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OCULAR SURFACE GROUP

CYCLOSPORINE A USING F4H5 AS LIQUID DRUG CARRIER IS EFFECTIVE IN TREATING EXPERIMENTAL DRY-EYE DISEASE

CECAD COLOGNE Excellent in Aging Research – Excellent in Alternsforschung

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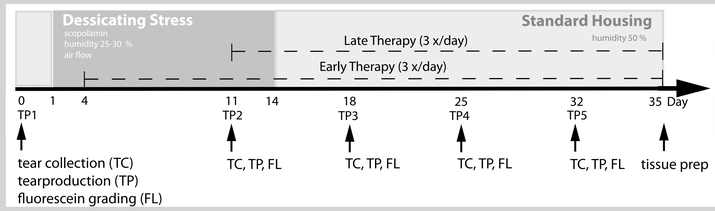
Background/ Purpose: Dry eye disease is an inflammatory disorder that can be treated effectively with topical Cyclosporine A (CsA). As CsA is complex to formulate in water based solutions, semifluorinated alkanes (SFAs) were used as a new delivery platform that enable easier and preservative-free formulation of lipophilic drugs such as CsA. This experimental study was designed to test the use of CsA using SFA as carrier (F4H5) for topical therapy in a mouse model of experimental dry eye disease (EDE) with respect to different treatment regimen and aging.

Materials/ Methods: EDE was induced in adult 10-12 week old and senescent 12 months old female C57BL/6 mice using a controlled environmental chamber for 14 days and treatment with scopolamine (Dessicating Stress Model: Dursun et al. 2002) and subsequent transfer to standard housing conditions until day 35. Topical therapy was performed 3x/day starting from day 4 or 11. Mice were distributed in four groups: (1) 0.05 % CsA/F4H5 (Novaliq, Germany), (2) F4H5 (Novaliq GmbH, Germany) and (3) Restasis® (Allergan Inc., USA). A control group (4) received no eye drops, but was kept under the same conditions as the therapy groups. Clinical readouts were undertaken weekly (amount of tear fluid, corneal epithelial staining) in combination with a final preparation of conjunctival tissue for counting goblet cell density at day 35. Tear samples were collected weekly by adding 1 µl of assay buffer to the eye. 1 µl of tear fluid and buffer was then collected with a glass capillary. Tear washings of both eyes of two mice were pooled for cytokine analysis. Inflammation markers were investigated using Multiplex system (Biorad, Bio-Plex®).

Experimental Set-Up:



1. CsA/F4H5 is effective in treating EDE CsA/F4H5 prevents development of EDE

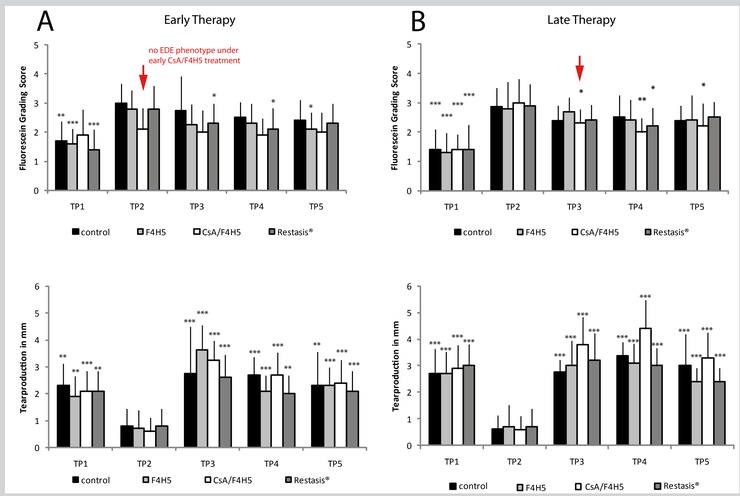


Fig. 1: Topical therapy with CsA/F4H5 resulted in significant earlier and stronger increase of tearproduction and earlier decrease of epithelial staining compared to control, F4H5 and Restasis® in 12 week old mice. Early Therapy from day 4 resulted in significant less epithelial staining in CsA/F4H5 group before the end of EDE (TP2), indicating a prophylactic effect of CsA/F4H5 . (n=5 mice/group, experiment was repeated twice with comparable results. (* indicating statistical differences compared to TP2 (end of EDE). Statistical analysis was performed using ANOVA, post hoc LSD. * p<0.05, ** p< 0.001, *** p<0.0001).

2. Goblet cell numbers remain normal following topical Early Therapy with CsA/F4H5

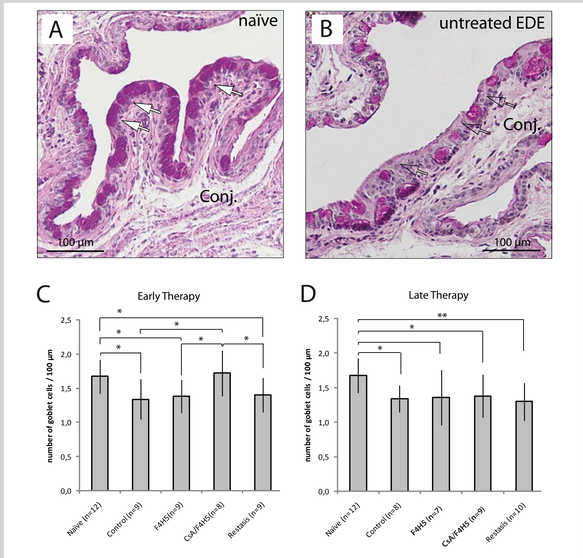


Fig. 2: EDE resulted in a significant loss of goblet cells at day 35 in untreated control mice (A+B). Following Early Therapy with CsA/F4H5 goblet cell numbers remain normal. Numbers of goblet cells were calculated from the lower cul-de-sac until the lid margin and presented per 100 µm (C+D). (A+B: PAS-staining. Conj.- conjunctiva).

3. Changes of tear film cytokines during topical therapy showed no statistical differences

Multiplex analysis of tear film cytokines (TNFα, IL-1, IL-2, IL-4, IL-10, IL-12, IL-13, IL-17, IFNγ) showed no statistical differences between groups, or between different timepoints of therapy.

4. Senescent mice develop a stronger phenotyp of EDE

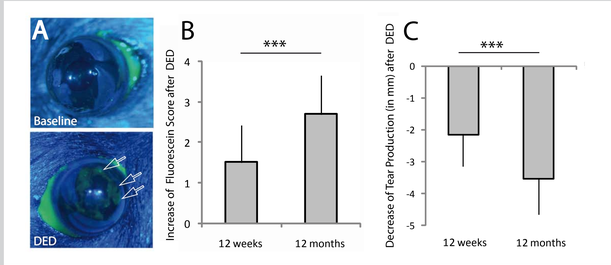


Fig. 4: Epithelial Staining and Tearproduction after EDE in 12 week and 12 months of age mice. Old mice develop a stronger DED phenotyp in the model used.

5. CsA/F4H5 is effective as EDE therapy in senescent mice

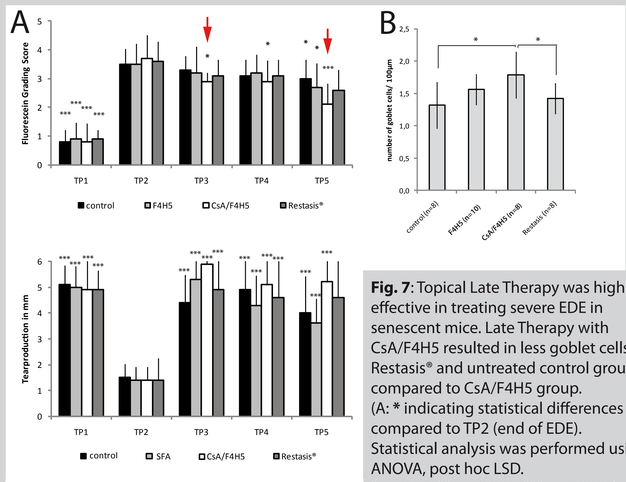


Fig. 7: Topical Late Therapy was highly effective in treating severe EDE in senescent mice. Late Therapy with CsA/F4H5 resulted in less goblet cells in Restasis® and untreated control groups compared to CsA/F4H5 group.
(A: * indicating statistical differences compared to TP2 (end of EDE).
Statistical analysis was performed using ANOVA, post hoc LSD.
* p<0.05, ** p< 0.001, *** p<0.0001).

Conclusion:

- CsA/F4H5 is highly effective in treating experimental dry-eye disease by reducing corneal staining and maintaining conjunctival goblet cells numbers.
- CsA/F4H5 was shown to be equally effective with Restasis®, but with a faster therapeutic response in adult and in senescent mice.
- Based on these results first applications in patients are on the way that may lead to a new therapeutic option in treating dry eye disease.

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