

Antimicrobial peptide delivery from self-assembling Hyaluronic acid Nanoparticles for tuberculosis treatment

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Abstract

Tuberculosis (TB), a disease caused by the highly virulent human pathogen *M. tuberculosis*, has recently joined HIV as the deadliest infectious diseases. In 2014, more than 9 million people worldwide were diagnosed with TB, 1.5 million of which died from the disease. Bacille Calmette Guérin (BCG) vaccine fails to prevent adult TB and current treatments rely on long-lasting, multiple antibiotic therapies that often result in treatment failure and in the current emergence of Multi-Drug Resistant (MDR) strains. Treatment costs can reach very high amounts (especially for MDR-TB) and the low patient compliance to the treatment regimen become crucial drawbacks to the therapy. For these reasons, new developments in TB therapy have become imperative.

In this context, AntiMicrobial Peptides (AMPs), commonly defined as small, cationic and amphipathic peptides that play a key role in the innate immune system, arise as promising candidates for TB treatment. The involvement of the only known human cathelicidin (a family of AMPs), LL37, in the intracellular killing of mycobacteria has been reported. Moreover, several analogues of LL37, including the more cationic and hydrophobic 18-mer LLKKK18 have been engineered to boost the therapeutic potential of LL37 [1]. Indeed, we recently showed the ability of this peptide to reduce the mycobacterial load of the opportunistic strain *M. avium* in axenic cultures [2].

We developed a new approach for TB treatment, based on the intra-tracheal administration of LLKKK18 loaded into self-assembling Hyaluronic Acid (HA) nanoparticles (NPs), previously developed at our lab [3]. These NPs may facilitate AMP targeting to activated macrophages since these express the CD44 receptor, which binds HA, thus enhancing its internalization.

This loaded peptide was internalized by bone marrow-derived macrophages, as indicated by labeling the peptide with a fluorescent tag, and it effectively co-localized with mycobacteria (demonstrated by confocal microscopy) within infected macrophages. This resulted in a significant reduction of the mycobacterial load in macrophages infected with either the opportunistic *M. avium* strain 2447 or the human pathogen *M. tuberculosis* H37Rv. More remarkable, the LLKKK18-loaded HA nanoparticles significantly reduced the infection levels of both *M. avium* and *M. tuberculosis* (Fig. 1) in infected mice after just a 5-administration regimen carried out over a period of 10 days. Nevertheless, further studies are currently being held to increase the peptide's effect.

Overall, we have developed a promising new approach towards anti-tuberculosis therapy, based on the high potential of LLKKK18 to fight mycobacteria. Additionally, the use of an AMP involves a much lower risk of acquired resistance by mycobacteria, while being comparatively cheaper and not requiring long-lasting treatments, as mandatory for MDR-TB.

References

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- [2] Santos JC, Silva-Gomes S, Silva JP, Gama FM, Rosa G, Gallo RL, Appelberg R. *Immunity, Inflammation and Disease* **2** (2014) 1-12.
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Figures

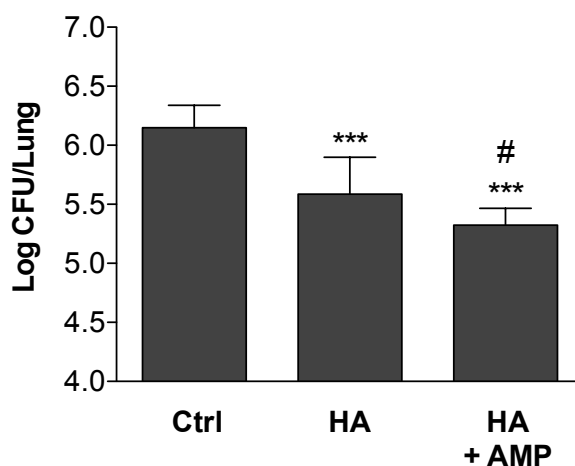


Fig. 1 - *In vivo* killing of *M. tuberculosis* induced by LLKKK18 (AMP)-loaded HA nanoparticles. C57BL/6 mice were infected with *M. tuberculosis* via the pulmonary route. After 3 months, five doses of the treatments were administered intra-tracheally every other day. Data represents the mean \pm SD for at least 6 mice per group. *** $p < 0.001$, compared to control. # $p < 0.05$, compared to HA.