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Preclinical evaluation of a ricinoleic acid poloxamer gel system for transdermal eyelid delivery

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ABSTRACT

Our previous study has shown that pluronic lecithin organogel (PLO gel) made of ricinoleic acid has the potential for use as a transdermal eyelid delivery system. The present study deals with the evaluation of ocular tissue concentrations of dexamethasone in a rabbit model following topical application of the gel formulation onto the eyelids. The PLO gel formulation containing dexamethasone was applied to the outside of the eyelid skin. Rabbits were sacrificed at regular time intervals of 2, 4, 8, 12, 20 and 24 h. Maxidex® eye drops were used as a control. Rabbits were sacrificed and dexamethasone concentrations were analyzed in anterior segment tissues such as the cornea, conjunctiva, aqueous humor, lens, and iris-ciliary body by liquid chromatography tandem mass spectrometry (LC–MS/MS). Rabbit eyes were also examined for ocular irritation and scored using the modified Draize scoring system. No significant irritation or redness was observed in the eyes as compared to the control rabbit eyes. PLO gel formulation resulted in constant dexamethasone concentrations in the anterior segment tissues for up to 24 h, which was equivalent or higher than Maxidex® eye drops. The findings of this investigation indicate that the ricinoleic acid PLO gel formulation may be clinically effective as a new treatment modality for anterior segment diseases.

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Ophthalmic infections in young children usually affect both eyes and pose unique challenges in diagnosis and treatment (Winfield et al., 1990). Most ocular infections are chronic in nature and characterized by inflammation of the cornea, conjunctiva, and eyelids. Inflammation is generally caused by a bacterial or viral infection or an allergic reaction (Viswalingam et al., 2005). Bacterial infections are commonly treated with compresses and anti-bacterial and steroidal drugs administered in the form of either eye drops or ointments (Cronau et al., 2010). Topical delivery in the form of eye drops is associated with poor patient compliance and low ocular bioavailability (<5%) due to non-productive absorption, tear production, transient residence time, and impermeable corneal epithelium (Boddu et al., 2010). Conjunctival blood vessels also play a major role in the systemic absorption of topically administered drugs. These factors collectively result in the elimination of 95% of the administered dose. Moreover, administering eye drops or ointment in the eyes can be a significant challenge, especially in young children who are reluctant to tolerate conventional instillation. Children intuitively resist putting any medication in the eye, and the necessity to administer the formulation three to six times a day adds to the problem (Fraunfelder, 1976). Despite intense research efforts, no alternative delivery system has become widely used. Though ophthalmic inserts and pH responsive hydrogels provide sustained delivery, there are several hurdles to commercialization (Aminabhavi et al., 2004; Achouri et al., 2012). Even nanocarrier systems made of chitosan and poly(lactic-co-glycolic acid) suffer from several disadvantages, including lack of particle size uniformity, poor formulation stability, burst release of drugs, and large-scale manufacturing difficulties (Agnihotri and Aminabhavi, 2007; Coelho et al., 2010; Valencia et al., 2012).

Pluronic lecithin organogels (PLO gels) are widely used for the transdermal delivery of both hydrophilic and lipophilic drug molecules. Though PLO gels have been known for a decade, their potential as vehicles for transdermal eyelid drug delivery has not been investigated. We have developed and characterized a ricinoleic acid pluronic lecithin organogel (PLO gel) for sustained delivery of drugs to infected ocular tissues following topical application onto the skin surface of the eyelids. Considering the sensitivity of eyelids, minimum concentrations of lecithin (1% w/v) and poloxamer (14% w/v) were used in the preparation of PLO gel (Boddu et al., 2014). Ricinoleic acid, which is known for its

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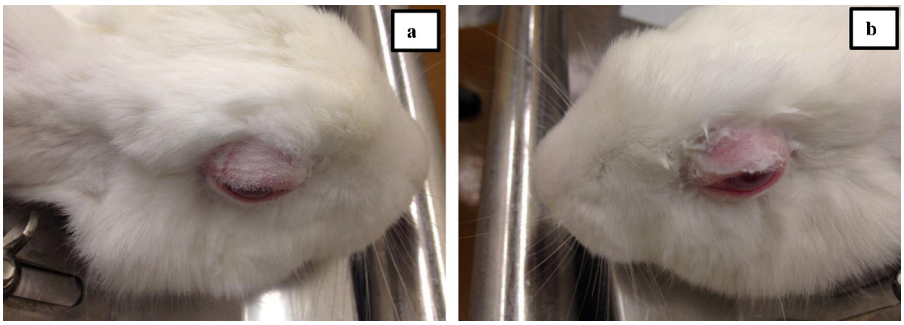

Fig. 1. Rabbit images before (a) and after (b) the application of ricinoleic acid PLO gel.

analgesic and anti-inflammatory properties, was used as oil phase instead of isopropyl palmitate (Vieira et al., 2000). Unlike the traditional ointment base, which binds to the drug and prevents its release, PLO gels exhibited better drug release and penetration properties (Boddu et al., 2014). In continuation of our previous study, the objective of the present study was to assess the skin irritation and ocular tissue concentrations of dexamethasone in rabbits following topical application of the PLO gel formulation to the eyelids.

Ricinoleic acid (lot 2DYB0), lecithin (lot RWUG0 IT), and sodium chloride (lot 123819) were obtained from Fisher Scientific (Pittsburgh, PA). Dexamethasone (lot C137572) and poloxamer 407 (lot 2433346) were procured from PCCA (Houston, TX). Butylated hydroxytoluene (lot U41659D01) was obtained from Amend Drug & Chemical, Irvington, CA. Distilled deionized water was used in the preparation of PLO gels. High performance liquid chromatography (HPLC) solvents, including methanol (lot 113904) and acetonitrile (lot 121151), were purchased from Fisher Scientific (Pittsburgh, PA).

New Zealand White (NZW) albino adult male rabbits, weighing between 2.0 and 2.5 kg, were obtained from the Robinson Services Incorporated (Mocksville, NC) and housed in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALACI) and US Department of Agriculture (USDA). The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Toledo. Studies performed were in accordance with the Association for Research in Vision and Ophthalmology Regulations and Standards (ARVORS) guidelines. Rabbits were divided into test and control groups, with 12 rabbits in the test group and 10 rabbits in the control group. Rabbits in the test groups were anesthetized with subcutaneous ketamine (35 mg/kg) and xylazine (3.5 mg/kg). Ten minutes after anesthesia, rabbits were restrained, and the hair on the upper and lower eyelids was removed with an electric razor. Eyelids were carefully examined to verify that the skin was free of scratches. Half an inch of 0.1% w/w PLO gel (0.3 g) formulation containing dexamethasone was applied to the outside of the left and right eyelids (area 3.5–4 cm^2) in the test group (Fig. 1). Both eyes of the animals were used in the present study. Two rabbits from the test group were sacrificed at 2, 4, 8, 12, 20 and 24 h with an intravenous administration of Beuthanasia (1 ml/4.5 kg) through the marginal ear vein. For the control group, Maxidex® eye drop suspension (100 μl) was administered in the cul-de-sac of the lower left and right eyelids of the control group. Two rabbits were sacrificed after 10, 30, 60, 120, and 180 min. Eyeballs were enucleated immediately and cleaned with an ice-cold phosphate buffer (pH 7.4) in order to remove any drug adsorbed onto the surface. Aqueous humor was withdrawn by limbal paracentesis. The enucleated eyeballs were cut open and the following tissues were collected into preweighed vials: cornea, conjunctiva, iris-ciliary body, lens, and aqueous humor. All tissue samples were stored at −80 °C until further analysis. Tissue samples were homogenized in 500 μl chilled (4 °C) phosphate buffer (pH 7.4) for about 4 min with an Ultra-Turrax homogenizer (Ika T10 Basic, Staufen, Germany) in an ice bath. Subsequently, 100 μl of the tissue homogenates (cornea, conjunctiva, iris-ciliary body, and lens) were collected for dexamethasone extraction. One hundred microliters of aqueous humor was used for extraction without further processing. Dexamethasone was extracted from ocular tissue homogenates by a simple liquid–liquid extraction, as per our published procedure, by liquid chromatography tandem mass spectrometry (LC–MS/MS) (Earla et al., 2010). Triamcinolone acetonide (10.0 μg/ml) was used as internal standard. The extraction recovery of this method was found to be greater than 85% and this was calculated in ocular tissues as the ratio between the peak areas of extracted and unextracted samples. A Varian

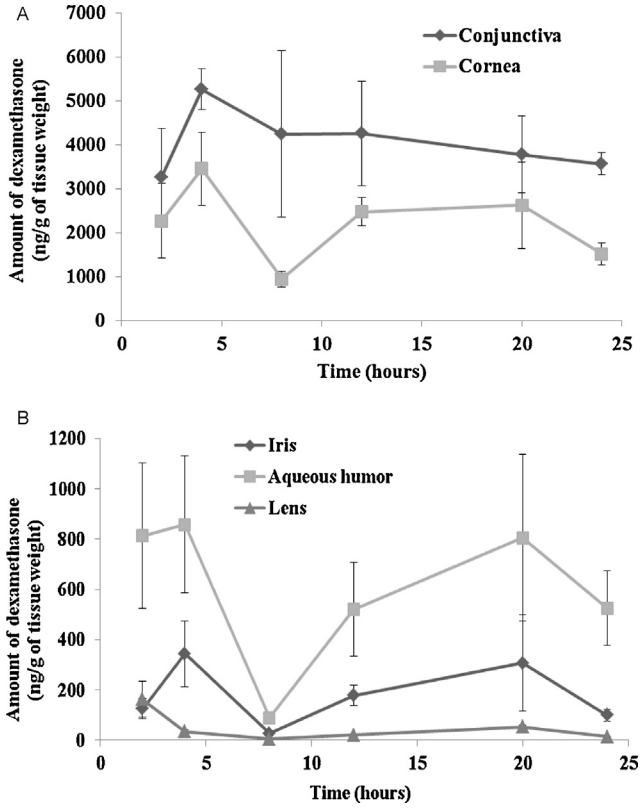

Fig. 2. Amounts of dexamethasone in the (A) cornea and conjunctiva, (B) aqueous humor, iris and ciliary body and lens after application of PLO gel formulation to the skin of the rabbit eyelid. The data represent the mean and standard error, $n = 3–4$. Invisible the error bars are smaller than the symbols.