

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^s) 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 (Ery^r) strains for which ABT MICs were low, ABT showed much greater activity (PD₅₀, 6.3 to 32.5 mg/kg) than ERY (PD₅₀, >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^r) 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 semi-synthetic 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.

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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^r) (8). *E. faecalis* TX0052 was isolated from the blood of an endocarditis patient and is resistant to ERY {Ery^r [erm(B)]}. *E. faecalis* V583 (13) is Ery^r [erm(B)] and Van^r

(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^r [erm(B)]; *E. faecium* TX0016.01 (DO cured of ERY resistance by novobiocin) (DO^c) (2, 15); *E. faecium* TX2465, a vanA-containing clinical isolate showing intermediate resistance to ERY (Eryⁱ, Van^r); *E. faecium* TX2597, a Van^r (vanA) isolate; and *E. faecium* TX4051(1464-74), showing moderate resistance to ERY but susceptibility to ABT. Both ERY (Erythrocin 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^s) when the MIC of ERY was ≤ 0.5 μ g/ml, intermediate when the MIC was between 1 and 4 μ g/ml, and resistant when the MIC was ≥ 8 μ g/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 $\sim 1,543 \times g$ 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 $10 \times$ the LD₅₀ of enterococci in SRFE, except in the initial stages, in which ABT

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

Organism (characteristics) ^d	MIC (μg/ml)		LD ₅₀ (CFU)	Treatment route	No. of doses	PD ₅₀ (mg/kg of body wt)	
	ABT	ERY				ABT	ERY
OG1RF (ATCC 47077) <i>E. faecalis</i> (Gel ⁺ , Fus ^r , Rif ^r)	0.062	0.5	1.2 × 10 ⁸	s.c.	1	4.1	31.5
TX0921 (HH22) <i>E. faecalis</i> Gel ⁺ , βla ⁺ , Gen ^r , Ery ^s	0.031	0.5	7.3 × 10 ⁶ –4.7 × 10 ⁷	p.o.	1	37.3	>200
TX0052 <i>E. faecalis</i> {Gel ⁺ , Str ^r , Gen ^r , Ery ^r [<i>erm</i> (B)], an endocarditis isolate}	≥128	1,024	1.3 × 10 ⁸	s.c.	1	>100	>200
V583 <i>E. faecalis</i> {Gel ⁺ , Str ^r , Gen ^r , Ery ^r [<i>erm</i> (B)], VAN ^r (<i>vanB</i>)}	≥128	512–1,024 ^a	<1.5 × 10 ⁸	s.c.	2	— ^c	>200
TX0860 (BE88) <i>E. faecalis</i> {Ery ^r [<i>erm</i> (B)]}	0.062	>512	2.3 × 10 ⁸	s.c.	1	32.5	>200
TX0641 (CH25) <i>E. faecalis</i> {Ery ^r [<i>erm</i> (B)]}	0.031	>512	2.1 × 10 ⁸	s.c.	1	6.3	>200 ^b
TX0016 (DO) <i>E. faecium</i> {Kan ^r , Str ^r , Ery ^r [<i>erm</i> (B)], Tet ^r , an endocarditis isolate}	≥128	1,024	3.7 × 10 ⁸	s.c.	1	>100	>200
TX0016.01 (DO) <i>E. faecium</i> [<i>erm</i> (B)-cured Ery ⁱ (<i>msrC</i>), Str ^r , Tet ^r]	0.062	2	3.7 × 10 ⁸	s.c.	1	16.2	27.7
TX2465 <i>E. faecium</i> [Ery ⁱ (<i>msrC</i>), Van ^r (<i>vanA</i>)]	0.062	2–4 ^a	2.5 × 10 ⁸	p.o.	1	16.6	>200
TX2597 <i>E. faecium</i> [Ery ^r (<i>msrC</i>), Van ^r (<i>vanA</i>)]	0.016	16	1.1 × 10 ⁹	s.c.	1	10.3	36.4
TX4051 <i>E. faecium</i> [Ery ^r (<i>msrC</i>)]	0.031	16	1.4 × 10 ⁹	s.c.	1	16.2	>200
				s.c.	1	9.1	>200

^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; βla⁺, β-lactamase producer.

^e —, not tested.

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 ERY only were considered to be iMLS_B.

For determination of PD₅₀s, both ABT and ERY were first tested by the s.c. and the p.o. routes against *E. faecalis* OG1RF-infected and *E. faecium* TX0016.01 (DO^c, Eryⁱ)-infected mice. The PD₅₀ of ABT for mice infected with OG1RF via the p.o. route was nine times higher than the PD₅₀ for those infected via the s.c. route. For *E. faecium* TX0016.01 (DO^c, Eryⁱ), the PD₅₀s of ABT administered by the p.o. (16.6 mg/kg of body weight) and s.c. (16.2 mg/kg) routes were similar.

ERY administered s.c. demonstrated a PD₅₀ of 31.5 mg/kg against OG1RF and 27.7 mg/kg against *E. faecium* TX0016.01 (DO^c, Eryⁱ), while orally administered ERY for OG1RF-infected and *E. faecium* TX0016.01 (DO^c, Eryⁱ)-infected mice showed no protection even at the highest dose of ERY (200 mg/kg). For this reason, the remainder of the study was performed using injections.

For OG1RF mice inoculated by the s.c. route, ABT displayed a PD₅₀ value 7.7 times lower than the PD₅₀ value found for ERY (ABT PD₅₀, 4.1 mg/kg; ERY PD₅₀, 31.5 mg/kg). For *E. faecalis* TX0921-infected mice, ABT displayed a PD₅₀ value (0.5 mg/kg) that was 31 times lower than the PD₅₀ value found for ERY (PD₅₀, 15.7 mg/kg). ABT displayed MICs of 0.062

and 0.031 μg/ml for OG1RF and TX0921, respectively, and ERY displayed MICs of 0.5 μg/ml for both strains.

Both ABT and ERY displayed high PD₅₀ values for mice infected with the highly ERY- and ABT-resistant *E. faecalis* strains TX0052 and V583 and *E. faecium* TX0016 (DO). The two-dose regimen for infected mice, consisting of one dose of 50 mg of ABT/kg of body weight at time zero and a second dose after a 4-h interval, presented better protection and survival than the 100-mg/kg regimen, suggesting that the higher level of mortality at 100 mg/kg was due in part to drug toxicity.

Unlike the nonprotective effect observed against strains highly resistant to ABT and ERY, an in vivo protective effect of ABT was seen against *E. faecalis* strains highly resistant to ERY but for which ABT MICs were low, with an ABT PD₅₀ of 32.5 mg/kg for *E. faecalis* TX0860 (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* TX0641 (ERY MIC, >512 μg/ml; ABT MIC, 0.031 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 PD₅₀s of 16.2 and 9.1 mg/kg, respectively.

It is of interest that while the ABT MICs were similar for the more ABT-sensitive (MICs, ≤0.062 μg/ml) *E. faecalis* and *E. faecium* strains, the PD₅₀s were lower against three of 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 Ery^s, 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 *msrC* (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

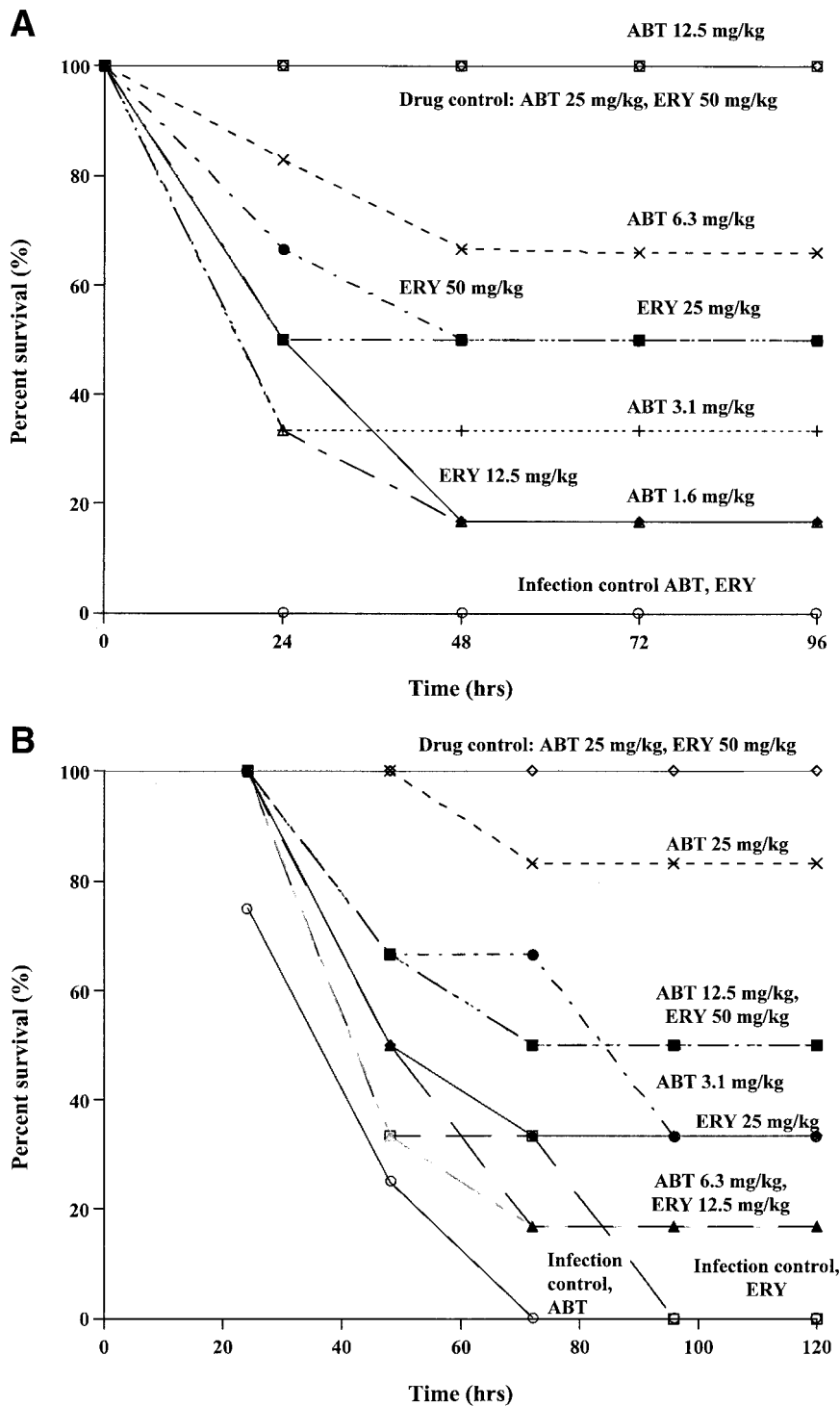


FIG. 1. Dose-response curves. (A) Drug-only (no bacteria) control animals showed 100% survival, and animals in the infection control (no antibiotic) group that were infected with *E. faecalis* strain OG1RF showed 0% survival. All of the mice received s.c. therapy with ABT or ERY for peritonitis caused by *E. faecalis* strain OG1RF. (B) Drug-only (no bacteria) control animals showed 100% survival, and animals in the infection control (no antibiotic) group that were infected with *E. faecium* strain TX2465 showed 0% survival. All the mice received s.c. therapy with ABT or ERY for peritonitis caused by *E. faecium* strain TX2465.

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 cMLS_B strains.

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