European Journal of Pharmaceutical Sciences 62 (2014) 115–123

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Proniosome-derived niosomes for tacrolimus topical ocular delivery: *In vitro* cornea permeation, ocular irritation, and *in vivo* anti-allograft rejection

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ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 23 March 2014
Accepted 26 May 2014
Available online 3 June 2014

Keywords:
Tacrolimus
Proniosome
Niosome
Ocular delivery
Allograft rejection

ABSTRACT

The objective of this study was to develop proniosome-derived niosomes for topical ophthalmic delivery of Tacrolimus (FK506). The FK506 loaded proniosomes containing poloxamer 188 and lecithin as surfactants, cholesterol as a stabilizer, and minimal amount of ethanol and trace water reconstituted to niosomes prior to use. The stability of FK506 loaded proniosomes was assessed, and the morphology, size, zeta potential, surface tension, and entrapment efficiency of the derived niosomes were characterized, indicating they were feasible for instillation in the eyes. The *in vitro* permeation of FK506 through the freshly excised rabbit cornea, the cumulative permeation amount of FK506 from niosomes, and the drug retention in the cornea all exhibited significant increase as compared to 0.1% FK506 commercial ointments. The *in vivo* ocular irritation test of 0.1% FK506 loaded niosomes instilled 4 times per day in rat eyes for 21 consecutive days showed no irritation and good biocompatibility with cornea. The *in vivo* anti-allograft rejection assessment was performed in a Sprague–Dawley (SD) rat corneal xenotransplantation model. The results showed treatment with 0.1% FK506 loaded niosomes delayed the occurrence of corneal allograft rejection and significantly prolonged the median survival time of corneal allografts to13.86 ± 0.80 days as compared with those treated with 1% Cyclosporine (CsA) eye drops, drug-free niosomes, or untreated. In conclusion, the proniosome-derived niosomes may be a promising vehicle for effective ocular drug delivery of FK506.

1. Introduction

Corneal allograft is clinically performed to improve the vision of patients who suffer from damaged or diseased corneas. However, corneal graft failure is commonly caused by allograft rejection (Coster et al., 2005). Although steroids are normally used to suppress the rejection reaction, the occurrence of allograft rejection still keeps high as 10–30% in the patients, and commonly followed with the secondary complications such as glaucoma, cataract, and systemic infection (Lindstrom, 1986). Tacrolimus (FK506), an immunosuppressive agent, can inhibit the action of enzyme calcineurin phosphatase and the transcription of IL-2 gene, resulting in suppression of T-lymphocyte response (Thomson et al., 1995). It can efficiently prevent post-transplant rejection in patients who are resistant to steroids and cyclosporine A (Scott et al., 2003). By far, FK506 has been topically administered to prevent the rejection of penetrating keratoplasty (Fei et al., 2008; Hikita et al., 1997; Reinhard et al., 2005; Sloper et al., 2001) and treat the intractable allergic conjunctivitis (Attas-Fox et al., 2008) and refractory inflammatory ocular surface diseases with immunologic causes (Lee et al., 2013).

At present, FK506 ointment (0.1% and 0.03%, w/w), which is known as Protopic® (Astellas Toyama Co., Ltd., Toyama Plant, Japan), has been approved by the US Food and Drug Administration (FDA) for treating moderate to severe atopic dermatitis (Ruzicka et al., 1997). But no ophthalmic preparation of FK506 is commercially available, and the dermatological FK506 ointment was clinically used instead of ophthalmic preparation in some reports (Attas-Fox et al., 2008; Lee et al., 2013; Miyazaki et al., 2008). Therefore, it is urgent to develop an ophthalmic delivery system for FK506 for its application in preventing and treating eye diseases.

Owing to its highly hydrophobic characteristic and high molecular weight (822.5 D), FK506 had difficulties in penetrating the cornea and reaching the effective therapeutic intraocular level.

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http://dx.doi.org/10.1016/j.ejps.2014.05.020
0928-0987/

Moreover, its poor water stability hinders formulating it with massive aqueous medium into eye drops. Therefore, the ideal delivery system for FK506 should not only enhance drug penetration through the cornea but also maintain its stability and reduce its undesirable side effects such as alterations in kidney function and glucose metabolism, neurotoxicity and susceptibility to infection or malignancy with systemic administration (Fung et al., 1991).

Some delivery systems including liposomes (Pleyer et al., 1993; Zhang et al., 2010; Dai et al., 2013), nanospheric suspensions (Fei et al., 2008), in situ nanosuspensions (Luschmann et al., 2013), nanoparticles (Nagarwal et al., 2012) and nanoemulsions (Garg et al., 2013) have been investigated for potential ocular application.

Niosomes which are made of nonionic surfactants and lipids can load either hydrophilic or hydrophobic drugs. They also possess many advantages over other vesicular drug delivery systems, such as low production cost, low toxicity, and ease of formulation without unacceptable solvents (Choi and Maibach, 2005; Sankar et al., 2010). Moreover, the nonionic surfactants used in niosomes can increase the bioavailability of poor water soluble drugs by enhancing the solubility and permeability through the biological membrane. It was reported that niosomes could provide a prolonged and controlled action at the corneal surface and prevent the drug metabolism by enzymes present at the tear/corneal surface (Abdelbary and El-Gendy, 2008). Several studies have demonstrated the successful use of niosomes as a potential ocular drug delivery system for drugs such as cyclopentolate, acetazolamide, timolol maleate, gentamicin, and naltrexone (Abdelbary and El-gendy, 2008; Abdelkader et al., 2012; Aggarwal and Kaur, 2005). However, niosomes also exhibit physical and chemical instability during storage, such as aggregation, sedimentation, fusion and leakage or hydrolysis of encapsulated drugs, which may affect the shelf life of dispersion (Hu and Rhodes, 1999). Therefore, the latest approach in vesicular delivery system is to take the provesicular approach to format "proniosomes" (Hu and Rhodes, 1999; Ammar et al., 2011; Sankar et al., 2010), which are liquid crystalline-compact niosomal hybrids and will convert into niosomes upon hydration with water prior to administration. Proniosomes are generally present in a stable semisolid gel structure, which can prevent the hydrolysis of encapsulated drug during storage and transport. Many studies have shown that proniosomes might be a promising drug delivery system via transdermal route (El-Laithy et al., 2011; Fang et al., 2001; Jukanti et al., 2011; Vora et al., 1998). Proniosomes can be hydrated by water from the skin to form niosomes, and both phospholipids and non-ionic surfactants in proniosomes can act as penetration enhancers (Ammar et al., 2011; Sankar et al., 2010). These properties may also make proniosomes potential vehicles for topical ophthalmic drug delivery.

Therefore, the aim of the present work was to develop a proniosome system to reconstitute niosomes for ophthalmic delivery of FK506. The stability of FK506 loaded proniosomes was evaluated, and the niosomes were characterized in terms of morphology, vesicle size and size distribution, zeta potential, surface tension, and entrapment efficiency. Permeation of the niosomes through rabbit cornea was evaluated *in vitro* and ocular irritation in rabbits was assessed *in vivo*. Furthermore, the *in vivo* anti-allograft rejection of the FK506 loaded niosomes was evaluated in a mode of corneal xenotransplantation.

2. Materials and methods

2.1. Chemicals

FK506 was purchased from Teva Czech Industries (S.R.O. Ostravska 29305, 74770 Opava-Komarov, Czech Republic). Soybean phosphatidylcholine (SPC, Lipoid S100, purity >98%) was obtained from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol (CHOL, Tian Ma Chemical Plant, Guangzhou), Poloxamer 188 (BASF, Ludwigshafen, Germany), 0.1% FK506 commercial ointment (Protopic®, Astellas Toyama Co., Ltd. Toyama Plant, Japan), and 1% Cyclosporine (CsA) eye drops (Zhongshan Ophthalmic Center, Guangzhou) were used in the study. Other reagents were of analytical grade.

2.2. Animals

New Zealand albino rabbits (male and female, weighing 2–3 kg) were provided by the Animal Experimental Center of Zhongshan Ophthalmology Center in Sun Yat-sen University. Wistar (female, weighing 200–220 g, 6–8 weeks old) and Sprague–Dawley (SD) rats (female, weighing 200–220 g, 6–8 weeks old) were obtained from Guangzhou Animal Testing Center. The animals were housed under standard condition (temperature of 25–28 °C, humidity of 40–60% RH, and light/dark cycle of 12/12 h) prior to operation and fed with a standard pellet diet and water at free. The protocols for animal use and care were approved by the Institutional Animal Care and Use Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University.

2.3. Preparation of FK506 loaded proniosomes and reconstruction of niosomes

Proniosomes were prepared according to a modified literature method (Vora et al., 1998). Briefly, poloxamer 188, lecithin, and cholesterol in a ratio of 9:9:1 (w/w/w) were mixed with the required amount of ethanol in a glass tube. Then the required amount of FK506 was added in and the tube was warmed in a water bath at 65 ± 2 °C for 10 min. Afterwards, the phosphate buffer saline (pH 7.4) was dropped into the tube which was kept in the water bath for about 5 min until a clear solution formed. The solution was cooled down at room temperature until the proniosomal gel formed. Blank proniosomes were prepared similarly but without addition of FK506. Prior to application, 0.1% FK506 loaded niosomes were reconstituted by adding 10 ml of normal saline solution to 0.2 g of proniosomes and shaking for 3 min.

2.4. Characterization of FK506 loaded niosomes

2.4.1. Transmission electron microscopy (TEM)

The morphological characteristics of the proniosome-derived niosomes were examined using transmission electron miroscropy (JEM1400, Tokyo, Japan). Briefly, a drop of niosomes was applied onto a collodion coated 300 mesh copper grid and remained for 1 min to allow the niosomes to adhere to the collodion. Then, a drop of 2% uranyl acetate solution was applied for staining, and the grid was air dried at room temperature and examined by TEM (Abd-Elbary et al., 2008).

2.4.2. Particle size, size distribution and zeta potential

The particle size, polydispersity index (PDI) and zeta potential of niosomes were determined at 25 °C by photon correlation spectroscopy at the scattering angle of 90° equipped with a Zetasizer (Malvern Instruments Nano ZS90, Worcestershire, UK). Each sample was measured in triplicate.

2.4.3. Surface tension

The surface tension of FK506 loaded niosomes was measured at room temperature using an interfacial tension meter (Hengping Instrument, Shanghai, China), and the FK506 aqueous suspension and normal saline solution were used as the controls. All measurements were carried out in triplicate.