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Efficacy of Telithromycin (HMR 3647) against Enterococci in a Mouse Peritonitis Model

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We used a mouse peritonitis model to evaluate the in vivo efficacy of telithromycin (HMR 3647) (TEL) and erythromycin (ERY) against four strains of *Enterococcus faecalis* and three strains of *Enterococcus faecium* with differing susceptibilities to TEL. TEL was highly active in vivo against Ery-susceptible (Ery^s) and -intermediate (Ery^i) strains (MIC of TEL = 0.015 to 0.062 μg/ml) and showed less efficacy against Ery-resistant (Ery^r) isolates (MIC of TEL = 4 to 16 μg/ml), although this was overcome in part by a second subcutaneous dose. Quinupristin-dalfopristin was also noted to have less efficacy against Ery^r versus Ery^s or Ery^i *E. faecium* strains, but this difference was reduced by intravenous administration. In conclusion, TEL was more potent in vivo against enterococci than was ERY; its activity was lowered by the presence of *erm*(B)-mediated Ery^r.

Infections caused by gram-positive organisms are a therapeutic concern because of their increased occurrence and the high rates of resistance among some isolates that cause serious infections (4, 12, 13, 19, 22, 25). Ketolides, including telithromycin (TEL; formerly HMR 3647), have been tested both in vitro and in vivo against gram-negative and gram-positive organisms (1, 3, 5, 8, 18, 23; P. Rajagopalan-Levasseur, E. Vallee, C. Agouridas, J. F. Chantot, and J. J. Pocidalo, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F173, 1995), but multiresistant enterococci have not been well represented in in vivo studies. In a published study, one *Enterococcus faecalis* and two *Enterococcus faecium* strains were tested in a mouse septicemia model which demonstrated the ability of a ketolide (HMR 3004) to prolong survival or protect after inoculation of organisms (1). In the present study, we describe the activity of TEL and the determination of the 50% protective doses (PD_{50}s) of TEL, erythromycin (ERY), and quinupristin-dalfopristin (Q-D) against enterococci in a mouse peritonitis model.

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Bacterial strains used in the study included the following four *E. faecalis* strains: TX0921 (HH22) (14), a β-lactamase producer (Bla^+) with high-level resistance to gentamicin (Gen^r); TX0052, an endocarditis isolate; OG1RF (ATCC 47077) (15), a well-known plasmid-free isolate used as a recipient in many laboratories; and V583 (9, 21), a *vanB*-containing isolate. The three *E. faecium* strains used in the study were TX16 (2), an endocarditis isolate; TX16.01-Ery^c (TX16 cured of ERY resistance [Ery^r] by novobiocin), provided by Robert M. Rakita, Houston, Tex.; and a *vanA*-containing clinical isolate, TX2465 (11; K. V. Singh et al., 38th ICAAC, abstr. B-13). TEL was obtained from Hoechst Marion Roussel, Romainville, France; ERY A was obtained from Abbott Laboratories, North Chicago, Ill.; and Q-D was obtained from Rhône-Poulenc Rorer, Vitry sur Seine, France. MICs were determined by following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (16, 17). The susceptibility breakpoints of ERY, according to the NCCLS guidelines (16, 17) for enterococci, were ≤0.5 μg/ml for Ery^s, 1 to 4 μg/ml for Ery^i, and ≥8 μg/ml for Ery^r. For in vivo testing, enterococci grown on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) plates with or without ERY were used to inoculate brain heart infusion broth, and preparation of inocula and CFU determination were done by following a previously published method (24). Female, 4- to 6-week-old outbred ICR mice (Harlan Sprague Dawley, Houston, Tex.) with a mean weight of 25 g were used. Each dosing group was composed of six animals. For the treatment groups, mice were injected intraperitoneally with 1 ml of premixed bacteria (cell density corresponding to 10 times the minimal lethal dose) in 50% sterile rat fecal extract (10, 24). TEL and other antibiotics were administered orally (p.o.) by gavage, subcutaneously (s.c.) or by the intravenous (i.v.) route immediately following the inoculation of mice. Animals were observed for up to 96 h for *E. faecalis* and 120 h for *E. faecium*. The PD_{50}s of TEL and other antibiotics were determined by the method of Reed and Muench (20); Kaplan-Meier survival curves were generated for some. Bacteria were recovered from the spleens of dead mice, and the identity of the organisms was confirmed by phenotypic characteristics or by using pulsed-field gel electrophoresis. Preapproved guidelines of the Animal Welfare Committee of the University of Texas Health Science Center at Houston were followed throughout the course of the animal experiments.

The results of MIC testing for isolates used in the antibiotic protection studies are presented in Table 1 along with PD_{50}s. ERY administered by the oral route showed no protection of OG1RF-inoculated mice even at the highest dose, while TEL demonstrated PD_{50}s of 29.7 mg/kg of body weight when administered by the p.o. route. When administered by the s.c. route (Table 1), TEL displayed a PD_{50} three times lower (PD_{50} = 9.4 mg/kg) than s.c. ERY (PD_{50} = 31.9 mg/kg) in OG1RF-inoculated mice. In TX0921-inoculated mice, the

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TABLE 1. PD_{50}s of telithromycin and other antibiotics for enterococci in the mouse peritonitis model

Antibiotic	Route of therapy[b]	PD_{50} (mg/kg) for[a]: *E. faecalis*				*E. faecium*		
		OG1RF (ATCC 47077)	TX0921 Bla^+ Chl^i Gen^r Tet^r	TX0052 Chl^i Ery^r [*erm*(B)] Gen^r Str^r	V583 Chl^i Ery^r [*erm*(B)] Tet^r Van^r (*vanB*)	TX16 Chl^i Ery^r [*erm*(B)] Gen^r Str^r Tet^r	TX16.01-Ery^c Gen^r Str^r Tet^r	TX2465 Amp^r Chl^i Ery^i Van^r (*vanA*)
TEL		**0.031**	**0.015**	**4–8**	**8**	**16**	**0.062**	**0.015–0.031**
	p.o., 1 dose	29.7	34.9	>200	>200	>200	12.5	38.6
	p.o., 2 doses	—	—	80	>200	168	9.35	—
	s.c., 1 dose	9.4	0.57	>50	>50	>50	13.6	10.4
	s.c., 2 doses	—	—	40	25	12.5	3.12	—
ERY		**0.5**	**0.5**	**1,024**	**512–1,024**	**1,024**	**2**	**2–4**
	p.o., 1 dose	>200	>200	>200	—	>200	>200	—
	s.c., 1 dose	31.9	12.5	>200	>200	>200	27.7	35.6
Q-D		**8**	**8**	**16**	**16**	**1**	**1**	**1**
	s.c., 1 dose	—	—	—	—	>200	27.9	—
	s.c., 2 doses	—	—	—	—	36.5	—	—
	i.v., 1 dose	—	—	—	—	32.1	15.2	10.2

[a] Values in bold are MICs, (in micrograms per milliliter). Ranges represent the results of different determinations. Chl, chloramphenicol; Str, streptomycin; Tet, tetracycline; Van, vancomycin. MICs were 1 to 4 μg/ml for Ery^i and 16 μg/ml for Chl^i. —, not tested.

[b] Single-dose administration of drug was performed at 0 h immediately after intraperitoneal inoculation of bacteria. Two-dose administration was performed at 0 and 4 h after infection.

PD_{50} of TEL administered by the p.o. route was 34.9 mg/kg, while p.o. ERY at 200 mg/kg did not show protection (Table 1). TEL displayed a PD_{50} ~21 times lower (PD_{50} = <0.57 mg/kg) than ERY (PD_{50} = 12.5 mg/kg) when administered by the s.c route. There was 100% survival of mice at doses of 3.12, 6.25, and 12.5 mg of TEL per kg compared with 60% survival with 12.5 mg of ERY per kg; s.c. ERY did not show any protection in TX0921-inoculated mice at 3.12 and 6.25 mg/kg (Fig. 1A). p.o. TEL and ERY showed no protection (Table 1) at 200 mg/kg against either *E. faecalis* TX0052 (ERY and TEL MICs of 1,024 μg/ml and 4 to 8 μg/ml, respectively) or V583 (ERY and TEL MICs of 512 to 1,024 μg/ml and 8 μg/ml, respectively) in inoculated mice. Two doses of TEL given by the p.o. route showed some protection (Table 1) against TX0052 (PD_{50} = 80 mg/kg) but not against V583 (PD_{50} = >200 mg/kg). One dose of s.c TEL (50 mg/kg) did not show any protection against these strains, but two doses of s.c. TEL showed protection (Table 1) against both (PD_{50}s = 25 to 40 mg/kg). Organisms recovered from the spleens of dead mice were retested for MICs by the agar dilution method, and the MICs of TEL for these isolates were consistent with those observed prior to the inoculation.

TEL and ERY administered p.o. showed no protection (Table 1) at 200 mg/kg against the *erm*(B)-containing *E. faecium* strain TX16 (ERY MIC = 1,024 μg/ml), but two doses of TEL (TEL MIC = 16 μg/ml) had a PD_{50} of 168 mg/kg. Similarly, one s.c. dose of TEL (50 mg/kg) did not show any protection against TX16, but two doses of s.c TEL showed a PD_{50} of 12.5 mg/kg (Table 1). No protection was seen in mice when one dose of Q-D was administered by the s.c. route, but two doses of Q-D showed protection (PD_{50} = 36.5 mg/kg) in mice (Table 1). One dose of Q-D administered by the i.v. route showed a PD_{50} of 32.1 mg/kg (Table 1) in TX16-inoculated mice. Against the *erm*(B)-lacking TX16.01, a single dose of s.c. TEL was highly effective and showed a PD_{50} twofold lower than ERY and Q-D (Table 1). The time course of survival following therapy of peritonitis with TEL and ERY (one dose given s.c.) is shown in Fig. 1B; TEL showed more protection than ERY at similar or lower doses. As was seen with TX16, i.v. Q-D again showed a lower PD_{50} than did s.c. Q-D (Table 1). The PD_{50} of p.o. TEL was 38.6 mg/kg (Table 1) in mice inoculated with TX2465, and s.c. TEL and s.c. ERY showed PD_{50}s of 10.4 and 35.6 mg/kg, respectively (Table 1). Q-D showed a PD_{50} of 10.2 mg/kg (Table 1) when administered by the i.v. route.

In this study we explored the in vitro and in vivo activities of TEL, ERY, and Q-D against *E. faecalis* and *E. faecium* strains. Similar to a previous study (23), we found that TEL inhibited Ery-susceptible (MIC = ≤0.5 μg/ml) and -intermediate (MIC = 1 to 4 μg/ml) enterococci at ≤0.031 μg/ml; for two *E. faecalis* (TX0052 and V583) and one *E. faecium* (TX16) strain for which the MICs of ERY were 1,024 μg/ml, the MICs of TEL were 4 to 8 μg/ml and 16 μg/ml, respectively. TEL displayed excellent in vivo activity against two Ery^s *E. faecalis* and two *E. faecium* strains (one Ery^s and one *vanA* Ery^i ampicillin-resistant [Amp^r] strain) when administered by the p.o. and s.c. routes, while ERY showed protection only when administered by the s.c. route, with higher PD_{50}s, these strains. Paralleling the increased MICs of TEL for the Ery^r enterococci, single doses of TEL and ERY failed to protect mice when administered by the p.o. or s.c. route, while two doses of s.c. TEL showed protection in mice against these strains. In vivo efficacy of HMR 3004 and HMR 3647 (TEL) was demonstrated earlier against other gram-positive bacteria, including one Ery^s and two Ery^i enterococci (1,6).

While the bactericidal and in vivo activities of Q-D against Ery^r *E. faecium* have been questioned previously (7), we also found that s.c. Q-D was ineffective when given as a single dose for an Ery^r *E. faecium* strain (TX16), while the PD_{50} was 27.9 mg/kg for a derivative of this strain that was cured of its Ery resistance. Based on the manufacturer's recommendation that the i.v. administration was more appropriate to achieve the desired ratios of the individual components, we also tested i.v. Q-D and found this route to be much more effective, with two doses by the s.c. route and one i.v. dose generating similar PD_{50}s (36.5 and 32.1 mg/kg, respectively) against the Ery^r strain TX 16.

In summary, the greater in vitro activity of TEL versus ERY against test bacteria was also reflected in its in vivo activity in a mouse peritonitis model against both *E. faecalis* and *E. fae-*

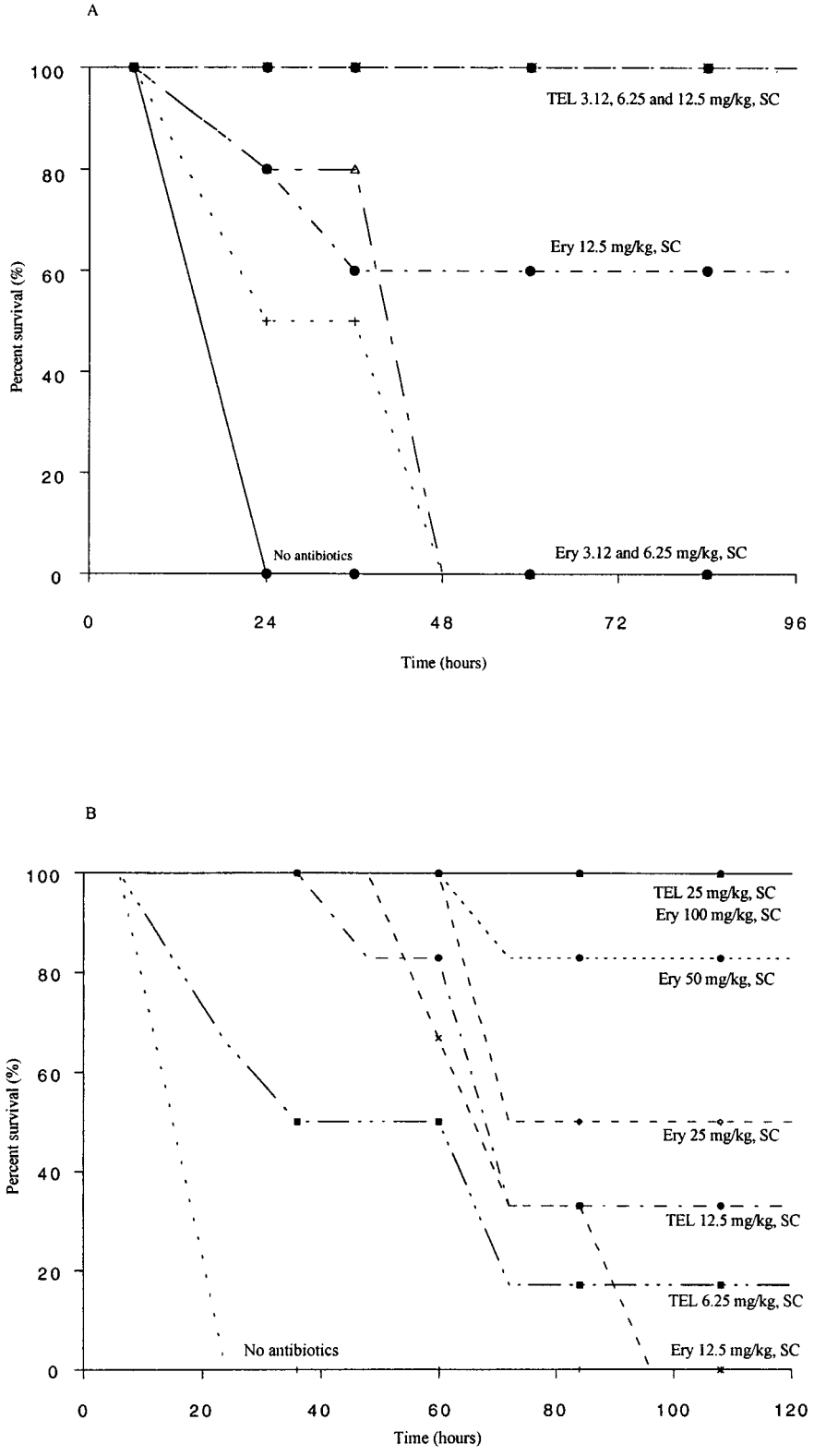

FIG. 1. (A) Survival and dose-response curve following therapy with TEL or ERY of peritonitis caused by *E. faecalis* strain TX0921 (HH22 Bla^+ Gen^r) given by the s.c. route (single dose). (B) Survival and dose-response curve following therapy with TEL or ERY of peritonitis caused by *E. faecium* strain TX0016.01-Ery^c given by the s.c. route (single dose).

cium strains, suggesting that this ketolide could be a promising drug for use against some multiresistant enterococci.

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