molecular pharmaceutics

Brief Article

Cite This: *Mol. Pharmaceutics* 2018, 15, 2883−2889

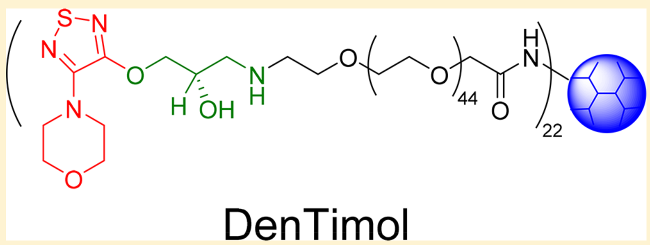
DenTimol as A Dendrimeric Timolol Analogue for Glaucoma Therapy: Synthesis and Preliminary Efficacy and Safety Assessment

Michael G. Lancina, III,[†,#] Juan Wang,[‡,#] Geoffrey S. Williamson,[†] and Hu Yang*[,‡,§,∥]

[†]Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, Virginia 23284, United States
[‡]Department of Chemical & Life Science Engineering, Virginia Commonwealth University, Richmond, Virginia 23219, United States
[§]Department of Pharmaceutics, Virginia Commonwealth University, Richmond, Virginia 23298, United States
[∥]Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia 23298, United States

Supporting Information

ABSTRACT: In this work, we report the synthesis and characterization of DenTimol, a dendrimer-based polymeric timolol analog, as a glaucoma medication. A timolol precursor (*S*)-4-[4-(oxiranylmethoxy)-1,2,5-thiadiazol-3-yl]morpholine (OTM) was reacted with the heterobifunctional amine polyethylene glycol acetic acid (amine−PEG−acetic acid, M_n = 2000 g/mol) via a ring opening reaction of an epoxide by an amine to form the OTM−PEG conjugate. OTM−PEG was then coupled to an ethylenediamine (EDA) core polyamidoamine (PAMAM) dendrimer G3 to generate DenTimol using the *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) coupling reaction. MALDI mass spectrometry, ^{1}H NMR spectroscopy, and HPLC were applied to characterize the intermediate and final products. Ex vivo corneal permeation of DenTimol was assessed using the Franz diffusion cell system mounted with freshly extracted rabbit cornea. The cytotoxicity of DenTimol was assessed using the WST-1 assay. Our results show that DenTimol is nontoxic up to an OTM equivalent concentration of 100 μM. DenTimol is efficient at crossing the cornea. About 8% of the dendrimeric drug permeated through the cornea in 4 h. Its IOP-lowering effect was observed in normotensive adult Brown Norway male rats. Compared to the undosed eye, an IOP reduction by an average of 7.3 mmHg (∼30% reduction from baseline) was observed in the eye topically treated with DenTimol (2 × 5 μL, 0.5% w/v timolol equivalent) in less than 30 min. Daily dosing of DenTimol for a week did not cause any irritation or toxicity as confirmed by the histological examination of ocular tissues, including the cornea, ciliary body, and retina.



KEYWORDS: *β-blocker, 3-amino-1,2-propanediol, nanomedicine, glaucoma, polymeric drug*

INTRODUCTION

Ocular hypertension, i.e., elevated intraocular pressure (IOP), is a characteristic of glaucoma, a chronic ocular disease that can lead to degenerative and irreparable vision loss. Although the pathophysiology leading to glaucoma is multifactorial, the immediate aim of treatment is to lower IOP back to normal levels and prevent disease progression. Pharmacologic therapies are usually the first treatment option because they are less costly and less invasive than surgical interventions. *β*-Adrenergic antagonists, commonly known as *β*-blockers, reduce IOP by slowing the production of the aqueous humor. Timolol maleate is a *β*-blocker developed in the 1970s.[1] It selectively binds to adrenergic receptors in the ciliary body, making it a more potent IOP-lowering drug than other *β*-blockers.[2] Despite the existence of these potent IOP-lowering drugs, glaucoma remains the second leading cause of blindness worldwide.[3] This is because the impact of current treatments is severely limited by inefficient drug delivery formulations. For example, when timolol is delivered as a topical solution, only about 5% of the total drug at best makes it to the target organ with each dose.[4] Because of this, the period after a dose where the drug is within its therapeutic concentration window in the anterior chamber is narrow, typically 4−5 h.[5,6] The remaining volume is flushed into systemic circulation where it can cause cardiovascular side effects of varying severity.[7−9]

New antiglaucoma drugs are being actively pursued by targeting different therapeutic targets to acquire IOP reduction and neuroprotective benefits, for example, via increasing optic nerve head blood flow or trabecular meshwork outflow.[10] Like current antiglaucoma drugs or other small molecule drugs, new drugs may eventually need an enabling delivery system to achieve sustained and effective delivery and improved bioavailability.[11] In our opinion, a compelling proactive approach for antiglaucoma drug development is to build molecules with a known therapeutic effect or established therapeutic potential into a polymeric backbone to form pharmacologically active polymeric drugs. While many advances have been made in the development of polymeric

Received: April 17, 2018
Revised: May 11, 2018
Accepted: May 16, 2018
Published: May 16, 2018

DOI: 10.1021/acs.molpharmaceut.8b00401
Mol. Pharmaceutics 2018, 15, 2883−2889

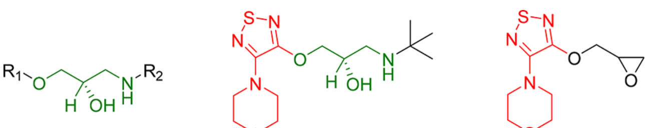
drugs for some diseases,[12−16] it is rarely used in the rational design of antiglaucoma drugs. This approach potentially accelerates new antiglaucoma therapy development by capitalizing on benefits of polymeric drugs, such as macromolecular multivalency and flexible structures, allowing for accommodation of additional moieties to have desirable physicochemical properties for delivery.

Polymeric vehicles that can improve the delivery efficiency of this drug could reduce either the necessary concentration or dosing regimen, representing a significant improvement in the clinical management of glaucoma. The study of nanoparticle vehicles to improve the bioavailability of topical ocular drugs has received considerable attention in the past decade.[17−19] Most drug loss from eye drops occurs due to precorneal mechanisms.[4] Generally, most drugs penetrate the corneal epithelium slowly relative to the rate of tear drainage and turnover.[20] Polyamidoamine (PAMAM) dendrimers have the potential to overcome these factors because they have appropriate size and mucoadhesiveness to both slow washout and increase cornea permeability.[21,22] PAMAM dendrimers have been complexed with other ocular therapeutics, resulting in improved drug delivery efficiency, but these methods rely on the formation of metastable dendrimer−drug complexes.[23−27] In the past, however, no success has been reported in developing polymeric drugs for glaucoma.[25,28] In this work, we synthesized and characterized a dendrimer-based polymeric timolol analog (DenTimol) as a glaucoma medication. Its efficacy and safety were assessed in normotensive adult Brown Norway male rats. Developing IOP-lowering polymeric drugs is complementary to traditional small molecule antiglaucoma drug development and offers unique features by simultaneously tackling pharmacological potency and physiological barriers to delivery. It is novel to develop DenTimol as a glaucoma medication.

■ EXPERIMENTAL SECTION

Synthesis. (*S*)-4-[4-(Oxiranylmethoxy)-1,2,5-thiadiazol-3-yl]morpholine (OTM) (Toronto Research Chemicals, Toronto, Canada, cat#O847080) was dissolved in dichloromethane (DCM) and reacted with an equimolar amount of amine polyethylene glycol acetic acid (amine−PEG−acetic acid, M_n = 2000 g/mol) (JenKem, Plano, TX) for 3 h at room temperature. The resulting OTM−PEG was recovered by rotary evaporation, purified by dialysis in water with a 3.5 kDa dialysis membrane, and lyophilized. OTM−PEG (32 equiv) was then reacted with an EDA core polyamidoamine dendrimer generation 3.0 (G3.0) (1 equiv) (Dendritech, Midland, MI) in DMSO overnight in the presence of a large molar excess of *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS). The product DenTimol was purified by dialysis with a 7.5 kDa dialysis membrane for 48 h and lyophilized.

Scheme 1. Chemical Structures of Common β-Blockers (1), Timolol (2), and OTM (3)[a]



1 2 3

[a]R_1 and R_2 in **1** stand for different groups.

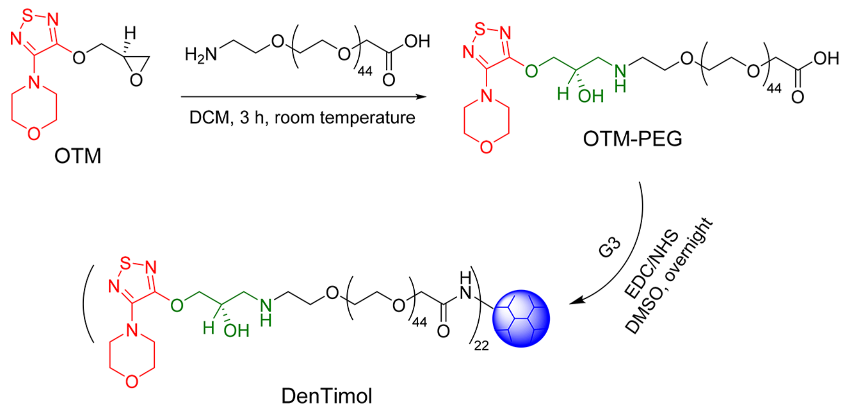
HPLC Analysis. The reactants and products were analyzed with a Waters reverse phase HPLC system equipped with a Waters 2487 dual absorbance detector and an XTerra C18 column (4.6 mm × 150 mm, particle size 5 μm). The mobile phase constituted water/acetonitrile (50:50, v/v). UV absorbance was monitored at 220 and 300 nm.

Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI MS). MALDI MS analysis was performed on an Applied Biosystems Voyager matrix-assisted laser desorption/ionization time-of-flight mass spectrometer. Spectra were calibrated internally for the AARS screening assay to 756.2352 (the mass of the 4-formylphenoxypropyl triphenylphosphonium AMP derivative). The reactant amine−PEG−acetic acid and the intermediate OTM−PEG were dissolved in deionized water and spotted on a MALDI plate before analysis.

^{1}H NMR Spectroscopy. ^{1}H NMR spectrum of DenTimol was collected on a Bruker 600 MHz NMR spectrometer. ^{1}H NMR (600 MHz, CD_3OD, δ (ppm)): 3.89 (s, 2H), 3.80−3.73 (m, 4H), 3.73−3.59 (m, 223H), 3.52 (d, *J* = 4.6 Hz, 2H), 3.47−3.35 (m, 5H), 3.27 (s, 3H), 3.18 (dd, *J* = 10.0, 4.7 Hz, 2H), 2.85 (d, *J* = 57.9 Hz, 9H), 2.60 (s, 3H), 2.38 (s, 7H).

Ex Vivo Corneal Permeation Study. Corneal permeability was determined using Franz diffusion cells (PermeGear, Hellertown, PA).[29] Corneas were extracted from fresh rabbit eyes and placed immediately into diffusion cells with the endothelial surface facing the acceptor chamber and the epithelial surface facing the donor chamber. The acceptor chamber was filled with 5 mL of glutathione-buffered Ringer's solution and the donor chamber with 100 μL of a solution containing 1 mg of DenTimol (3.7 mM OTM equivalent). For comparison, permeation of OTM and OTM−PEG across the cornea were also tested at the same OTM equivalent concentration. The diffusion cells were placed in a water bath at 37 °C. At various

Scheme 2. Synthesis of DenTimol



DOI: 10.1021/acs.molpharmaceut.8b00401
Mol. Pharmaceutics 2018, 15, 2883−2889

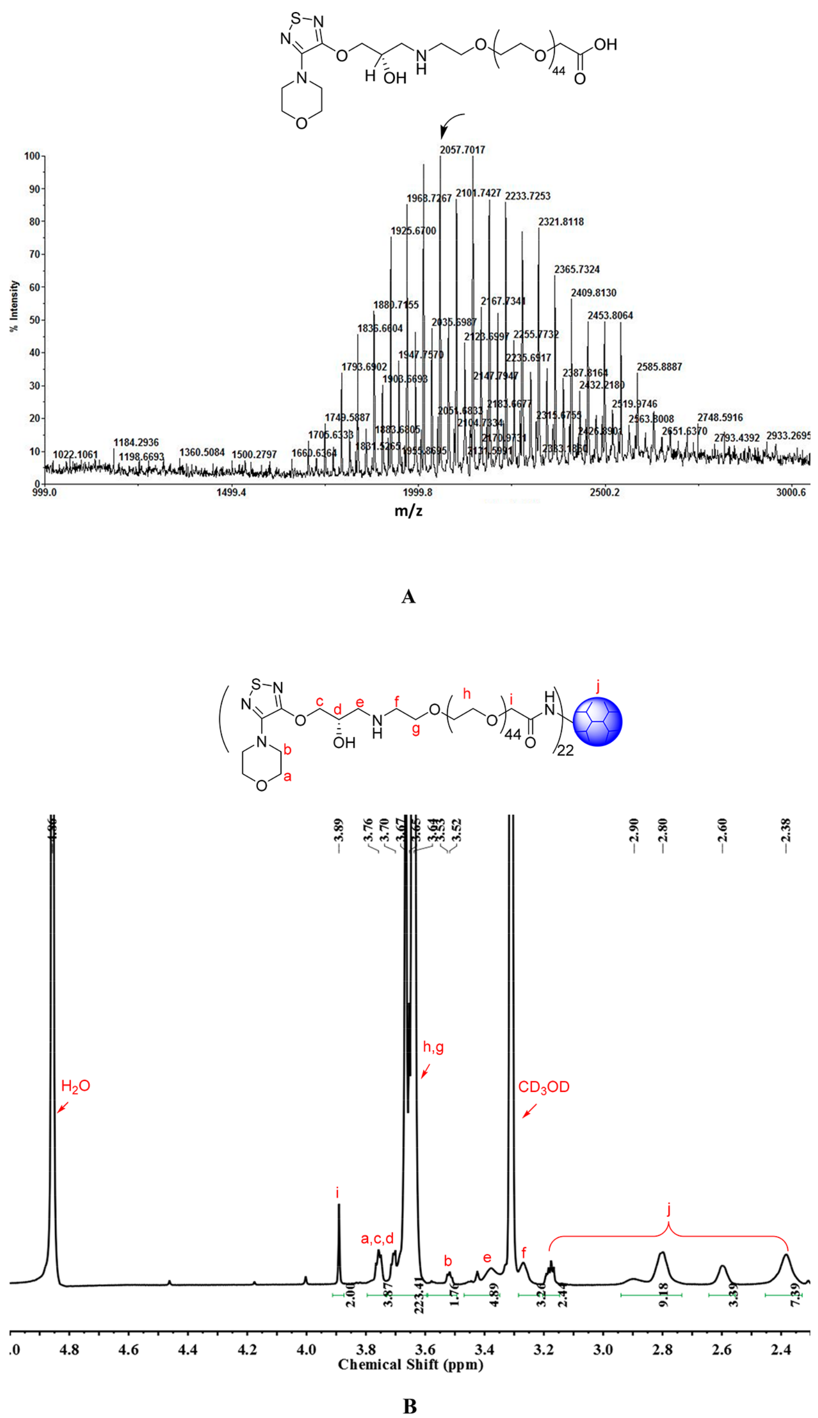


Figure 1. Structural characterization of the intermediate product OTM−PEG and the final product DenTimol. (A) MALDI mass spectrum of OTM−PEG. (B) ^{1}H NMR spectrum of DenTimol.

time points, 250 μL samples were withdrawn from the acceptor chamber, and drug concentrations were measured using the reverse phase HPLC system.

Cytotoxicity Study. NIH 3T3 fibroblasts were seeded in a 96-well plate at 5000 cells per well and allowed 24 h to attach. The cells were maintained in DMEM medium supplemented with 10% serum, streptomycin (100 U/mL), and penicillin (100 U/mL) at 37 °C in 95% air/5% CO_2. Upon the removal of the medium, the cells were incubated with a fresh DMEM medium (control) or a fresh medium containing various amounts of OTM, OTM−PEG, or DenTimol (n = 8) for 24 h. The cell viability relative to untreated cells was determined using the WST-1 cell proliferation assay.

In Vivo Efficacy Assessment. Normotensive adult brown Norway rats (Charles River Laboratories, Wilmington, MA) were used for all animal experiments in this study. They were housed under proper conditions at Virginia Commonwealth University (VCU). The rats were kept under a cycle of 12-h light and 12-h dark. The animal procedures were approved by the VCU IACUC. Drug efficacy was measured in vivo by dosing brown Norway rats (n = 4) in the right eye with a timolol solution (2 × 5 μL, 0.5% w/v) or an equivalent DenTimol

DOI: 10.1021/acs.molpharmaceut.8b00401
Mol. Pharmaceutics 2018, 15, 2883−2889

solution. IOP was measured using a TonoLab rebound tonometer (Icare, Finland) at various time points in both eyes. Change in IOP was referenced to the contralateral (left) eye. No anesthetics or chemical restraints were employed during the measurements. All measurements were taken by the same operator at the same location using the hand corresponding to that eye. Time 0 for all experiments (and baseline IOP readings) occurred at approximately 10 a.m. The reported IOP values are the average of 18−30 individual instrument readings of each eye.

In Vivo Safety Assessment. To assess in vivo ocular tolerance, rats received OTM, timolol, or DenTimol (2 × 5 μL, 0.5% w/v OTM equivalent) in the right eye once daily for 7 days. At the conclusion of the experiment, the rats were euthanized. The eyes were enucleated and immediately fixed in Davidson's solution, and 5 μm sections were prepared for histological evaluation.

Statistical Analysis. All of the data are expressed as means ± standard deviation. A Student's *t*-test was performed for comparison. A value of $p < 0.05$ was considered statistically significant.

■ RESULTS AND DISCUSSION

Synthesis and Characterization. Most β-blockers contain 3-amino-1,2-propanediol (APD) (**1** in Scheme 1), a key structural element responsible for antiglaucoma effects. Timolol (**2**) is a β-blocker commonly prescribed as a glaucoma medication. However, it lacks reactive groups that can directly conjugate with a polymer to form a polymeric antiglaucoma drug. To make a polymeric (dendrimer-based) timolol drug, we used a timolol precursor (*S*)-4-[4-(oxiranylmethoxy)-1,2,5-thiadiazol-3-yl]morpholine (OTM, **3**),[30] which not only has the same key chemical structure as timolol (highlighted in red) but also a reactive epoxy end-group for functionalization. As shown in Scheme 2, the terminal amine group of the heterobifunctional PEG spacer amine−PEG−acetic acid was reacted with the epoxy of OTM to generate the OTM−PEG conjugate containing an APD moiety. MALDI mass spectrometry analysis shows that the *m*/*z* value is 2057.7 (Figure 1A), suggesting a successful one-to-one coupling of OTM to PEG (PEG *m*/*z* = 1880.0, Figure S1). OTM−PEG was then conjugated to PAMAM dendrimer G3 via the NHS/EDC coupling reaction. HPLC analysis (Figure S2) confirmed that DenTimol was purified successfully, and there was no unreacted OTM in the final conjugate. The ^{1}H NMR spectrum (Figure 1B) shows that DenTimol has both PEG and OTM on the surface, and an average of 22 OTM−PEG were coupled to a dendrimer according to integrals.

In Vitro Assessment. Covering a large portion of the dendrimer surface with PEG chains significantly improves dendrimer cytocompatibility.[31] Indeed, our cytotoxicity results indicate that DenTimol shows no signs of cytotoxicity at the OTM equivalent concentration of 100 μM (Figure 2A). Because of the high drug payload per dendrimer, this suggests DenTimol can be more effective in maintaining therapeutic concentrations for timolol.

OTM has similar bioactivity to timolol, but it is hydrophobic and has even lower ocular bioavailability than timolol maleate.[32] Coupling to a linear hydrophilic polymer, such as PEG, can solubilize the compound, but as our corneal permeability results show, this structure does not readily penetrate the cornea (<2% in 2 h), likely due to its high hydrophilicity (Figure 2B). We found that DenTimol is efficient at crossing the cornea: 8%

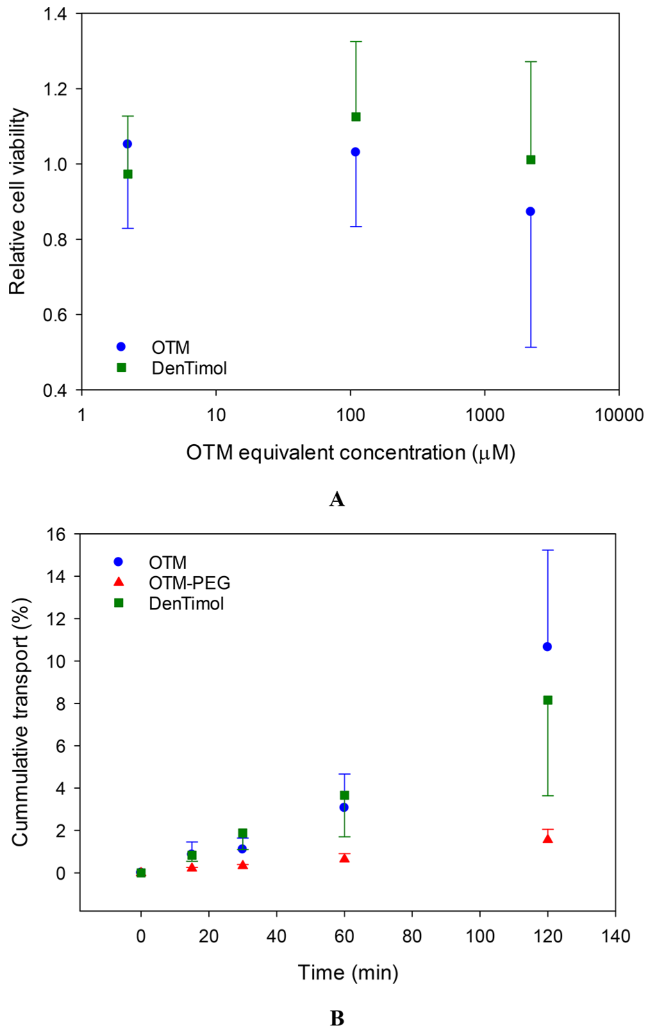


Figure 2. In vitro assessment of DenTimol. (A) Dose-dependent cytotoxicity of OTM and DenTimol. (B) Ex vivo permeation of OTM, OTM−PEG, and DenTimol across a rabbit cornea.

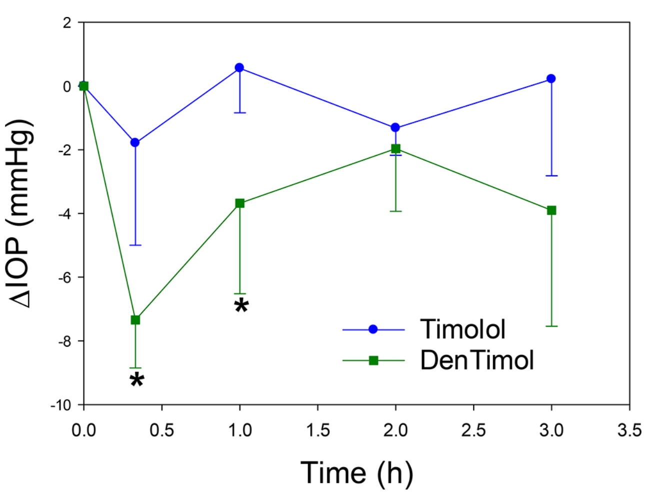


Figure 3. In vivo IOP-lowering assessment of DenTimol in normotensive rats after a one-time topical administration.* indicates $p < 0.05$ vs timolol PBS eye drops.

of the drug permeated through the cornea in 4 h, which was 2.3-fold and 4-fold higher than timolol (3.5% in 4 h[33]) and OTM−PEG (<2% in 2 h) (Figure 2B), respectively. DenTimol's

DOI: 10.1021/acs.molpharmaceut.8b00401
Mol. Pharmaceutics 2018, 15, 2883−2889

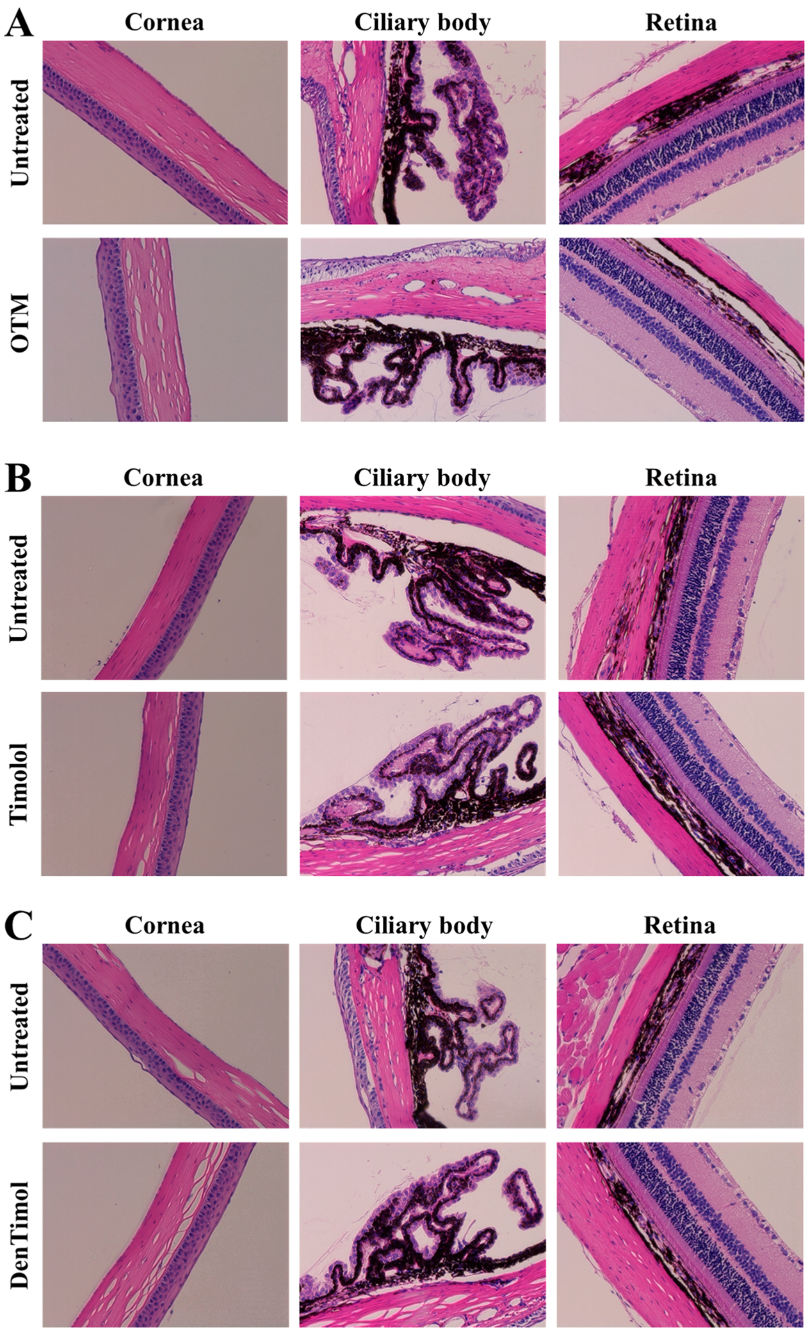


Figure 4. Ocular tissues stained with H&E following a chronic application of OTM (A), timolol (B), or DenTimol (C) (magnification 200×).

high corneal permeation is attributed to its hydrophilic–lipophilic balance with timolol being the outermost layer. PAMAM dendrimers are also highly hydrophilic, but they are also compact and strongly cationic. This unique architecture allows them to penetrate the cornea relatively easy, even when covered with drug moieties. Direct coupling of OTM to dendrimer without a spacer is theoretically possible, and would likely result in an even higher level of bioavailability.[34] However, without the flexibility provided by the polymeric linker, it is likely bioactivity of each prodrug moiety would be reduced, either due to steric hindrance from the dendrimer or the folding-in of the dendrimer end groups toward the core of the molecule.[35]

In Vivo Efficacy and Safety Assessment. A one-time topical application of DenTimol (10 μL of 0.5% w/v timolol) in normotensive adult Brown Norway male rats resulted in an IOP reduction by an average of 7.3 mmHg (~30%) in less than 30 min, which was significantly stronger than timolol PBS eye drops (Figure 3) (both showed a peak effect in less than 30 min). The repeated application of DenTimol did not show any signs of toxicity or ocular irritation in vivo. H&E stained sections of ocular tissues confirmed that the repeated dosing of DenTimol, OTM, or OTM–PEG did not alter the structures of the cornea, ciliary body, and retina (Figure 4).

Although the use of fully awake animals provides a more philologically relevant model, it produces more noise in the measurement of pressures by rebound tonometry. This noise makes it unclear at this point if potency is increased over timolol. The IOP response profile observed for DenTimol is roughly equivalent to literature reported values for timolol in rodents.[36] The effect of the chronic application has also not yet

DOI: 10.1021/acs.molpharmaceut.8b00401
Mol. Pharmaceutics 2018, 15, 2883–2889

been studied. Clinical IOP lowering treatments rely on an additive effect of repeated drug dosing. We expect, at minimum, for this phenomenon to be present with DenTimol treatment if not greater due to the mucoadhesive quality of the dendrimeric drug. Future work will be focused on building a complete pharmacokinetic profile for DenTimol. The efficacy and safety of DenTimol will be validated in animal models of glaucoma. We believe DenTimol can extend the therapeutic window for timolol sufficiently to reduce the dosing frequency to once a day or less. DenTimol has three distinct structural features: (1) it retains the effective structure of timolol, thus gaining the potency as an antiglaucoma drug; (2) the incorporation of a PEG spacer can enhance the biocompatibility and water solubility of the new polymeric drug; and (3) DenTimol is a novel self-transporting "smart" drug, as PAMAM dendrimers can serve as a powerful ocular drug delivery vehicle with adaptable features and cornea transport into in the anterior chamber once applied to the eye.

■ CONCLUSIONS

In this work, we developed DenTimol, a dendrimer-based polymeric timolol analog, as a glaucoma medication. DenTimol is highly water-soluble and shows no signs of toxicity or ocular irritation in vitro or in vivo. We observed its IOP-lowering effect, supporting us to fully examine its PK/PD and study how to use the multivalency of dendrimers to improve its potency and safety. Optimization of drug loading and spacer length may further improve its IOP-lowering effect. Such studies will be conducted in future work.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.8b00401.

HPLC analysis and a MALDI mass spectrum (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: hyang2@vcu.edu.

ORCID

Hu Yang: 0000-0003-3030-004X

Author Contributions

#M.G.L. and J.W. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported, in part, by the National Institutes of Health (R01EY024072). The facility is supported, in part, by funding from the NIH-NCI Cancer Center Support Grant P30CA016059.

■ REFERENCES

(1) Franciosa, J. A.; Freis, E. D.; Conway, J. Antihypertensive and hemodynamic properties of the new beta adrenergic blocking agent timolol. *Circulation* **1973**, *48* (1), 118–24.

(2) Zimmerman, T. J.; Kaufman, H. E. Timolol. A beta-adrenergic blocking agent for the treatment of glaucoma. *Arch. Ophthalmol.* **1977**, *95* (4), 601–604.

(3) Quigley, H. A.; Broman, A. T. The number of people with glaucoma worldwide in 2010 and 2020. *Br. J. Ophthalmol.* **2006**, *90* (3), 262.

(4) Davies, N. M. Biopharmaceutical considerations in topical ocular drug delivery. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27* (7), 558.

(5) Coakes, R. L.; Brubaker, R. F. The mechanism of timolol in lowering intraocular pressure. In the normal eye. *Arch. Ophthalmol.* **1978**, *96* (11), 2045–8.

(6) Büker, E.; Dinç, E. A New UPLC Method with Chemometric Design-Optimization Approach for the Simultaneous Quantitation of Brimonidine Tartrate and Timolol Maleate in an Eye Drop Preparation. *J. Chromatogr. Sci.* **2017**, *55* (2), 154–161.

(7) Nieminen, T.; Lehtimäki, T.; Mäenpää, J.; Ropo, A.; Uusitalo, H.; Kähönen, M. Ophthalmic timolol: plasma concentration and systemic cardiopulmonary effects. *Scand. J. Clin. Lab. Invest.* **2007**, *67* (2), 237–45.

(8) Rana, M. A.; Mady, A. F.; Rehman, B. A.; Alharthy, A.; Huwait, B.; Riaz, A.; Aletreby, W. T. From Eye Drops to ICU, a Case Report of Three Side Effects of Ophthalmic Timolol Maleate in the Same Patient. *Case Rep. Crit Care* **2015**, *2015*, 714919.

(9) Korte, J.-M.; Kaila, T.; Saari, M. K. Systemic bioavailability and cardiopulmonary effects of 0.5% timolol eyedrops. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2002**, *240* (6), 430.

(10) Donegan, R. K.; Lieberman, R. L. Discovery of Molecular Therapeutics for Glaucoma: Challenges, Successes, and Promising Directions. *J. Med. Chem.* **2016**, *59* (3), 788–809.

(11) Sheybani, N. D.; Yang, H. Pediatric ocular nanomedicines: Challenges and opportunities. *Chin. Chem. Lett.* **2017**, *28* (9), 1817–1821.

(12) Zhang, K.; Yang, P.-P.; Zhang, J.-P.; Wang, L.; Wang, H. Recent advances of transformable nanoparticles for theranostics. *Chin. Chem. Lett.* **2017**, *28* (9), 1808–1816.

(13) Qi, X.; Qin, J.; Fan, Y.; Qin, X.; Jiang, Y.; Wu, Z. Carboxymethyl Chitosan-Modified Polyamidoamine Dendrimer Enables Progressive Drug Targeting of Tumors via pH-Sensitive Charge Inversion. *J. Biomed. Nanotechnol.* **2016**, *12* (4), 667–78.

(14) Qu, Y.; Chu, B.; Shi, K.; Peng, J.; Qian, Z. Recent Progress in Functional Micellar Carriers with Intrinsic Therapeutic Activities for Anticancer Drug Delivery. *J. Biomed. Nanotechnol.* **2017**, *13* (12), 1598–1618.

(15) Zhang, H.; Zhu, J.; Chu, B.; Chen, L.; Shi, K.; Zhang, L.; Chen, X.; Qian, Z. Preparation, Characterization and *In Vivo* Antitumor Evaluation of a Micellar Formulation of Camptothecin Prodrug. *Nanosci. Nanotechnol. Lett.* **2017**, *9* (11), 1755–1766.

(16) Li, J.; Yu, F.; Chen, Y.; Oupický, D. Polymeric drugs: Advances in the development of pharmacologically active polymers. *J. Controlled Release* **2015**, *219*, 369–382.

(17) Zimmer, A.; Kreuter, J. Microspheres and nanoparticles used in ocular delivery systems. *Adv. Drug Delivery Rev.* **1995**, *16* (1), 61.

(18) Diebold, Y.; Calonge, M. Applications of nanoparticles in ophthalmology. *Prog. Retinal Eye Res.* **2010**, *29* (6), 596.

(19) Almeida, H.; Amaral, M. H.; Lobao, P.; Frigerio, C.; Sousa Lobo, J. M. Nanoparticles in Ocular Drug Delivery Systems for Topical Administration: Promises and Challenges. *Curr. Pharm. Des.* **2015**, *21* (36), 5212–24.

(20) Ibrahim, M. M.; Abd-Elgawad, A. H.; Soliman, O. A.; Jablonski, M. M. Natural Bioadhesive Biodegradable Nanoparticle-Based Topical Ophthalmic Formulations for Management of Glaucoma. *Transl Vis Sci. Technol.* **2015**, *4* (3), 12.

(21) Bravo-Osuna, I.; Woodward, A.; Argueso, P.; Martínez, I. T. M.; Gómez, R.; de la Mata, F. J.; Navarro, M. M.; Noiray, M.; Ponchel, G.; Herrero-Vanrell, R. Linear Polymers Versus PAMAM Dendrimers In The Interaction With Transmembrane Ocular Mucins: Analysis By Biosensor Technology. *Invest. Ophthalmol. Visual Sci.* **2012**, *53* (14), 1845.

(22) Kannan, R.; Nance, E.; Kannan, S.; Tomalia, D. Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. *J. Intern. Med.* **2014**, *276* (6), 579–617.

(23) Yao, W.; Sun, K.; Mu, H.; Liang, N.; Liu, Y.; Yao, C.; Liang, R.; Wang, A. Preparation and characterization of puerarin–dendrimer complexes as an ocular drug delivery system. *Drug Dev. Ind. Pharm.* **2010**, *36* (9), 1027.

DOI: 10.1021/acs.molpharmaceut.8b00401
Mol. Pharmaceutics 2018, 15, 2883–2889

(24) Yavuz, B.; Pehlivan, S. B.; Vural, I.; Unlu, N. In Vitro/In Vivo Evaluation of Dexamethasone−PAMAM Dendrimer Complexes for Retinal Drug Delivery. *J. Pharm. Sci.* **2015**, *104* (11), 3814−23.
(25) Kambhampati, S. P.; Mishra, M. K.; Mastorakos, P.; Oh, Y.; Lutty, G. A.; Kannan, R. M. Intracellular delivery of dendrimer triamcinolone acetonide conjugates into microglial and human retinal pigment epithelial cells. *Eur. J. Pharm. Biopharm.* **2015**, *95*, 239−249.
(26) Mishra, V.; Jain, N. K. Acetazolamide encapsulated dendritic nano-architectures for effective glaucoma management in rabbits. *Int. J. Pharm.* **2014**, *461* (1−2), 380−90.
(27) Lancina, M. G.; Singh, S.; Kompella, U. B.; Husain, S.; Yang, H. Fast Dissolving Dendrimer Nanofiber Mats as Alternative to Eye Drops for More Efficient Antiglaucoma Drug Delivery. *ACS Biomater. Sci. Eng.* **2017**, *3* (8), 1861−1868.
(28) Lancina, M.; Yang, H. Dendrimers for ocular drug delivery. *Can. J. Chem.* **2017**, *95* (9), 897−902.
(29) Wang, J.; Williamson, G.; Lancina, M.; Yang, H. Mildly Cross-Linked Dendrimer Hydrogel Prepared via Aza-Michael Addition Reaction for Topical Brimonidine Delivery. *J. Biomed. Nanotechnol.* **2017**, *13* (9), 1089−1096.
(30) Bredikhin, A. A.; Zakharychev, D. V.; Gubaidullin, A. T.; Fayzullin, R. R.; Pashagin, A. V.; Bredikhina, Z. A. Crystallization Features of the Chiral Drug Timolol Precursor: The Rare Case of Conglomerate with Partial Solid Solutions. *Cryst. Growth Des.* **2014**, *14* (4), 1676−1683.
(31) Wang, W.; Xiong, W.; Wan, J.; Sun, X.; Xu, H.; Yang, X. The decrease of PAMAM dendrimer-induced cytotoxicity by PEGylation via attenuation of oxidative stress. *Nanotechnology* **2009**, *20* (10), 105103.
(32) Pech, B.; Duval, O.; Richomme, P.; Benoit, J. A timolol prodrug for improved ocular delivery: Synthesis, conformational study and hydrolysis of palmitoyl timolol malonate. *Int. J. Pharm.* **1996**, *128* (1−2), 179−188.
(33) Holden, C. A.; Tyagi, P.; Thakur, A.; Kadam, R.; Jadhav, G.; Kompella, U. B.; Yang, H. Polyamidoamine dendrimer hydrogel for enhanced delivery of antiglaucoma drugs. *Nanomedicine* **2012**, *8* (5), 776−83.
(34) Sk, U. H.; Kambhampati, S. P.; Mishra, M. K.; Lesniak, W. G.; Zhang, F.; Kannan, R. M. Enhancing the efficacy of Ara-C through conjugation with PAMAM dendrimer and linear PEG: a comparative study. *Biomacromolecules* **2013**, *14* (3), 801−10.
(35) Menjoge, A. R.; Kannan, R. M.; Tomalia, D. A. Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications. *Drug Discovery Today* **2010**, *15* (5), 171.
(36) Akaishi, T.; Odani-Kawabata, N.; Ishida, N.; Nakamura, M. Ocular hypotensive effects of anti-glaucoma agents in mice. *J. Ocul. Pharmacol. Ther.* **2009**, *25* (5), 401−8.

DOI: 10.1021/acs.molpharmaceut.8b00401
Mol. Pharmaceutics 2018, 15, 2883−2889