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Clinical Candidate AA139 - Efficacy in a Neutropenic Thigh Model caused by *E. coli* AID#172

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Abstract

Background
AA139 is a novel Antimicrobial Peptide (AMP) derived from the natural AMP Arenicin-3. It is potently active against Gram-negative ESKAPE pathogens and is currently in pre-clinical development. In this study we like to present its efficacy in an *E. coli* thigh infection model in the neutropenic mouse.
Methods
Mice were rendered neutropenic by a -4 and -1 day treatment with cyclophosphamide. Mice were inoculated intramuscularly with 0.05 ml of a suspension of freshly prepared overnight colonies of approximately 1-5x10^7 CFU/ml of a multi resistant E.coli clinical isolate. Groups of 5 mice were treated IV, 1 and 7 hrs post infection, with 0.63-15 mg/kg AA139, 5 mg/kg polymyxin B or vehicle. Mice were sacrificed by cervical dislocation 25 hrs post infection and the left hind leg from the hip joint to the hock was collected and homogenized in 5 ml saline. Each sample was then 10 fold diluted in saline and 20-µl spots were applied on 5 % blood agar plates incubated 18-24 hrs at 35°C in ambient air.
Results
The *E.coli* isolate was highly virulent resulting in a 2.3 $\log_{10}$ increase in bacterial loads in the thighs during the 24 h study period. The maximum effect of AA139, E_{max}, was 3.79 $\log_{10}$ CFU. The ED_{50} was calculated in GraphPad Prism using Sigmoidal dose-response (variable slope) and was determined to 4.8 mg/kg. The 1 log killing, was estimated to be 8.2 mg/kg.
The clinical score during the experiment correlates well with the bacterial loads. At time 22 – 24 hours post treatment the mice in the vehicle and the 0.63 mg/kg dosing groups showed moderate to severe clinical signs of infection. The 1.25 and 2.5 mg/kg dosing groups showed mild clinical signs of infection and the 5- 15 mg/kg dosing groups showed no signs of infection.
Conclusions
Clinical candidate AA139 showed a potent antibacterial effect against a multiresistant *E.coli* in the neutropenic thigh infection model after dosing twice with 5-15 mg/kg. The antibacterial effect of 5-15 mg/kg AA139 was comparable or slightly better than the effect of 5 mg/kg polymyxin B. The ED_{50} of 2 doses of AA139 was determined to 4.8 mg/kg

Background

The Arenicin family consists of three members: Arenicin-1 and -2, which were characterized by a Russian research group (Ovchinnikova et al., 2004), and Arenicin-3 which is a novel member of the family. Arenicin-3 was isolated from the marine lugworm *Arenicola marina*; it contains 21 natural amino acid residues constrained in an amphipathic beta hairpin structure by two disulfide bridges between Cys3, Cys20 and Cys7, Cys16. Four positively charged arginines, and 9 hydrophobic residues contribute to the amphipathic characteristics of the peptide.

In this study we present the efficacy of clinical candidate AA139 in a thigh infection model caused by multidrug-resistant *E. coli* AID#172 .

Method

Mice were rendered neutropenic by a -4 and -1 day treatment with cyclophosphamide. Mice were inoculated intramuscularly with 0.05 ml of a suspension of freshly prepared overnight colonies of approximately 1-5x10^7 CFU/ml of a multi resistant *E. coli* clinical isolate. Groups of 5 mice were treated IV, 1 and 7 hrs post infection, with 0.63-15 mg/kg AA139, 5 mg/kg polymyxin B or vehicle. Mice were sacrificed by cervical dislocation 25 hrs post infection and the left hind leg from the hip joint to the hock was collected and homogenized in 5 ml saline. Each sample was then 10 fold diluted in saline and 20-µl spots were applied on 5 % blood agar plates incubated 18-24 hrs at 35°C in ambient air.

Results

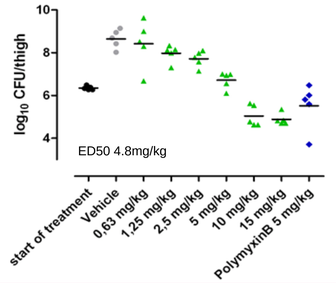
The ED50 values for the *E. coli* strain AID#172 was calculated to 4.8 mg/kg.(Fig. 1). The dose resulting in a 1 log killing effect was determined to 8.9 mg/kg and Emax was calculated to 3.8 log10 CFU/ml.

The bacterial loads in the thighs at start of treatment was determined to 6.35 $\log_{10}$ CFU/mouse and increased significantly ($p<0.001$) to 8.65 $\log_{10}$ CFU/mouse in mice treated with vehicle. A similar increase in bacterial loads was observed also for the 0.63 – 2.5 mg/kg dosing groups ($p<0.001$, $p<0.01$).

Results-Continued

The 5 mg/kg dosing group resulted in a static bacterial load whereas the 10 and 15 mg/kg dosing groups resulted in significantly reduced bacterial loads as compared to the start of treatment ($p<0.01$). The 5-15 mg/kg dosing groups all showed significantly ($p<0.001$) lower bacterial loads compared to vehicle treatment at 24 hrs after treatment start.

Figure 1. Dose-response curve of activity of AA139 against a multi-resistant strain of *E. coli* in thighs five hours after treatment in a neutropenic thigh infection model



Conclusion

- Clinical candidate AA139 showed a potent antibacterial effect against multidrug-resistant *E.coli* AID#172 in the neutropenic thigh infection model after two drug from 5 to 15 mg/kg.
- The antibacterial effect of 5-15 mg/kg AA139 was comparable or slightly better than the effect of 5 mg/kg polymyxin B.
- The ED_{50} of 2 doses of AA139 was determined to 4.8 mg/kg.

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