Antimicrobacterial Activity of Synthetic Cationic Peptides

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ABSTRACT

Mycobacterium tuberculosis, the causative agent of tuberculosis, infects approximately nine million people every year. The number of sufferers totals one third of the world's population. Antibiotic treatment of tuberculosis has met challenges due to the rise of multi drug resistance. Synthetic cationic peptides similar to naturally occurring peptides produced by platelets have been shown in previous studies to have antimicrobial activity against a variety of microorganisms. In this study we have hypothesized that these synthetic peptides have antimicrobial activity against members of the mycobacterium genes. To test our hypothesis we employed the mycobacterial model organism *Mycobacterium smegmatis*, which has a short generation time and low pathogenicity as a surrogate for *M. tuberculosis*. We incubated diluted cultures of *M. smegmatis* with five different peptides, 0, 0.5, 1.0, 2.0, and 4.0, on 8.0µg/ml of peptide that were used in the experiment. The samples were incubated for 0, 3 ,6, and 24 hours at pH 7.3 or pH 5.5. After incubation with the peptide, *M. smegmatis* was diluted and plated to produce countable colonies on the 7H10 agar plates. The plates were counted and compared to control zero peptide samples. Four out of the five peptides have shown antimicrobial activity against *M. smegmatis* at pH 7.3 solution and three out of the five had activity at pH 5.5. *Mycobacterium tuberculosis* occupies acidic locations within the human body, such as acidified phagosomes of macrophages. Peptides active at acidic pH may have increased antimicrobial activity within the human body.

INTRODUCTION

Tuberculosis is a life threatening disease that has killed hundreds of millions of people and continues to infect people every year. There are several treatments available but the bacterium, *Mycobacterium tuberculosis*, has evolved and some bacteria have a multi-drug resistance. The bacterium, *M. tuberculosis*, typically lives in phagosomes in macrophages. It has been shown in studies that *M. tuberculosis* has developed several mechanisms to enter macrophages, even though these environments can kill it. To mimic the properties of *M. tuberculosis* in our experiment, *Mycobacterium smegmatis* was used. *M. smegmatis* has a short generation time and is non- toxic, making it an ideal model organism for the experiment. Synthetic cationic peptides are produced by platelets and act the same as natural peptides. It has been shown that in several studies synthetic cationic peptides has had antimicrobial activity against microbial genes.

OBJECTIVE

To demonstrate that synthetic cationic peptides have antimicrobial activity against members of the mycobacterium genus.

METHOD and MATERIALS

Media: 7H9 broth and ADC, 7H10 and ADC plates

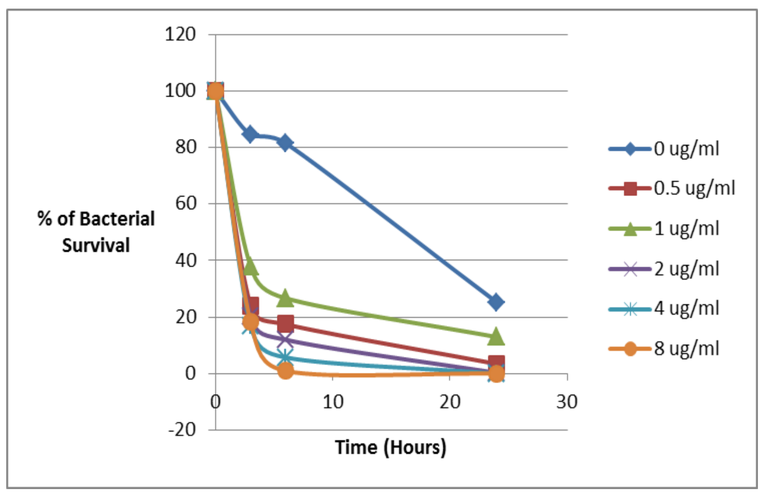
Peptide: 6W RP. 1, RP 13, RP 11, RP .1, AA RP.1

Buffer: 10 mM 7.3 Pipes buffer, 2mM Mes pH 5.5

Method : First the dilutions of *Mycobacterium smegmatis* were carried out in buffer solutions of 7.3 pH or 5.5 pH. Then these known dilutions of *Mycobacterium smegmatis* were mixed with selected synthetic cationic peptides. The peptides were at different concentrations levels (.5 ug/ml, 1ug/ml, 2 ug/ml, 4 ug/ml, 4 ug/ml, and 8 ug/ml). One tube of *M. smegmatis* was set aside without peptide for control. Then the mixture was incubated. After specific interval of time (3hours, 6 hours, or 24 hours) the mixture was serial diluted and plated on 7H10 plates. The plates with different concentrations of peptide were then compared with the control; to see the bactericidal effects if any from the peptide.

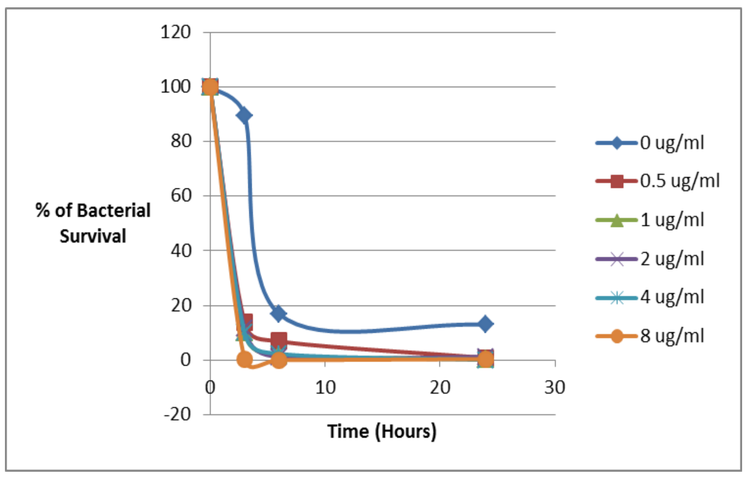
RESULTS

M. smegmatis was exposed to various concentrations of the synthetic cationic peptides in buffer at pH 7.3 or 5.5 and we determined the bactericidal effects of the peptides as described above. Plates containing serial dilutions of bacteria exposed to peptides or not exposed to peptides were counted for colony numbers. The graphs below show peptides that decreased the percent bacterial survival. Furthermore, as the concentration of some peptides increased the percent survival continued to decrease. The percent of bacterial survival was calculated by dividing the colony count after exposure to peptides by the colony count of the peptide free control.



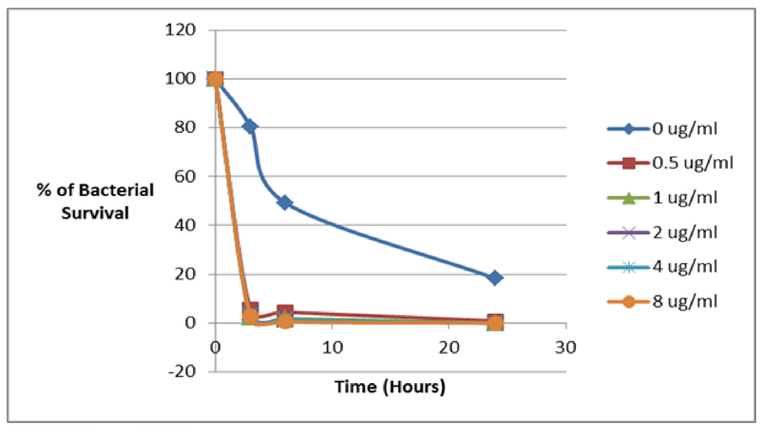
RP.1 at pH 5.5

Referring to figure 1, *M. smegmatis* was exposed to RP.1 peptide in buffer at 5.5, at various concentrations of the peptide. The data graphed in figure 1 is the averages of all the trials. The graph shows RP.1 decreased the percent bacterial survival. As the concentration of the peptide increased the percent survival continued to decrease.



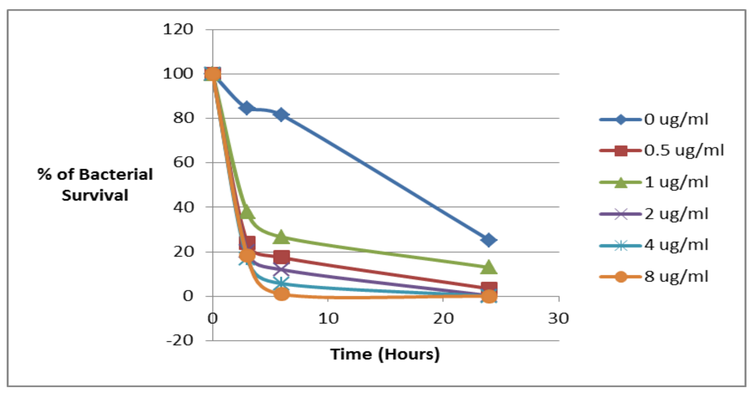
RP. 1 at pH 7.3

When *M. smegmatis* was exposed to RP.1 peptide in buffer at 7.3, at various concentrations of the peptide, the data was calculated and graphed in figure 2. The data graphed shows the averages of all the trials at pH 7.3 for RP.1. The graph shows RP.1 decreased the percent bacterial survival.



AA RP.1 at pH 7.3

Using different concentrations of the peptide, we calculated a significant decrease in bacterial survival compared to the control. The graph shows that AA RP.1 decreased the percent of bacterial survival. As the concentration of the peptide increased the percent survival continued to decrease. There is around a 20% bacterial survival difference between the control and those exposed to peptide. This figure shows the effect AA RP.1 has on the bacterial survival of *M. smegmatis* at pH 7.3.



6W RP.1 at pH 5.5

The average percentage of bacterial survival of *M. smegmatis* when it was exposed to 6W RP. 1 at pH 5.5 was graphed. The graph shows that 6W RP.1 decreased the percent bacterial survival. As the concentration of the peptide increased the percent survival continued to decrease. There is around a 20% bacterial survival difference between the control and those exposed to the peptide.

CONCLUSIONS

- Our evidence demonstrates that at pH 7.3, the peptides (AA RP. 1 and RP. 1), show a negative effect on bacterial survival.
- Also our evidence demonstrates that at pH 5.5, the peptides (6W RP. 1, RP. 1, RP .11) show a negative effect on bacterial survival.
- The peptides could potentially be used for future therapeutic purposes for bacteria resistant to current treatments.

FUTURE STUDIES

- Examine the effect of synthetic cationic peptides on *M. tuberculosis*.
- Determine the effect of different pH buffers on *M. tuberculosis.*
- Determine the effects of peptides on static cultures. Static cultures are bacteria cultures that stop dividing.

REFERENCES

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