OKYO-0101, an agonist of G-protein coupled receptor (GPCR), ameliorates inflammation in an experimental model of dry eye disease in mice

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BACKGROUND

Dry-eye disease (DED) also referred to as 'Keratoconjunctivitis Sicca' is one of the most common ophthalmic conditions encountered in clinical practice. DED is a multifactorial disorder caused by lack of lubrication and moisture that significantly lowers the quality of life of affected individuals. The evidence form over 40 years of scientific literature suggests inflammation as the most common underlying cause of the disease. Dry eye syndrome represents a major economic burden in public healthcare accounting to total annual cost for the management of disease including lost productivity due to symptoms to the US economy more than $50 billion annually. Symptoms of dry eye include constant discomfort and irritation accompanied by visual impairment and potential damage to ocular surface. The infiltration of T cells in the conjunctiva and lacrimal glands and increase in the levels of inflammatory cytokines in both conjunctiva and tears is known to cause the chronic inflammation associated with the DED. Therefore, development of new therapeutic agents that targets inflammatory pathways is crucial in improving symptoms in DED patients.

The chemerin receptor (CMKLR1 or ChemR23) is a chemokine like G protein-coupled receptor (GPCR) expressed on select populations of cells including inflammatory mediators as well as epithelial cells[1-3]. Activation of CMKLR1 has been shown to resolve the inflammation in animal models of asthma[4] and modulate the inflammation environment in autoimmune diseases by recruiting regulatory T cells (Tregs)[5]. In this study, we investigated the effects of OKYO-0101, an agonist of CMKLR1, in improving dry eye symptoms using murine dry eye model. We also evaluated ocular tolerance of OKYO-0101 following repeated ocular instillation in rabbits followed by clinical ophthalmic observations.

METHODS

Induction of Dry Eye Disease (DED): DED was induced in 6-8 week old, female C57BL/6J mice by exposure to desiccating stress (DS). Animals were acclimated at 55% relative humidity (RH) for at least one week prior to start of the study. DS was induced by subcutaneous injection of scopolamine hydrobromide (0.5 mg/0.2 ml, four times per day throughout the study, except Day 5 when a single injection was given 1 hour prior to Oregon Green Dextran (OGD) staining. Mice were placed in a cage with constant airflow and RH was controlled at 20%, and the temperature was maintained at 25°C. Control mice were maintained in a nonstressed (NS) environment at 55% RH without exposure to air draft.

Assessment of Corneal Barrier Function: Corneal staining was measured by penetration of OGD. For baseline assessment, 0.5 µL of OGD was instilled on the cornea of both eyes, and the mice were housed in the dark for 1 min before washing with balanced saline solution (BSS) and imaging. For corneal permeability assessment at Day 5, 0.5 µL of OGD was instilled on the cornea of both eyes, and the mice were immediately housed in the dark for one minute, followed by euthanization and immediate imaging. Eyes were washed with 2 mL of BSS. Digital images were captured, and the mean fluorescence intensity was measured with NIS Elements imaging software (Nikon).

Conjunctival goblet cells (GC) measurement: Enucleated mouse eyes with intact conjunctiva were fixed in 10% formalin and embedded in paraffin. Six-micrometer sections were stained with periodic acid–Schiff reagent. GC density in the superior and inferior conjunctiva was measured using Nikon imaging software.

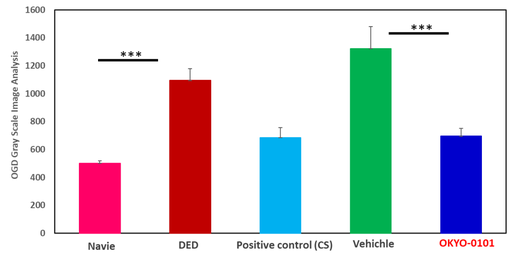
Immunohistochemistry to detect CD4+ T cells: Enucleated mouse eyes with intact conjunctiva were suspended in optimal cutting temperature (OCT) compound and flash frozen in liquid nitrogen. Immunohistochemistry was performed on six-micrometer frozen sections to detect and count the number of cells in conjunctival epithelium that stained positively for CD4, a biotinylated secondary antibody and NovaRED peroxidase. Positively stained cells were counted in the conjunctiva using Nikon imaging software.

Rabbit ocular tolerance (Draize assessment) test: New Zealand White rabbits, female (n = 6) were used for ocular tolerance test. The rabbits were administered with OKYO-0101 twice daily on Days 1-4 to the left eye only, (40 µl per instillation). Draize scoring was performed by EyeCRO scientists on Days 1-3 at 30 minutes post instillation, and the ophthalmic exam was performed by a consulting Professor of Ophthalmology on Day 4 at 30 min post-instillation, and Day 5 at 24 h post-instillation. Draize and McDonald-Shadduck scores were compiled at the end of study.

RESULTS

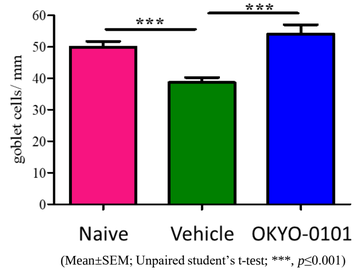
MICE DRY EYE STUDIES

Potency of OKYO-0101 in reducing corneal permeability was comparable to cyclosporine



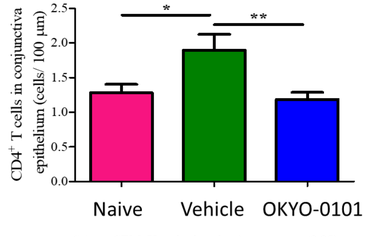
(Mean±SEM; ANOVA with Tukey's post-test; **, $p \leq 0.01$; ***, $p \leq 0.001$)

Goblet Cell density, reduced due to DED, was normalized by OKYO-0101



(Mean±SEM; Unpaired student's t-test; ***, $p \leq 0.001$)

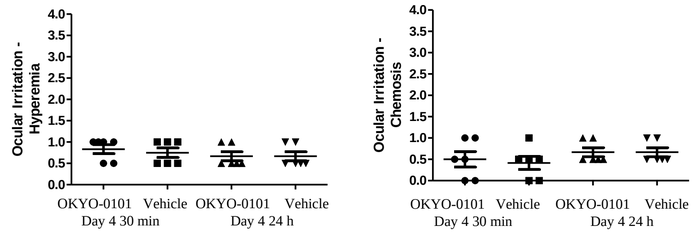
OKYO-0101 normalized inflammatory CD4+ T cells in the conjunctival epithelium of DED eyes



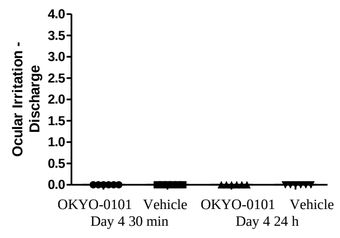
(Mean±SEM; Unpaired student's t-test; *, $p \leq 0.05$)

RABBIT SAFETY STUDIES

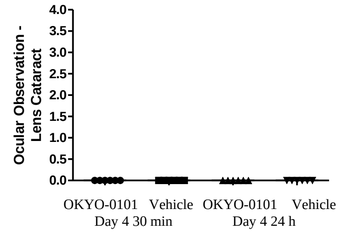
Ocular Irritation Scores



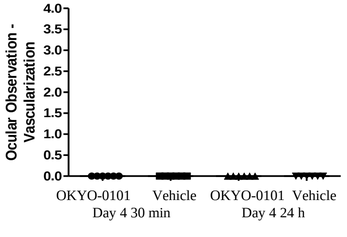
- Mild hyperemia and chemosis seen with OKYO-0101 was similar to vehicle group and not directly correlated with the drug
- Clinical ophthalmic exam showed no adverse signs in the OKYO-0101 treated rabbit eyes



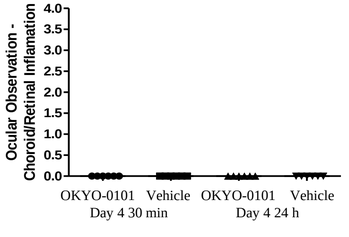
No ocular discharge was seen after OKYO-0101 treatment



No lens cloudiness was observed after OKYO-0101 treatment



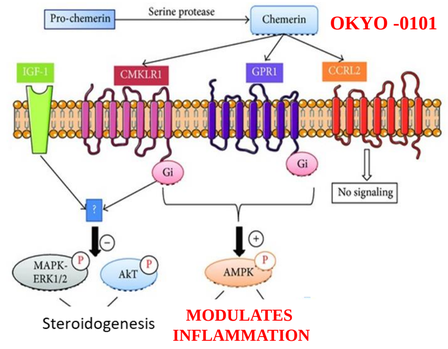
No corneal/retinal vascularization was seen after OKYO-0101 treatment



No ocular inflammation was observed after OKYO-0101 treatment

MECHANISM

Chemerin GPCR signaling
Anti-inflammatory action of OKYO-0101



CONCLUSIONS

- Increase in corneal permeability due to dry eye was reduced significantly by OKYO-0101 compared to vehicle group.
- Potency of OKYO-0101 in reducing corneal permeability was comparable to cyclosporine, an active ingredient of Restasis® (Allergan).
- OKYO-0101 normalized the dry eye induced loss of goblet cell density
- OKYO-0101 reduced the dry eye induced-enhancement of $CD4^+$ T-cells, which are known biomarkers of inflammation.
- Rabbit Ocular tolerance test using OKYO-0101 showed no adverse signs such as inflammation, chemosis or hyperemia and no signs of local irritation.
- Clinical ophthalmic exam of rabbit eyes after topical application of OKYO-0101 for 4 days (twice daily) showed no discharge, cloudiness, vascularization, edema, inflammation or retinal hemorrhage.

REFERENCES

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5. Stevenson et al. (2012) Arch Ophthalmol. 130, 90–100.

DISCLOSURES

Raj Patil is an employee of OKYO Pharma and Kunwar Shailubhai is on Board of Directors of OKYO Pharma.