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ARTICLE

Efficacy of cecropin A-melittin peptides on a sepsis model of infection by pan-resistant *Acinetobacter baumannii*

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Abstract Pan-resistant *Acinetobacter baumannii* have prompted the search for therapeutic alternatives. We evaluate the efficacy of four cecropin A-melittin hybrid peptides (CA-M) in vivo. Toxicity was determined in mouse erythrocytes and in mice (lethal dose parameters were LD_0, LD_{50}, LD_{100}). Protective dose 50 (PD_{50}) was determined by inoculating groups of ten mice with the minimal lethal dose of *A. baumannii* (BMLD) and treating with doses of each CA-M from 0.5 mg/kg to LD_0. The activity of CA-Ms against *A. baumannii* was assessed in a peritoneal sepsis model. Mice were sacrificed at 0 and 1, 3, 5, and 7-h post-treatment. Spleen and peritoneal fluid bacterial concentrations were measured. CA(1–8)M(1–18) was the less haemolytic on mouse erythrocytes. LD_0 (mg/kg) was 32 for CA(1–8)M(1–18), CA(1–7)M(2–9), and Oct-CA(1–7)M(2–9), and 16 for CA(1–7)M(5–9). PD_{50} was not achieved with non-toxic doses ($\leq LD_0$). In the sepsis model, all CA-Ms were bacteriostatic in spleen, and decreased bacterial concentration ($p<0.05$) in peritoneal fluid, at 1-h post-treatment; at later times, bacterial regrowth was observed in peritoneal fluid. CA-Ms showed local short-term efficacy in the peritoneal sepsis model caused by pan-resistant *Acinetobacter baumannii*.

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Introduction

Acinetobacter baumannii is a Gram-negative coccobacillus that causes an increasing number of mainly nosocomial infections, with pneumonias, bacteremias, urinary tract infections, surgical site infections, and meningitis as the most frequent ones [1]. This pathogen has relevant clinical implications as it survives on almost every surface and develops resistance to all available antibiotics [2]. Thus, several outbreaks associated with a high morbidity and mortality have been caused by multi-drug resistant or even pan-resistant *A. baumannii* strains [3, 4], with a dramatic reduction in the available antimicrobial armamentarium, including the last gold-standard imipenem [5, 6], and colistin [7]. Therefore, an urgent need exists for new agents to treat MDR *A. baumannii* nosocomial infections.

Eukaryotic antimicrobial peptides (EAPs) are important components of the innate immune response of higher organisms against invasive pathogens [8]. Despite their diverse size and structures, nearly all EAPs have a strong cationic character and fold into amphipathic structures, two features that are critical for bacterial killing. This is achieved

by disruption of the pathogen membranes, by stoichiometric interaction of the EAP with the anionic phospholipids on the extracellular leaflet of the cell membrane of prokaryotes and lower—though not higher—eukaryotes [9–12]. As a result, EAPs show broad spectrum activity, fast killing rates, and scarce induction of resistance. Other advantages are their anti-endotoxic properties [13–15] and potential synergism with classical antibiotics. On the downside are their rather short life in biological fluids, poor tissue penetration, and high production costs [9, 10]. Being gene-encoded, EAPs show simple, if any posttranslational modifications, which makes optimization of their microbicidal activity easily achievable by genetic or chemical manipulation of the primary structure [16, 17]. In this regard, the cecropin A-melittin (CA-M) hybrid peptides are paradigmatic. CA-M peptides are formed by juxtaposing the cationic N-amino terminus of cecropin A (CA) to the hydrophobic N-terminus of melittin (M) [18, 19]. The result is a significant reduction of the toxicity with respect to M, and a substantial improvement of the antibiotic activity on Gram-positive bacteria, fungi and protozoa relative to CA [19–22].

In a previous work we demonstrated the in vitro antibiotic efficacy of four CA-M hybrids against four pan-resistant *A. baumannii* strains by membrane permeabilization, regardless of the resistance pattern of the strain [23, 24]. The present study aims to determine the in vivo potential of CA-M peptides as chemotherapeutic alternative on *A. baumannii*, evaluated on an experimental model of peritoneal sepsis.

Material and methods

Bacterial strains

The clinical pan-resistant *A. baumannii* strain 208628 (Ab208628), resistant to cefotaxime, ceftazidime, imipenem, amoxicillin, amikacin, piperacillin/tazobactam, doxycycline and colistin, was chosen from a previous work by its high colistin MIC (64 μg/mL) [23]. The isolate was stored at −70°C until required.

Antimicrobial peptides

Peptides (Table 1) were synthesized by solid phase methods using Fmoc chemistry as described [21]. They were purified by reverse phase HPLC (>98%), characterized by MALDI–TOF mass spectrometry and quantified by tryptophan absorbance [21].

Animals

For the sepsis animal model, immunocompetent C57BL/6 female mice [25] (16–20 g), obtained from the animal facility of Seville University were used. Animals had pathogen-free sanitary status and assessed genetic authenticity. For haemolytic activity assays, BALB/c mice (22–28 g, from the animal facility at Centro de Investigaciones Biológicas, Madrid, Spain) were chosen due to their high blood volume (10.75 mL/100 g) [26]. Animals were housed in regulation cages with food and water ad libitum. Surviving mice were sacrificed after 7 days with a 5% (wt/vol) overdose of thiopental. Clearance from the Ethics and Clinical Research Committee of the Virgen del Rocío University Hospitals and Centro de Investigaciones Biológicas were obtained.

Haemolytic activity

Haemolysis was chosen as a parameter for peptide cytotoxicity on mice cells. Erythrocytes from blood withdrawn by cardiac puncture were washed twice in Hank's medium (140 mM NaCl, 4.0 mM Na_2HPO_4, 1.0 mM KCl, 4.8 mM $NaHCO_3$, 10 mM D-glucose, pH 7.2) to eliminate serum and free haemoglobin. The resulting erythrocytes were resuspended in the same medium at 4×10^7 cells/mL; 50 μL aliquots were transferred into Eppendorf tubes. An equal volume of the peptide in Hank's was added. After incubation (4 h, 37°C), the suspension was centrifuged (Hettich, Mikro 200, 3 min, 3000 x *g*). An aliquot (90 μL) of the supernatant, containing the released haemoglobin, was carefully withdrawn, transferred into a 96-microwell plate, and measured in a BioRad 680 plate reader at 550 nm. Full haemolysis (100%) was achieved by lysis with Triton X-100 (0.1%, final concentration) [27]. Experiments were repeated at least twice using samples by triplicate.

In vivo toxicity

The Reed and Munch method [28] was applied. Groups of six C57BL/6 mice were intraperitoneally (i.p.) inoculated with a single 250 μL dose of peptide, starting at 0.5 mg/kg and serially duplicated until 100% mortality was reached. Maximum tolerated dose (LD_0), lethal dose 50 (LD_{50}), and lethal dose 100 (LD_{100}), were defined as those causing 0%, 50%, and 100% mortality, respectively. Other signs of toxicity (piloerection, handicapped motility) were additionally monitored.

Determination of bacterial minimal lethal dose (BMLD) and bacterial lethal dose 50 (BLD_{50})

To assess BMLD (minimal inoculum causing a 100% mortality) and BLD_{50}, groups of ten C57BL/6 mice were inoculated i.p. with 250 μL of a ten-fold serially diluted inoculum, starting at 10^8 CFU/mL [28], obtained from a

Table 1 In vivo toxicity of cecropin A-melittin hybrid peptides in C57BL/6 mice

Peptide	Sequence	MW	LD_0[a]	LD_{50}[a]	LD_{100}[a]
CA(1–8)M(1–18)	KWKLFKKIGIGAVLKVLTTGLPALIS-NH_2	2793.78	32.0	64.0	128.0
CA(1–7)M(2–9)	KWKLFKKIGAVLKVL-NH_2	1770.19	32.0	45.2	64.0
Oct-CA(1–7)M(2–9)	$^1N^{\alpha}$-octyl -KWKLFKKIGAVLKVL-NH_2	1898.50	32.0	45.2	64.0
CA(1–7)M(5–9)	KWKLFKKVLKVL-NH_2	1544.07	16.0	39.4	128.0

LD_0 maximum tolerated dose, *LD_{50}* lethal dose 50, *LD_{100}* lethal dose 100

[a] Expressed as mg/kg

24-h Ab208628 culture in Mueller-Hinton II broth, cation adjusted (MHBCA; Becton Dickinson, Cockeysville, MD) at 37ºC. Dilutions were done with saline solution plus 5% (wt/vol) porcine gastric mucin (Sigma Chemical Co., St Louis, MO, USA). The animals were observed for 7 days and their cumulative survival rates recorded.

As a control group, ten mice were inoculated with 250 μL of porcine mucin as above, to discard an intrinsic toxic effect of mucin.

Determination of protective dose

The protective dose for 50% of the population (PD_{50}) was determined for each peptide [28]. Groups of ten mice were infected i.p. with a BMLD inoculum. Four hours later, animals were treated i.p. with two-fold increasing doses of each peptide (0.5 mg/kg to LD_0), in physiological serum (0.5 mL final volume), and observed for 7 days to measure cumulative survival rates.

Influence of serum on activity of CA(1–8)M(1–18)

Two complementary experiments were carried out: (1) CA (1–8)M(1–18) at 25 μg/mL (80% haemolysis) was mixed with serum at different concentrations (0–20% vol/vol) and incubated (15 min, 37°C) prior to its addition to the erythrocyte suspension. Afterwards, the haemolysis was assayed as above. (2) Variation of haemolysis with CA (1–8)M(1–18) concentration (0–200 μg/mL), was checked at a fixed 10% serum concentration. CA(1–8) M(1–18) was then incubated with 10% autologous mouse serum (15 min, 37°C), prior to its addition to erythrocyte suspension, and haemolysis assayed by the standard procedure.

The inhibition by serum of the bactericidal activity of CA(1–8)M(1–18) and its all-D enantiomer was tested at a concentration equal to their MIC calculated in the absence of serum (4 μg/mL). To this end, peptide was incubated with serum (0–20% vol/vol) for 15 min at 37°C before its addition to bacterial suspension (initial inoculum $5x10^5$ CFU/mL, final volume 100 μL). The bacteria were allowed to grow 24 h at 37°C and read at 600 nm. The intrinsic effect of different amounts of serum on bacterial growth was corrected, inhibition percentages being referred to their respective growth rate.

Experimental peritoneal sepsis in mice

Groups of 25 female C57BL/6 mice were i.p. inoculated with BMLD. After 4 hours, animals were i.p. injected with a single dose of each peptide (16 mg/kg) diluted in physiological serum (0.5 mL final volume). This dose corresponds to the LD_0 for CA(1–7)M(5–9) and the dose previous to LD_0 for the other peptides. A group of 25 infected but untreated mice was used as a control (control A).

Groups of five mice were sacrificed at 4, 5, 7, 9, and 11 h after bacterial inoculation (0, 1, 3, 5 and 7 h post-treatment). The CFUs in peritoneal fluid were determined by i.p. injection of 2 mL sterile saline serum and, after mild abdominal massage, 1 mL of peritoneal fluid was aspirated with a sterile pipette tip. One hundred μL aliquots of serial ten-fold dilutions were plated on Columbia sheep blood agar and incubated at 37°C. CFUs were counted after 24 h and expressed as median (P_{25}, P_{75}) of $\log_{10}$ CFU/mL. Spleens were aseptically removed and homogenized (2 min) in 2 mL of sterile saline solution (Stomacher 8, Tekmar Co., Cincinnati, OH). After ten-fold dilution of the homogenate, 100 μL aliquots were plated on Columbia sheep blood agar and CFUs determined as above and expressed as median (P_{25}, P_{75}) of the $\log_{10}$ CFU/g of tissue.

Results

Haemolytic activity

The results of the haemolytic activity of the CA-M hybrid peptides on mouse erythrocytes are shown in Fig. 1. Haemolysis rises steeply with peptide concentration, reaching >60% at 5 μg/mL, except for CA(1–8)M (1–18) with a less pronounced increase. Interestingly, despite the increase in overall hydrophobicity caused by

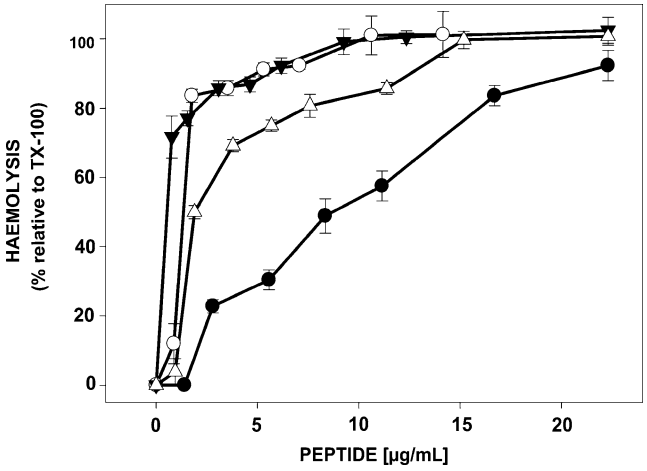

Fig. 1 Haemolytic activity of CA-M peptides on BALB/c mouse erythrocytes. Peptides were incubated with BALB/c mouse erythrocytes (4 h, 37°C) and the haemoglobin released measured. Full permeabilization (100%) was considered as that achieved with 0.1% Triton X-100. *Closed circle* CA(1–8)M(1–18), *empty circle* CA(1–7)M(2–9), *empty triangle up* Oct-CA(1–7)M(2–9), *filled triangle down* CA(1–7)M(5–9)

octanoylation of CA(1–7)M(2–9), the haemolysis of Oct-CA(1–7)M(2–9) is moderately lower than its non acylated parental peptide.

In vivo toxicity

Lethal parameters LD_0, LD_{50}, and LD_{100} of each peptide are summarized in Table 1. LD_0 ranged between 16 and 32 mg/kg, and LD_{100} between 64 and 128 mg/kg. As adverse effects, transitory movement disorders and muscle spasms were observed with dose-dependent duration, from 4 mg/kg except for CA(1–8)M(1–18), which starts at 8 mg/kg.

Determination of bacterial minimal lethal dose (BMLD) and bacterial lethal dose 50 (BLD_{50})

The BMLD of Ab208628 was 2.5×10^6 CFU. This value was used for the rest of animal assays. BLD_{50} was 5×10^5 CFU. A 2.5×10^4 CFU inoculum did not cause mortality.

Protective dose determination

Four hours after the i.p. inoculation with the BMLD of Ab208628, peptides ranging from 0.5 mg/kg up to the respective LD_0 (Table 1) were administered to the animals. PD_{50} was not achieved at non-toxic doses of any peptide. Results are shown in Fig. 2.

Influence of serum on activity of CA(1–8)M(1–18)

The haemolytic activity of CA(1–8)M(1–18) at 25 µg/mL was severely reduced with increasing serum concentration (Fig. 3a). Additionally, the haemolysis extent for CA(1–8)M(1–18) at 10% serum was considerably lower than in absence of serum (Fig. 3b).

The bactericidal activity of CA(1–8)M(1–18) and its all-D enantiomer, assayed at their MICs on Ab208628, was inhibited by preincubation with serum in a concentration-dependent manner. It is worth noting that the percentage of inhibition for the all-D enantiomer, impervious to proteolytic activity, was consistently lower than the all-L peptide (Fig. 3c).

Experimental peritoneal sepsis in mice

The bacterial concentration in the peritoneal fluid was consistently reduced ($p<0.05$, Mann-Whitney U test) by

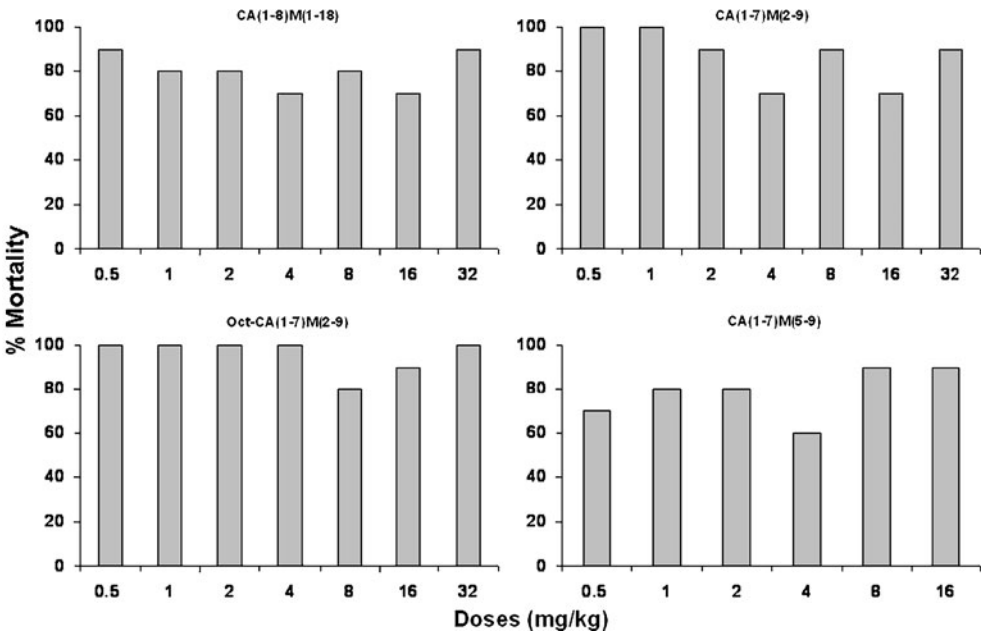

Fig. 2 Protective dose 50 (PD_{50}) results. Groups of ten mice were inoculated with Ab208628 BMLD. Four hours later, animals were treated with increasing in $\log_2$ doses of each peptide (from 0.5 mg/kg to LD_0)

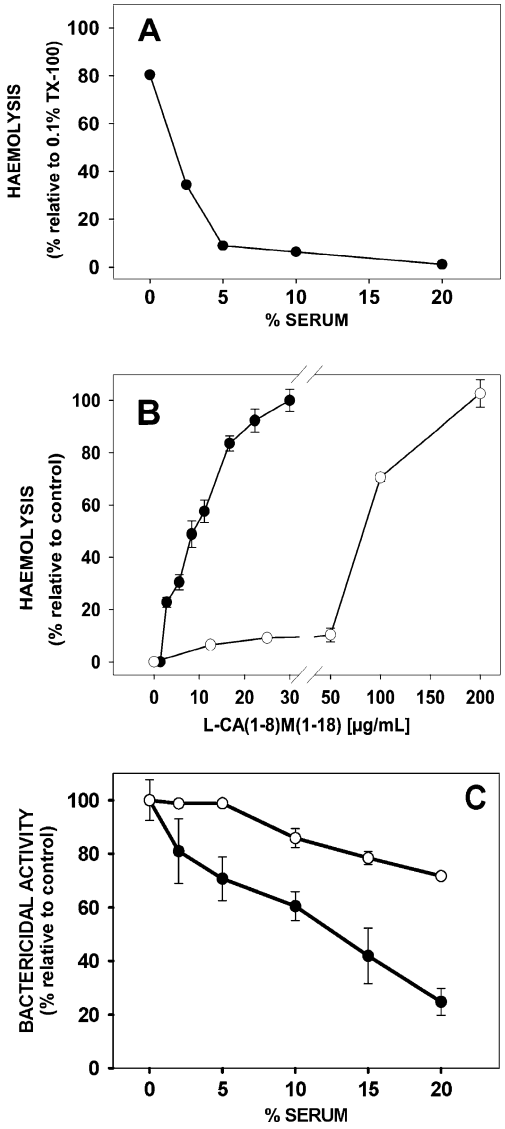

Fig. 3 Influence of mouse serum on the biological activities of CA (1–8)M(1–18). **a** Inhibition of haemolysis caused by L-CA(1–8)M (1–18) by serum at a constant peptide concentration of 25 µg/mL. **b** Variation of the haemolytic activity of L-CA(1–8)M(1–18) in presence (*open circle*) or absence (*closed circle*) of 10% heat inactivated autologous serum. **c** Serum inhibition of microbicidal activity of L-CA(1–8)M(1–18) (*closed circle*), and its all-D-enantiomer (*empty circle*), at a constant peptide concentration equivalent to their MIC in absence of serum (4 µg/mL)

CA-M peptides with respect to 4-h values in the control group. Nevertheless, a later bacterial re-growth ensued for all CA-M hybrids (Fig. 4). In the spleen, the increase of approximately 1 log of spleen CFUs observed at later times in untreated mice was abolished by all the peptides. Furthermore, a slight reduction in original CFUs was observed for Oct-CA(1–7)M(2–9). This decrease is delayed with respect to that for peritoneum with a maximal reduction at 7-h post infection (3 h after peptide inoculation) (Fig. 4).

Discussion

The lack of optimal alternatives for treatment of MDR *A. baumannii* infections has prompted the search of new therapeutic options, especially for nosocomial infections [2, 29]. EAPs may present a feasible alternative against the ever-raising multiresistance of microorganisms [30, 31]. A number of EAPs have been tested in vitro on *Acinetobacter* with in vitro MICS in the µg/mL range, even in the presence of large serum percentages [32]. CA-M hybrids and their parent peptides, CA, and M, exhibit good activity against *A. baumannii* [33, 34], including colistin- and pan-resistant strains [23, 24].

In this work, after CA-M peptide administration, all peptides caused an early drop of the bacterial load in the peritoneal cavity, CA(1–8)M(1–18) being the most effective. Of all peptides, only Oct-CA(1–7)M(2–9) caused a transitory decrease in CFUs in the spleen, whereas the other three remaining peptides, including the non-acylated parent peptide CA(1–7)M(2–9), only halted the bacterial load increase to about 1 log order, relative to untreated animals. Thus, the singularity of Oct-CA(1–7)M(2–9) appears to derive from its additional acyl chain. Other EAPs with similar replacements are resistant to degradation, either by shielding of the cleavage site, or by promoting conformations less accessible to peptidases [35].

In this work we sought to assay CA-M peptides under stringent conditions, similar to those at the onset of clinical treatment; the inoculum size consisted of a lethal dose of bacteria, and peptide administration was delayed for 4 h after inoculation, thus forcing peptide activity on an ongoing, well-established and disseminated infection. Other EAPs halt infection in animals, but administered with short delays (1–2 h, even simultaneously) after inoculation [14, 22, 32, 36]. Proteolytic degradation or sequestration by serum are two major hurdles for in vivo EAP application. For CA-M peptides, trypsin or serum led to inactivation of microbicidal activity, as shown by comparison between the two enantiomers of CA(1–8)M(1–18). Thus, substantial degradation and inactivation of CA-M peptides appears to occur after their injection, accounting for the inability to attain a PD_{50} in the murine sepsis model.

We have also chosen to work on CA-M peptides composed exclusively of proteinogenic amino acids, to reduce the cost of chemical synthesis and to allow eventual production by recombinant methods [37]. The inclusion of D- or β-amino acids, or the replacement of peptide bonds by peptidomimetics, is a powerful strategy to increase EAP proteolytic resistance [38]. Thus, protection of mice against *A. baumannii* infection was not achieved by all-L K_6L_9, whereas one of its diastereomer analog, with five D-residues, was effective [32]. In our study, even the peptide with the highest toxicity, CA(1–7)M(2–5) (LD_0 16 mg/kg)

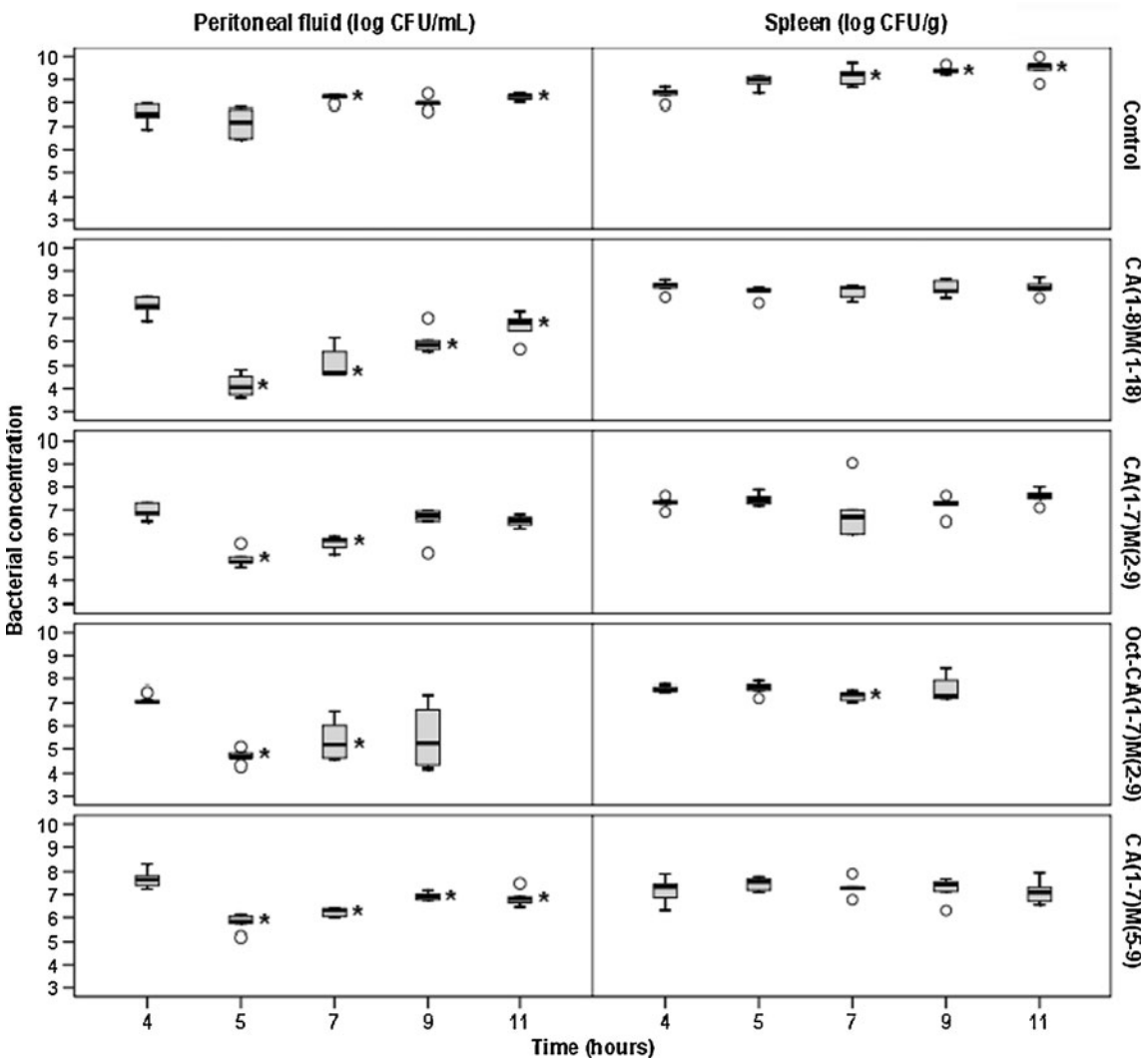

Fig. 4 Experimental peritoneal sepsis in mice. After i.p. inoculation of Ab208628 (t=0) at its BMLD, peptide (16 mg/kg) was injected 4 h later. Samples removed from either peritoneum or spleen for CFU determination as described in Material and Methods. For Oct-CA(1–7)M(2–9) data at 11 h were omitted as mice died earlier. * $p<0.05$ respect to 4 h, Mann-Whitney U test

compares favorably the with K_6L_9 diastereomer (80% exitus at 9 mg/kg) [32].

While, taken together, our results allow moderate optimism on the use of CA-M hybrids to fight sepsis caused by *A. baumannii*, it is clear that additional fine-tuning of the prototypes is required. Thus, it seems worthwhile to concentrate efforts on developing analogs more resistant to in vivo inactivation, either by strategic replacement of vulnerable residues or by inclusion of D-residues, as shown by the comparison between the enantiomers of CA(1–8)M(1–18), in tune with data from K_6L_9 diastereomers [32]. A further concern is dosage; our assumption that a single shot of peptide would achieve full pathogen elimination was indeed naïve; rather, an increased and, if possible, steady level of peptide delivery at the higher sub-toxic dose at least for several hours appears to be desirable. While this obviously increases the peptide requirements, they can be nonetheless diminished by the choice of shortened, proteolysis-resistant analogs. Another alternative for reducing peptide requirements could be synergy with conventional antibiotics. For CA-M hybrids, synergy was reported on other Eubacteria such as *Escherichia coli* [39] or *P. aeruginosa* [40]; more specifically, for *A. baumannii*, in vitro synergy between CA(1–7)M(2–9) and β-lactams including imipenem was reported [33].

In summary, the findings of the present work, used as a proof-of-principle, allow for a moderately optimistic view on the efficacy of EAPs in this regard. The CA-M peptides of this work, bactericidal against pan-resistant *A. baumannii* [23], show a local efficacy in the *A. baumannii* peritoneal sepsis model, but are only bacteriostatic on the spleen. Thus, several drawbacks must be overcome before EAPs become

useful antimicrobials, such as to improve the feasibility of their systemic administration, in contrast with the hitherto demotion of EAPs to topical or mucosal administration.

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References

1. Rodriguez-Bano J, Cisneros JM, Fernandez-Cuenca F, Ribera A, Vila J, Pascual A, Martinez-Martinez L, Bou G, Pachon J (2004) Clinical features and epidemiology of *Acinetobacter baumannii* colonization and infection in Spanish hospitals. Infect Control Hosp Epidemiol 25:819–824
2. Vila J, Pachon J (2008) Therapeutic options for *Acinetobacter baumannii* infections. Expert Opin Pharmacother 9:587–599
3. Fournier PE, Richet H (2006) The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin Infect Dis 42:692–699
4. Villegas MV, Hartstein AI (2003) *Acinetobacter* outbreaks, 1977–2000. Infect Control Hosp Epidemiol 24:284–295
5. Poirel L, Nordmann P (2006) Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 12:826–836
6. Towner KJ, Levi K, Vlassiadi M (2008) Genetic diversity of carbapenem-resistant isolates of *Acinetobacter baumannii* in Europe. Clin Microbiol Infect 14:161–167
7. Valencia R, Arroyo LA, Conde M, Aldana JM, Torres MJ, Fernandez-Cuenca F, Garnacho-Montero J, Cisneros JM, Ortiz C, Pachon J, Aznar J (2009) Nosocomial outbreak of infection with pan-drug-resistant *Acinetobacter baumannii* in a tertiary care university hospital. Infect Control Hosp Epidemiol 30:257–263
8. Ganz T (2004) Defensins: antimicrobial peptides of vertebrates. C R Biol 327:539–549
9. Giuliani A, Nicoletto SF (2007) Antimicrobial peptides: an overview of a promising class of therapeutics. Cent Eur J Biol 2:33
10. Jenssen H, Hamill P, Hancock RE (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19:491–511
11. Khandelia H, Ipsen JH, Mouritsen OG (2008) The impact of peptides on lipid membranes. Biochim Biophys Acta 1778:1528–1536
12. Matsuzaki K (2009) Control of cell selectivity of antimicrobial peptides. Biochim Biophys Acta 1788:1687–1692
13. Rivas L, Andreu D (2003) Cecropin-melittin hybrid peptides as versatile templates in the development of membrane active antibiotics agents. In: Menestrina G, Dalla Serra M (eds) Pore-forming peptides and protein toxins. Harwood Academic Publishers, Reading, Berkshire, United Kingdom, pp 215–259
14. Giacometti A, Cirioni O, Ghiselli R, Mocchegiani F, D'Amato G, Del Prete MS, Orlando F, Kamysz W, Lukasiak J, Saba V, Scalise G (2003) Administration of protegrin peptide IB-367 to prevent endotoxin induced mortality in bile duct ligated rats. Gut 52:874–878
15. Ghiselli R, Giacometti A, Cirioni O, Orlando F, Mocchegiani F, Pacci AM, Scalise G, Saba V (2001) Therapeutic efficacy of the polymyxin-like peptide ranalexin in an experimental model of endotoxemia. J Surg Res 100:183–188
16. Giuliani A, Pirri G, Bozzi A, Di Giulio A, Aschi M, Rinaldi AC (2008) Antimicrobial peptides: natural templates for synthetic membrane-active compounds. Cell Mol Life Sci 65:2450–2460
17. Zelezetsky I, Tossi A (2006) Alpha-helical antimicrobial peptides—using a sequence template to guide structure-activity relationship studies. Biochim Biophys Acta 1758(9):1436–1449
18. Andreu D, Ubach J, Boman A, Wahlin B, Wade D, Merrifield RB, Boman HG (1992) Shortened cecropin A-melittin hybrids. Significant size reduction retains potent antibiotic activity. FEBS Lett 296(2):190–194
19. Boman HC, Boman IA, Andreu D, Li ZQ, Merrifield RB, Schlenstedt G, Zimmermann R (1989) Chemical synthesis and enzymic processing of precursor forms of cecropins A and B. J Biol Chem 264(10):5852–5860
20. Cavallarin L, Andreu D, San Segundo B (1998) Cecropin A-derived peptides are potent inhibitors of fungal plant pathogens. Mol Plant Microbe Interact 11(3):218–227
21. Chicharro C, Granata C, Lozano R, Andreu D, Rivas L (2001) N-terminal fatty acid substitution increases the leishmanicidal activity of CA(1–7)M(2–9), a cecropin-melittin hybrid peptide. Antimicrob Agents Chemother 45(9):2441–2449
22. Friedrich C, Scott MG, Karunaratne N, Yan H, Hancock RE (1999) Salt-resistant alpha-helical cationic antimicrobial peptides. Antimicrob Agents Chemother 43(7):1542–1548
23. Rodriguez-Hernandez MJ, Saugar J, Docobo-Perez F, de la Torre BG, Pachon-Ibanez ME, Garcia-Curiel A, Fernandez-Cuenca F, Andreu D, Rivas L, Pachon J (2006) Studies on the antimicrobial activity of cecropin A-melittin hybrid peptides in colistin-resistant clinical isolates of *Acinetobacter baumannii*. J Antimicrob Chemother 58(1):95–100
24. Saugar JM, Rodriguez-Hernandez MJ, de la Torre BG, Pachon-Ibanez ME, Fernandez-Reyes M, Andreu D, Pachon J, Rivas L (2006) Activity of cecropin A-melittin hybrid peptides against colistin-resistant clinical strains of *Acinetobacter baumannii*: molecular basis for the differential mechanisms of action. Antimicrob Agents Chemother 50(4):1251–1256
25. C57BL/6 Datasheet. Harland. http://www.harlan.com/research_models_and_services/research_models_by_product_type/inbred_mice/c57bl6j_inbred_mice.hl. Accessed 13 December 2010.
26. BALBc Datasheet. Harland. http://www.harlan.com/research_models_and_services/research_models_by_product_type/inbred_mice/balbc.hl. Accessed 13 December 2010.
27. Solanas C, de la Torre BG, Fernandez-Reyes M, Santiveri CM, Jimenez MA, Rivas L, Jimenez AI, Andreu D, Cativiela C (2009) Therapeutic index of gramicidin S is strongly modulated by D-phenylalanine analogues at the beta-turn. J Med Chem 52(3):664–674
28. O'Reilly T, Cleeland R, Squires EL (1996) Evaluation of antimicrobials in experimental animal infections. In: Lorian V (ed) Antibiotics in laboratory medicine. Williams and Wilkins, MD, USA, pp 604–765
29. Pachon J, Vila J (2009) Treatment of multiresistant *Acinetobacter baumannii* infections. Curr Opin Investig Drugs 10(2):150–156
30. Pereira HA (2006) Novel therapies based on cationic antimicrobial peptides. Curr Pharm Biotechnol 7(4):229–234
31. Zhang L, Falla TJ (2006) Antimicrobial peptides: therapeutic potential. Expert Opin Pharmacother 7(6):653–663
32. Braunstein A, Papo N, Shai Y (2004) In vitro activity and potency of an intravenously injected antimicrobial peptide and its DL amino acid analog in mice infected with bacteria. Antimicrob Agents Chemother 48(8):3127–3129

33. Giacometti A, Cirioni O, Kamysz W, D'Amato G, Silvestri C, Del Prete MS, Lukasiak J, Scalise G (2003) Comparative activities of cecropin A, melittin, and cecropin A-melittin peptide CA(1–7)M (2–9)NH2 against multidrug-resistant nosocomial isolates of *Acinetobacter baumannii*. Peptides 24(9):1315–1318
34. Saugar JM, Alarcon T, Lopez-Hernandez S, Lopez-Brea M, Andreu D, Rivas L (2002) Activities of polymyxin B and cecropin A-, melittin peptide CA(1–8)M(1–18) against a multiresistant strain of *Acinetobacter baumannii*. Antimicrob Agents Chemother 46(3):875–878
35. Avrahami D, Shai Y (2003) Bestowing antifungal and antibacterial activities by lipophilic acid conjugation to D, L-amino acid-containing antimicrobial peptides: a plausible mode of action. Biochemistry 42(50):14946–14956
36. Gough M, Hancock RE, Kelly NM (1996) Antiendotoxin activity of cationic peptide antimicrobial agents. Infect Immun 64(12):4922–4927
37. Zhang L, Falla T, Wu M, Fidai S, Burian J, Kay W, Hancock RE (1998) Determinants of recombinant production of antimicrobial cationic peptides and creation of peptide variants in bacteria. Biochem Biophys Res Commun 247(3):674–680
38. Seebach D, Beck AK, Bierbaum DJ (2004) The world of beta- and gamma-peptides comprised of homologated proteinogenic amino acids and other components. Chem Biodivers 1(8):1111–1239
39. Zhang L, Benz R, Hancock RE (1999) Influence of proline residues on the antibacterial and synergistic activities of alpha-helical peptides. Biochemistry 38(25):8102–8111
40. Piers KL, Brown MH, Hancock RE (1994) Improvement of outer membrane-permeabilizing and lipopolysaccharide-binding activities of an antimicrobial cationic peptide by C-terminal modification. Antimicrob Agents Chemother 38(10):2311–2316