Small compounds targeting autophagy protect primary retinal pigment epithelial cells

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Introduction

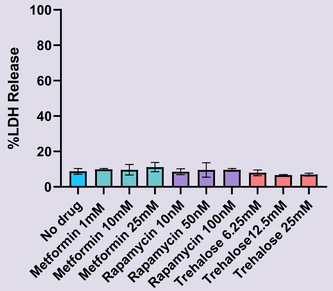
Age-related macular degeneration (AMD) is a **leading cause of irreversible blindness in adults over 50**.[1] Dysfunction of retinal pigmented epithelium (RPE) is an early pathological change during AMD development.[2] Autophagy is a cellular stress-response for waste clearance and maintenance of cell homeostasis. It involves **autophagosome fusion with lysosomes and finally, autophagolysosome** formation. **Impaired autophagy in the RPE** has been associated with AMD pathogenesis.[3,4] We aimed to study if **manipulating autophagy using small compounds could protect RPE cells *in vitro*.**

Methods

Primary mouse RPE cells were treated with different concentrations of metformin, rapamycin or trehalose. Cell cytotoxicity was determined by LDH assay. LC3B tandem sensor (GFP and RFP), Lysotracker and live cell imaging were used to measure the autophagy flux. Hoechst staining was used to quantify cell number. **Oxidative stress was induced by chronic, pulsed exposure to hydrogen peroxide (H_2O_2).**

Results 1

Acute (24 hours) treatment with all three small compounds did not induce toxicity in primary RPE cells.



^Fig. 1. LDH release by RPE cells in control and compound samples.

Acute (24 hours) treatment with all three small compounds did not show cytotoxicity to primary RPE cells when compared to a control sample as demonstrated in figure 1.

Results 2

All 3 compounds variably induced the autophagic activities in the cells, demonstrated by enhanced autophagosome and autophagolysosome vacuoles, and maintained lysosomes.

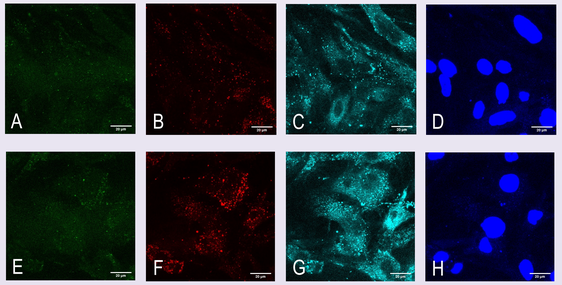
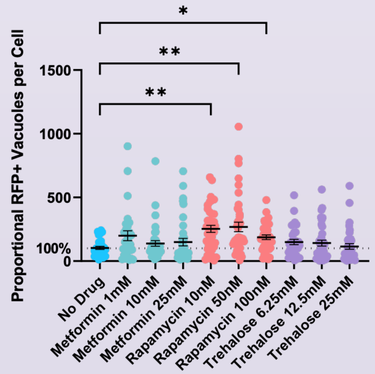


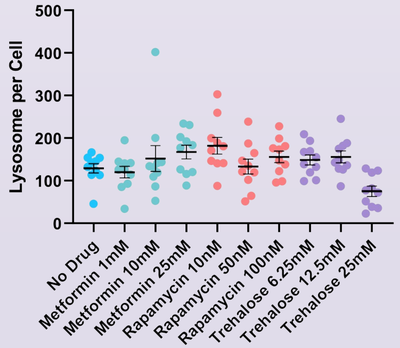
Fig. 2 shows visibly increased GFP fluorescence (autophagosomes) and RFP fluorescence (autophagolysosomes) in Trehalose 12.5mM samples (E, F) compared to control (A, B). Lysotracker fluorescence (lysosomes) is similar in both samples (C, G) showing maintained lysosomes. Hoechst staining (cell nuclei) was used to quantify cell number (D, H)

^Fig. 2. Live cell imaging using confocal microscopy at 63X magnification. Control samples (A-D) and Trehalose 12.5mM (E-H).



^Fig. 3. RFP+ vacuoles per cell in control and compound samples.

The proportion of autophagolysosomes per cell were greater in all compound samples compared to controls. *P<0.05, **P<0.01.

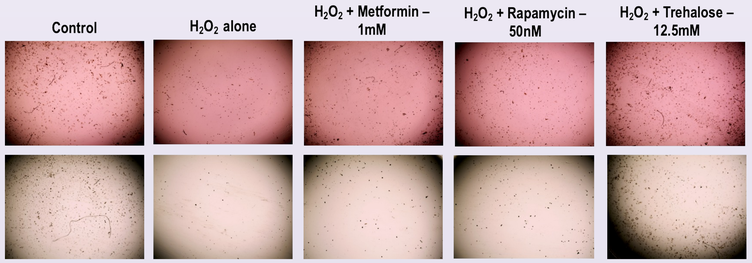


^Fig. 4. Lysotracker+ vacuoles per cell in control and compound samples.

There were maintained numbers of lysosomes in all compound samples compared to controls, indicating health of the downstream pathway.

Results 3

Upregulation of autophagy by the 3 compounds protects against oxidative stress induced cell death.



^Fig. 5. Live cell imaging at 4X magnification after 3 days (top) and 9 days (bottom) of pulsed, chronic oxidative stress treatment.

Figure 5 demonstrates the effect of autophagy induction on RPE cells exposed to 3 days (top) and 9 days (bottom) of pulsed chronic oxidative stress. Pulsed chronic H_2O_2 treatment visibly induced RPE cell death in the control sample. Oxidative stress induced cell death was largely inhibited by the 3 autophagy inducing compounds. Trehalose exerted greatest protection, compared to the other two molecules.

Conclusion

Metformin, rapamycin and trehalose all show promising effects to combat oxidative stress induced cell death in primary RPE cells. The compounds were non-toxic at the dosages tested. **All 3 compounds successfully upregulated autophagy flux.** Subsequently, **enhanced autophagy reduced RPE cell death during oxidative stress**. *In vivo* studies are required to assess the therapeutic potential for AMD.

Financial disclosures

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