

Efficacy of the new cephalosporin ceftaroline in the treatment of experimental methicillin-resistant *Staphylococcus aureus* acute osteomyelitis

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Objectives: To evaluate the activity of a new cephalosporin, ceftaroline, in comparison with other antistaphylococcal drugs (linezolid and vancomycin) at projected human therapeutic doses against methicillin-resistant *Staphylococcus aureus* (MRSA) and glycopeptide-intermediate *S. aureus* (GISA) strains.

Methods: Using a rabbit experimental model of acute osteomyelitis, efficacy was assessed following 4 days of treatment by colony counts of infected bone tissues (joint fluid, femoral bone marrow and bone).

Results: Although vancomycin remains the standard treatment for MRSA osteomyelitis, it was ineffective against the MRSA strain and poorly active against GISA infections in this model. Ceftaroline and linezolid demonstrated significant activity in bone marrow and bone, and were significantly better than vancomycin treatment. However, ceftaroline was the only drug to exhibit significant activity against MRSA in infected joint fluid.

Conclusions: The present study supports ceftaroline as a promising therapeutic option for the treatment of severe MRSA infections, including osteomyelitis.

Keywords: MRSA, bone, linezolid, vancomycin

Introduction

Osteomyelitis is an infective process in bone and bone marrow, requiring prolonged antibiotic and surgical treatment, and is associated with chronic morbidity. *Staphylococcus aureus*, the most common pathogen in hospital-acquired infections, is also the most common causative organism of osteomyelitis.¹ A class of antibiotics with reasonable efficacy against methicillin-resistant *S. aureus* (MRSA) strains, i.e. glycopeptides, have been extensively used in the treatment of bone and joint infections, but resistance of staphylococci to glycopeptides has already been described.² New alternatives are needed to overcome the increasing resistance of *S. aureus* strains and to improve the antimicrobial therapy of bone and joint infections.

Ceftaroline is a novel broad-spectrum cephalosporin with potent activity against MRSA strains because of a strong affinity for *S. aureus* penicillin-binding proteins (PBPs), including PBP2A, the additional protein responsible for the methicillin resistance

mechanism.^{3–5} In contrast to other classic and new antistaphylococcal drugs, ceftaroline also exhibits antibacterial activity against common Gram-negative pathogens.

The aim of the present study was to assess the *in vivo* efficacy of ceftaroline compared with that of other antistaphylococcal drugs, linezolid and vancomycin, using a rabbit model of acute experimental osteomyelitis with projected human therapeutic dosages.

Materials and methods

Bacterial strains

Two strains of MRSA were used in this study. The MRSA strain (originally designated BCB8) was isolated from a blood culture and exhibited heterogeneous low-level methicillin resistance (methicillin MIC = 16 mg/L).⁶ The glycopeptide-intermediate *S. aureus* strain (GISA) is the well-known reference strain, Mu50.²

Susceptibility testing

The MICs of ceftaroline, linezolid and vancomycin were determined by the CLSI reference broth microdilution method.⁷

Plasma concentration determination

HPLC was used to determine the concentrations of linezolid (lower detection limit, 0.1 mg/L; coefficient of variation, <10%) and ceftaroline (detection threshold, 0.1 mg/L; coefficient of variation, 6.8%). Vancomycin assays were performed by an immunoenzymatic method using a COBAS MIRA[®] unit and EMIT[®] reagents (Behring Diagnostics Inc., Cupertino, CA, USA) (detection threshold, 2.5 mg/L; coefficient of variation, <10%).

Therapeutic regimens

A computer-controlled pump was employed to allow the simulation of human pharmacokinetics of ceftaroline and linezolid in this rabbit model.^{8,9} For each MRSA strain, animals were randomly assigned to four groups: no treatment (controls); ceftaroline regimen mimicking the human dose of 10 mg/kg given twice daily (600 mg 12 hourly); linezolid regimen mimicking the human dose of 10 mg/kg given twice daily (600 mg 12 hourly); and vancomycin administered by a constant intravenous (iv) infusion to reach a serum steady-state concentration of 20× the MIC (mimicking the human dose of 30 mg/kg given once daily).¹⁰

Acute osteomyelitis model

Female New Zealand white rabbits (weight, 2.0–2.5 kg), housed in individual cages with free access to food and water, were used for this study. The Committee of Animal Ethics of the University of Nantes approved all animal experimentation in this study. Advice was taken from a professional vet and from a surgeon with regard to the management of analgesia and surgical procedures. Fentanyl analgesia (Durogesic[®], fentanyl transdermal patch, 12 µg/h) was used in the management of pain. Due to the delay in its action (~12 h), the patch was applied the night before the start of experimentation and continued for 7 days following surgery. The procedures used in this experimental model were as previously described.¹¹ On day 0, we used a percutaneous transarticular approach to perform a femoral trepanation of the right knee using a Jamshidi bone marrow biopsy needle (8 gauge) under general anaesthesia (ketamine, 20 mg/kg iv, and xylazine, 1 mg/kg iv). The Jamshidi needle was inserted between the two femoral condyles through the epiphysis, physis and metaphysis to reach the medullar canal. Following needle removal, the skin incision was closed. A bacterial suspension of 1 mL of *S. aureus* adjusted to 10⁸ cfu/mL was injected into the knee cavity. Infection was allowed to develop for 3 days, and then a surgical debridement of the infected tissues was performed followed by an articular wash using 50 mL of 0.9% saline buffer. Samples of infected joint fluid, bone marrow and bone were removed, placed immediately on ice, weighed, homogenized in 0.5 mL of saline buffer, and then spread on TS plates using a spiral system. Treatment was started 72 h after inoculation, and antibiotics were administered via the marginal ear vein for a 4 day course.

At the end of the 4 day regimen, animals were euthanized, and infected joint fluid, epiphyseal bone samples and femoral bone marrow were obtained. Dilutions at 10⁻¹, 10⁻² and 10⁻⁴ were performed to eliminate potential carryover effects. Bacterial counts were determined after 48 h of incubation at 37°C. The efficacy measurement was made by comparing the bacterial load before (day 3 after infection) and after (day 7 after infection) antibacterial therapy. The lower detection limit for this method is 1 cfu/50 µL of undiluted tissue homogenate. To determine whether antibiotic regimens could induce the selection of *in vivo*

resistant variants, undiluted vegetation homogenates were spread on agar plates containing ceftaroline, linezolid or vancomycin at concentrations corresponding to 4-fold the MIC.

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 methylmethacrylate. Longitudinal sections in a sagittal plane were cut at 5 µm and slices were stained with Masson–Goldner stain for histological analysis.

Statistics

Statistical analyses were performed with GraphPad Prism[®] v4.0 (GraphPad Software, San Diego, CA, USA). For each strain studied, analysis of variance was used to compare the effects between the different groups, followed by a Student–Newman–Keuls (SNK) test to compare treated groups two by two. A *P* value ≤0.05 was considered significant.

Results

MICs

MICs for the MRSA and GISA strains were 1 mg/L for ceftaroline, 1 and 8 mg/L for vancomycin, and 2 and 1 mg/L for linezolid, respectively.

Efficacy in the osteomyelitis model

The *in vivo* outcomes after a 4 day treatment regimen are shown in Tables 1 and 2 for the MRSA and GISA strains, respectively. Results of treatment with vancomycin were not significantly different from results observed in the (untreated) control group against the MRSA strain. Ceftaroline and linezolid demonstrated significant activity in bone marrow and bone. Ceftaroline was the only drug able to exhibit significant activity in MRSA-infected joint fluid. As previously noted for the MRSA strain, no difference was observed between vancomycin treatment and the untreated control group for GISA in bone marrow and bone. Poor activity

Table 1. Bacterial titres in MRSA-infected tissues after 4 days of treatment

Treatment (no. of animals)	Mean ± SD Δlog ₁₀ cfu/g of tissue (day 7 – day 3) ^a		
	joint fluid	bone marrow	bone
Controls (8)	0.09 ± 0.59	0.20 ± 0.59	0.11 ± 0.81
Ceftaroline (10)	-1.98 ± 1.00 ^{b,c,d}	-2.95 ± 0.44 ^{e,f}	-2.83 ± 1.50 ^{e,f}
Linezolid (8)	-0.77 ± 1.39	-2.69 ± 1.92 ^{b,c}	-2.25 ± 1.55 ^{b,c}
Vancomycin (10)	-0.19 ± 1.19	-0.39 ± 1.60	-0.52 ± 0.69

^aThe efficacy measurement was made by comparing the bacterial load before (day 3 after infection) and after (day 7 after infection) antibacterial therapy.

^b*P* < 0.05 versus controls.

^c*P* < 0.05 versus vancomycin.

^d*P* < 0.05 versus linezolid.

^e*P* < 0.001 versus controls.

^f*P* < 0.01 versus vancomycin.

Table 2. Bacterial titres in GISA-infected tissues after 4 days of treatment

Treatment (no. of animals)	Mean \pm SD Δ log ₁₀ cfu/g of tissue (day 7 – day 3) ^a		
	joint fluid	bone marrow	bone
Controls (8)	0.86 \pm 0.30	0.63 \pm 0.57	0.23 \pm 0.41
Ceftaroline (8)	-1.55 \pm 0.52 ^b	-2.02 \pm 0.93 ^{b,c}	-2.01 \pm 0.90 ^{b,c}
Linezolid (8)	-1.10 \pm 1.15 ^d	-2.38 \pm 1.02 ^{b,c}	-2.23 \pm 1.08 ^{b,c}
Vancomycin (8)	-0.68 \pm 0.34 ^d	-0.41 \pm 0.43	-0.57 \pm 0.44

^aThe efficacy measurement was made by comparing the bacterial load before (day 3 after infection) and after (day 7 after infection) antibacterial therapy.

^b $P < 0.001$ versus controls.

^c $P < 0.01$ versus vancomycin.

^d $P < 0.05$ versus controls.

was demonstrated for vancomycin in the infected joint fluid. Ceftaroline and linezolid were significantly better than no treatment and vancomycin treatment in infected joint fluid, marrow and bone. The mortality rate was equivalent between ceftaroline and vancomycin groups with the MRSA strain (i.e. 17%), but was higher in the linezolid group (39%). For the GISA strain, no mortality was observed in the ceftaroline and linezolid groups, but a mortality rate of 27% was observed in the vancomycin group. The mortality in this model is thought to be due to *S. aureus* bacteraemia episodes, which could explain the high rates observed in this study.

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. Chronic inflammation was mild, and occasional bone necrosis was noticeable (data not shown).

Discussion

The osteomyelitis experimental model is a demanding model, and the eradication of bacteria from the bone represents a very difficult challenge.¹² In experimental studies, viable bacteria may be retrieved from the bone despite a prolonged antibiotic treatment of up to 4 weeks. Common problems in experimental models of osteomyelitis include a low success rate in reproducing osteomyelitis, the elimination of the causative bacteria by the immune system and the need for use of sclerosing agents such as sodium morrhuate.¹³ The aim of development of the present acute osteomyelitis model was to obtain high bacterial counts in infected joint fluid, femoral bone marrow and bone with maintenance of the MRSA infection for the duration of the experimentation period (7 days). Our protocol allowed bacterial densities to reach 6–9 log₁₀ cfu/g of infected tissue and confirm this experimental model as a useful model to assess the early *in vivo* activity of antibacterial agents in bone infections and to provide additional information relative to studies utilizing chronic infection models.

Although vancomycin remains the standard treatment for MRSA osteomyelitis,¹⁴ it was ineffective against the MRSA strain and demonstrated poor activity against the GISA strain in this experimental model. No difference was observed between vancomycin therapy and the control group (no treatment) for MRSA-infected joint fluid, marrow and bone after 4 days of treatment. Similar results were observed with the GISA strain, with the exception of infected joint fluid in which modest activity was observed. It is important to note that the lack of efficacy of vancomycin in bone tissues is not directly attributable to the relatively short treatment used in this study (i.e. 4 days). Despite its time-dependent bactericidal activity, we have previously shown that vancomycin is effective *in vivo* against MRSA in a rabbit experimental endocarditis model after 2–4 days of treatment.^{8,15} The extrapolation of results from animal experimental studies to humans must be done cautiously; nevertheless, the results obtained in the present study do not support the use of vancomycin for the treatment of osteoarticular infections caused by MRSA, and are in agreement with reports of vancomycin treatment failure.¹⁶

Linezolid treatment reduced the bacterial counts in marrow and bone against the MRSA strain but not in infected joint fluid, where only ceftaroline was effective. For infections with the GISA strain, linezolid, like ceftaroline, exhibited significant *in vivo* activity in the three infected tissues and was significantly better than vancomycin therapy. Although linezolid was the only bacteriostatic agent among the drugs studied, this protein synthesis inhibitor demonstrated significant activity and was superior to vancomycin. One explanation could be a better ability to penetrate into infected bone tissues, as suggested by Rana *et al.*,¹⁷ although several studies would argue against this hypothesis.^{18,19}

In clinical Phase II studies of complicated skin and skin-structure infections, ceftaroline was safe, well tolerated, and no major safety issues were observed, as expected for a member of the cephalosporin class of antimicrobials.²⁰ Ceftaroline therapy resulted in a 6 log₁₀ cfu/g decrease against MRSA and GISA strains after 4 days of treatment in a rabbit endocarditis model.⁹ In the present osteomyelitis study, after 4 days of treatment, ceftaroline (600 mg 12 hourly) was the only drug to demonstrate homogeneous *in vivo* activity against both MRSA and GISA strains in all three infected tissues. These data suggest a sufficient penetration of ceftaroline in bone with effective concentrations at the infection site.

In the present study, ceftaroline, linezolid and vancomycin did not induce the development of resistance in the surviving bacteria at the end of 4 days of therapy, although this may not be surprising because the treatment period was relatively short to detect such resistance induction. For ceftaroline, previous *in vitro* passage studies have indicated that there is a low rate of development of resistance for ceftaroline in staphylococci.²¹

Conclusions

Despite the clinical use of vancomycin for the treatment of MRSA osteomyelitis, vancomycin was surprisingly ineffective against MRSA and GISA strains in our experimental rabbit model of acute osteomyelitis. Ceftaroline, a new broad-spectrum cephalosporin, demonstrated significant *in vivo* activity against both MRSA and GISA strains, and the antibacterial activity was uniformly effective for the different infected tissues (joint fluid, femoral marrow and

bone). Ceftaroline appears to be a reasonable therapeutic option for the treatment of severe MRSA infections. Moreover, the broad spectrum of ceftaroline allows coverage against most of the pathogens found in orthopaedic infections.

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Transparency declarations

D. B. is an employee of Cerexa, Inc., a subsidiary of Forest Laboratories, Inc. (New York, NY, USA), which is developing ceftaroline. Y. G. was an employee of Cerexa at the time these studies were carried out. D. B. holds stock and stock options in Forest Laboratories, Inc. All other authors: none to declare.

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