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Preparation of bromfenac-loaded liposomes modified with chitosan for ophthalmic drug delivery and evaluation of physicochemical properties and drug release profile

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ABSTRACT

The purpose of this study was to design a submicron-sized liposomal non-steroidal anti-inflammatory drug (NSAID) preparation that targets the retina via topical instillation of eye drops. Bromfenac (BRF)-loaded liposomes were prepared using the calcium acetate gradient method. Liposome sizes and encapsulation efficiencies were optimized by screening several liposome formulations of lipid, drug concentration, and buffer solution. BRF entrapment efficiency was greater than 90% using this method, and was low using conventional hydration methods. High initial BRF loading using the pH gradient method caused aggregation of liposomes. To circumvent aggregation, the negatively charged lipid dicetylphosphate was incorporated into liposomes, which formed anion layer preventing coalescence. Release of BRF from liposomes was sustained for several hours depending on lipid concentration, inner water phase, initial drug amounts, and surface properties. Surface modification with chitosan (CS), a mucoadhesive cationic polymer, was achieved using electrostatic interactions of negatively charged liposomes. The optimal concentration of CS for evasion of liposome aggregation was 0.15%.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat severe inflammations and related diseases of the ocular posterior segment, such as retinal and choroidal neovascularization and cystoid macular edema [1–3]. NSAIDs have the potential to be safe and effective alternatives to corticosteroids in topical treatments of ocular inflammation.

Intravitreal injections are commonly used to deliver these drugs to the back of the eye, though multiple injections can induce complications such as cataracts, vitreous hemorrhages, and retinal detachment [4–6]. Topical application with

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eye drops is safe and easy to use. However, corneal and conjunctival epithelia, along with tear film, serve as biological barriers that protect the eye from potentially harmful substances and drugs. Therefore, conventional eye drops do not allow the retention of sufficient drug in the posterior area.

Several colloidal drug delivery systems have enhanced ocular bioavailability of eye drop-administered drugs [7,8]. In our previous study, we revealed that submicron-sized (100 nm) liposomes are potential drug carriers eye drop preparations for targeting the retina [9]. Previously, we identified physico-chemical properties of liposomes, such as particle size, surface properties, and composition, significantly influence the fates of the drug and carrier [10]. In particular, surface modification using cationic mucoadhesive polymers such as chitosan (CS) may improve delivery efficiency to the retina [11]. Furthermore, we have reported that topical liposomal treatments achieved retinal delivery of NSAID by promoting non-corneal drug penetration [12].

In the present study, several liposomal formulations were prepared for NSAID delivery. Sodium bromfenac (BRF) was used as a model NSAID drug and was encapsulated in liposomes using the calcium acetate gradient method. We evaluated the effects of lipid composition and CS-modified surface properties on *in vitro* release profiles.

2. Materials and method

2.1. Materials

L-α-Distearoylphosphatidylcholine (DSPC) was purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). BRF was provided by the Wakamoto Pharmaceutical Co., Ltd. (Tokyo, Japan). Cholesterol and Dicetylphosphate (DCP) were obtained from Sigma–Aldrich (St. Louis, MO, USA). CS of around 1000 Da [13] was supplied by Katakura Chikkarin Co., Ltd. (Tokyo, Japan). 2-Morpholinoethanesulfonic acid monohydrate (MES) was purchased from Nacalai Tesque (Kyoto, Japan). Hank's balanced salt solution (HBSS) was purchased from GIBCO BRL (Grand Island, NY, USA). All other chemicals were commercial products of reagent grade.

2.2. Preparation of liposomes using the acetate salt pH gradient method

DSPC, DCP, and cholesterol (lipid concentrations: 20.4 or 40.8 mM) were dissolved in a small amount of chloroform in a round-bottom flask and were dried in a rotary evaporator under reduced pressure at 40 °C to form a thin lipid film. The film was dried in a vacuum oven overnight to ensure complete removal of the solvent. Subsequently, the lipid film was hydrated at 70 °C with acetate salt solutions (120-mM calcium acetate or 150-mM sodium acetate) by vortexing. The resulting multilamellar vesicles were frozen and thawed 4 times using a freezer and a water bath maintained at 40 °C. Submicron-sized liposomes were prepared using an extruder (LipoFast™-Pneumatic; Avestin, Inc., Ottawa, Canada) with a size-controlled polycarbonate membrane (0.1-μm membrane filter pore size). Extrusion was performed 41 times under nitrogen pressure (200 psi). To create an acetate salt concentration gradient across the liposomal membrane, the acetate salt of the external liposome medium was replaced with HBSS–MES buffer (pH 6.0) at 4 °C in two dialysis steps. BRF powder was mixed with liposomes to final concentrations of 1–5 mg/ml. Remote loading was achieved by incubation of the liposomes at 37 °C.

Surface modification of negatively charged liposomes by positively charged CS was mediated by electrostatic interactions. CS polymers were dissolved in HBSS–MES buffer. Surface modification of prepared liposomes was accomplished by mixing aliquots of the liposomal suspension with equal volumes of CS solution.

2.3. Characterization of liposomes

Particle size of liposomes was measured in aliquots of liposomal suspension diluted in large volumes of distilled water using the dynamic light scattering method (Zetasizer Nano ZS, Malvern, Worcestershire, UK). Liposome zeta potentials were measured using the laser Doppler method (Zetasizer Nano ZS). To determine the efficiency of liposomal BRF, BRF-loaded liposomes were separated from free BRF using ultracentrifugation (231,000 g, 45 min) at 4 °C. BRF concentrations in the supernatants were determined by HPLC using a COSMOSIL $5C_{18}$-MS-II column (Nacalai Tesque, Tokyo, Japan) with a mobile phase containing Milli-Q/methanol = 3/7 and 0.04% acetic acid at 275 nm. Entrapment efficiency of liposomes was calculated using the following equation: % entrapment efficiency = (Atotal − Afree)/Atotal × 100, where Atotal was the total drug amount in liposomes when methanol was added, and Afree was the amount of free drug in liposome solutions.

BRF-loaded liposomes were observed using transmission electron microscopy (TEM). The samples were absorbed to a 400 mesh carbon-coated grid and were put on 2% phosphotungstic acid solution (pH 7.4) for a few seconds for negative staining. The liposomes were observed by a TEM (JEM-1200EX; JEOL, Tokyo, Japan) at an acceleration voltage of 80 kV. Digital images were taken with a Veleta TEM CCD camera (Olympus Soft Imaging System, Munster, Germany).

2.4. Release test

The BRF release study was conducted in phosphate buffered saline (PBS) at pH 7.4, which simulated tear fluid. BRF-loaded liposomes (0.5 ml) were transferred into dialysis bags. The bags were then soaked in PBS at 37 °C (49.5 ml). At a predetermined time, 0.2 ml of receptor phase was removed and replaced with an equal volume of fresh PBS. Released BRF was quantified using HPLC.

3. Results and discussion

3.1. Preparation of BRF-loaded liposomes using the calcium acetate gradient method

Rigid liposomes generally exhibit higher stability and are capable of maintaining entrapped substances. Our previous study confirmed that DSPC-liposomes with higher phase transition temperatures are more effective than egg phosphatidylcholine (EPC) at delivering drugs to the retina [9].