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Human Beta Defensin 1 as Potential Antimycobacterial Agent against Active and Dormant Tubercle Bacilli

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Abstract : Counteracting the deadly pathogen Mycobacterium tuberculosis (M. tb) effectively is still a global challenge. Scrutinizing alternative weapons like antimicrobial peptides to strengthen existing tuberculosis artillery is urgently required. Considering the antimycobacterial potential of Human Beta Defensin 1 (HBD-1) along with isoniazid, the present study was designed to explore the ability of HBD-1 to act against active and dormant M. tb. HBD-1 was screened in silico using antimicrobial peptide prediction servers to identify its short antimicrobial motif. The activity of both HBD-1 and its selected motif (Pep B) was determined at different concentrations against actively growing M. tb in vitro and ex vivo in monocyte derived macrophages (MDMs). Log phase M. tb was grown along with HBD-1 and Pep B for 7 days. M. tb infected MDMs were treated with HBD-1 and Pep B for 72 hours. Thereafter, colony forming unit (CFU) enumeration was performed to determine activity of both peptides against actively growing in vitro and intracellular M. tb. The dormant M. tb models were prepared by following two approaches and treated with different concentrations of HBD-1 and Pep B. Firstly, 20-22 days old M. tbH37Rv was grown in potassium deficient Sauton media for 35 days. The presence of dormant bacilli was confirmed by Nile red staining. Dormant bacilli were further treated with rifampicin, isoniazid, HBD-1 and its motif for 7 days. The effect of both peptides on latent bacilli was assessed by colony forming units (CFU) and most probable number (MPN) enumeration. Secondly, human PBMC granuloma model was prepared by infecting PBMCs seeded on collagen matrix with M. tb(MOI 0.1) for 10 days. Histopathology was done to confirm granuloma formation. The granuloma thus formed was incubated for 72 hours with rifampicin, HBD-1 and Pep B individually. Difference in bacillary load was determined by CFU enumeration. The minimum inhibitory concentrations of HBD-1 and Pep B restricting growth of mycobacteria in vitro were 2µg/ml and 20µg/ml respectively. The intracellular mycobacterial load was reduced significantly by HBD-1 and Pep B at 1µg/ml and 5µg/ml respectively. Nile red positive bacterial population, high MPN/ low CFU count and tolerance to isoniazid, confirmed the formation of potassium deficienybaseddormancy model. HBD-1 (8µg/ml) showed 96% and 99% killing and Pep B (40µg/ml) lowered dormant bacillary load by 68.89% and 92.49% based on CFU and MPN enumeration respectively. Further, H&E stained aggregates of macrophages and lymphocytes, acid fast bacilli surrounded by cellular aggregates and rifampicin resistance, indicated the formation of human granuloma dormancy model. HBD-1 (8µg/ml) led to 81.3% reduction in CFU whereas its motif Pep B (40µg/ml) showed only 54.66% decrease in bacterial load inside granuloma. Thus, the present study indicated that HBD-1 and its motif are effective antimicrobial players against both actively growing and dormant M. tb. They should be further explored to tap their potential to design a powerful weapon for combating tuberculosis.

Keywords : antimicrobial peptides, dormant, human beta defensin 1, tuberculosis
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