In Vivo Activity of a Novel Anti-Methicillin-Resistant *Staphylococcus aureus* Cephalosporin, Ceftaroline, against Vancomycin-Susceptible and -Resistant *Enterococcus faecalis* Strains in a Rabbit Endocarditis Model: a Comparative Study with Linezolid and Vancomycin[∇]

Cédric Jacqueline,¹ Jocelyne Caillon,^{1*} Virginie Le Mabecque,¹ Anne-Françoise Miègeville,¹ Yigong Ge,² Donald Biek,² Eric Batard,¹ and Gilles Potel¹

Université de Nantes, Nantes Atlantique Universités, Thérapeutiques Cliniques et Expérimentales des Infections, EA3826, UFR Médecine, Nantes, France,¹ and Cerexa, Inc., Oakland, California²

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We assessed the in vitro and in vivo efficacy of the novel parenteral broad-spectrum cephalosporin ceftaroline against *Enterococcus faecalis* in time-kill experiments and in a rabbit endocarditis model with simulated human dosing. Ceftaroline was more active than either vancomycin or linezolid against vancomycin-sensitive and -resistant isolates of *E. faecalis*.

In recent years, enterococci have become significant pathogens due to their ability to resist most available antibiotics, including β -lactams, aminoglycosides, and glycopeptides, through intrinsic and/or acquired mechanisms of resistance (12). The lack of effective antimicrobial therapy for patients infected with glycopeptide-resistant enterococci has led to the need for new therapeutic agents. Ceftaroline is a broad-spectrum bactericidal cephalosporin that demonstrates time-dependent killing activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* and multi-drug-resistant *Streptococcus pneumoniae*, as well as common gram-negative pathogens (6, 10, 16). Ceftaroline is currently in phase 3 trials for complicated skin and skin structure infections and community-acquired pneumonia. Ceftaroline is active in vitro against *Enterococcus faecalis*, including vancomycin-resistant (Van^r) strains, although like other cephalosporins, it is inactive against *Enterococcus faecium* (6, 16).

In view of the limited number of antibiotics available for treating infections involving *E. faecalis*, it was of interest to us to investigate the efficacy of ceftaroline against this significant pathogen. The aim of the present study was to evaluate the in vitro and in vivo efficacy of ceftaroline, compared with that of linezolid and vancomycin, against *E. faecalis* strains by using time-kill experiments and a rabbit endocarditis model with simulated human dosing.

The *E. faecalis* strains employed in these studies (EF 12704 and EF NJ1) were isolated from blood cultures. EF 12704 is susceptible to vancomycin (Van^s), and EF NJ1 exhibits a Van^r VanA phenotype. To assess in vitro activity, MICs were determined according to Clinical and Laboratory Standards Institute reference broth microdilution methods (1,

3). The bactericidal activity of each drug was evaluated by using time-kill experiments with an inoculum of 5×10^6 CFU/ml (13).

The in vivo efficacy of ceftaroline was assessed by using a rabbit endocarditis model (4, 14). All animal studies were approved by the Committee of Animal Ethics of the University of Nantes. Catheters were placed into the left ventricles of anesthetized New Zealand White rabbits. Twenty-four hours later, endocarditis was induced with an inoculum of 10^8 CFU *E. faecalis*. Treatment was started 24 h after inoculation, with antibiotics delivered via the marginal ear vein by a computer-controlled pump as described elsewhere (2, 8). After 4 days of treatment, 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. To evaluate whether ceftaroline, linezolid, or vancomycin 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. Bacterial counts were determined after 48 h of incubation at 37°C.

The human pharmacokinetic profiles of linezolid and ceftaroline were simulated as previously described (8, 9). For ceftaroline, the study was designed to simulate pharmacokinetic parameters observed in healthy volunteers after a 1-h infusion of 600 mg ceftaroline fosamil (ca. 10 mg/kg) (7). For each *E. faecalis* strain, animals were randomly assigned to receive no treatment (control), ceftaroline mimicking a human dose of 10 mg/kg/12 h (600 mg every 12 h), linezolid mimicking a human dose of 10 mg/kg/12 h (600 mg every 12 h), or vancomycin by continuous intravenous infusion in order to reach a 20- to 25-mg/liter steady-state concentration in serum (17). Statistical analyses were performed with GraphPad Prism v4.0 (GraphPad Software, San Diego, CA). For each strain, analysis of variance was used to compare the effects between different groups, followed by Bonferroni's test to compare treated groups two by two. A *P* value of ≤ 0.05 was considered significant.

* Corresponding author. Mailing address: UPRES EA 3826, UER de Médecine, 1 rue Gaston Veil, 44035 Nantes Cedex 01, France. Phone and fax: (33) 240-41-2854. E-mail: jocelyne.caillon@univ-nantes.fr.

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FIG. 1. Killing curves for different concentrations of ceftaroline, linezolid, and vancomycin against strains EF 12704 and EF NJ1. Circles, control; white squares, ceftaroline at 4 mg/liter; black squares, ceftaroline at 20 mg/liter; white triangles, linezolid at 2 mg/liter; black triangles, linezolid at 12 mg/liter; crosses, vancomycin at 25 mg/liter.

The MICs of ceftaroline, linezolid, and vancomycin for strains EF 12704 and EF NJ1 were 2 and 1 mg/liter, 2 and 1 mg/liter, and 2 and >256 mg/liter, respectively. As shown in the time-kill curves (Fig. 1), linezolid at 12 mg/liter reduced the initial inoculum by less than 1 log after 24 h. Vancomycin similarly failed to exhibit bactericidal activity against either strain. In contrast, ceftaroline showed time-dependent killing and bactericidal activity at a clinically achievable concentration of 20 mg/liter against both the Van^s and Van^r strains.

In the in vivo study, linezolid significantly reduced bacterial counts of both *E. faecalis* strains in aortic valve vegetations from rabbits after 4 days of treatment but failed to demonstrate bactericidal activity (a <3-log reduction in CFU counts; Table 1) (15). Vancomycin achieved a significant reduction in bacterial counts compared with those of the untreated control group but was not bactericidal against EF 12704 (only a 2-log reduction), despite the susceptibility of this strain. Vancomycin was ineffective against Van^r (VanA phenotype) strain EF NJ1.

At the simulated human dosage, ceftaroline was efficacious ($P < 0.001$ versus the control) against both *E. faecalis* isolates, decreasing the titers of Van^s EF 12704 in vegetations by an additional 1 log compared with vancomycin and linezolid and producing 4- and 3-log decreases in the titers of Van^r EF NJ1 in vegetations compared with those produced by vancomycin and linezolid treatments, respectively. Nevertheless, for these two isolates, the in vivo activity of ceftaroline seemed to parallel the in vitro MICs for the strains; a 4.5-log decrease in EF NJ1 (MIC = 1 mg/liter) was observed, whereas an about 3-log decrease in EF 12704 (MIC = 2 mg/liter) was demonstrated.

None of the agents tested selected for resistant variants of *E. faecalis* in vivo, as assessed by the absence of colonies following plating of vegetation homogenates on agar containing the study drugs at four times the MIC and incubation for 48 h at 37°C.

In this study, simulated human dosages of ceftaroline demonstrated greater in vivo activity against the *E. faecalis* strains than did linezolid and vancomycin, which is consistent with the more potent activity of ceftaroline observed in time-kill studies. As expected for an agent with a static mode of action, linezolid showed only modest time-dependent activity against both strains. Vancomycin failure against strain EF NJ1 (MIC, ≥ 256 mg/liter) was expected, but it was not expected against susceptible strain EF 12704 at vancomycin concentrations corresponding to the steady-state concentration in plasma (during continuous infusion). Entenza et al. (5) also observed a lack of in vitro vancomycin activity in time-kill studies with two Van^s *E. faecalis* strains (MICs = 0.25 and 2 mg/liter), despite the use of 36 mg/liter of vancomycin. Our findings of limited activity of vancomycin against *E. faecalis* in this rabbit endocarditis model are consistent with a previous study by Lafaurie et al. (11), who observed a similar activity (a log₁₀ 2-CFU/g decrease) against two Van^s *E. faecalis* strains in a rabbit endocarditis model following intramuscular administration of vancomycin 50 mg/kg/12 h for 5 days.

In summary, the significant in vitro and in vivo activities of ceftaroline against *E. faecalis* suggest that this new cephalosporin could be a therapeutic option for patients with *E. faecalis* infections. Based on the success of this study, further investigation elucidating the in vivo efficacy of ceftaroline against this pathogen alone and in combination with other agents is suggested.

TABLE 1. Bacterial titers in vegetations after 4 days of treatment

Treatment	Mean log ₁₀ CFU/g of vegetation $\pm$ SD (no. of animals)	
	Van ^s EF 12704	Van ^r EF NJ1
Control	8.56 $\pm$ 0.74 (8)	8.60 $\pm$ 0.54 (9)
Ceftaroline (10 mg/kg/12 h) ^a	5.68 $\pm$ 0.49 (7) ^{b,c}	3.98 $\pm$ 0.85 (9) ^{b,d}
Linezolid (10 mg/kg/12 h) ^a	6.88 $\pm$ 0.70 (7) ^b	6.88 $\pm$ 0.77 (9) ^{b,e}
Vancomycin	6.70 $\pm$ 0.25 (8) ^b	8.01 $\pm$ 0.76 (8)

^a Simulated dose for humans.

^b $P < 0.001$ versus controls.

^c $P < 0.05$ versus linezolid and vancomycin.

^d $P < 0.001$ versus linezolid and vancomycin.

^e $P < 0.05$ versus vancomycin.

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REFERENCES

1. Amsterdam, D. 1996. Susceptibility testing of antibiotics in liquid media, p. 52–111. In V. Lorian (ed.), *Antibiotics in laboratory medicine*, 4th ed. The Williams & Wilkins Co., Baltimore, MD.
2. Bugnon, D., G. Potel, J. Caillon, D. Baron, H. B. Drugeon, P. Feigel, and M. F. Kergueris. 1998. In vivo simulation of human pharmacokinetics in the rabbit. *Bull. Math. Biol.* **60**:545–567.
3. Clinical and Laboratory Standards Institute. 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 7th ed. M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
4. Durack, D. T., and P. B. Beeson. 1972. Experimental bacterial endocarditis. II. Survival of bacteria in endocardial vegetations. *Br. J. Exp. Pathol.* **53**:50–53.
5. Entenza, J. M., T. Callandra, Y. Moosmann, R. Malinverni, and M. P. Glauser. 1992. Teicoplanin versus vancomycin for prophylaxis of experimental *Enterococcus faecalis* endocarditis in rats. *Antimicrob. Agents Chemother.* **36**:1256–1262.
6. Ge, Y., D. Biek, G. H. Talbot, and D. F. Sahm. 2008. In vitro profiling of ceftaroline against a collection of recent bacterial isolates from across the United States. *Antimicrob. Agents Chemother.* **52**:3398–3407.
7. Ge, Y., R. Redman, L. Floren, S. Liao, and M. Wikler. 2006. The pharmacokinetics and safety of ceftaroline (PPI-0903) in healthy subjects receiving multiple-dose intravenous infusions, abstr. A-1937. In *Abstr. 46th Intersci. Conf. Antimicrob. Agents Chemother.* American Society for Microbiology, Washington, DC.
8. Jacqueline, C., E. Batard, L. Perez, D. Boutoille, A. Hamel, J. Caillon, M. F. Kergueris, G. Potel, and D. Bugnon. 2002. In vivo efficacy of continuous infusion versus intermittent dosing of linezolid compared to vancomycin in a methicillin-resistant *Staphylococcus aureus* rabbit endocarditis model. *Antimicrob. Agents Chemother.* **46**:3706–3711.
9. Jacqueline, C., J. Caillon, V. Le Mabecque, A. F. Miègeville, A. Hamel, D. Bugnon, J. Y. Ge, and G. Potel. 2007. In vivo efficacy of ceftaroline (PPI-0903), a new broad-spectrum cephalosporin, against methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus*: comparison with linezolid and vancomycin in a rabbit endocarditis model. *Antimicrob. Agents Chemother.* **51**:3397–3400.
10. Jones, R. N., T. R. Fritsche, Y. Ge, K. Kaniga, and H. S. Sader. 2005. Evaluation of PPI-0903M (T91825), a novel cephalosporin: bactericidal activity, effects of modifying in vitro testing parameters and optimization of disc diffusion tests. *J. Antimicrob. Chemother.* **56**:1047–1052.
11. Lafaurie, M., B. Perichon, A. Lefort, C. Carbon, P. Courvalin, and B. Fantin. 2001. Consequences of VanE-type resistance on efficacy of glycopeptides in vitro and in experimental endocarditis due to *Enterococcus faecalis*. *Antimicrob. Agents Chemother.* **45**:2826–2830.
12. Moellering, R. C. 2000. *Enterococcus* species, *Streptococcus bovis*, and *Leuconostoc* species, p. 2147–2156. In G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), *Principles and practice of infectious diseases*. Churchill Livingstone, New York, NY.
13. Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman. 1980. Method for reliable determination of minimal lethal antibiotic concentrations. *Antimicrob. Agents Chemother.* **18**:699–708.
14. Perlman, B. B., and L. R. Freedman. 1971. Experimental endocarditis. II. Staphylococcal infection of the aortic valve following placement of a polyethylene catheter in the left side of the heart. *Yale J. Biol. Med.* **44**:206–213.
15. Peterson, L. R., and C. J. Shanholtzer. 1992. Tests for bactericidal effects of antimicrobial agents: technical performance and clinical relevance. *Clin. Microbiol. Rev.* **5**:420–432.
16. Sader, H. S., T. R. Fritsche, K. Kaniga, Y. Ge, and R. N. Jones. 2005. Antimicrobial activity and spectrum of PPI-0903M (T-91825), a novel cephalosporin, tested against a worldwide collection of clinical strains. *Antimicrob. Agents Chemother.* **49**:3501–3512.
17. Wysocki, M., F. Delatour, F. Faurisson, A. Rauss, Y. Pean, B. Misset, F. Thomas, J. F. Timsit, T. Similowski, H. Mentec, L. Mier, and D. Dreyfuss. 2001. Continuous versus intermittent infusion of vancomycin in severe staphylococcal infections: prospective multicenter randomized study. *Antimicrob. Agents Chemother.* **45**:2460–2467.