In Vivo Efficacy of the Ketolide ABT-773 (Cethromycin) against Enterococci in a Mouse Peritonitis Model

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Using six Enterococcus faecalis and five Enterococcus faecium strains, the ketolide ABT-773 (ABT), now known as cethromycin, was found to have in vivo efficacy against both erythromycin (ERY)-susceptible (Ery+) and -intermediate (Eryi) enterococci (ABT 50% protective doses [PD50s], 0.5 to 4.1 and 10.3 to 16.2 mg/kg of body weight, respectively). Against four highly Ery-resistant (Eyr) strains for which ABT MICs were low, ABT showed much greater activity (PD50s, 6.3 to 32.5 mg/kg) than ERY (PD50s, >200 mg/kg) but was not protective for strains for which ABT MICs were high. In conclusion, ABT-773 showed in vivo efficacy and considerably greater activity than ERY in a mouse peritonitis model.

Enterococci are important causes of nosocomial infections, including infective endocarditis, urinary tract infections, and bacteremia (7, 14), and are problematic because of increasing antibiotic resistance. Although two agents (quinupristin-dalfopristin and linezolid) have been approved for use against vancomycin-resistant (Van+) enterococci since 1999, emergence of resistance to these agents among vancomycin-resistant enterococci and/or adverse events have continued the need for new antibiotics (5, 6). ABT-773 (cethromycin [ABT]) is a new semisynthetic ketolide that differs from the natural macrolide erythromycin (ERY), with an 11,12-position cyclic carbamate group in addition to the 3-keto group. ABT has a broad spectrum of activity against some gram-positive, gram-negative, and intracellular bacteria (1, 3, 4, 17, 18), but there is no published information regarding in vivo activity against enterococci. In the present study, we evaluated the activity of ABT against Enterococcus faecalis and Enterococcus faecium strains with various susceptibilities to ERY in a mouse peritonitis model and found that the in vivo efficacy of ABT was considerably greater than that of ERY.


Six E. faecalis isolates were selected for the present study based on their varied antibiotic susceptibility profiles (Table 1). E. faecalis OG1RF (ATCC 47077) is a commonly used strain that is plasmid free (9). E. faecalis TX0921 (HH22) is a β-lactamase-producing strain with high-level resistance to gentamicin (Gen+) (8). E. faecalis TX0052 was isolated from the blood of an endocarditis patient and is resistant to ERY (Ery+ [erm(B)]) and Van+ (vanB). Both E. faecalis TX0860 and E. faecalis TX0641 are highly resistant to ERY but susceptible to ABT.

The E. faecium strains studied included TX0016 (also known as DO) (for a partial sequence, see http://www.hgsc.bcm.tmc.edu/microbial/efaecium/) (2), an endocarditis isolate that is Ery+ [erm(B)]; E. faecium TX0016.01 (DO cured of ERY resistance by novobiocin) (DO+) (2, 15); E. faecium TX2465, a vanA-containing clinical isolate showing intermediate resistance to ERY (Ery+, Van'); E. faecium TX2597, a Van (vanA) isolate; and E. faecium TX4051 (1464-74), showing moderate resistance to ERY but susceptibility to ABT. Both ERY (Erytrocin I.V [erythromycin lactobionate]) and ABT were obtained from Abbott Laboratories, Chicago, Ill. The antibiotics were appropriately reconstituted and the stocks were stored according to the manufacturer’s instructions. MIC tests were performed according to the recommended guidelines for susceptibility testing of the National Committee for Clinical Laboratory Standards (NCCLS) (9, 10) by agar dilution with Mueller-Hinton agar II (Becton Dickinson and Company, Cockeysville, Md.) and using E. faecalis ATCC 29212 as a control strain. Enterococci were considered susceptible (Ery+) when the MIC of ERY was ≤0.5 μg/ml, intermediate when the MIC was between 1 and 4 μg/ml, and resistant when the MIC was ≥8 μg/ml (10, 11).

Female, 4- to 6-week-old, outbred ICR mice (Harlan Sprague Dawley, Houston, Tex.) with a mean weight of 25 g were used in the study. The 50% lethal dose (LD50) of enterococci for mice was determined as described earlier (15), with a 12.5% concentration of sterile rat fecal extract (SRFE). SRFE was prepared using crushed, dried rat feces by mixing with 2 volumes of 0.9% (wt/vol) saline and autoclaving at 121°C and 15 lb of pressure for 15 min. The autoclaved sample was centrifuged at ~1,543 × g at a temperature of 4°C, and the supernatant (100% SRFE) was reautoclaved under the conditions described above. Table 1 shows the LD50s observed. To determine the 50% protective doses (PD50s), both ABT and ERY were administered by subcutaneous (s.c.) injection immediately following intraperitoneal inoculation of 10 × the LD50 of enterococci in SRFE, except in the initial stages, in which ABT

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was also administered orally (p.o.) by gavage. When two doses were used, they were administered at 0 and 4 h after infection. The dose ranges studied were between 3.12 and 100 mg/kg of body weight. The PD50s of ABT and ERY were determined by the method of Reed and Muench (12). Six mice/dose/drug were used to generate LD50 and PD50 values and dose-response curves. In both the LD50 and PD50 experiments, mouse spleen homogenates were used to recover and confirm the identity of the lethal organism either by phenotypic characteristics or by using pulsed-field gel electrophoresis.

MICS of the antibiotics are shown in Table 1 along with the LD50 and PD50 values, and dose-response curves are shown in Fig. 1. Strains with erm(B) that were highly resistant to ABT and ERY were classified as cMLS B, whereas those highly resistant to ABT and ERY, an in vivo protective effect observed against strains highly resistant to ABT and ERY, an in vivo protective effect observed against strains highly resistant to ERY but for which ABT MICs were low, with an ABT PD50 of 32.5 mg/kg for E. faecalis TX8060 (ERY MIC, >512 μg/ml; ABT MIC, 0.062 μg/ml)-infected mice and an ABT PD50 value of 6.25 mg/kg for E. faecalis TX0016 (ERY MIC, >512 μg/ml)-infected mice. Protective effects were also seen with ABT for E. faecium TX2597 (ABT MIC, 0.016 μg/ml; ERY MIC, 16 μg/ml)-infected mice and E. faecium TX4051 (ABT MIC, 0.031 μg/ml; ERY MIC, 16 μg/ml)-infected mice, with ABT PD50 of 16.2 and 9.1 mg/kg, respectively.

It is of interest that while the ABT MICs were similar for the ABT-sensitive (MICs, ≤0.062 μg/ml) E. faecalis and E. faecium strains, the PD50 values were lower against the four E. faecalis strains than those against the four E. faecium strains; the reason for this in vivo-in vitro difference is not known. Two of these E. faecalis strains were Eryr, and the other two were erm(B)+ with the iMLS B phenotype. None of the E. faecium strains for which the ABT MICs were very low were erm(B)+, but for each strain, the ERY MIC was 2 to 16 μg/ml, a result Perhaps related in part to the presence of msr C (16). The in vivo activity (both intra- and interspecies) of ABT against ERY-susceptible and intermediate resistance enterococci was also observed to be similar to that of telithromycin (HMR

**TABLE 1. MICs, LD50s, and PD50s of ABT and ERY against enterococci in the mouse peritonitis model**

<table>
<thead>
<tr>
<th>Organism (characteristics)</th>
<th>MIC (μg/ml)</th>
<th>LD50 (CFU)</th>
<th>Treatment route</th>
<th>No. of doses</th>
<th>PD50 (mg/kg of body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABT</td>
<td>ERY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OG1RF (ATCC 47077) E. faecalis (Gel+, Fus', Rif')</td>
<td>0.062</td>
<td>0.5</td>
<td>1.2 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX9021 (HH22) E. faecalis Gel+, Bla+, Gen', Ery'</td>
<td>0.031</td>
<td>0.5</td>
<td>7.3 × 10^-4 - 4.7 × 10^7</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX0852 E. faecalis (Gel+, Str', Gen', Ery' [erm(B)], an endocarditis isolate)</td>
<td>≥128</td>
<td>1,024</td>
<td>1.3 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>V583 E. faecalis (Gel+, Str', Gen', Ery' [erm(B)], VanA (van8))</td>
<td>≥128</td>
<td>512-1,024^*</td>
<td>&lt;1.5 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX8600 (BES8) E. faecalis (Ery' [erm(B)])</td>
<td>0.062</td>
<td>&gt;512</td>
<td>2.3 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX0641 (CH25) E. faecalis (Ery' [erm(B)])</td>
<td>0.031</td>
<td>&gt;512</td>
<td>2.1 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX0816 (DO) E. faecium (Kan', Str', Ery' [erm(B)], Tet', an endocarditis isolate)</td>
<td>≥128</td>
<td>1,024</td>
<td>3.7 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX0816.01 (DO) E. faecium [erm(B)-cured Ery' (msrC), Str', Tet'])</td>
<td>0.062</td>
<td>2</td>
<td>3.7 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX2465 E. faecium [Ery' (msrC), Van' (van4)]</td>
<td>0.062</td>
<td>2-4^*</td>
<td>2.5 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX2597 E. faecium [Ery' (msrC), Van' (van4)]</td>
<td>0.016</td>
<td>16</td>
<td>1.1 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
<tr>
<td>TX051 E. faecium [Ery' (msrC)]</td>
<td>0.031</td>
<td>16</td>
<td>1.4 × 10^9</td>
<td>s.c.</td>
<td>1</td>
</tr>
</tbody>
</table>

a Values represent results of different determinations.
b The PD50 of erythromycin for this strain was inadvertently determined using an inoculum of more than 10 times the LD50.
c VanA and vanB results were derived on the basis of PCR or hybridization to PCR products.
d Fus, fusidic acid; Gen, gentamicin; Kan, kanamycin; Rif, rifampin; Str, streptomycin; Tet, tetracycline; β-lactamase producer.

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*References*:
1. Vol. 47, 2003 NOTES 2707
2. MICs, LD50s, and PD50s of ABT and ERY against enterococci in the mouse peritonitis model. Table 1.
3. The in vivo activity (both intra- and interspecies) of ABT against ERY-susceptible and intermediate resistance enterococci was also observed to be similar to that of telithromycin (HMR...
(3647) in the mouse peritonitis model, which also showed some efficacy when two doses were given s.c., indicating that it might be possible to achieve an effect even against more resistant organisms (15).

In conclusion, ABT showed in vivo efficacy against ERY-susceptible and ERY-intermediate enterococci and against some highly ERY-resistant enterococci that were inhibited by low concentrations of ABT. As with in vitro results, ABT was...
found to be more potent than ERY in the mouse peritonitis model but was not protective against cMLSb strains.

This study was supported by a grant from Abbott Laboratories.

REFERENCES


