The effect of ULK1 inhibition on corneal epithelial cells during *Pseudomonas aeruginosa* infection

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INTRODUCTION

Pseudomonas aeruginosa (PA) is a gram negative bacterium and the leading causative agent in all culture positive cases of microbial keratitis. It is an opportunistic pathogen, with the majority of cases associated with contact lens wear or injury to the eye.[1,2,3]

Studies in our lab have shown that PA infection induces autophagy, a major cellular degradation process, in corneal epithelial cells. Further stimulation or the inhibition of autophagy both reduced intracellular PA survival. Consistent with this, we also showed that the inhibition of autophagy by the Unc-51-like kinase (ULK1), an enzyme that mediates formation of the autophagosome, reduces intracellular levels of PA.

More recently we demonstrated that PA infection negatively impacts host mitochondria. While ULK1/2 has been reported to translocate to mitochondria to mediate mitophagy, a role for ULK1/2 in mitochondrial homeostasis during infection has not yet been explored.[4]

PURPOSE

This study investigated the effects of the inhibition of Ulk1/2 during PA infection on mitochondria in corneal epithelial cells.

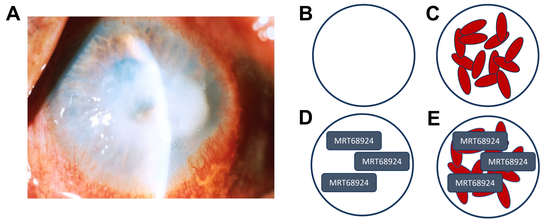


Figure 1: (A) Severe *Pseudomonas aeruginosa* keratitis. **(B-E)** Experimental overview: **(B)** Control – PBS treatment; **(C)** PA01; **(D)** MRT68924 inhibitor control; and **(E)** PA01 + MRT68924 inhibitor.

MATERIALS AND METHODS

Cell culture: Telomerase-immortalized human corneal epithelial (hTCEpi) cells were maintained in serum-free keratinocyte growth medium (KGM).

Pseudomonas aeruginosa (PA): A standard invasive test strain of PA, strain PA01, was used in this study.

Mitochondrial polarization: JC1 was used to assess mitochondrial polarization. Fluorescence was visualized using confocal microscopy.

Inflammatory cytokines: Cytokine levels were measured using an enzyme-linked immunoassay (ELISA).

Protein expression: Relative protein expression was assessed by Western blot.

Transmission electron microscopy (TEM): Mitochondrial morphology was assessed using TEM at the UTSW Electron Microscopy Core Facility.

Metabolomics: Untargeted metabolomics was performed using mass spectrometry at the UTSW Metabolomics Core facility.

RESULTS

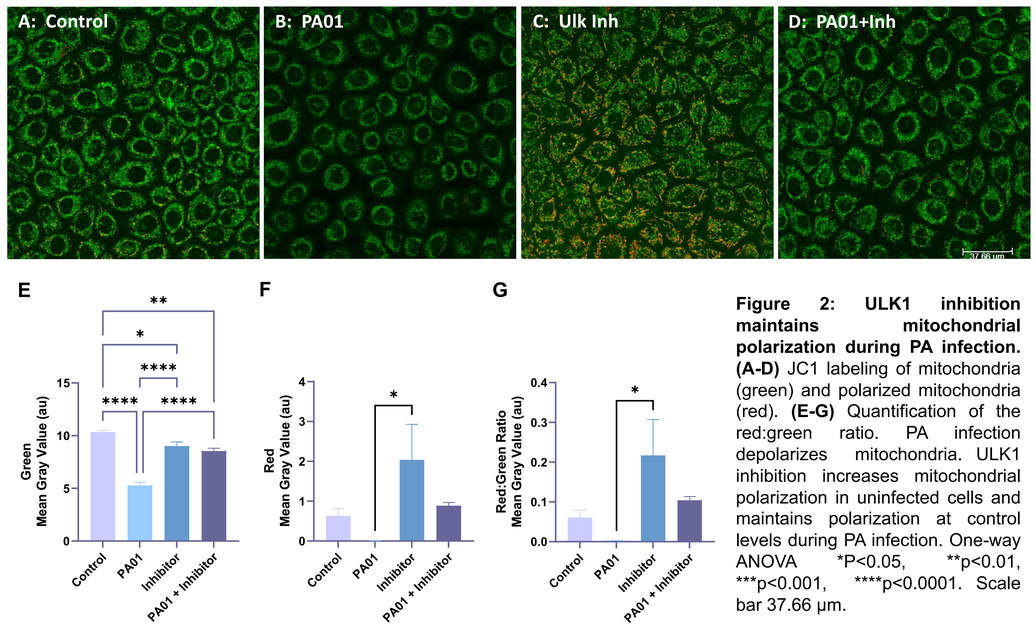


Figure 2: ULK1 inhibition maintains mitochondrial polarization during PA infection. (A-D) JC1 labeling of mitochondria (green) and polarized mitochondria (red). **(E-G)** Quantification of the red:green ratio. PA infection depolarizes mitochondria. ULK1 inhibition increases mitochondrial polarization in uninfected cells and maintains polarization at control levels during PA infection. One-way ANOVA *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Scale bar 37.66 µm.

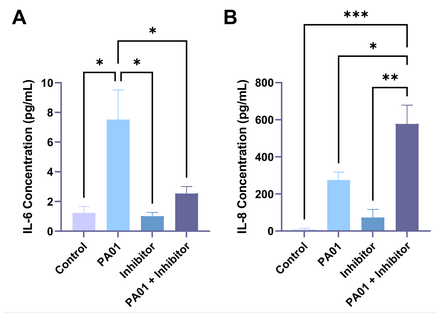
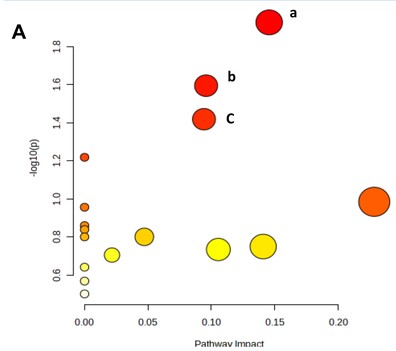


Figure 3: ULK1 inhibition decreased IL-6 but increased IL-8 in PA-infected corneal epithelial cells. (A) IL-6 secretion was decreased to control levels in PA infected cells treated with MRT68924. **(B)** In contrast to IL-6, MRT68924 increased expression of IL-8 in PA-infected cells. Consistent with this, ULK treated cells were highly chemotactic for neutrophils (data not shown). N=3. One-way ANOVA *P<0.05, **p<0.01, ***p<0.001.

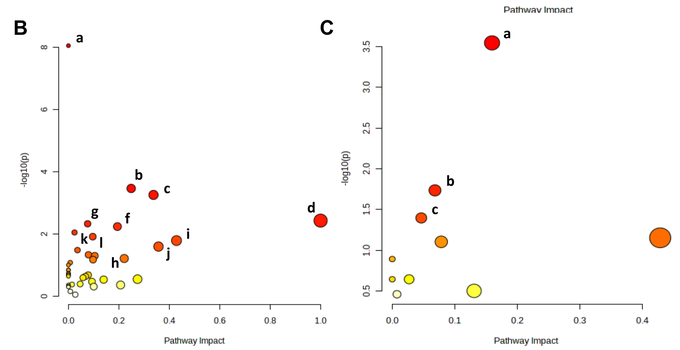


A: Pathways significantly upregulated during PA infection of corneal epithelial cells compared to the uninfected control

a. Purine metabolism
b. Glycine, serine and threonine metabolism
c. Tryptophan metabolism

B: Pathways significantly downregulated due to the ULK1/2 inhibitor compared to control

a. Aminoacyl tRNA biosynthesis
b. Pyrimidine metabolism
c. Alanine, aspartate and glutamate metabolism
d. Phenylalanine, tyrosine and tryptophan biosynthesis
e. Arginine biosynthesis
f. Nicotinate and nicotinamide metabolism
g. Glycine, serine and threonine metabolism
h. Glycerophospholipid metabolism
i. Taurine and hypotaurine metabolism
j. Phenylalanine metabolism
k. Glutathione metabolism
l. Glyoxylate and dicarboxylate metabolism



C: Pathways significantly downregulated due to ULK1/2 inhibitor in PA infected cells compared to non-inhibitor treated PA infected corneal epithelial cells

a. Pyrimidine metabolism
b. Purine metabolism
c. Glycerophospholipid metabolism

Figure 4 (above): ULK1/2 inhibition attenuates the PA-induced increase in purine metabolism. Untargeted metabolomics showing up- and downregulated pathways during PA infection with and without ULK1/2 inhibition. P<0.05 for all listed pathways.

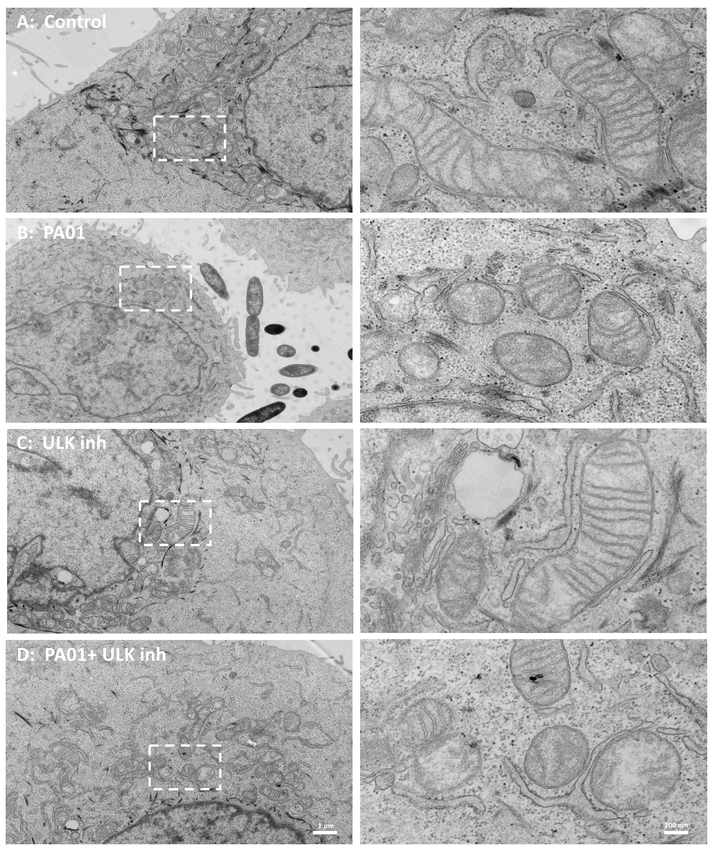


Figure 5 (left): Ulk1/2 inhibition enhances cristae morphology in PA infected corneal epithelial cells. (**A-D**) TEM showing ultrastructural changes in mitochondria in each treatment group. Left column showing zoom of rectangular area from right column.

DISCUSSION AND FUTURE DIRECTIONS

In corneal epithelial cells, infection with an invasive isolate of PA induces mitochondrial dysfunction and cellular inflammation by increasing mitochondrial depolarization, fission and secretion of pro-inflammatory cytokines. This is associated with an increase in purine metabolism. ULK1/2 inhibition not only maintained mitochondrial polarization during infection, but enhanced cristae architecture, attenuated levels of IL-6, and downregulated purine metabolism in host cells. Further studies in the rabbit animal model are underway to evaluate the effects of ULK1/2 inhibition on corneal inflammation and infection *in vivo*.

REFERENCES

1. Robertson et al. *Sci Rep* 2017.
2. Hilliam et al. *J Med Microbiol* 2020.
3. Willcox et al. *Optom Vis Sci* 2007.
4. Zachari et al. *Essays Biochem* 2017.

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