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Ion-paired pirenzepine-loaded micelles as an ophthalmic delivery system for the treatment of myopia

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Abstract

Myopia is one of the most common ocular disorders for which standard treatments, such as refractive surgery, often involve invasive procedures. Pirenzepine (PRZ), a muscarinic receptor antagonist, has been recognized as a promising candidate for the treatment of myopia, but possesses poor ocular bioavailability. The overall objective of this study was to prepare PRZ-sorbic acid complexes suitable to be encapsulated into micelles with high efficiency for optimal ophthalmic delivery. The results demonstrated that sorbic acid, used as the counter ion, had the most significant effects in increasing octanol–water distribution coefficient of PRZ as well as improving its corneal permeability *in vitro* among various counter ions tested. *In vivo* absorption results showed that a 1.5 times higher bioavailability was achieved by the addition of sorbic acid at 1:1 ratio. Cytotoxicity study *in vitro* and the biocompatibility study *in vivo* indicated that the micelles did not cause significant toxicities on the eyes.

Key words: PRZ; Ion-pair formation; Micelles; And ophthalmic delivery

Myopia is one of the most common ocular disorders around the world and is becoming more prevalent among younger generations. Asia, in particular, has seen a rapid growth of occurrence with prevalence reaching a whopping 60% in recent years.[1] The progression of myopia could lead to some complications including maculopathy, cataract, glaucoma and retinal detachment.[2] More importantly, high myopia is one of the leading causes of blindness in developed countries.[3] Although traditional eyeglasses and refractive surgeries are able to correct the visual abnormalities caused by myopia, these treatments are still far from satisfactory for complete recovery of myopia in the long term.[4] Therefore, it is utterly important to identify proper treatments for children with myopia.[1–3] Recently, several controlled clinical trials have provided evidence that atropine, a classic muscarinic antagonist that binds potently to both M_3 (accommodation and mydriasis) and M_1 muscarinic receptors (putative myopia),[5,6] can slow down myopia progression in children. However, the clinical use of atropine as a therapeutic has been limited due to serious ocular side effects such as mydriasis and cycloplegia because of undesirable binding to the M_3 receptor.[7] Alternatively, pirenzepine (PRZ, Figure 1) is a muscarinic receptor antagonist that is selective only to the M_1 receptor,[8,9] and thus is less likely to cause mydriasis and

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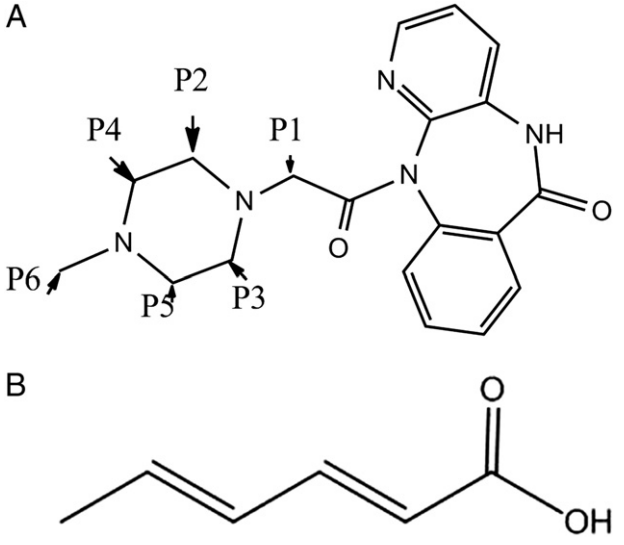

Figure 1. Structure of (**A**) PRZ and (**B**) sorbic acid.

cycloplegia than atropine. PRZ has also been shown to inhibit the development of deprivation-induced myopia and the axial elongation of eyes,[10,11] and PRZ solutions of up to 2% did not elicit any systemic side effects in adult volunteers according to previous phase-I trials on safety and tolerability.[12] As a hydrophilic compound, however, PRZ often suffers from very low transcorneal permeability and poor ocular bioavailability, which will decrease its anti-myopia effect.[13]

To overcome this delivery challenge, micellar systems had been widely investigated as carriers for PRZ to facilitate the internalization process *via* endocytosis and endosomal permeation and have been reported to increase ocular availability of PRZ by two-fold without causing any corneal damage, which is typically associated with free suspension of the drug. A material that is of particular interest in the current study is the amphiphilic block co-polymer mPEG-PDLLA. It has been reported that micelles self-assembled from mPEG-PDLLA display minimal cytotoxicity to both tumor and healthy mammalian cells[14–17] and are characterized by a unique core-shell structure with uniform size distribution. The spatial distribution of the drugs within the micelles depends on their polarities. In an aqueous environment, nonpolar molecules will be entrapped in the core, polar molecules will be adsorbed onto the surface, and substances with intermediate polarity will be distributed in certain intermediate positions.[18] However, the loading efficiency of PRZ into the micelles will be limited by its hydrophilicity, which makes it harder to be encapsulated into the hydrophobic core of micelles. Fortunately, many techniques can be utilized to further improve loading capacity as well as the corneal permeation of therapeutics.[19]

Ion pair formation, especially the organic acid ion pair formation, is one of the most promising strategies for improving loading capacity. An ion-pair is a pair of oppositely charged ions interacting with each other *via* Coulombic attractions instead of forming a covalent bond. As a result, they will behave like a single unit. Kato et al[19] reported that the ion pair formation between the drug and the organic acid could significantly increase the hydrophobicity of the drug and therefore effectively improve loading efficiency as well as eventually, bioavailability in the eyes.

Among the organic acids, sorbic acid (SA, Figure 1), an unsaturated fatty acid with six carbon atoms, might have the potential to help increase the hydrophobicity of the drug while maintaining suitable water solubility. Higashiyama et al discovered that SA could increase the oil–water distribution coefficient of timolol and its permeability across the cornea. At the optimal molar ratio of 2:1 (SA:timolol), the maximum concentration (C_{max}) and the area under the curve (AUC) were found to increase by 3.15 and 2.17-fold, respectively, as compared to the reference group, meaning significant enhanced permeability across the cornea.[20] In addition, the safety of SA for oral and ophthalmic use has been evaluated extensively, which is included in the China Pharmacopeia (volume IV) and approved by the State Food and Drug Administration (SFDA) for use.[21] Therefore, SA could be a promising candidate to be used as the counter ions to optimize lipophilicity as well as to enhance safety of the drug.

Thus, for all of the above reasons, the objective of the current *in vitro* and *in vivo* study was to design, characterize and optimize an SA/PRZ encapsulated micellar system made from an amphiphilic block co-polymer for ophthalmic delivery. In our previous work,[18] PRZ alone was adsorbed onto the mPEG corona of the micelles and PRZ was only present on the outer surface of the micelles, which limited drug loading efficiency. The addition of SA in the current study increased the hydrophobicity of PRZ, which led to a higher drug loading efficiency in the hydrophobic core of the micelles. Additionally, the complexation between SA and PRZ in mPEG-PDLLA micelles could lower the polarity of the resulting complexes and lead to marked alterations to both ocular penetration and the bioavailability of PRZ *in vivo*. Specifically, the impact of different amounts of SA on PRZ, including their effects on ocular permeability as well as on the octanol–water distribution coefficient (DC_{app}) of PRZ in the micelle systems was extensively investigated. To demonstrate its applicability *in vivo*, the ocular pharmacokinetics of PRZ micelles using SA as the counter ion were also evaluated.

Methods

Materials

Pirenzepine dihydrochloride (purity >99.5%) was obtained from Wanlian Pharmaceutical Co. (Ningbo, China). Methoxyl poly (ethylene glycol)-poly(D,L-lactic acid) (mPEG-PDLLA, Mw = 5000, molar ratio of mPEG/PDLLA = 40/60) was purchased from Xi'an ruixi Biological Technology Co. Hydroxypropyl methylcellulose (HPMC) (Methocel K100 M) was obtained from Colorcon (Shanghai, China). All organic acids were purchased from WanQing Chemical Glassware Instrument (Nanjing, China). All the reagents were analytical grade and used without further purification.

Corneal epithelial cell culture

Human corneal epithelial (HCE-2) cells purchased from the American Type Culture Collection (ATCC®number CRL-11135) were maintained in 175 cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM)/F12 (Gibco, Invitrogen, Carlsbad, Calif., USA) containing 10% fetal calf serum, 100 U/ml penicillin G, and 100 μg/ml streptomycin sulfate in a 37 °C, humidified, 5%