl) Quinoline as a Promising Therapeutic Agent
Licensed Content Author	Angélica S. Reis et al
Licensed Content Date	Sep 1, 2020
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	full article/chapter
Will you be translating?	no

P

Circulation/distribution	1 - 29
Author of this Springer Nature content	yes
Title	Implicações do envelhecimento na neuropatia induzida por oxaliplatina: 7-cloro-4-(fenilseleno)quinolina como estratégia terapêutica
Institution name	UFPel
Expected presentation date	Sep 2021
Requestor Location	Mrs. Angélica Reis Av. Pinheiro Machado 506 Bloco D ap 303 Pelotas, RS 96040-500 Brazil Attn: UFPel
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:*** Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

8. 2. For content reuse requests that qualify for permission under the STM Permissions Guidelines, which may be updated from time to time, the STM Permissions Guidelines supersede the terms and conditions contained in this licence.

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from **[the Licensor]**: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from **[the Licensor]**: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from **[the Licensor]**: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from **[the Licensor]**: on behalf of Cancer Research UK: : **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The **[the Licensor]**: on behalf of Cancer Research UK: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from **[the Licensor]**: **[Book Publisher** (e.g. Palgrave Macmillan, Springer etc) **[Book Title]** by **[Book author**(s)] **[COPYRIGHT]** (year of publication)

Other Conditions:

Version 1.3

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

ANEXO E

Permissão para rebublicação

CCC | Marketplace™

Order Number: 1141472
Order Date: 18 Aug 2021

Payment Information

Angélica Reis
ge_schiavon@hotmail.com
Payment method: Invoice

Billing Address:
Mrs. Angélica Reis
Av. Pinheiro Machado 506
Bloco D
ap 303
Pelotas, RS 96040-500
Brazil

+55 (53)981030173
ge_schiavon@hotmail.com

Customer Location:
Mrs. Angélica Reis
Av. Pinheiro Machado 506
Bloco D
ap 303
Pelotas, RS 96040-500
Brazil

Order Details

1. Brain research bulletin

Billing Status: Open

Article: Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity: Correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline.

Order License ID	1141472-1	**Type of use**	Republish in a thesis/dissertation
Order detail status	Completed		
ISSN	1873-2747	**Publisher**	ELSEVIER SCIENCE
		Portion	Chapter/article

0.00 USD
Republication Permission

LICENSED CONTENT

Publication Title	Brain research bulletin	**Rightsholder**	Elsevier Science & Technology Journals
Article Title	Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity: Correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline.	**Publication Type**	e-Journal
		Start Page	282
		End Page	290
		Volume	162
		URL	http://www.sciencedirect.com/science/journal/03619230
Author/Editor	International Behavioral Neuroscience Society.		
Date	01/01/1995		
Language	English		
Country	United States of America		

REQUEST DETAILS

Portion Type	Chapter/article	**Distribution**	Other territories and/or countries
Page range(s)	282	**Enter territories/countries**	Brazil
Total number of pages	290	**Translation**	Original language of publication
Format (select all that apply)	Print,Electronic	**Copies for the disabled?**	No
Who will republish the content?	Author of requested content	**Minor editing privileges?**	No
Duration of Use	Life of current edition	**Incidental promotional use?**	No
Lifetime Unit Quantity	Up to 499	**Currency**	USD
Rights Requested	Main product		

NEW WORK DETAILS

Title	Implicações do envelhecimento na neuropatia induzida por oxaliplatina: 7-cloro-4-(fenilseleno)quinolina como estratégia terapêutica	**Institution name**	UFPel
		Expected presentation date	2021-09-03
Instructor name	Angélica Schiavom dos Reis		

ADDITIONAL DETAILS

The requesting person / organization to appear on the license	Angélica Schiavom dos Reis

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	article	**Title of the article/chapter the portion is from**	Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity: Correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline.
Editor of portion(s)	Reis, Angélica S.; Paltian, Jaini J.; Domingues, William B.; Costa, Gabriel P.; Alves, Diego; Giongo, Janice L.; Campos, Vinicius F.; Luchese, Cristiane; Wilhelm, Ethel A.	**Author of portion(s)**	Reis, Angélica S.; Paltian, Jaini J.; Domingues, William B.; Costa, Gabriel P.; Alves, Diego; Giongo, Janice L.; Campos, Vinicius F.; Luchese, Cristiane; Wilhelm, Ethel A.
Volume of serial or monograph	162	**Publication date of portion**	2020-09-01
Page or page range of portion	282-290		

Elsevier Science & Technology Journals Terms and Conditions

Elsevier publishes Open Access articles in both its Open Access journals and via its Open Access articles option in subscription journals, for which an author selects a user license permitting certain types of reuse without permission. Before proceeding please check if the article is Open Access on http://www.sciencedirect.com and refer to the user license for the individual article. Any reuse not included in the user license terms will require permission. You must always fully and appropriately credit the author and source. If any part of the material to be used (for example, figures) has appeared in the Elsevier publication for which you are seeking permission, with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder. Please contact permissions@elsevier.com with any queries.

Elsevier Science & Technology Journals Special Terms and Conditions

Posting of the full article/ chapter online is not permitted. You may post an abstract with a link to the Elsevier website www.elsevier.com , or to the article on ScienceDirect if it is available on that platform.

Total Items: 1

Subtotal: 0.00 USD

Order Total: 0.00 USD