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The potential use of novel chitosan-coated deformable liposomes in an ocular drug delivery system

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ABSTRACT

In this study, novel chitosan-coated deformable liposomes (DL-CS) were proposed as an ocular drug delivery system to prolong pre-corneal retention, and improve transcorneal penetration and absorption. Flurbiprofen-loaded deformable liposomes (FP-DL) were prepared by a modified ethanol injection method and then coated with chitosan. Both DL and DL-CS exhibited a homogeneous particle size distribution, high encapsulation efficiency and good stability. After coating with 0.1% CS, the zeta potential was shifted from negative to positive. The apparent permeability coefficient of FP-DL-0.1% CS evaluated using isolated rabbit corneas was 1.29-, 1.95- and 4.59- fold greater than that of uncoated FP-DL, conventional liposomes and FP solution ($P < 0.01$), respectively. The *in vivo* pre-corneal retention time and elimination dynamics were assessed using gamma scintigraphy technology. The area under the remaining activity-time of FP-DL-0.1% CS was prolonged 2.84- and 1.53-fold compared with that of the FP solution and FP-DL groups, respectively. Moreover, the ocular irritation test *in vivo* revealed that DL-0.1% CS produced no ocular damage or abnormal clinical signs. These results indicate that DL-CS appears to be a novel ophthalmic drug delivery strategy with the potential to overcome the limitations of conventional eye drops.

1. Introduction

Ophthalmic solutions are usually selected for the treatment of eye diseases, especially when a topical effect is required [1]. However, traditional ocular drug delivery systems have a lower bioavailability, due to the limited eye capacity, blinking reflex, lachrymal fluid erosion, nasolacrimal drainage, and the biological barrier of the corneal and conjunctival epithelia, which reduce the amount of effective drug, shorten the retention time in the eyes and produce less absorption in the intraocular area [2]. Therefore, in clinical applications, frequent instillations of eye drops are often required to obtain the expected therapeutic efficacy, which may lead to problems with patient compliance, cellular damage and other side effects associated with nasolacrimal absorption [3].

To overcome the disadvantages of traditional eye drops, several ocular drug delivery systems have been developed in recent years, such as in-situ gels [4], microemulsions [5], microspheres [6], liposomes [7], solid lipid nanoparticles (SLN) [8], and nanostructured lipid carriers (NLC) [9]. Among them, liposomes have a great potential for penetration enhancement and high biocompatibility [10,11]. Deformable liposomes (DL), as a special form of liposomes, have many favorable properties. DL consists of phospholipids and edge activators (surfactants with a high radius of curvature and mobility) [16], and a higher degree of penetration of the mucous membrane compared with conventional liposomes (CL) as has been proved in many studies of their ophthalmic use [2,12]. In addition, DL are highly deformable and/or flexible and can cross pores of a size smaller than the conventional liposomal diameter without disassembling, because the lipid bilayers are affected by edge activators and exhibit a higher curvature [13].

In addition to penetration enhancement, the drug retention on the corneal surface is also a key factor for improving ocular bioavailability. Chitosan (CS) is a natural cationic polysaccharide with favorable biocompatibility, biodegradability and mucoadhesiveness [14] and several studies have proved the potential application

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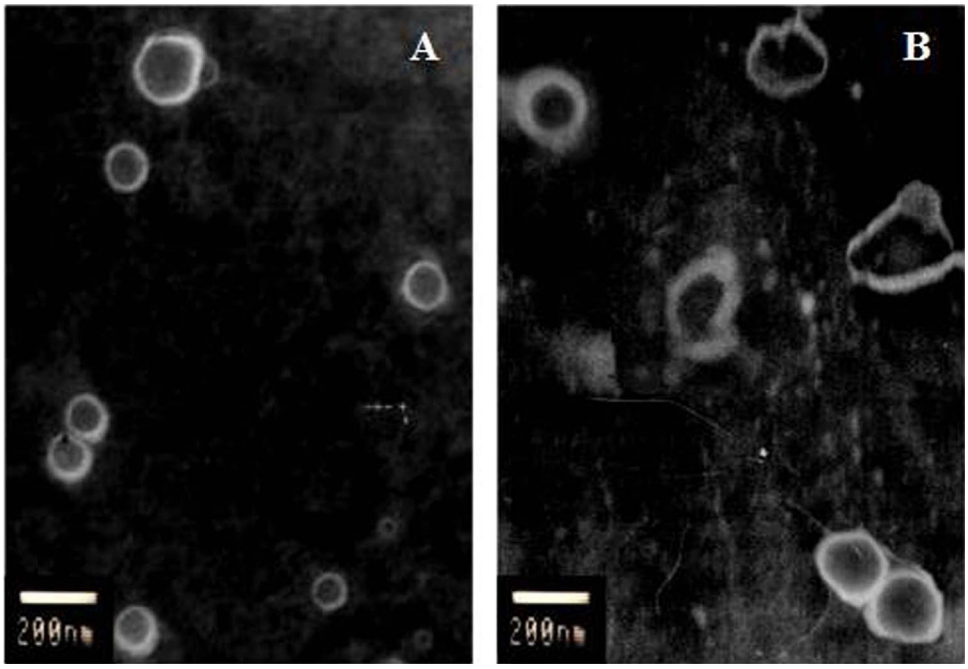

Fig. 1. TEM morphology of (A) FP-DL and (B) FP-DL-0.1% CS. Scale bar = 200 nm.

of CS as an absorption-enhancing agent because of its high affinity for cell membranes [15]. Due to its bioadhesive properties, CS has also received great attention for use in novel bioadhesive delivery systems [16,17]. For example, it has been reported that the CS is positively charged at a physiological pH (pH 7.4), and it may develop molecular attraction forces by electrostatic interactions with the negative charge of the cornea to increase the drug retention time [3,18]. Currently, there are a number of reports of CS being extensively used as a mucoadhesive in ocular drug delivery systems [19–21].

In this study, a novel form of CS-coated deformable liposomes (DL-CS) was proposed for ocular drug delivery by combining CS with a molecular weight of 50 kDa and negatively charged DL. The linking of CS on the surface of the liposomes resulted in a prolonged retention time, increased penetration, and increased ocular bioavailability. Flurbiprofen (FP), which has a low penetration across the cornea and produces ocular irritation, was chosen as a model drug. Our hypothesis is that this novel DL-CS could improve the drug penetration into the intraocular area and increases its ocular delivery efficiency.

2. Materials and methods

2.1. Materials and animals

Egg phosphatidylcholine (EPC) PL 100 M was purchased from Q.P. Corporation (Japan), Solutol HS-15 (polyoxyethylene esters of 12-hydroxystearic acid) was obtained as a gift from BASF (Germany). FP was supplied by Hangzhou Keben Chemical Co., Ltd. (Zhejiang, China). CS (molecular weight 50KD, with a deacetylation degree of 95%) and CS Oligosaccharides were provided by Tianjin Hiromi Biotechnology Development Co., Ltd. (Tianjing, China). Reduced glutathione was purchased from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). Technetium-99^{m} (99mTc)—diethylenetriaminepentaacetic acid (DTPA) was supplied by the Department of Nuclear Medicine at the General Hospital of Shenyang Military Region (Shenyang, China). All other chemicals and reagents used were of analytical grade.

Albino New Zealand rabbits without any ocular damage was obtained from Shenyang Pharmaceutical University Animal Center (Shenyang China.). All animal studies were conducted in accordance with the Principles of Laboratory Animal Care and approved by the Animal Ethical Committee of Shenyang Pharmaceutical University.

2.2. Preparation of FP-loaded conventional and deformable liposomes

DL was formed when the constituents were in a suitable ratio and the temperature was higher than the phase transition temperature of the constituents. To obtain a higher encapsulation of drug, investigations of the preparation temperature and time were needed. In our study, a series of ratios of EPC to Solutol HS-15 were studied, and different preparation temperatures and times were also examined. In our optimal formulation, FP-CL was composed of EPC and Cholesterol, while FP-DL contained EPC and Solutol HS-15 in the same ratio of 7.5:1 (w/w). FP-CL and FP-DL were both prepared by a modified ethanol injection method [22]. Briefly, FP (3 mg) and the required amount of mixed lipids were dissolved in ethanol, which was then evaporated under nitrogen. Following this, 5 ml water was added to the vial at 55 °C with constant magnetic stirring for about 30 min to obtain a coarse lipid suspension. Finally, sonication was carried out at 4 °C (JY-92-II, ultrasonic processor, Xinzhi, China) to obtain liposomes with a small particle size.

2.3. Preparation of CS-coated liposomes

CS solutions were prepared by using an appropriate amount of CS dissolved in dilute hydrochloric acid solution at pH 5.5–6.0. For the preparation of CS-coated liposomes, 5 ml of the above DL was added drop wise to 5 ml of CS solutions (0.1%, 0.2% and 0.4%, w/v) under continuous magnetic stirring for 20 min at room temperature to obtain the final formulation of FP-DL-0.05%CS, FP-DL-0.1%CS and FP-DL-0.2%CS.