The in vitro and in vivo efficacy of A3-APO, a new antibacterial peptide against multiresistant bacteria

Doctoral (Ph.D.) thesis

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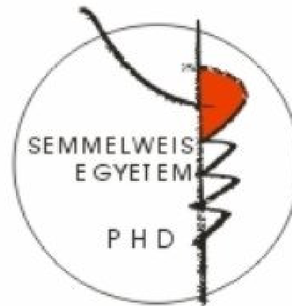

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1. Introduction

One of the major problems of current antimicrobial research is the increased rate of bacterial resistance in humans compared to the discovery and development of new antimicrobial agents. Pyrrhocoricin is a naturally occurring antibacterial peptide, isolated from the European sap-sucking bug, *Pyrrhocoris apterus*. A number of pyrrhocoricin analogs were designed from which a dimeric version named A3-APO emerged as the most attractive preclinical and clinical candidate. As most other antibacterial peptides, A3-APO selectively destabilizes the bacterial membrane structure without having major effects on eukaryotic cells. In addition, as a special feature of some insect-originated antibacterial peptides, A3-APO inhibits DnaK, the 70 kDa bacterial heat shock protein. Peptide A3-APO is resistant against the proteolytic digestion activity of body fluids.

Encouraged by positive in vitro activity of A3-APO against resistant pathogens, we measured of in vivo efficacy in various mouse models of human infections. Toxicological tests showed that the safest administration is intramuscular (im), where we did not find any effect at a 50 mg/kg dose. A brief pharmacokinetic assay, using trichloroacetic acid to precipitate serum proteins, did not yield clues to the optimal dose.

Nevertheless, at 5 mg/kg im A3-APO has proven effective in different systemic *Escherichia coli* and *Acinetobacter baumannii* infections, and in *A. baumannii* burn wound infections in mouse models. Based on these in vivo efficacy assays, A3-APO has the potential to represent a new antibiotic family against multidrug-resistant bacteria.

2. Aims

1. Our aim was to compare the in vitro antibacterial spectrum and efficacy of A3-APO and of its parent compound, pyrrhocoricin.
2. With pharmacokinetic assays, we planned to characterize the blood levels of A3-APO after im administration in mice.
3. Toxicity tests were performed to determine the maximum administered A3-APO dose which causes no systemic adverse effects.
4. Our aim was to determine the effective intraperitoneal (ip) dose of A3-APO. The readout of success was the reduction in blood bacterial counts to a level similar to that achieved by 40 mg/kg imipenem in systemic *E. coli* mouse infections. Meeting these criteria means that A3-APO can serve as a new alternative treatment against multidrug-resistant, ESBL-producing *E. coli* infections.
5. Our aim was to examine the efficacy of iv or im administration of A3-APO in systemic infections caused by an MBL-producing *A. baumannii* strain.
6. We tried to cure *A. baumannii* wound infections and the consequential systemic infection with im administered A3-APO.
7. Our objective was to show that A3-APO administered daily once im at 5 mg/kg protects mice from nosocomial infections, whether we induced these experimentally with *A. baumannii*, or the causative agents were other contaminating bacteria found in the assay environment.

3. Materials and methods

3.1. Used bacterial strains

For the in vitro MIC measurements we used 13 different *E.coli,* 13 *Klebsiella pneumoniae,* 2 *Salmonella enterica* serovar Typhimurium*,* 5 *Enterobacter cloacae,* 9 *A. baumannii,* 5 *Proteus vulgaris,* 3 *Enterococcus faecalis* and 8 *Pseudomonas aeruginosa* strains. Some of these strains were purchased from the American Type Culture Collection, others were clinical isolates.

In systemic infections the ATCC strains, *E. coli* 5770 and Neumann, or *A. baumannii* BAA 1805 were used. This latter strain was also used in our burn wound infection models. *E. coli* 5770 produces ESBL, *A. baumannii* BAA 1805 produces MBL.

3.2. In vitro antibacterial assays

Two different antibacterial growth inhibition assays were performed. In the first set of assays, in 96-well polypropylene plates 50 µl of suspension of midlogarithmic phase bacterial cultures diluted to $5x10^5$ cfu/ml in Müller-Hinton broth was added to 50 µl of serially diluted peptides. Cultures were incubated at 37 °C for 16-20 hours without shaking. Growth inhibition was measured by recording the absorbance at 600 nm using a microplate reader. MICs were identified as the lowest antimicrobial doses when the 600 nm absorbance did not exceed the value obtained for media only. In the second round, we confirmed the results obtained with automatic plate readers using a readout resembling to more conventional MIC assays. A3-APO was two-fold serially diluted from 256 to 0,5 mg/l in Müller-Hinton broth. 100 µl of each dilution was inoculated with 10 µl of 0,5 McFarland bacterial suspension.

Incubation was performed at 37 °C for 24 hours without shaking, and determination of MIC was made with the naked eyes.

3.3. In vivo assays

3.3.1. Animals

Female CD-1 mice of 20-25 g (Charles River Hungary, Budapest) were used for the in vivo efficacy studies. Mice were housed in plastic type 2 cages, three to five mice per cage on softwood granules as bedding. The room was kept between 21 °C and 25 °C with 12: 12 h light: dark cycles. The animals had free access to tap water and pelleted rodent food. The assays were initiated after a three-week acclimatization period. Upon completion of the experiments, the mice were euthanized with 150 mg/kg sodium barbiturate (Sigma Aldrich, Budapest, Hungary). The location and handling of animals were consistent with the guidelines of the Government Decree 243/1998 about animal testing, were in compliance with the protocol approved by the Animal Experimental Committee of Semmelweis University, and the Animal Experimental Work Commission, with a license number: 399/003/2005.

3.3.2. Toxicity assays

We investigated the acute toxicity of A3-APO after 10, 25 and 50 mg/kg ip single doses. The repeated dose toxicity tests used the same doses of peptides, as the efficacy assays, 10, 20 and 40 mg/kg ip three times in every four hours.

Iv toxicity values were determined after a single dose of 10, 25 and 50 mg/kg A3-APO.

The im toxicity analysis was done in two consecutive assays. We started with 20, 40, and 60 mg/kg bolus doses, and later expanded the scope to 25, 50, 75, and 100 mg/kg bolus doses.

3.3.3. Pharmacokinetic assay

For the pharmacokinetic analysis 5 mg/kg A3-APO was injected im into CD-1 mice. One hundred µl of blood was taken from the eye at 0,5, 15, 45, 90 and 120 min after peptide administration. Cells were centrifuged at 5000 rpm and 20 µl of aqueous 15% trichloracetic acid was added per 60 µl of plasma. After repeated centrifugation at 12000 rpm, 0,5 µl of supernatant was loaded on to a matrix-assisted laser ionization/desorption time-of-flight tandem mass spectrometer, using α-cyano-4-hydroxycinnamic acid as the matrix. In addition, a combined volume of 210 µL supernatant was loaded to a C18 high performance liquid chromatography column that had previously been calibrated with 50 ng, 200 ng, 500 ng and 1 µg peptide A3-APO dissolved in PBS. Absorbance was measured at 214 nm.

3.3.4. Efficacy assays in the treatment of *E. coli* systemic infections

First 1,1 x 10^7 CFU/g *E. coli* Neumann was injected ip into CD-1 mice. Forty mg/kg imipenem, or 2,5, 10 or 20 mg/kg A3-APO was administered ip at 30 min, 4 h and 8 h after infection. The survival rate was recorded 5 days post treatment.

For efficacy assay two, mice were challenged with $1,6x10^8$ CFU/g mouse of *E. coli* 5770 in physiological dextran solution. At 4, 8 and 12 hour after challenge, 2,5 mg/kg, 5 mg/kg or 10 mg/kgA3-APO or 40 mg/kg imipenem was administered ip.

In assay 3, three days prior to challenge mice were pre-treated with a single 18 mg/kg dose of cisplatin. An amount of $1,2x10^8$ CFU/g *E. coli* 5770 was injected ip. At 4, 8 and 12 hours after infection 10 mg/kg or 20 mg/ kg A3-APO or 40 mg/kg imipenem was administered ip.

In assay 2 and 3 the survival rate was monitored until 5 days post infection. Prior to drug administration at all three time points and 4 h later, 10 µl of blood was taken from the tail vein of 3-3 mice to determine blood bacterial counts.

3.3.5. Efficacy assays in the treatment of *A. baumannii* systemic infections

In the intravenous assay $4x10^7$ cfu/g *A. baumannii* BAA-1805 was injected ip into CD-1 mice. A3-APO or imipenem were administered iv at a 2,5 mg/kg dose at 4, 8 and 12 hours post-infection.

In the first im assay mice were infected ip with $2x10^6$ CFU/g *A. baumannii* BAA-1805. Four, 8 and 12 hours after infection 5 mg/kg A3-APO or 40 mg/kg imipenem were administered im.

In the second im assay mice were challenged ip with $3x10^7$ CFU/g *A. baumannii* BAA-1805, and 4 and 8 hours after infection 5 mg/kg A3-APO or 40 mg/kg imipenem were administered im.

In all three assays the survival rate was monitored until 24 hours post-infection. Prior to drug administration at all three time points and 4 h later, 10 µl of blood was taken from the tail vein of mice to determine blood bacterial counts.

3.3.6. Efficacy assays in wound infections

In each experiment we created a standard size wound on the back of the mice. The animals were anesthetized and burn injury was inflicted by pressing a 1 g hot metal weight (diameter 11 mm, temperature 90 ^{o}C) to their shaved dorsal skin. The weight was pressed to skin for 2 min. Three hours after burn injury, *A. baumannii* BAA-1805 was injected into the wound.

A assay: An amount of $2x10^{3}$CFU/wound/day of *A. baumannii* was injected into six mice on 5 consecutive days. Three mice were treated with 5 mg/kg A3-APO on days 0,1,2,3 and 4 immediately after infection. Blood was taken from the tail vein 3 hours after the first inoculation, and on the second and fourth days. On day 5 the mice were killed by diethyl ether inhalation, their wounds were excised and their wound bacterial load was counted.

B assay: An amount of $7,2x10^{9}$CFU/wound *A. baumannii* was injected once into mice. Mice were treated immediately after infection with a single im dose of 5 mg/kg A3-APO, 5 mg/kg colistin or 40 mg/kg imipenem. Blood was taken from the tail vein and blood bacterial counts were determined 1, 2, 3 and 4 hours after challenge. Surviving mice were photographed on day 5 and killed by diethyl ether inhalation, their wounds were excised and the bacterial load in the wound was counted.

C assay: An extremely high dose, $2x10^{11}$ CFU/wound *A. baumannii* was injected into the mice. Immediately after infection and in 4 consecutive days, the animals were treated with 5 mg/kg A3-APO, 5 mg/kg colistin or 40 mg/kg imipenem. On day 5 the mice were killed by diethyl ether inhalation, their wounds were excised and their wound bacterial load was counted.

Effect of A3-APO on uninfected burn wounds: Four mice did not receive any medical intervention after burn injury and four mice received 5 mg/kg A3-APO im 3 hours later, and on 4 consecutive days. On day 5 the mice were killed by diethyl ether inhalation, and their wounds were removed and fixed in 10% buffered formaldehyde pH, 7,4. After 24 h fixation, the specimens were dehydrated in graded ethanol and placed in methylbenzoate for 1h then transferred to xylene and embedded in paraffin. Six μm thick paraffin sections were deparaffinized and stained with haematoxylin and eosin. Photomicrographs were taken using a Zeiss light microscope. The final magnification of the picture was 100-fold.

4. Results

4.1. In vitro antibacterial efficacy

The MIC of A3-APO against 5 different *P. vulgaris*, 3 *E. faecalis* and 7 *P. aeruginosa* strains was higher than 64 mg/l. Against one *P. aeruginosa* strain the MIC value was 64 mg/l. A3-APO exhibited a MIC activity range of 2 mg/l to 64 mg/l against the *E. coli* and *K. pneumoniae* strains.

A3-APO exhibited 8 mg/l MIC value against one of the two tested *S. enteritica* serovar *typhimurium* strains and 32 mg/l against the other.

The measured MIC values against the 5 *E. cloacae* strains were: 4, 8, 8, 8 and 16 mg/l respectively.

The MIC of 9 different *A. baumannii* strain were the following: 32 mg/l (3), 64 mg/l (3), and >64mg/l (3).

4.2. Results of the in vivo assays

4.2.1. Results of the toxicity tests

The No Observed Adverse Effect Level (NOAEL) after ip administration was determined at 20 mg/kg, after a single iv challenge at 10 mg/kg, and after a single im dose at 50 mg/kg, respectively. While at 50 mg/kg A3-APO did not cause any toxic effect im it was lethal to the mice when administered at the same dose ip or iv. After im challenge 100 mg/kg A3-APO caused only general malaise.

4.2.2. Result of the pharmacokinetic assay

We failed to detect any free A3-APO in mouse sera, using TCA as detergent, in the time period 5 min to 2 h after im administration.

4.2.3. Results of the treatments of *E. coli* systemic infections

At time intervals 30 min, 4 and 8 h after the development of systemic infection triggered by *E. coli* Neumann ip challenge, the antimicrobials were administered also ip. This 30 min time-interval was enough for the equable distribution of bacteria all over the body. While untreated animals died within 8-10 h and 2,5 mg/kg dose of peptide was inactive, the 10 and 20 mg/kg peptide doses were highly active with long-term (5 days) survival rates of 80 % and 100 %, respectively. The 40 mg/kg imipenem treatment was also effective with a 100 % survival rate. Imipenem, 20 mg/kg A3-APO, as well as 10 mg/kg peptide improved the

survival time statistically significantly (p=0,00467). However no significant difference was measured between 10 mg/kg A3-APO and 40 mg/kg imipenem treatment (p= 0,94058).

Humans are rarely treated immediately after pathogen acquisition. Thus in our second animal model treatment was started 4 h after infection. 1,6x10^8 CFU/g *E. coli* 5770 bacteria were inoculated ip in the presence of dextran in order to feed up peritoneal macrophages and slow down the development of sudden sepsis and septic shock. Twenty and 10 percent of the negative control, untreated mice survived for 19 and 25 h, respectively. The 5-day survival rate of the imipenem treated animals was 90%. A3-APO treatment was successful in a dose-dependent manner, the early (19 h post infection) survival rate was 20%, 50% and 80% at 2,5, 5 and 10 mg/kg doses, respectively. The survival curves of A3-APO 10 mg treatment and of the untreated group were significantly different (p=0,02222). Examining the impact on early survival of 10 mg/kg A3-APO or 40 mg/kg imipenem treatment, there was no significant difference (p= 0,60495). Less impressive was the late survival rate (25 h), which was determined to 10%, 30% and 20% at 2,5 mg/kg, 5 mg/kg and 10 mg/kg doses of A3-APO. Owing to rapid endotoxin release that kills animals during antimicrobial therapy, blood bacterial counts are more sensitive indicators of the efficacy. In the infected non-treated animals, blood bacterial counts increased to 10^7 CFU/ml by 4h and to 10^8 CFU/ml by 12 h after infection. The first dose of 10 mg/kg A3-APO decreased the bacterial count to the sub-10^5 CFU/ml range. The second dose of peptide further decreased the bacterial count below the detection limit. Long term bacterial counts were around 10^4 CFU/ml values. After the first dose of 40 mg/kg imipenem treatment the bacterial counts decreased to 10^5 CFU/ml, after the second dose decreased further to 10^4 CFU/ml. Compared to the untreated group, 10mg/kg A3-APO statistically significantly decreased the bacterial counts (p=0,0109). There was no significant difference between the 40 mg/kg imipenem or 10 mg/kg A3-APO treated mice (p=0,1204).

Peptide drugs are subject to kidney clearance, which is 8-10-fold faster in mice than in primates. To make the mouse model more suitable for testing the efficacy of peptide antibiotics, in the third assay mice were pretreated with a single dose of cisplatin 3 days prior to bacterial challenge. This exercise reduces the kidney clearance rate of mice to approximately the levels found in humans. However, the nephrotoxic cisplatin pre-treatment made the animals generally sick. Whilst the infection was as successful as without cisplatin, (10% survival of untreated mice 48 h post infection), the antibacterial treatments were not as successful. The 40 mg/kg imipenem dose protected only 83%, and 56 % mice after cisplatin instead of 90% without this pre-treatment, at 24 and 48 h, respectively. Peptide A3-APO added at 10 mg/kg was quite efficacious, with 70% and 40% survival rates at 24 and 48 h. Increasing the A3-APO dose to 20 mg/kg did not result better results, survival rates were 60% and 20% at 24 h and 48 h post infection. Blood bacterial counts of untreated animals continuously increased and reached 10^7 CFU/ml after 12 h. The first dose of imipenem reduced blood bacterial counts to 10^5 CFU/ml, the second dose reduced counts below the detection limit of 10^3 CFU/ml. The first dose of A3-APO resulted in a 40- and 60-fold reduction in bacterial counts at 10 mg/kg and 20 mg/kg, respectively. After the second dose the percentage of mice without bacterium in blood was 100% and 40% at 10 mg/kg and 20 mg/kg, respectively. At 8h after infection, the decrease of bacterial counts was equally significant for 40 mg/kg imipenem (p=0,01808), 10 mg/kg (p=0,01298) or 20 mg/kg (p=0,01271) A3-APO treatment.

4.2.4. Results of the treatments of A. baumannii systemic infections

After ip infection, iv administered 2,5 mg/kg A3-APO reduced the blood bacterial counts by 0,5 $\log_{10}$ units after 2 doses at 4 and 8 h after infection, and 1,5 $\log_{10}$ units after the

third dose at 12 h. The peptide prolonged the early survival rate better than 2,5 mg/kg imipenem. At 12 h after infection, while all the seven A3-APO treated mice were alive, from the imipenem treated or untreated animals 3 already succumbed to bacteremia.

When comparing the efficacies after im drug administration, for imipenem we used the 40 mg/kg dosing recommended for clinical applications. Imipenem reduced the blood bacterial counts to the 10^6 CFU/ml and 10^3 CFU/ml range at 8 and 12 h after infection. Although A3-APO decreased the bacterial counts only to 10^5 CFU/ml or 10^4 CFU/ml range at this time, the peptide still prolonged the survival better than imipenem. At 18 h the number of survival mice was 5/7 in the peptide-treated group and only 3/7 in the imipenem-treated group.

In the third systemic infection assay we treated the animals im at 4 and 8 h after infection with 40 mg/kg imipenem or 5 mg/kg A3-APO. Imipenem reduced the blood bacterial counts by 2 $\log_{10}$ units, and the number of surviving mice was only 2 at 8h post-infection. Although A3-APO reduced the bacterial counts only by 1-1 $\log_{10}$ units at 4 and 8 h after infection, all the seven mice were alive at 8h post-infection from this treatment group.

4.2.5. Results of the treatments of burn infections

Assay A: Due to the low daily bacterial inocula, no bacterium was found in the blood 3 h after the first inoculation or 2 or 4 days later regardless of treatment status. On day 5 the wound bacterial counts of A3-APO treated mice reduced to $3,4x10^1$ CFU/mg, compared to the untreated group which exhibited wound bacterial counts of $1,5x10^4$ CFU/mg.

Assay B: Two (from 7) of the untreated, control mice succumbed to bacterial sepsis and died on day 2 reflecting the increased bacterial load. At the time period of 1-3 h after infection mice receiving no drug, or dosed with imipenem or colistin were sick but later

recovered. The A3-APO treated animals had no visible signs of discomfort. The blood bacterial counts immediately after infection mirrored the behavioral signs. One hour after the first dose of antimicrobials, the bacterial load in the blood of untreated, colistin-treated and imipenem-treated animals exceeded 10^6 CFU/ml. In contrast, the blood bacterial counts of A3-APO-treated mice were in the low 10^3 CFU/ml range. After 5 days, surviving mice still had wounds on their backs, but the wound sizes were dependent upon the treatment they received. Untreated mice carried the most extensive lesions. The colistin-treated and imipenem-treated animals had smaller wound sizes but the damage was not localized to the burn site. Mice receiving A3-APO treatment had small wounds with clear surroundings. The wounds of untreated animals contained the highest bacterial load (10^7CFU/mg), followed by imipenem-treated (10^6CFU/mg) and colistin-treated (10^5CFU/mg) mice. A3-APO-treated mice had wound bacterial loads close to the lowest detection limit of 1,000 CFU/mg tissue. There were significant differences between the wound bacterial counts of untreated and A3-APO-treated animals, p=0,0367, and between the colistin-treated and A3-APO-treated wounds, p=0,0020.

Assay C: The very high infectious dose had a major effect on the gross health condition of the mice. Two (from 7) animals from the control group died on day 2. The imipenem-treated mice survived but were generally very sick, and the colistin-treated mice were sick after each treatment although they recovered after 1 h. Mice receiving A3-APO-treatment looked healthy throughout the experiment. The higher bacterial inocula compared to Assay B was more than compensated for with the daily antibiotic treatment. By day 5 imipenem reduced the wound bacterial counts by 100-fold to 10^5CFU/mg tissue and colistin managed to further reduce it to the mid-10^3 CFU/mg range. A3-APO-treated mice had less than 1,000 CFU/mg *A. baumannii* in their wounds.

Promotion of wound healing without direct pathogen inoculation: Burn wounds are often infected with invading environmental microbes. We also wanted to examine whether peptide A3-APO can improve wound healing when bacteria reach the injury site via environmental contamination. In one group of mice the burn injury was untouched for 5 days and in another group mice were treated five times with A3-APO after injury infliction. The wound tissues were removed after 5 days. In the tissue of untreated mice, more than half of the epithelial region was excoriated. Under the epithelium, a homogeneous eosinophilic and necrotic material was observed that contained polymorphonuclear pus cells and was surrounded with loose granulation tissue. In contrast, in the tissue of A3-APO-treated mice there were clear signs of a regenerated epithelial layer under the necrotic surface, without pus cells and with decreased levels of inflamed granulation.

5.Conclusions

1. Based on the MIC determination against 57 bacterial strains, the designer proline-rich peptide A3-APO retained, or exceeded the in vitro efficacy of the parent native proline-reach antibacterial peptides. A3-APO is especially effective against bacteria of the *Enterobacteriaceae* family, with an exception of the *Proteus vulgaris* species. The peptide exhibits medium efficacy against *A. baumannii,* but it is almost ineffective against *Pseudomonas aeruginosa* and *Gram*-positive bacteria. The low level of activity against these bacteria is due to the effect on the bacterial membrane, while against the other A3-APO-sensitive bacteria this effect is enhanced by inactivating DnaK, an intracellular chaperone protein.

2. With our pharmacokinetic assays, we were unable to detect A3-APO in the blood after im dosing in 0-90 min interval after the challenge and we were unable to quantitate either the rate of elimination or the maximum achievable serum levels. In vitro in serum long half-life was measured, but perhaps after im administration the peptides bound to a carrier protein. After the binding the antibacterial effect remains strong, the toxicity parameters improve, but the peptide is undetectable after TCA precipitation of serum proteins. It is also possible, that peptide binds to a cellular element, it is effective in vivo against infections, but undetectable from the cell-free sera. Modified pharmacokinetic assays and in vitro cell culture measurements with various blood cells may answer these questions.
3. With our toxicity assays we determined the No Observed Adverse Effect Level (NOAEL) of A3-APO after ip, iv or im administration. After a single ip dose of 10 mg/kg peptide challenge we did not observe any adverse effect, but at a single dose of 25 mg/kg the animals were extremely sick. At repeated ip dosage we determined the NOAEL at 20 mg/kg. The NOAEL of a single iv dose is 10 mg, and increasing the dose to 25 mg/kg we already observed systemic toxicity. After im administration the NOAEL was determined as 50 mg/kg after bolus peptide administration, with increasing the dose to 75 mg/kg A3-APO resulting transient toxic effects. Both iv and ip doses of 50 mg/kg A3-APO was lethal.
4. With comparative studies in *E. coli* systemic infections, 40 mg/kg imipenem reduced the bacterial counts as much as 10 mg/kg A3-APO did. Unfortunately, A3-APO is likely to induce a sudden release of large amounts of endotoxin, an event that reduces the chances of mice to survive. In the treatment of infections triggered by multidrug resistant ESBL-producing *E. coli*, A3-APO represents a viable alternative treatment

option. To prevent the sudden endotoxin release, A3-APO is recommended to be used in lower doses, in combination with synergistic non-peptide antibiotics.

5. In systemic infections with an MBL-producing *A. baumannii* strain, iv administered A3-APO at 2,5 mg/kg or im administered A3-APO at 5 mg/kg decreased not only the blood bacterial counts, but increased the survival rate of mice compared to the untreated group.
6. Two different amounts of multidrug-resistant *A. baumannii* inoculums were used to develop wound infections. In the less heavy infection regimen, A3-APO administered at 5 mg/kg not only prevented bacteremia better than similar treatment using 40 mg/kg imipenem or 5 mg/kg colistin, but also reduced the wound bacterial count significantly more effectively. Even a heavier systemic infection and the coexisting wound infection were successfully treated with a daily A3-APO treatment applied for 5 days.
7. We proved, that A3-APO protects mice from infections, whether we induced it experimentally with *A. baumannii*, or the causative agent were other contaminant bacteria found in the assay environment. With this study we confirmed that daily once im administered A3-APO at 5 mg/kg protects mice from nosocomial infections.

6. Publications

6.1.Publications, which form the basis of the thesis

1. Rozgonyi F, Szabo D, Kocsis B, **Ostorhazi E,** Abbadessa G, Cassone M, Wade JD, Otvos L, Jr. (2009) The antibacterial effect of a proline-rich antibacterial peptide A3-APO. Curr Med Chem, 16: 3996-4002. **IF: 4,708**

2. Szabo D[1], **Ostorhazi E[1]**, Binas A, Rozgonyi F, Kocsis B, Cassone M, Wade JD, Nolte O, Otvos L, Jr. (2010) The designer proline-rich antibacterial peptide A3-APO is effective against systemic Escherichia coli infections in different mouse models. Int J Antimicrob Agents, 35: 357-361. **IF: 3,032**
3. **Ostorhazi E**, Rozgonyi F, Szabo D, Binas A, Cassone M, Wade JD, Nolte O, Bethel CR, Bonomo RA, Otvos L, Jr. (2010) Intramuscularly administered peptide A3-APO is effective against carbapenem-resistant acinetobacter baumannii in mouse models of systemic infections. Biopolymers, 96: 126-129. **IF: 2,605**
4. **Ostorhazi E**, Rozgonyi F, Sztodola A, Harmos F, Kovalszky I, Szabo D, Knappe D, Hoffmann R, Cassone M, Wade JD, Bonomo RA, Otvos L, Jr. (2010) Preclinical advantages of intramuscularly administered peptide A3-APO over existing therapies in *Acinetobacter baumannii* wound infections. J Antimicrob Chemother, 65: 2416-2422. **IF: 4,352**

6.2. Publication related to the thesis

1. **Ostorhazi E**, Holub MC, Rozgonyi F, Harmos F, Cassone M, Wade JD, Otvos L, Jr. (2011) Broad-spectrum antimicrobial efficacy of peptide A3-APO in mouse models of multidrug-resistant wound and lung infections cannot be explained by in vitro activity against the pathogens involved. Int J Antimicrob Agents, 37: 480-484. **IF: 3,032**

6.3. Further publications

1. Rozgonyi F, **Ostorhazi E**, Marodi CL, Ghidan A. (2001) Resistance to beta-lactams and glycopeptides in staphylococci and streptococci. Acta Microbiol Immunol Hung, 48: 359-391.
2. Ponyai K, Marschalko M, Schoffler M, **Ostorhazi E**, Rozgonyi F, Varkonyi V, Karpati S. (2009) Analysis of syphilis and gonorrhoea cases, based on data from the National STD Centre, Department of Dermatology and Venerology, Semmelweis University (2005-2008). Orv Hetil, 150: 1765-1772.
3. Pinter G, Batta G, Keki S, Mandi A, Komaromi I, Takacs-Novak K, Sztaricskai F, Roth E, **Ostorhazi E**, Rozgonyi F, Naesens L, Herczegh P. (2009) Diazo transfer-click reaction route to new, lipophilic teicoplanin and ristocetin aglycon derivatives with high antibacterial and anti-influenza virus activity: an aggregation and receptor binding study. J Med Chem, 52: 6053-6061. **IF: 4,802**
4. Pinter G, Bereczki I, Batta G, Otvos R, Sztaricskai F, Roth E, **Ostorhazi E**, Rozgonyi F, Naesens L, Szarvas M, Boda Z, Herczegh P. (2010) Click reaction synthesis of carbohydrate derivatives from ristocetin aglycon with antibacterial and antiviral activity. Bioorg Med Chem Lett, 20: 2713-2717. **IF: 2,650**
5. Pinter G, Bereczki I, Roth E, Sipos A, Varghese R, Udo EE, **Ostorhazi E**, Rozgonyi F, Phillips OA, Herczegh P. (2011) The effect of systematic structural modifications on the antibacterial activity of novel oxazolidinones. Med Chem, 7: 45-55. **IF: 1,642**
6. Toth V, Hornyak C, Kovacs T, Toth B, Varallyay G, **Ostorhazi E**, Koles J, Bereczki D, Marschalko M, Karpati S. (2011) Meningovascular neurosyphilis as the cause of ischemic cerebrovascular disease in a young man. Orv Hetil, 152: 763-767.