Eperezolid modulates autophagy to control the growth of intracellular mycobacteria

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ABSTRACT

The emergence of drug resistance Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M. tb*) remains a significant threat to global health. Therefore, alternative host-directed strategies have been explored to combat the pathogen and boost anti-TB immunity inside the host. Among the host-directed therapies, autophagy, a well-established host mechanism, has been studied extensively to regulate the growth of mycobacteria. Hence, we mainly focus on the induction of autophagy by using pharmaceutical compounds to target the deadly disease. Our ongoing study analyzed the anti-mycobacterial effect of Eperezolid, a pharmacologically active compound from the LOPAC library. We dissected the non-cytotoxic dose of the compound via MTT assay, which was found to be 1µM. Further, time kinetics experiments were done to check the autophagic induction potential of the compound in dTHP-1 cells. We found the optimum conversion of LC3-I to LC3-II and more LC3 puncta formation after 24h of treatment. Similarly, MDC staining also signified more autophagic vacuole formation after 24h drug treatment. In addition, to check the effect of Eperezolid on the intracellular viability of mycobacteria, CFU assays were performed. We observed a sharp reduction in the viability of *M. smegmatis* in Eperezolid treated cells as compared to control cells which described its mycobacterial growth inhibition capacity. Further experiments are underway to deduce the autophagy induction potential of Eperezolid in mycobacteria-infected cells.

INTRODUCTION

Mycobacterium tuberculosis (*M. tb*), the causative agent of pulmonary tuberculosis (TB), is a successful human pathogen that infects one-third of the world's population. The emergence of multidrug-resistant (MDR) and extremely drug-resistant (XDR) strains and inadequate protection imparted by *M. bovis* BCG has further aggravated the problem. *M. tb* primarily infects the lungs and resides in alveolar macrophages. Macrophages act as a principal reservoir of *M. tb*, trying to kill the bacteria, thereby clearing the infection at the primary stage. Internalization of bacteria results in the formation of a phagosome, which, upon maturation, fuses with the lysosome, resulting in the degradation of the bacteria. However, *M. tb* employs different strategies to inhibit this fusion and other important host's antimicrobial mechanisms like generating reactive oxygen and nitrogen intermediates (ROI and RNI), apoptosis and autophagy. Autophagy is an intracellular degradation system wherein unwanted cargo, such as old or damaged organelles, unneeded proteins, as well as pathogenic agents, are digested, and the macromolecular contents from the digestion are released back into the cytosol. It involves the sequestration of cell organelles and cytoplasmic material into double-membrane vesicles called autophagosomes and their subsequent delivery to the lysosomes for degradation by the lysosomal hydrolases. A number of proteins called autophagy-related (Atg) genes like Beclin-1, Atg 3, Atg 7, and many more have been discovered that have been shown to coordinate this process. Various studies have already reported that induction of autophagy in infected macrophages compels *M. tb* loaded phagosome to fuse with the lysosome, killing bacteria. Recently pharmacological modulation of autophagy has been shown to suppress intracellular *M. tb* proliferation in infected macrophages by inhibiting a major negative regulator of autophagy. So, induction of autophagy by pharmaceutical compounds might improve TB therapy since it involves a major player of the host response in order to drive the host-pathogen interaction to a favourable outcome against the bacteria. In the present study, we examined the autophagy-inducing potential of Eperezolid, a pharmaceutical compound from the LOPAC library, in dTHP-1 cells, as well as its effect on intracellular mycobacterial growth.

METHODS

THP1 cells were treated with Phorbol 12-myristate 13-acetate (PMA, 50nM) overnight, allowing the differentiation of monocyte into macrophages. The next day, cells were treated with varying concentrations of Eperezolid. The effect of Eperezolid on the viability of THP1 cells was studied using an MTT assay. A time kinetics experiment was then performed with the non-cytotoxic concentration of Eperezolid to see the autophagic potential of Eperezolid by studying the expression of different autophagic marker proteins through western blotting. Eperezolid-mediated autophagy was also confirmed by studying LC3 puncta formation in cells at different time points using confocal microscopy. The cells were also stained with MDC to observe autophagic vacuole formation in response to Eperezolid induce autophagy. The effect of autophagy induction on the viability of mycobacteria was then studied by CFU plating the control and Eperezolid treated sample on 7H11 agar plates.

RESULTS

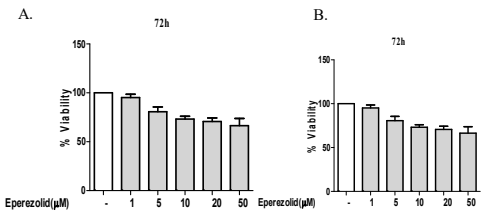

Figure 1 Effect of different concentrations of Eperezolid on cell viability of dTHP-1 cells. (A), dTHP-1 cells were treated with varying concentrations of Eperezolid for 72h. At the end of treatment, MTT (10µl/well) was added to each well, and the plate was incubated for 3h before adding lysis buffer. The absorbance of the colored product formed was taken at 562nm. Data shown represent the percentage of cell viability over control cells (B), dTHP-1 cells were treated with various concentrations of Eperezolid, and after 72h, cells were harvested and counted using a hemocytometer after mixing them with an equal volume of trypan blue. Data shown reveals the percentage of cell viability over control cells.

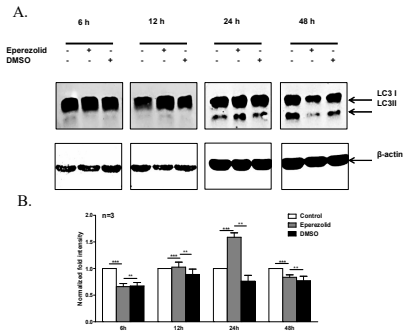

Figure 2 Effect of non-cytotoxic dose of Eperezolid on autophagy in dTHP-1 cells
(A) dTHP-1 cells were treated with Eperezolid for different time points. Whole-cell lysates prepared in RIPA buffer from the treated cells were subjected to western blotting. (B), Statistical analysis of western blotting replicates showing normalized fold intensity in comparison to the control cells.

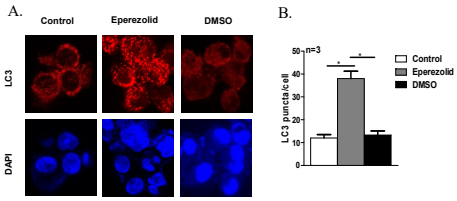

Figure 3. Effect of Eperezolid on LC3 puncta formation
(A) dTHP-1cells were treated with Eperezolid at indicated time points and stained with anti LC3 antibody. Cells were visualized under confocal microscope. (B), Statistical analysis of LC3 puncta in different confocal fields.

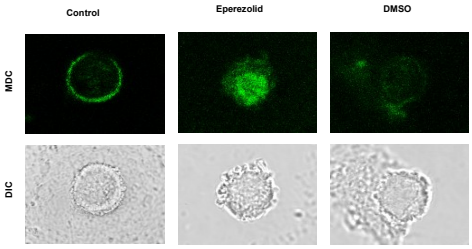

Figure 4. Visualisation of autophagic vacuoles through MDC staining
Eperezolid-treated dTHP-1 cells were stained with MDC, followed by fixation and observation under the confocal microscope.

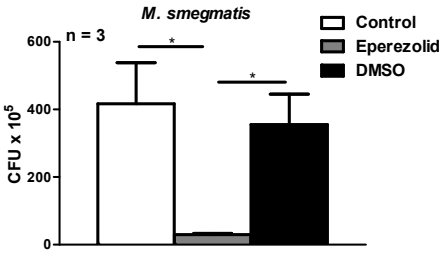

Figure 5. Effect of Eperezolid on intracellular mycobacterial viability in infected macrophages
Infected dTHP-1cells were treated with Eperezolid. After 2 days of treatment cells were lysed and lysates were log diluted and plated on 7H11 agar plates.

CONCLUSIONS

1. Eperezolid above 1µg/ml is cytotoxic to PMA dTHP-1 cells.
2. The non-cytotoxic concentration of Eperezolid induced autophagy in THP-1 cells.
3. Eperezolid induced autophagy decreased the intracellular viability of mycobacteria.

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