Ac-93253 inhibits intracellular growth of mycobacteria in human macrophages by inducing apoptosis in mitochondrial-dependent manner

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INTRODUCTION

Tuberculosis (TB) is a global health hazard caused by the pathogen *M. tb* and is responsible for millions of deaths worldwide every year. According to a 2020 global TB report by the world health organization, 10 million new infection cases were reported in 2019, with 1.4 million deaths. TB is an airborne infectious disease, where pathogens enter the lung's distal area through aerosol. Macrophages engulf these infectious particles by phagocytosis and release different chemokines and cytokines in response to *M. tb* infection to recruit monocytes, neutrophils, and innate lymphocytes at the site of infection. Host immune cells form a protective structure called a granuloma, a histological hallmark of the disease that helps the pathogen to propagate. The alveolar macrophages are the principal reservoir of *M. tb* that first tries to kill the pathogen by the innate immune defense mechanisms. *M. tb* has developed several strategies to protect itself against the host's innate immune response. These strategies include blockage of phagosome maturation and endolysosome formation, the inflection of host cytokines production and inhibition of autophagy, interference with antigen presentation and resistance to reactive oxygen and nitrogen radicals, and modification of host programmed cell death pathways. Previously publised studies with 2-[3-dihydro-(1,3,3-trimethyl-2H-indol-2-ylidene)-1-1propenyl]-3-ethyl-benzothiazolium iodide (Ac-93253) have reported its anticancer effect towards non-small cell lung cancer (NSCLC) with an IC50 of 0.1 μM. Ac-93253 is a novel SRC inhibitor that induces apoptosis in lung cancer cell lines, PC9 and H358, respectively. Since, the induction of apoptosis limits the mycobacterial growth within the macrophage, the present study was undertaken to investigate the effect of Ac-93253 on intracellular mycobacteria and the interplay of apoptosis in its anti-mycobacterial property.

METHODOLOGY

We have checked the expression of apoptosis-inducing genes and proteins through qRT-PCR and western blotting in Ac-93253 treated and mycobacterial-infected dTHP-1 cells. To confirm the apoptosis, We isolate DNA fragments and analyze them through agarose gel electrophoresis. To validate the above findings, we have used Annexin V and propidium iodide staining to check the accumulation of phosphatidylserine in the plasma membrane's outer leaflet. BacTiter-Glo microbial cell viability kit (Promega Corporation; WI, USA) was used for ATP production analysis. To quantify the loss of $\Delta\Psi m$ we used Rhodamine 123 staining, and stained cells were analyzed through flow cytometry. To validate the role of $\Delta\Psi m$, we used cyclosporine A1 and checked its effect on apoptosis and anti-mycobacterial activity. To understand the canonical pathway of apoptosis, we checked the secretion of TNFα through sandwich ELISA.

RESULTS

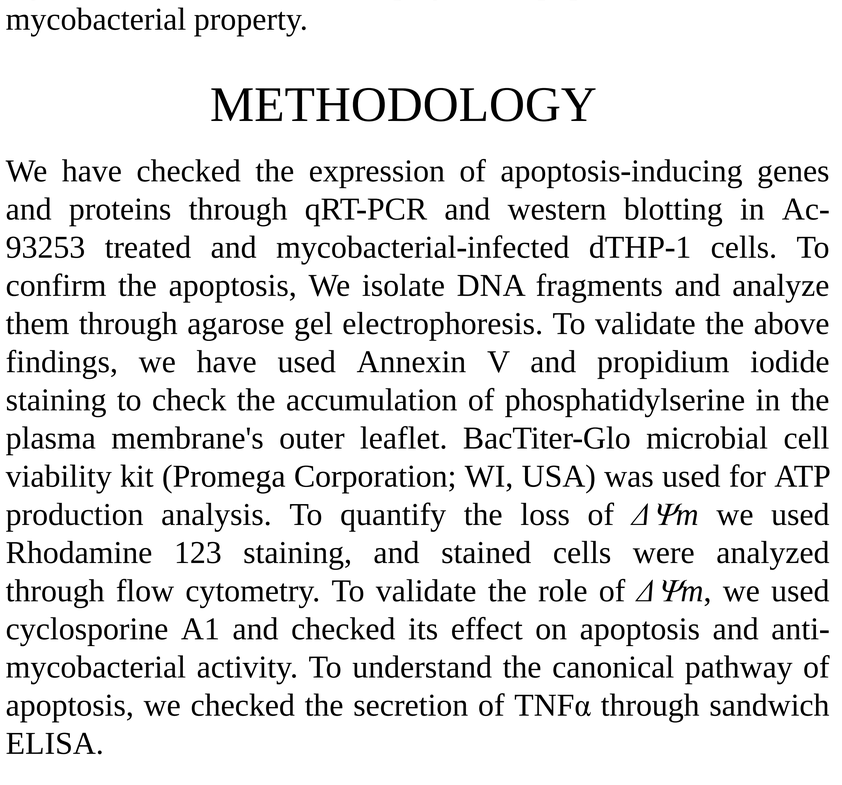

Fig. 1. Effect of Ac-93253 on apoptosis in dTHP-1 cells.

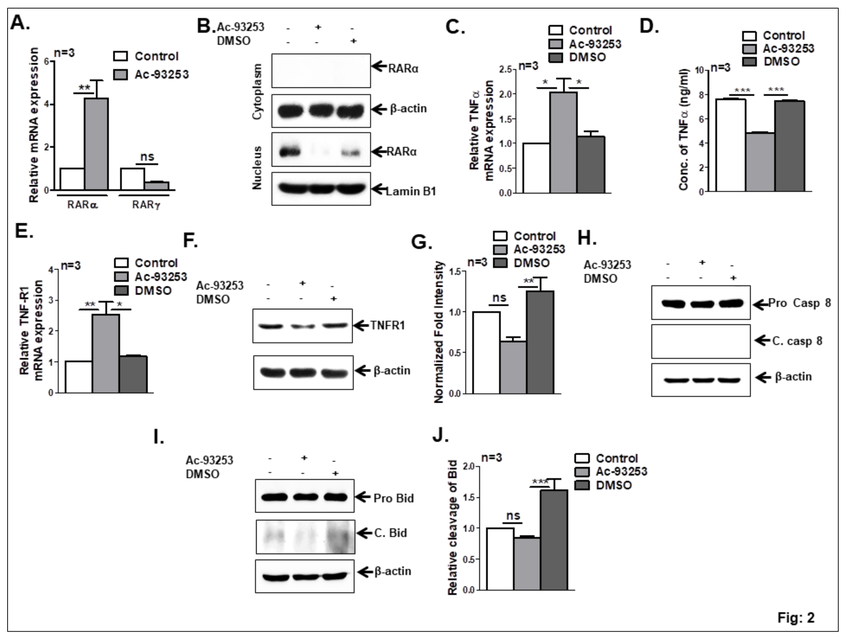

Fig. 2. Ac-93253 induces apoptosis in dTHP-1 cells, independent of TNFα mediated signaling.

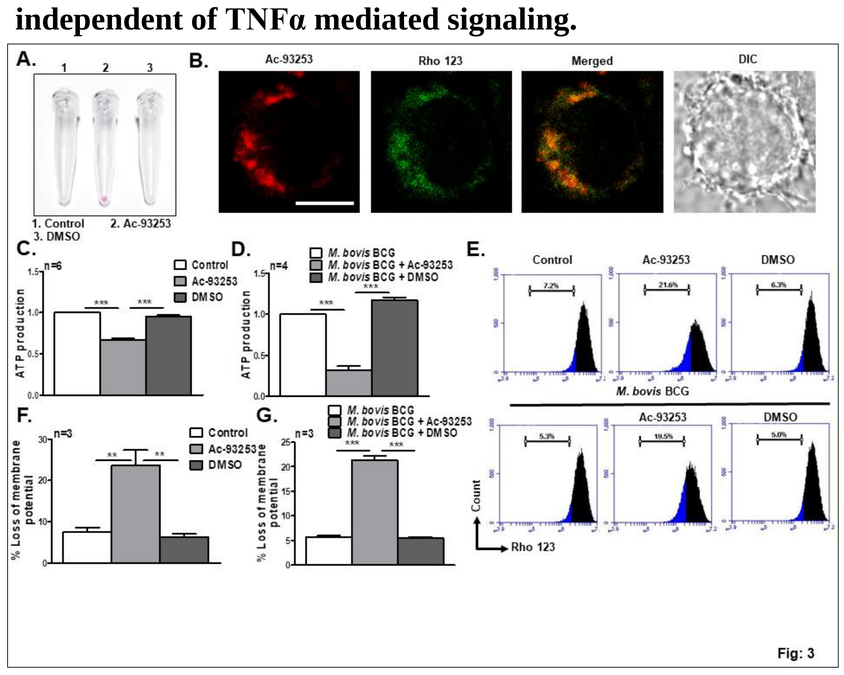

Fig. 3. Ac-93253 directly interacts with mitochondria to activate apoptosis in THP-1 macrophages.

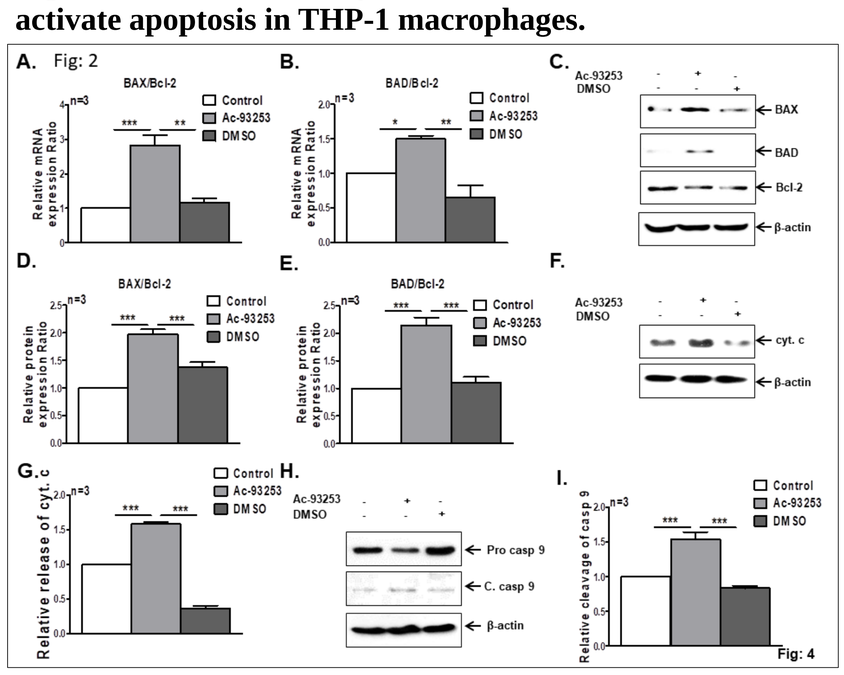

Fig. 4. Ac-93253 induces apoptosis in dTHP-1 cells followed the intrinsic pathway.

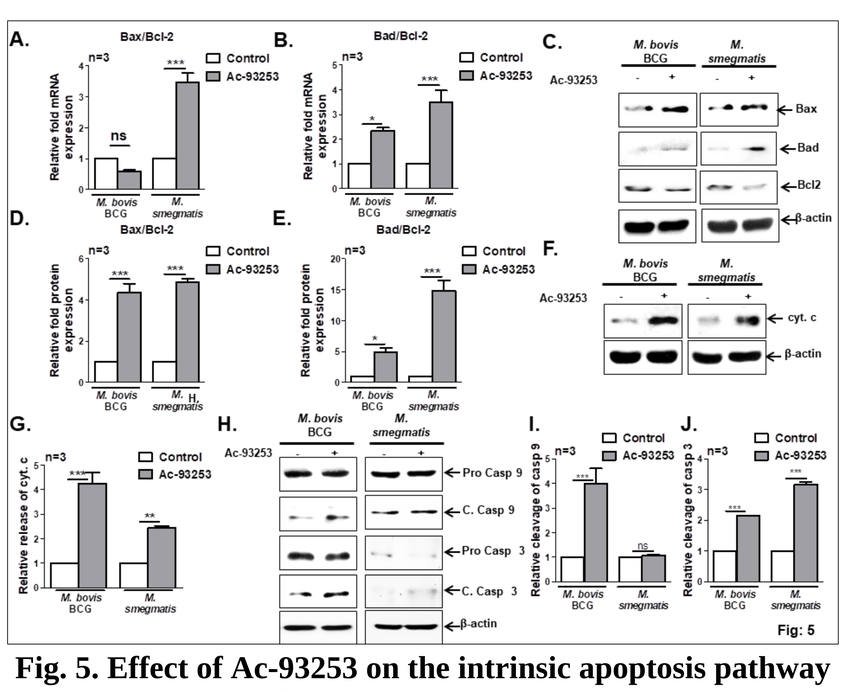

Fig. 5. Effect of Ac-93253 on the intrinsic apoptosis pathway in mycobacterial infected dTHP-1 cells.

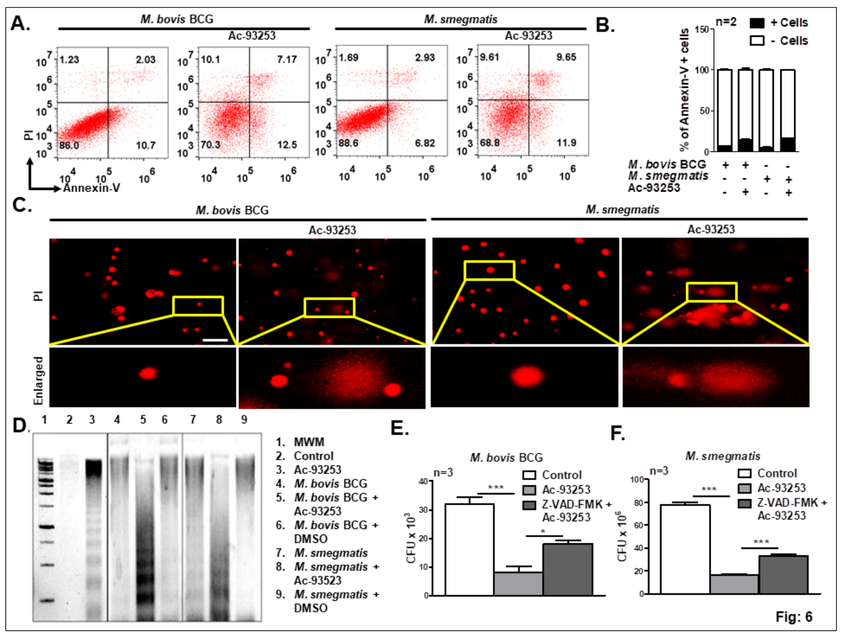

Fig. 6. Effect of Ac-93253 on apoptosis in mycobacterial infected dTHP-1 cells.

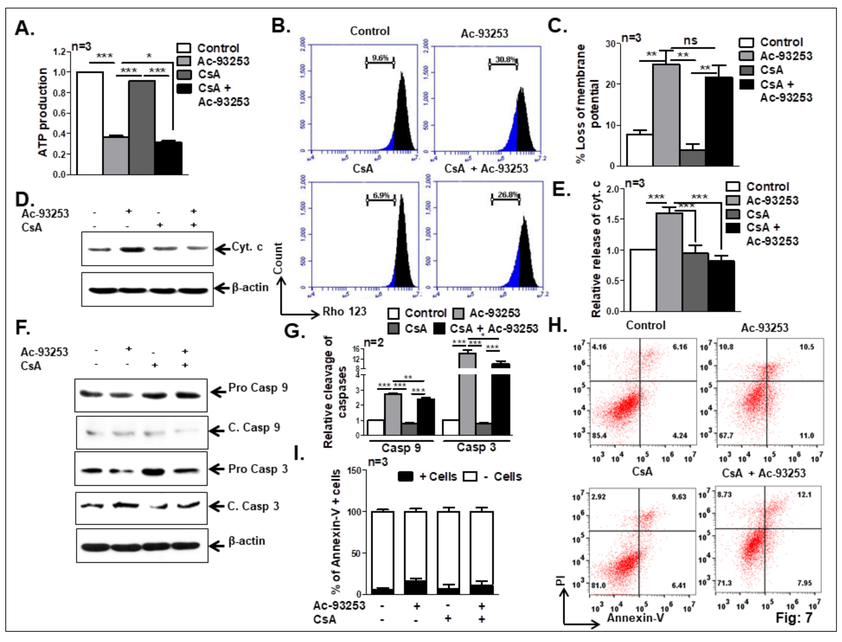

Fig. 7. Ac-93253 treatment reduces the mitochondrial membrane potential to activate apoptosis in human macrophages.

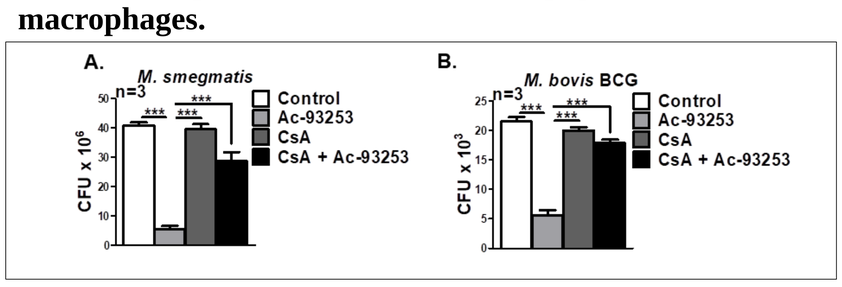

Fig. 8. Loss of Mitochondrial membrane potential mediated to Ac-93253 treatment is responsible for Anti-mycobacterial activity.

CONCLUSIONS

1. Through MTT and trypan blue exclusion assay, 0.5 μM of Ac-93253 was found to be non-cytotoxic even after 72 hours of treatment in dTHP-1 cells.
2. Significant up-regulation in the expression of various pro-apoptotic genes like Bcl-2, Bax, and Bad and the cleaved caspase 3 was observed upon treatment with a non-cytotoxic dose of Ac-93253.
3. Ac-93253 treatment also leads to DNA fragmentation and increased phosphatidylserine accumulation in the outer leaflet of the plasma membrane.
4. Ac-93253 treatment reduced ATP production and loss of $\Delta\Psi_m$
5. Ac-93253 treatment also effectively reduced the intracellular growth of mycobacteria in infected dTHP-1 cells, and Z-VAD-FMK broad-range apoptosis inhibitor significantly brought back the intracellular mycobacterial growth in Ac-93253 treated macrophages.

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