European Journal of Pharmaceutics and Biopharmaceutics 88 (2014) 123–128

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Semifluorinated alkanes as a liquid drug carrier system for topical ocular drug delivery

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ARTICLE INFO

Article history:
Received 17 October 2013
Accepted in revised form 9 May 2014
Available online 15 May 2014

Keywords:
Semifluorinated alkanes
Ocular drug delivery
Cyclosporine
Ocular pharmacokinetics
Ex vivo model
EVEIT

ABSTRACT

Semifluorinated alkanes (SFA, e.g. perfluorobutylpentane F4H5, perfluorohexyloctane F6H8) are inert, non-toxic fluids capable of dissolving lipophilic drugs. The aim of this study to assess the bioavailability and safety of SFAs as drug solvents for the topical ocular application of Cyclosporin A (CsA). A commercially available CsA formulation (Restasis®, 0.05% CsA in castor oil) was tested against two novel formulations of 0.05% CSA in (a) F4H5 containing Ethanol (0.5 w/w%) and (b) F6H8 containing Ethanol (0.5 w/w%) with 0.05% CsA. Formulations were tested on rabbit corneas cultured on an artificial anterior chamber with a constant flow of an aqueous humour supplement (Ex Vivo Eye Irritation Test (EVEIT) system). Anterior chamber fluids were sampled at multiple time points to analyse the CsA concentration following single and repeated application regimes by HPLC. Photographs of fluorescein sodium-stained corneas were recorded for corneal toxicity evaluation. The impact of the formulations on the integrity of the corneal barrier function was tested after drug application by fluorescein sodium corneal diffusion experiments. The influence on the corneal metabolism was evaluated by analysis of the metabolic markers glucose and lactate.

Restasis® did not pass the corneal barrier after short term application, CsA in ethanolic F4H6 reached a maximum of 152.95 ng/ml in anterior chamber fluid samples whilst CsA in ethanolic F6H8 reached a maximum of 15.12 ng/ml. After repeated applications for 8 h, Restasis® reached 21.07 ng/ml compared to 247.62 ng/ml and 174.5 ng/ml for F4H5 and F6H8, respectively. No corneal toxicity was observed in following application of any of the formulations.

In contrast to the commercially available castor oil-based formulation, CsA dissolved in SFAs reached therapeutic inner ocular concentrations after topical administration, possibly leading to the replacement of systemic applications of CsA for inflammatory ocular disease.

1. Introduction

The cornea is a complex barrier for ocular drug delivery consisting of a lipophilic core encased by inner and outer hydrophilic layers. In addition, tears and blinking facilitate rapid clearance of topically applied substances. To prolong the exposure of topically applied drugs, viscous formulae have been developed [1]. Viscous liquids such as polyethylene glycol, glycerine and hyaluronic acid are now widely used as solvents/vehicles for topically applied ophthalmic therapeutics [2,3]. Since all the aforementioned are effective solvents for hydrophilic drugs, a solvent for lipophilic drugs such as CsA is much-needed. Restasis®, the only commercially available CsA formulation for topical ocular application uses emulsified mineral oil as a lipophilic solvent. The limited ocular bioavailability of Restasis® restricts its usefulness for application to ocular surface disease such as the dry eye syndrome [4].

This present work focuses on a novel strategy for ocular lipophilic drug delivery using semi-fluorinated alkanes. Semi-fluorinated alkanes (e.g. perfluorohexyloctane, F6H8) are amphiphilic, non-aqueous solvents. Their inert, non-toxic properties make them ideal candidates as solvents for lipophilic drugs for ocular drug delivery [5]. Using the R_FR_H type of solvents, drugs can be dissolved in their base form, which is of special interest for drugs such as 5-fluoruracil that are commonly applied as their

Abbreviations: CsA, Cyclosporin A; SFAs, semifluorinated alkanes; EVEIT, Ex Vivo Eye Irritation Test; HPLC, high-performance liquid chromatography.

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http://dx.doi.org/10.1016/j.ejpb.2014.05.009
0939-6411/

inactive water-soluble hydrochloride salt [6]. Hardung et al. dissolved diclofenac in F6H8. Interestingly, no penetration of F6H8 through the skin was observed although a good penetration of diclofenac could be shown [7]. SFAs are further found as ingredients of blood substitutes. Chemically, these blood substitutes are fluocarbon emulsions using phospholipids as surfactants and SFAs as co-surfactants. As co-surfactants, SFAs are enriched at the fluocarbon/water interface leading to smaller and more stable droplets. Clinically, this results in a higher vascular persistence and less side effects of fluocarbon emulsions [8,9].

In ophthalmology, F6H8 is used during vitreo-retinal surgery for intraoperative flattening of the retina as well as a replacement of the vitreous body [10]. Since early reports on the safety of F6H8 as a long term endotamponade showed side effects such as photophobia, ocular pain and hypotony and the development of fibrinous membranes its use is questioned [11].

Here, we used the SFAs F4H5 and F6H8 as solvents for CsA for comparison with commercially available Restasis® (CsA in castor oil) to ascertain their toxicity and intraocular bioavailability after topical corneal application. To determine how and to which extent the Ex Vivo Eye Irritation Test (EVEIT) system is suitable for corneal permeation and ocular bioavailability studies, we performed initial fluorescein sodium permeation experiments before dosing with the test formulations.

2. Materials and methods

2.1. EVEIT

To study the corneal diffusion behaviour and ocular bioavailability of CsA in different formulas, we made use of the EVEIT (Fig. 1). This is a non-animal test that simulates the anterior ocular chamber with a physiological corneal barrier for testing corneal drug permeation and corneal toxicity. This test has been described in detail previously [12,13]. Briefly, EVEIT consists of a culture of rabbit corneas obtained from slaughterhouse rabbits used for human food supply. The eyes are separated, the corneas excised and placed in an artificial anterior ocular chamber for long term nutrition (Fig. 1) with the preparation and cultivation being performed within 8 h post mortem. For nutrition, the chamber is supplied with a culture medium containing Earle's salts and HEPES buffer (Minimal Essential Medium Eagle (MEM), HEPES buffer 5.9 g/l). The medium is free of any serum components. Three corneas per formulation were used in the experiments (measurements in triplicates). The incubation occurred at 32 °C with a humidity of more than 95% through all experimental procedures and there was no additional moisturising with culture medium. Aqueous humour was changed by means of a continuous flow process having a flow rate of 6.44 µl/min, which imitates physiological conditions in the eye. The flow rate was controlled by a flexible infusion pump module as customarily used in intensive care units (Ismatec IPC, IDEX Health & Science GmbH, Wertheim, Germany).

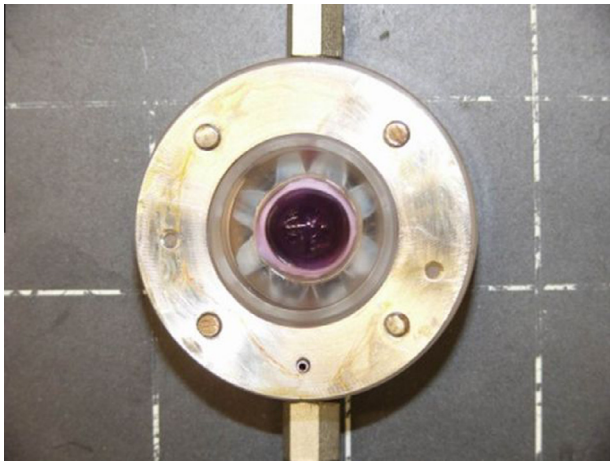

Fig. 1. Image of the EVEIT system. A rabbit cornea is centred on top of a fluid filled chamber with a continuous flow-through of culture medium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Test substances

The test formulations are specially designed to incorporate and deliver the highly lipophilic Csa. Restasis® is a mixture of 0.05% CsA in a vehicle comprising glycerine, castor oil, Polysorbate 80, Carbomer 1342, and sodium hydroxide. The pH is adjusted to between 6.5 and 8, and the osmolality of the solutions ranges between 230 and 320 mOsmol/kg.

The two test formulations studied here are 0.05 w/w% solutions of CsA in 0.5 w/w% ethanol in F4H5 and 0.05 w/w% ethanol in F6H8/ethanol. As these are non-aqueous solutions, pH and osmolality are not defined. Aliquots of the culture medium MEM applied to each of three corneas served as a non-toxic reference. To ensure accurate dosing, all samples were applied directly by pipetting a volume of 100 µl onto the corneal vertexes.

2.3. Toxicity assessment

A corneal vitality assessment was performed by demonstration of metabolic activity. For this purpose, the concentrations of glucose and lactate in the eluted anterior chamber fluid were quantified photometrically (Fluostar optima microplate reader, BMG LABTECH GmbH, Offenburg, Germany). The glucose/lactate concentrations were analysed before, during and after test formulations were applied to the corneal surface as specified in Fig. 2. In addition, the morphology of the corneal epithelium was recorded in photographs of the unstained and fluorescein sodium-stained corneas using a camera (KY-F1030U, JVC, Bad Vilbel, Germany) mounted on a Z16 APO Microscope (Wetzlar, Germany) controlled by DISKUS software, (Aachen, Germany). Finally, corneal morphology was histologically evaluated using a standard haematoxylin and eosin staining method.

2.4. Ocular bioavailability

The corneal diffusion characteristics of CsA were determined using HPLC analysis of the eluting artificial anterior chamber fluid. The flow rate within the system was kept constant with 6.44 µl/min so with an artificial anterior chamber volume of 200 µl, the total exchange of the anterior chamber volume was accomplished within 200 µl/6.44 µl/min = 31 min. Thus, any drug diffusing across the corneal surface into the anterior chamber would be detected in the eluting medium within 31 min of application. For sampling at specified time points, the medium was removed from the system immediately behind the EVEIT chamber and placed into a sterile, silicon-free 1 ml tuberculin syringe (Injekt®-F Solo, B. Braun, Melsungen, Germany).

The CsA samples were analysed according to the validated HPLC/mass spectrometry method from the Fraunhofer ITEM Study No. 15N12002. Before delivery of the samples on dry ice to the Fraunhofer Institute, Hannover, Germany, they were stored in sterile tubes at −80 °C.

2.5. Experimental procedure

Day 1: Corneas were prepared for drug application as described for the EVEIT procedure.