Calcimycin induced autophagy decreases mycobacterial growth in THP-1 cells through P2RX7 dependent pathway mediated by intracellular calcium

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ABSTRACT

Introduction – Calcimycin, calcium ionophore, show *in vitro* antimicrobial activity against gram-positive bacteria and fungi. In our study we found that it is bactericidal in action against *Mycobacterium tuberculosis* (*M.tb)* and it induces autophagy as well as kill intracellular mycobacteria at a low concentration due to which we want to decipher the mechanism of autophagy induction and its effect on survival of intracellular mycobacteria in THP-1 cells.

Methods – We checked the expression of major autophagy genes in calcimycin treated and infected samples by western blotting. To understand the mechanism of autophagy induction we check the expression of ATP receptor, purinergic receptor P2X7 (P2RX7), in calcimycin treated samples by qRT-PCR and measure extracellular ATP release by fluorescence to validate P2RX7 activation. We checked intracellular calcium level by fluorescence and confocal microscopy. We also checked the interaction of calcimycin with P2RX7 by performing docking experiment. To validate P2RX7 role in autophagy induction we use P2RX7 inhibitor, 1-[N,O-bis(5-Isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62) or intracellular chelator, 1,2-Bis(2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid tetra (acetoxy-methyl) ester (BAPTA-AM) and checked the expression of major autopghay markers by western blotting and confocal microscopy.

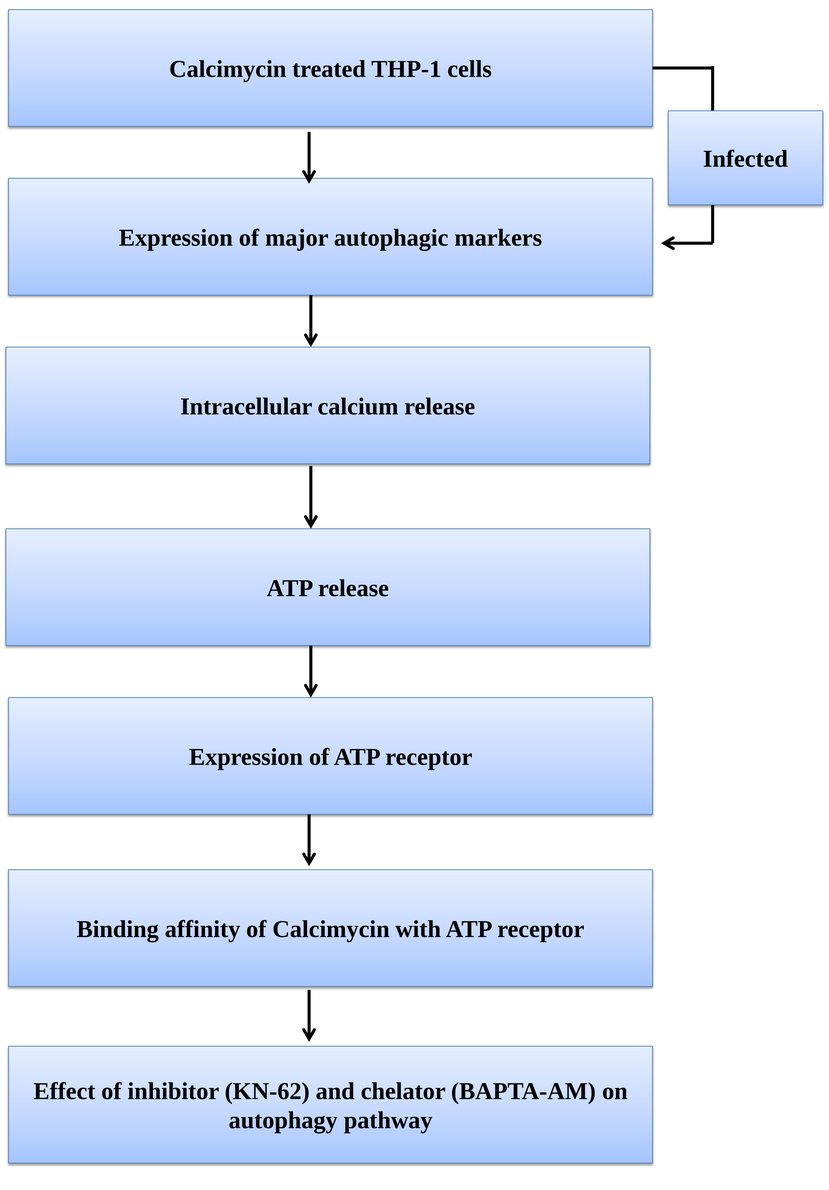
Results - We noticed that treatment with calcimycin led to up-regulation of major autophagy markers like Beclin-1, autophagy-related gene (Atg) 7, Atg 3 and enhanced microtubule-associated protein 1A/1B-light chain 3-I (LC3-I) to LC3-II conversion in infected macrophages. We also demonstrate that calcimycin binding with P2RX7 led to increase in intracellular calcium level that regulates the extracellular release of ATP. Blocking of either P2RX7 expression by KN-62 or reducing intracellular calcium levels by BAPTA-AM abrogated the antimycobacterial activity of calcimycin.

Conclusion - Calcimycin exerts its antimycobacterial effect by regulating intracellular calcium-dependent ATP release that induces autophagy in a P2RX7 dependent manner.

INTRODUCTION

M. tb , the notorious pathogen , that causes deadly infectious disease tuberculosis (TB) have evolved various strategies to combat host defenses. *M. tb* enters the human body through inhalation where alveolar macrophages internalize and degrade the pathogen by employing various defence mechanisms including autophagy. During infection, autophagy acts as an innate defence mechanism and play an important role in modulating intracellular mycobacterial growth. The recent finding of calcium-dependent autophagy induction in an adenosine triphosphate (ATP)-dependent pathway is associated with the reduction of this intracellular pathogen. Therefore, we intended to study the effect of novel calcium-inducing compounds like calcimycin, an ionophorous, polyether antibiotic from *Streptomyces chartreusensis* on autophagy of infected macrophages and its repercussions on intracellular viability of mycobacteria.

METHODS



RESULTS

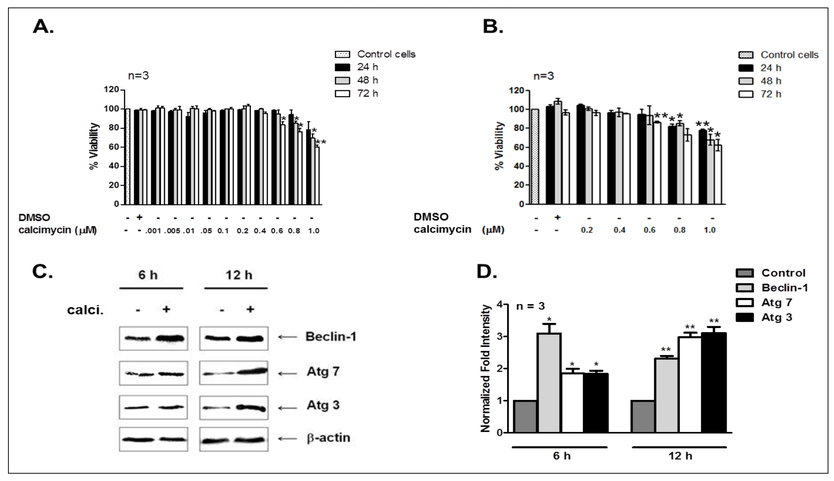


Fig. 1. Cumulative % cell viability of THP-1 cells treated with varying concentration of calcimycin at different time points and effect of calcimycin (0.4 μM) on autophagy in differentiated THP-1 cells.

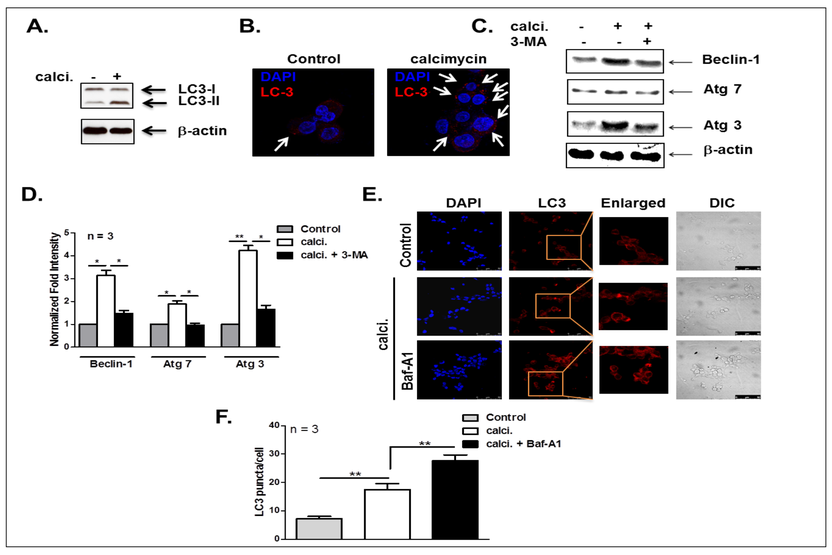


Fig. 2. Effect of calcimycin on LC3 conversion and effect of 3-MA on autophagy in differentiated THP-1 cells.

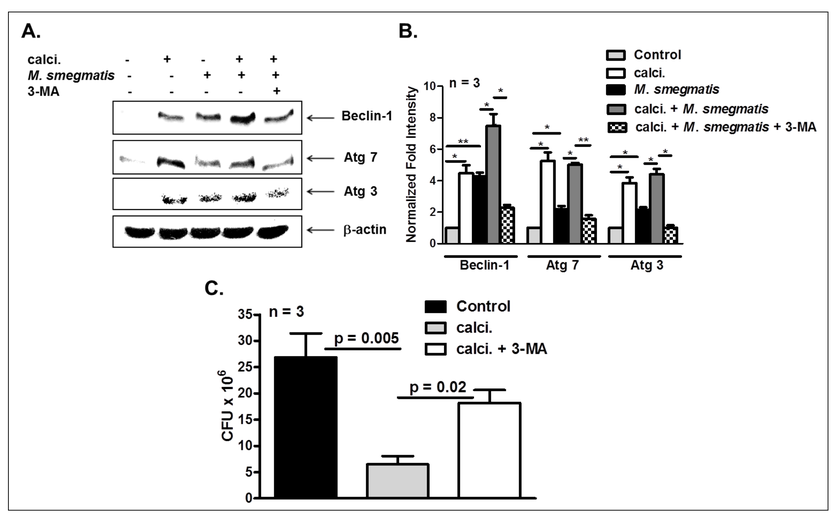


Fig. 3. Effect of calcimycin on autophagy and intracellular *M. smegmatis* viability in infected macrophages.

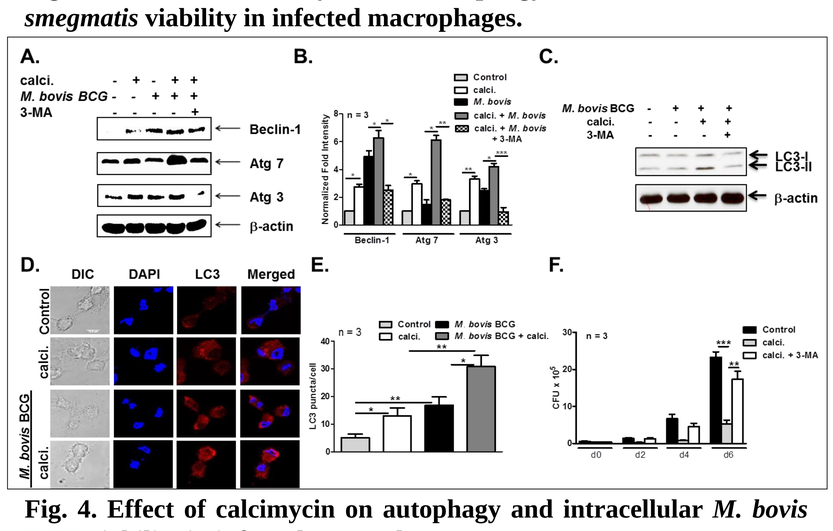


Fig. 4. Effect of calcimycin on autophagy and intracellular *M. bovis* BCG viability in infected macrophages.

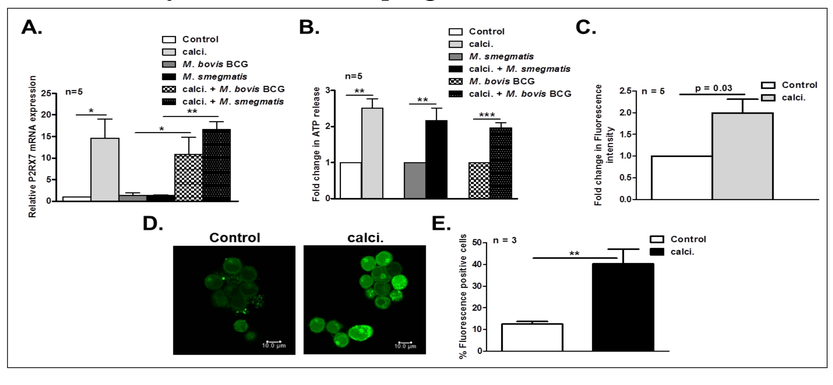


Fig. 5. Effect of calcimycin on P2RX7 mRNA expression, ATP release and intracellular Calcium level in THP-1 cells.

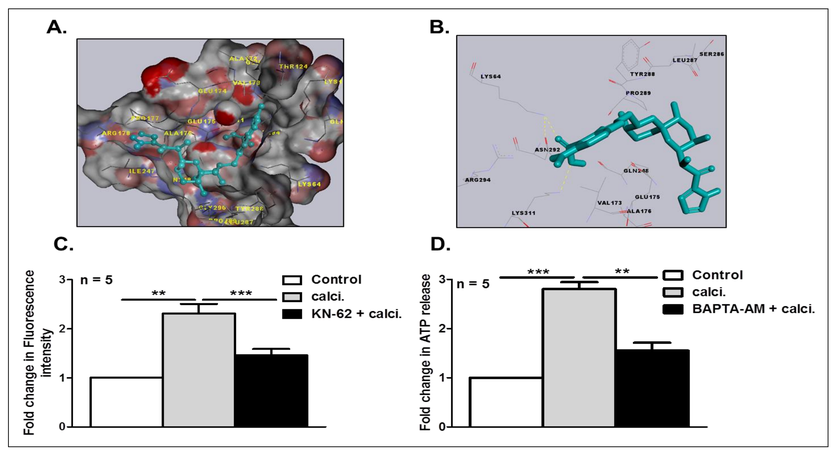


Fig. 6. The binding affinity of calcimycin with P2RX7 and effect of P2RX7 or intracellular calcium inhibition on intracellular calcium level or ATP release.

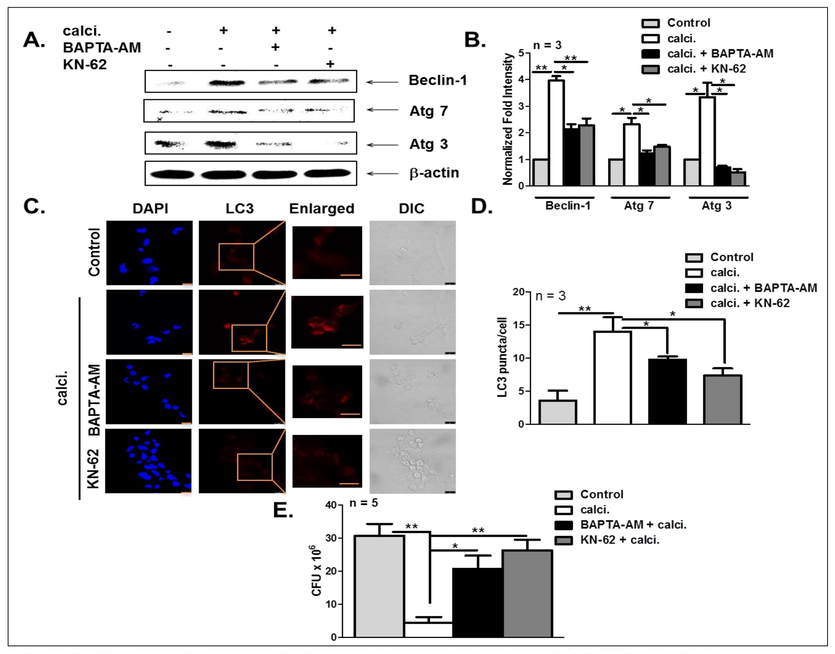


Fig. 7. Effect of intracellular calcium chelation, P2RX7 inhibition on autophagy and intracellular mycobacterial viability in calcimycin treated THP-1 cells.

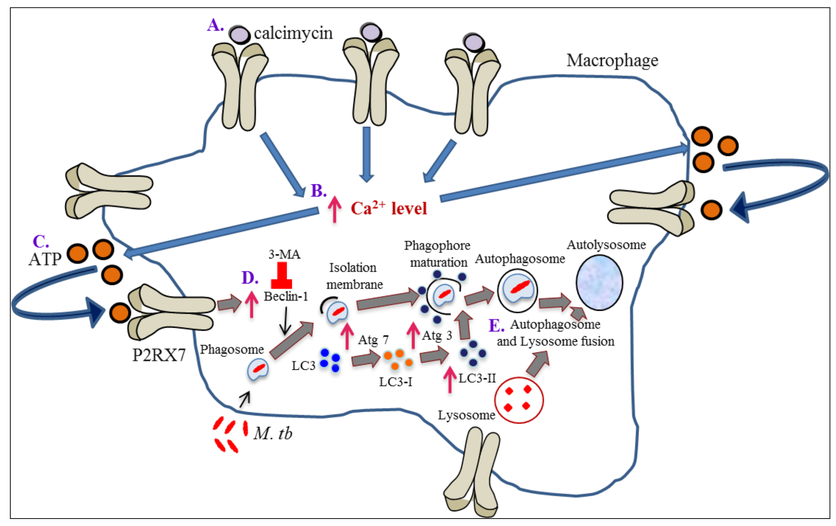


Fig. 8. ATP-dependent P2RX7 regulation of autophagy in calcimycin treated THP-1 cells by modulating intracellular calcium level.

CONCLUSIONS

1. Calcimycin induced autophagy showed antimycobacterial effect in THP-1 cells.
2. Calcimycin treatment led to increased P2RX7 mRNA expression, ATP release and intracellular calcium level in THP-1 cells.
3. Calcimycin regulated increase in intracellular calcium level is partially controlled by ATP through P2RX7 in an autocrine fashion.
4. Intracellular calcium chelation or P2RX7 inhibition abrogated the calcimycin induced autophagy leading to increased intracellular mycobacterial viability.
5. Calcimycin interaction with P2RX7 led to enhancement in intracellular calcium level that regulated ATP dependent autophagy thereby killing intracellular mycobacteria.

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