Evaluation of ceftobiprole medocaril against *Enterococcus faecalis* in a mouse peritonitis model

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Objectives: Ceftobiprole is a novel broad-spectrum cephalosporin with good *in vitro* activity against methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis*. The objective of this study was to assess the *in vivo* activity of ceftobiprole against four strains of *E. faecalis*, including β-lactamase-producing (Bla+) and vancomycin-resistant strains.

Methods: Mice were infected intraperitoneally with strains of *E. faecalis*: (i) the Bla+ strain HH22; (ii) two vancomycin-resistant strains (TX2484 and V583); and (iii) OG1RF (a laboratory strain), using 10× the LD50 for each strain. Ceftobiprole doses of 25, 12.5 and 6.25 mg/kg (single doses) and ampicillin 50, 25, 12.5 and 6.25 mg/kg (single and double doses) were administered subcutaneously immediately after bacterial challenge and mice were monitored for 96 h.

Results: All four *E. faecalis* had ceftobiprole MICs ≤0.5 mg/L. Despite being susceptible *in vitro* at the standard inoculum, ampicillin (single and double doses) did not protect mice against intraperitoneal challenge with Bla+ *E. faecalis* HH22, with a 50% protective dose (PD50) of >100 mg/kg, whereas ceftobiprole was protective (PD50 of 2 mg/kg). Ceftobiprole PD50s for vancomycin-resistant isolates TX2484 and V583 were 7.7 and 5.2 mg/kg, respectively, similar to those of single dose ampicillin (12.5 and 16.4 mg/kg, respectively). For OG1RF, both ampicillin and ceftobiprole protected all mice at doses of 25 and 12.5 mg/kg, respectively, with a PD50 of 4.2 and 8 mg/kg for ceftobiprole and ampicillin, respectively.

Conclusions: Ceftobiprole had comparable *in vivo* activity to that of ampicillin against vancomycin-resistant and ampicillin-susceptible strains of *E. faecalis* in the mouse peritonitis model. Ceftobiprole was superior *in vivo* to ampicillin against the Bla+ strain HH22. Our data support the further study of ceftobiprole as a therapeutic agent in humans infected with *E. faecalis*.

Keywords: enterococci, cephalosporins, animal model

Introduction

The current clinical challenges posed by enterococci include the increased incidence of nosocomial infections and the lack of enterococcal activity of several compounds due either to intrinsic resistance or the acquisition of resistance genes. Moreover, although still rare, resistance to recently approved antimicrobial agents with anti-enterococcal activity (e.g. daptomycin)1–4 has been documented in *Enterococcus faecalis* and other enterococcal species indicating that new therapeutic options for enterococcal infections might be needed in the future.

Ceftobiprole is a novel broad-spectrum, β-lactamase-stable, parenteral cephalosporin with high affinity for the penicillin binding proteins (PBPs) of Gram-positive cocci,5 including PBP2a of *Staphylococcus aureus* and PBP2x of pneumococci.6 The *in vitro* activity of ceftobiprole includes β-lactamase-producing and vancomycin-resistant *E. faecalis*7 and ampicillin-susceptible...
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*Enterococcus faecium*, Ceftobiprole medocaril (formerly BAL5788) is a water-soluble prodrug that has been used previously in several animal models including a mouse model of sepsis for Gram-positive and Gram-negative bacteria, a mouse abscess model of methicillin-resistant *S. aureus* (MRSA) and *S. aureus* with intermediate resistance to vancomycin (VISA) and a mouse pneumonia model with *Haemophilus influenzae*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. Rat and rabbit models of MRSA and VISA endocarditis have also evaluated the activity of ceftobiprole medocaril in these infections. The tissue cage model (which is a foreign body infection model in which bacteria are injected into pre-implanted Teflon tissue cages) has also been established to assess the activity of ceftobiprole against MRSA. In general, ceftobiprole has comparable or superior activity versus the comparators (vancomycin, linezolid or other β-lactams) in all models with a good correlation between *in vitro* susceptibility and *in vivo* activity. Clinical data on complicated skin and soft tissue infections indicated that ceftobiprole was as effective and safe as vancomycin in treating patients with these conditions. Phase III clinical trials of ceftobiprole medocaril in the treatment of hospital- and community-acquired pneumonia are currently underway.

The mouse peritonitis model has been extensively used in the past to evaluate the *in vivo* antibiotic activity against enterococci but its use to assess the activity of ceftobiprole against *E. faecalis* isolates has not been reported. Although ceftobiprole has shown good *in vitro* activity against *E. faecalis*, *in vivo* data are lacking. Moreover, its *in vitro* spectrum indicates that it would be active against β-lactamase producers and vancomycin-resistant isolates of *E. faecalis*. Therefore, our main goal was to evaluate the *in vivo* activity of ceftobiprole medocaril against isolates of *E. faecalis*, including ones exhibiting either vancomycin resistance or production of β-lactamase, in the mouse peritonitis model. We also sought to determine if the presence of β-lactamase in *E. faecalis* produced an *in vivo* effect in this model, despite the *in vitro* susceptibility at the standard inoculum for laboratory testing.

### Materials and methods

#### Bacterial isolates

Four well characterized isolates of *E. faecalis* were included in this study (Table 1): (i) HH22 (TX0921), a Houston isolate from 1981, which was the first enterococcal isolate found to produce β-lactamase; (ii) TX2484, also recovered in Houston in 1994 from the blood of a patient and harbouring the vanB gene cluster; (iii) V583, a vancomycin-resistant isolate (whose genome has been sequenced) recovered from the bloodstream of a patient and (iv) OG1RF, a laboratory strain of *E. faecalis* that exhibits rifampicin and fusidic acid resistance used extensively for evaluation of *E. faecalis*.

#### Antibiotics and *in vitro* susceptibility testing

For *in vitro* testing, ceftobiprole was diluted in 9.9% glacial acetic acid and 1% high quality dimethyl sulphoxide as recommended by the manufacturer (Johnson & Johnson, Raritan, NJ, USA). Vancomycin and ampicillin were obtained from Sigma, St Louis, MO, USA. The MICs of each antibiotic were determined by the agar dilution method using Mueller–Hinton (MH) agar-II (Becton–Dickinson and Company, Cockeysville, MD, USA) following the recommendations of the CLSI. *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 strains were included as controls. High inoculum MICs in broth (using 10⁷ cfu/mL of ampicillin and ceftobiprole) were determined for isolate *E. faecalis* HH22 in cation-adjusted MH broth (Becton–Dickinson and Company) following the recommendations of the CLSI.

#### Mouse peritonitis model

Female, 4- to 6-week-old, outbred ICR mice (Harlan Sprague–Dawley, Houston) weighing between 19.1 and 25 g were used as described previously. Each dosing group was composed of six animals. Bacteria for inoculation were grown on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, MI, USA) and subsequently inoculated in BHI broth at 37°C for 24 h. Mice were injected intraperitonely with 1 mL of a solution containing the *E. faecalis* strain in 12.5% suspended sterile rat faecal extract with an inoculum of ≥10 x 10⁷ cfu/mL. For isolate TX2484, LD₅₀ was determined before the therapeutic assay. Antibiotics (ceftobiprole medocaril and ampicillin dissolved in water) were given subcutaneously as a single dose. Ampicillin doses of 100, 50, 25, 12.5 and 6.25 mg/kg were administered. For ceftobiprole, doses of 25, 12.5, 6.25, 3.12 and 1.56 mg/kg were used. To compensate for differences in half-life between ceftobiprole and ampicillin, an additional experiment was performed administering a second dose of ampicillin subcutaneously to animals infected with strain HH22 (TX0921), 2 h after

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristics</th>
<th>Reference(s)</th>
<th>Bla+</th>
<th>VAN resistance</th>
<th>Ampicillin PD₅₀ (mg/kg)</th>
<th>Cefotaxime PD₅₀ (mg/kg)</th>
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<td>HH22 (TX0921)</td>
<td>First reported β-lactamase-producing (Bla+) strain</td>
<td>15</td>
<td>yes</td>
<td>no</td>
<td>&gt;100</td>
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<td>yes</td>
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<td>7.7</td>
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<tr>
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<td>no</td>
<td>yes</td>
<td>16.4</td>
<td>5.2</td>
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<tr>
<td>OG1RF</td>
<td>Laboratory strain, FUS and RIF resistance</td>
<td>19</td>
<td>no</td>
<td>no</td>
<td>8</td>
<td>4.2</td>
</tr>
</tbody>
</table>

VAN, vancomycin; FUS, fusidic acid; RIF, rifampicin.

₁₀⁻¹² PD₅₀ calculated administering single doses of ampicillin and ceftobiprole

*E. faecalis* ATCC 29212 and *S. aureus* 29213 were used as controls for MIC determinations and were within the stipulated range.
bacterial inoculation. Based on previous observations, 23 mice were monitored every 24 h for 96 h. The LD$_{50}$ and 50% protective dose (PD$_{50}$) were determined by the method described by Reed and Muench. All animal experiments followed the pre-approved guidelines of the Animal Welfare Committee of the University of Texas Health Science Center at Houston. Comparison of the survival curves of the ceftobiprole- and ampicillin-treated groups at similar doses was performed using a log-rank test with Prism for Windows (version 4.00 GraphPad Software). A value of $P < 0.05$ was considered significant.

Results and discussion

In vitro activity of ceftobiprole

All four E. faecalis isolates had ceftobiprole MICs <0.5 mg/L, whereas vancomycin MICs were 0.5, 1, 128 and >512 mg/L for HH22, OG1RF, V583 and TX2484, respectively. Ampicillin MICs at standard inoculum were 1 mg/L for all four isolates. Use of a high inoculum increased the ampicillin MIC for isolate HH22 to 512 mg/L but produced only a minor rise in the ceftobiprole MIC (from 0.5 to 1 mg/L). These results confirmed the stability of ceftobiprole against β-lactamase-producing enterococci and its good in vitro activity against the vancomycin-resistant E. faecalis isolates.

In vivo activity of ceftobiprole against β-lactamase-producing E. faecalis

Figure 1 shows the dose–response curves for the β-lactamase-producing E. faecalis HH22 (Figure 1a) and the laboratory strain OG1RF (Figure 1b). Ampicillin did not protect mice against intraperitoneal challenge with E. faecalis HH22 (inoculum of 1×10$^9$ cfu/mL, which was ~10× the calculated LD$_{50}$ for the strain) at the administered doses (PD$_{50}$ >100 mg/kg) (Table 1) with lethality similar to the control group (without antibiotic) (Figure 1a), demonstrating an in vivo effect of the β-lactamase enzyme in the peritonitis model when a high bacterial inoculum is used. In contrast, ceftobiprole medocaril protected 100% and 83.3% of mice against intraperitoneal challenge with strain HH22 at a dose of 6.25 and 3.12 mg/kg, respectively (Figure 1a), with a PD$_{50}$ of 2 mg/kg body weight (Table 1). In contrast, for OG1RF, both ceftobiprole and ampicillin were protective for all mice at doses of 12.5 and 25 mg/kg, respectively (Figure 1b) (P = 1.0, log-rank test). Similarly, no difference in mouse survival was found between ceftobiprole and ampicillin at lower doses (83.3% versus 66.6% survival at 96 h at doses of 12.5 mg/kg of ampicillin versus 6.25 mg/kg of ceftobiprole, respectively; $P = 0.55$) with a PD$_{50}$ of 4.2 and 8 mg/kg for ceftobiprole and ampicillin (single dose), respectively (Table 1).

We also evaluated survival of mice after peritoneal challenge with strain HH22 (β-lactamase-producing) after administering two doses of ampicillin (given subcutaneously, 2 h apart) and compared survival with that of the ceftobiprole group. We found no difference in mortality between administering one or two doses of ampicillin (PD$_{50}$ >100 mg/kg for both groups) (Figure 1a). These findings confirm the in vivo effect of the enterococcal β-lactamase in the peritonitis model and demonstrate the good in vivo activity of ceftobiprole against this β-lactamase-producing strain.

The enterococcal β-lactamase enzyme is identical to the class A staphylococcal enzyme and ceftobiprole is a poor substrate for class A enzymes (particularly the staphylococcal penicillinase PC1). In a previous study, the rate of ceftobiprole hydrolysis of the class A staphylococcal PC1 enzyme was only 0.93 mol of substrate hydrolysed/mol of enzyme/min, whereas for penicillin it was 10 000 mol of substrate hydrolysed/mol of enzyme/min. Therefore, it is likely that the activity of ceftobiprole against β-lactamase-producing E. faecalis is due to its inherent stability to hydrolysis by this enzyme.

![Figure 1](https://academic.oup.com/jac/article-abstract/60/3/594/734011/596)

**Figure 1.** Survival and dose–response curves for mice infected intraperitoneally with (a) E. faecalis HH22 (a β-lactamase producer) and (b) OG1RF. Ceftobiprole (BPR) (dotted lines) and ampicillin (AMP) (continuous lines) were given subcutaneously. All animal groups received the same dose range of antibiotics (see the Materials and methods section) and only the lowest doses of ceftobiprole or ampicillin that protected 100% of mice at 96 h are depicted (doses above the one shown had the same effect). For HH22, ampicillin was administered as a single dose ($\times$ 1) or in two doses given 2 h apart ($\times$2). Only single dose ampicillin is shown for OG1RF.
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Figure 2 shows the dose–response curves for strains TX2484 (Figure 2a) and V583 (Figure 2b) (VanB vancomycin-resistant isolates) after intraperitoneal challenge with $1.7 \times 10^8$ and $2.1 \times 10^9$ cfu/mL, respectively ($\sim 10 \times$ the calculated PD$_{50}$ for the strain). Both ampicillin (25 mg/kg × 1) and ceftobiprole (12.5 mg/kg × 1) protected 100% of mice ($P = 1.0$, log-rank test). The PD$_{50}$s of ceftobiprole and ampicillin were 7.7 mg/kg and 12.5 mg/kg, respectively, for TX2484 (Table 1), and 5.2 and 16.4 mg/kg, respectively, for V583 (Table 1). The results indicate that the efficacy of ceftobiprole in the mouse peritonitis model is independent of vancomycin resistance. Also, our findings support the fact that the activity of ceftobiprole is comparable to that of ampicillin in the mouse peritonitis model for non-β-lactamase-producing ampicillin-susceptible strains of E. faecalis, a unique characteristic of ceftobiprole amongst the cephalosporins.

The affinity of ceftobiprole for PBP2a of staphylococci is notable (IC$_{50}$ for competition with fluorescein-labelled ampicillin was reported to be 0.87 μM for ceftobiprole, as compared with 115 μM and $>$500 μM for ceftriaxone and methicillin, respectively). It is plausible that members of the pyrrolidinone-3-ylidenemethyl cephems (to which ceftobiprole belongs) also exhibit high affinities for the E. faecalis PBPs and this feature may explain its in vivo and in vitro activity. As a caveat, ceftobiprole lacks affinity for PBP5 of E. faecium$^3$ (IC$_{50}$ $>$ 500 μM) which is commonly overexpressed and/or mutated$^{26}$ in multi-resistant isolates of E. faecium, indicating that this compound will not be useful in the treatment of resistant E. faecium infections.

In conclusion, we found an in vivo effect of the E. faecalis β-lactamase when ampicillin was used as therapy in the mouse peritonitis model that is probably related to the high inoculum used, similar in some aspects to the high density of organisms found in cardiac vegetations where the presence of this enzyme in E. faecalis also showed a biological effect when ampicillin was used, despite in vitro susceptibility at standard inoculum. However, the dose–response curves and PD$_{50}$s of ceftobiprole for HH22 and non-β-lactamase-producing isolates showed no evidence of an in vivo effect of the enterococcal β-lactamase against ceftobiprole. The in vivo data presented here support the activity of ceftobiprole against isolates of E. faecalis including vancomycin-resistant and β-lactamase-producing strains. Our findings indicate that ceftobiprole is a promising novel alternative for multidrug-resistant E. faecalis infections and support its further exploration as a therapeutic agent in humans.

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Transparency declarations

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