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Research review paper

# Antimicrobial peptides as novel anti-tuberculosis therapeutics

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ABSTRACT

Tuberculosis (TB), a disease caused by the human pathogen *Mycobacterium tuberculosis*, has recently joined HIV/AIDS as the world's deadliest infectious disease, affecting around 9.6 million people worldwide in 2014. Of those, about 1.2 million died from the disease.

Resistance acquisition to existing antibiotics, with the subsequent emergence of Multi-Drug Resistant mycobacteria strains, together with an increasing economic burden, has urged the development of new anti-TB drugs. In this scope, antimicrobial peptides (AMPs), which are small, cationic and amphipathic peptides that make part of the innate immune system, now arise as promising candidates for TB treatment. In this review, we analyze the potential of AMPs for this application. We address the mechanisms of action, advantages and disadvantages over conventional antibiotics and how problems associated with its use may be overcome to boost their therapeutic potential. Additionally, we address the challenges of translational development from benchside to bedside, evaluate the current development pipeline and analyze the expected global impact from a socio-economic standpoint.

The quest for more efficient and more compliant anti-TB drugs, associated with the great therapeutic potential of emerging AMPs and the rising peptide market, provide an optimal environment for the emergence of AMPs as promising therapies. Still, their pharmacological properties need to be enhanced and manufacturing-associated issues need to be addressed.



**Contents**



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## 1. Introduction: tuberculosis - a global emergency

Tuberculosis (TB) recently joined HIV/AIDS on the top rank of the deadliest infectious diseases, being actually responsible for one fourth of HIV-related deaths. According to the latest data from World Health Organization, around 9.6 million people were diagnosed with TB in 2014, having about 1.2 million of those died from the disease (WHO, 2015a). Globally, TB incidence remains highest in Africa, in terms of new cases per inhabitants, but new TB occurrences are also increasing in Southeast Asia and Western Pacific regions.

As a result of the implementation of the *Millennium Development Goals* in 2000 (WHO, 2015b), which particularly focused on reducing TB incidence, around 37 million lives were saved between 2000 and 2013 due to effective diagnosis and treatment and since 2007 the treatment success rate has been at or above 85%. Despite all efforts to fight this disease, its death toll remains elevated and multi-drug resistant TB (MDR-TB) strains are emerging mostly as a result of overuse or misuse of antimicrobial agents (*e.g.* antibiotics). By definition, MDR-TB is resistant to, at least, isoniazid and rifampicin, the two most powerful, first-line (or standard) anti-TB drugs (Onyebujoh et al., 2005). Also, extensively drug-resistant TB (XDR-TB), an even more severe form of MDR-TB, resistant to even more available medicines, has emerged. XDR-TB strains are usually resistant to at least isoniazid, rifampicin or any fluoroquinolone, and to any of the three second-line injectables (amikacin, capreomycin, and kanamycin). Noteworthy, about 480,000 people developed MDR-TB in 2013, being estimated that around 9% of those cases were XDR-TB. Nonetheless, the term XDR-TB, as well as totally drug-resistant TB (TDR-TB), have not been clearly defined by WHO due to technical challenges and limitations of *in vitro* drug susceptibility testing.

The approval of the *Beijing Call for Action* in 2009 and the *World Health Assembly Resolution 62.15* by UN Member States represented a major commitment towards MDR-TB treatment and control (WHO, 2009). Still, MDR-TB represents a major public health concern within the European Union (EU), as only a third of MDR-TB patients are successfully treated in the EU, one of the lowest rates in the world. This has led EU members to implement an Action Plan against antimicrobial resistance, which started in 2011 (European Commission, 2011).

Within this context of multi-drug resistance strain emergence, a new class of drugs – antimicrobial peptides (AMPs) – arises as promising candidates for TB treatment.

## 2. Mycobacteria: made to resist

Although over 170 species and subspecies of mycobacteria have been reported (http://www.bacterio.cict.fr/m/mycobacterium.html) only a few are described as pathogenic, namely *Mycobacterium tuberculosis*, *Mycobacterium leprae* and *Mycobacterium ulcerans* (Gaspar et al., 2008). Mycobacterial species are Gram-positive, non spore-forming, aerobic bacteria, which feature a characteristic thick cell wall that confers them a unique impermeability to many molecules, namely antimicrobials, and comprising several distinct layers (Jarlier and Nikaido, 1994; Neyrolles and Guilhot, 2011). The innermost is composed of peptidoglycan. External to the peptidoglycan is a covalently linked polymer of sugars, arabinogalactan, to which mycolic acids are esterified. Finally, a variable mixture of glycolipids and lipoglycans are thought to interact *via* their acyl groups with the mycolic acids through hydrophobic interactions. Fig. 1 schematizes this unique cell wall and shows how it compares with the cell walls of Gram-negative and Gram-positive bacteria. A capsule composed of non-covalently linked loosely associated glycans, lipids and proteins has been shown to decorate the outer surface of the mycobacterial envelope. Noteworthy, the prevalence of mycolic acid molecules covalently linked to arabinogalactan in the intermediate layer confers its high hydrophobicity and decreased permeability to external compounds (Gaspar et al., 2008; Jarlier and Nikaido, 1994; Neyrolles and Guilhot, 2011).

In addition to the intrinsic basis of antimicrobial resistance of mycobacteria related to their peculiar cell wall, both life-style and pathological consequences of infection dictate additional levels of difficulty in obtaining effective chemotherapeutical drugs. Mycobacteria are able to replicate inside the macrophage.

In the case of lung infections by *M. tuberculosis*, mycobacteria are first phagocytized by alveolar macrophages and quickly spread locally in the lungs and eventually to other organs *via* lymphatic and blood circulation (Guirado et al., 2013). Once inside phagosomes, mycobacteria impair the recruitment of proteins and phosphoinositides, required for intracellular trafficking, to the phagosomal membrane, which results in phagosome maturation arrest (Guirado et al., 2013; Hmama et al., 2015). Through this process, mycobacteria avoid the subsequent phagosomal fusion with lysosomes and the contact with potent hydrolytic enzymes and antigen-presenting organelles within the host macrophage (Fratti et al., 2004). At tissue level, both infected and non-infected macrophages will be organized within granulomas, which frequently undergo central necrosis (caseous necrosis) or may be found scattered in the alveolar spaces in pneumonic forms (Hunter, 2011). The heterogeneity of the lesions in human tuberculosis will certainly impact on the bioavailability of anti-tubercular drugs, as recently observed by Prideaux et al. (2015). Finally, freely replicating mycobacteria have been found in biofilms lining the aerial side of cavities (Orme, 2014), further complicating the issue of the access of the drugs to their targets.

## 3. The TB drugs pipeline

### 3.1. Standard treatments

Mycobacterial infections are very difficult to treat. Bacille Calmette-Guérin (BCG) vaccine, a live attenuated strain of *Mycobacterium bovis*, is the only vaccine available. Although quite effective in the prevention of childhood TB, adults can have new infections (Roy et al., 2014; WHO, 2012).

Current therapeutics rely mostly on the use of antibiotics (antimicrobials, by definition) of natural or chemical origin, that kill or inhibit the growth of infectious agents (O'Toole, 2003). Indeed, the discovery of streptomycin in 1944 (Bugie and Waksman, 1944) brought forth the first anti-tuberculosis drug. Soon after, many other drugs have been developed, including para-aminosalicylic acid, thiacetazone and isoniazid (Fox et al., 1999). Together with streptomycin, these drugs constituted the first TB treatment regimen (Stehr et al., 2014). However, long-lasting treatment (18–24 months), along with painful injections and toxic effects deterred the use of this regimen, until rifampicin appeared around 1959, reducing therapy length to 6 months (Sensi et al., 1959).

Current standard treatments for non-resistant TB are based on an intensive 2-month administration of a multi-drug cocktail consisting of isoniazid, pyrazinamide, rifampicin and ethambutol, followed by a second 4-month treatment of rifampicin and isoniazid (first-line therapy). These four drugs combine different actions: both isoniazid and ethambutol inhibit cell wall synthesis, rifampicin causes the inhibition of RNA synthesis and pyrazinamide disrupts the plasma membrane and energy metabolism (Somoskovi et al., 2001). However, despite being highly active against replicating mycobacteria, these drugs (especially isoniazid) are ineffective against mycobacteria in stationary phase or with very low proliferation rates (Onyebujoh et al., 2005; Sosnik et al., 2010). In addition, lack of patient compliance with the 6-month treatment, along with adverse drug reactions and interactions, resulted in the emergence of MDR-TB (Gaspar et al., 2008). Treatment of MDR-TB is based on the administration of pyrazinamide together with second-line drugs, such as ethionamide, prothionamide, cycloserine, capreomycin or fluoroquinolones (Mukherjee et al., 2004). Standard recommendations for TB therapy, including the treatment duration, according to the resistance pattern of each strain, are summarized in