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Semifluorinated alkane based systems for enhanced corneal penetration of poorly soluble drugs

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ABSTRACT

Semifluorinated alkanes (SFAs) are amphiphilic liquids that can dissolve hydrophobic drugs to form clear solutions. This study evaluated the potential of two SFAs to act as vehicle for topical ocular drug delivery. After confirming ocular safety, an *ex vivo* corneal penetration model was developed to determine drug distribution and corneal bioavailability. Hydrophobic dye distribution in the different corneal layers was visualised under a confocal microscope. Corneal bioavailability of cyclosporine A (CsA) dissolved in perfluorobutylpentane (F4H5) or perfluorohexyloctane (F6H8) was compared to commercially available CsA ophthalmic emulsions, Restasis® and Ikervis®. Precorneal residence of the four test vehicles containing the hydrophobic dye was also compared using an *ex vivo* corneal tissue model. Preferential accumulation of the hydrophobic dye in the corneal epithelium was observed with higher amounts detectable when delivered via the SFAs compared to Restasis or Ikervis. A significant improvement in corneal CsA penetration was observed after application of a single dose of 0.05% CsA in F4H5 and F6H8 when compared to Restasis with the area under curve over 4 h ($AUC_{(0–4h)}$) being at least 8-fold greater for both SFAs ($p < .0001$). Moreover, the $AUC_{(0–4h)}$ of 0.1% CsA in F4H5 was almost 5-fold greater than Ikervis ($p < .0001$). Finally, the precorneal residence time of both SFA solutions was significantly longer than that of the commercial emulsions with the $AUC_{(0–60min)}$ being 2- to 11-fold greater. This study demonstrated that SFAs can significantly improve the local bioavailability of hydrophobic drugs by increasing corneal penetration as well as prolonging precorneal residence. They therefore offer a promising new platform for topical drug delivery to the eye.

1. Introduction

Topical application is the most preferred route for ocular drug administration; however, the bioavailability of topically applied drugs is usually very low due to the well-developed defence mechanisms of the eye. The cornea is a formidable barrier composed of a hydrophilic stroma sandwiched between a lipophilic endothelium on the basal side and a several layer thick lipophilic epithelium on the apical side, forming the primary barrier to entry of foreign substances. Moreover, rapid precorneal clearance due to tear production and lid blinking further limits the exposure of topically applied drugs and allows only a fraction of the dose to be absorbed. Precorneal residence time and ocular bioavailability of topical eye drops are usually increased using viscous aqueous solutions (Meseguer et al., 1996). However, the formulation of poorly water soluble drugs into aqueous eye drops remains particularly difficult with such drugs usually solubilised or emulsified in aqueous systems using large quantities of surfactants and other additives that have frequently been associated with ocular toxicity (Yang and Acosta, 1994).

Semifluorinated alkanes (SFAs) are R_FR_H diblock compounds containing a perfluorocarbon and a hydrocarbon segment (Fig. 1) and are physically, chemically and physiologically inert. Due to their biocompatibility and excellent solubilisation potential, SFAs have extensively been used in the preparation of artificial blood substitutes (Meinert and Knoblich, 1993). In ophthalmology, SFAs have previously been used as temporary endotamponades (Kim et al., 2005), as intraoperative tools in retinal translocation procedures (Herbert et al., 2003) and for removal of silicone oil remainders from intraocular lenses

Abbreviations: SFAs, semifluorinated alkanes; F4H5, perfluorobutylpentane; F6H8, perfluorohexyloctane; CsA, cyclosporine A; HET-CAM, Hen's Egg Test – Chorioallantoic Membrane; BCOP, bovine corneal opacity and permeability; STF, simulated tear fluid; HBSS, Hank's Balanced Salt solution; AUC, area under the curve

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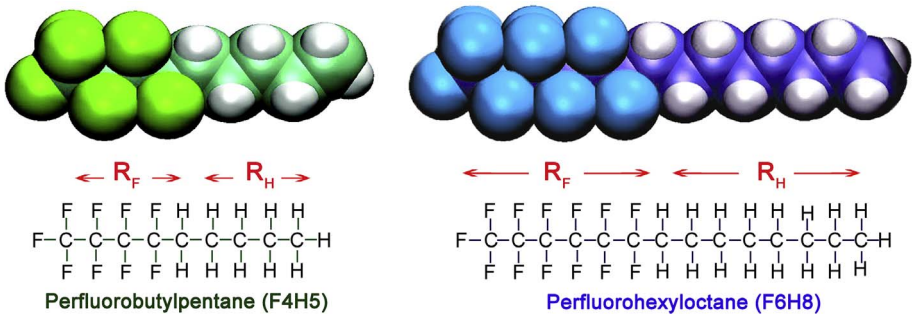

Fig. 1. Chemical structure of the SFAs, perfluorobutylpentane (F4H5, left) and perfluorohexyloctane (F6H8, right). Image adapted from (Morgado et al., 2011).

and the vitreous cavity (Liang et al., 2008). However, their application in the management of topical anterior segment conditions is relatively new. A dry eye therapy (NovaTears®, Novaliq GmbH) based on F6H8 has recently been introduced in the European market for relief of hyperevaporative dry eye symptoms (Steven et al., 2015).

Due to their amphiphilic nature, SFAs can also be used to solubilise poorly water soluble molecules, such as Cyclosporine A (CsA), which has previously been reported to have a water solubility of only 7.3 µg/ml in water of 37 °C (Ismailos et al., 1991). CsA is an immunomodulatory agent commonly used for its anti-inflammatory activity in dry eye disease and other ocular inflammatory conditions. Currently, Restasis® (0.05% ophthalmic emulsion, Allergan) is the only FDA approved CsA eye drop on the market; however, the ocular bioavailability of CsA from this anionic emulsion is generally low and long term therapy is usually needed to relieve dry eye symptoms (Barber et al., 2005; Sall et al., 2000). Moreover, a high incidence of ocular adverse events has often been reported after application of Restasis (29% of 293 subjects) with significant adverse events also being evident upon application of the drug-free emulsion (18.5% of 192 subjects), suggesting that the vehicle plays the predominant role in the manifestation of ocular toxicity (Sall et al., 2000). Recently, Ikervis® (0.1% ophthalmic emulsion, Santen), which is a cationic nanoemulsion of CsA, was launched in Europe and has been reported to result in higher ocular bioavailability of CsA than from Restasis, possibly due to the presence of the positive charge in the vehicle (Lallemand et al., 2012), which increases electrostatic interactions with the corneal epithelium as well as ocular surface mucins. Due to its low viscosity, this system reportedly does not blur vision despite its opacity; however, the incidence of adverse events continue to remain high. In a double masked Phase III clinical trial, adverse events were reported by 35.9% of 221 subjects treated with Ikervis and 20.3% of 161 subjects treated with the vehicle alone (EMEA, 2015; Leonardi et al., 2015).

Thus, there is an urgent demand for a suitable vehicle to increase the ocular bioavailability of CsA in dry eye patients without further exacerbating ocular discomfort. SFAs, being non-aqueous physiologically inert liquids, can help in overcoming the limitations of currently marketed CsA formulations as no preservatives, surfactants or pH modifiers (which are frequently implicated in ocular toxicity) are required to prepare SFA based eye drops. Also, since SFAs are only slightly polar, potential toxicity associated with charged emulsion-vehicles is eliminated.

Recently, application of a F4H5 based formulation containing CsA was found to significantly reduce corneal fluorescein staining scores and improve goblet cell density compared to commercial CsA formulations in a mouse model (Gehlsen et al., 2017). F4H5 and F6H8 have also been found to enhance the penetration of CsA across rabbit corneas into the aqueous humour (Dutescu et al., 2014). However, the therapeutic benefit of CsA in ocular surface disorders is mainly attributed to its anti-inflammatory and immune modulating effects in corneal epithelial cells, where it reduces apoptosis and therefore, corneal staining which is a primary marker of disease (Wolffsohn et al., 2017). CsA is also believed to limit the release of matrix metalloproteins and inflammatory cytokines in epithelial cells (Shetty et al., 2015; Tepelus et al., 2017), thus inhibiting an inflammatory cascade and further exacerbation of ocular surface diseases (Baudouin et al., 2013). Therefore, CsA absorption and localisation into the cornea, rather than penetration across it and into the aqueous humour, is critical for improved therapeutic outcomes in ocular surface disorders.

In this study, the corneal bioavailability and distribution of a model hydrophobic dye as well as CsA from SFAs was compared to that of the commercial formulations, Restasis and Ikervis, using *ex vivo* models in an attempt to explain the improved therapeutic benefits of SFA based CsA formulations. Significant anatomical and histological differences exist between human and rabbit eyes used in previous studies (Agarwal and Rupenthal, 2016), therefore, we used anatomically more relevant porcine eyes to minimise interspecies variations and simulate the clinical conditions more closely. Additionally, the *ex vivo* precorneal residence time of all four vehicles was compared to estimate the corneal drug exposure time and thus the potential for prolonged drug delivery *in vivo*.

2. Materials and methods

2.1. Materials

SFAs (F4H5 and F6H8) were kindly provided by Novaliq GmbH (Germany). BODIPY® 493/503 (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene) was purchased from Thermofisher Scientific (USA). CsA was obtained from Euticals SpA (Italy), while the internal standard n-octyl-4-hydroxybenzoate (OHB) was purchased from Alfa Aesar (UK). Restasis (Allergan, USA) and Ikervis (Santen, France) were obtained on prescription. Tissue-Tek® O.C.T. compound was purchased from Sakura® Finetek (USA) while 4′,6-diamidino-2-phenylindole (DAPI) was purchased from Sigma-Aldrich (USA). Gradient HPLC grade acetonitrile, purchased from Merck (Germany), and Milli-Q water, obtained by reverse osmosis using a Millipak system (0.22 µm; Millipore, USA), were used for preparation of the mobile phase and diluents. Sodium fluorescein was purchased from Sigma-Aldrich (USA). All other chemicals, including sodium hydroxide (ECP Labchem, New Zealand), ethanol (Merck, Germany), potassium chloride (Scharlau Chemicals, Spain), sodium chloride (Scharlau Chemicals, Spain), calcium chloride dihydrate (Scharlau Chemicals, Spain), magnesium chloride (ECP Labchem, New Zealand) and sodium hydrogen carbonate (ECP Labchem, New Zealand), were of reagent grade and were used as received.

2.2. Preparation of test formulations

SFA based test formulations were prepared by dissolving 0.05% w/v of CsA in F4H5 and F6H8, respectively. An F4H5 solution containing 0.1% w/v of CsA was also prepared to evaluate if corneal penetration was dose dependent. The commercial formulations, Restasis and Ikervis, were used as received. Restasis is an anionic emulsion containing 0.05% w/v of CsA in glycerol, castor oil, Polysorbate 80,