Abstract

Mankind is fighting against *Mycobacterium tuberculosis* since the early sixteen century and still helpless even with the current available drug regime. Tuberculosis (TB) was declared a global health emergency by the World Health Organization in 1993 and currently claims approximately 1.7 million lives per annum, more than can be attributed to any other bacterial infection. It is estimated that one-third of the world’s population is infected with the causative agent, the obligate human pathogen *Mycobacterium tuberculosis* with around 9 to 10 million new cases of TB being reported each year. Since the discovery of rifampicin, a broad-spectrum antibiotic is the main drug of current anti-TB chemotherapy by virtue of being active against *M. tuberculosis* in exponential growth phase as well as possessing activity against nonreplicating persistent bacilli. Rifampicin inhibits bacterial RNA polymerase activity by binding to the β-subunit encoded by *rpoB* gene and forming a stable drug-enzyme complex. The mechanism by which *M. tuberculosis* develops resistance to rifampicin has been the subject of intensive research and there may be several ways. The first consists of a mutational alteration(s) of the *rpoB* gene. Such a mutation(s) can result in the continued function of this essential mycobacterial enzyme despite the presence of concentration of rifampicin lethal to wild-type strains. The second mechanism may involve the efflux of rifamcin from the cell which generally results in a lower level of resistance than that seen in *rpoB* mutants. The analysis of genome sequences has shown that *M. tuberculosis* has many open-reading frames encoding putative efflux. Rv1258c is one such efflux pump gene which is reported to be upregulated in the clinical isolates of *M. tuberculosis* which are resistant to rifampicin. Piperine, a trans-trans isomer of 1-piperoyl-piperidine isolated from black pepper. It is reported to be bioavailability enhancer and has potential immunomodulatory activity. It has been previously characterized as an inhibitor of NorA efflux pump of *S. aureus* resulting in ciprofloxacin resistance. This study was performed with as objective to study the *in-vitro* effect of piperine with rifampicin in combination studies against *M. tuberculosis*. Additionally it was also evaluated for its immunomodulatory activity to enhance the efficacy of rifampicin in a murine model of *M. tuberculosis* infection. Piperine in combination with rifampicin reduced the minimum inhibitory concentration (MIC) of rifampicin by four folds in *Mycobacterium tuberculosis* H37Rv including rifampicin resistant *M. tuberculosis* and clinical isolates. The mutation prevention concentration (MPC) of rifampicin was >80 μg/ml which was reduced to 2 μg/ml with
the combination of rifampicin and piperine. Moreover, piperine effectively enhanced the bactericidal activity of rifampicin in time kill studies and also significantly extended its post antibiotic effect (PAE). In the presence of rifampicin, *M. tuberculosis* rif showed 3.6 fold over-expression of Rv1258c. The 3-D structure of Rv1258c using in-silico modeling was analyzed to elucidate the binding of piperine to the active site.

Piperine was also further tested as an immunomodulator *in-vitro* and *in-vivo* (both prophylactic and therapeutic). Piperine efficiently up-regulated the immune response. This was evidenced from our findings that *in-vitro* piperine increased T and B cell proliferation and also stimulated IFN-γ expression at 1 μg/ml and 10 μg/ml. Piperine demonstrated the up-regulation of TH1 immune response by stimulating the expression of IL-2, IFN-γ and CD4+/CD8+ ratio at a dose of 10 mg/kg PO for 4 month (treatment started 2 month before infection till 2 month after infection). The lung biopsies of the mice treated with piperine at 10 mg/kg showed enhanced immune response and epitheloid cell granuloma formation during histopathological studies. Piperine 10 mg/kg when tested in combination with rifampicin 10 mg/kg against *M. tuberculosis* in murine model for 4 to 8 weeks resulted in 1.4 to 0.8 log additional reduction of the bacterial load in the lungs with respect to rifampicin alone. The above results suggested that combination of rifampicin and piperine could be quite useful for the treatment of immuno-compromised population of TB patients.