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A potential carrier based on liquid crystal nanoparticles for ophthalmic delivery of pilocarpine nitrate

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ABSTRACT

Poor corneal penetration and short preocular retention of a clinical hydrophilic drug, pilocarpine nitrate (PN), for the treatment of open-angle glaucoma and acute angle-closure glaucoma, limit its ocular application. The purpose of this study was to investigate the potential of liquid crystal nanoparticles (LCNPs) for ocular delivery of PN. LCNPs were developed by a top-down method using glyceryl monoolein (GMO) and water in the presence of stabilizer Poloxamer 407. They were characterized by transmission electron microscopy (TEM) and small angle X-ray diffraction (SAXS). The size of LCNP is 202.28 ± 19.32 nm and the encapsulation efficiency reached 61.03%. The *in vitro* release profiles indicated that PN could keep sustained release from PN-loaded LCNPs for 8 h. An *ex vivo* corneal permeation study revealed that the apparent permeability coefficient of PN-loaded LCNPs was 2.05-fold higher than that of commercial eye drops. In addition, the topical administration test showed that PN-loaded LCNPs had a prolonged effect on decreasing intraocular pressure (IOP) of rabbits compared with commercial drug and physiological saline. In conclusion, LCNPs had been demonstrated to be potential for controlled-release ocular drug delivery.

1. Introduction

Glaucoma is the most common cause of irreversible blindness, which will affect 79.6 million people in 2020 worldwide (Quigley and Broman, 2006). The causes of glaucoma include increased intraocular pressure (IOP), oxidative stress and impaired ocular blood flow. As a miotic agent, pilocarpine nitrate (PN) has been used for the treatment of chronic open-angle glaucoma and acute angle-closure glaucoma for over 100 years (Nair et al., 2012). However, the high hydrophilicity of PN usually possesses several limitations such as poor corneal penetration, precorneal tear clearance and short preocular retention which sharply reduce the ocular bioavailability of PN (less than 5% or even below 1%) (Nagarwal et al., 2009). Therefore, it is required for frequent administration of a large quantity of PN, which will induce several undesirable side effects, such as myopia and miosis (Ticho et al., 1979). To overcome these shortcomings of the conventional remedy, ointments, gel, or ocusert have been developed as new topical formulations with prolonged ocular residence time (Anumolu et al., 2009; Miller and Donovan, 1982; Shell, 1984; Sieg and Robinson, 1979). However, gel and ointments with high viscosity might adversely accelerate the blinking frequency, leading to a discomfort feeling. Non-erodible inserts of ocusert are considered to be a technical breakthrough even though they also limit patient compliance, due to the requirement of weekly insertion and difficulty of their removal. To enhance the ocular residence time on the cornea and prolong the drug's pharmacological activity, colloidal dosage formulations of drug delivery systems (DDSs) such as liposomes (Li et al., 2009), nanoparticles (Ibrahim et al., 2010), nanocapsules (Desai and Blanchard, 2000), microspheres (Gavini et al., 2004), and microemulsions (Vandamme, 2002) could be used for glaucoma therapy. Liquid crystalline phases of both the bulk and dispersed forms with multidimensional structures (Mulet et al., 2013), based on the underlying crystal lattices, are increasingly recognized as offering more desirable properties than other nano-carriers. Despite the intense interest in these systems, liquid crystalline phases are still over-shadowed by the other colloidal formulations particularly for the investigation of their application in ophthalmic drug delivery.

GMO, a nontoxic, biodegradable, and biocompatible amphiphilic lipid, swells in the water, and then spontaneously form well-ordered liquid crystalline phases. The most common liquid crystalline phases are lamellar phase (L_α), inverted hexagonal phase (H_2), and cubic phase (V_2) (Bansal et al., 2012; de Campo et al., 2004; Yaghmur and Glatter, 2009a). These phases can be readily dispersed into nanoparticles through high energy input

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methods, which is influenced by temperature and water content (Thadanki et al., 2011). It has been reported that LCNPs associated with their bulk phase could retain their internal structure, morphology and stability, and show some unique properties such as small particle size, low viscosity and good biocompatibility (Guo et al., 2010). The structure of LCNPs in terms of their high internal interfacial area is separated into hydrophilic and hydrophobic domains, which gives a possibility of encapsulating hydrophilic, lipophilic as well as amphiphilic drugs (Vandoolaeghe et al., 2006). Therefore, PN may be well trapped into LCNPs. Taking into account of nontoxicity, bioadhesive nature and sustained-release behavior (Drummond and Fong, 1999), LCNPs may be a good candidate for PN ocular delivery. Furthermore, because of the similarity to biological membrane, LCNPs may enhance the drug delivery topically (Gan et al., 2010; Lopes et al., 2006) as well as orally and intravenously (Chung et al., 2002; Johnsson et al., 2006).

In this study, the PN-loaded LCNPs were constructed by top-down approach (Guo et al., 2010) and characterized by TEM and SAXS. The *in vitro* release profiles of PN were studied using a dynamic dialysis method. An *ex vivo* penetration study was performed using freshly excised rabbit cornea. The irritation and safety of the PN-loaded LCNPs were evaluated by Draize (Draize et al., 1944a) method, corneal hydration levels and historical examination. Finally, the *in vivo* efficacy of IOP reduction was evaluated.

2. Materials and methods

2.1. Materials

Glyceryl monoolein (GMO) with glyceryl monoolein purification of ≥80% was purchased from Aladdin Reagent Co. Ltd (Shanghai, China). Poloxamer 407 (F127) was obtained by BASF (Ludwigshafen, Germany). Pilocarpine nitrate (PN) was purchased from Leawell International Ltd (Brazil). Double distilled water was purified using Milli-Q (Gradient). All other chemicals and reagents used in the study were of analytical grade.

2.2. Animals

Adult New Zealand white rabbits (3.0–3.5 kg) were supplied by Animal Experimental Center of Nanjing Medical University (Nanjing, China). They were acclimated at 25 °C and 55% of humidity under natural light/dark conditions for 1 week before experiment. All animal experiments were performed in accordance with guidelines evaluated and approved by the ethics committee of Nanjing Medical University (Nanjing, China).

2.3. Preparation of PN-loaded LCNPs

Bulk phase was prepared through top-down method (Guo et al., 2010) by adding PN solution at an appropriate concentration (35% w/w) to the molten GMO (2.0 g) and F127 (0.22 g) lipid at 60 °C. The mixture was heated to 60 °C, vortexed, centrifuged at 1500 *g* and repeated this cycle three times. Afterwards, the sample was kept at room temperature until its homogenous state was achieved.

To prepare PN-LCNPs, the homogenous state mixture was melted to low viscosity at 60 °C and added into water using an 18-gauge needle. The concentration of the bulk phase in the dispersion was approximately 10% (w/w). The crude dispersion was subsequently fragmented by intermittent probe sonication (Scientz-IID, Shanghai, China) for 10 min in a pulse mode (2 s pulses interrupted by 9 s breaks) at 10% of maximum power (95 W) energy input at 25 °C. The resulting dispersion was extruded through high-pressure homogenizer (Avestin Em-C3, Ottawa, Canada) (Yang et al., 2004) by five cycles at 500 bar to form an opalescent dispersion for particle size reduction. Finally, the free drug was removed by centrifugal filter devices. Then the final concentration of PN was adjusted to 0.5 and 1.0% (w/v). The LCNP dispersion was stored at room temperature for further analysis or use.

2.4. Characterizations of PN-LCNPs

2.4.1. Morphology

The morphology of PN-loaded LCNPs was viewed by transmission electron microscope (TEM) (JEM-1010). The samples were prepared by loading a 5 μL droplet of the formulations onto a 300-mesh carbon-coated copper grid, and then the excess fluid was removed by an absorbent paper.

2.4.2. Small-angle X-ray scattering (SAXS) measurement

The SAXS measurements were carried out on a Bruker Nanostar SAXS camera, with pinhole collimation for point focus geometry. The SAXS camera was fitted with a Hi-star 2D detector (effective pixel size 100 μm). The optics and sample chamber were under vacuum to minimize air scatter. The liquid samples were contained in 0.5 mm glass capillaries and temperature controlled by Peltier system accurate to 25 °C ± 0.1 °C. Samples were equilibrated for 30 min prior to measurement. Scattering patterns were acquired over 60 min for dispersions.

2.4.3. Particle size, pH and osmotic pressure

The particle size (PS) and polydispersity index (PI) of PN-LCNPs were determined by photon correlation spectroscopy using a photo correlation spectroscopy with Malvern Zetasizer 3000 (Malvern Instruments, Malvern, UK) after dilution with double distilled water at 25 °C.

The pH was established at 25 °C using a Model PHS-3C pH-meter (Shanghai precision & Scientific Instrument Co., Ltd, China). The Osmotic pressure was estimated by the freezing-point method using a Model FM-9X Osmometer (Instrumental Factory of Shanghai Medical University, Shanghai, China).

2.4.4. Determination of encapsulation efficiency and drug loading

The drug encapsulation efficiency (EE %) and drug loading (DL %) were determined by ultra-filtration method using Microcon centrifugal filter devices (Millipore, Billerica, MA, USA). 0.5 mL PN-LCNPs solution were transferred to the upper chamber of the centrifuge tube with molecular weight cut-off of 10 kDa followed by centrifugation at 5000 rpm for 40 min. The PN concentration of the filtrate and original sample preparation (before filtration) were determined using high performance liquid chromatography (HPLC) at a wavelength of 215 nm with a UV-2401 spectrophotometer (Shimadzu, Tokyo, Japan). Methanol/water (70/30, v/v) with pH 4.5 was used as the mobile phase at a flow rate of 1.0 mL/min. PN was separated using a C18 column (5 μm, 4.6 mm × 150 nm; Hanbon, Jiangsu, China). The linearity range of the calibration curve was within 0.5–15 μg/mL with a correlation coefficient of 0.9996. The drug encapsulation efficiency and drug loading were calculated according to:

$$\mathrm{EE\%} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100\%$$

$$\mathrm{DL\%} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}} - W_{\text{free drug}} + W_{\text{emulsifier}} + W_{\text{lipid}}} \times 100\%$$

where $W_{\text{initial drug}}$, $W_{\text{free drug}}$, $W_{\text{emulsifiers}}$ and W_{lipid} were the total amount of the drug in the nanoparticles, the amount of the drug in the filtrate and the amount of lipid and the amount of the emulsifiers, respectively.