Herpes Simplex Virus type 1 (HSV-1) induced keratitis: nanoparticles and antiviral peptides as a novel topical treatment

Inés Ruedas-Torres[1], Yolanda Jiménez-Gómez[2], Laura Ortega-Llamas[2], Jaime Gómez-Laguna[1], Miguel González-Andrades[2], May Griffith[3] and LiQD Cornea Consortium[4]

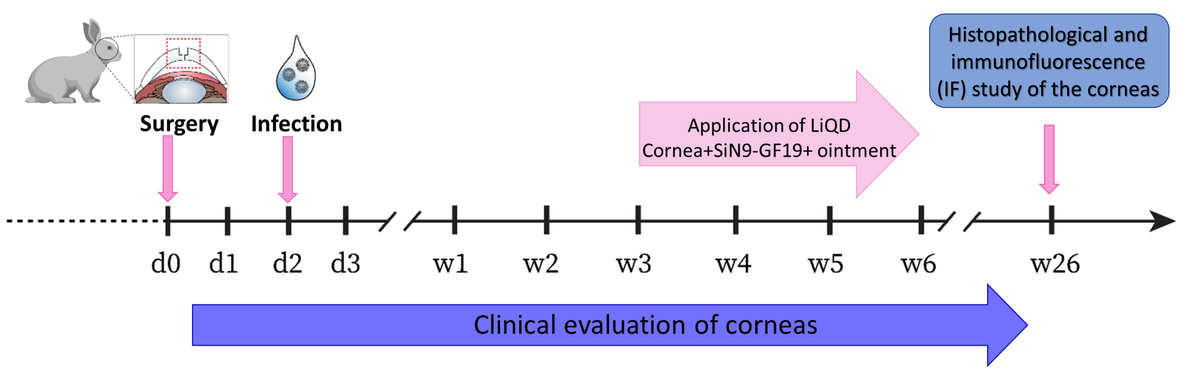
[1]Department of Anatomy and Comparative Pathology and Toxicology, Pathology and Immunology Group (UCO-PIG), UIC Zoonosis y Enfermedades Emergentes ENZOEM, University of Cordoba, Spain [2]Department of Ophthalmology, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital and University of Cordoba, Spain. [3]Department of Ophthalmology and Institute of Biomedical Engineering and Maisonneuve-Rosemont Hospital Research Centre, Montreal, Canada. [4]Department of Ophthalmology, Vilnius University Hospital, Lithuania and Department of Microbiology, Infectiology and Immunology, University of Montreal, Canada.

INTRODUCTION

Herpes simplex type 1 (HSV-1) is a global health problem, and can cause blindness due to uncontrolled inflammation that damages corneal tissue. The objective of this work is to study the effect of four formulations in a rabbit cornea model infected with HSV-1.

MATERIAL & METHODS

Punctures were made in the left eyes of 24 rabbits (6 rabbits/group) and after two days they were infected with HSV-1.



Each group was treated for 6 months with different formulations:

- Cyanoacrylate adhesive **(GROUP 1)**
- LiQD Cornea (liquid hydrogel matrix with collagen peptides, polyethylene glycol and fibrinogen) **(GROUP 2)**
- LiQD Cornea+SiN9-GF19 (nanoparticles coated with the antiviral peptide GF19) **(GROUP 3)**
- LiQD Cornea+SiN9-GF19+ointment (with SiN9-GF19) **(GROUP 4)**

RESULTS

Compared with the corneas of GROUP 4 and CONTROL, the corneas of GROUPS 1, 2, and 3 showed greater severity in histopathological lesions, mainly stromal neovascularization **(FIGURE 1B)** and the degree of inflammatory infiltrate **(FIGURE 2A and B)**, especially the corneas of GROUP 1 **(TABLE 1).**

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
Inflammatory infiltrate	I	2/6	-	1/6	-
	II	1/6	1/6	-	-
	III	-	-	1/6	-
Neovascularization		4/6	-	1/6	-
Stromal disarrangement	I	3/6	1/6	5/6	4/6
	II	1/6	1/6	-	1/6
	III	1/6	3/6	1/6	-
Lipid keratopathy		2/6	-	-	-
Stromal thinning		2/6	4/6	4/6	4/6
Epithelial hyperplasia		4/6	4/6	4/6	4/6
Swelling of epithelial cells		4/6	4/6	4/6	-

TABLE 1. Main histopathological lesions observed.

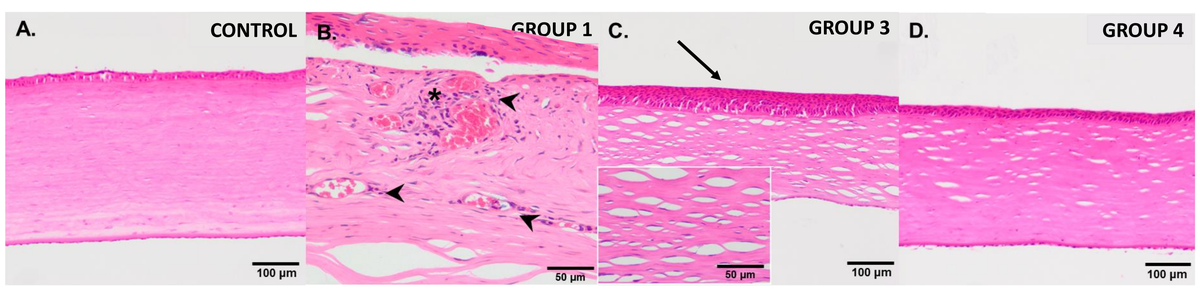


FIGURE 1. A. Non-infected cornea (CONTROL). **B.** GROUP 1 cornea with neovascularization (arrowheads) and perivascular inflammatory infiltrate (asterisk). **C.** GROUP 3 cornea with moderate stromal disarrangement and epithelial hyperplasia (arrow). **D.** GROUP 4 cornea with mild stromal disarragement.

All the corneas of the infected animals presented different degrees of disorganization of the corneal layers **(FIGURE 1C and D).**

RESULTS

Other lesions found were lipid keratopathy **(FIGURE 2C and E)**, epithelial hyperplasia **(FIGURE 2D)**, epithelial cell swelling **(FIGURE 2E)** and stromal thinning **(FIGURE 2F)**.

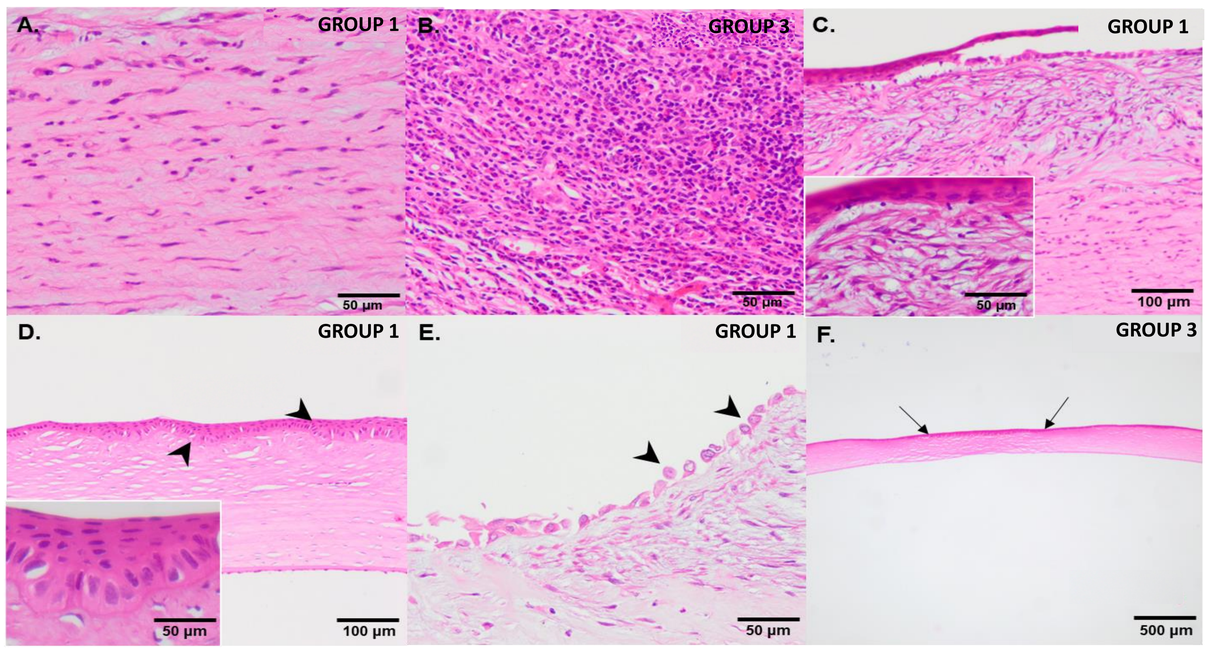


FIGURE 2. A. GROUP 1 cornea with mild inflammatory infiltrate. **B.** GROUP 3 cornea with intense inflammatory infiltrate. **C.** GROUP 1 cornea with lipid keratopathy. **D.** GROUP 1 cornea with epithelial hyperplasia (arrowheads). **E.** GROUP 1 cornea with lipid keratopathy and epithelial cell swelling (arrowheads). **F.** GROUP 3 cornea with thinning of the stroma (arrows).

On the other hand, a regeneration of the corneal epithelium (CK3+) was observed in the treated groups that contained LiQD Cornea in their formulation (GROUPS 2, 3, 4) **(FIGURE 3A-E)**. The expression of α-SMA was significantly lower in the corneas of the animals that contained only LiQD Cornea (GROUP 2) as treatment **(FIGURE 3F-J)**, while that of βIII tubulin was lower in GROUPS 2, 3 and 4, showing immature neurons in GROUPS 3 and 4 **(FIGURE 3N and Ñ)**.

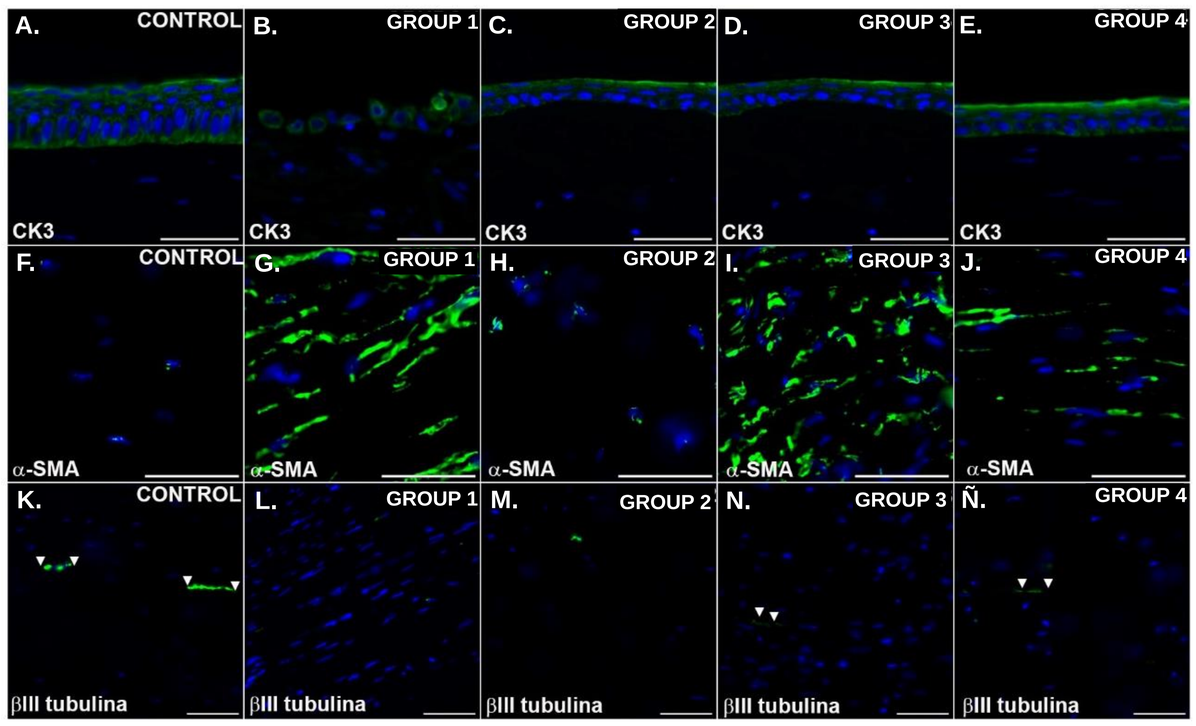


FIGURE 3. A. IF against CK3 (green) in uninfected cornea (CONTROL). IF against CK3 (green) in infected cornea of GROUP 1 **(B)**, GROUP 2 **(C)**, GROUP 3 **(D)** and GROUP 4 **(E)**. **F.** IF against α-SMA (green) in uninfected cornea (CONTROL). IF against α-SMA (green) in infected cornea of GROUP 1 **(G)**, GROUP 2 **(H)**, GROUP 3 **(I)** and GROUP 4 **(J)**. **K.** IF against βIII tubulin (green) in uninfected cornea (CONTROL). IF against βIII tubulin (green) in infected cornea of GROUP 1 **(L)**, GROUP 2 **(M)**, GROUP 3 **(N)** and GROUP 4 **(Ñ)**. Scale bars, 50 µm.

Viral antigen analysis by IF showed that HSV-1 was present in the corneas of GROUPS 1 and 2, while it was not detected in those of animals belonging to GROUPS 3 and 4 **(FIGURE 4A-E).**

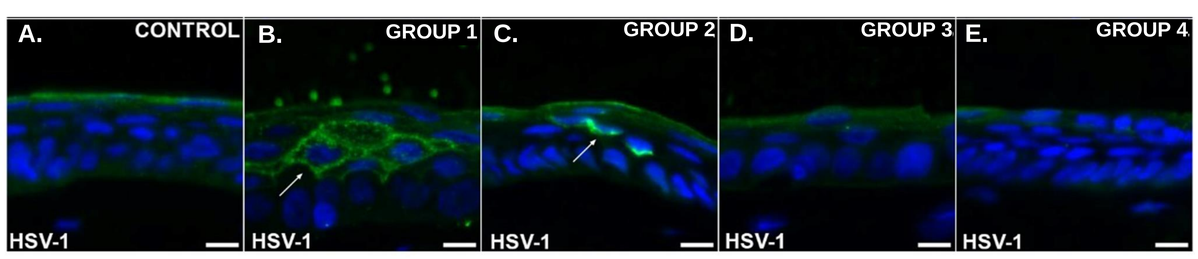


FIGURE 4. **A**. IF against HSV-1 (green) in uninfected cornea (CONTROL). IF against HSV-1 (green) in infected cornea of Group 1 **(B)**, GROUP 2 **(C)**, GROUP 3 **(D)**, and GROUP 4 **(E)**. Scale bars, 10 µm.

CONCLUSION

Our results suggest a greater efficacy of the formulation "LiQD Cornea+SiN9-GF19+ointment" capable of stopping the viral infection and regenerating the corneal tissue, structurally and functionally approaching a healthy cornea 6 months after the operation.