pharmaceutics

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Supplementary Materials: Poly(Lactic-Co-Glycolic Acid) Nanoparticles Encapsulating the Prenylated Flavonoid, Xanthohumol, Protect Corneal Epithelial Cells from Dry Eye Disease-Associated Oxidative Stress

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Xanthohumol does not inhibit the drug efflux transporter, P-glycoprotein 1

Drug uptake into corneal epithelial cells and tissue levels depend on among other drug efflux transporters. With particular relevance to dry eye disease, cyclosporine is a potent inhibitor of P-glycoprotein 1 (P-gp), which is highly expressed in HCE-T cells. To determine whether xanthohumol exerts inhibitory effects on P-gp, we performed an *in vitro* drug efflux transporter assay.

To this end, HCET-T cells seeded in 96-well plates were exposed to a dose-range of either xanthohumol (10 nM – 10 μM) or to cyclosporine A (5 nM – 100 μM) for 30 min. Calcein-AM (2 μM) was added and fluorescence (excitation λ = 495 nm; emission λ = 525 nm) was measured in a Cytation 5 plate reader (BioTek Instruments, Inc.; Winooski, VT, USA) every 5 min for a 30 min period. The slope of the response was calculated and plotted over the drug concentration. Data were fitted using a Hill equation in Prism 9.0 (GraphPad, Inc., La Jolla, CA, USA).

Xanthohumol had no effect on P-gp drug transporter function. The slope did not change with an increasing concentration of Xanthohumol (Suppl. Fig. 1) suggesting the absence of an inhibitory effect on P-gp. In contrast, cyclosporine resulted in a dose-dependent increase in the slope that could be fitted with a Hill equation, confirming the inhibitory effect of cyclosporine on P-gp (supplementary material figure S1).

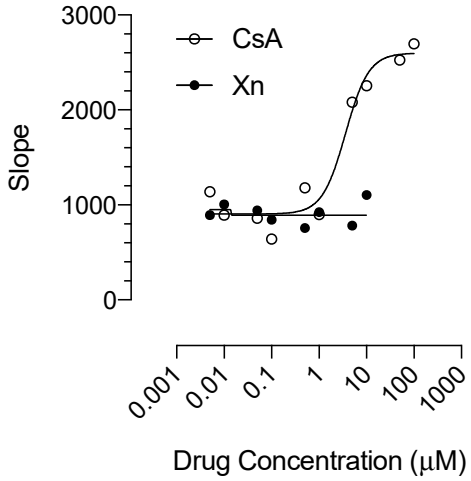

Figure S1. Xanthohumol had no effect on P-gp drug transporter function. Xanthohumol (filled circles) had no effect on the slope, while cyclosporine (open circles) showed a dose-dependent increase that was fitted using a Hill equation. Data were calculated from 8 technical replicates per concentration and are presented as mean.

Given the potent effects of cyclosporine on P-gp, care should be taken when co-administering ocular topical drugs together with cyclosporine, as this may results in increased concentrations in the corneal epithelial cells due to inhibition of P-gp.