
Abstract

Tuberculosis, a serious type of infectious disease, is caused by the *Mycobacterium tuberculosis*. The medications against TB are becoming limited due to the XDR-TB and MDR-TB. In this context, host-directed-therapy (HDT) can be a novel approach to treat TB as it targets the TB infected macrophages, instead of the pathogen, through differential macrophage activation i.e. M1/M2 macrophage polarization. In this context, this study has been conducted to investigate macrophage polarization grade during *M. tuberculosis* infection and the role of STK11-isolated from nutshell of *Cocos nucifera* L. on it.

We have found that 6 hrs of H37Ra infection could induce M2 macrophage polarization enhancing the expressions of Arg-1, YM-1, FIZZ-1, CD206, IL-4, & IL-10 significantly ($p < 0.05$). Similarly, H37Rv infection upregulated these M2 markers and diminished M1 markers (iNOS2, iNOS2, CD68, IL-6) in macrophages, while, BCG and heat-killed H37Ra-challenge brought about reverse results. Hence, accomplishment of the cure of *M. tuberculosis* might depend on M1 polarization that could be achieved using a modest immunomodulator.

In this context, we have selected STK11 (46 kDa glycoprotein), isolated from the nutshell of *C. nucifera* L., that earlier proved its potential for macrophage M1 activation. Results indicated that STK11 (200 $\mu\text{g/ml}$) could restrict up to 44.83 % bacilli growth in direct killing assay, while STK11 (10 $\mu\text{g/ml}$) significantly ($p < 0.001$) decreased 62.86 % of intracellular bacilli growth within macrophages highlighting the advantages of HDT using it. Additionally, STK11 boosted M2 markers and reduced M1 markers significantly ($p < 0.05$) in H37Ra-infected macrophages. Hence, STK11 might enforce the M1 polarization to restrict the bacilli growth within the host cells.

Further, STK11 interacted with Ras isoforms and potentiate the nitrite dependent killing of intracellular H37Ra bacilli.

The efficacy of the STK11 have been examined against latent state of *M. tuberculosis* also. Herein, the results indicated that STK11 (200 µg/ml) inhibited the latent H37Ra bacilli by 49.10% in the resazurin assay indicating the direct killing potential of it. STK11 (10 µg/ml) could kill the latent H37Ra bacilli by 73.53% within the macrophages with concomitant nitrite release. This subsequently increased the M1 markers and diminished the M2 markers in latent H37Ra-challenged macrophages. Besides, mRNA expression of TLRs supported the interaction of STK11 with it, which might be involved in STK11-mediated M1 polarization in infected macrophages.

Finally, we have examined the combinatorial effect of STK11 (200 µg/ml) with rifampicin (0.12 µg/ml) against *M. tuberculosis* (H37Ra). This could restrict the growth of H37Ra bacilli by 63.19%. Interestingly, in the presence of STK11 (2.5 µg/ml), rifampicin (0.1 µg/ml) could kill the intracellular bacilli up to 72.20%, which indicated that STK11 could enhance the efficacy of the rifampicin. Further the combinatorial treatment with rifampicin and STK11 also endorsed the M1 markers and suppressed the M2 markers in H37Ra-infected macrophages with prompt nitrite generation, highlighting the effectiveness of combination therapy over their individual effect.

Altogether, STK11 alone or in combination with rifampicin, could be considered as a potent immunomodulator interacting with TLRs and Ras isoforms that might be acquainted for the treatment of tuberculosis through HDT in the near future. However, further studies are required to enlighten this work.

Keywords: *Mycobacterium tuberculosis*; Active TB; Latent TB; Macrophage polarization; STK11; Rifampicin; Host-directed therapy.
