



# Efficacy evaluation of a cationic emulsion of cyclosporine in a mouse model of dry eye

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## Introduction

Dry eye disease (DED) is a complex multifactorial pathology characterized by corneal epithelium lesions and inflammation. The severity of these assaults is often correlated to the severity of the disease. DED is estimated to affect approximately 25-30 million people in the United States. Patients with severe DED lack a convenient and effective therapy to treat their signs (corneal fluorescein staining (CFS) assessing keratitis), and alleviate their symptoms and protect their ocular surface.

## Purpose

The aim of the present study was to evaluate the efficacy of a cationic oil-in-water emulsion<sup>1</sup> of cyclosporine (CsA): Ikervis<sup>®</sup>, in a mouse model that mimics severe dry eye.

Table 1. Summary of the physico-chemical parameters of the cationic oil-in-water emulsion of CsA.

Parameters	Cationic oil-in-water emulsion Ikervis <sup>®</sup>
Aspect	White opaque to slightly translucent
pH	6.0
Osmolality (mOsmol/kg)	270
Mean droplet size (nm) <sup>a</sup>	170 (100%)
Zeta potential (mV) <sup>b</sup>	Positive (+ 40 mV)
Sterility	Sterile

*Note:* <sup>a</sup> Mean droplet size was determined by dynamic light scattering (HPPS, Malvern Instruments), and <sup>b</sup> zeta potential by electrophoretic mobility measurement (Zetasizer 2000, Malvern Instruments).

## Methods

Eight to 12-week-old female C57BL6 mice with tail patches of scopolamine (replaced every other days) were housed in controlled environment chambers (CEC) to induce dry eye<sup>2,3</sup>. 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 oil-in-water emulsion, a 0.1% CsA-loaded cationic oil-in-water emulsion, and 1% methylprednisolone (positive control), or left untreated. Aqueous tear production, CFS score, Goblet cell density in the conjunctiva and CD11b<sup>+</sup> cell count in flat mounted cornea were evaluated at the end of the treatment period (at day 10).

## Results

Figure 1. Tear secretion over time of mice (n=10/group) placed in a controlled environmental chamber and subjected to different eye drops treatments. The error bars represent standard errors.

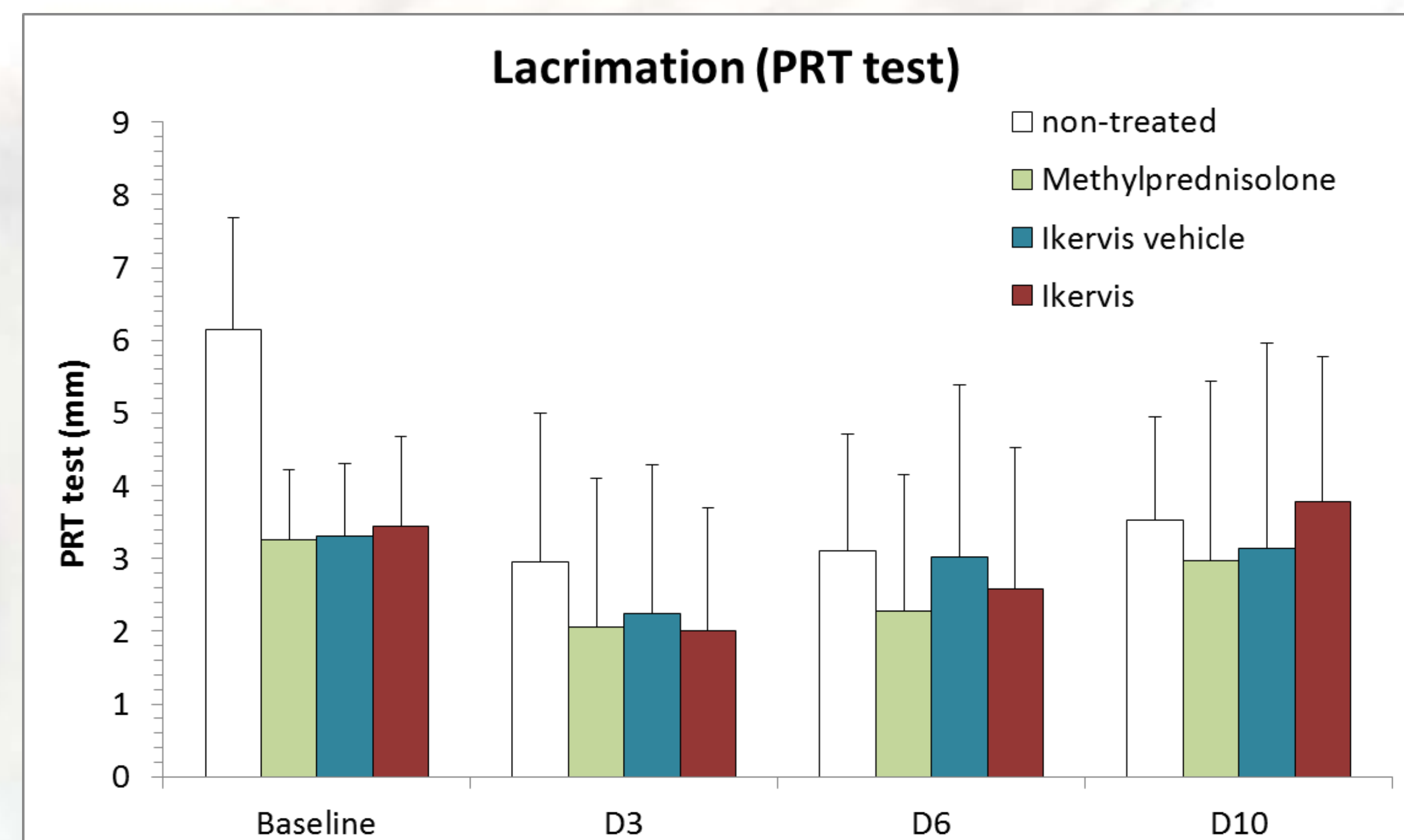


Figure 2. Corneal fluorescein staining over time of mice (n=10/group) placed in a controlled environmental chamber and subjected to different eye drops treatments. The error bars represent standard errors.

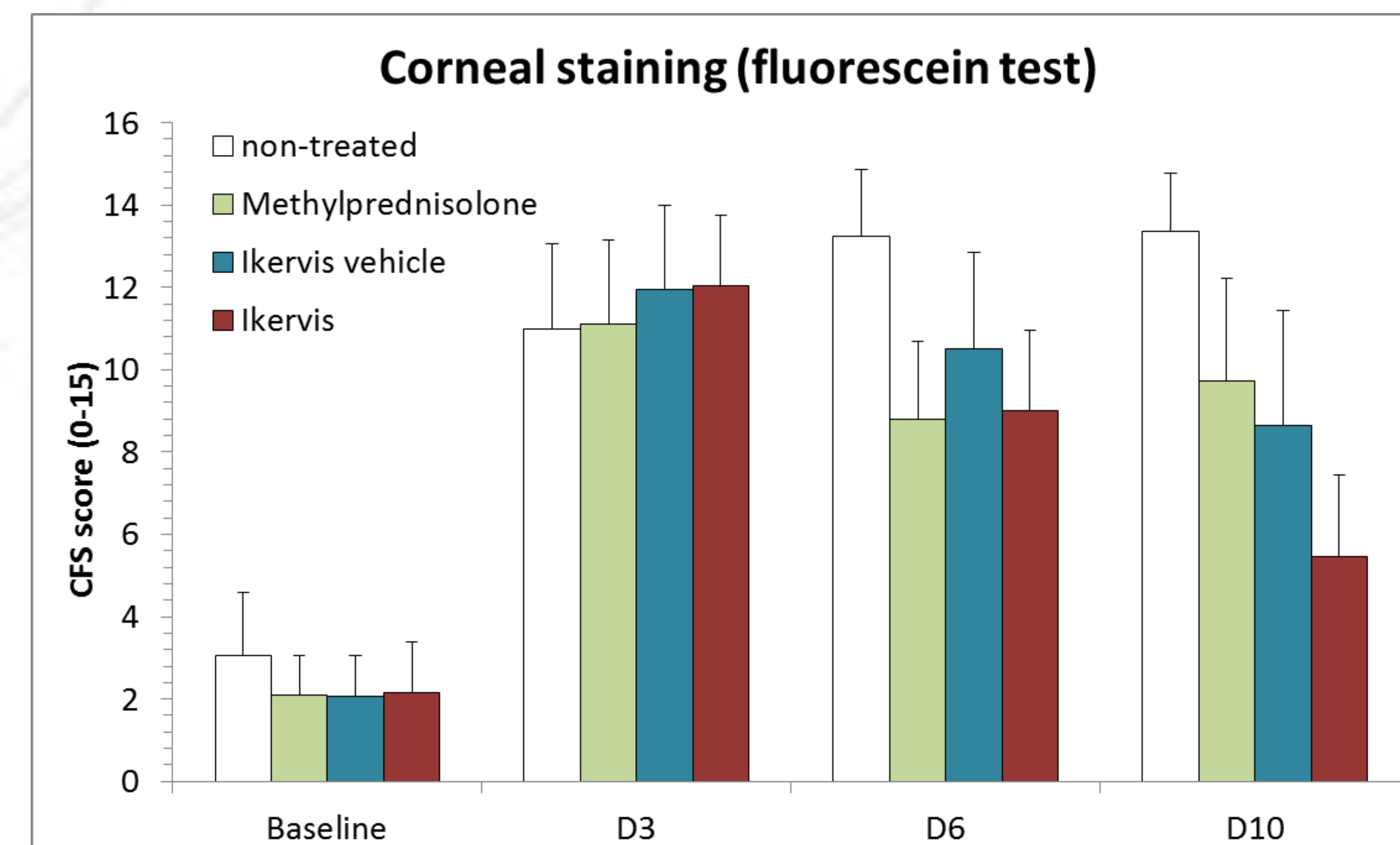
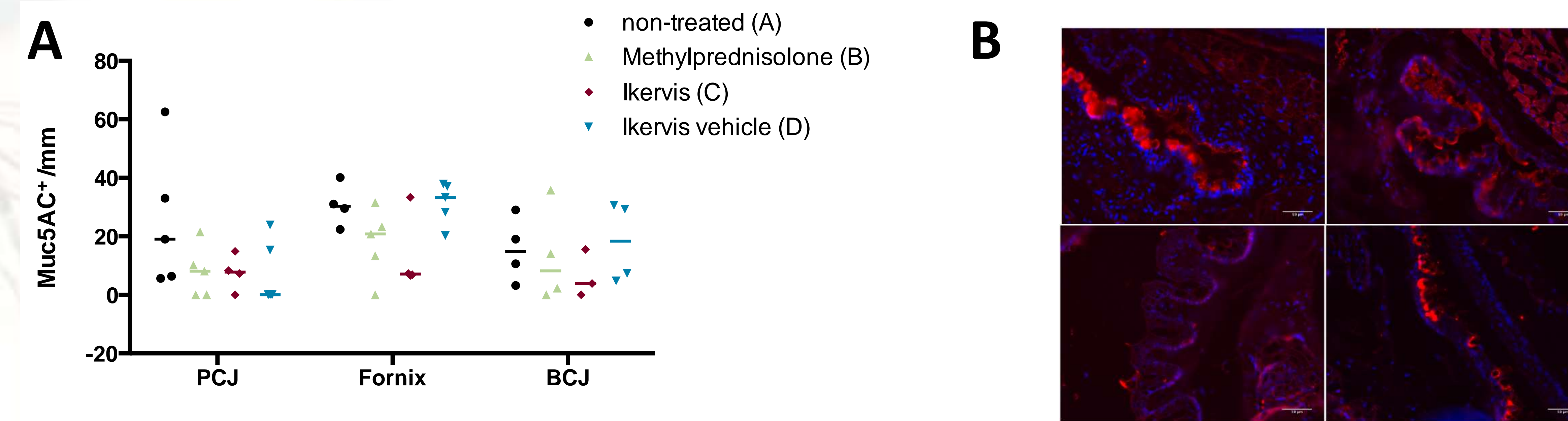
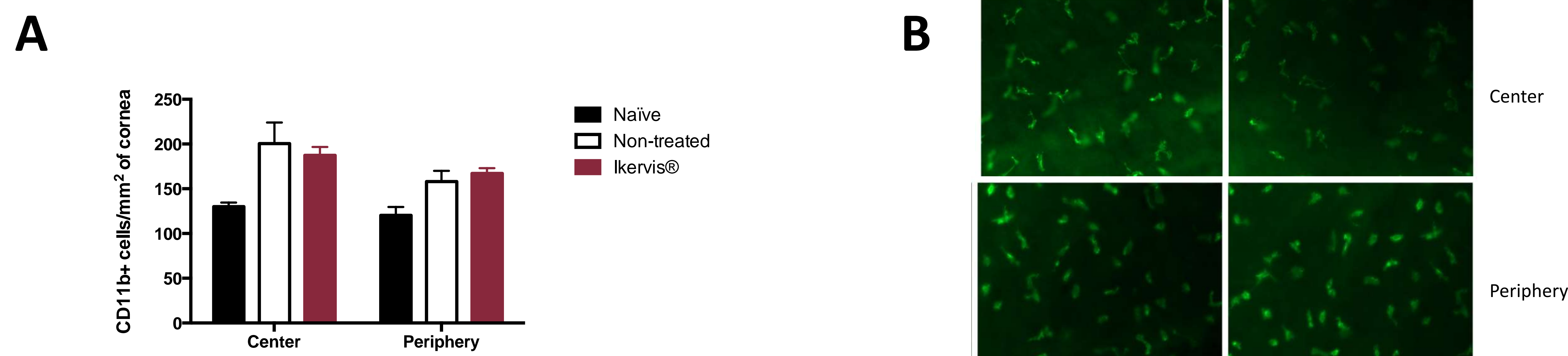


Figure 3. (A) Goblet cells count into the conjunctiva (n=5) of mice subjected to different eye drop treatments, and (B) representative images of forniceal conjunctival sections immunostained with anti-MUC5AC PE (nuclei stained with DAPI).



PCJ: Palpebral conjunctiva, BCJ: bulbar conjunctiva

Figure 4. (A) CD11b<sup>+</sup> cell count in flat mounted cornea (n=5) of mice subjected to different eye drop treatments, and (B) representative images of whole mounted immunostained with anti-CD11b FITC.



## Conclusion

The PRT lacrimation test confirmed the scopolamine-induced decrease in aqueous production by the lacrimal gland. After 7 days of treatment, the CFS score was reduced by 59% with the 0.1% CsA-loaded cationic emulsion (CFS score at D3 before treatment: 12.1 ± 1.7; vs D10: 5.5 ± 2.0). The beneficial effect of the cationic oil-in-water emulsion vehicle itself on keratitis was also clearly evidenced by its better performance over the 1% methylprednisolone eye drop, -36%, vs. -28%, respectively.

The Goblet cell density and the tear production were not markedly improved by any treatment in this model. A slight reduction (not significant) in CD11b<sup>+</sup> cells was also observed for the 0.1% CsA-loaded cationic emulsion in cornea when compared to the untreated group. An enumeration of CD4<sup>+</sup> T cells into the conjunctiva and cornea is currently in progress.

This study indicates that the cationic emulsion of cyclosporine (0.1%) was a very effective formulation for the management of corneal epithelium lesions (as evidenced by CFS scores) in a severe dry eye disease mouse model. In addition, it performed better than a potent glucocorticosteroid (1% methylprednisolone)<sup>4</sup>. This cationic emulsion of cyclosporine (0.1%) represents a promising new treatment strategy for the management of the signs of dry eye<sup>5,6</sup>.

**Note:** In March 2015 Ikervis<sup>®</sup> (IKERVIS 1 mg/mL eye drops, emulsion) Marketing Authorization was granted by the European Commission based on EMA's January 2015 positive opinion.

Ikervis<sup>®</sup>, once a day at bed time, is indicated for "Treatment of severe keratitis in adult patients with dry eye disease, which has not improved despite treatment with tear substitutes".

## References

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