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INTERACTION OF ANTIMICROBIAL PEPTIDES AS AN ALTERNATIVE TREATMENT OF TUBERCULOSIS

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Abstract:

Introduction: *Tuberculosis (TB) is still one of the most deadly communicable diseases worldwide. In 2013, an estimated 9 million people developed TB and 1.5 million people died from TB. The global trend is a decrease of incidence, prevalence, and mortality.*

Objectives of the study: *The basic aim of the study is to analyze the interaction of antimicrobial peptides as an alternative treatment of tuberculosis.*

Methodology of the study: *This cross sectional study was conducted at Allied Hospital Faisalabad during July 2019 to December 2019. The patients of both genders were selected for this study. Those patients who suffered from TB from last one year was included in this study. In this analyzes we compare the different drugs of TB which are available in the market as compared to antimicrobial peptides.*

Results: *In general the three methods led to the same results. Linde et al. and other groups assayed the antimycobacterial activity by the BACTEC radio-metric method, which detected growth of mycobacteria by measurement of CO_2 released as a consequence of bacterial catabolism.*

Conclusion: *It is concluded that Many AMPs have good or moderate activities against mycobacteria, however, in general the activities are lower as compared to the activities found against other Gram-negative or Gram-positive bacteria.*

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INTRODUCTION:

Tuberculosis (TB) is still one of the most deadliest communicable diseases worldwide. In 2013, an estimated 9 million people developed TB and 1.5 million people died from TB [1]. The global trend is a decrease of incidence, prevalence, and mortality. However, with the emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) *Mycobacterium tuberculosis* (*Mtb*) strains, new classes of anti-mycobacterial agents are urgently needed. MDR strains are resistant at least against isoniazid (INH) and rifampicin (RIF) and XDR strains are resistant against INH, RIF, fluoroquinolone, and one of amikacin, kanamycin, or capreomycin [2].

Mycobacterium tuberculosis, the causative agent of TB, was discovered by Robert Koch at the end of the nineteenth century. This infectious killer agent currently is one of the most common diseases, infecting approximately one-third of the world's population. According to the WHO survey, it is estimated that there were 8.6 million people infected with TB in 2012. In addition, there are an estimated 450000 MDR-TB cases and 170000 fatalities occur as a result [3]. The development of new cases, adverse events in response to anti-TB drugs, co-morbidity with HIV and the lack of an effective vaccine pose great problems in the search for new anti-TB drugs.

Due to continuous failure of antibiotics against drug resistant bacteria, pharmaceuticals industries are looking for alternative strategy. One of the possible alternatives to antibiotics (traditional drug mainly based on small molecules) is peptide based therapeutics [4]. A significant and vast majority of peptides from plant, bacteria and fungus sources proven to have anti-microbial action and these can be used as a supplement or alternate for conventional antibiotics. In last few decades, researchers have screened several peptides effective against *Mycobacterium*, which has been used for TB treatment in different therapeutic strategy like as single anti-TB agents, in combination with conventional drugs and synergistic effect with traditional antibiotic therapy [5].

Objectives of the study:

The basic aim of the study is to analyze the interaction of antimicrobial peptides as an alternative treatment of tuberculosis.

METHODOLOGY OF THE STUDY:

This cross sectional study was conducted at Allied Hospital Faisalabad during July 2019 to December 2019. The patients of both genders were selected for this study. Those patients who suffered from TB from last one year was included in this study. In this analyzes we compare the different drugs of TB which are available in the market as compared to antimicrobial peptides. No novel drugs have been on the market against TB in the past 40 years except the novel anti-TB drug bedaquiline, which was approved in 2012. A reasonable approach to find new therapeutics against the high number of MDR and XDR strains is to find molecules having a different killing mechanism. It has been shown that naturally occurring antimicrobial peptides (AMPs), also called host defense peptides (HDPs), are an important part of the innate immunity of humans.

Three potential ways of using AMPs in TB infections are discussed in literature: (i) stimulation of the endogenous production of AMPs for direct bacterial killing, (ii) administration of AMPs also for direct killing, and (iii) modulation of the innate immune response by endogenous and externally applied AMPs. Two TB conditions have to be distinguished: the acute and the latent phase. In the latent phase persons do not have any symptoms.

Presence of AMPs in the lung:

In the last 20 years, most of the AMPs which are expressed in the lung are tested against mycobacteria. Furthermore, many other AMPs including synthetic compounds were investigated. Most of the authors determined the activity against the *Mtb* H37Rv strain, which helps to compare the results. On the other hand, the assays as well as the conditions used are quite diverse. Miyakawa et al. compared three different techniques for the determination of the activity of the AMPs: CFU counting, radiometric method, and radial diffusion assay.

RESULTS:

In general the three methods led to the same results. Linde et al. and other groups assayed the antimycobacterial activity by the BACTEC radio-metric method, which detected growth of mycobacteria by measurement of CO_2 released as a consequence of bacterial catabolism. Other groups use resazurin as an indicator of residual bacterial viability or imaged the growth in 96 well plates using light microscopy.

Table 01: Activities of various AMPs including the mycobacterial strains and conditions used in the respective experiments.

AMP	Bacterial strain	Conditions	Activity
hNP-1, hNP-2, hNP-3	M. avium–M. intracellulare	CFU counting, 37 °C, 1:100 Middlebrook 7H10, 2.5×10^6 or 2.5×10^8 bacteria, AMP conc.	Between 34.2 and 87.2% at 50 μg/ml hNP-1
hNP-1, hNP-2, hNP-3, sNP-1, NP-2, PG-1	Mtb H37Ra and clinical isolates	CFU counting, radiometric method, radial diffusion assay, 37 °C, 1:100 Middlebrook, 5.1×10^5 or 4.5×10^7bacteria, Incubation time (6 to 48 h), AMP concentration (5 and 50 μg/ml), Incubation time (6 to 48 h), pH (between 2 and 8), different salt concentrations	Between 85.9 and 97.5% at 50 μg/ml after 24 h incubation time
Granulysin	Mtb H37Rv	CFU counting, radial diffusion assay, 37 °C, Middlebrook 7H9 with OADC, 2×10^6 bacteria, Incubation time (72 h), AMP conc. (between 1 and 30 μM), Activity against extra- and intracellular Mtb	Extracellular: 90% at 30 μM Intracellular: Only in presence of perforin
PG-1, hBD-1	Mtb H37Rv (ATCC 27294) and the MDR RM22 strain	CFU counting, Middlebrook 7H9, 1×10^3bacteria Incubation time (0 and 3 days), AMP conc. (between 4 and 128 μg/ml)	PG-1: 68.4% at 64 μg/ml and 96.7% at 128 μg/ml hBD-1: 49.9% at 128 μg/ml Synergistic effect with isoniazid
PR-39 (hNP-1, PG-1, cecropin P1, LL-37)	Mtb H37Rv ATCC 25618, Mtb E1380/94 and P35/95 (MDR clinical isolates) M. avium ATCC 26518	CFU counting, radiometric assay, 37 °C, Middlebrook 7H12, 1.5×10^6 bacteria, Incubation time (24 h), AMP conc. (50, 100 μg/ml)	PR-39: 60% inhibition at 50 μg/ml against H37Rv and 39 and 49% against E1380/94 and P35/95 Comparable activity of HNP-1 and PG-1, no activity of LL-37 and cecropin P1
hNP1–3, LL-37, lipocalin 2	M. bovis-BCG, Mtb H37Rv	CFU counting, 37 °C, Middlebrook 7H9, 6×10^5 bacteria, Incubation time (168 h and 192 h), effect of iron	Iron dependent activity of lipocalin 2 (5 μg/ml)
NK-lysin, peptides based on NK-lysin and granulysin	Mtb H37Rv	BACTEC radiometric assay, PBS, 7.5×10^5 bacteria Incubation time (8 days), AMP conc. (3, 30 μM)	90% growth inhibition of the peptides at 30 μM, 60% of native NK-lysin
LL-37, mCRAMP, E2, E6, CP26	Mtb H37Rv, MDR strain	Resazurin assay, 37 °C, Middlebrook 7H9 with OADC,	MIC values between 2 and 10 μg/ml

		Incubation time (5 days), AMP conc. (0.4–12.8 µg/ml)	
LL-37	M. marinumNJB0419	CFU counting, Middlebrook 7H9 with OADC, AMP conc. (10 µg/ml)	No activity at 10 µg/ml
LL-37 (L and D enantiomer)	Mtb H37Rv and Mtb Vertulo (MDR)	CFU counting, 37 °C, Middlebrook 7H9, 1×10^6 bacteria/ml, AMP conc. (1, 10, 100 µg/ml)	No activity of the L- and low activity of the D-enantiomer at 100 µg/ml

DISCUSSION:

Lactoferricins are naturally occurring peptides, formed by the cleavage of the highly cationic N1 terminal domain of the iron-binding protein lactoferrin[6]. All peptides showed an antimycobacterial activity with LD50 values between 10 and 40 µM. The stronger activity was found in the bovine-based peptides. A significant difference in the activity against three different *M. avium* strains could not be found [7].

PR-39, a proline–arginine rich AMP isolated from pig intestine, has activity against drug-susceptible as well as MDR clinical isolates of *Mtb*. At 50 µg/ml 80% growth inhibition of *Mtb* H37Rv could be achieved. Martineau et al. investigated the role of iron concentration on the activity of lipocalin 2. Lipocalin 2–induced suppression of *Mtb* CFU was greater in iron-depleted broth (10 nM iron) than in iron-replete broth (150 µM Fe) with values of 60% versus 45%, respectively [8].

Azurophil granule protein (AZP) are active against *M. smegmatis* and *M. bovis* BCG [73]. Jena et al. investigated this mixture of molecules and identified elastase and lysozyme to be important for mycobacterial killing [9]. Elastase reduced *M. smegmatis*growth by approximately 60% and lysozyme by approximately 75% at 10 µg/ml after 6 h.

Two very promising new antimycobacterial peptides were found in screens of extracts from uncultured species. From *Lentzea kentuckyensis* the posttranslationally modified 16 amino acid long peptide lassomycin was purified [10].

CONCLUSION:

It is concluded that Many AMPs have good or moderate activities against mycobacteria, however, in general the activities are lower as compared to the activities found against other Gram-negative or Gram-positive bacteria. One big advantage of AMPs is that the active ones showed almost same activities against *Mtb* strains being sensitive against conventional antibiotics and also against MDR and XDR strains.

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