European Journal of Pharmaceutical Sciences 91 (2016) 256–264

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Topical delivery of a Rho-kinase inhibitor to the cornea via mucoadhesive film

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ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form 29 April 2016
Accepted 14 May 2016
Available online 16 May 2016

Keywords:
ROCK inhibitor
Ocular delivery
Cornea
Cryoprobe
Diffusional release
Mucadhesive film
Topical delivery

ABSTRACT

The application of inhibitors of the Rho kinase pathway (ROCK inhibitors) to the surface of the eye in the form of eyedrops has beneficial effects which aid the recovery of diseased or injured endothelial cells that line the inner surface of the cornea. The aim of this study was to test the plausibility of delivering a selective ROCK inhibitor, Y-27632, to the cornea using a thin polymeric film. Mucoadhesive polymeric thin films were prepared incorporating Y-27632 and diffusional release into PBS was determined. Topical ocular delivery from the applied film was investigated using freshly excised porcine eyes and eyedrops of equivalent concentration acted as comparators; after 24 h the formulations were removed and the corneas extracted. Drug-loaded thin polymeric films, with high clarity and pliability were produced. ROCK inhibitor Y-27632 was weakly retained within the film, with release attaining equilibrium after 1 h. This in turn facilitated its rapid ocular delivery, and an approximately three-fold greater penetration of Y-27632 into cryoprobe-treated corneas was observed from the thin film ($p < 0.01$) compared to eyedrops. These findings support the further development of ROCK inhibitor delivery to the cornea via release from thin mucoadhesive films to treat vision loss cause by corneal endothelial dysfunction.

1. Introduction

The cornea of the eye, which is just over 0.5 mm thick in humans, owes its transparency to the characteristic spatial arrangement of its constituent collagens and other extracellular matrix components which make up much of its thickness (Knupp et al., 2009; Meek and Knupp, 2015). Lining the inner surface of the cornea is a single layer of metabolically active endothelial cells which separate the corneal matrix from the adjacent aqueous humor and prevent the ingress of fluid into the cornea (Hodson and Miller, 1976). If the endothelial cell layer is compromised the cornea swells and becomes oedematous, scatters light and loses its transparency. This results in severe vision loss and in most cases the only option is corneal transplant surgery. Typically, corneal endothelial dysfunction occurs because of an inherited disease called Fuch's endothelial corneal dystrophy (FECD) in which corneal endothelial cells deteriorate or are lost over the years. It can also occur as a result of accidental damage to the corneal endothelium during cataract surgery to remove or replace the lens of the eye, and this condition is often referred to as bullous keratopathy. Either way, the endothelial cell damage causes cornea to imbibe excess fluid and swell, making the cornea appear hazy (Fig. 1) clouding vision (Fig. 2) (Heiting, 2015; Royal National Institute of Blind People, 2015).

Techniques to treat vision loss caused by corneal endothelial dysfunction include an anterior corneal micropuncture and laser treatment to puncture the corneal epithelium. Although these procedures can be effective they carry a high risk of rejection and sometimes result in complications including corneal perforation and scarring (Rahman et al., 2008). But, by far the most common treatment for corneal endothelial dysfunction is a corneal graft, and the numbers of surgeries performed indicate the scale of the problem. The prevalence of FECD, generally, is estimated to be around 4% of individuals over the age of 40, but in inbred American, Singaporean and Icelandic populations this rises to 22%, 7% and 9% respectively (Zoega et al., 2006; Eghrari et al., 2012; Kitagawa et al., 2002).

In terms of drug therapy, the ROCK signaling pathway has received recent attention in light of the diverse therapeutic potential of changing cell behaviour in various diseases such as hypertension, vasospasm, and glaucoma (Arnold et al., 2013); and more recently FECD (Koizumi et al., 2013; Okumura et al., 2009, 2011, 2013, 2015; Nakagawa et al., 2015). ROCK inhibitors are protein serine/threonine kinases with various functions throughout the body (Riento and Ridley, 2003). Out of several different ROCK inhibitors with different therapeutic effects (Liao et al., 2007; Wang and Chang, 2014), a selective ROCK inhibitor Y-27632 was reported to have promoted the proliferation of corneal endothelial cells in vitro (Okumura et al., 2009) and the healing of the corneal endothelium in vivo (Okumura et al., 2011). Sufficient corneal endothelial cell density is crucial for the ionic pump and barrier functions (Okumura et al., 2013), which in FECD would lead to an increased overall cell size and cell shape alteration, resulting in endothelial

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http://dx.doi.org/10.1016/j.ejps.2016.05.016

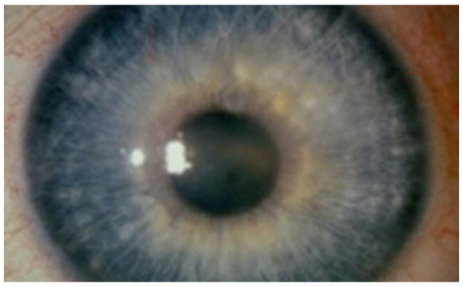

Fig. 1. Corneal haze of Fuchs Dystrophy.

dysfunction, and it was reported that Y-27632 was able to enhance cell density to the normal levels and resume the pump and barrier function, providing a functional recovery of the cornea. Koizumi et al. (2013) reported that some patients with FECD could be treated with ROCK inhibitor Y-27632 eye drops subsequent to transcorneal freezing to destroy damaged corneal endothelial cells.

The delivery of drugs to the eye is basically divided into three routes; topical, systemic and intraocular. The intraocular route involves an injection into the eye or use of implants, which is surgically fairly invasive, carries the risk if infection and is seen undesirable by patients. Systemic delivery is inefficient with potential unwanted side effects. The topical delivery of drugs to the eye is thus judged to be an attractive route. Examples of topical delivery include the use of eye drop solutions, ointments, suspensions and emulsions. Eye drops are widely used, but are easily washed out by blinking and nasolacrimal drainage; this means that only a small amount of the applied dose is likely to penetrate the cornea. Ointment, suspension and emulsion formulations are widely reported to cause ocular adverse effects, which include irritation and visual disturbance (Patel et al., 2013). Studies have been carried out on alternative topical approaches such as the OCUSERT® system, Topical Opthalmic Drug Delivery Device, medicated contact lenses and intraocular lenses (Morrison, 2015). However, none of these approaches are available commercially on the market. In this study, we investigated an alternative approach based upon drug-eluting thin polymeric muacoadhesive films that affix to the cornea, in an effort to improve the drawbacks of current methods. Our approach could conceivably lead to a more efficacious and user-friendly device capable of targeted delivery of ROCK inhibitors to attain improved clinical effect. Such therapy would also minimize wastage of costly ROCK inhibitor and avoid potential toxicity from dosing other tissues unnecessarily, in particular the tear duct and nasal cavity. Therefore, the hypothesis of this study is that topical delivery of ROCK inhibitor Y-27632 from thin mucoadhesive films is a superior alternative to eye drops for trans-corneal drug delivery. This study aimed to test the plausibility of topical delivery to the cornea from polymeric films by the fabrication of appropriate films, determination of drug release and finally the determination of trancorneal drug delivery in vitro.

Fig. 2. The hazy and clouded vision of a Fuchs patient (left) as compared to normal vision.

2. Materials and methods

2.1. Materials

ROCK inhibitor Y-27632 dihydrochloride (MW 247.3) was obtained from ApexBio Technology LLC (Houston, US). Acetonitrile (HPLC grade), water (HPLC grade) and phosphate buffered saline (PBS) tablets were obtained from Fisher Scientific (Loughborough, UK). Methylene blue (MW 319.9), polyethylene glycol 400 (PEG 400) and trifluoroacetic acid (≥99.0%) were from Sigma-Aldrich Company Ltd. (Poole, UK). Carbopol 917 (CP) was a gift from Noveon Inc. (Cleveland, U.S.) and hydroxyproplymethyl cellulose (HPMC) was a gift from Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan). Porcine eyeballs were obtained from a local abattoir by blunt dissection, immediately following slaughter.

2.2. Preparation of thin films

Ingredients were weighed into a 250 mL conical flask as detailed in Table 1 to provide a 1% w/v polymeric solution loaded with either 1.7, 5.2 or 10 mg Y-27632·2HCl; the aqueous solubility of Y-27632·2HCl is reported as 14 mg/mL (Santa Cruz Biotechnology) and that of methylene blue 43.6 mg/L (Pubchem). The mixtures were stirred overnight on a magnetic stirrer at room temperature. The following day, the mixtures were placed in an ultrasonic bath for 2 h to further assist particle comminution, and to degas the solution. Next, 50 mL of the solutions were poured into Petri dishes and left to dry in an oven overnight set at 60 °C. Once completely dry, the clear films were carefully removed from the petri dish and checked for any imperfections – those that had bubbles or crystals were discarded.

2.3. Diffusional release from thin films

Ten 0.5 × 0.5 cm patches were excised from each drug loaded film and each patch was accurately weighed before being immersed in 1 mL of PBS solution in an Eppendorf tube for various durations (10 s, 20 s, 30 s, 1 min, 2 min, 5 min, 30 min, 1 h, 3 h and 6 h). The patches were then carefully removed from the PBS solution with forceps. The remaining solutions were transferred to autosampler vials, prior to analysis by HPLC (Section 2.8). The diffusional release was carried out over the timescale of up to 6 h to broadly simulate an overnight dosage

Table 1
Working formulae to produce methylene blue and ROCK inhibitor Y-27632 films.

Film	Working formula	
Methylene blue	HPMC	0.175 g
	PEG 400	0.25 g
	CP	0.075 g
	Methylene blue	0.01 g
	DI H_2O	to 50 mL
ROCK inhibitor Y-27632 10 mg	HPMC	0.175 g
	PEG 400	0.25 g
	CP	0.075 g
	Y-27632·2HCl	10 mg
	DI H_2O	to 50 mL
ROCK inhibitor Y-27632 5.2 mg	HPMC	0.175 g
	PEG 400	0.25 g
	CP	0.075 g
	Y-27632·2HCl	5.2 mg
	DI H_2O	to 50 mL
ROCK inhibitor Y-27632 1.7 mg	HPMC	0.175 g
	PEG 400	0.25 g
	CP	0.075 g
	Y-27632·2HCl	1.7 mg
	DI H_2O	to 50 mL