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Efficacy of Ceftobiprole Medocaril against *Enterococcus faecalis* in a Murine Urinary Tract Infection Model

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We evaluated ceftobiprole against the well-characterized *Enterococcus faecalis* strain OG1RF (with and without the β-lactamase [Bla] plasmid pBEM10) in a murine urinary tract infection (UTI) model. Ceftobiprole was equally effective for Bla$^+$ and Bla$^-$ OG1 strains, while ampicillin was moderately to markedly (depending on the inoculum) less effective against Bla$^+$ than Bla$^-$ OG1 strains. These data illustrate an *in vivo* effect on ampicillin of Bla production by *E. faecalis* and the stability and efficacy of ceftobiprole in experimental UTI.

Enterococci cause various infections, most commonly urinary tract infections (UTIs) (13, 16, 18, 20, 34). Ceftobiprole (BAL9141) is a new cephalosporin with broad *in vitro* activity against Gram-positive cocci, including *Enterococcus faecalis* (2, 4, 9, 15), and ceftobiprole medocaril (prodrug; BAL5788) has been shown to be active against vancomycin-resistant and β-lactamase-positive (Bla$^+$) (penicillinase-producing) *E. faecalis* strains in a mouse peritonitis model and against staphylococci in endocarditis models (1, 7, 10, 11). Among pyrrolidinone-3-ylidenemethyl cephems, ceftobiprole exhibits good affinities for *E. faecalis* PBPs, which explains its *in vivo* and *in vitro* activity (1, 14). However, the efficacy of ceftobiprole against *E. faecalis* infection in a mouse UTI model has not been evaluated. The major goal of the present study was to evaluate the efficacy of ceftobiprole compared to that of ampicillin against strains of *E. faecalis* with and without a Bla-encoding plasmid and to assess a possible *in vivo* inoculum effect with ampicillin, which would suggest lower efficacy of ampicillin in a high-bacterial-density infection sites against a Bla$^+$ strain and large amounts of Bla at the same infection sites. We also sought to determine if ceftobiprole would suffer an effect from large amounts of Bla at the same site(s).

OG1RF (referred to herein as Bla$^-$ OG1) (6, 26) is a rifampin- and fusidic acid-resistant strain of *E. faecalis*, and Bla$^+$ OG1 contains the plasmid pBEM10 (25), encoding Bla and high-level gentamicin resistance. These strains were used in order to compare effect of Bla in the same *E. faecalis* host background. Ceftobiprole (BAL 9141), used for *in vitro* MICs, and ceftobiprole medocaril, used for *in vivo* experiments, were obtained from Johnson & Johnson (Raritan, NJ), and vancomycin and ampicillin were obtained from Sigma (St. Louis, MO). MICs were determined by following CLSI guidelines (8), with *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 as controls. MICs of ampicillin and ceftobiprole for a standard inoculum (10^5 CFU/ml) and a high inoculum (10^7 CFU/ml) were also determined. All animal manipulations and 50% infective dose (ID_{50}) determinations were done by our previously described methods (32, 33). For *in vivo* antibiotic testing, our standard inoculum of 10^5 CFU/mouse (≥100 times the calculated ID_{50}) was used for Bla$^-$ OG1 and Bla$^+$ OG1, and in the case of Bla$^+$ OG1, a high inoculum of 10^7 CFU/mouse (10,000 times the calculated ID_{50}) was also used to determine an *in vivo* "inoculum effect" against the beta-lactam antibiotics, i.e., ampicillin and ceftobiprole. Subcutaneous (s.c.) therapy commenced at 1 h postinoculation (1 hpi) based on reports showing that 1 h postinoculation is sufficient for kidney colonization and intracellular bacterial community formation in mouse bladders (19). Single doses of ceftobiprole medocaril and vancomycin (2-fold range from 6.25 to 50 mg/kg of body weight) were given 1 hpi, i.e., equivalent to 4.3 to 34.2 mg/kg of ceftobiprole (parent drug); this is similar to doses previously used for s.c. ceftobiprole in mice (3, 12) and generates concentrations achievable in humans with standard human dosing (31). Two doses of ampicillin (2-fold range from 12.5 to 200 mg/kg, s.c., 1 hpi and 2 hpi) were used to avoid any potential bias for ceftobiprole; levels achieved with 80 mg/kg, s.c., 1-h dosing interval has previously been shown (with ampicillin-sulbactam) to simulate ampicillin human doses of 3 g (24). An untreated but infected group of animals served as controls for each test bacterium, and the numbers of CFU of bacteria in kidneys and bladders obtained 48 h postinfection were compared between untreated and treatment groups (5, 21). The minimum detection limit of bacteria in these experiments was 10^2 CFU/gm. The 50% protective doses (PD_{50}) were determined by the method of Reed and Muench (29), and protection was defined as no recovery of bacteria from kidney or bladder homogenates. Randomly selected colonies recovered from organs were tested by nitrocefin and/or by pulsed-field gel electrophoresis to confirm that they were the inoculated strains. The $\log_{10}$ CFU per gram of bacteria in tissues (kidneys and bladders) were analyzed for significance by the unpaired *t* test using Graph Pad Prism version 4.0 (GraphPad Software, San Diego, CA). The guidelines stipulated by the animal welfare committee of the University of Texas Health Science Center at Houston were followed (protocol HSC-AWC-09-023).

The MICs of ceftobiprole against Bla$^-$ OG1 and Bla$^+$ OG1 with 10^5 CFU/ml were 1 μg/ml and 0.5 μg/ml, while the ampicillin MICs were 1 and 4 μg/ml with 10^5 CFU/ml, respectively (Table 1).

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TABLE 1 PD_{50}s of ceftobiprole and other antibiotics against *E. faecalis* strains Bla$^-$ OG1 and Bla$^+$ OG1 in a mouse UTI model

Strain	Inoculum (CFU)	Antibiotic	MIC (μg/ml)	No. of doses (time [h] postinoculation)	PD_{50} (mg/kg body wt)	
					Kidney	Bladder
Bla$^-$ OG1	10^5	Ceftobiprole[a]	1	1 (1)	10	18
		Ampicillin	1	2 (1, 2)	7	19
		Vancomycin	1	1 (1)	31	36
Bla$^+$ OG1	10^5	Ceftobiprole	0.5	1 (1)	7	15
		Ampicillin	4	2 (1, 2)	44	66
		Vancomycin	1	1 (1)	27	38
Bla$^+$ OG1	10^7	Ceftobiprole	1	1 (1)	33	31
		Ampicillin	>128	2 (1, 2)	>200	>200
		Vancomycin[b]				

[a] Ceftobiprole (BAL 9141) was used for *in vitro* MIC determinations, and ceftobiprole medocaril (prodrug; BAL5788) was used for *in vivo* experiments.
[b] Vancomycin was not tested at the higher inoculum, since it is not known to be affected by *E. faecalis* Bla and the purpose was to evaluate an *in vivo* effect of Bla on ampicillin and test the stability of ceftobiprole.

The MICs of vancomycin against Bla$^-$ OG1 and Bla$^+$ OG1 with 10^5 CFU/ml were 1 μg/ml. At 10^7 CFU/ml, ampicillin MICs were 1 and >128 μg/ml against Bla$^-$ OG1 and Bla$^+$ OG1, respectively, and the ceftobiprole MIC was 1 μg/ml against both strains (Table 1). Since vancomycin is not a substrate for Bla, we did not test it at the higher inoculum.

In mice inoculated with Bla$^-$ OG1 (10^5 CFU), ampicillin (two doses) and ceftobiprole (one dose) showed almost equal PD_{50}s, while vancomycin showed 3- to 4-times-higher PD_{50}s for kidneys (Table 1). In mice inoculated with Bla$^+$ OG1 (10^5 CFU), PD_{50}s of ampicillin (two doses) were 4 to 6 times higher than those of ceftobiprole (Table 1) and those for Bla$^-$ OG1, while the PD_{50} for vancomycin was the same. Data for bladder were generally in agreement with those from kidneys but are not shown further here, since we and others have observed greater variability in bladder colonization than kidney colonization (22, 23, 33). For mice inoculated with Bla$^+$ OG1 (10^7 CFU), ampicillin (two doses) PD_{50}s were >6 times higher than those of ceftobiprole (Table 1). While ceftobiprole and ampicillin were equally effective against Bla$^-$ OG1 at 10^5 CFU, there was a 2- to 3-fold decrease in PD_{50} with ampicillin against 10^5 CFU of Bla$^+$ OG1 versus Bla$^-$ OG1 and a 10- to >20-fold difference with ampicillin against 10^7 CFU versus 10^5 CFU of Bla$^+$ OG1.

The reduction in CFU in kidneys with ceftobiprole and ampicillin is shown in Fig. 1A. In mice inoculated with Bla$^-$ OG1 (10^5 CFU), both ceftobiprole and ampicillin resulted in significantly reduced CFU in kidneys versus untreated animals at doses of 12.5 mg/kg (data not shown) and 25 mg/kg ($P < 0.001$ for ceftobiprole and ampicillin) (Fig. 1A) and were not significantly different from each other ($P = 0.9$). An *in vivo* effect on ampicillin was seen in Bla$^+$ OG1-inoculated mice (Fig. 1B). In mice inoculated with 10^5 CFU of Bla$^+$ OG1, ampicillin at 25 and 50 mg/kg showed nonsignificant differences in the number of CFU/g ($P > 0.3$) in kidneys versus untreated mice (Fig. 1B), while ceftobiprole showed a significant CFU/g reduction ($P < 0.0001$ and < 0.002) at both doses (Fig. 1B). Vancomycin showed significant CFU/g reduction ($P < 0.002$) in kidneys at 50 mg/kg versus untreated mice (Fig. 1B), even though this dose is lower than the dose reported to simulate concentrations achieved in humans (12, 30); data for 25 mg/kg

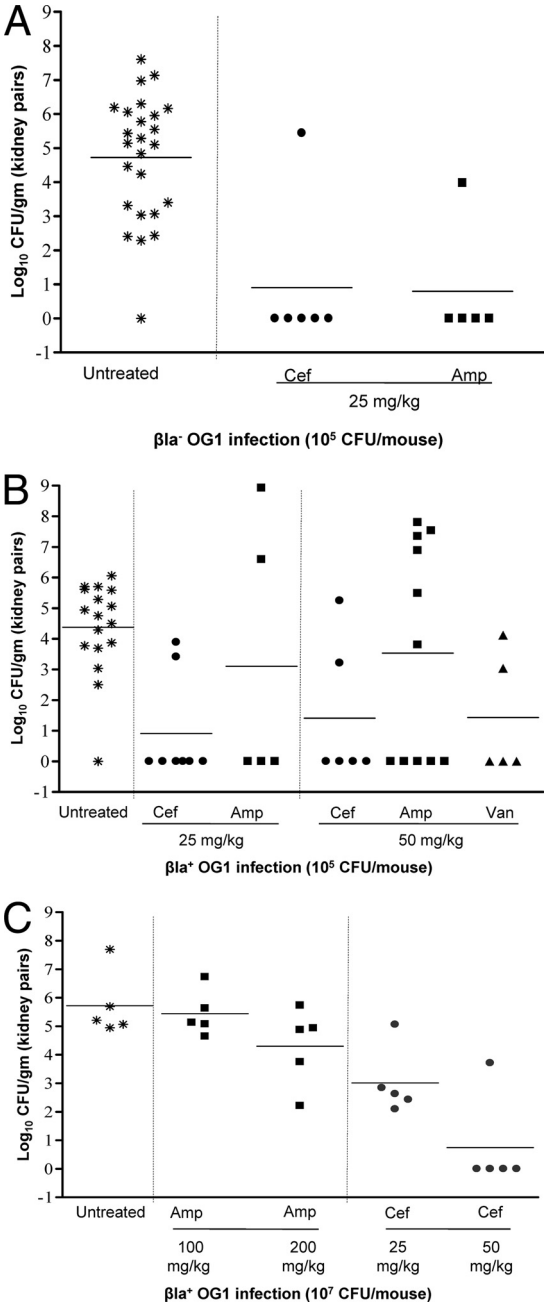

FIG 1 Dose and inoculum effect in a mouse UTI model. (A) Bla$^-$ OG1 at an inoculum of 10^5 CFU. Bacterial counts from kidneys of mice treated with ceftobiprole (single 25-mg/kg dose) and ampicillin (two 25-mg/kg doses) and untreated controls are shown. Horizontal bars represent the geometric means ($P < 0.001$ for ceftobiprole and ampicillin at 25 mg/kg for all treated versus untreated control mice). (B) Bla$^+$ OG1 at an inoculum of 10^5 CFU. Bacterial counts from kidneys of mice treated with ceftobiprole (single doses of 25 and 50 mg/kg), ampicillin (two doses of 25 and 50 mg/kg each), and vancomycin (single dose of 50 mg/kg) and untreated controls are shown. Horizontal bars represent the geometric means ($P < 0.0001$ and < 0.002 for ceftobiprole at 25 and 50 mg/kg, respectively, $P > 0.3$ for ampicillin at 25 and 50 mg/kg, and $P < 0.002$ for vancomycin at 50 mg/kg for all treated versus untreated control mice). (C) Bla$^+$ OG1 at an inoculum of 10^7 CFU. Bacterial counts from kidneys of mice treated with ceftobiprole (single doses of 25 mg and 50 mg/kg) and ampicillin (two doses of 100 and 200 mg/kg each) and untreated controls are shown. Horizontal bars represent the geometric means ($P < 0.005$ for ceftobiprole at 25 mg/kg versus ampicillin at 100 mg/kg and $P < 0.005$ and 0.006 for ceftobiprole at 50 mg/kg versus ampicillin at 100 mg/kg and 200 mg/kg, respectively).

vancomycin are not shown, since this is lower than the PD_{50}. With 10^7 of Bla$^+$ OG1, 100 and 200 mg/kg ampicillin showed nonsignificant differences in numbers of CFU/g ($P = 0.1$ and > 0.6, respectively) in kidneys versus untreated mice (Fig. 1C), while ceftobiprole showed significant CFU/g reduction versus ampicillin ($P < 0.005$ for 25 mg/kg ceftobiprole versus 100 mg/kg ampicillin; $P < 0.005$ and 0.006 for 50 mg/kg ceftobiprole versus 100 mg/kg and 200 mg/kg ampicillin, respectively) (Fig. 1C).

We previously showed that the β-lactamase enzyme in *E. faecalis* is identical to the type A staphylococcal enzyme (25, 35), and ceftobiprole has been reported to be a poor substrate for type A *S. aureus* enzyme (PC1) (28). Our recently published study using ceftobiprole and various cephalosporins against 98 clinical methicillin-susceptible *S. aureus* strains, representing four types of Bla, showed lower high- and standard-inoculum MICs of ceftobiprole than of other cephalosporins (27), reflective of the stability of ceftobiprole to staphylococcal β-lactamases, including type A. The failure of ampicillin against high inocula of Bla$^+$ OG1 is similar to an observation made in a rat endocarditis model, where high Bla$^+$ *E. faecalis* density in vegetations showed a biological effect with ampicillin therapy, even though the bacteria were susceptible *in vitro* at a standard inoculum (17).

In conclusion, we observed an *in vivo* effect of the *E. faecalis* β-lactamase and ampicillin treatment failure in the mouse UTI model, while ceftobiprole was efficacious in animals even when a high inoculum of Bla$^+$ *E. faecalis* was used. Our findings suggest that ceftobiprole may have potential against urinary tract infections caused by antibiotic-resistant *E. faecalis* strains and support its further investigation against such infections.

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