Furamidine-induced autophagy exerts an anti-mycobacterial effect in a SIRT 1-FOXO3a-dependent manner by elevation of intracellular Ca^{2+} level expression

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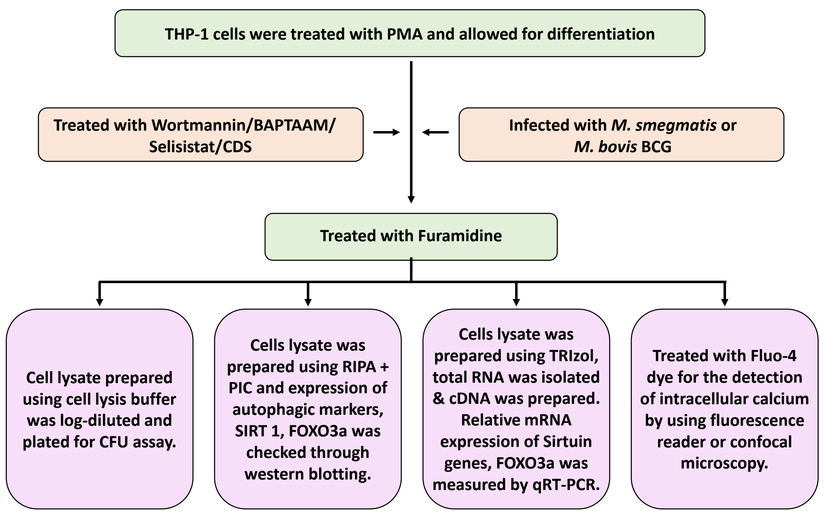
ABSTRACT

Mycobacterium tuberculosis (*M. tb*), the causative agent of human tuberculosis (TB), continues to be a significant cause of mortality worldwide. The latest findings have highlighted autophagy as a host-defence mechanism that eradicates many invading bacteria, including *M. tb.* Thus, novel approaches like the stimulation of autophagy using various pharmaceutical drugs can be undertaken to deal with this noxious pathogen. The present study has been formulated to evaluate the anti-mycobacterial potential of Furamidine, a pharmaceutical drug from the LOPAC library. Initially, a non-cytotoxic concentration of Furamidine (10µM) was used to evaluate its effect on intracellular mycobacterial viability in dTHP-1 cells. Furamidine treatment compromised intracellular mycobacterial fitness compared to control cells. Autophagy, a well-known host defensive strategy was investigated as a possible contributor to reveal the mechanism of action. Multiparametric approaches were employed to study autophagic response that conclusively suggested the autophagy induction potential of Furamidine in dTHP-1 cells. Further, elevated LC3-II expression and increased autophagic vacuole accumulation in the presence of Baf-A1 demonstrated the positive regulation of autophagic flux upon Furamidine treatment. Pre-treatment of Wortmannin abrogated autophagy in treated cells, indicating the specificity of autophagy induction. Mechanistic investigations showed increased intracellular Ca^{2+} level expression, SIRT 1 and FOXO3a activation upon its treatment. Inhibition of Ca^{2+} level expression suppressed calcium-mediated-FOXO3a level in Furamidine-treated cells. Furthermore, the administration of various inhibitors hampered the Furamidine-induced autophagy, reducing intracellular mycobacteria clearance. These results conclude that Furamidine triggered the Ca^{2+}/SIRT 1/FOXO3a pathway, causing less mycobacterial load in dTHP-1 cells.

INTRODUCTION

TB control has to be enhanced in several areas, including point-of-care diagnostics, shorter and safer drug regimens, and preventative vaccination to effectively combat this pandemic. Drug-resistant strains of *M. tb* remain a global concern, and novel host-directed therapeutics are urgently required to address this problem. DNA minor groove binders (MGBs) are a class of compounds that have shown promising outcomes in a broad spectrum of illnesses. Furamidine is a pharmaceutical drug that belongs to the MGBs family and has anti-parasitic, anti-bacterial, and anti-cancer properties. However, its anti-mycobacterial role is yet to be explored to the best of our knowledge. In this study, we investigated the anti-TB potential of Furamidine and its effect on the autophagy to eradicate the intracellular mycobacterial burden in human macrophages, and its underlying mechanisms.

METHODS



RESULTS

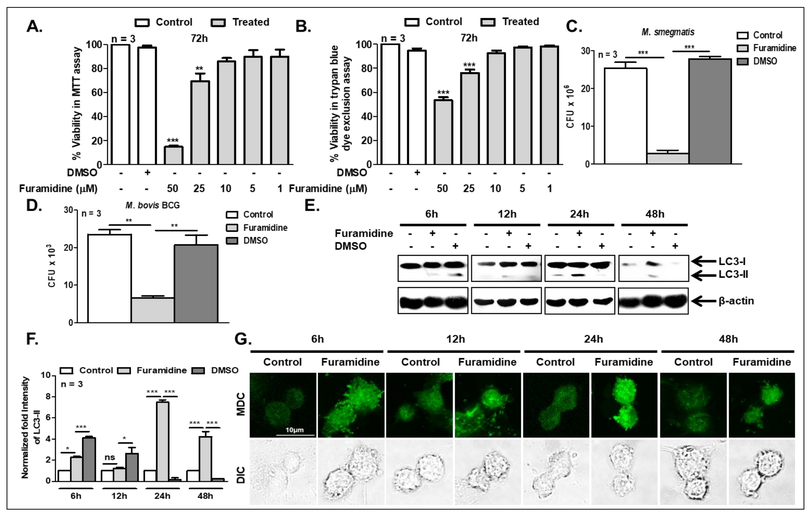


Fig 1. Effect of Furamidine on cell viability and intracellular mycobacterial load

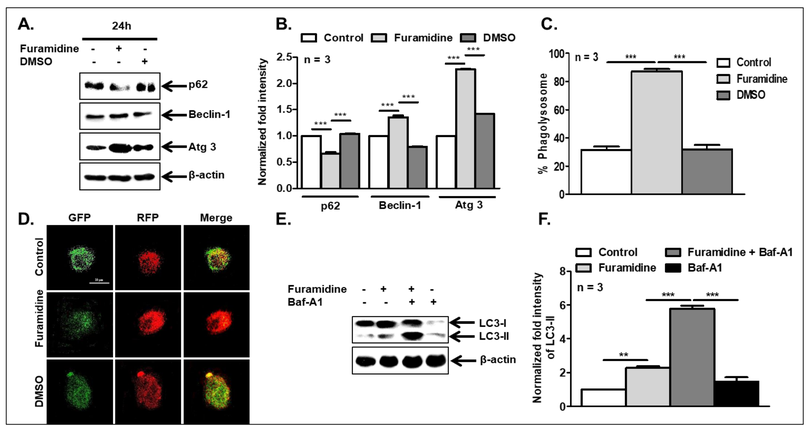


Fig 2. Effect of Furamidine on autophagy and autophagic flux in dTHP-1 cells

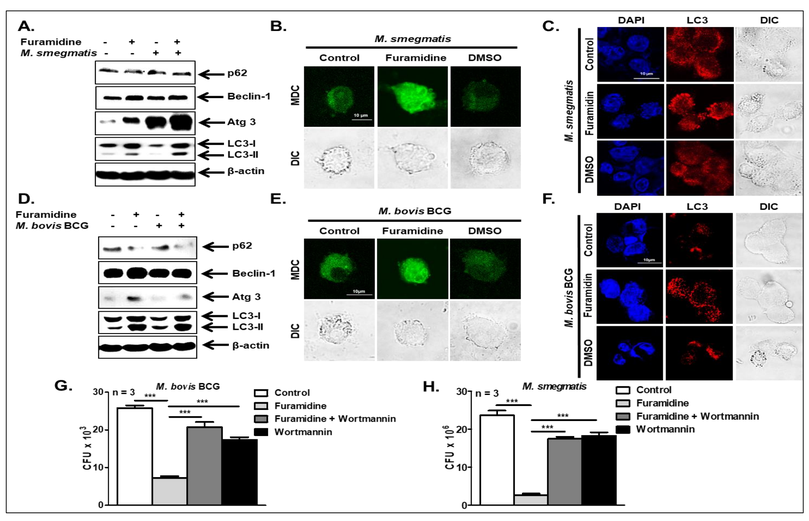


Fig 3. Effect of Furamidine-induced autophagy in mycobacteria-infected dTHP-1 cells

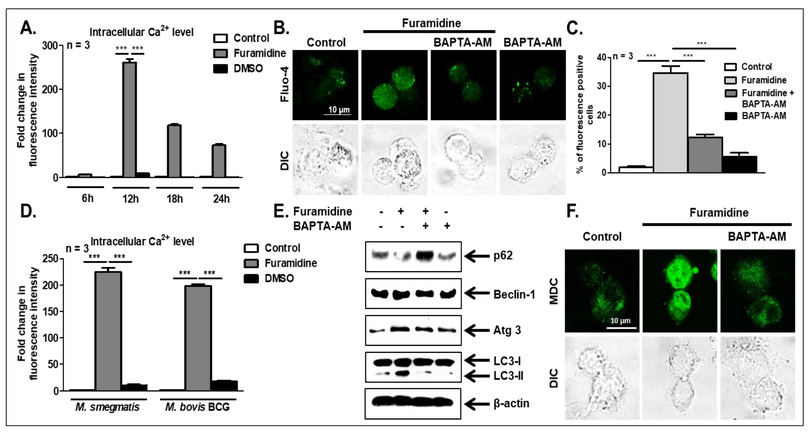


Fig 4. Effect of Furamidine treatment on intracellular calcium levels in dTHP-1 cells

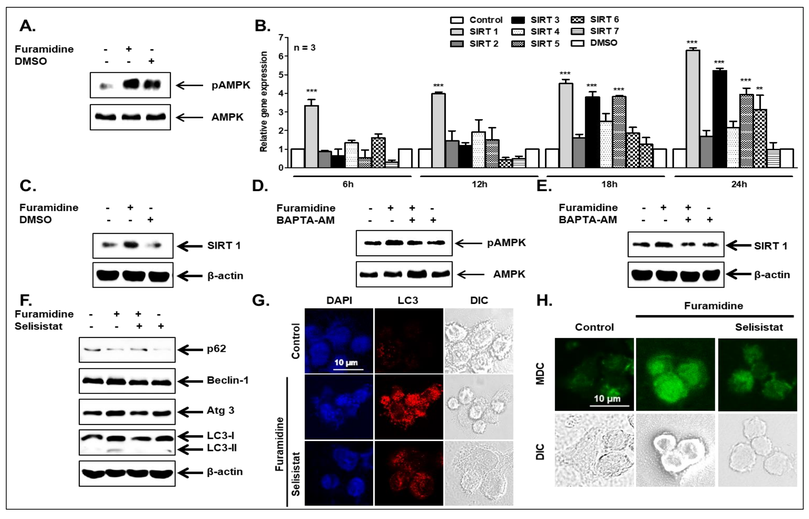


Fig 5. Effect of Furamidine on fuel sensing molecules such as AMPK and SIRT 1

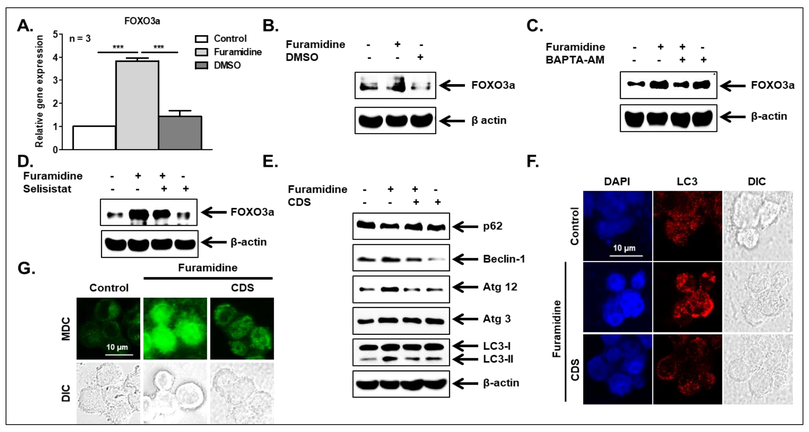


Fig 6. Effect of Furamidine on activation of transcription factor FOXO3a

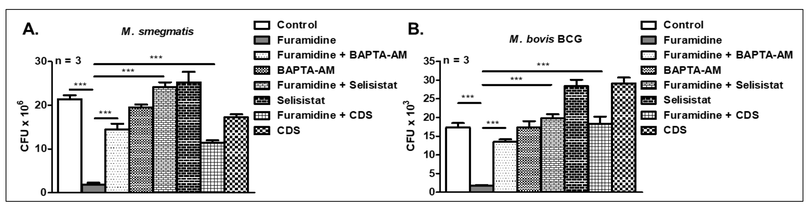


Fig 7. Effect of different inhibitors on intracellular mycobacterial viability in dTHP-1 cells

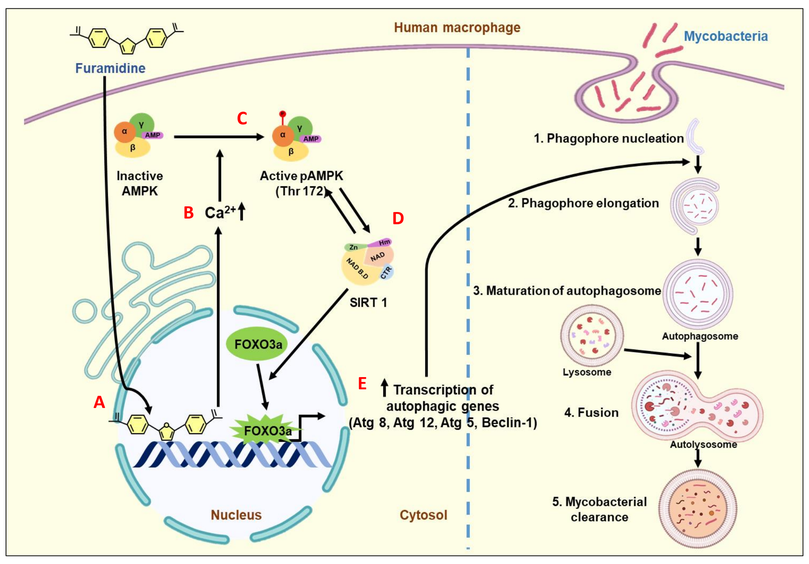


Fig 8. Overall Summary

CONCLUSION

- Non-cytotoxic concentration of Furamidine (10µM) treatment significantly reduced intracellular viability of mycobacterial species in dTHP-1 cells
- Furamidine induced autophagy led to enhanced killing of intracellular mycobacteria
- Furamidine treatment triggered elevated intracellular Ca^{2+} level in dTHP-1 cells
- Furamidine mediates its anti-mycobacterial effect through Ca^{2+}/SIRT 1/FOXO3a pathway dependent manner

ACKNOWLEDGEMENT

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