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Short Communication

Daptomycin as adjunctive treatment for experimental infection by *Acinetobacter baumannii* with resistance to colistin

Garyfallia Poulakou[a], Georgios Renieris[b], Labros Sabrakos[b], Olympia Zarkotou[c], Katherine Themeli-Digalaki[c], Efstathia Perivolioti[d], Eleni Kraniotaki[d], Evangelos J. Giamarellos-Bourboulis[b,*], Nikolaos Zavras[e]

[a] *3rd Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Athens 115 27, Greece*
[b] *4th Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Athens 12462, Greece*
[c] *Department of Microbiology, Tzaneio Hospital, Piraeus 185 36, Greece*
[d] *Department of Microbiology, Evangelismos Athens General Hospital, Athens, Greece*
[e] *Department of Children's Surgery, National and Kapodistrian University of Athens, Medical School, Athens 124 62, Greece*

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ABSTRACT

The emergence of *Acinetobacter baumannii* with resistance to colistin (ABRC) led to the investigation of daptomycin as an adjunctive to colistin for these isolates. In this study, one ABRC carbapenemase-producing bloodstream isolate was examined. Minimum inhibitory concentrations (MICs) were >512, >512 and 8 μg/mL for imipenem, daptomycin and colistin, respectively. First, a 'humanised' model of the pharmacokinetics of daptomycin and colistin was developed in 18 male C57BL/6 mice. Then, 112 mice were infected by intraperitoneal injection of the ABRC isolate and were randomly assigned into four groups of once-daily treatment for 7 days: group A, controls treated with saline; group B, treated with 20 mg/kg colistin; group C, treated with 50 mg/kg daptomycin; and group D, treated with both agents. Survival was recorded for 7 days in ten mice per group. The remaining mice were sacrificed at regular time intervals following bacterial challenge and the bacterial outgrowth in the liver, lung and right kidney was determined. Mean serum concentrations of daptomycin at 15, 30 and 60 min post-dose were 121.8, 110.3 and 100.4 μg/mL, respectively. The respective concentrations of colistin were 13.9, 9.1 and 7.5 μg/mL. The 7-day mortality in groups A, B, C and D was 100%, 50%, 100% and 0%, respectively. Tissue outgrowth of the right kidney was significantly decreased in group D compared with group B after 72 h. Daptomycin used in combination with colistin leads to prolonged survival in an experimental infection by ABRC. Failure of colistin alone is probably related to rebound of tissue outgrowth.

1. Introduction

Acinetobacter spp. have become one of the most common aetiologies of hospital-acquired infections, causing ventilator-associated pneumonia, skin and wound infections, bacteraemia and urinary tract infections [1]. *Acinetobacter baumannii* prevails, particularly in intensive care units (ICUs). A worrisome trait is the emergence of resistance of this species to colistin. An analysis of the resistance patterns of *A. baumannii* isolated from the bloodstream of patients with ICU-acquired sepsis in Greece showed an increase in resistance to colistin from 0% during the period 2006–2009 to >30% during the period 2010–2013 [2].

Colistin is widely used nowadays for the treatment of nosocomial pneumonia and sepsis caused by multidrug-resistant Gram-negative bacteria [3,4]. Its widespread use has led to the emergence of *A. baumannii* with resistance to colistin (ABRC) [5].

In the case of infection by ABRC, therapeutic options are limited as these isolates are usually resistant to carbapenems. Frequently clinicians are prompted to use paradoxical combinations [6]. One proposed combination for the treatment of ABRC is the combination of colistin and daptomycin [7]. In a study using 10 ABRC isolates from patients in tertiary hospitals in Athens (Greece), 10 μg/mL daptomycin was synergistic with 0.25–0.5 μg/mL colistin after 24 h of growth in one-half of the isolates [7]. However, the reported efficacy needs to be validated in an in vivo setting. To investigate the in vivo interaction of colistin and daptomycin against ABRC, an animal model delivering 'humanised' pharmacokinetics of both drugs is warranted. In the present study, a two-step approach was used. In the first step, a 'humanised' dosing model delivering

* Corresponding author. Present address: 4th Department of Internal Medicine, ATTIKON University Hospital, 1 Rimini Street, 12462 Athens, Greece. Tel.: +30 210 58 31 994; fax: +30 210 53 26 446.
E-mail address: egiamarel@med.uoa.gr (E.J. Giamarellos-Bourboulis).

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maximum serum concentrations of daptomycin close to 10 μg/mL and of colistin close to 4 μg/mL was developed. Then, the utility of this model for the treatment of experimental infection by ABRC was studied in a second step.

2. Materials and methods

All experiments were conducted in the unit of animals for medical scientific purposes of University General Hospital 'Attikon' (Athens, Greece) according to EU Directive 2010/63/EU for animal experiments and to the Greek law 2015/2001, which incorporates the Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of the Council of Europe (code of the facility EL 25BIO014, approval no. 1853/2015). Colistin sulphate salt was purchased from AppliChem GmbH (Darmstadt, Germany). Daptomycin was purchased from Novartis Pharma AG (Basel, Switzerland).

A clinical ABRC isolate from the bloodstream of a patient was used. Minimum inhibitory concentrations (MICs) of colistin, meropenem and daptomycin were 8, >512 and >512 μg/mL, respectively. Following PCR for *mcr-1* (forward primer, 5′-CGG TCA GTC CGT TTG TTC-3′; reverse primer, 5′-CTT GGT CGG TCT GTA GGG-3′), $bla_{\text{OXA-51}}$ (forward primer, 5′-TAA TGC TTT GAT CGG CCT TG-3′; reverse primer, 5′-TGG ATT GCA CTT CAT CTT GG-3′), $bla_{\text{OXA-23}}$ (forward primer, 5′-GAT CGG ATT GGA GAA CCA GA-3′; reverse primer, 5′-ATT TCT GAC CGC ATT TCC AT-3′), $bla_{\text{OXA-24}}$ (forward primer, 5′-GGT TAG TTG GCC CCC TTA AA-3′; reverse primer, 5′-AGT TGA GCG AAA AGG GGA TT-3′) and $bla_{\text{OXA-58}}$ (forward primer, 5′-AAG TAT TGG GGC TTG TGC TG-3′; reverse primer, 5′-CCC CTC TGC GCT CTA CAT AC-3′), it was found that the isolate did not express the *mcr-1* gene but that it expressed OXA-23-like and OXA-51-like carbapenemases.

A 'humanised' model of the pharmacokinetics for daptomycin and colistin was developed in C57BL/6 male mice ($n = 3$ for each agent per time of sacrifice) with a median weight of 25 g. Analgesia was achieved by subcutaneous (s.c.) administration of meloxicam 5 mg/kg. At the selected timepoint, mice were killed using isoflurane. The studied doses of colistin and daptomycin were 20 mg/kg and 50 mg/kg, respectively. Antibiotics were reconstituted in sodium chloride 0.9% and were administered subcutaneously. Blood was sampled following animal sacrifice via the lower vena cava for measurement of drug levels.

Daptomycin was measured following methanol extraction and analysis by a high-performance liquid chromatography (HPLC) system (Agilent 1100 Series; Agilent Technologies Deutschland GmbH, Waldbronn, Germany) as previously described [8]. The lower limit of detection (LLOD) was 6.25 μg/mL and the interday co-efficient of variation of the assay was 4.7%. For the measurement of colistin, standard microbiological assays (Sensititre™; TREK Diagnostics, East Grinstead, UK) were used where 0.2 mL of serum was plated on the selected assays using a standard curve of known concentrations on a semi-logarithmic scale.

A log-phase culture (9×10^5 CFU/mL) was exposed over time in tubes with Muller–Hinton II broth (Becton Dickinson, Franklin Lakes, NJ) at a final volume of 10 mL containing 10 μg/mL colistin, 10 μg/mL daptomycin, or both agents for 24 h. At baseline and following incubation at 37 °C in a shaking water-bath, bacterial counts were measured after four serial 1:10 dilutions of a 0.1 mL aliquot and plating onto MacConkey agar (Becton Dickinson) at 35 °C. The results were expressed as $\log_{10}$ CFU/mL. All experiments were conducted twice.

For the animal studies, adult male C57BL/6 mice (median weight 25 g) were studied. Animals were housed in cages under constant temperature (21 °C) and humidity with a constant 12-h light/dark cycle. All animals had ad libitum access to food and water. Analgesia was achieved by s.c. administration of meloxicam 5 mg/kg. A total of 130 mice were used, including 18 mice for the pharmacokinetic model and 112 mice for the survival and sacrifice study. All mice were infected by intraperitoneal (i.p.) injection of 10^6 CFU/mice of the ABRC isolate and were randomly assigned into four groups of once-daily s.c. treatment for 7 days, as follows:

- group A: controls, injected with two injections of 0.1 mL of 0.9% normal saline (N/S) s.c. in the neck;
- group B: injected with 20 mg/kg colistin in 0.9% N/S at a final volume of 0.1 mL in the neck; they were also injected with 0.1 mL of 0.9% N/S s.c. in the neck;
- group C, injected with 50 mg/kg daptomycin in 0.9% N/S at a final volume of 0.1 mL in the neck; they were also injected with one simultaneous injection of 0.1 mL of 0.9% N/S s.c. in the neck; and
- group D, combination, injected with two injections of colistin and daptomycin as described above.

Survival was recorded for 7 days for ten mice per group. The remaining mice were sacrificed 24, 48 and 72 h after bacterial challenge and bacterial outgrowth was assessed. The sacrifice procedure involved i.p. administration of ketamine (300 mg/kg) and xylazine (30 mg/kg) followed by cervical dislocation. Tissue samples were collected through an abdominal incision under sterile conditions. Tissue segments were weighted, were added into sterile tubes with 1 mL of 0.9% N/S and were homogenised. Bacterial growth was then quantitatively measured by four serial 1:10 dilutions of 0.1 mL aliquots of each homogenate in 0.9% N/S and plating of 0.1 mL aliquots of each dilution onto MacConkey agar. Plates were read after 24 h of incubation at 35 °C. The results were expressed as $\log_{10}$ CFU/mL. The LLOD was 10 CFU/mL.

Results were expressed as mean ± standard error (SE). Comparisons were done by Mann–Whitney *U*-test. Survival was analysed by Kaplan–Meier analysis and comparisons were done by the log-rank test. A *P*-value of <0.05 after Bonferroni correction for multiple comparisons was considered statistically significant.

3. Results

Mean serum daptomycin concentrations at 15, 30 and 60 min post-injection were 121.8, 110.3 and 100.4 μg/mL, respectively; the respective concentrations for colistin were 13.9, 9.1 and 7.5 μg/mL. These concentrations are in accordance with the measured concentrations in patients' blood samples [9,10].

Daptomycin alone did not affect in vitro bacterial growth. Colistin alone rapidly decreased bacterial growth but re-growth emerged after 24 h. However, re-growth was prevented with the drug combination (Fig. 1).

The 7-day survival of groups A, B, C and D was 0%, 50%, 0% and 100%, respectively (Fig. 2), showing a significant survival benefit with the combination of colistin and daptomycin compared with colistin alone.

Tissue outgrowth of the kidney, liver and lung at 24 h and 48 h after bacterial challenge is shown in Fig. 3. No mice in groups A and C survived after 48 h to allow tissue bacterial outgrowth after 72 h. This happened only for animals in groups B and D; after 72 h, the mean ± SE $\log_{10}$ bacterial outgrowth was 2.34 ± 0.48 CFU/g for group B and 1 ± 0 CFU/g for group D in the kidney ($P = 0.035$) and 1.86 ± 1.35 CFU/g and 1 ± 0 CFU/g, respectively, in the lung ($P = 0.089$).

4. Discussion

In this mouse study of humanised pharmacokinetics, it was shown that daptomycin acted synergistically with colistin preventing bacterial tissue re-growth and leading to a survival benefit in experimental infection by ABRC. One major finding of the study

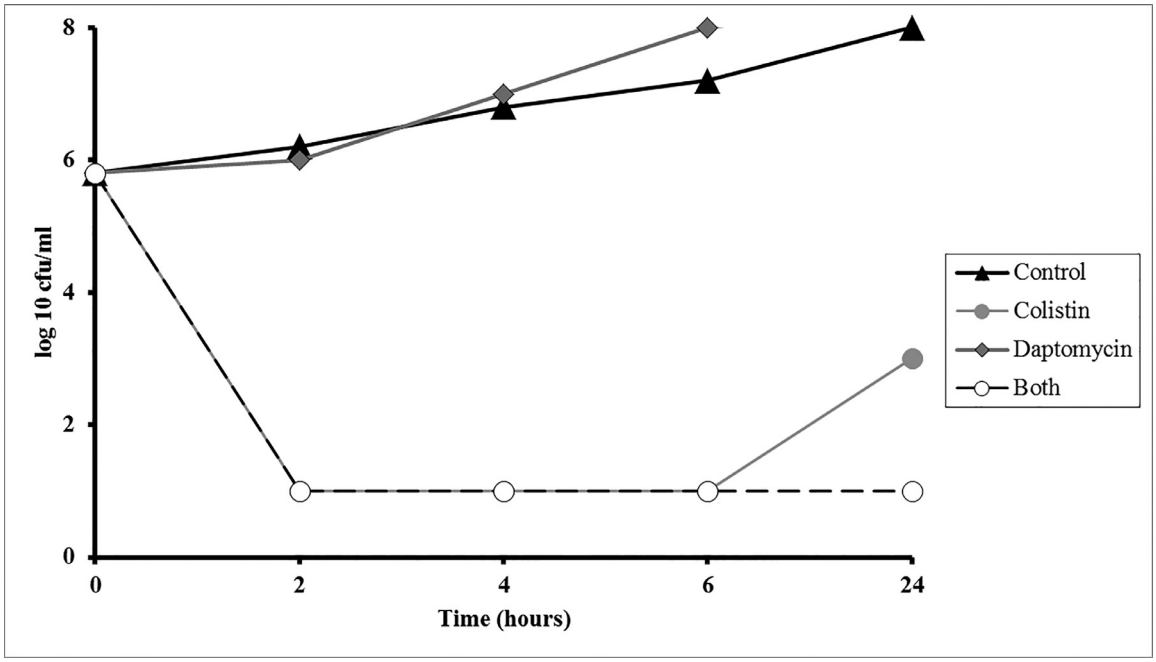

Fig. 1. Growth over time of the studied isolate in the presence of colistin, daptomycin and their combination. Synergy was defined as a >2 $\log_{10}$ decrease in bacterial count by the combination of colistin and daptomycin compared with the most effective single agent.

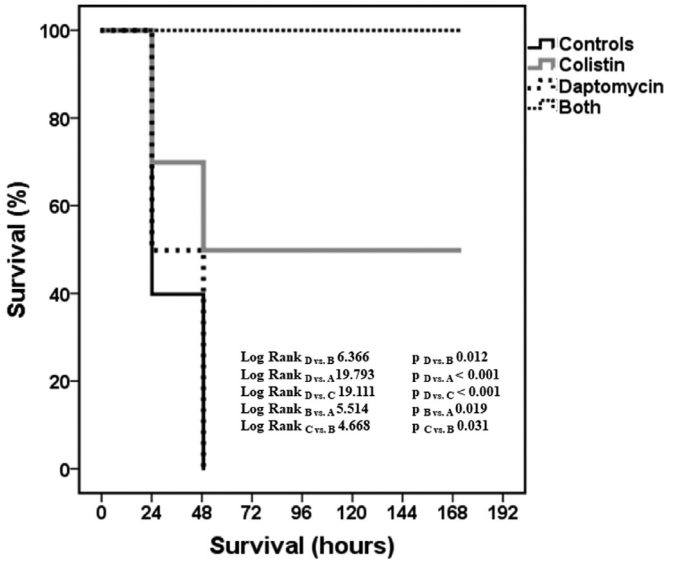

Fig. 2. Survival curves of the studied groups of mice: group A, controls; group B, treated with colistin; group C, treated with daptomycin; and group D, treated with colistin and daptomycin. Log-rank tests of the indicated comparisons and the *P*-values are provided.

was the 50% survival of colistin-treated mice despite their status of colistin resistance. This is probably due to the isolate being resistant but with an MIC of only 8 μg/mL; this concentration was exceeded by the humanised model of delivery of colistin.

Acinetobacter baumannii has become an important health problem owing to treatment failures caused by multiple antibiotic resistances. The emergence of resistance to colistin generated concerns for the selection of the most appropriate antimicrobial agent(s) for these isolates. A recent nationwide epidemiological study of *A. baumannii* in Greece demonstrated a colistin susceptibility of 72.7% [11]. In that study, the majority of carbapenem-resistant isolates in Greek hospitals almost uniformly expressed the OXA-23 carbapenemase and belonged mainly to International Clone 2 [11]. Several unconventional antimicrobial agents have been studied in vitro to explore potential synergy with colistin against ABRC. Interactions with rifampicin and azithromycin [12], vancomycin and teicoplanin [13,14], telavancin and trimethoprim [15,16] have been studied with variable reported efficacy.

One of the promising combinations for the treatment of ABRC is the combination of colistin with daptomycin. This has been explored in recent in vitro and a few in vivo experiments. Galani et al. investigated the in vitro effect of the combination of colistin with daptomycin against colistin-susceptible and -resistant carbapenemase-producing *A. baumannii*. Their study showed a synergism in 50% of the tested carbapenemase-producing *A. baumannii*; however, the combination of colistin + daptomycin was particularly beneficial against colistin-susceptible multidrug-resistant *A. baumannii* [7]. Cirioni et al. confirmed the in vitro data in in vivo settings [17]. They used two clinical isolates of *A. baumannii*, one with an MIC of 1 μg/mL and another with an MIC of 2 μg/mL, to infect 120 mice (60 mice for each isolate), followed by treatment with colistin, daptomycin, teicoplanin or their combination. In the colistin + daptomycin group, mortality was 10% by the isolate with an MIC of 1 μg/mL and 15% by the isolate with an MIC of 2 μg/mL. Both previous studies did not provide a clear answer for the problem of ABRC. According to Clinical and Laboratory Standards Institute (CLSI) criteria, resistance to colistin is characterised by an MIC of ≥4 μg/mL. The selected isolate in the study by Cirioni et al. with a MIC of 2 μg/mL does not comply with these criteria. This is the strength of the current study using an isolate with an MIC of 8 μg/mL for animal challenge.

The combination of daptomycin and colistin had a bactericidal effect against ABRC in vitro, whereas treatment with colistin alone led to a rebound growth effect after 6 h. Daptomycin used in combination with colistin in vivo led to eradication of the experimental infection by ABRC. The synergistic action is thought to be mediated by an increase in the permeability of the bacterial membrane by colistin, which allows the large daptomycin molecule to pene-

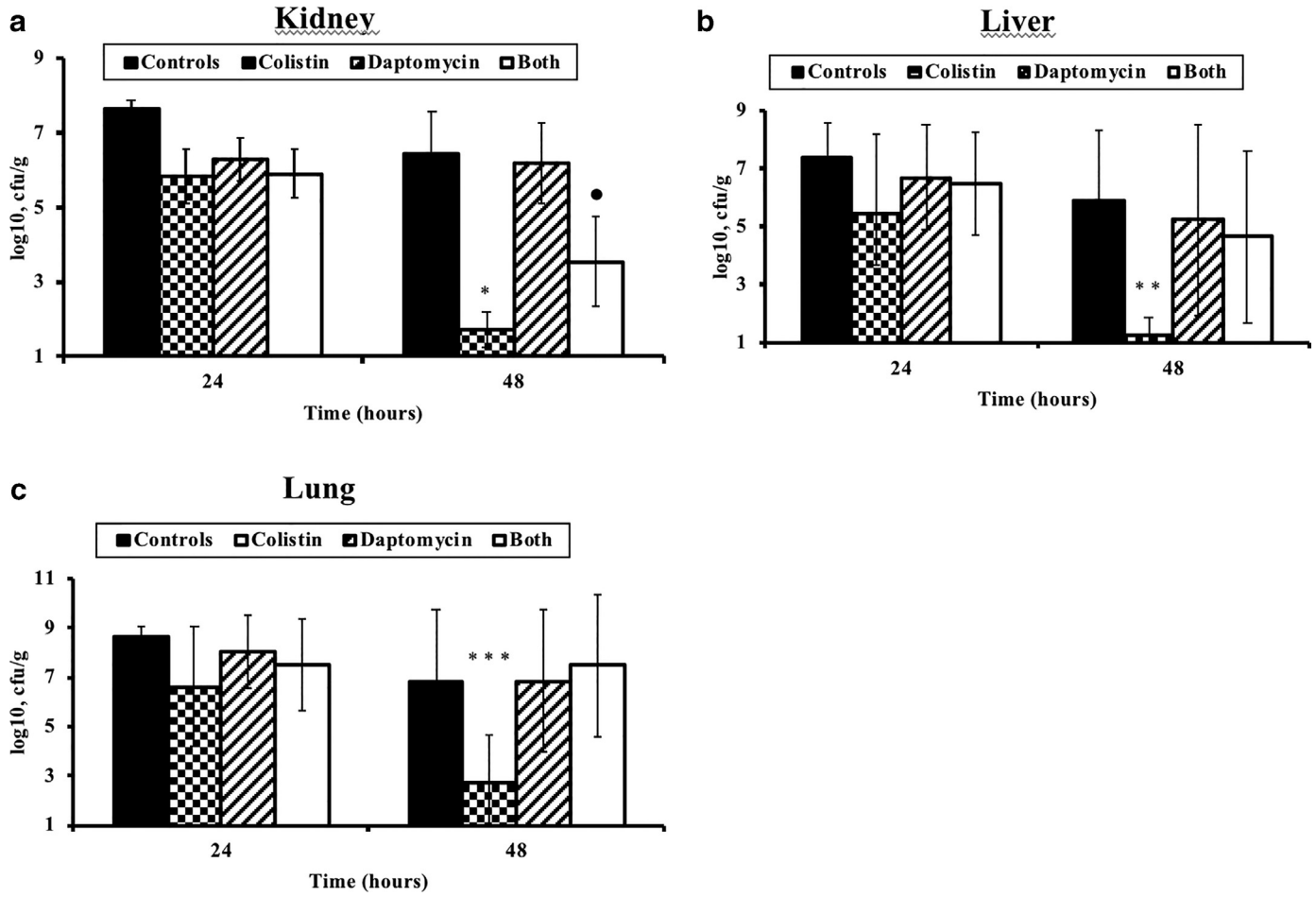

Fig. 3. Bacterial outgrowth at 24 h and 48 h after bacterial challenge in (a) kidney, (b) liver and (c) lung tissue in the studied groups of mice: group A, controls; group B, treated with colistin; group C, treated with daptomycin; and group D, treated with colistin and daptomycin. Statistically significant differences: group A vs. group B, * $P < 0.001$; ** $P = 0.001$; *** $P = 0.015$; group A vs. group D •$P = 0.01$.

trate the bacterial membrane. Failure of colistin alone is probably related to rebound of tissue outgrowth that is restrained by the addition of daptomycin.

The studied ABRC isolate expressed the most common plasmid-borne carbapenemase (OXA-23-like) that is characteristic of the I and II clone lineages. The isolate also expressed the OXA-51-like carbapenemase, which is a chromosomal enzyme [18]. Combinations of antimicrobials have been tested in small-scale trials. The majority of published studies remain inconclusive since they are single-arm without comparators [19]. The major limitation of future exploitation for the proposed daptomycin + colistin combination is the failure of daptomycin to penetrate the lung as it is hydrolysed by lung surfactant, making the results inapplicable in the setting of lung infection.

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Competing interests

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Ethical approval

The animal studies were approved by the Greek veterinary directorate [protocol no. 1853/2015].

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