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Topical delivery of aqueous micellar resolvin E1 analog (RX-10045)

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ABSTRACT

Purpose: The primary objective of this study were to optimize aqueous micellar solution of isopropyl ester prodrug of resolvin (RX-10045), study *in vivo* ocular compatibility and tissue distribution following topical administration.
Methods: An optimized ratio of hydrogenated castor-oil and octoxynol-40 (1.0:0.05 wt%) was prepared to entrap RX-10045 in the hydrophobic core of micelles. RX-10045 aqueous micelles were subjected to characterization. *In vitro* stability studies were performed at 4 °C, 25 °C and 40 °C. *In vivo* studies were conducted in New Zealand albino rabbits following topical drop administration.
Results: Aqueous RX-10045 micellar solutions were successfully prepared. Micelles had a mean diameter of ~12 nm with low negative surface charge. RX-10045 demonstrated high stability in citrate buffer (0.01 M) at 40 °C. Hackett–McDonald ocular irritation scores were extremely low comparable to negative control. No significant difference in intraocular pressure was noted. Electroretinography studies did not reveal any retinal damage after multiple dosing of RX-10045 micellar solution. Ocular tissue distribution studies demonstrated appreciable drug concentrations in anterior ocular tissues. Moreover, RX-10008 (active metabolite of RX-10045) was detected in retina/choroid upon topical drop instillation.
Conclusions: A clear, stable, aqueous 0.1% RX-10045 micellar formulation was successfully prepared. Micellar solution was well-tolerated and did not have any measurable tissue damage in rabbit ocular tissues. Micelles appear to follow conjunctival/scleral pathway to reach back-of-the-eye tissue (retina). Topical aqueous formulations may be employed to treat posterior ocular diseases. Such micellar topical formulations may be more patient acceptable over invasive routes of administrations such as intravitreal injection/implants.

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1. Introduction

The cornea is a translucent tissue devoid of blood and lymphatic vessels, which is an essential feature for its transparency. It may lose stromal limpidity due to infection, injury or surgery (Wilson, 2012). Acute inflammation helps the host to defend against infections by generating pro-inflammatory lipid mediators (prostaglandin E2 and leukotriene B4) and by upregulating neutrophils (Buckley et al., 2014; Serhan, 2014; Serhan et al., 2000). On the other hand, chronic ocular surface inflammations may develop from dry eye syndrome, allergic conjunctivitis and contact lens intolerance (Dana and Hamrah, 2002; Thakur and Willcox, 2000). Such inflammatory processes may cause significant ocular epithelia damage (corneal and conjunctival) subsequently leading to blindness (Cortina and Bazan, 2011). During corneal wound healing, some important biological events occur. Biological events include proliferation of corneal myofibroblast, decline in corneal crystalline expression by corneal cells and production of abnormal extracellular matrix (Jester et al., 1999; Mohan et al., 2003; Netto et al., 2006). Such events may cause development of corneal haze or opacity.

Current treatment strategies for ocular surface inflammations include topical administration of corticosteroids, immunomodulatory agents and/or nonsteroidal anti-inflammatory drugs (NSAID). Corticosteroids such as dexamethasone have been recommended as first line of treatment. However, chronic use of steroids is limited due to development of severe side effects such as sub-capsular cataract and elevation of intraocular pressure (Jobling and Augusteyn, 2002; Pleyer et al., 2013). In steroid responders, second line of treatment includes immunomodulatory drugs like cyclosporine A (CsA). At present, the only FDA approved and

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commercially available drug product is Restasis® (0.05% CsA emulsion). Topical application of Restasis® is associated with development of hyperemia, stinging and foreign body sensation, epiphora, pain and redness of the eye and visual disturbance (Schermer et al., 1986) causing patient noncompliance and leading to discontinuation. On the other hand, NSAIDs exhibit limited anti-inflammatory activity and may delay corneal wound healing (Kim et al., 2010). Topically applied drug/formulation may not only reach anterior tissues but also to back-of-the-eye tissues (Cholkar et al., 2012). There are different pathways proposed for ocular drug disposition (Hughes et al., 2005), which may depend on physiochemical properties of drugs such as hydrophilicity and hydrophobicity. Physicochemical properties of drug such as high lipophilicity or hydrophilicity may impede drug absorption. Higher lipophilic property of drug may have higher corneal epithelial layer permeation. However, hydrophilic corneal stroma may impede further drug permeation and lead to drug accumulation (Prausnitz and Noonan, 1998). Similarly, drug with very low lipophilicity may encounter lipophilic corneal epithelium as a barrier and prevent drug permeation. Therefore, subtle balance of hydrophobicity and hydrophilicity *i.e.*, hydrophilic-lipophilic balance is required for drug disposition across ocular tissues.

Resolvins are lipid mediators identified in the resolution phase of inflammation. These molecules are endogenously generated from essential dietary omega-3-polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid) following lipoxygenation. Such polyunsaturated fatty acids are classified as E-series (RvE) and D-series (RvD). These endogenous molecules belong to a class of potent lipid mediators that are known to be beneficial in regulating and causing reversal of inflammatory response (Serhan, 2007). The biological functions of E-series resolvins include, counter regulation of superoxide anion generation and proinflammatory gene expression. Other functions include shielding tissues from leukocyte mediated injuries, blocking pro-inflammatory cytokine expression, preventing trans-endothelial neutrophil migration and attracting non-inflammatory monocytes and macrophages to inflamed site and promote neutrophil clearance (Serhan, 2014; Schwab and Serhan, 2006; Hasturk et al., 2006). Moreover, RvD and RvE may prevent conjunctival goblet cell mucous secretion (Dartt et al., 2011). Resolvins, being endogenous molecules are studied for the treatment of ocular inflammatory conditions. These compounds appear to be to be effective in experimental models of ocular inflammatory diseases such as dry eye (Li et al., 2010), herpes simplex virus induced ocular inflammation (Rajasagi et al., 2011), uveitis (Settimio et al., 2012) and retinal angiogenesis (Hunt, 2007). Resolvyx (RX-10045) is a synthetic isopropyl ester prodrug of the resolvin E1 analog (RX-10008). This novel molecule is highly effective in accelerating tear production and corneal tissue repair. This compound is also known to reduce corneal inflammation, prevent epithelial damage and inhibit release of pro-inflammatory mediators from corneal epithelial cells (Pan et al., 2008). Physically RX-10045 is a yellow viscous oil with very poor aqueous solubility. Moreover, this molecule is light sensitive, highly unstable and easily prone to degradation at room temperature. Aqueous solution of RX-10045 was prepared using propylene glycol as a solubilizing agent. This formulation was evaluated in murine models for the treatment of dry eye with topical drop. Results demonstrated that RX-10045 was highly efficacious in treating dry eye syndrome. In Phase II clinical trials RX-10045 was found to be safe and well-tolerated but produced somewhat nonequivalent efficacy results. One of the reasons may be that RX-10045 being recognized by efflux transporters expressed on corneal and conjunctival membrane which may cause lower drug levels in the cytoplasm. Recently, we demonstrated for the first time that RX-10045 is a substrate/inhibitor of MRP2 and BCRP (Cholkar et al., 2015a). Interestingly, results also demonstrated that RX-10045 inhibited organic cation (OCT-1) influx transporter (Cholkar et al., 2015a). Such interactions of RX-10045 with influx and efflux transporters may limit intracellular permeability causing therapeutic failure. In the present study we hypothesized that encapsulation of RX-10045 inside the lipid core of micelles may help to enhance RX-10045 aqueous solubility, improve drug stability, prevent interactions with influx and efflux proteins and deliver high drug concentrations in the cytoplasm.

2. Materials and methods

Purified RX-10045 (physical state-viscous yellow oil; lot number: NR13799-969-143) was obtained from PPD. Hydrogenated castor oil-40 (HCO-40) of pharmaceutical grade was procured from Barnet Products, USA and octoxynol-40 (Oc-40 or Igepal CA-897) was purchased from Rhodia Inc., New Jersey, USA. Ethyl acetate (HPLC grade) was purchased from Fischer Scientific, USA. PVP-K90 (lot #56943447G0) was obtained from BASF Aktiengesellschaft 67056 Ludwigshafen Germany. Benzalkonium chloride was obtained from Sigma chemical Co. St. Louis, Missouri, USA. Disodium EDTA, sodium chloride and sodium citrate were purchased from Fischer Chemicals, USA. Citric acid was purchased from Sigma Chemical Co., USA. For buffer and formulation preparation double distilled deionized water was used. HPLC grade methanol was procured from Fisher Scientific, USA.

2.1. HPLC analysis

In vitro analysis of RX-10045 was performed by a reversed phase RP-HPLC method with a Shimadzu HPLC pump (Shimadzu, Shimadzu Scientific instruments, Columbia, MD), Alcott autosampler (model 718 AL), Shimadzu UV/visible detector (Shimadzu, SPD-20A/20AV, USA), ODS column (5 μm, 150 $\times$ 4.6 mm) thermostated at $40 \pm 1\,^{\circ}$C and Hewlett Packard HPLC integrator (Hewlett Packard, Palo Alto, CA). The mobile phase was comprised of methanol (MeOH), water and trifluoroacetic acid (TFA) (70:30:0.05% v/v) which was set at a flow rate of 0.5 mL/min. Detection wavelength was set at 272 nm. The sample tray temperature was maintained at 4 °C. Calibration curve (0.5–5 μg/mL) for RX-10045 (injection volume 10 μL) was prepared by making appropriate dilutions from stock solution in 2-propanol.

2.2. UPLC analysis

The stability study samples were analyzed with the Ultra Pressure Liquid Chromatographic (UPLC) system. The system consisted of Waters UPLC Acquity H Class with UPLC TUV & PDA Detector (230 nm) and data system: Waters Empower 2. Waters Acquity CSHTM C18 (1.7 μM, 2.1 $\times$ 50 mm) column was used for separation. Column temperature was maintained at 50 °C and autosampler temperature was set at 4 °C. The mobile phase was composed of phase A: 0.1% formic acid in water and Phase B: 0.1% formic acid in methanol and UPLC TUV Detector set at 230 nm. Injection volume was 2 μL. A gradient solvent system was employed for separation and analysis.

2.3. LC–MS/MS analysis

All *in vivo* ocular tissues, fluids and blood samples were analyzed with liquid chromatography–tandem mass spectrometry (LC–MS/MS). LC–MS/MS comprised a triple quadrupole mass spectrometer with SCIEX API 4000TM (API 4000; Applied Biosystems/MDI SCIEX) coupled to a liquid chromatography system (Shimadzu LC-10 AD, USA) and reversed phase ACE 5

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