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Research article

Efficacy of a new topical cationic emulsion of cyclosporine A on dry eye clinical signs in an experimental mouse model of dry eye

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ABSTRACT

Dry eye disease (DED) is a complex, multifactorial pathology characterized by corneal epithelium lesions and inflammation. The aim of the present study was to evaluate the efficacy of a cationic emulsion of cyclosporine A (CsA) in a mouse model that mimics severe dry eye. Eight to 12-week-old female C57BL/6N mice with tail patches of scopolamine were housed in controlled environment chambers to induce dry eye. At day three, following dry eye confirmation by corneal fluorescein staining (CFS, score 0–15) and phenol red thread (PRT) lacrimation test, the mice (n = 10/gp) were either treated 3 times a day in both eyes with drug-free cationic emulsion, a 0.1% CsA cationic emulsion, or 1% methylprednisolone (positive control), or non-treated. Aqueous tear production and CFS scores were evaluated at baseline and throughout the treatment period. The lacrimation test confirmed the scopolamine-induced decrease in aqueous production by the lacrimal gland. A reduction of 59% in induced-CFS was observed following topical treatment with 0.1% CsA. The beneficial effect of the cationic emulsion vehicle itself on keratitis was also clearly evidenced by its better performance over 1% methylprednisolone, -36%, vs. -28% on the CFS scores, respectively. This study indicates that the cationic emulsion of CsA (0.1%) was a very effective formulation for the management of corneal epithelium lesions in a severe DED mouse model. In addition, it performed better than a potent glucocorticosteroid (1% methylprednisolone). This cationic emulsion of CsA (0.1%), combining CsA and a tear film oriented therapy (TFOT), i.e. with vehicle properties that mechanically stabilize the tear film, represents a promising new treatment strategy for the management of the signs of dry eye.

1. Introduction

Dry eye disease (DED) is a complex multifactorial pathology characterized by corneal epithelium lesions, inflammation of ocular surface and symptoms of discomfort (DEWS, 2007). Taken individually, the severity of the lesions (signs) or symptoms are often correlated with the severity of the disease (Amparo et al., 2015; Baudouin et al., 2014), while taken together the severity of signs and symptoms do not necessarily correlate (Bartlett et al., 2015; Schmidl et al., 2015). DED is therefore difficult to treat as both signs and symptoms, which do not correlate well and are modulated upon treatment within different timeframes, need to be improved concomitantly. Artificial tears, with their good safety profile, represent the first line therapy for the management of DED patients, and were demonstrated to be able to alleviate mild to moderate symptoms of DED (Alves et al., 2013; Moshirfar et al., 2014; Williamson et al., 2014). The mechanical restoration towards a normal precorneal tear film (TF) with tear substitutes using various technical strategies like water retention gels, or tear film lipid layer (TFLL) restoration (for example with cationic emulsion) was sufficient to temporarily improve the ocular discomfort symptoms of DED patients (Amrane et al., 2014; Amrane and Lambert, 2008; McCann et al., 2012). While both strategies are well accepted by DED patients, TFLL restoration with cationic emulsion is a new approach and allows for the restoration of a structurally more natural precorneal TF with its three layers (TFLL, aqueous layer and glycocalyx). Interestingly, it seems that TFLL restoration approach possesses better outcomes for DED patients in

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the long term, especially for Meibomian gland dysfunction (MGD) patients (Amrane et al., 2014; Ismail et al., 2014). Tear film instability (http://www.dryeye.ne.jp/en/tfot/index.html) (Yokoi et al., 1996, 2015) and inflammation (Wei and Asbell, 2014) of the ocular surface have both been recognized as the core mechanism of DED. Inflammation was indeed demonstrated to have a central role in the DED vicious cycle (Baudouin, 2007), and to be a major contributor to the severity of both signs and symptoms (Bron et al., 2014; Calonge et al., 2010). As such, anti-inflammatory drugs, such as methylprednisolone or the immunosuppressant drug cyclosporine A (CsA), with anti-inflammatory properties, have been proven useful for the management of DED patients (Byun et al., 2012; Marsh and Pflugfelder, 1999; Pflugfelder, 2004; Stonecipher et al., 2005). It has been demonstrated experimentally, in *in vitro* and animal models, that both drugs are effective at reducing inflammation in corneal epithelial cells. Recently, cationic emulsion-based artificial tears were developed as a new tear substitute, for the treatment of DED symptoms (Amrane et al., 2014; Ismail et al., 2014), and as a vehicle for CsA (Daull et al., 2013), to improve the ocular drug delivery through its peculiar electrostatic interactions with the negatively charged corneal epithelium (Daull et al., 2014; Lallemand et al., 2012). The aim of the present study using a validated murine model of dry eye (Barabino et al., 2005) was to assess the efficacy of the cationic emulsion of CsA (Ikervis, 1 mg/ml CsA), and its vehicle in the management of DED-induced ocular surface damages.

2. Methods

2.1. Animals

Forty pigmented C57BL/6N mice aged 8–11 weeks (Centre d'élevage JANVIER, Le Genest Saint Isle, France) were used in this study. Iris Pharma Internal Ethics Committee approved the protocol. All animals were treated according to the Directive 2010/63/UE European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and to the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Experimental procedure

Mice were placed in a controlled environmental chamber (CEC) for 10 days (temperature: 20–22 °C; relative humidity:<25%; airflow: 15 L/min), and treated with transdermal scopolamine patch administration (0.5 mg/72h, Scopoderm® TTS, Novartis, Reuil-Malmaison, France) on Day 1, 3, 5, 7 and 9 adapted from previously described model (Barabino et al., 2005).

The mice were then randomly assigned to 4 groups, including a control group according to the corneal fluorescein staining (CFS) values at baseline (before any treatment and induction). All treatments were preservative-free and prepared under a laminar flow hood in 250 μL aliquots. The aliquots were stored at room temperature and one fresh aliquot was used each day for each treatment group. Ten mice per group received one of the tested solutions: 1% methylprednisolone in 0.9% NaCl solution (Methylprednisolone®, Sigma-Aldrich, Saint-Quentin Fallavier, France), 0.1% cyclosporine A (Ikervis®, Santen, Evry, France) and Ikervis® vehicle (Santen, Evry, France). Three microliters of the test items were instilled three times a day in both eyes from Day 4–10. The control group did not receive any eye drop treatment. The treatments were encoded, and the group allocation was blinded to the technician administering the treatment, and to the researcher assessing the outcome of the experiment. Group identification was uncovered at the end of the analysis.

Tear volume was measured with phenol red thread (PRT) test (Zone-Quick, Lacrimedics, Eastsound, WA, USA), as described previously (Barabino et al., 2014). Corneal fluorescein staining (evaluated using the National Eye Institute scheme) was performed before dry eye induction (Day 0), and during the experiment at days 3, 6 and 10 according to a previously published protocol (Barabino et al., 2014). Briefly, 0.5 μL of a 0.5% fluorescein sodium solution (Fluoresceine Faure, 0.4-mL unit-dose vials, Novartis Pharma SAS, France) was instilled into the inferior conjunctival sac using a micropipette. The cornea was examined through a biomicroscope by light passing through a cobalt blue filter. The stained area was assessed and graded using the grading system from the NEI/Industry Workshop guideline (Lemp, 1995). The system provided a stepwise categorization of the cornea, by dividing it into five sectors; with each one of them scored on a 0–3 scale, for a total maximal score of 15.

2.3. Statistical analysis

The statistical analysis was performed using the software GraphPad Prism 6.0b. Data were first evaluated for normality with D'agostino-Pearson normality test. Appropriate parametric or non-parametric statistical tests were used to make comparisons between groups or time-points. Within group, changes from baseline were analyzed with Friedman's test for repeated measures. The drug effect was assessed on the corneal fluorescein staining which was considered as the primary outcome. The comparison of treated groups was performed with dry eye-induced and non-treated group. To take into account the baseline PRT, the CFS was analyzed between groups using Cochran-Mantel-Haenszel method with baseline PRT as stratification variable. Statistical significance was set at a p value of 0.05. Results are presented as mean ± SD.

3. Results

3.1. Improvement of DED clinical signs: lacrimation

DED was induced in C57BL/6N mice receiving a new scopolamine patch every other day and placed in a controlled environment chamber (CEC, ie. in a dry and constantly ventilated environment (Barabino et al., 2005)). For all groups, the tear volume decreased at day 3 when compared to the baseline values (Fig. 1A). The mean tear volume values (±SD) at day 3 post DED induction was 2.9 ± 1.5, 2.0 ± 0.6, 2.2 ± 0.6 and 2.0 ± 0.6 for untreated, 1% methylprednisolone, Ikervis vehicle and Ikervis groups, respectively. These PRT values were in the range of PRT values for mouse DED with the environmental chamber + scopolamine model (historical data), thus confirming DED induction. The PRT measurement variability is relatively important which may limit its usefulness as an efficacy endpoint. Upon treatment, tear volume tend to gradually increase to baseline values, with day 10 mean lacrimation values being not statistically different from baseline in the animals of the three treatment groups (Ikervis, Ikervis vehicle and 1% methylprednisolone), as a consequence of the low PRT baseline value for these groups. Compared to 1% methylprednisolone or cationic emulsion (Ikervis vehicle), the CsA-containing cationic emulsion (Ikervis) seemed to show a better improvement in tear volume (Fig. 1b). However those improvements were not statistically significant among the groups.

3.2. Improvement of DED clinical signs: corneal fluorescein staining (CFS)

CFS scores increased dramatically as soon as two days after dry eye induction. The change was statistically significant for all groups