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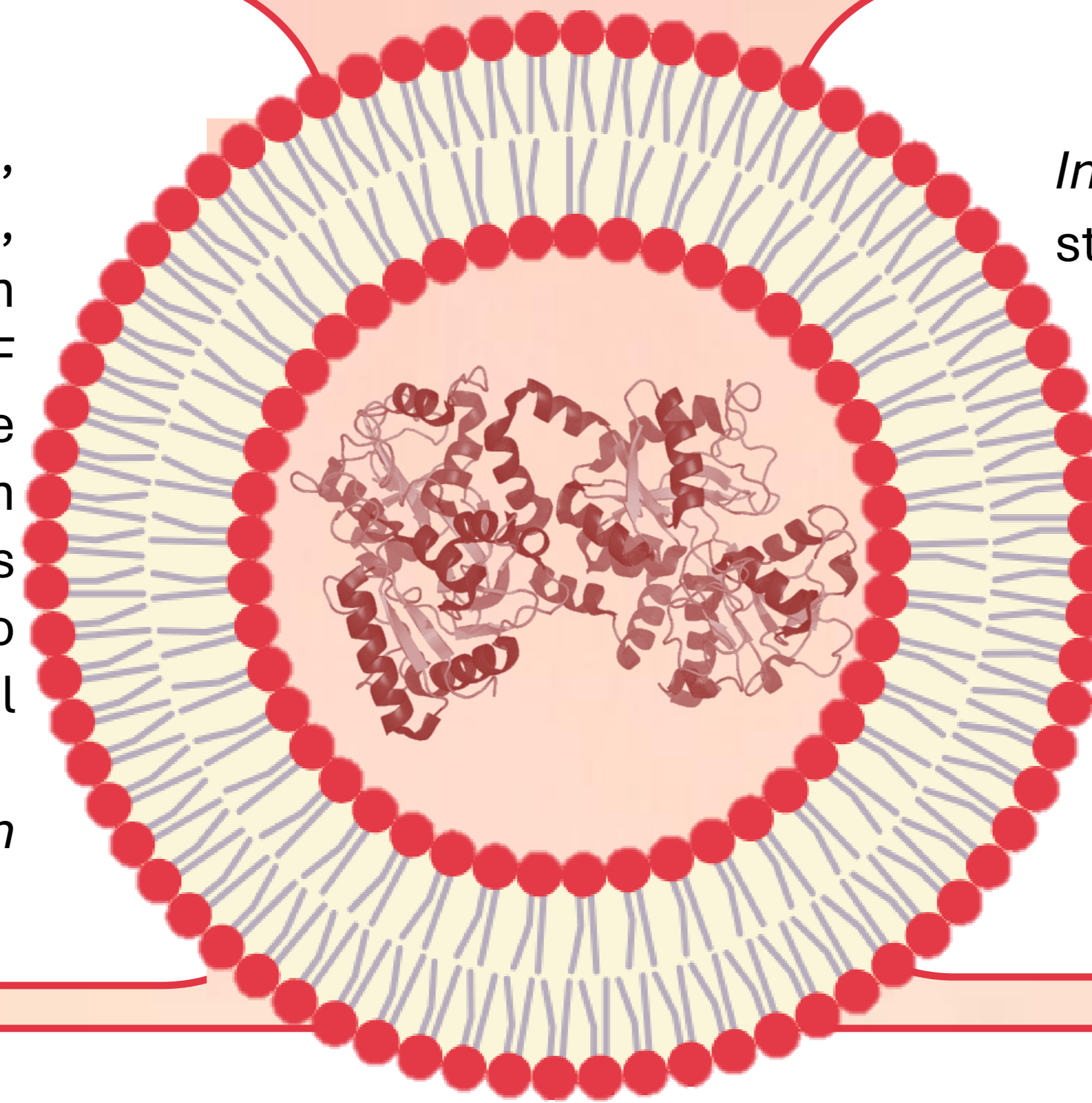
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Purpose

LTF Lactoferrin (LTF) is an antimicrobial, immunomodulatory and anti-inflammatory protein, and it has been advocated that LTF plays an important role at the ocular surface. Therefore, LTF liposomes have been produced in order to be applied as eyedrops and exert an immunomodulatory ocular response. LTF liposomes used in prophylaxis of cataract surgery showed to improve the physiological defenses and control bacterial ocular surface contamination [1].

Now, to evaluate its role in the immune response *in vitro* and *in vivo* assays were performed.



Methods

In vitro inflammation prevention was assed on human corneal epithelial cells (HCE-2). LPS was employed as inflammatory stimulus (10 µg/mL) to produce an immune response. Afterwards, treatments were applied for 24 h as prevention therapy and LPS was added for 24 h. Supernatants were collected, and IL-8 and Monocyte Chemoattractant Protein-1 (MCP-1) were analyzed.

In vivo inflammation prevention was carried out using New Zealand albino rabbits (n = 3/group). LTF liposomes activity was compared with free LTF and NaCl 0.9 %. Firstly, each formulation was administered, and after 30 min of exposure sodium arachidonate (SA) was instilled as an inflammatory stimulus. The evaluation of prevention and treatment of each formulation were carried out from the first application up to 210 min, according to the Draize modified test scoring system.

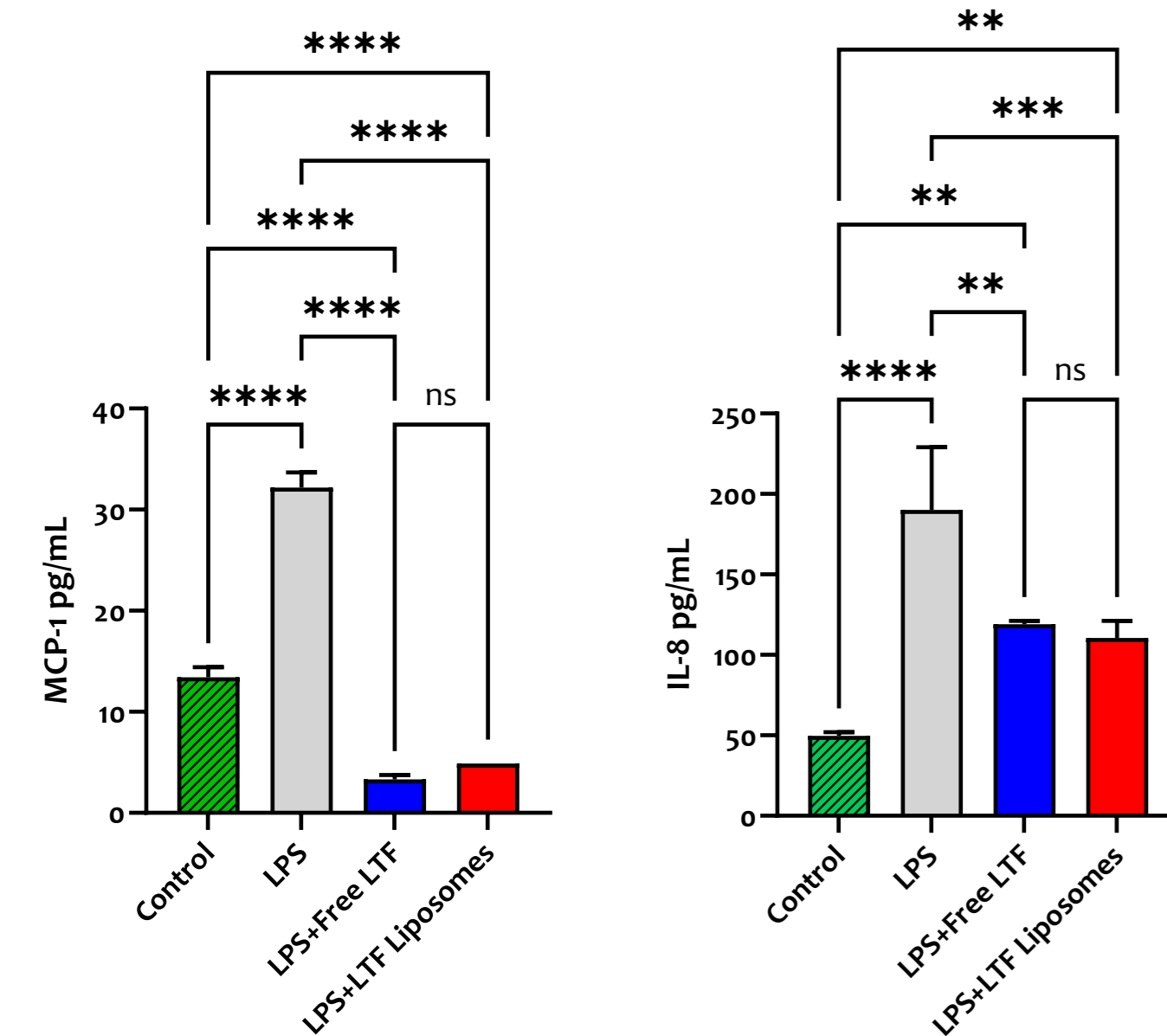
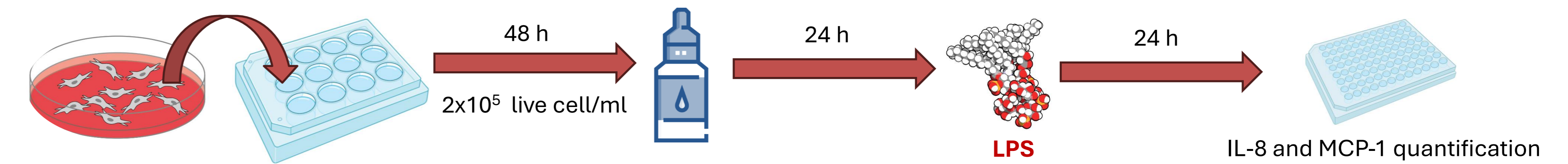


Figure 1. Quantification of secreted MCP-1 and IL-8 in LPS-stimulated HCE-2 cells. Values are expressed as mean \pm SD; **p < 0.01 and ***p < 0.001 and ****p < 0.0001.

Results

IL-8 and MCP-1 were quantified and compared against free LTF (Figure 1). Regarding IL-8, both free LTF and LTF liposomes showed statistical differences against LPS (p<0.01 and p<0.005, respectively). No differences were obtained between free and encapsulated LTF in IL-8 values thus confirming that both possess ability to modulate IL-8 secretion. In addition, both free LTF and LTF liposomes decreased significantly MCP-1 against both LPS (p<0.0001) as well as against the healthy cells (p<0.0001). No differences were obtained between free and encapsulated LTF confirming that *in vitro* both are able to modulate the cellular response to LPS stimuli, inhibiting the secretion of the pro-inflammatory cytokine IL-8 and MCP-1.

In vivo inflammatory prevention test showed significant differences between the degree of inflammation treated with LTF formulations or NaCl 0.9 % (Figure 2). Nevertheless, eyes treated with LTF liposomes presented a faster swelling reduction rather than free LTF, mainly owing to tear clearance in case of free LTF and the improved ocular surface adherence of liposomes, thus presenting longer residence time in the cornea [2].

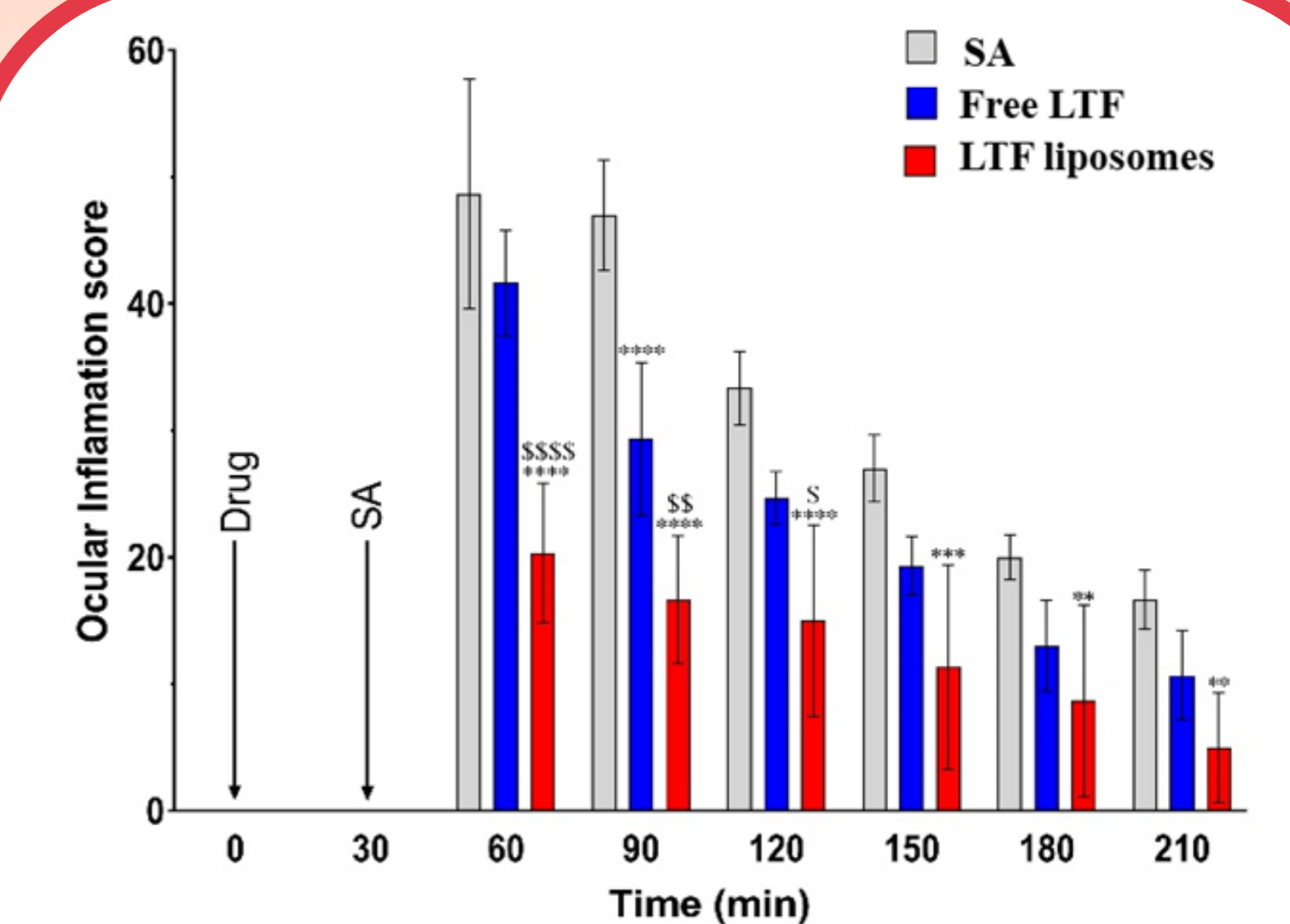


Figure 2. Ocular inflammation prevention. Values are expressed as mean \pm SD; **p < 0.01 and ***p < 0.001 and ****p < 0.0001 significantly lower than the inflammatory effect induced by SA; \$p < 0.05, \$\$p < 0.01 and \$\$\$p < 0.0001 significantly lower than the inflammatory effect induced by free LTF.

Conclusions

LTF liposomes possess an immunomodulatory response *in vitro* by preventing the release of inflammatory molecules such as IL-8 and MCP-1. Furthermore, LTF liposomes were able to prevent the inflammation caused by SA *in vivo*.

Although further investigations are needed to assess the expression and modulation of other mediators involved and to better define the action of liposomal LTF at the ocular level, this study reports the immunomodulatory capacity of LTF liposomes and their capacity to control ocular inflammation processes.

References

- [1] Giannaccare G, et al. Effect of Liposomal-Lactoferrin-Based Eye Drops on the Conjunctival Microflora of Patients Undergoing Cataract Surgery. *Ophthalmol Ther.* 2023 Apr;12(2):1315-1326.
- [2] *Colloids Surfaces B: Biointerfaces.* 2016;145:241–250. doi: 10.1016/j.colsurfb.2016.04.054.

Acknowledgements

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