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Fabrication of a composite system combining solid lipid nanoparticles and thermosensitive hydrogel for challenging ophthalmic drug delivery

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ABSTRACT

The purpose of this study was to explore a composite thermosensitive in situ gelling formulation using the distribution of solid lipid nanoparticles (SLNs) among poloxamer-based hydrogels as a potential carrier for novel ocular drug delivery. SLNs containing the model drug Resina Draconis were prepared using a melt-emulsion ultrasonication method. A central composite design (CCD) was adopted to screen the thermosensitive hydrogel (THG) formulation. After aqueous SLNs were dispersed into the THG matrices, the physicochemical properties of the SLNs were characterized before and after their incorporation into hydrogels. The *in vitro* corneal penetration experiment, ocular irritant test and transcorneal mechanism across the cornea have been previously described to predict the feasibility for the proposed ophthalmic application. Finally, the optimal THGs consisted of 27.8% (w/v) poloxamer 407 and 3.55% (w/v) poloxamer 188. The particle size of the SLNs remained within the colloidal range. *In vitro* corneal penetration studies revealed a nearly steady sustained drug release. The hen's egg test-chorioallantoic membrane (HET-CAM) test indicated that all of the tested polymer systems were non-irritant. Coumarin-6 labeled SLNs formulated into THGs displayed a more homogeneous fluorescence with a deeper penetration intensity into the cornea at various times. Taken together, these results suggest that the SLN-based THG system can be used as a potential vehicle for ocular application.

1. Introduction

Topical ophthalmic application of drugs is generally regarded as the most popular and well-accepted approach for the treatment of various eye diseases [1]. However, the efficient protective mechanisms of the eye render it difficult to achieve a desired drug concentration at the target site. The unique physiological constraints of the eye, such as the blinking reflex, lachrymal secretion and nasolacrimal drainage, contribute to this organ's exquisitely imperviousness to foreign substances. Moreover, the anatomy and safeguard barrier of the cornea compromise the rapid absorption of drugs [2].

Currently, eye drops are a common type of topical ocular medication in clinical practice, where a drop of an ophthalmic solution, irrespective of the instilled volume, often rapidly eliminates irritants after administration, despite the small amount that actually reaches the intraocular tissue [3]. Thus, this method cannot provide and maintain an adequate concentration of drug in the precorneal area [4]. Consequently, various routine ophthalmic vehicles, such as viscous solutions, ointments, gels, or polymeric inserts, have been devised to enhance ocular bioavailability and the duration of drug action. In consideration of these preparations, these limitations of conventional dosage forms cannot address the problem of low bioavailability presented in actual ophthalmic administration. Thus, an ideal dosage form is needed for ophthalmic drug delivery that not only increases the drug's corneal penetration capability but also prolongs the retention time of the vehicle on the ocular surface [5].

The emergence of colloidal delivery systems, which offers a valuable route for enhancing the potency of corneal penetration, are preferable methods toward a settlement of the former strategy [6], such as liposomes, cubosomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and microemulsions. These nanocarriers help to overcome anatomical barriers and delivers the drug to the desired site, minimizing systemic exposure and severe adverse effects [7].

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Among these various nanocarriers for ocular administration, the introduction of SLNs loaded with an active ingredient to enhance the penetration capability across the cornea has gained increasing interest in the field of ocular drug delivery within the past few years, due to the obvious advantages of SLNs, such as its ability to encapsulate and protect the lipophilic drug, prevent tear wash out, enhance ocular tolerance, improve penetration efficiency and increase corneal uptake [8,9]. Nevertheless, SLNs are still aqueous dispersal systems, which display low viscosity as a result of the difficult retention capability on the ocular surface. To overcome this issue, SLNs can be incorporated into traditional semi-solid systems (e.g., hydrogels) to increase the consistency of the final formulations and to improve the long-term stability of the incorporated nanoparticles [10,11].

Another alternative method to prolong the precorneal residence time may be achieved using delivery systems based on in situ gel forming solutions, which consist of phase transition systems when instilled in a liquid form that shifts to the gel or solid phase. According to the different factors that cause sol-to-gel phase transitions on the eye surface, the phase transition is triggered by the pH of tears, the temperature at the eye surface or the electrolytes present in the tear film [1]. Several in situ gelling systems have been developed to prolong the precorneal residence time of a drug and to improve ocular bioavailability [12].

Poloxamers are nonionic triblock copolymers consisting of a central hydrophobic chain of polyoxypropylene oxide (PPO) flanked by two hydrophilic chains of polyoxyethylene oxide (PEO) and are known to exhibit the phenomenon of reverse thermal gelation under a specific concentration and temperature. Because of its unique thermoreversible gelation properties, poloxamer analogs have become one of the most extensively investigated temperature-responsive materials [13].

Resina Draconis (also called “Dragon’s blood” in China), a deep red resin, has been used in traditional medicine since ancient times in many cultures. Pharmacological studies have shown that it demonstrates beneficial effects on the treatment of blood stasis syndrome, trauma, tumors, inflammation, gynecopathy, allergic dermatitis, among others. Taken together, these results indicated that Resina Draconis has enormous potential for further study [14]. However, the poor solubility of Resina Draconis limits its therapeutic efficacy and clinical application. It is generally accepted that SLNs have been proposed as a significant drug carrier system, thus loading Resina Draconis into the SLNs carrier may be regarded as a potential approach for ocular administration to improve its bioavailability.

The aim of the present study was to fabricate a composite hybrid thermosensitive in situ gelling formulation using the distribution SLNs among poloxamer-based hydrogels as a potential carrier for novel ocular drug delivery. This composite SLNs-based thermosensitive hydrogel (THG) system combines the advantage of SLNs as a drug carrier with the virtue of in situ gelling delivery systems to address the problem of low ocular bioavailability caused by poor aqueous solubility of the drug and rapid nasolachrymal drainage. SLNs containing the drug Resina Draconis were prepared using the melt-emulsion ultrasonication and low temperature solidification method. After prepared aqueous SLNs were dispersed into the THG matrices, their physicochemical properties were characterized before and after their incorporation into the hydrogels. A central composite design (CCD) was performed for the optimization and development of THG formulation containing poloxamer 407 in combination with poloxamer 188. The *in vitro* corneal penetration experiment, ocular irritant test and transcorneal mechanism across the cornea using confocal laser scanning microscopy of the same optimal formulations have also been described to predict their feasibility for the proposed ophthalmic application.

2. Materials and methods

2.1. Materials and animals

Resina Draconis was purchased from Hunan Dongtian Pharmaceutical Co. Ltd. (China). GMS was provided by Shanghai Chemical Reagent Co., Ltd. (China). Poloxamer 188 and 407 were purchased from Beijing Fengli Jingqiu Commerce and Trade Co., Ltd. (China). Coumarin-6 (C_6) was obtained from Sigma–Aldrich (USA). Acetonitrile was of high performance liquid chromatography (HPLC) grade. All other reagents and solvents were of analytical reagent grade.

New Zealand albino rabbits free of any ocular damage were obtained from the Taishan Medical University Animal Center (Taian, China). All animal studies were handled according to the Principles of Laboratory Animal Care, and the protocols were approved by the Taishan Medical University Animal Ethical Committee.

2.2. Preparation of Resina Draconis-loaded SLNs

The Resina Draconis-loaded SLNs were prepared using the melt-emulsion ultrasonication and low temperature-solidification method as previously described with some modification [15]. Briefly, the lipid matrix was melted at 5–10 °C above its melting point. The Resina Draconis was dissolved in the melt lipid phase. The hot lipid phase was dispersed into a hot water-surfactant solution at the same temperature, then the pre-emulsion was formed under constant mechanical agitation (DC-40, Hangzhou Electrical Engineering Instruments, China) at 1000 rpm for 15 min at 70 °C. The original warm emulsion was further treated for 5 min (work 2 s and stand 3 s) using a Lab ultrasonic cell pulverizer (JY92-II, Ningbo Scientz Biotechnology Co., Ltd. China) at 600 W to form a nanoemulsion. The emulsion was rapidly cooled by immersing the beaker into ice-cold water (0 °C). Agitation continued until the nanoemulsion yielded a uniform dispersion of nanoparticles.

The drug concentration in the supernatant was analyzed using the HPLC method. The chromatography system consisted of a Shimadzu LC-10AT solvent delivery pump (Kyoto, Japan) equipped with a 20 μL loop and a UV visible detector. The eluate was monitored at 280 nm. The mobile phase was acetonitrile and water (33:67, v/v) with a flow speed of 1.2 mL min^{-1} at room temperature.

The entrapment efficiency (EE) of the Resina Draconis incorporated in the SLNs was determined after centrifugation (CS120GXL, Hitachi, Japan) at 50,000 rpm for 15 min. EE was performed after 48 h of storage at 4 °C to evaluate the drug leakage following storage as a colloidal dispersion. To perform confocal laser scanning microscopy (CLSM), C_6-labeled SLNs were also prepared following the procedure previously described.

2.3. Preparation of SLNs-based thermosensitive hydrogels

THGs consisting of Poloxamer 407 and Poloxamer 188 were prepared using the cold process described in previously published studies [16]. For preparation of this composite hybrid system, a specific amount of polymers (P407 and P188) were dispersed in a cool nanosuspension and stored in the refrigerator until the polymer completely dissolved to form a clear solution. Other excipients, such as glycerin and benzalkonium chloride, were added as an isotonicity agent and preservative, respectively. The concentration of the isotonicity adjustment agent that rendered the formulations isotonic with eye fluid was calculated using the freezing point depression method on a STY-2 osmometer (Tianjin, China). The resulting formulation was kept at 4 °C for further study.