The Effect of an Ocular Surface Modulator in an *In Vitro* Model of Inflammatory Dry Eye

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BACKGROUND

Dry eye syndrome (DES) is a common condition that can significantly interfere with patients' quality of life, negatively affecting everyday activities such as reading, using a computer, working, and driving.[1] DES treatment with substitute tears only has shown some limitations because of the *lack of a treatment that can interact with the ocular surface system and modulate inflammation.*

T-Lysyal (T-LYS) is a supramolecular compound containing lysine hyaluronate, thimine, and sodium chloride that form longer chains than hyaluronic acid (HA) alone and a 3D structure with nanotubes. While hyaluronic acid binds water but it is not able to move it, T-LYS can attract water and has the capacity to move it, modulating growth factors expression and inflammatory mediators.[2] Furthermore, T-LYS is more resistant to the lytic enzyme hyaluronidase than HA because of the presence of lysine hyaluronate and thimine on the target sites of the enzyme. This fact accounts for a superior stability of the product that may exert a repairing activity for a longer period. In vitro T-LYS was able to repair corneal epithelial cells damaged by dry conditions.[3]
In our project we tested the hypothesis that T-LYS can control the inflammatory response in an in vitro model of inflammatory dry eye.

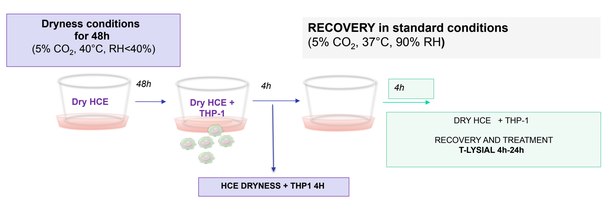
PURPOSE

An in vitro model of inflammatory dry eye based on 3D reconstructed human corneal epithelium (HCE) with infiltrating THP-1 cells has been used to evaluate the efficacy of an ocular surface modulator (T-Lysyal) to inhibit immune cells infiltration and to control the inflammatory response.

METHODS

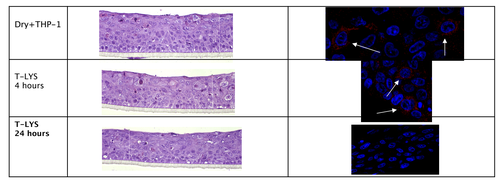
The EPISKIN Reconstituted Human Corneal epithelium of 0,5 cm^2 is produced by Episkin-Lyon(F) Epithelial human Immortalized cells (HICEC) are deposed on a polycarbonate filter and cultured at the air-liquid interface for 5 days in a chemically defined medium in order to form a structured epithelium. The HCE model (standard inserts)[4] has been validated by EURL ECVAM (EPISKIN HCE Eye Irritation Test) as alternative method to identify chemicals not requiring classification and labelling for eye irritation or serious eye damage (OECD TG 492, Revised July 2016).
The density and the diameter of the pores of the membrane of the HCE standard model allows medium diffusion for cell nutrition but are too small for cells circulation. On the contrary the migratrory insert (HCE-CMM) used in this project allows cells circulation.[5]
The HCE model was exposed to dryness conditions (T=40°C and Rh<40%) for 48 hours and then co-cultured with monocytes THP-1 at defined density cells for 4 hours. Thirty μl of T-Lysyal were applied on corneal surface for 4 hours and 24 hours. Tissue morphology by H&E staining and immuno-histochemistry of CD14, CD86, Aquaporin 3, ICAM-1 were performed after 4 and the 24 hours in treated and control HCE. As negative controls were used HCE exposed to the same conditions but treated with saline solution in presence of THP-1 cells. Gene expression was evaluated for Aqp3, TLR4, MMP-9.

For the immuno-staining the following primary and secondary antibodies have been used:
-Anti-AQP3 ABCAM Ab153694 (rabbit)
-Anti ICAM-1 ABCAM Ab171123 (mouse)
-Anti CD14 SIGMA HPA002127 (rabbit)
-Anti CD86 ABCAM Ab53004 (rabbit)
- Alexa Fluor 555 Donkey anti rabbit Life technology A31572
- SIMPLE STAIN MULTI (AP) + new fuchsin chromogen

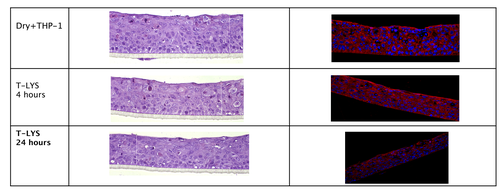


RESULTS

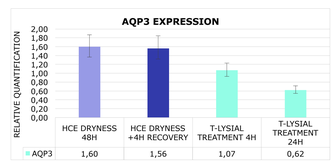
CD 86:the protein expression level was significantly reduced after 24 hours of treatment, but not after 4 hours

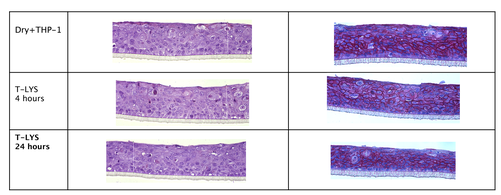


CD 14: the protein expression level was significantly reduced after 24 hours of treatment, but not after 4 hours

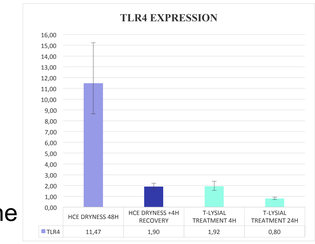


Aquaporin 3 was significantly reduced after 4 and 24 hours of treatment

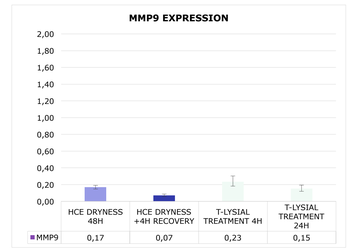




The TLR4 gene expression was significantly upregulated in presence of THP1 compared to control HCE.
The 24h T-Lysial treatment was effective in down regulating the gene expression showing a positive activity in rebalancing the corneal epithelium innate response



The MMP9 gene expression was not upregulated in our model



CONCLUSION

Dry eye syndrome is a common disease characterized by an activation of the immunological response of the ocular surface. By using an in vitro model that closely mimics the immune-activation we demonstrated that the T-Lysyal molecule is able to partially control this mechanisms. Further studies are necessary to confirm these results, but the concept of controlling inflammation over time in dry eye is certainly an important therapeutic target.

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5. De Servi, et al. immunocompetent cells infiltration into reconstructed human corneal epithelium: application to dry eye syndrome. ARVO abstract 2017.

DISCLOSURES: STEFANO BARABINO: SILDEHA SWISS CODE R, DEMETRIO MANENTI SILDEHA SWISS (PERSONAL FINANACIAL INTEREST)