F-259

Clinical Candidate AA139 - Efficacy in two Neutropenic Bacteraemia/Peritonitis Models Caused by *E. coli* Strains with Different MIC Values

S. Lociuro**, S. Neve*, C.V. Lundberg** J.M. Andersen**, and P. Nordkild*.

*Adenium Biotech, Ole Maaloesvej 3 , DK-2200 Copenhagen, Denmark

** Statens Serum Institut, Ørestads Boulevard 5, DK-2300 Copenhagen, Denmark

Contact information
slo@adeniumbiotech.com
Phone +39 349 501 9411

Abstract

Background

AA139 is a novel Antimicrobial Peptide (AMP) derived from natural AMP Arenicin-3. It is potently active against Gram-negative ESKAPE pathogens and is currently in pre-clinical development. In this report we present the efficacy of AA139 in two studies of bacteraemia /peritonitis in the neutropenic mouse induced by 2 multi resistant strains of *E. coli* with MICs to AA139 of 0.12 (AID#172 - Study 1) and 1 µg/ml (AID#106-09 - Study 2).

Methods

Mice were rendered neutropenic with cyclophosphamide and inoculated intraperitoneally with 0.5 ml of a suspension of freshly prepared overnight colonies of one or the other *E. coli* strain of approximately $2x10^6$ CFU/ml in sterile saline. Groups of 3 mice were treated IV, 1 h post infection, with doses of AA139 (Study 1: 0.06-7.5 mg/kg; Study 2: 0.28 -18mg/kg), meropenem (study 1: 40 mg/kg), polymyxin B (Study 2: 5 mg/kg) or vehicle. Mice were anesthetized, blood was collected by axilliary cut down and then mice were sacrificed by cervical dislocation 5 hrs after treatment. 2 ml sterile saline was injected i.p. and peritoneal fluid sampled with a pipette. The bacterial loads in the samples were determined with the spot CFU method on blood agar plates.

Results

	Study 1 (*E. coli* AID#172; MIC 0.12 µg/ml)		Study 2 (*E. coli* AID#106-09; MIC 1 µg/ml)	
	Peritoneal fluid	Blood	Peritoneal fluid	Blood
ED_{50}	1.85 mg/kg	1.55 mg/kg	1.8 mg/kg	1.7 mg/kg
1 log killing	1.22 mg/kg	--	1.45 mg/kg	1.05 mg/kg
2 log killing	1.78 mg/kg	0.89 mg/kg	1.91 mg/kg	1.74 mg/kg
3 log killing	2.28 mg/kg	1.69 mg/kg	2.70 mg/kg	

In both study 1 and 2 a highly significant reduction in CFU levels compared to vehicle treatment ($p<0.001$) could be measured in both the peritoneal fluid and in blood. ED_{50}, 1, 2 and 3 log killing, were estimated using GraphPad Prism.

Conclusions

Clinical candidate AA139 showed a potent antibacterial effect in the bacteraemia / peritonitis infection model in neutropenic mice. ED_{50}s, and 1, 2 and 3 log killing data appeared to be only marginally affected by the MIC of the strain used in the experimental infection model.

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 the clinical candidate AA139 in two studies of bacteraemia/peritonitis in the neutropenic mouse infection model using *E. coli* strains with different MIC.

Method

Dose-response (ED50) Bacteraemia/peritonitis study

Neutropenia was induced by dosing mice intraperitoneally (i.p.) with cyclophosphamide on Day -4 (200 mg/kg) and on Day -1 (100 mg/kg).

On day 0 at Time =0 hrs:

Study 1. Mice were inoculated i.p. with a bacterial suspension of 2 x 10E6 CFU/ml of *E. coli AID172* (clinical isolate, 2003, from a human wound; multi-resistant to ampicillin, ceftazidime, aztreonam, gentamicin and ciprofloxacin). MICs: AA139: 0.12 mg/L; meropenem: 0.25 mg/L.

Study2. Mice were inoculated i.p. with a bacterial suspension of 2 x 10E6 CFU/ml of *E. coli EC106-09* (clinical isolate, 2009, from a human blood stream infection; multi-resistant (ciprofloxacin, sulfamethoxazole, trimethroprim). MICs: AA139: 1 mg/L; polymyxin B: 0.125 mg/L

Time =1 hrs:

Mice (n=4) were treated with single doses of AA139 i.v. ranging from 0.06 – 7.5 mg/kg in study 1 and with 0.28-18mg/kg in study 2. Control antibiotics were single dose meropenem (40 mg/kg) and polymyxin B (5 mg/kg), respectively.

Time=6 hrs:

The mice were anaesthetized and blood was collected. Subsequently, mice were sacrificed and a peritoneal wash performed. Quantitative bacterial counts were determined in both blood and peritoneal fluid and compared to untreated controls before start of treatment (T=0 hrs) and six hours later.

The 50% effective dose (ED50) is defined as the dose required to obtain 50% of the maximum log CFU difference compared to start of treatment. The ED50 was calculated in GraphPad Prism using a sigmoidal dose-response curve (Hill's regression) with variable slope.

Results

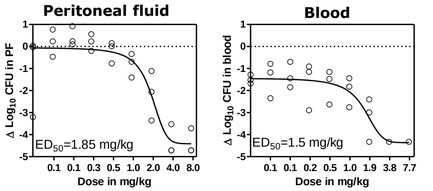
The purpose of these two studies was to determine the dose-response relationship following intravenous (i.v.) administration of a single dose of AA139.

Study 1: the ED50 values were determined to be 1.85 mg/kg in the peritoneal fluid and 1.55 mg/kg in the blood. The 1, 2 and 3 log killings in the peritoneal fluid was estimated to be 1.22, 1.78 and 2.28 mg/kg, respectively; 2 and 3 log killings of 0.89 and 1.69 mg/kg, respectively was instead estimated in blood.

Results-Continued

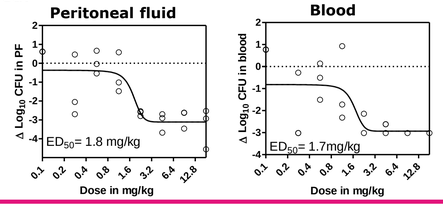
Control antibiotic meropenem produced 1 and 2 log killing in peritoneal fluid and blood, respectively at the dose used.

Figure1: Dose response curve of AA139 in peritoneal fluid (left) and blood (right) tested against *E. coli* AID#172 in the neutropenic murine peritonitis infection model.



Study 2: the ED50 values were determined to be 1.8 mg/kg in the peritoneal fluid and 1.7 mg/kg in the blood. The 1, 2 and 3 log killings in the peritoneal fluid was estimated to be 1.45, 1.91 and 2.70 mg/kg, respectively; 1 and 2 log killings of 1.05 and 1.74 mg/kg, respectively was instead estimated in blood. Control antibiotic polymyxin B produced a 3 log killing both in the peritoneal fluid and in blood at the dose used.

Figure2: Dose response curve of AA139 in peritoneal fluid (left) and blood (right) tested against *E. coli* EC109-09 in the neutropenic murine peritonitis infection model.



Conclusion

- Clinical candidate AA139 showed a potent antibacterial effect in the bacteraemia / peritonitis infection model in neutropenic mice.
- In these two studies, strains with different resistance profile and with a 8-fold difference in MIC were compared.
- The higher MIC did not seem to affect efficacy of AA139 as ED_{50}'s, and 1, 2 and 3 log killing data appeared to be only marginally affected.

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