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Bioadhesive chitosan-loaded liposomes: A more efficient and higher permeable ocular delivery platform for timolol maleate

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ABSTRACT

The aim of this study was to develop and characterize a novel colloidal system, namely, timolol maleate chitosan coated liposomes (TM-CHL) to enhance the ocular permeation, precorneal residence time and bioavailability. The resulting TM-CHL was the most promising formulation with a mean particle size of 150.7 nm and an EE% of 75.83 ± 1.61%. In vitro release of the TM-CHL showed an extended drug release profile. The TM-CHL exhibited significant mucin adhesion and compared with commercial eye drops, TM-CHL produced a 3.18-fold increase in the apparent permeability coefficient (Papp), resulting in a significant enhancement of corneal permeation. In addition, the gamma scintigraphic study and the pharmacokinetic study showed that TM-CHL could be retained at the corneal surface for longer time compared with eye drops. The ocular irritation study indicated that the developed liposomes produced no significant irritant effects. Furthermore, pharmacodynamics results showed that the maximum intraocular pressure(IOP) produced by TM-CHL was (19.67 ± 1.14) mmHg compared with the (23.80 ± 1.49) mmHg for TM eye drops, revealing that TM-CHL was more effective in reducing the IOP. These results demonstrate that CHL is a potentially useful carrier for ocular drug delivery, which could improve the efficacy of TM.

1. Introduction

Ocular drug delivery presents a major challenge in the development of effective drug systems due to its unique physiological structure and environment for pharmaceutical person. Most topically applied drugs are eliminated by the rapid tear turnover, lacrimal fluid and blinking [1,2]. Moreover, the cornea, the most anterior layer of the eye, is a mechanical barrier which limits the entry of exogenous substances such as drugs into the eye [3,4]. Considering patient acceptance and compliance, topical application of drugs to the eye is the most popular route of administration [5,6]. However, owing to the anatomical barriers and the clearance mechanisms present in the eye, conventional drug delivery systems, such as eye drops, have poor ocular bioavailability (<5%) [7–9]. Consequently, there is a high clinical demand to increase the efficient delivery of therapeutic drugs to the eye, and the main approaches involve increasing drug transport through the corneal barriers and extending the precorneal drug residence time [10,11].

Researchers have investigated various approaches to improve drug delivery. The novel drug delivery systems, for example, microemulsions [12], microspheres [13], nanoparticles [14], hydrogels [15,16], and liposomes [17], have been designed to increase ocular bioavailability. Liposomes are promising drug carriers for ocular application, which provide benefits including high biocompatibility, superior corneal penetration, longer clearance times, lack of immunogenicity, and reduced toxicity [18]. In addition, the liposomes are easy to apply, and are localized and maintain drug activity at the site of action [19]. Also, the simplicity of preparation and the versatility of their physical characteristics make liposomes very suitable for ocular drug delivery [20].

These findings encouraged us to develop ocular liposomes with the aim to achieving a higher bioavailability. However, liposomes composed of phospholipids and cholesterol are limited by their clearance in tears, especially in the case of neutral and negatively charged liposomes [20,21]. It has been reported that cationic lipo-

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somes have a higher binding affinity for the corneal surface and can increase drug retention time and absorption [22]. Nevertheless, cationic liposomes used for ocular drug delivery usually involve cationic lipids such as stearylamine to produce a positive surface charge but these have been proved to be toxic to cells and irritating to the eye [23]. An important approach to optimize liposomes is the use of mucoadhesive polymers, which have shown not only great potential to increase the bioavailability of the applied drugs, but also have protective and healing properties to epithelial cells are concerned [24]. So, mucoadhesive polymers have been used to coat or modify liposomes, improving their bioadhesion properties to further increase bioavailability.

Chitosan (CH) is a cationic polysaccharide, known for its unique biological properties such as bioadhesion to the corneal surface, which has been widely used in ophthalmic preparations and the major driving force of chitosan bioadhesion appears to be the electrostatic attraction [25–27]. Apart from mucoadhesion, CH has penetration-enhancing property that is mainly due to the opening of the tight junctions located in epithelial cells, and this property has attracted a lot of attention as a potential enhancer of absorption across the mucosal epithelia [25,28]. Moreover, certain authors have reported the possibility of additional intracellular pathways which may contribute to the increase in cellular permeability attributed to CH [29].

Taking this information into account, we selected chitosan as the coating for liposomes and Timolol maleate (TM), an anti-glaucoma medication, was chosen as a model drug. The objective of our work was to design TM chitosan coated liposomes (TM-CHL), which could combine with negatively charged corneal mucin to effectively prolong the precorneal residence time, increase corneal penetration and improve ocular bioavailability.

2. Materials and methods

2.1. Materials

Timolol maleate(TM) was supplied by the first pharmaceutical factory in Suzhou (Jiangsu, China). Soybean phosphatidylcholine (PC) that contained approximately 80% phosphatidyl was purchased from Aikang Chemical Co., Ltd. (Shanghai, China). Cholesterol (Chol) was obtained from the National Medicine Co., Ltd. (Shanghai, China). Chitosan (CH) was provided by Haidebei Biochemical Corp. (Shandong, China). All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of TM-containing liposomes

The method of an ammonium sulfate gradient coupled with a pH-gradient was used for the preparation of TM-containing liposomes [30]. Soybean phosphatidylcholine (PC) (170 mg) and cholesterol (34 mg) were dissolved in chloroform, and a rotary evaporator was used to remove the organic solvent and an uniform lipid film was formed in a pear-shaped bottle. Then, the resulting lipid film was hydrated with appropriate 250 mM ammonium sulfate solution, stirring mechanically to elute lipid film and, subsequently, sonicated in a probe-ultrasonic cell disruptor for 3 min. Unencapsulated ammonium sulfate was eliminated from the liposomal suspension by dialysising in 0.9% (w/v) NaCl for 24 h. Then, TM solution was added to the blank liposomes, and the pH was adjusted with appropriate NaOH solution, following by incubation on a water bath.

2.2.2. Preparation of TM chitosan coated liposomes (TM-CHL)

The equivalent drug-loaded liposomes were added dropwise into the chitosan solution (0.5%, w/v) with moderate magnetic stirring at room temperature. The obtained solution was stirred for 30 min to obtain homogeneous suspensions. The effect of the concentration of chitosan on the particle size (PS) and zeta potential was studied.

2.2.3. Characterization of formulations

2.2.3.1. Particle size distribution and zeta potential. The particle size distribution was analyzed using a laser particle size analyzer (LS230, Beckman-Coulter, USA) and the zeta potential was determined using a zeta potential analyzer (Delsa 440SX, Beckman-Coulter, USA).

2.2.3.2. Determination of entrapment efficiency. The entrapment efficiency (EE%) of TM liposomes and TM-CHL was determined by an ultrafiltration centrifugation-HPLC method. Liposome solution was added to an ultrafiltration centrifuge tube (Millipore, USA, molecular weight cut-off 10,000), then centrifuged for 45 min at 4000 rpm. The liposome solution in the centrifuge tube was broken down with anhydrous ethanol, using HPLC to measure its amount. The mobile phase was a mixture of methanol-water- triethylamine (1:1:0.1%), and the pH was adjusted to 3.5 with phosphoric acid. The flow rate was set at 1.0 ml/min and the wavelength was 295 nm. A Dimonsil-C18 column was used and the encapsulation efficiency (EE%) was calculated according to the following equation:

$$EE\,(\%) = \frac{amount\ drug\ in\ liposomes}{total\ amount\ of\ drug} \times 100$$

2.2.3.3. Transmission electron microscopy (TEM). Samples were placed on a copper grid for 2–3 min, and then phosphotungstic acid (PTA, 1%) was added for negative staining about 2–3 min, using filter paper to remove excess liquid. The samples were dried at room temperature and an electron microscope (JEM-1200EX, Tokyo Japan) was used for the analyses with an acceleration voltage of 60 kV.

2.2.3.4. Differential scanning calorimetry(DSC). DSC analysis was performed on a DSC-60 differential scanning calorimeter (Shimadzu, Japan). Appropriate amounts of samples, previously lyophilized, were placed in an aluminum pans and sealed with a lid. An empty pan was used as a reference, and the heating rate was 10 °C/min.

2.2.4. In vitro release

The in vitro release of drug from the TM eye drops, TM liposomes and TM-CHL was carried out in phosphate buffered saline solution (PBS, pH 7.4). Briefly, 2.0 ml samples were placed in dialysis bags (molecular weight cut-off 8000–14,000), then immersed in 200.0 ml of release medium with magnetic stirring (50 rpm) at 34 ± 0.5 °C. At predetermined times (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h), 0.5 ml samples were withdrawn and immediately replaced with an equal volume of fresh release medium. The amount of TM in the samples was measured by HPLC as described above. Each assay was carried out in triplicate.

2.2.5. In vitro transcorneal permeation studies

New Zealand white rabbits (all animals were supplied by the Animal Experiment Center of Shenyang Pharmaceutical University) weighing 2.5–3.0 kg were used. All animal studies were in accordance with the Principles of Laboratory Animal Care (NIH publication No. 92–93, revised in 1985), and were performed in conformity with the guidelines of the institutional Animal Ethics Committee. The excision of cornea was carried out according to Schoenwald and Huang [31]. The dissected cornea with a nearly 2 mm sclera ring were mounted over modified Franz-type diffusion chambers which consisted of a donor and a receptor compartment