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Efficacy of daptomycin combined with rifampicin for the treatment of experimental meticillin-resistant *Staphylococcus aureus* (MRSA) acute osteomyelitis

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ABSTRACT

Daptomycin exhibits rapid bactericidal activity against Gram-positive organisms, including meticillin-resistant *Staphylococcus aureus* (MRSA). Daptomycin in combination with rifampicin needs to be assessed in bone infection. An MRSA acute osteomyelitis model was used. Daptomycin and vancomycin were compared, alone or in combination with rifampicin, over 4 days. Surviving bacteria were counted in bone, bone marrow and joint fluid. Vancomycin and daptomycin as single therapies were ineffective, but both combinations were significantly more effective than the corresponding monotherapy. Combination of daptomycin and rifampicin could prevent *S. aureus* from developing resistance. This combination could be a useful alternative to treat MRSA osteomyelitis at an early stage.

1. Introduction

Orthopaedic infections are among the most difficult to treat, involving surgical procedures and prolonged antibiotherapy [1]. Clinical guidelines are lacking in this area of infectious disease and most of the recommendations, such as the French recommendations [2], are simply based on expert opinion as there are insufficient data to support a high level of evidence. Glycopeptides remain the first-line recommended therapy for the treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) orthopaedic infections, in combination with rifampicin when the microorganism is susceptible [3]. When MRSA is multiresistant, only a few therapeutic alternatives are available. Daptomycin is the first approved lipopeptide antibiotic and provides potent bactericidal activity against a broad range of Gram-positive bacteria, including MRSA [4–6]. The relevance of its combination with rifampicin has recently been demonstrated in animal models, particularly in foreign-body infection models [7]. Daptomycin showed similar efficacy to vancomycin in previous experimental osteomyelitis studies but has not been evaluated at the early stage of treatment or in combination with rifampicin [8–10]. Therefore, this new antibiotic needs to be appraised in combination with rifampicin in an acute osteomyelitis model.

The present study aimed to assess the efficacy of daptomycin, compared with vancomycin, in combination with rifampicin in a rabbit model of experimental MRSA acute osteomyelitis.

2. Materials and methods

2.1. Bacterial strain

MRSA strain BCB8 isolated from blood culture was used in the study.

2.2. Antibiotics

Daptomycin powder was supplied by Cubist Pharmaceuticals (Lexington, MA).

2.3. Susceptibility testing

Minimum inhibitory concentrations (MICs) of daptomycin, vancomycin and rifampicin were determined by the microdilution method in cation-adjusted Mueller–Hinton broth. For daptomycin, the test medium was supplemented with 50 mg/L Ca^{2+} according to Clinical and Laboratory Standards Institute (CLSI) guidelines [11].

2.4. Animals

Study animals were female New Zealand White rabbits. This study was approved by the animal research committee of the University of Nantes (France). Fentanyl analgesia (fentanyl transdermal patch, 12 μg/h) was used for pain management.

2.4.1. Human pharmacokinetic simulation studies

A first step in the pharmacokinetic studies consisted of investigating the parameters allowing simulation of the kinetics of

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Table 1
Bacterial counts in bone, bone marrow and joint fluid (difference between Day 7 and Day 3).

Treatment	Mean ± S.D. $\Delta\log_{10}$ CFU/g of tissue (no. of sterile samples/total no.)		
	Bone	Bone marrow	Joint fluid
Control (n = 8)	0.11 ± 0.80 (0/8)	0.20 ± 0.59 (0/8)	0.10 ± 0.60 (0/8)
Daptomycin (n = 8)	−0.85 ± 1.08 (0/8)	−0.69 ± 0.67 (0/8)	−1.06 ± 0.99 (0/8)
Vancomycin (n = 14)	−0.75 ± 0.81 (0/14)	−0.61 ± 1.50 (0/14)	−0.72 ± 1.39 (0/14)
Daptomycin + rifampicin (n = 9)	−4.51 ± 0.81*,** (9/9)	−5.00 ± 1.16*,** (8/9)	−4.568 ± 1.32*,**,‡ (4/9)
Vancomycin + rifampicin (n = 8)	−3.85 ± 1.83*,** (1/8)	−4.24 ± 1.98*,** (1/8)	−2.46 ± 1.34*,† (1/8)

S.D., standard deviation; CFU, colony-forming units.
* P < 0.01 versus untreated controls.
** P < 0.001 versus corresponding monotherapy.
† P < 0.05 versus corresponding monotherapy.
‡ P < 0.01 versus vancomycin + rifampicin.

daptomycin in human serum. Blood samples were taken from three healthy rabbits at 0, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 24 h after administration of an intravenous (i.v.) bolus of daptomycin at 6 mg/kg body weight to determine spontaneous drug kinetics. Pharmacokinetic data were processed and were compared with those of humans [12]. A computer-controlled system was then used to obtain the human kinetic profiles for daptomycin in rabbits. Pharmacokinetic parameters were intended to simulate those observed in healthy volunteers following administration of 6 mg/kg i.v. daptomycin every 24 h [13]. The infusion was delivered by a computer-controlled pump that allowed the flow to be adjusted to a profile mathematically defined in time. To validate the simulation, plasma concentrations were determined in five rabbits.

Vancomycin was given as a continuous infusion at a dose of 100 mg/kg body weight/day so that the steady-state serum level was equivalent to the usual target in humans (ca. 25 mg/L) [14]. Rifampicin 20 mg/kg was injected intramuscularly every 12 h to simulate human 10 mg/kg twice-daily oral administration [15].

2.4.2. Osteomyelitis model

The experimental procedure used in this work has been published recently [16]. A transarticular aperture was drilled in the cortical bone between the condyles on the right femur of the rabbits under general anaesthesia (ketamine 20 mg/kg i.v. and xylazine 1 mg/kg i.v.). An 8 G needle was introduced into the medullary canal and was then removed. One millilitre of a bacterial suspension of MRSA adjusted to 10^8 colony-forming units (CFU)/mL was injected into the knee cavity. Three days after inoculation, debridement and irrigation of the infected joint with 50 mL of sterile saline was performed. Samples of infected joint fluid, bone marrow and cortical bone were removed, weighed and homogenised in 500 μL of sterile saline and then serial dilutions were plated on trypticase soy agar. Following overnight incubation at 37 °C, the number of viable bacteria was determined. After lavage and debridement, rabbits were randomly assigned to five regimens: no treatment (controls); monotherapy arms (daptomycin or vancomycin); and combination arms with rifampicin (daptomycin or vancomycin). For each group, eight animals were required to validate the results. At the end of a 4-day course of antibiotics, animals were euthanised and infected joint fluid, epiphyseal bone sample and femoral bone marrow were obtained. Dilutions at 10^{-1}, 10^{-2} and 10^{-4} were performed to avoid any carry-over effect. Bacterial counts were determined after 48 h of incubation at 37 °C. The efficacy measurement was performed by comparing the bacterial load before (Day 3 after infection) and after (Day 7 after infection) antibacterial therapy. The lower limit of detection was 20 CFU/mL. The endpoint was expressed as the mean difference ± standard deviation in log CFU/g of infected tissue between Day 3 and Day 7 (Δlog CFU/g).

2.4.3. Emergence of resistance

To determine whether antibiotic regimens could induce the selection of in vivo resistant variants, undiluted samples were spread on agar plates containing daptomycin or rifampicin at concentrations corresponding to 4× MIC. Bacteria recovered after 48 h of incubation at 37 °C were tested to determine the MIC of daptomycin or rifampicin by the broth microdilution method.

2.4.4. Histopathology

Samples of the distal half of the femur bone were fixed in neutral-buffered formalin solution, dehydrated in a graded alcohol solution and embedded in methyl methacrylate. Longitudinal sections in a sagittal plane were cut at 5 mm and slices were stained with Masson–Goldner stain for histological analysis.

2.5. Statistical analysis

Mean loads from each group were compared by Newman–Keuls test after analysis of variance (ANOVA) using GraphPad Prism v4.0 (GraphPad Software Inc., San Diego, CA). A P-value <0.05 was considered significant.

3. Results

3.1. Susceptibility testing

MICs for MRSA strain BCB8 were 0.5 μg/mL for daptomycin, 1 μg/mL for vancomycin and 0.008 μg/mL for rifampicin.

3.2. Pharmacokinetic data of daptomycin in serum

Pharmacokinetic parameters of daptomycin obtained with the human-simulated dose of 6 mg/kg were close to those observed in humans: mean half-life ($T_{1/2}$), 7.8 ± 1.0 h; peak concentration (C_{max}), 86.4 ± 7.1 mg/L; and area under the concentration–time curve (AUC), 705 ± 67 mg h/L.

3.3. Osteomyelitis model

All of the animals infected with MRSA exhibited positive joint fluid, bone marrow and bone smear cultures, with mean bacterial counts 3 days after infection of 7.7 ± 0.4, 7.9 ± 0.3 and 8.4 ± 0.5 log CFU/g, respectively.

Following treatment, none of the animals in the daptomycin or vancomycin groups had sterile bone and the Δlog CFU/g was not significantly different from that observed in the control group. In both antibiotic combination groups, the mean bacterial counts in the three osteoarticular samples were significantly lower than those in the control and in both monotherapy groups (P < 0.05). The combination of daptomycin plus rifampicin gave better results,

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with a greater reduction in the bacterial load (statistically significant only in the joint fluid, $P < 0.01$) and a greater number of sterile bone samples (Table 1).

3.4. Antibiotic resistance

No variant resistant to rifampicin was detected in the group treated with daptomycin plus rifampicin, but resistant mutants were detected in one animal in the group treated with vancomycin plus rifampicin. The rifampicin MIC of mutants was >32 mg/L compared with an initial MIC of 0.008 mg/L. Resistant mutants to daptomycin were detected in five samples from three animals treated with daptomycin alone; the MICs of mutants were 2 mg/L and 4 mg/L.

3.5. Histopathology

In the inoculated femur, haematopoietic cells in the marrow spaces were preserved. Minimal to mild acute inflammation with intramedullary abscess was observed in the medulla of the metaphysis. Occasional bone necrosis was noticeable (data not shown).

4. Discussion

In this acute osteomyelitis model, daptomycin and vancomycin alone were ineffective at reducing the bacterial count significantly. Both combinations were more effective than the corresponding monotherapy. Bacterial eradication was achieved more often with the combination of daptomycin and rifampicin compared with the combination of vancomycin and rifampicin. No emergence of resistance to rifampicin or daptomycin was observed in the daptomycin/rifampicin group, whereas resistance to daptomycin was detected in the monotherapy group.

Since daptomycin is used to treat infections caused by Gram-positive cocci resistant to β-lactams, its efficacy in the treatment of wound infections, especially in orthopaedic surgery, is of interest. This new antimicrobial could be a first step in the treatment of osteomyelitis and other orthopaedic infections. However, there are few data regarding its efficacy in treating such infections. Some experimental studies have compared daptomycin with vancomycin, showing a possible benefit of daptomycin [8–10,17]. The potential interest of daptomycin for the treatment of orthopaedic infections is supported by certain specific features of this antibiotic. Indeed, daptomycin has recently been shown to demonstrate activity against intracellular meticillin-susceptible *S. aureus* and MRSA in monocyte-derived macrophages, to inhibit slime synthesis and to induce slime disruption in vitro [18,19]. These properties could explain the potency of the association of daptomycin with rifampicin in such infections.

In this acute osteomyelitis model, no efficacy was achieved with either monotherapy regimen. Moreover, in another model, vancomycin and daptomycin monotherapy regimens were unable to cure any cage-associated infection [7]. In this model, daptomycin at high dose in combination with rifampicin showed the highest activity against planktonic and adherent MRSA.

The rabbit model used here was similar to human osteoarticular infection because of the mechanism of inoculation, the high bacterial load in bone without spontaneous cure, the combination of medical and surgical treatment, and the simulation of human pharmacokinetics. This acute osteomyelitis model is of interest in assessing efficacy of antibiotics at an early stage of treatment. The other models were different owing to a more moderate bacterial count in bone tissues leading to a spontaneous cure rate, or mimicking of haematogenous osteomyelitis [8,20].

These results and recent literature highlight the importance of adding rifampicin to daptomycin in difficult-to-treat staphylococcal bone infections. This combination could be a promising and easier treatment option for orthopaedic infections caused by MRSA.

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Ethical approval: In vivo studies were approved by the Animal Study Committee of the University of Nantes, France.

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