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Ionic Liquid Pilocarpine Analog as an Antiglaucoma Drug Candidate

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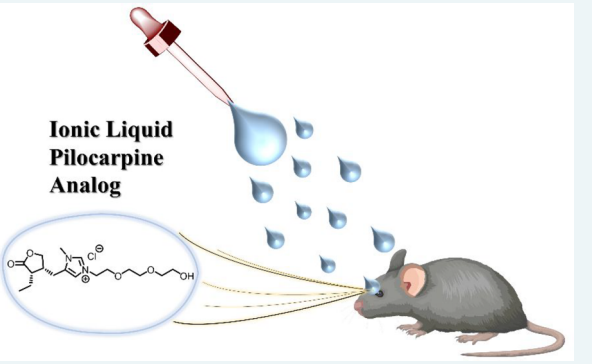
ABSTRACT: Ionic liquid pilocarpine analog [Pilo-OEG]Cl, the first-of-its-kind ionic liquid antiglaucoma drug candidate, is synthesized and reported. To synthesize [Pilo-OEG]Cl, pilocarpine was reacted with the oligo-polyethylene glycol chloride, 2-[2-(2-chloroethoxy)ethoxy] ethanol, to form an ionic liquid molecule with an imidazole cation and a chloride anion. The chemical structure of [Pilo-OEG]Cl was confirmed with ^{1}H NMR spectroscopy. Compared with pilocarpine (Pilo) and pilocarpine hydrochloride (PiloHCl), [Pilo-OEG]Cl has improved structural stability according to pH measurements and LC-MS analysis. The corneal permeability coefficient of [Pilo-OEG]Cl is 2-fold higher than that of Pilo and 8-fold higher than that of PiloHCl. [Pilo-OEG]Cl does not show apparent toxicity to human corneal epithelial cells over a broad range of concentrations up to 50 mM. The in vivo efficacy of PiloHCl and [Pilo-OEG]Cl was tested in normotensive adult Brown Norway female rats. [Pilo-OEG]Cl is found to be more potent than PiloHCl in lowering the intraocular pressure (IOP). [Pilo-OEG]Cl is more cytocompatible than PiloHCl based on the in vitro ELISA and hemolysis tests and in vivo histological analysis. Taken together, [Pilo-OEG]Cl has enhanced stability, corneal permeability, better IOP-lowering efficacy, and improved biocompatibility, thus making it a promising new drug candidate for antiglaucoma therapy. Transforming old antiglaucoma drugs to pharmaceutically active ionic liquids represents an innovative approach to developing glaucoma medications.



KEYWORDS: *ionic liquid, glaucoma, pilocarpine, corneal permeability, intraocular pressure reduction*

INTRODUCTION

Drugs may have unpredictable polymorphic conversion, poor water solubility, and low bioavailability, collectively limiting their therapeutic benefits.[1] Converting active pharmaceutical ingredients (APIs) to salts has proven to be effective in improving their physicochemical properties such as solubility without compromising the therapeutic activities.[1−5] An estimated 50% of APIs are produced in the salt form.[5] Recently, liquid salts have attracted increasing consideration for their potential use in drug development and improvement.[6] Ionic liquids (ILs) are a class of ionic, salt-like substances that appear in the liquid form at ambient temperatures.[7,8] Owing to their unique physical and chemical features, ILs have been broadly studied as solvents, energetic materials, catalysts, and so on.[9] Shamshina pointed out that medicinal ILs would be the future direction for the development of ILs.[6] The application of ILs as pharmaceuticals has been explored.[6,10−16] IL drugs maintain the active ingredients of the drugs themselves. The common synthesis strategies of IL APIs include the reaction of the imidazole ring with chlorine compounds and combining cations and anions in an "anti-crystal engineering" approach.[16] For instance, the IL form of lidocaine docusate avoided the homogeneous polycrystalline transformation issue associated with lidocaine and gained controlled solubility and improved thermal stability.[16] Davis et al. reported the first IL API derived from the antifungal drug miconazole.[17] This new IL API was made through the reaction between the imidazole ring and alkyl iodides followed by an anion metathesis. Not only do IL APIs have improved water solubility[11] but also they become thermodynamically stable, avoiding the polymorphic conversion issue. IL drugs possess both the therapeutic activities and the structural features of ILs. The dual functionality is appealing and is a new strategy for restructuring and reformulating old drugs.[18]

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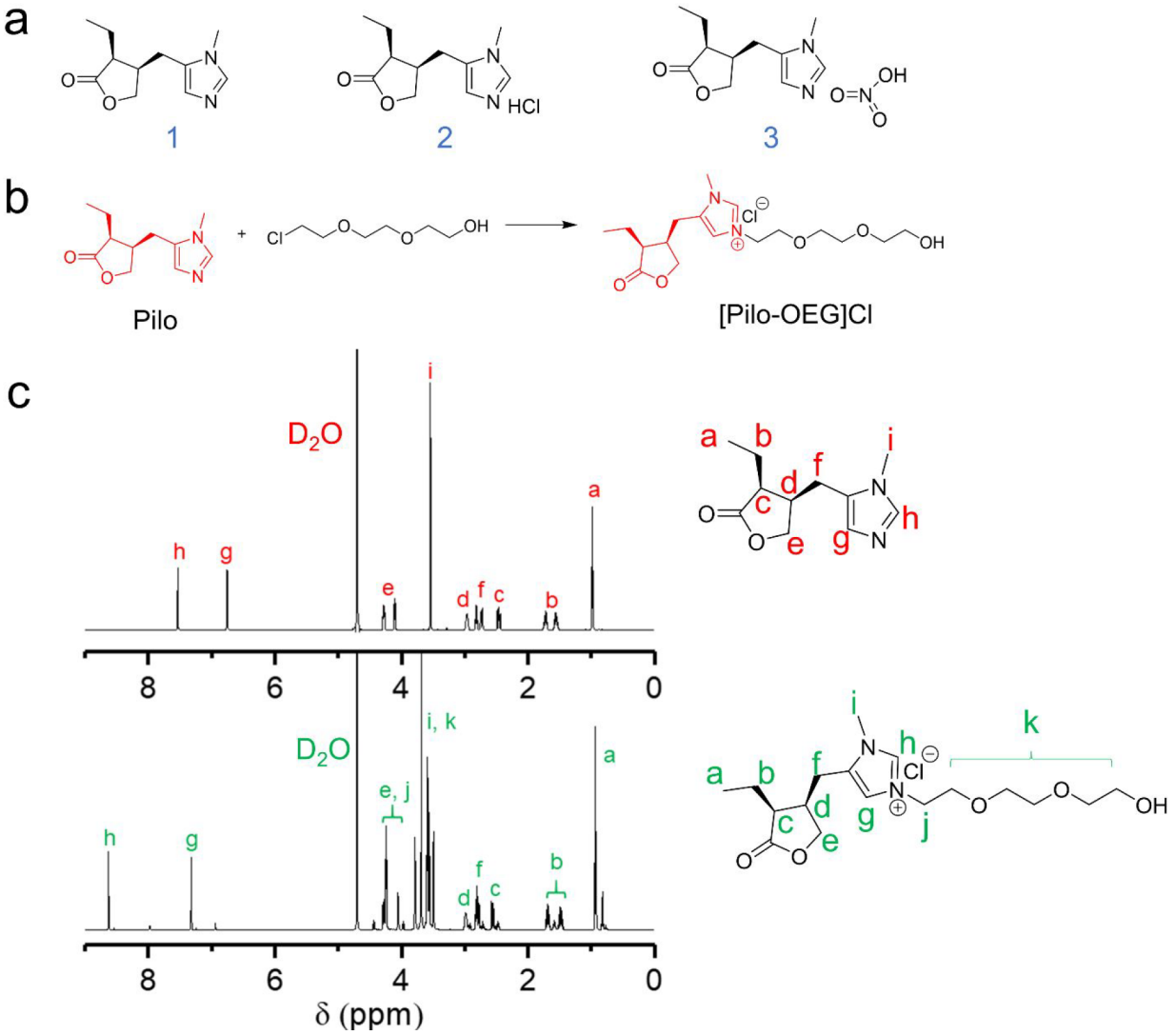

Figure 1. (a) Chemical structures of pilocarpine (**1**), pilocarpine hydrochloride (**2**), and pilocarpine nitrate (**3**). (b) Synthetic route for the synthesis of the ionic liquid pilocarpine analog [Pilo-OEG]Cl. (c) ^{1}H NMR spectra of pilocarpine and [Pilo-OEG]Cl in D_2O.

Current glaucoma medications lower the intraocular pressure (IOP) by either reducing the production of aqueous humor or increasing its outflow. Alpha-adrenergic agonists, beta blockers, and carbonic anhydrase inhibitors reduce the production of the fluid.[19] On the other hand, prostaglandins, rho kinase inhibitors, nitric oxides, and miotic or cholinergic agents help fluid drain from the eye.[19] Pilocarpine is a cholinergic drug that has been used as glaucoma medication for years.[20] Pilocarpine induces miosis to facilitate aqueous humor outflow as a means to reduce IOP and mitigate vision loss.[21,22] Pilocarpine has stability issues as it hydrolyzes to pilocarpine acid (Pilo acid) and/or epimerizes to isopilocarpine (IsoPilo) in aqueous solution and eventually to isopilocarpine acid (IsoPilo acid).[23] IsoPilo, Pilo acid, and IsoPilo acid are all pharmacologically inactive. To improve stability, the commonly used pilocarpine APIs in eye drops are pilocarpine hydrochloride and pilocarpine nitrate, both in salt form (Figure 1a). Like many clinically used antiglaucoma medications, pilocarpine in eye drops also suffers from low bioavailability.[24] For more efficient IOP reduction, effort has been expanded to develop new drugs, discover novel therapeutic targets, and explore combination therapy. In addition, delivery systems have been actively investigated to improve ocular drug bioavailability. It is interesting that ILs can effectively overcome the skin barrier and enhance transdermal penetration of payloads.[25] Therefore, it is likely that IL antiglaucoma pharmaceuticals are capable of self-promoted delivery across physiological barriers and have improved ocular bioavailability. In this work, we report the synthesis and characterization of an IL pilocarpine analog, [Pilo-OEG]Cl, the first-of-its-kind IL form antiglaucoma API. We assessed its biocompatibility, corneal permeability, and IOP-lowering effect and compared it with clinically used pilocarpine hydrochloride. Our results suggest that this newly developed IL pilocarpine analog is a promising drug candidate to treat glaucoma.

EXPERIMENTAL SECTION

Materials. Pilocarpine (Pilo), pilocarpine hydrochloride (PiloHCl), 2-[2-(2-chloroethoxy) ethoxy] ethanol, triethylamine (TEA), and WST-1 assay kit were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Phosphate-buffered saline (PBS, 10×) was purchased from Fisher Scientific (Pittsburgh, PA) and diluted to 1× before use. Human IL-6

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and TNF-α ELISA kits were purchased from Beyotime Biotechnology (Shanghai, China).

Cell Lines. HCE-2 human corneal epithelial cells were purchased from Mingzhou Biotechnology Co., Ltd. (Ningbo, China). The medium for HCE-2 (ATCC CRL-11135, epithelial Adenovirus 12-SV40 hybrid transformed) was Keratinocyte-serum-free medium supplemented with 0.05 mg/mL bovine pituitary extract (BPE), 5 ng/mL epidermal growth factor (EGF), 500 ng/mL hydrocortisone, and 5 μg/mL insulin.

Animals. Normotensive adult Brown Norway female rats (Charles River Laboratories), 8 months old, were purchased from SPF Biotechnology Co., Ltd. (Beijing, China) and used in this work. The rats were given free access to food and water and a 12 h light and dark cycle in a temperature-controlled room (18−24 °C). The procedures conducted were approved by the Institutional Animal Care and Treatment Committee of Sichuan University (Chengdu, China).

Synthesis of Ionic Liquid Pilocarpine Analog ([Pilo-OEG]Cl). Pilo (0.5 mmol, 104 mg) was mixed with 2-[2-(2-chloroethoxy)ethoxy] ethanol (0.53 mmol, 92 mg), and the mixture was heated to 120 °C to allow reflux. ^{1}H NMR (Bruker 600 MHz spectrometer) spectroscopy was used to monitor the reaction progress. The reaction was not stopped until the proton peaks at 6.75 and 7.57 ppm disappeared. [Pilo-OEG]Cl was obtained without further purification. ^{1}H NMR (600 MHz, D_2O) δ 8.63 (s, 1H), 7.32 (s, 1H), 4.26 (dt, J = 20.1, 4.3 Hz, 4H), 4.05 (dd, J = 9.6, 3.2 Hz, 1H), 3.85−3.42 (m, 13H), 2.99 (t, J = 5.4 Hz, 1H), 2.80 (td, J = 15.9, 5.8 Hz, 2H), 2.56 (dd, J = 16.2, 11.3 Hz, 1H), 1.69 (s, 1H), 1.48 (dd, J = 8.1, 6.2 Hz, 1H), 0.93 (s, 3H).

Stability Test. Fresh water solutions of Pilo, PiloHCl, and [Pilo-OEG]Cl (5 mM) were prepared, and their pH at 0, 0.75, 4, 8, and 24 h was measured with a pH meter (PHS-3E, INESA). The samples at 0 and 24 h were further analyzed with LC-MS/MS using a Surveyor Plus HPLC System and triple-stage quadrupole (TSQ) Quantum Ultra Mass Spectrometer (Thermo Fisher Scientific, USA) equipped with an autosampler and electrospray ionization (ESI) source. Separation was carried out on an Hypersil Gold column (150 mm × 2.1 mm, 5 m, Thermo) maintained at 25 °C. The mobile phase consisted of 0.1% (v/v) formic acid water phase (A) and methanol phase (B). The gradient elution program was as follows: 95% of A at 0−5 min and back to 95% of A at 23.1 min, reconditioning of the column for 8 min. The flow rate was 0.2 mL/min. A capillary temperature of 350 °C, spray voltage of 4500 V, sheath gas of 30 arb, and auxiliary gas of 10 arb were maintained as the optimal instrument conditions. Unit resolution was used for Q1 (fwhm 0.7). Nitrogen was used as both sheath gas and auxiliary gas.

pK_a Measurements. The UV−vis spectra of Pilo, PiloHCl, and [Pilo-OEG]Cl in a series of different pH phosphate buffers (pH = 2.2, 5.3, 5.8, 6.6, 7.2, 7.6, and 8.0; concentration = 0.15 mM) were measured on a spectrophotometer (U-3010, Hitachi). The peak absorptions of Pilo, PiloHCl, and [Pilo-OEG]Cl were plotted as a function of pH, and the pK_a was then calculated by Boltzmann fitting analysis.[26,27]

Cytotoxicity Assessment. HCE-2 human corneal epithelial cells were seeded in a 96-well plate at a density of 1 × 10^4 cell/well. After attachment, the cells were incubated with various concentrations of Pilo, PiloHCl, and [Pilo-OEG]Cl for 24 h, and cell viability was determined using a WST-1 assay.

Pro-inflammatory cytokines TNF-α and IL-6 released from HCE-2 were also quantified. Briefly, HCE-2 was seeded in a 24-well plate (1 × 10^5 cells/well) and cultured for cell attachment. The medium in each well was replaced with fresh medium containing Pilo, PiloHCl, and [Pilo-OEG]Cl at different concentrations (1, 2.5, or 5 mM). Following 4 h incubation, the culture media were collected and centrifuged. The supernatants were subjected to ELISA analysis following the manufacturer's instructions.

Hemolysis Toxicity Test. Hemolysis testing followed a method reported previously.[28] Specifically, fresh rabbit blood (2 mL) was centrifuged (3500 rpm, 10 min). The resulting pellet (erythrocytes) was collected and washed with PBS (pH 7.4) to ensure no cell lysis. Erythrocytes were then resuspended in 10 mL of sterile PBS (pH 7.4). To 750 μL of erythrocyte suspension, 50 μL of Pilo, PiloHCl, and [Pilo-OEG]Cl (0.25 mM), PBS (negative control), and Triton X-100 (1% w/v, positive control) were added and incubated for various lengths of time, i.e., 1, 2, 4, and 8 h, under mild shaking at 37 °C. At the end of incubation, the suspensions were centrifuged and 100 μL of the supernatant from each treatment group was withdrawn. The absorbance of each supernatant sample at 450 nm was read using a microplate reader (VERSAmax, Molecular Devices).

Ex Vivo Corneal Permeation Studies. Corneas were extracted from fresh rabbit eyes and mounted immediately in a Franz diffusion cell system with the epithelial surface facing the donor chamber. In each test, Pilo, PiloHCl, or [Pilo-OEG]Cl (200 μL, 10 mg/mL) was loaded to the donor chamber. The receiver chamber was filled with 5 mL of PBS. At predetermined time points within the first 4 h, aliquots of 1 mL from the receiver chamber were collected via syringe and analyzed with a UV−vis spectrophotometer (U-3010, Hitachi). Fresh PBS (1 mL) was added to the receiver chamber following each sampling. All of the experiments were conducted in triplicate. The permeability coefficient, P, was then determined based on the following equation

$$P = \frac{dQ/dt}{AC}$$

where dQ/dt is the steady-state slope of a cumulative flux curve (in this study, the data in 1 h is used for dQ/dt), C is the drug concentration in the donor chamber, and A is the effective cross-sectional area (0.629 cm^2) available for diffusion.[29]

In Vivo Efficacy Assessment. Rats were randomly grouped into two groups of four (n = 4). The right eye of each rat was instilled with PiloHCl or [Pilo-OEG]Cl PBS solution (2 × 5 μL, 82 mM pilocarpine equivalent), one dose at 8:00 am and the second dose at 12:00 pm. The IOPs of both eyes were measured at 8:00 am (before dosing), 8:45 am, 12:00 pm (before the second dosing), 12:45 pm, and 16:00 pm using an Icare TONOLAB tonometer TV02. At the end of the experiment, we randomly selected one rat from each group. The selected rats were euthanized. The eyes were enucleated, immediately fixed in Davidson's solution, and then processed to obtain hematoxylin−eosin (HE) staining tissue slices. Tissue images were taken under a Pannoramic 250 digital slide scanner (3DHISTECH, Hungary).

Statistical Analysis. The data were expressed as means ± standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA). For pH, cytotoxicity, cytokine, and hemolysis results, Student−Newman−Keuls q (SNK-q) tests were applied to analyze the multiple comparison after

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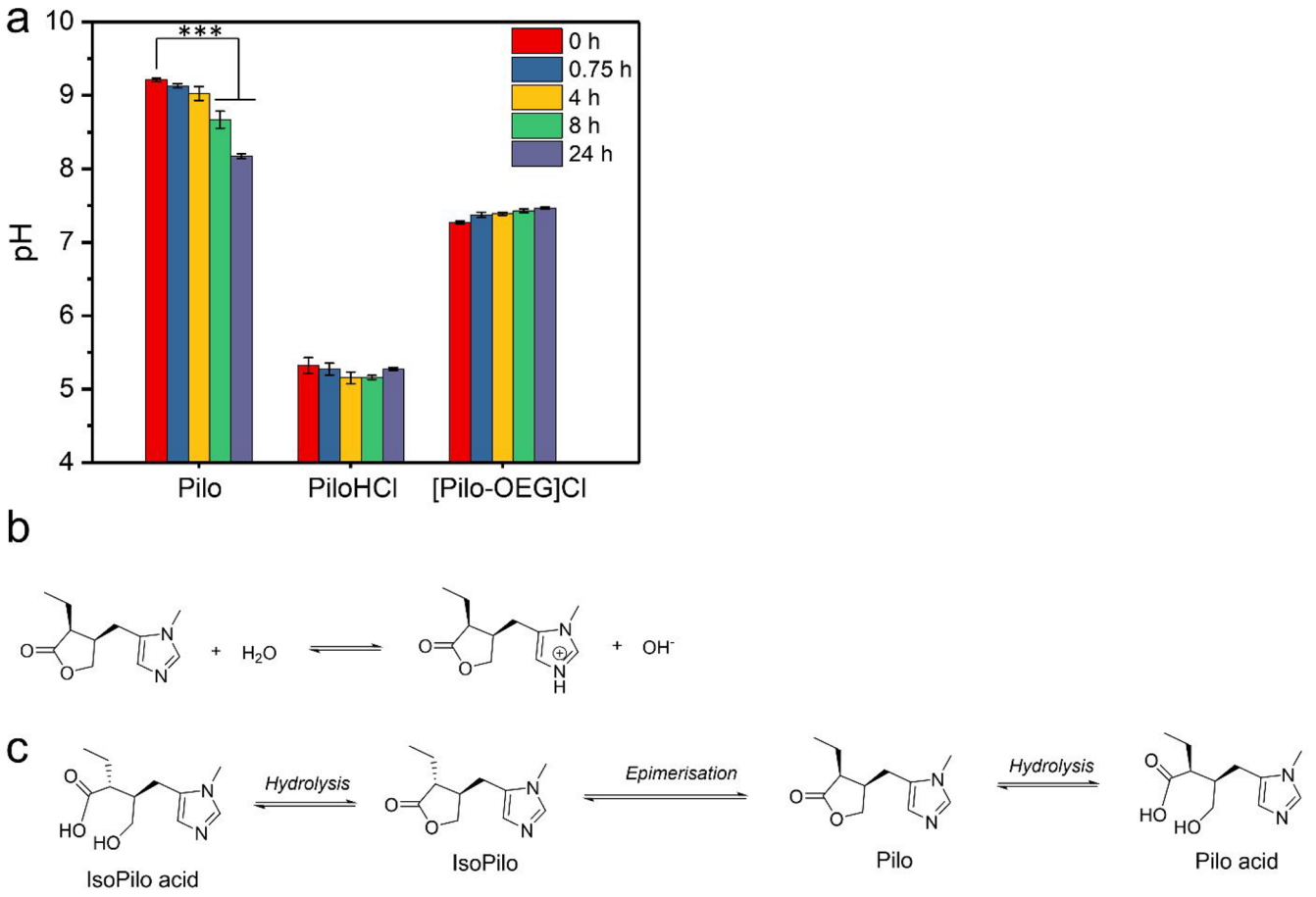

Figure 2. (a) pH changes of Pilo, PiloHCl, and [Pilo-OEG]Cl aqueous solution (5 mM) over a course of 24 h. (***) $P < 0.001$. (b) Dissociation of Pilo in water. (c) Hydrolyzation and degradation pathways of Pilo.

ANOVA. For IOP data, a two-sample *t*-test was adopted. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Structure Identification and Stability of the Ionic Liquid Pilocarpine Analog [Pilo-OEG]Cl. IL pilocarpine analog [Pilo-OEG]Cl was obtained from the reaction between oligo-polyethylene glycol chloride and pilocarpine at the imidazole ring (Figure 1b). Comparing the spectrum of [Pilo-OEG]Cl with the ^{1}H NMR spectrum of Pilo (Figure 1c), the chemical shifts of the protons in the imidazole ring (g and h) of [Pilo-OEG]Cl shifted from 6.75 and 7.57 ppm to 7.32 and 8.63 ppm. These shifts as well as the appearance of the proton peaks of OEG at 3.85−3.42 ppm verified the successful synthesis of [Pilo-OEG]Cl. [Pilo-OEG]Cl is composed of organic cations ($[Pilo\text{-}OEG]^+$) and inorganic anions (Cl^-), and it is liquid at room temperature, confirming its IL characteristics.

The pH values of freshly prepared Pilo, PiloHCl, and [Pilo-OEG]Cl solutions are 9.2, 5.3, and 7.3, respectively (Figure 2a), at the same concentration of 5 mM. Pilo is a weak alkaline drug and it dissociates in water (Figure 2b). In contrast, as a strong acidic−weak alkaline salt, PiloHCl hydrolyzes in water. Nonetheless, [Pilo-OEG]Cl is an IL, and it does not alter the pH of water upon addition, a well-recognized property of ILs.[30] We monitored the pH values of their solutions as a means to check their structural stability. Within 24 h, the pH values of PiloHCl and [Pilo-OEG]Cl aqueous solutions remain relatively stable. However, the pH of Pilo solution drops to 8.7 at 8 h and 8.2 at 24 h, significantly deviating from its initial pH value of 9.2. The hydrolysis of both Pilo and IsoPilo leads to lactone ring opening (Figure 2c). A carboxyl group appears as a result. Acting as a proton donor, the carboxyl group contributes to the pH decrease of the solution. Neither PiloHCl solution nor [Pilo-OEG]Cl solution experienced pH reduction, indicating their structural stability. In a clinical eye drop formulation of PiloHCl, the pH is controlled by adding hydrochloride or sodium hydroxide. In this work, we aim to compare the property of the traditional salt form drug with the newly developed IL salt form drug candidate. As shown in Figure 2a, [PiloOEG]Cl and PiloHCl have better pH stability compared with Pilo, demonstrating that the conversion of Pilo to IL can improve the stability similar to conversion to its traditional salt form. In addition, the pH value of [PiloOEG]Cl is close to that of the normal physiological environment. Therefore, by converting pilocarpine into an ionic liquid, not only was the stability improved but also the pH value was close to normal physiological conditions without using any pH regulator.

Our LC-MS analysis further ascertains the structural instability of Pilo and PiloHCl. In the Pilo solution, IsoPilo formed (eluted at 2.02 min, m/z = 209.06) and further hydrolyzed into acids (m/z = 227.06) (Figure 3a). IsoPilo also formed in the PiloHCl solution, although no hydrolyzed Pilo acid or IsoPilo acids was detected (Figure 3b). In contrast, neither IsoPilo nor Pilo and IsoPilo acids formed in the [Pilo-

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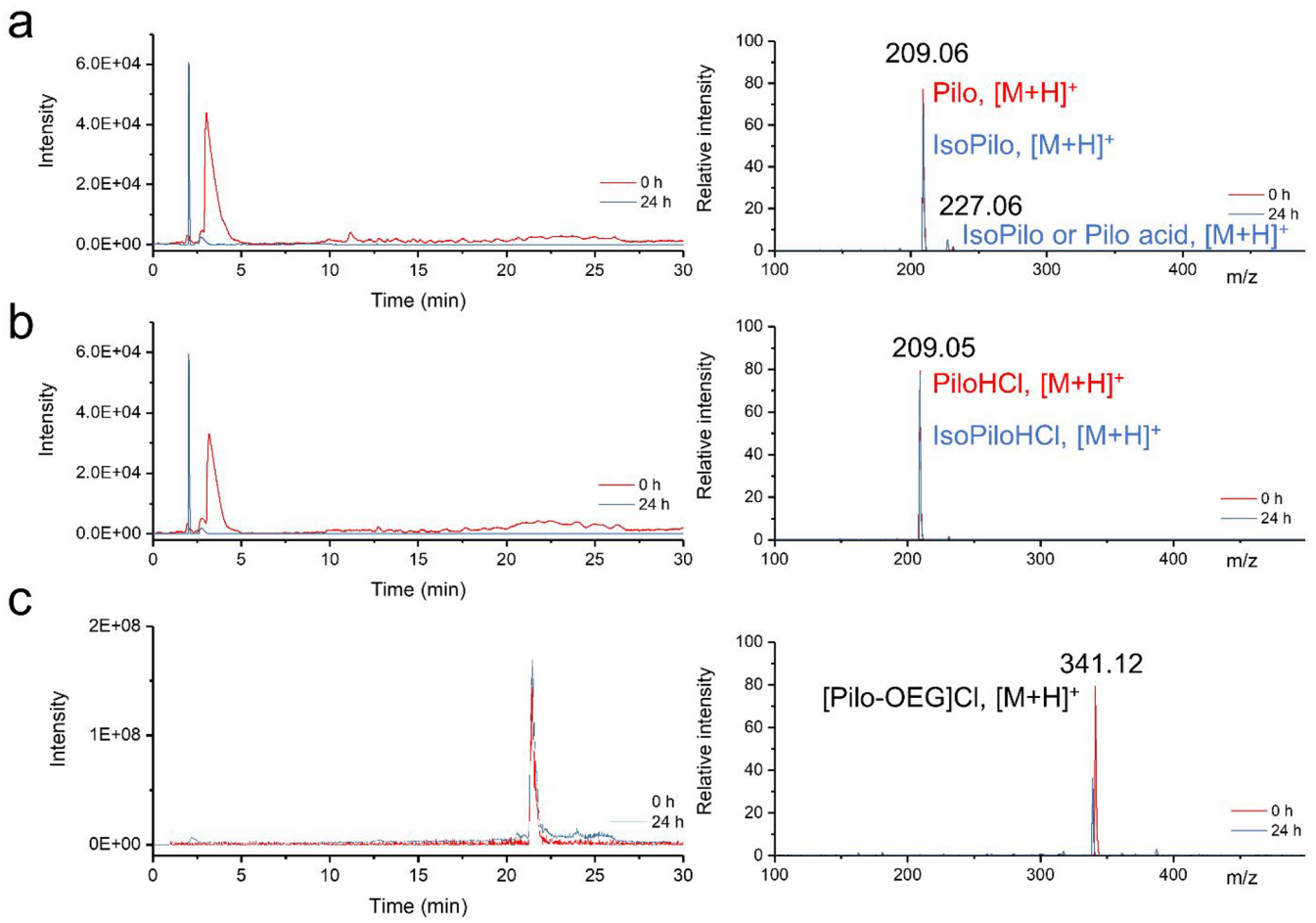

Figure 3. LC-MS chromatograms of Pilo (a), PiloHCl (b), and [Pilo-OEG]Cl (c): (left) LC chromatogram; (right) MS spectra of the eluents.

OEG]Cl solution (Figure 3c). Since IsoPilo, Pilo acid, and IsoPilo acid are pharmacologically inactive, the superior stability of [Pilo-OEG]Cl compared with Pilo and PiloHCl becomes a huge advantage.

Cytocompatibility. The cytotoxicity of ILs has been a topic of debate, delaying their entry into the biomedical field. ILs were initially considered as nontoxic "green" solvents because of their nonvolatility.[7] A recent study revealed the toxic potential of ILs.[31] Their cytotoxicity depends on the molecular structures and dose. It is possible to design ILs with low cytotoxicity by integrating the structures of the cations and anions of ILs.[32] At nontoxic concentrations, ILs could enhance drug solubility, enable a high drug content in topical formulations, and maintain drug stability.[33] Therefore, it is important to evaluate the cytotoxicity of ILs when used as pharmaceutics. In this study, we examined the cytotoxicity of the newly formed [Pilo-OEG]Cl to HCE-2 human cornea epithelium cells (Figure 4a). PiloHCl shows strong dose-dependent cytotoxicity. Its IC_{50} was determined to be 25 mM, suggesting high tolerance by cells. Within a wide range of concentrations (from 1 mM to 50 mM), both Pilo and [Pilo-OEG]Cl are cytocompatible. Since corneal inflammation such as superficial keratitis is one of the likely side effects of pilocarpine eye drops, it is necessary to test the inflammatory response of Pilo, PiloHCl, and [Pilo-OEG]Cl. In this work, we examined the inflammatory response in HCE-2 cells by assessing two cytokines IL-6 and TNF-α. Compared with the control and Pilo groups, both PiloHCl and [Pilo-OEG]Cl induce about 100 pg/mL TNF-α secretion especially at 2.5 and 5 mM (Figure 4b). However, there is no significant statistical difference between PiloHCl and [Pilo-OEG]Cl, indicating that [Pilo-OEG]Cl has a compatible pro-inflammatory property related with TNF-α with PiloHCl. At the tested doses, all the three groups (Pilo, PiloHCl, and [Pilo-OEG]Cl) did not stimulate IL-6 production (Figure 4c). Compared to the negative and positive controls, none of the tested groups showed hemolytic behavior, indicating the excellent blood compatibility of Pilo, PiloHCl, and [Pilo-OEG]Cl (Figure 4d).

Corneal Permeation Property. We studied the permeation behavior of Pilo, PiloHCl, and [Pilo-OEG]Cl across the rabbit cornea. As shown in Figure 5a, within 4 h, 27.8 ± 14.7% of Pilo and 8.1 ± 1.8% of PiloHCl permeated through the cornea while as high as 51.6 ± 8.1% of [Pilo-OEG]Cl (6-fold higher than PiloHCl) crossed the cornea. The permeability coefficient of [Pilo-OEG]Cl was calculated to be 1.3×10^{-5} cm/s, while the permeability coefficients of Pilo and PiloHCl are 6.6×10^{-6} and 1.5×10^{-6} cm/s, respectively. The permeability coefficient of [Pilo-OEG]Cl is 2-fold higher than that of Pilo and 8-fold higher than that of PiloHCl.

The dissociation constants (pK_a) of Pilo, PiloHCl, and [Pilo-OEG]Cl were determined to be 6.99, 8.38, and 5.77, respectively (Figure 5b--d). The obtained pK_a value of Pilo is consistent with the value reported in *DrugBank* (6.78) and the *Merck Index* (7.15). The intrinsic physicochemical factors of pharmaceutics including the pK_a and molecular weight are crucial for its passive diffusion across the cornea.[34] In this

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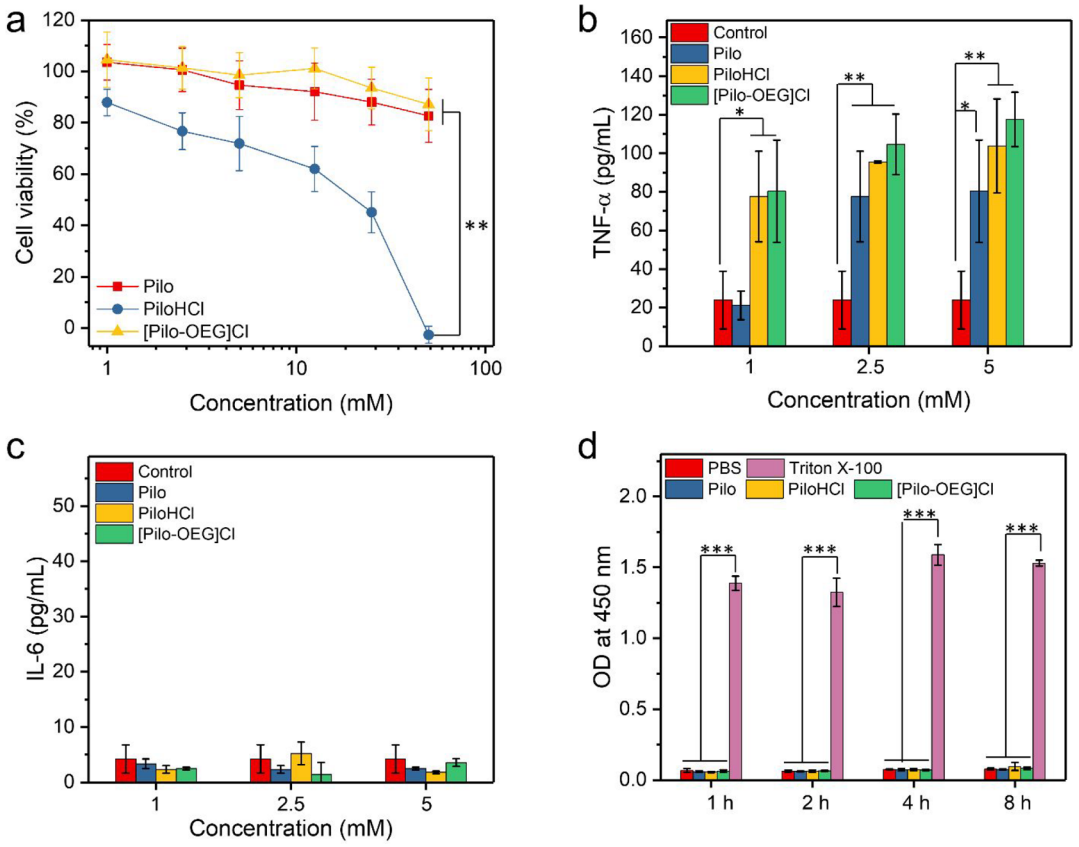

Figure 4. (a) Cytotoxicity of Pilo, PiloHCl, and [Pilo-OEG]Cl on HCE-2 cells, $n = 7$. (b and c) Cytokines of TNF-α and IL-6 expression on HCE-2 after 4 h incubation, $n = 3$. (d) Hemolysis of Pilo, PiloHCl, and [Pilo-OEG]Cl, $n = 6$. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$.

study, the effect of pK_a is particularly important since there is almost no difference in the molecular weights of Pilo, PiloHCl, and [Pilo-OEG]Cl. On the basis of the Henderson−Hasselbalch equation and the pK_a values, about 38.1% of Pilo, 3.58% of PiloHCl, and 93.8% of [Pilo-OEG]Cl are expected to be in their nonionized form in precorneal tear film (pH ≈ 7.2). As ionized drugs are minimally lipid soluble, passive diffusion through the cornea is not favored at all, thus justifying the superior permeability values of [Pilo-OEG]Cl to those of Pilo and PiloHCl.[34,35]

In Vivo Efficacy and Safety. While the improved in vitro transport properties of [Pilo-OEG]Cl are encouraging, it is also critical for [Pilo-OEG]Cl to show IOP-lowering effects. We examined the in vivo IOP-lowering effects of [Pilo-OEG]Cl in normotensive adult Brown Norway female rats. The normotensive adult Brown Norway rats have normal IOP and are broadly used as models for the investigation of neurodegenerative diseases, including glaucoma. PiloHCl was included as a positive control. Similar to PiloHCl, the highest IOP reduction of [Pilo-OEG]Cl is seen at 0.75 h postadministration (Figure 6a). Although the IOP reduction caused by [Pilo-OEG]Cl (2.75 mmHg) was larger than that caused by PiloHCl (1.25 mmHg), there was no statistical difference. Therefore, [Pilo-OEG]Cl is not considered to be more efficient in lowering the IOP than PiloHCl at 0.75 h. A second dose was applied 4 h after the first dose. The second dose of PiloHCl kept the IOP reduction of ∼1 mmHg. In contrast, a second dose of [Pilo-OEG]Cl induced a stronger IOP reduction of 2.625 mmHg. The IOP reduction by [Pilo-OEG]Cl was significantly deeper than that of PiloHCl at 4.75 h by statistical analysis. We noticed that [Pilo-OEG]Cl did not decay the rebound of the IOP compared with PiloHCl. Overall, [Pilo-OEG]Cl was shown to be more effective than PiloHCl. There is no abnormal tearing caused by either PiloHCl or [Pilo-OEG]Cl, indicating that there is no apparent irritation induced by the new IL pilocarpine analog (Figure 6b). Further in vivo safety was evaluated by histologic analysis of the whole ocular globe (Figure 6c). The histologic whole globe tissue images treated by both PiloHCl and [Pilo-OEG]Cl showed that there was no visible structural or pathologic changes caused to ocular tissues when compared with the blank control group (left eyes). The corneal tissues treated by the PiloHCl and [Pilo-OEG]Cl groups exhibited a well-defined layered morphology except slight corneal exfoliation. The cells in the uppermost layer of the corneal epithelium are continuously desquamated from the surface and replaced by cell proliferation. This is a normal process for renewal and regeneration of the corneal epithelium.[36] Due to the strong regenerative capacity of corneal epithelial cells, this slight cell exfoliation would not cause corneal injury.[36,37] Given the widespread use of PiloHCl in clinical practice, the rapid regeneration capacity of corneal epithelial cells, and the assessment by clinicians, slight cell exfoliation is within the acceptable safety range. Besides, there was no infiltration of inflammatory cells, loss of tissue integrity, or edema observed for the cornea, retina, and the drug-targeting tissue, ciliary body.

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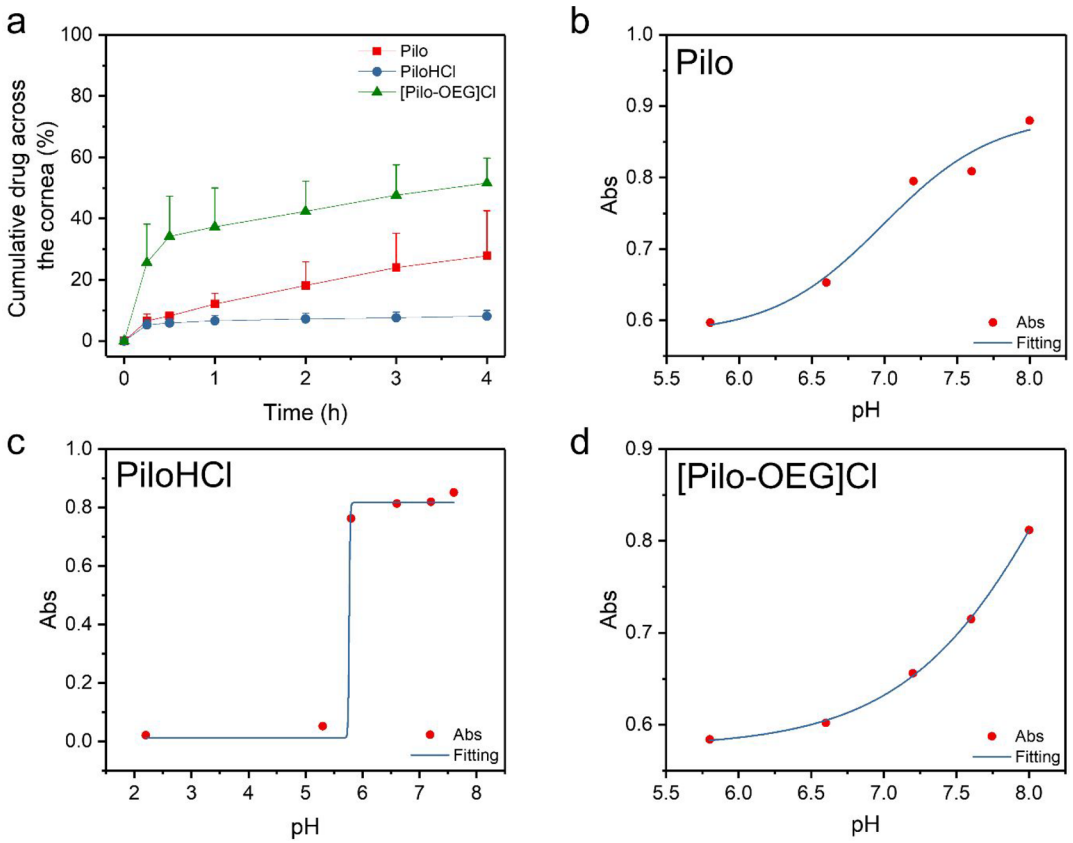

Figure 5. (a) Permeability of Pilo, PiloHCl, and [Pilo-OEG]Cl across the cornea. (b–d) pK_a fitting based on the plot of the peak absorbance of Pilo, PiloHCl, and [Pilo-OEG]Cl vs pH.

CONCLUSIONS

We synthesized an ionic liquid pilocarpine analog [Pilo-OEG]Cl. Compared to PiloHCl, [Pilo-OEG]Cl shows enhanced stability, cytocompatibility, corneal permeability, and in vivo IOP-lowering efficacy. This work represents a new way to develop effective analogs for old glaucoma drugs. [Pilo-OEG]Cl exhibits enhanced stability in aqueous media, cytocompatibility, and cornea permeability without compromising water solubility. It is probably not necessary to add thickeners, pH-adjusting agents, and other inactive ingredients using IL API to prepare a clinical formulation. Besides the improved physical and biological properties owing to its unique IL form, this new antiglaucoma drug candidate, [Pilo-OEG]Cl, could be further tailored to have additional features. The chloride anion could be altered by anion metathesis readily. The moiety oligo-PEG could be substituted with other chloride-containing compounds or drugs. The incorporation of the OEG moiety endows [Pilo-OEG]Cl with the possibility for further chemical modification via its hydroxyl end group.

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Author Contributions

J.W., B.L., and H.Y. proposed and designed the research. J.W. performed most of the experiments. J.T. worked on the histological analysis. L.Q. worked on the pK_a measurement and in vivo experiments. X.Q. conducted the hemolysis toxicity study. N.X. conducted the cytotoxicity tests. J.W. and H.Y. read and approved the manuscript. All of the authors have read and approved the final manuscript.

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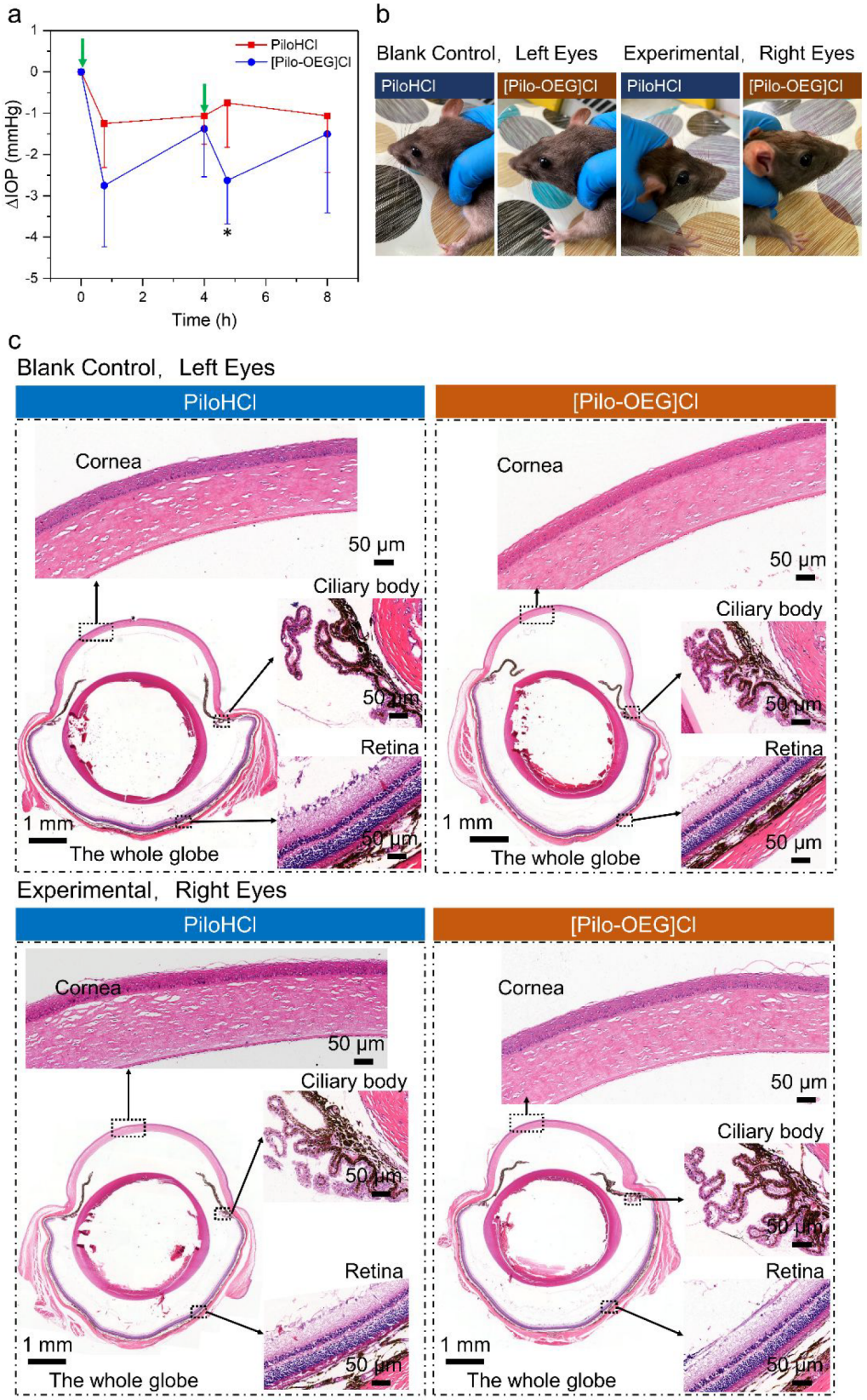

Figure 6. In vivo IOP-lowering effects and compatibility evaluation. (a) IOP-lowering effects of PiloHCl and [Pilo-OEG]Cl in normotensive rats following two-time topical instillation. Green arrows mean instillation. $n = 4$; (*) $P < 0.05$, PiloHCl vs [Pilo-OEG]Cl. (b) Images of rat eyes immediately after instillation. (c) Ocular tissues of rat eyes at the end of experiments.

Notes

The authors declare no competing financial interest.

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