

## Comparison of ceftaroline fosamil, daptomycin and tigecycline in an experimental rabbit endocarditis model caused by methicillin-susceptible, methicillin-resistant and glycopeptide-intermediate *Staphylococcus aureus*

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**Objectives:** The aim of this study was to compare the *in vivo* activities of the new antistaphylococcal drugs ceftaroline fosamil, daptomycin and tigecycline at projected human therapeutic doses against methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) and glycopeptide-intermediate *S. aureus* (GISA) strains in a rabbit model of endocarditis.

**Methods:** The efficacy of therapeutic regimens in our model was evaluated following 4 days of treatment by determining colony counts of infected vegetations. Emergence of resistant variants during therapy was assessed.

**Results:** Using this model of infective endocarditis, ceftaroline fosamil and daptomycin demonstrated high bactericidal *in vivo* activity (reduction of  $>5 \log_{10}$  cfu/g of vegetation) after a 4 day treatment against MSSA, MRSA and GISA strains. Both drugs were more efficacious than tigecycline, which showed moderate activity but failed to exhibit a bactericidal effect. Ceftaroline was superior to daptomycin in terms of sterilization of the vegetations. Emergence of resistant variants during daptomycin therapy was observed in two animals (one in the MSSA group and one in the MRSA group) but was not observed in ceftaroline- or tigecycline-treated animals.

**Conclusions:** The novel  $\beta$ -lactam agent ceftaroline fosamil was the most active bactericidal drug in this model and is a promising therapeutic option for the treatment of severe *S. aureus* infections, including those caused by MRSA and GISA strains.

**Keywords:** antistaphylococcal drugs, cephalosporins, MRSA

### Introduction

Infective endocarditis is a severe disease associated with high morbidity and mortality rates. *Staphylococcus aureus* is the most common cause of endocarditis worldwide and methicillin-susceptible *S. aureus* (MSSA) isolates are found in up to two-thirds of cases.<sup>1</sup>  $\beta$ -Lactam agents are the standard therapy for MSSA endocarditis, but currently available  $\beta$ -lactams are generally ineffective against methicillin-resistant *S. aureus* (MRSA) strains. High rates of clinical failure have been reported with vancomycin therapy for MRSA endocarditis,<sup>2</sup> and the emergence of glycopeptide-intermediate *S. aureus* (GISA) strains highlights the urgent need for new therapeutic options for treatment of infections by isolates not susceptible to methicillin and glycopeptides.

Among the new options, tigecycline is the first member of a new class of broad-spectrum antibacterials, the glycylcyclines, which have been specifically developed to overcome the two major mechanisms of tetracycline resistance.<sup>3</sup> Daptomycin is a cyclic lipopeptide antimicrobial agent with *in vitro* activity against *S. aureus*, including MRSA strains. Daptomycin is a potential alternative to vancomycin for the treatment of severe MRSA infections, with benefits such as once-daily dosing, the lack of need for monitoring serum concentrations and FDA approval for the treatment of right-sided endocarditis.<sup>4</sup> Ceftaroline fosamil, recently approved by the FDA for use in treating acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia, is a novel broad-spectrum cephalosporin prodrug that is rapidly converted to

the microbiologically active drug, ceftaroline, in plasma following parenteral administration. Ceftaroline demonstrates bactericidal time-dependent killing activity. In contrast to other classic and new antistaphylococcal drugs, ceftaroline exhibits antibacterial activity against common Gram-negative pathogens, which could allow clinicians to avoid the use of combination therapy for empirical treatment of certain infections.

The purpose of the present study was to assess and compare the *in vivo* activity of ceftaroline fosamil with that of daptomycin and tigecycline against three *S. aureus* strains (methicillin susceptible, methicillin resistant and glycopeptide intermediate) in a rabbit model of aortic valve endocarditis with projected human therapeutic doses.

## Materials and methods

### Animals

The animals used for this study were female New Zealand white rabbits (weight, 2.2–2.5 kg) housed in individual cages with free access to food and water. Animals were treated in accordance with institutional policies and the guidelines stipulated by the animal welfare committee. The Committee of Animal Ethics of the University of Nantes approved all animal experimentation in this study.

### Bacterial strains

We studied three *S. aureus* strains: MSSA, strain ATCC 29213; MRSA, strain P9 (clinical strain isolated from blood cultures); and a methicillin-resistant GISA strain (Mu50).

### Antimicrobial agents

Clinical forms of daptomycin (Cubist Pharmaceuticals, Lexington, MA, USA) and tigecycline (Wyeth, Paris, France) were used in this study. Ceftaroline fosamil powder was provided by Forest Laboratories, Inc. (New York, NY, USA). The drugs were prepared according to the manufacturer's recommendations.

### Susceptibility testing

The MICs of ceftaroline, daptomycin and tigecycline for the three strains were determined in cation-supplemented Mueller–Hinton (MH) broth by the CLSI microdilution technique.<sup>5</sup> For daptomycin, the test medium was supplemented with  $\text{Ca}^{2+}$  to 50 mg/L according to the CLSI guidelines.<sup>6</sup> Overnight MH broth cultures were used to prepare inocula of  $10^5$  cfu/mL. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation at 37°C.

### Pharmacokinetic studies

The human pharmacokinetic profiles of ceftaroline and daptomycin were simulated as previously described.<sup>7,8</sup> For tigecycline, the first step in the pharmacokinetic studies consisted of investigating the parameters that would allow simulation of the kinetics of tigecycline in human serum. Blood samples were taken from three healthy rabbits at 0, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8 and 24 h after administration of a 30 min infusion of tigecycline (1 mg/kg) to determine spontaneous drug kinetics. The pharmacokinetic data were analysed and compared with those for humans.<sup>9</sup> Simulation was intended to provide pharmacokinetic parameters close to those observed in healthy volunteers after administration of tigecycline 50 mg twice daily. The infusion was delivered by a

computer-controlled pump that allowed the flow to be adjusted to a profile mathematically defined in time.

### Animal model (endocarditis)

The procedures used in the experimental endocarditis model were as previously described.<sup>10,11</sup> A catheter was placed into the left ventricle of anaesthetized New Zealand white rabbits. Twenty-four hours later, each animal was inoculated intravenously with 1 mL of a bacterial solution (adjusted to  $10^8$  cfu/mL) with the MSSA, MRSA or GISA strain. Treatment began 24 h after inoculation using a computer-controlled pump, as described elsewhere.<sup>9</sup> The animals were euthanized by using an intravenous bolus of thiopental at the beginning of the treatment period (controls) or at the end of the 4 day regimen. Aortic valve vegetations were excised, weighed, homogenized in 0.5 mL of saline buffer and used for quantitative cultures on agar for 24 h at 37°C. If there was no growth of undiluted vegetation homogenates spread on agar plates after 48 h of incubation at 37°C, the sample was considered sterile and the lower detection limit for the method was assigned (i.e. 1 cfu per 50  $\mu\text{L}$  of undiluted vegetation homogenate).

To evaluate whether ceftaroline, daptomycin or tigecycline treatment could induce the selection of resistant variants, undiluted vegetation homogenates were spread on agar plates containing the study drugs at concentrations corresponding to four times the MIC (with adjustment of calcium to 50 mg/mL for daptomycin testing). Bacterial counts were determined after 48 h of incubation at 37°C.

### Therapeutic regimens

For each strain, animals were randomly assigned to either no treatment (controls), ceftaroline fosamil mimicking the human dose of 10 mg/kg/12 h (600 mg twice daily), daptomycin mimicking the human dose of 6 mg/kg once daily or tigecycline mimicking the human dose of 100 mg initially, followed by 50 mg twice daily.

### Statistics

Statistical analyses were performed with GraphPad Prism<sup>®</sup> 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 Bonferroni's test to compare treated groups two by two. A *P* value of  $\leq 0.05$  was considered significant.

## Results

### MICs

MICs for the MSSA, MRSA and GISA strains are shown in Table 1. The three *S. aureus* strains were susceptible to ceftaroline, daptomycin and tigecycline, with MICs  $\leq 1$  mg/L.

**Table 1.** MICs of ceftaroline, daptomycin and tigecycline for MSSA, MRSA and GISA isolates

Strain	MICs (mg/L)		
	ceftaroline	daptomycin	tigecycline
MSSA	0.5	0.5	0.25
MRSA	1	0.5	0.5
GISA	1	0.5	0.5

**Table 2.** Bacterial titres in vegetations after 4 days of treatment

Treatment	Mean $\pm$ SD log <sub>10</sub> cfu/g of vegetation (n) <sup>d</sup>		
	MSSA	MRSA	GISA
Controls	9.63 $\pm$ 0.80 (0/8)	8.80 $\pm$ 0.33 (0/10)	8.51 $\pm$ 0.39 (0/8)
Ceftaroline (HE 10 mg/kg/12 h)	$\leq$ 2.44 $\pm$ 0.27 (8/8) <sup>a,b</sup>	$\leq$ 2.59 $\pm$ 0.12 (8/8) <sup>a,b</sup>	$\leq$ 2.48 $\pm$ 0.12 (8/8) <sup>a,b</sup>
Daptomycin (HE 6 mg/kg/24 h)	3.85 $\pm$ 2.43 (5/8) <sup>a,b</sup>	3.52 $\pm$ 1.98 (4/7) <sup>a,b</sup>	$\leq$ 2.57 $\pm$ 0.31 (8/8) <sup>a,b</sup>
Tigecycline (HE 50 mg/12 h)	6.89 $\pm$ 1.83 (0/6) <sup>c</sup>	7.11 $\pm$ 1.18 (0/5) <sup>c</sup>	7.20 $\pm$ 1.27 (0/6) <sup>c</sup>

HE, human equivalent.

<sup>a</sup>P < 0.001 versus controls; Bonferroni's test after analysis of variance.

<sup>b</sup>P < 0.01 versus tigecycline; Bonferroni's test after analysis of variance.

<sup>c</sup>P < 0.05 versus controls; Bonferroni's test after analysis of variance.

<sup>d</sup>n = no. of sterile vegetations (below the limit of detection)/total no. of vegetations.

### Computer-controlled pharmacokinetic simulation of tigecycline

The mean peak concentration, area under the curve and half-life after administration of a dose simulating a 50 mg dose in humans were: 783.8  $\pm$  42.5 ng/mL; 4.9  $\pm$  0.4  $\mu$ g h/mL; and 12.5  $\pm$  0.6 h (as compared with 819  $\pm$  113 ng/mL, 2.2  $\pm$  0.3  $\mu$ g h/mL and 11.8  $\pm$  2.5 h in humans).<sup>12</sup>

### Efficacy in endocarditis model

*In vivo* results after a 4 day treatment regimen are shown in Table 2. For all strains, the ceftaroline fosamil regimen (600 mg twice daily) demonstrated high bactericidal activity (defined as  $\geq$ 3 log<sub>10</sub> reduction in bacterial titre over untreated controls at the time of antibiotic dosing) after a 4 day treatment in this experimental endocarditis model, with reductions >5 log<sub>10</sub> cfu/g of vegetation. Daptomycin (6 mg/kg once daily) was also bactericidal against the *S. aureus* strains tested in this study, but ceftaroline was the only drug that achieved 100% sterilization of the infected vegetations for all three strains in all animals (compared with 62%, 57% and 100% achieved with daptomycin for animals infected with MSSA, MRSA and GISA strains, respectively).

Bacterial counts in aortic valve vegetations from rabbits treated with tigecycline were significantly reduced for the three strains compared with the controls; however, tigecycline failed to exhibit bactericidal activity.

Agar plates containing ceftaroline or tigecycline at four times the MIC showed no *S. aureus* colonies after plating undiluted vegetation homogenates and incubating for 48 h at 37°C. Agar plates containing daptomycin at four times the MIC showed daptomycin-resistant mutants isolated from one animal of the MSSA group and one animal of the MRSA group. Daptomycin MICs were determined to be 2 mg/L for these resistant variants.

### Discussion

During recent years, new drugs active against *S. aureus*, including methicillin-resistant strains, have reached the market (i.e. daptomycin, tigecycline and telavancin) or, like ceftaroline, will be available soon. The FDA has recently approved ceftaroline fosamil for the treatment of community-acquired bacterial pneumonia and for acute bacterial skin and skin structure infections (ABSSSI), including those caused by MRSA.<sup>13</sup>

Prescribing doctors should be able to use clinical trial data as a major source of information for evidence-based medicine for the treatment of infectious diseases.<sup>14</sup> However, the difficulty in performing clinical trials in severe types of infection such as endocarditis has resulted in a lack of clinical information for many new antibiotics regarding use in treating severe infections. Experimental animal models are one method used to assess the *in vivo* activity of new antimicrobials in the treatment of severe infections. In the present study, we used three resistant phenotypes of *S. aureus* (MSSA, MRSA, GISA) in a head-to-head comparison of new therapeutic options for the treatment of severe *S. aureus* infection.

Ceftaroline fosamil (simulated human dosing of 600 mg twice daily) clearly demonstrated highly bactericidal activity against MSSA, MRSA and GISA strains in this rabbit endocarditis experimental model (reductions >5 log<sub>10</sub> cfu/g of vegetation). These results confirm the data from a previous study with two different MRSA strains,<sup>7</sup> and are consistent with results obtained for the  $\beta$ -lactam ceftobiprole, as demonstrated by Tattevin *et al.*<sup>15</sup> using a similar experimental model. Ceftaroline achieved 100% sterilization of the vegetations infected by the MSSA, MRSA or GISA strains, whereas daptomycin sterilized 62%, 57% and 100% of the vegetations, respectively. Tigecycline exhibited 0% sterilization, as expected for a drug with a bacteriostatic mode of action. Despite *in vivo* bactericidal activity, the emergence of daptomycin-resistant variants after only 4 days of therapy is a concern. This suggests the possible need for combination therapy for daptomycin treatment of *S. aureus* infections. The 6 mg/kg daptomycin dosage regimen was not able to prevent the emergence of resistance in two animals (one in the MSSA group and one in the MRSA group). Moreover, the detection of these resistant variants was correlated with a failure of daptomycin in treating the infection in these particular rabbits. These data support the use of daptomycin dosages exceeding 6 mg/kg to increase bacterial killing and limit the risk of emergence of resistant variants during daptomycin therapy. Case reports involving daptomycin at doses up to 12 mg/kg have been described and shown to be safe and well tolerated.<sup>16,17</sup> No resistant variants were detected from vegetations during ceftaroline or tigecycline treatments.

Tigecycline demonstrated moderate activity (reduction <2 log<sub>10</sub> cfu/g compared with the controls) against MRSA and

GISA strains in this model. This moderate activity could be improved by the use of a partner drug in combination with tigecycline, but few studies have investigated the activity of combinations with the glycolcycline antibiotic against MRSA strains. Recently, it was observed that the addition of gentamicin significantly improved the killing activity of tigecycline in biofilm-forming *S. aureus* using an *in vitro* pharmacodynamic model.<sup>18</sup> Studies conducted in both animals and humans have demonstrated that tigecycline distributes widely into various tissues and body fluids.<sup>19</sup> Nevertheless, peak serum concentrations do not exceed 1 mg/L, which may limit its utility in the treatment of bacteraemia and endocarditis.<sup>20,21</sup> The recommended dosage of tigecycline could be too low for the treatment of severe infections and the assessment of higher doses in severe experimental animals models could be useful.

### Conclusions

Both ceftaroline fosamil and daptomycin demonstrated highly bactericidal activity against MSSA, MRSA and GISA strains after a 4 day treatment. These data support continued interest of these new therapeutic options in the management of severe MRSA infections. Nevertheless, the emergence of daptomycin non-susceptible *S. aureus* during therapy strongly suggests the need for considering combination therapy with other antibacterial agents and for evaluating higher dosages.

These data support the novel cephalosporin ceftaroline as a promising addition for the treatment of severe *S. aureus* infections, including those caused by MRSA and GISA strains.

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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. D. B. holds stock and options in Forest Laboratories, Inc. All other authors: none to declare.

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