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Liposome coated with low molecular weight chitosan and its potential use in ocular drug delivery

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ABSTRACT

In this study liposome coated with low molecular weight chitosan (LCH) was proposed and investigated its in vitro and in vivo properties, and its potential use in ocular drug delivery was evaluated. LCH with a molecular weight of 8 kDa was prepared and coated on liposome loaded with diclofenac sodium. The LCH coating changed the liposome surface charge and slightly increased its particle size, while the drug encapsulation was not affected. After coating, the liposome displayed a prolonged in vitro drug release profile. LCH coated liposome also demonstrated an improved physicochemical stability at 25 °C in a 30-day storage period. The ocular bioadhesion property was evaluated by rabbit in vivo precorneal retention, and LCH coated liposome achieved a significantly prolonged retention compared with non-coated liposome or drug solution. The LCH coating also displayed a potential penetration enhancing effect for transcorneal delivery of the drug. In the ocular tolerance study, no irritation or toxicity was caused by continual administration of LCH coated liposome in a total period of 7 days. In conclusion, the LCH coating significantly modified the properties of liposome and brought a series of notable advantages for ocular drug delivery.

1. Introduction

Ocular drug delivery system requires a series of specified characteristics according to the physiological structure of the eye. Human eye is an organ highly sensitive to exogenous substances such as debris, microorganisms and drugs. To treat the local ophthalmic diseases, liquid eye drop is the most desirable dosage form when considering convenience of administration and clinical compliance of the patients. However, conventional eye drops, most of which present in the drug solution form, usually have quite a limited therapeutic efficiency due to the low bioavailability. In clinical use of eye drops, frequent instillations are often required to get the expected therapeutic effect, and this leads to rising inconvenience and adverse effects.

The low bioavailability of eye drops is due to the quick elimination in the extraocular area. After instillation, the formulation is quickly diluted by the tear film and drained into the nasolacrimal duct. On the other hand, corneal and conjunctival epithelia of human eye, along with the tear film, construct a compact barrier preventing the drug absorption into the intraocular area.

In recent years, studies on novel ocular drug delivery systems have been reported, such as in situ gel, microemulsion, microspheres, solid lipid nanoparticles (SLN) and liposomes (Qi et al., 2007; Chan et al., 2007; Gavini et al., 2004; Cavalli et al., 2002). Generally, ocular drug delivery systems are expected to prolong the pre-ocular retention and promote the absorption of the drug. Meanwhile, the adverse effects, such as ocular toxicity, irritation, or vision interference of the delivery system should be taken into serious consideration. Liposomes serving as ocular drug delivery systems have been a promising perspective in the last decade of years. Liposome is a highly biocompatible and biodegradable drug carrier. In ocular drug delivery, it offers advantages such as prolonged drug retention and improved drug absorption (Kaur et al., 2004). Nevertheless, efforts are still needed to improve the drug delivery efficiency and to extent the application range of ocular liposomes. It has been reported that positively charged liposomes had a higher binding affinity to the corneal surface than the neutral and negatively charged vesicles as a result of interaction of positively charged liposomes with the polyanionic corneal and conjunctival surfaces, and therefore increase the drug retention and absorption (Fresta et al., 1999). However, positively charged liposomes for ocular delivery were commonly using cationic lipid such as stearylamine as positive charge substance which may lead to irritation and potential toxic effect to the eye (Taniguchi et al., 1988).

Chitosan is a natural-sourced cationic polymer with unique biological properties including favorable biocompatibility and mucoadhesiveness, and has been extensively studied in drug

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delivery research. However, chitosan is water-insoluble under physiological pH value, which largely constrains its application. When the molecular weight (MW) of chitosan is decreased by physical, chemical or enzymatic depolymerization, a marvelous improvement of its water-solubility is achieved as a result of the decrease in intramolecular hydrogen bonds (Kubota et al., 2000). Solubility of low molecular weight chitosan (LCH) increases with the decrease in molecular weight (Li et al., 2005). In recent years LCH has attracted much interest in the field of life and health sciences for not only its favorable water-solubility but also a series of biological properties which are distinct from its high molecular weight precursor (Seyfarth et al., 2008).

In this study, LCH coated liposome (LCHL) was prepared and evaluated for ocular drug delivery. Diclofenac sodium (DS) was encapsulated in the liposome as model molecule. Aqueous solution containing 0.1% (w/v) DS (used as eye drops) is clinically effective to treat postoperative ocular inflammation and pain after photorefractory keratectomy and cataract surgery (Diestelhorst et al., 1996). LCH with an appropriate molecular weight was coated on negatively charged liposome. The linking of LCH on the surface of lipid bilayer could presumably modify the action mechanism of liposome and improve its efficiency in ocular drug delivery.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree 97% and molecular weight 540 kDa) was purchased from Haidebei Biochemical Corp. (Shandong, China). Hydrogenated soy phosphatidylcholine (PC) was supplied by Lipoid (Ludwigshafen, Germany). Phosphatidylserine (PS) was a product of Avanti Polar Lipids (USA). Cholesterol was produced by Shanghai Guoyao Corp. (Shanghai, China). Diclofenac sodium (DS) was purchased from Wujing Pharm. Corp. (Hubei, China). All other reagents were of analytical grade.

2.2. Preparation of LCH

LCH with different MWs was prepared by oxidative degradation with hydrogen peroxide (H_2O_2) (Qin et al., 2002). Briefly, 1 g of chitosan was dissolved in 80 mL of 0.05 M hydrochloride to form a uniform solution, and then added 10 mL of 30% H_2O_2. The solution was kept at 70 °C with magnetic stirring for an appropriate time to process the degradation. Subsequently the solution was concentrated at 50 °C under reduced pressure to a volume of 10 mL. The resulting solution was neutralized by 5% NaOH to pH 7.0 and then mixed with 40 mL ethanol to precipitate the product. The precipitate was collected by filtration, washed thoroughly with ethanol and dried in vacuum. Its average MW was determined by a viscosity method (Kumar et al., 2007) using an Ubbelohde viscometer (Shenli Instrument Co., Shanghai, China). The solubility of LCH in pure water was evaluated as follows: an excess amount of LCH was dispersed in water and sonicated in a bath-type sonicator (Kunshan Electronics, Jiangsu, China) for 20 min. Afterward, the solution was centrifugated at 4000 rpm for 20 min, and the precipitate was dried in vacuum and weighed to calculate the amount of dissolved LCH.

2.3. Preparation of DS loaded liposomes

Liposomes containing 0.1% (w/v) DS were prepared by an injection method. Briefly, PC, cholesterol, PS and DS in a molar ratio of 3:1:0.1:0.2 were dissolved in an appropriate amount of ethanol. The solution was injected slowly by a 21-gauge syringe needle into an appropriate amount of 105 mM calcium acetate solution, which was kept stirring at 60 °C under a nitrogen flow. After the injection, the suspension was kept under stirring for 20 min at 60 °C and then for another 20 min at 25 °C to evaporate the ethanol. The obtained suspension was homogenized under 10^4 psi (700 bar) for 6 circles by a high pressure homogenizer (NS1001L, Niro, Italy). Then the liposome was dialyzed with 0.9% NaCl for 12 h to create a calcium acetate gradient across the lipid bilayer. The resulting suspension was incubated at 50 °C for 10 min to complete the drug loading.

2.4. Coating of liposome

LCHL with different LCH concentrations was prepared as follows. Firstly LCH was dissolved in 0.9% NaCl to form a 5% (w/v) solution. Then the above mentioned DS liposome was slowly added to an appropriate portion of 5% LCH solution, followed by magnetic agitation at 25 °C for 20 min, and the volume was adjusted by adding 0.9% NaCl in order to keep the DS concentration (0.1%). Then it was sonicated for 5 min in a bath-type sonicator for particle homogenization.

2.5. Encapsulation efficiency (EE) measurement

The DS loaded liposome and non-encapsulated drug were separated by a size-exclusion separation. Briefly, Sephadex G-50 flushed with instilled water was loaded in a 5 mL syringe and then centrifugated at 2000 rpm for 5 min to obtain a dehydrated column. Subsequently, 0.5 mL of liposome was applied onto the column and centrifugated (2000 rpm, 5 min), and then the centrifugation process was repeated for 7 runs, before each run 0.5 mL of water was applied onto the column as eluent. After centrifugation, aliquots of DS loaded liposome were obtained, and the amount of encapsulated DS was determined by HPLC. Prior to HPLC analysis, the liposome was dissolved with ethanol. HPLC conditions were as follows: a Diamasil® C18 column (200 mm × 4.6 mm, 5 μm, Dikma, China) was used. The mobile phase was a mixture of methanol, water and glacial acetic acid (80:20:0.5). The flow rate was 1.0 mL min^{-1} and the column temperature was 35 °C.

EE was calculated as: EE (%) = (encapsulated drug/total drug) × 100, where the total drug represents the addition of drug encapsulated in liposome and non-encapsulated in outer water phase.

The recovery of DS in the formulation was calculated as: recovery (%) = (total drug/initially added drug) × 100.

2.6. LCH binding efficiency

The LCH binding efficiency to the liposome was evaluated by ultrafiltration. Briefly, 0.5 mL of LCHL was applied onto an ultrafilter (Amicon Ultra, Millipore Co., USA, MWCO 30 kDa) set in a centrifuge tube, followed by centrifugating at 4000 rpm for 30 min. The amount of LCH in the ultrafiltrate was determined by a colorimetric method (Muzzarelli, 1998), in which an anionic dye, Cibacron Brilliant Red 3B-A (Sigma, USA), was used to react with the amino groups in the LCH molecules. Briefly, a 0.0075% (w/v) dye solution in citrate buffer saline (pH 3.2) was prepared. Prior to analysis, 3 mL of dye solution was mixed with 0.3 mL of the above mentioned ultrafiltrate and then incubated in a 30 °C water bath for 5 min. Then the absorption at 575 nm was measured using a UV–visible spectrophotometer (UV-9100, Shanghai, China). The LCH binding efficiency was calculated as follows:

$$\text{binding efficiency}\,(\%) = \frac{\text{LCH}_{\text{total}} - \text{LCH}_{\text{free}}}{\text{LCH}_{\text{total}}} \times 100\%$$

where $\text{LCH}_{\text{total}}$ was the initial LCH amount added to the formulation.