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Efficacy of dual carbapenem treatment in a murine sepsis model of infection due to carbapenemase-producing *Acinetobacter baumannii*

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Objectives: To evaluate the *in vivo* efficacy of a dual carbapenem combination containing imipenem plus meropenem against carbapenem-resistant *Acinetobacter baumannii* producing carbapenemases OXA-23 or OXA-58.

Methods: An experimental model of peritonitis using C57BL/6J female mice was developed and the minimum lethal doses were calculated for infections due to OXA-23 or OXA-58 producers of *A. baumannii* clinical isolates. The efficacies of the carbapenems in monotherapy and in combination were tested.

Results: Meropenem was better than imipenem in mice infected with either of the carbapenem-resistant *A. baumannii* (CRAb) strains. The combination of meropenem plus imipenem significantly improved the clearance of CRAbs from spleen compared with non-treated groups. The carbapenem-containing combination was better than imipenem for treating mice infected with both carbapenemase producers. In blood, the carbapenem combination significantly decreased the bacterial load of the OXA-23 producers compared with imipenem or meropenem used in monotherapy.

Conclusions: These results suggest that dual carbapenem combination could be an option for the treatment of infections due to carbapenemase-producing *A. baumannii* such as OXA-23 and OXA-58 producers.

Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAb) is becoming a major public health concern,[1] as stated by Tacconelli *et al.*[2] who pointed to CRAb as one of the critical pathogens for which new research and development of antibiotics is needed. Moreover, almost all CRAb isolates produce carbapenemases[3] belonging to different Ambler classes, such as mostly class D (oxacillinases), A and B (metallo-β-lactamases).[4] Additionally, CRAb isolates are usually resistant to other antibiotics commonly used for these infections making it difficult to treat patients, who sometimes requires combined antibiotic treatment, with variable results.[4] The most common carbapenemases in *A. baumannii* are OXA-23, OXA-40 and OXA-58.[5]

A recent study evaluated a dual-carbapenem combination *in vitro* (imipenem and meropenem), against a collection of non-clonally related *A. baumannii* isolates recovered from clinical samples in different countries, all of them being carbapenem-resistant according to the EUCAST breakpoints (http://www.eucast.org/). A synergic activity of imipenem plus meropenem was shown for 25% of the tested isolates, almost all of them carrying Ambler class D β-lactamases (with the acquired enzymes OXA-23- and OXA-58-type) and one isolate expressing a class B metallo-β-lactamase of the NDM type.[6] Therefore, our goal was to evaluate the *in vivo* efficacy of imipenem plus meropenem in a murine sepsis model against two CRAb isolates.

Materials and methods

Bacterial isolates

Two non-clonally related carbapenemase-producing *A. baumannii* clinical isolates were used, *A. baumannii* R824 (OXA-58) and *A. baumannii* R625 (OXA-23). Both isolates were resistant to imipenem and meropenem. MIC values were 16 and 16 mg/L, respectively, for the OXA-58 isolate and 32

and 32 mg/L, respectively, for the OXA-23 isolate.[6] Moreover, imipenem plus meropenem showed *in vitro* synergy against these two CRAb isolates by chequerboard assay.[6]

Antimicrobials

Clinical formulations of antimicrobials were used: imipenem (Fresenius Kabi, Barcelona, Spain) and meropenem (Ranbaxy, Barcelona, Spain).

Animals

Immunocompetent C57BL/6J female mice of 20 g (7–9 weeks old) were used (Production and Experimentation Animal Centre, University of Seville, Seville, Spain). Animals had a sanitary status of murine pathogen free and were assessed for genetic authenticity. Mice were housed in an individually ventilated cage system under specific pathogen-free conditions, with water and food *ad libitum*. The study was carried out following the recommendations of the Guide for the Care and Use of Laboratory Animals.[7] *In vivo* experiments were approved by the Committee on the Ethics of Animal Experiments of the University Hospital Virgen del Rocío and the Ministry of *Agricultura, Pesca y Desarrollo Rural* (06/03/2018/022), Spain. Procedures were performed under ketamine/xylazine (Pfizer, Parke-Davis S.L. Spain/ Bayer Hispania S.L. Spain), anaesthesia. Mice were sacrificed using sodium thiopental (B. Braun Medical S.A., Spain) and all efforts were made to minimize suffering.

Murine experimental model

The minimum lethal doses (MLDs) were determined by inoculating groups of six mice intraperitoneally (IP) with 0.5 mL of decreasing concentrations of each strain, from 7.8 to 6.05 $\log_{10}$ cfu/mL for *A. baumannii* R824 (OXA-58) strain and from 7.98 to 6.96 $\log_{10}$ cfu/mL for *A. baumannii* R625 (OXA-23) strain, and monitoring the survival of the mice for 7 days.

A murine peritoneal sepsis model was then established by IP inoculation of mice with the MLD previously characterized for strain *A. baumannii* R824 (OXA-58) and *A. baumannii* R625 (OXA-23), corresponding to ($\log_{10}$ cfu/mL): 6.05 and 7.98 for *A. baumannii* R824 and *A. baumannii* R625 strains, respectively.

Efficacy studies

Briefly, groups of 15 C57BL/6 female mice were infected by IP injection of the MLD of each strain. Treatments were initiated 2 h post-inoculation and lasted 24 h. For mice infected with each strain, they were randomly included into four different therapeutic groups: (a) controls (untreated); (b) imipenem 30 mg/kg/q4h administered intramuscularly (IM); (c) meropenem 300 mg/kg/q2h IP; and (d) imipenem plus meropenem, using the same dosing schedule as in monotherapy. The antimicrobial dosages were based on the pharmacokinetic/pharmacodynamic data, and their proven efficacy, alone and in combination, in previous murine models of infection.[8,9] Samples were extracted and processed immediately after the death of mice. The survivor mice were sacrificed (with sodium thiopental) at 24 h. Aseptic thoracotomies were carried out, and blood samples were obtained for qualitative blood cultures. Results were expressed as positive ($\geq$1 cfu present in the plate) or negative and quantitative cultures were determined ($\log_{10}$ cfu/mL). Spleens were aseptically extracted, weighed, and homogenized in sterile saline (Stomacher 80; Tekmar Co., Cincinnati, OH, United States) and quantitative cultures were determined by plating onto Columbia agar with 5% sheep blood plates.[10]

Statistical analysis

Mortality and positive blood cultures were expressed as percentages. Bacterial spleen concentrations ($\log_{10}$ cfu/g) and bacterial blood concentrations ($\log_{10}$ cfu/mL) were expressed as means±SD. Differences in bacterial concentrations in spleen and blood were compared by analysis of variance (ANOVA) and the Dunnet and Tukey *post hoc* tests. Mortality and blood sterility rates between groups were compared by use of the two-tailed Fisher's test. A $P < 0.05$ value was considered as statistically significant. SPSS v22.0 was used (SPSS Inc., Chicago, IL, USA).

Results

Peritoneal sepsis model

The MLD for the CRAb strains producing OXA-58 and OXA-23 were 6.05 and 7.98 $\log_{10}$ cfu/mL, respectively. The efficacies of the antimicrobials alone or in combination are shown in Table 1.

Mortality

Mortality in both control groups (non-treated) was 100% within the first 24–42 h post-infection. Meropenem and its combination with imipenem reduced mortality in animals infected with the OXA-58 strain to 0% in both treatment groups. Meropenem and the combination of carbapenems also reduced the mortality compared with controls and the imipenem group in mice infected with the OXA-23 strain (to 27% and 13%, respectively).

Bacterial clearance

Both imipenem- and meropenem-containing monotherapies and the carbapenem combination significantly improved the clearance of bacteria from spleen compared with the control for mice infected with strains producing OXA-58 or OXA-23 (Table 1). Meropenem monotherapy and the combination were also better than imipenem alone in mice infected with the OXA-58 producer, whereas only the combination improved the bacterial clearance compared with imipenem in mice infected with the OXA-23-producing isolate.

In blood, both carbapenem monotherapies and the combination significantly decreased the bacterial load of the OXA-58 producers compared with non-treated animals, as well as meropenem alone and the combination as compared with imipenem monotherapy. Meropenem alone or in combination significantly decreased the bacterial load in blood of the OXA-23 producer as compared that of non- treated and imipenem-treated groups. Finally, the blood load of the OXA-23 producer was significantly decreased by the combination of antibiotics as compared with meropenem as monotherapy.

Discussion

To the best of our knowledge, dual carbapenem combinations have never been tested *in vivo* against carbapenemase-producing *A. baumannii*. In this study we found that the combination of imipenem and meropenem was efficacious in the murine sepsis model with OXA-23- or OXA-58 producers.

We found than meropenem in monotherapy regardless of its resistance pattern was able to reduce the mortality and bacterial loads in spleen and blood compared with controls for both OXA-23 and OXA-58 producers. Moreover, we found that meropenem plus imipenem was able to improve bacterial clearance in both spleen and blood of mice infected with the OXA-23 and OXA-58 producers. The mortality was significantly reduced with meropenem and with the combination against both strains, all mice surviving in

Table 1. Efficacy of imipenem and meropenem, alone and in combination, in the experimental peritoneal sepsis models with carbapenem-resistant *A. baumannii* isolates

Isolate	Treatment group	*n*	Doses (mg/kg)	Spleen (log_{10} cfu/g)	Blood (log_{10} cfu/mL)	Bacteraemia (%)	Mortality (%)
OXA-58	Control	15	–	8.97±0.72	7.39±2.23	93	36
	IPM	15	30	5.55±1.94[a]	3.86±2.85[a]	87	7
	MEM	15	300	1.93±2.59[a,b]	0.46±0.93[a,b]	27[a,b]	0[a]
	IPM+MEM	15		1.73±2.26[a,b]	0.23±0.64[a,b]	13[a,b]	0[a]
OXA-23	Control	15	–	8.96±1.13	5.55±1.94	100	79
	IPM	15	30	8.12±0.53[a]	6.56±1.10	100	67
	MEM	15	300	5.44±0.74[a]	3.84±0.98[a,b]	100	27[a,b]
	IMP+MEM	15		4.66±1.66[a,b]	1.59±0.99[a,b,c]	80	13[a,b]

Control received no antimicrobial treatment; IPM, imipenem; MEM, meropenem.
[a]$P \leq 0.05$ with respect to Control group.
[b]$P \leq 0.05$ with respect to IPM group.
[c]$P \leq 0.05$ with respect to MEM group.

the OXA-58 groups in parallel with the deep reduction in spleen and blood bacterial concentrations.

The therapeutic effects of meropenem in monotherapy are probably related to its serum concentration exceeding the MIC for more than the 50% of the time between doses.[9] In addition, the *in vitro* synergy of meropenem plus imipenem against the OXA-58 and OXA-23 producers[6] is in accordance with the *in vivo* results found with the combination of the carbapenems. This combination was better in reducing the blood bacterial concentration against the OXA-23 strain, compared with both carbapenems in monotherapy. We believe that this therapeutic difference could be due to the specific carbapenemases produced by each isolate; we have also observed differences in other experimental models of infection caused by *Klebsiella pneumoniae* strains producing different carbapenemases.[10]

The use of combination therapy is an attractive approach and is justified by the high mortality rates associated with these infections, the lack of proven valid therapeutic options, and the rapid development of antimicrobial resistance.[11] The combination of two synergistic carbapenems (ertapenem plus meropenem, doripenem, or imipenem), alone or combined with other antibiotics, has been suggested as treatment for infections produced by carbapenem-resistant and carbapenemase-producing *Klebsiella pneumoniae*.[12] As suggested previously,[12] the dual mechanisms of action of carbapenems on carbapenemase-producing organisms may be related to the binding of one of the carbapenem molecules to the active site of the enzyme, acting as a suicide substrate, while the other carbapenem molecule delivers the efficacy.

In conclusion, our results suggest that dual carbapenem combination might be an option for the treatment of infections such as a peritoneal infection due to CRAb producers. Clinical studies exploring the efficacy of this combination in *A. baumannii* infections would be of interest, owing to the paucity of drugs available for treating CRAb-related infections.

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Transparency declarations

None to declare.

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