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Liposome incorporated ion sensitive in situ gels for opthalmic delivery of timolol maleate

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ABSTRACT

This study was aimed to design a liposomal based ion-sensitive in situ ophthalmic delivery system of timolol maleate (TM). The TM liposome was produced by the reverse evaporation technique coupled with pH-gradients method (REVPR), and then was incorporated into deacetylated gellan gum gels. The TM liposome was demonstrated to be a round and uniform shape in TEM pictures. Compared with the TM eye drops, the TM liposome produced a 1.93 folds increase in apparent permeability coefficients (P_{app}), resulting in a significant increase of the corneal penetration. The TM-loaded liposome incorporated ion sensitive in situ gels (TM L-ISG) showed longer retention time on corneal surface compared with the eye drops using gamma scintigraphy technology. Draize testing showed that TM L-ISG was non-irritant for ocular tissues. The biggest efficacy of TM L-ISG occurred 30 min after eye drops administration, and efficacy disappeared after 240 min. Then, compared with the eye drops, the optimal TM L-ISG could quickly reduce the intraocular pressure and the effective time was significantly longer ($P \leq 0.05$). These results indicate that liposome incorporated ion sensitive in situ gels have a potential ability for the opthalmic delivery.

1. Introduction

Most conventional ophthalmic dosage forms for treating various eye diseases, such as eye drops, are simplistic. They usually have a low bioavailability, typically less than 5% (Paulsson et al., 1999; Urtti, 2006; Gan et al., 2013), for the corneal tissue because of eye drops rapidly drained by tear, impermeability of the corneal epithelium membranes, momentary residence in the fornix conjunctiva and non-productive absorption in nasal cavity. To improve the effectiveness of the drugs, a considerable amount of research groups have attempted several methods including suspensions, ointments, inserts, hydrogels, polymeric micelles, and lipid-based nanocarriers (Sasaki et al., 1996; Gershkovich et al., 2008; Shen et al., 2009; Gan et al., 2013). Most of these modifications offer some improvement over conventional liquid vehicles, but the shortcomings of blurred vision and lack of patient compliance are the main reasons that they have not universally accepted (Jung et al., 2012; Gallarate et al., 2013).

Glaucoma is an ocular disease characterized by the increasing of the intraocular pressure (IOP), optic nerve head changing and decreasing of retinal sensitivity that ultimately lead to the loss of vision (Rathore et al., 2010; Jung et al., 2013). Timolol, a non-selective beta blocker, is to be used as an anti-glaucoma agent and decreases the IOP by reducing the aqueous humor fluid through blocking of the beta receptors in the ciliary body (Jung et al., 2013; Mohamed et al., 2014). Efficacy of the marketed ocular timolol maleate (TM, Fig. 1) products is limited by extremely low bioavailability, frequent instillation and concomitant patient compliance (Schenker et al., 1999; Shafaa et al., 2011; Bochot et al., 1998; Urtti, 2006; Dave and Paliwal, 2014; Tuomela et al., 2014).

During the past decades, various delivery methods have been researched, including two main strategies: to prolong the contact time of the formulations on the ocular surface and to increase the corneal permeability of the drugs (Järvinen et al., 1995; Sasaki et al., 1996; Rupenthal et al., 2011; Almeida et al., 2013). The former

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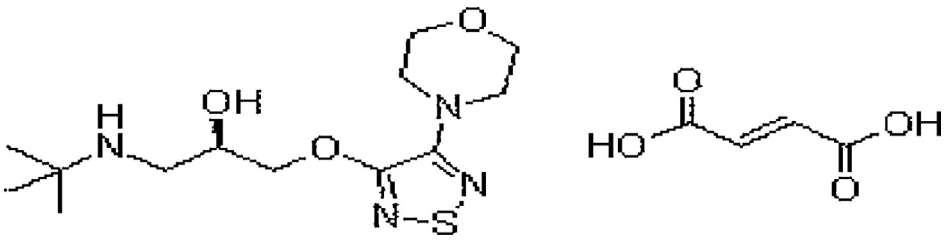

Fig. 1. Chemical structure of timolol maleate.

strategies mainly contain in situ gelling systems, suspensions, ointments and contact lenses, the latter are achieved by the colloidal delivery systems including nanoparticles (Diebold and Calonge, 2010), liposomes (Meisner and Mezei, 1995) and emulsions, the use of prodrugs and penetration enhancers (Asasutjarit et al., 2011; Rupenthal et al., 2011; Almeida et al., 2013). Therefore, to overcome these drawbacks of the TM eye drops, the choice of the dosage form that should lengthen the contact time of TM in the eye, improve the bioavailability and decrease the non-productive absorption is the critical step. Many researches have shown that liposomes used as ocular drug carrier have many features including traversing cornea easily, good histocompatibility, non-toxic, no immunogenicity (Shafaa et al., 2011; Lajunen et al., 2014; Petralito et al., 2014). However, because of the low viscosity of liposomes that does not have sufficient contact time of the drug in the eye, so the method needs to be improved. One strategy used to decrease the precorneal drainage rate of liposomes is dispersing the liposomes into an ion-sensitive gel (Budai et al., 2007; He et al., 2013; B. Zhang et al., 2013; W. Zhang et al., 2013; Almeida et al., 2013). In 1992, the USFDA approved gellan gum to be used in the food industry because the polysaccharides are biocompatible polymers and environmentally degradable (Kang et al., 1993). Due to its good rheological characteristics, which exhibit pseudoplastic flow properties to decrease the interference with blinking (Deasy and Quigley, 1991; El-Kamel, 2002; Bradbeer et al., 2015), the DGG is a bacterial polysaccharide with great commercial potential for pharmaceuticals (Miyazaki et al., 1999; Kubo et al., 2003; Zhang et al., 2015). Deacetylated gellan gum (DGG) that can lengthen the mucosal contact time, enhance the bioavailability of nasal and ophthalmic formulations, is an anionic polysaccharide secreted by pseudomonas elodea. DGG must be in the presence of cationic, which can be finished a rapid sol–gel transition that produces a strong gel for an optimal contact time (Paulsson et al., 1999; Agrawal et al., 2012; Prezotti et al., 2014; Osmałek et al., 2014). Compared to native gellan, the deacetylated gellan gum could provide high gel strength and thermal stability (Kang et al., 1993; Bradbeer et al., 2015). Using gel- and liposome-supported formulation is expected as promising ocular carriers due to their potential to minimize tear-driven dilution in the conjunctival sac and allows sufficient retention time of the dosage form (Budai et al., 2007).

In this study, we attempted to find a preparation method that dispersed liposomes containing TM into DGG to enhance drug histocompatibility and bioavailability.

2. Materials and methods

2.1. Materials

Timolol maleate (TM) was kindly supplied by the first pharmaceutical factory in Suzhou (Jiangsu, China). Soybean phosphatidylcholine (PC) that contained approximately 80% of the phosphatidyl was purchased from Aikang Chemical Co., Ltd. (Shanghai, China). Cholesterol (Chol) was obtained from the National Medicine Co., Ltd. (Shanghai, China). Tris(hydroxymethyl) aminomethane–HCl (Tris–HCl) was the product of Bodi Chemical Co., Ltd. (Tianjin, China). Deacetylated gellan gum (DGG) was kindly supplied by Zhongbai Entrepreneurial Chemical Industry Co., Ltd. (Beijing, China). Rhodamine B was purchased from TCI Shanghai Co., Ltd. (Shanghai, China). Other chemicals and reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of TM liposomes

The timolol maleate liposomal vesicles were prepared by pH-gradients method coupled with reverse evaporation technique (Dos Santos et al., 2004; Zalba et al., 2012). Soybean phosphatidylcholine (0.8 wt%, 80 mg) and cholesterol (0.3 wt%, 30 mg) were dissolved in ether (10 mL) to form the transparent and uniform oil phase. Then, pH 6.0 Tris–Hcl buffer (2 mL) containing TM (0.1 wt%, 10 mg) and the oil phase were blended and sonicated in a water bath for 10 min at room temperature to become a steady w/o emulsion. The solvent was removed under moderate stirring at 40 °C to obtain a viscous gel, and the transmembrane pH gradient was implemented by adjusting the external pH of liposomes with the Tris–Hcl buffer to 9.2. The obtained coarse emulsion was ultrasonic 3 min by a probe-ultrasonic cell disruptor (JY-92-II; Xinzhi, Ningbo, China) and the osmotic pressure was regulated by suitable amount of mannitol to 260–330 mOsm. Finally, the dispersion was adjusted the pH to 7.0.

2.2.2. Characterization of TM liposomes

2.2.2.1. Particle size and zeta potentials. The mean particle size (PS) was observed by Laser Particle Size Analyzer (LS230, USA). The zeta potential (ZP) was measured by Malvern Instrument at room temperature.

2.2.2.2. Liposomal morphology. The morphology of TM liposomes was acquired by transmission electron microscopy (TEM, JEM-1200EX JEOL, Tokyo, Japan). The sample was prepared by retention on copper grid 2–3 min, then negative staining with 1% phosphotungstic acid 2–3 min, using filter paper to remove excess liquid, natural drying for viewing (B. Zhang et al., 2013; W. Zhang et al., 2013).

2.2.3. Determination of entrapment efficiency

Entrapment efficiency of TM liposomes was measured by gel permeation chromatography on a Sephadex G-50 column (Li et al., 2008; Di Venosa et al., 2008). Quantitative liposome was added to the top of gel column and eluted continuously with the pH 7.0 Tris–Hcl buffer at a speed of 1.0 mL/min. The eluant was collected respectively in different beakers to separate liposome entrapped drug and freed drug. Afterwards, put the eluate together and added anhydrous ethanol (buffer:ethanol = 1:4, v/v) to destroy the