Cellular fluorescein hyperfluorescence is dynamin-dependent and is increased with Tetronic 1107 treatment

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Introduction

We have previously shown that treatment with SICS-inducing MPS causes hyperfluorescence in human corneal (HC) cells and mouse fibroblast cultures (L929 cells). We have also shown that fluorescein staining is an active process not associated with necrosis[1]. In this work, we investigated the role of dynamin, a protein implicated in membrane remodelling during endocytosis[2] (active uptake of extracellular materials), in fluorescein staining in L929 and HC cultures. Tetronic 1107 is a surfactant commonly found in SICS-inducing MPS, and we compared the effect of this on fluorescein staining, relative to that for a generic laboratory surfactant (Triton X-100).

Methods

MPS/Dynasore treatment: L929 cells were treated with all test MPS for 2 hours (Table 1). L929 and HC cells were also exposed to P-PQ1-1107 and P-1107 for 2 hours before treatment with or without dynasore (an inhibitor of the protein dynamin) for 30 minutes; control cells were incubated with media alone. Cultures were then treated with fluorescein (0.01% w/v) for 10 minutes.

Surfactant treatment: Cells were treated with Tetronic 1107 at concentrations 0.1%, 1%, and 10% w/v, and all test MPS for 2 hours. HC cells were similarly exposed to Triton X-100 at concentrations 0.00125%-1.25% v/v for 2 hours. All cultures were then treated with fluorescein (0.01% w/v) for 10 minutes.

Quantification of fluorescein fluorescence: Fluorescence uptake was quantified using a high content analysis (HCA) microscopy platform.

MPS	Manufacturer	Formulation
Biotrue	Baush+Lomb	P-PQ1-1107
ReNu Sensitive	Baush+Lomb	P-1107
Opti-Free RepleniSH	Alcon	PQ1-Aldox-1304
Complete Revitalens	AMO	PQ1-Alex-904

Table 1: MPS formulations used in this study

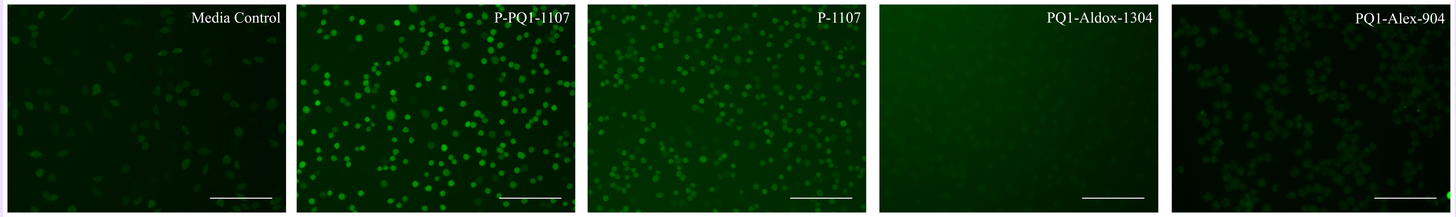

Figure 1: Fluorescein uptake in L929 cell after treatment with MPS. Scale bars represent 100µm.

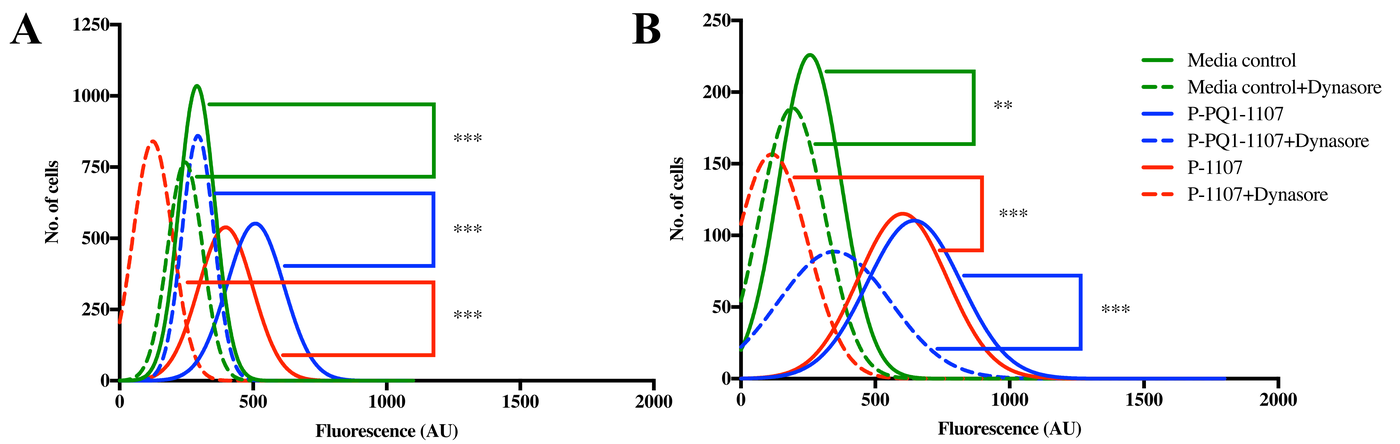

Figure 2: Fluorescein uptake in (A) L929 and (B) HC cells treated with hyperfluorescence inducing MPS P-PQ1-1107 and P-1107, with or without dynasore; **p=0.01 and ***p≤0.001.

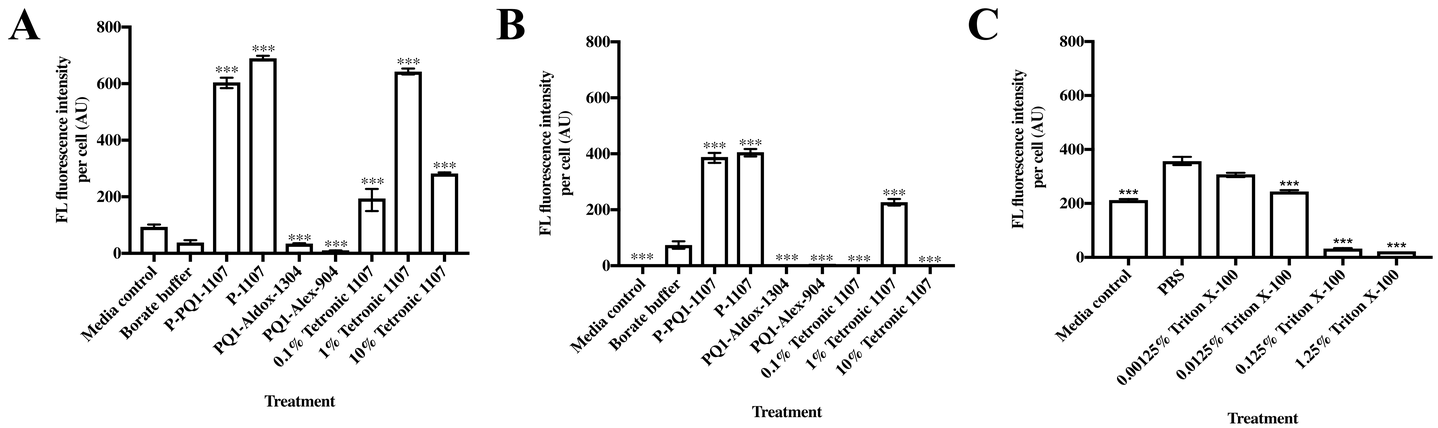

Figure 3: Fluorescein uptake in (A) L929 and (B) HC cells after Tetronic 1107 treatment, and (C) in HC cells after Triton X-100 treatment. Error bars represent 95% CI limits and ***p≤0.001.

Results

P-PQ1-1107 and P-1107 treatments in L929 cells increased fluorescence uptake in comparison to control, as typically seen clinically. Similarly, minimal fluorescein uptake was observed after PQ1-Aldox-1304 and PQ1-Alex-904, two MPS clinically known to cause little staining (Figure 1). However, with addition of dynasore, fluorescein intensity significantly decreased across all treatments and cell types (Figure 2A and B).

Furthermore, treatment with 1% w/v Tetronic 1107 in L929 cells caused hyperfluorescence greater than P-PQ1-1107, and also exceeded media control, borate buffer (carrier control) PQ1-Aldox-1304 and PQ1-Alex-904 (Figure 3A). Similarly, after Tetronic 1107 treatment in HC cells, an increase in hyperfluorescence was only observed at 1% w/v concentration relative to media and carrier control treatments (Figure 3B). Treatment with Triton X-100 at all concentrations did not cause hyperfluorescence beyond that observed with PBS, the carrier control (Figure 3C).

Conclusions

Our *in vitro* model shows fluorescein uptake is a dynamin-associated process in both cell types. Furthermore, 1% w/v Tetronic 1107 treatment in both cell lines exceeded the levels of hyperfluorescence of the media and carrier control, whereas Triton X-100 did not. This suggests that Tetronic 1107 is associated with MPS-induced hyperfluorescence and that perhaps hyperfluorescence is surfactant specific.

References

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TK was supported through a PhD CASE Award by the Biotechnology and Biological Sciences Research Council and CooperVision