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 (produg; 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 cephehms, ceftobiprole exhibits good affinities for 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 Blaencoding 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 (10⁶ CFU/ml) and a high inoculum (10⁷ CFU/ml) were also determined. All animal manipulations and 50% infective dose (ID₅₀) determinations were done by our previously described methods (32, 33). For in vivo antibiotic testing, our standard inoculum of 10⁶ CFU/mouse (≥100 times the calculated ID₅₀) was used for Bla⁻ OG1 and Bla⁺ OG1, and in the case of Bla⁺ OG1, a high inoculum of 10⁷ CFU/mouse (10,000 times the calculated ID₅₀) 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 10² CFU/gm. The 50% protective doses (PD₅₀) 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 log₁₀ 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 10⁶ CFU/ml were 1 µg/ml and 0.5 µg/ml, while the ampicillin MICs were 1 and 4 µg/ml with 10⁷ CFU/ml, respectively (Table 1).

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concentrations achieved in humans (12, 30); data for 25 mg/kg even though this dose is lower than the dose reported to simulate 0.002) in kidneys at 50 mg/kg versus untreated mice (Fig. 1B), the higher inoculum. Since vancomycin is not a substrate for Bla, we did not test it at Bla.

While ceftobiprole and ampicillin were equally effective against Bla at 105 CFU, there was a 2- to 3-fold decrease in PD50 for kidneys with ceftobiprole and ampicillin against 105 CFU of Bla (Table 1). In mice inoculated with Bla, PD50s were similar even though the inoculum.

In mice inoculated with Bla− OG1 (105 CFU), ampicillin (two doses) and ceftobiprole (one dose) showed almost equal PD50s, while vancomycin showed 3- to 4-times-higher PD50s for kidneys (Table 1). In mice inoculated with Bla+ OG1 (105 CFU), PD50s of ampicillin (two doses) were 4 to 6 times higher than those of ceftobiprole (Table 1) and those for Bla− OG1, while the PD50 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 (105 CFU), both strains showed significant differences in the number of CFU/g (P < 0.001 for ampicillin and ceftobiprole at 25 mg/kg for all treated versus untreated control mice). 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/107 CFU and 0.3) in kidneys.

The MICs of vancomycin against Bla− OG1 and Bla+ OG1 with 105 CFU/ml were 1 μg/ml. At 107 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 (105 CFU), ampicillin (two doses) and ceftobiprole (one dose) showed almost equal PD50s, while vancomycin showed 3- to 4-times-higher PD50s for kidneys (Table 1). In mice inoculated with Bla+ OG1 (105 CFU), PD50s of ampicillin (two doses) were 4 to 6 times higher than those of ceftobiprole (Table 1) and those for Bla− OG1, while the PD50 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 (105 CFU), both strains showed significant differences in the number of CFU/g (P < 0.001 for ampicillin and ceftobiprole at 25 mg/kg for all treated versus untreated control mice). 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). 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 < 0.001 for ampicillin and 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 107 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 < 0.005 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).

The MICs of vancomycin against Bla− OG1 and Bla+ OG1 with 105 CFU/ml were 1 μg/ml. At 107 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.
vancomycin are not shown, since this is lower than the PD₅₀. With 10⁷ 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 vegetables 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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