Poster
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Efficacy of preservative-free cyclosporine emulsion eye drops in a mouse model of dry eye

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Introduction

Dry eye disease (DED) is a multifactorial disease in which uncontrolled inflammation can lead to severe corneal epithelium lesions and symptoms of discomfort. Anti-inflammatory eye drops are effective in the management of DED clinical signs and inflammation[1,2].

DED-induced modifications in cornea, conjunctiva and lacrimal gland inflammatory pathways are not yet clearly established, and it might be possible that different pathways are modulated in different ocular surface tissues[3]. In addition, the precise efficacy profile of cyclosporine eye drops on those inflammatory pathways in these tissues is not yet clearly established.

References: 1. Leonardi et al. Efficacy and safety of 0.1% cyclosporine A cationic emulsion in the treatment of severe dry eye disease: a multicenter randomized trial. EJO. 2016;26:287-96. 2.Sall et al. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. Ophthalmology. 2000;107(4):631-9. 3. Daull et al. Modulation of Inflammation-Related Genes in the Cornea of a Mouse Model of Dry Eye upon Treatment with Cyclosporine Eye Drops. Curr Eye Res. 2019 Jan 21:1-10. doi: 10.1080/02713683.2018.1563197.

Purpose

The aims of the present study were:

- to compare the efficacy of two cyclosporine-based anti-inflammatory emulsions in a mouse model of dry eye with severe corneal epithelium lesions,
- and explore the genes expression profiles in ocular tissues following DED induction and anti-inflammatory CsA-based treatments.

Methods

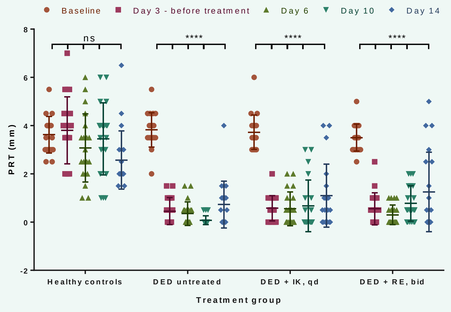
Six to 9-week-old female C57BL/6N mice with tail patches of scopolamine were housed in a controlled environment room (CER) to induce dry eye. At day three (dry eye baseline), following dry eye confirmation by corneal fluorescein staining (CFS, score 0-15) and phenol red thread (PRT) lacrimation test, the mice (n=10/gp) were instilled in both eyes with: QD 0.1% CsA cationic emulsion (Ikervis, Santen, France), BID 0.05% CsA anionic emulsion (Restasis, Allergan, USA), or left untreated. A group of healthy mice with no experimental DED was used as control.

Aqueous tear production and corneal epithelium lesions were assessed at baseline and day 3, 6, 10 and 14.

At the end of the experimental period left eyes were sampled, fixed and stained with hematoxylin/Eosin/Safran for histology analysis of the ocular surface, while the cornea, conjunctiva and lacrimal gland of right eyes were sampled, stored at -80°C for gene expression analysis with the NanoString® technology. Following mRNA extraction, the Mouse immunology panel was used to characterize the gene expression profile of DED mice and CsA-treated mice.

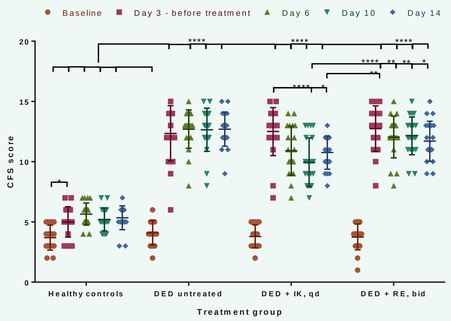
Results

Figure 1. Tear secretion over time following DED induction and treatment with cyclosporine.



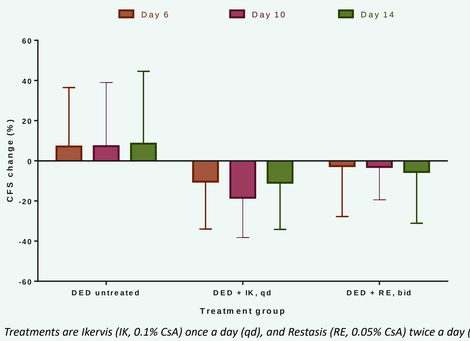
*Treatments are Ikervis (IK, 0.1% CsA) once a day (qd), and Restasis (RE, 0.05% CsA) twice a day (bid). ns, not significant, * p<0.05.*

Figure 2. Cornea Fluorescein Staining (CFS) score over time following DED induction and treatment with cyclosporine.



*Treatments are Ikervis (IK, 0.1% CsA) once a day (qd), and Restasis (RE, 0.05% CsA) twice a day (bid). ns, not significant, * p<0.05.*

Figure 3. CFS change over time.



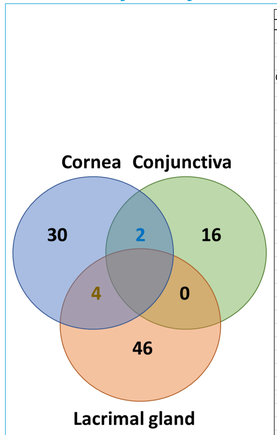
Treatments are Ikervis (IK, 0.1% CsA) once a day (qd), and Restasis (RE, 0.05% CsA) twice a day (bid).

Table 1. Summary of histology results at day 14.

	DED untreated	DED + IK, qd	DED + RE, bid
Palpebral conjunctiva epithelium	Thinned (n=2) Squamous metaplasia (n=2)	NO	NO
Bulbar conjunctiva epithelium	Thinned (n=2)	NO	NO
Conjunctival Goblet cells	Reduced number (n=2)	NO	NO
Corneal epithelium	Thinned or hyperplastic (n=7) Squamous metaplasia (n=2) Beginning of neovascularization (n=2)	NO	Squamous metaplasia (n=1)
Corneal stroma		NO	NO
Limbus epithelium	Thinned (n=7) Squamous metaplasia (n=2)	Thinned (n=2)	Thinned (n=4)
Mice with no pathologic signs	n=3	n=8	n=6

Treatments are Ikervis (IK, 0.1% CsA) once a day (qd), and Restasis (RE, 0.05% CsA) twice a day (bid). NO, no observation.

Figure 4. Venn diagram and list of the genes significantly modulated by the dry environment (CER + scopolamine).



Cornea (n=36)		Conjunctiva (n=18)		Lacrimal gland (n=50)	
gene	FC vs HC	gene	FC vs HC	gene	FC vs HC
Ikzf3	0.64	Smad5	0.82	Emr1	0.69
Nox4	0.66	Ncf4	1.50	Itga6	1.31
Fadd	0.83	Mapk1	0.86	Mif	1.36
Ceacam1	1.49	Npc1	0.83	Cd164	1.24
Ifih1	0.86	Hcst	1.65	Dpp4	1.19
Lif	1.45	Ccl11	0.65	Maf	0.67
Ptpn6	0.85	Mapkapk2	0.77	Itgam	0.58
Bcl3	1.55	S100a9	2.17	Chuk	1.18
Cx3cl1	0.81	Litaf	1.24	H60a	0.61
Il1r2	2.15	Ifnar2	1.21	Il1rl1	0.65
Ifngr1	1.16	Cfi	1.94	Cd81	1.32
Plau	1.29	Ptgs2	1.54	Itgb1	1.11
Nod2	1.64	Stat1	0.77	C1qb	0.71
Nfkb2	1.26	Cd55	0.72	Csf1	1.36
Ifna1	0.54	Il6st	0.81	Ccl6	0.57
Il1rn	1.55	Itga2b	0.70	Socs1	0.62
Nt5e	1.75	Gata3	0.74	Phlpp1	1.42
Kit	0.78	Il4ra	0.63	Fadd	1.19
Cd40	0.61			Ptk2	1.28
Il23r	1.47			Klrk1	0.70
Il18rap	0.66			Fcer1g	0.78
Plaur	1.96			Ccl25	1.27
Cd79b	1.44			Tgfbr1	1.15
Arhgdib	1.29			Tgfbi	0.73
Smad5	0.90			Mr1	1.54
Klra8	0.52			Clu	0.49
Mme	0.51			Fcgr4	0.70
Tnfrsf14	0.84			Fcgr3	0.68
Stat1	0.82			Cd40lg	0.68
Abl1	0.85			Cd46	0.73
Cxcl1	2.00			Traf6	0.86
Cd164	0.90			Ikbkap	1.20
Itga4	0.64			Vcam1	0.73
Il27	0.65			Cd24a	1.13
Ifi35	0.92			Tgfb2	1.67
Tnf	1.88			Ltbr	1.17
				C1qa	0.80
				Cd80	0.72
				Cxcl1	1.76
				Map4k4	0.87
				Cxcr4	0.53
				Il33	0.68
				Ccr5	0.76
				Il6ra	0.62
				H2-Eb1	0.69
				Fcamr	0.60
				Ifitm1	0.74
				Nt5e	1.21
				Cd14	0.75
				App	0.84

Top to botom, from the lowest p-value to p<0.05.

Figure 5. Genes significantly modulated by CsA in the different tissues.

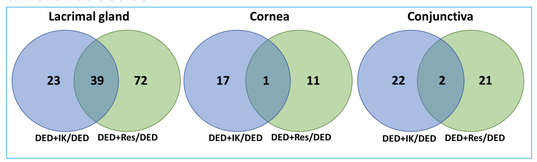


Table 2. Effects of CsA on the genes significantly modulated by the dry environment.

Cornea		Conjunctiva		Lacrimal gland	
By Ikervis	By Restasis	By Ikervis	By Restasis	By Ikervis	By Restasis
n=12/36	n=4/36	n=6/18	n=5/18	n=25/50	n=27/50
Ikzf3	Ikzf3	Smad5	Smad5	Emr1	Emr1
Nox4	Nox4	Ncf4	Ncf4	Itga6	Itga6
Fadd	Fadd	Mapk1	Mapk1	Mif	Mif
Ceacam1	Ceacam1	Npc1	Npc1	Cd164	Cd164
Ifih1	Ifih1	Hcst	Hcst	Dpp4	Dpp4
Lif	Lif	Ccl11	Ccl11	Maf	Maf
Ptpn6	Ptpn6	Mapkapk2	Mapkapk2	Itgam	Itgam
Bcl3	Bcl3	S100a9	S100a9	Chuk	Chuk
Cx3cl1	Cx3cl1	Litaf	Litaf	H60a	H60a
Il1r2	Il1r2	Ifnar2	Ifnar2	Il1rl1	Il1rl1
Ifngr1	Ifngr1	Cfi	Cfi	Cd81	Cd81
Plau	Plau	Ptgs2	Ptgs2	Itgb1	Itgb1
Nod2	Nod2	Stat1	Stat1	C1qb	C1qb
Nfkb2	Nfkb2	Cd55	Cd55	Csf1	Csf1
Ifna1	Ifna1	Il6st	Il6st	Ccl6	Ccl6
Il1rn	Il1rn	Itga2b	Itga2b	Socs1	Socs1
Nt5e	Nt5e	Gata3	Gata3	Phlpp1	Phlpp1
Kit	Kit	Il4ra	Il4ra	Fadd	Fadd
Cd40	Cd40			Ptk2	Ptk2
Il23r	Il23r			Klrk1	Klrk1
Il18rap	Il18rap			Fcer1g	Fcer1g
Plaur	Plaur			Ccl25	Ccl25
Cd79b	Cd79b			Tgfbr1	Tgfbr1
Arhgdib	Arhgdib			Tgfbi	Tgfbi
Smad5	Smad5			Mr1	Mr1
Klra8	Klra8			Clu	Clu
Mme	Mme			Fcgr4	Fcgr4
Tnfrsf14	Tnfrsf14			Fcgr3	Fcgr3
Stat1	Stat1			Cd40lg	Cd40lg
Abl1	Abl1			Cd46	Cd46
Cxcl1	Cxcl1			Traf6	Traf6
Cd164	Cd164			Ikbkap	Ikbkap
Itga4	Itga4			Vcam1	Vcam1
Il27	Il27			Cd24a	Cd24a
Ifi35	Ifi35			Tgfb2	Tgfb2
Tnf	Tnf			Ltbr	Ltbr
				C1qa	C1qa
				Cd80	Cd80
				Cxcl1	Cxcl1
				Map4k4	Map4k4
				Cxcr4	Cxcr4
				Il33	Il33
				Ccr5	Ccr5
				Il6ra	Il6ra
				H2-Eb1	H2-Eb1
				Fcamr	Fcamr
				Ifitm1	Ifitm1
				Nt5e	Nt5e
				Cd14	Cd14
				App	App

Yellow: common to both treatments
Blue: specific to Ikervis (0.1% CsA, qd)
Green: specific to Restasis (0.05%, bid)

- A different set of genes appear to be modulated by the dry environment in the ocular tissues (Fig 4).
- The formulation used to deliver CsA seems to have an impact on the expression profile of the drug product, not only the active principle (CsA) (Fig 5).
- The CsA formulations exert their beneficial effects by modulating different ocular surface tissue genes (Table 2).

Conclusion

This study demonstrates that both CsA emulsions were effective at reducing CFS scores, with the 0.1% CsA (qd) cationic emulsion being slightly better than bid instillations of 0.05% CsA anionic emulsion. DED animals treated with the 0.1% CsA cationic emulsions also presented fewer DED-induced ocular surface lesions. These data confirmed the improved potency of CsA when formulated in a cationic emulsion.

Interestingly, this study suggests that ocular tissues do not respond in the same way to the dry environment, and that a different set of genes are modulated by the CsA formulations in the ocular tissues.

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