

In Vitro Activity of TR-700, the Antibacterial Moiety of the Prodrug TR-701, against Linezolid-Resistant Strains[∇]

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Received 27 June 2008/Returned for modification 8 September 2008/Accepted 28 September 2008

TR-701 is the orally active prodrug of TR-700, a novel oxazolidinone that demonstrates four- to eightfold-greater activity than linezolid (LZD) against *Staphylococcus* and *Enterococcus* spp. In this study evaluating the in vitro sensitivity of LZD-resistant isolates, TR-700 demonstrated 8- to 16-fold-greater potency than LZD against all strains tested, including methicillin-resistant *Staphylococcus aureus* (MRSA), strains of MRSA carrying the mobile *cfr* methyltransferase gene, and vancomycin-resistant enterococci. The MIC₉₀ for TR-700 against LZD-resistant *S. aureus* was 2 µg/ml, demonstrating the utility of TR-700 against LZD-resistant strains. A model of TR-700 binding to 23S rRNA suggests that the increased potency of TR-700 is due to additional target site interactions and that TR-700 binding is less reliant on target residues associated with resistance to LZD.

Oxazolidinone antibiotics are one of the newest classes of antibiotics developed within the past 30 years, with linezolid (LZD) representing the only marketed member of this class. In 2000, LZD (Zyvox) was granted approval for the treatment of infections associated with vancomycin-resistant *Enterococcus faecium*, nosocomial pneumonia, community-acquired pneumonia due to *Streptococcus pneumoniae* and methicillin-sensitive *Staphylococcus aureus* (MSSA), and complicated skin and skin structure infections, including cases due to methicillin-resistant *Staphylococcus aureus* (MRSA) (1). Later approvals included pediatric use, pneumonia due to multidrug-resistant *S. pneumoniae*, and treatment of diabetic foot infections, without osteomyelitis, caused by gram-positive bacteria. These approvals represent important milestones for the novel oxazolidinone class in the treatment of serious infections.

Oxazolidinones have been shown to bind to the 50S ribosomal subunit and inhibit protein translation (31). A model of the binding of LZD to the 23S rRNA peptidyl transferase region has been previously published, based upon in vivo cross-linking experiments (18). This model predicts that LZD would specifically interfere with the binding of the amino acid portion of the aminoacyl tRNA to the ribosomal A site. The recent crystal structure of LZD bound to the 50S ribosomal subunit confirms these findings and suggests that the mechanism of inhibition involves competition with the incoming A site substrates (13). Mutations in the 23S rRNA central loop of domain V, the peptidyl transferase center (PTC), are associated with the development of LZD resistance.

LZD-resistant *S. aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, and *Enterococcus faecalis* mutants have been

isolated infrequently in the clinic and can be selected for in the laboratory (2, 9, 14, 27, 31). The most common mutation observed both clinically and from serial passage experiments is the G2576U mutation (*Escherichia coli* 23S rRNA numbering) (11, 31, 37).

Although this residue in the 23S rRNA does not directly interact with the oxazolidinone, G2576 N1 hydrogen bonds to the nonbridging oxygen of U2506 and stabilizes this region (G2505 and U2506), which surrounds a portion of the oxazolidinone ring (18). This interaction is lost in the G2576U substitution in the rRNA. Other mutations observed clinically include U2500A (22), or the laboratory-selected G2447U mutation (33, 34). These two residues also do not appear to interact directly with LZD but may rather act as a secondary shell to stabilize the peptidyl transferase site (18).

A novel plasmid-borne resistance mechanism was first observed in animal isolates (16, 30) but was also reported in the chromosome of CM/05, a clinical strain of MRSA from Colombia (35). This *cfr* (chloramphenicol-florfenicol resistance) methyltransferase gene confers resistance to a subset of important, structurally diverse antimicrobial agents that target the peptidyl transferase region, including chloramphenicol, clindamycin, tiamulin, quinupristin-dalfopristin, and LZD, through modification of 23S rRNA at residue A2503 (17, 20). This methyltransferase gene was shown to be located on the chromosome in *S. aureus* CM/05, downstream of the *ermB* gene and promoter (35). In addition, it is adjacent to regions with a high degree of sequence homology to a transposase as well as a gene responsible for plasmid replication, both suggesting the mobile nature of this genetic element (35). More recently, the *cfr* gene has been identified in two additional unrelated strains of MRSA, as well as in two strains of *S. epidermidis* (10, 23). In all four of these strains, the *cfr* gene has been demonstrated to be plasmid borne.

During clinical trials, LZD-resistant strains, although rare, were documented primarily in enterococci and in patients receiving prolonged therapy (9). The SENTRY Antimicro-

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[∇] Published ahead of print on 6 October 2008.

bial Surveillance Program characterized eight LZD-resistant strains from 2001 (six *Enterococcus* and two *Staphylococcus* spp.), which included five strains from patients with no prior oxazolidinone treatment (24). The results of the 2006 LEADER survey of U.S. clinical isolates have revealed a low but increasing rate of strains characterized as LZD nonsusceptible or resistant (14). Of 14,573 gram-positive pathogens isolated from 50 U.S. medical centers (2003 to 2006), the percentage of LZD-nonsusceptible or -resistant organisms has increased over time, as follows: 0.14% (2004), 0.24% (2005), and 0.45% (2006) (14). This was largely driven by the increasing incidence of resistance in coagulase-negative staphylococci and enterococci but was also apparent in *S. aureus*, where three nonsusceptible or resistant isolates were identified in 2005 and in 2006 (14). Two additional MRSA strains carrying a plasmid-borne *cfi* gene, identified in the 2006 survey from unrelated locations (10, 23), suggest a potentially troublesome dissemination of LZD resistance through the MRSA population in the future.

TR-701 (DA-7218, DA-70218), a new oxazolidinone antibiotic in clinical development, is a phosphate prodrug of the microbiologically active molecule TR-700 (DA-7157, DA-70157) (39). The prodrug TR-701 is orally absorbed and readily converted to the active form TR-700. Previous in vitro studies have shown that TR-700 is four- to eightfold more potent than LZD against recent gram-positive clinical isolates from the United States and Europe (28), as well as against bacterial clinical isolates that originated in South Korea (4, 19). MRSA or MSSA MIC₉₀s for TR-700 and LZD were 0.5 µg/ml and 4 µg/ml, respectively, for the U.S. and European isolates and 0.5 µg/ml (TR-700) versus 2 µg/ml (LZD) for both *E. faecium* and *E. faecalis* (28). To determine whether the superior in vitro potency of TR-700 versus LZD is maintained against LZD-resistant strains, we investigated the activity of TR-700 and LZD against LZD-nonsusceptible and -resistant U.S. clinical isolates with both defined and undefined mutations, as well as laboratory-derived, LZD-resistant strains.

(This study was presented in part at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 17 to 20 September 2007.)

MATERIALS AND METHODS

Antibiotics and susceptibility testing. TR-700 was synthesized at Dong-A Pharmaceuticals Co. (Seoul, Korea). The comparator agents were LZD (ChemPacific Corp., Baltimore, MD), vancomycin, oxacillin, and penicillin G (Sigma-Aldrich Corp., St. Louis, MO). MICs were determined using the broth microdilution method for bacteria that grow aerobically, as described by the CLSI (8). Mueller-Hinton II broth (Becton Dickinson, Sparks, MD) was utilized for MIC testing. The CLSI quality control strains *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were tested in each set of assays to ensure the proper performance of the assay.

Bacterial strains. Test organisms for the assay included recent clinical isolates from diverse geographical centers as well as laboratory-derived, oxazolidinone-resistant mutants. Strains with characterized mutations included the following: LZD-resistant *S. aureus* strain CM/05 (Colombia) containing the *cfi* rRNA methyltransferase gene on the chromosome (J. Quinn, Rush University, Chicago, IL); *S. aureus* 004-737X (OH) and 131-6952X (Belgium) containing a plasmid-borne *cfi* rRNA methyltransferase gene (R. N. Jones, JMI Laboratories, IA) (14, 23); six *S. aureus* strains harboring either the G2500A or G2576U mutation in the PTC; five MRSA strains from the Network on Antimicrobial Resistance in *S. aureus* (NARSA) repository; and two laboratory-derived *S. aureus* strains (G2447U) (Micromyx, Kalamazoo, MI). *Enterococcus* sp. isolates including

TABLE 1. In vitro activity of TR-700, LZD, and comparator drugs against LZD-resistant *S. aureus* strains

Strain derivation ^a	Mutation	MIC (µg/ml) ^b				
		TR-700	LZD	VAN	OXA	PEN
1651	G2576U	2	16	0.5	>32	16
1652	U2500A	4	32	1	32	32
NRS127 MRSA	Non-23S rRNA	1	8	1	32	16
NRS119 MRSA	G2576U	4	64	ND	ND	ND
NRS120 MRSA	G2576U	8	64	ND	ND	ND
NRS121 MRSA	G2576U	4	64	ND	ND	ND
NRS271 MRSA	G2576U	2	32	ND	ND	ND
3067 CM/05	Chrom <i>cfi</i> ^c	1	16	1	>32	ND
004-737X	Plasmid <i>cfi</i> ^c	0.5	8	1	>2	ND
131-6952X	Plasmid <i>cfi</i> ^c	1	32	1	>2	ND
ATCC 29213	Wild type	1	4	1	0.12	1
425/LZD-Res devel	G2447U	8	32	2	0.12	1
480/LZD-Res devel	G2447U	16	>32	1	<0.03	0.25

^a Strains 1651 and 1652 were obtained from the Beth Israel Deaconess Medical Center, Boston, MA; NRs strains were obtained from the NARSA repository; CM/05 was obtained from the Chicago Infectious Disease Research Institute; 004-737X and 131-6952X were obtained from JMI Laboratories, North Liberty, IA; 425 and 480 represent transfers no. 18 and no. 19, respectively, from a spiral plating experiment derived from the host strain ATCC 29213 (31).

^b VAN, vancomycin; OXA, oxacillin; PEN, penicillin; ND, not determined.

^c Resistance due to *cfi* gene acquisition. Chrom *cfi*, the resistance gene has been shown to be in the chromosome of *S. aureus*; plasmid *cfi*, the resistance gene is plasmid borne.

seven from *E. faecalis* and one from *E. faecium*, all containing the G2576U mutation, were included (Micromyx, Kalamazoo, MI). A collection of 94 uncharacterized LZD-resistant U.S. isolates was tested (2003 to 2006), which included 17 *S. aureus*, 19 *S. epidermidis*, 16 *E. faecalis*, 36 *E. faecium*, 1 *Staphylococcus hominis*, and 5 *Staphylococcus sciuri* isolates (Eurofins Medinet, Herndon, VA).

Docking of TR-700 to the PTC *E. coli* ribosome site. The structural model developed previously (18) was used to model the interactions with the PTC of the *E. coli* ribosome. Using the program LigandFit (38), the binding site was defined using LZD as the “docked” ligand and TR-700 was docked in flexible mode. The poses retrieved by LigandFit were visually examined, and the pose with maximum overlap (0.37 Å) in the oxazolidinone and fluorophenyl rings was selected as the best fit. Computational algorithms are available from Discovery Studio 2.0 from Accelrys (<http://www.accelrys.com/products/dstudio/>).

RESULTS

Activity of TR-700 and LZD against defined LZD-resistant *S. aureus* isolates. Table 1 provides the MICs for five clinically isolated strains of MRSA that carry the prevalent G2576U mutation. NRS119, NRS120, and NRS121 are closely related strains from the United States (2001) (37), whereas NRS271 is an unrelated United Kingdom strain (40). The 8- to 16-fold-greater potency of TR-700 versus LZD that has been observed against MRSA (28) was maintained in these LZD-resistant MRSA isolates. Clinical isolate 1652 carrying the U2500A mutation demonstrated an eightfold-greater susceptibility to TR-700 than LZD.

Three strains which carried the *cfi* methyltransferase gene also demonstrated an ~16-fold-greater susceptibility to TR-700 than to LZD (Table 1). CM/05 is the original MRSA clinical isolate from Colombia (35), and strains 004-737X (OH) and 131-6952X (Belgium) are more recent isolates from the 2006 U.S. LEADER and European surveillance programs

TABLE 2. In vitro activity of TR-700, LZD, and comparator drugs against LZD-resistant *Enterococcus* spp. strains

Strain derivation ^a	Mutation	MIC (μg/ml) ^b				
		TR-700	LZD	VAN	OXA	PEN
<i>E. faecalis</i>						
411	G2576U	2	16	1	>32	4
412	G2576U	2	16	1	16	2
413	G2576U	4	32	1	>32	4
414	G2576U	4	32	32	16	1
1172	G2576U	4	16	>32	32	4
3124	G2576U	4	16	>32	8	ND
3128	G2576U	4	16	>32	2	ND
<i>E. faecium</i>						
854	G2576U	2	16	>32	>32	ND

^a Strains 411 and 412 were obtained from JMI Laboratories, North Liberty, IA; 413 and 414 were obtained from the Beth Israel Deaconess Medical Center, Boston, MA; 1172 was obtained from Clarian Health Partners, Indianapolis, IN; 3124, 3128, and 854 were obtained from the University of California at Los Angeles.

^b VAN, vancomycin; OXA, oxacillin; PEN, penicillin; ND, not determined.

(14, 23). The *cfp* gene is on the chromosome in CM/05, whereas the latter two strains have been demonstrated by PCR amplification and sequencing to contain the identical *cfp* gene on a plasmid (10, 23).

In a previous study, G2447U mutants were isolated from sequential transfers from a spiral plating experiment using *S. aureus* reference strain ATCC 29213 (31). Against these laboratory-derived isolates, TR-700 retained the fourfold superior potency observed in the wild-type parental strain (Table 1).

Activity of TR-700 and LZD against defined LZD-resistant *Enterococcus* isolates. TR-700 was eightfold more active than LZD against LZD-resistant enterococci (Table 2). MICs for seven G2576U clinical isolates of *E. faecalis* and one *E. faecium* G2576U isolate ranged from 2 to 4 μg/ml versus 16 to 32 μg/ml for LZD (Table 2). This collection included five strains that were vancomycin resistant.

Activity of TR-700 and LZD versus LZD-nonsusceptible and -resistant clinical isolates. Microdilution assays of TR-700, LZD, daptomycin, vancomycin, and oxacillin were performed against a collection of clinically relevant LZD-nonsusceptible strains, including 5 *S. aureus*, 5 vancomycin-resistant *S. aureus*, 5 daptomycin-nonsusceptible *S. aureus*, 25 coagulase-negative *Staphylococcus* (2003 to 2006), 15 *E. faecalis* (2003 to 2005), and 35 *E. faecium* (2003 to 2005) isolates. The data in Table 3 show the comparative activities of TR-700 and LZD against these isolates. The eightfold difference in the MIC₉₀ for LZD versus TR-700 for *E. faecalis*, *E. faecium*, and MRSA and the ≥8-fold difference in the MIC₉₀ versus *S. epidermidis* demonstrate that the greater potency of TR-700 was maintained against LZD-nonsusceptible and -resistant strains, including vancomycin-resistant and daptomycin-nonsusceptible organisms (data not shown).

Model of TR-700 in the peptidyl transferase site. Based upon in vivo cross-linking data using photoreactive oxazolidinone derivatives, a model of the binding of LZD to the 23S rRNA peptidyl transferase region has been previously published (18) using the 3.5 Å crystallographic structure of the *Escherichia coli* ribosome (29). Using this structure, we docked TR-700 into the LZD binding site using LigandFit from Ac-

TABLE 3. In vitro activity of TR-700 and LZD against clinical isolates resistant to LZD

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml) ^a		
		Range	50%	90%
<i>S. aureus</i> (17)	TR-700	0.12 to 8	0.25	2
	LZD	1 to 64	2	16
<i>S. epidermidis</i> (19)	TR-700	2 to >64	4	8
	LZD	16 to >128	32	>128
<i>E. faecalis</i> (16)	TR-700	2 to 4	4	4
	LZD	8 to 32	32	32
<i>E. faecium</i> (36)	TR-700	0.5 to 8	2	4
	LZD	4 to >128	32	64
<i>S. hominis</i> (1)	TR-700	2 to 2	NA	NA
	LZD	16 to 16	NA	NA
<i>S. sciuri</i> (5)	TR-700	2 to 4	NA	NA
	LZD	16 to 32	NA	NA

^a NA, not applicable; the number of isolates was less than 10.

celrys. Figure 1 shows a comparison of the structures of the published models for LZD (18) and our model of TR-700 binding to the peptidyl transferase site. In both models, there is an anchoring hydrogen bond interaction between a hydrogen bond donor of the oxazolidinone and the 5' oxygen of the phosphate group of 23S rRNA residue G2505. For LZD, this interaction is proposed to be mediated by the amide hydrogen of the acetamide. For TR-700, the hydrogen of the primary alcohol is the proposed hydrogen bond donor. Additionally, the models suggest that the hydrophobic interactions between the oxazolidinone B ring and A2451 and C2452 for LZD (18) are conserved in the binding of TR-700. The C and D rings of TR-700 [the 5-(2-methyl-2H-tetrazol-5-yl)-pyridin-3-yl group] are predicted to add two new hydrogen bonds to the sugar backbone of residues A2451 and U2584. We hypothesize that these additional interactions may contribute to the increased potency of TR-700, as reflected by 3.4-fold-greater inhibition in vitro (50% inhibitory concentration in an *E. coli*-coupled transcription-translation assay) (4), antimicrobial activity (28), and in vivo potency (5, 7).

DISCUSSION

TR-701, the orally active prodrug of TR-700, is a promising new oxazolidinone antibacterial agent currently in development. TR-700 has a four- to eightfold-greater in vitro potency than LZD against MSSA, MRSA, *E. faecalis*, *E. faecium*, and *S. pneumoniae*, including strains resistant to a variety of medically important antibacterial agents (28). In this study, the in vitro activity of TR-700 was evaluated against clinical isolates of gram-positive bacteria with defined LZD resistance mutations as well as a collection of recent clinical isolates where the mechanism of LZD resistance had not been evaluated. Here we show that TR-700 was active against LZD-resistant strains, including *S. aureus* with a variety of 23S rRNA mutations. MICs of 2 to 8 μg/ml were obtained against strains with either of the most commonly identified mutations (G2576U or U2500A). TR-700 was active to a lesser extent against isolates

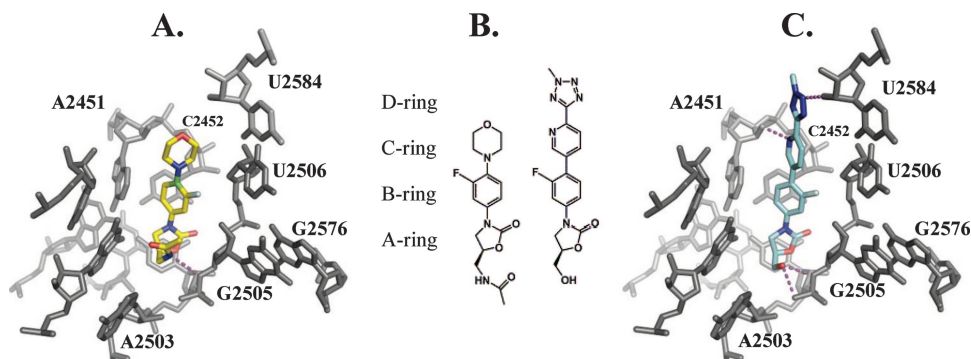


FIG. 1. Model of TR-700 versus LZD in the peptidyl transferase binding site (PTS). (A) LZD (yellow) in the PTS (18). (B) Structure of LZD and TR-700. (C) TR-700 (cyan) in the PTS. Critical hydrogen bond interactions are shown in purple. The *E. coli* 23S numbering system is used.

harboring laboratory-derived mutations at G2447U. In these laboratory mutants, no effect on growth was observed in vitro (unpublished observation). However, this mutation has not been detected in LZD-resistant clinical isolates, possibly due to the modest effect on in vitro growth phenotypes previously observed (34), resulting in decreased fitness or virulence in vivo. TR-700 was active in the 2 to 4 $\mu\text{g/ml}$ range for LZD-resistant enterococci harboring the G2576U mutation. TR-700 was 16-fold more potent than LZD against strain CM/05, which contains the transposon-associated *cfr* (methyltransferase) gene, as well as two newly isolated *cfr*-containing clinical isolates. The *cfr* methylase confers multidrug resistance to several structurally unrelated classes of protein synthesis inhibitors (chloramphenicol, clindamycin, tiamulin, quinupristin-dalfopristin, and oxazolidinones); therefore, the use of any of these agents in a clinical or veterinary setting is likely to promote the spread of plasmids and strains carrying this gene. Importantly, because the MIC of TR-700 ranged from 0.5 to 1 $\mu\text{g/ml}$ against the *cfr* MRSA isolates, it is likely that this drug will achieve clinical concentrations that may permit treatment of patients with these LZD-resistant infections. The plasmid and transposon genetic environment of this gene suggests that this mechanism of resistance is likely to continue to be problematic in the future. The current study demonstrates that the increased potency of TR-700 versus LZD is maintained against LZD-nonsusceptible and -resistant clinical isolates of MRSA and vancomycin-resistant *E. faecium* and *E. faecalis*.

The models of TR-700 and LZD provide a rationalization for the increased potency of TR-700 against LZD-resistant strains. The substitution in the A ring of a smaller alcohol group of TR-700 for the acetamide of LZD should be less affected by the increased steric bulk of the methylated A2503 residue generated by posttranscriptional modification in strains expressing a *cfr* methyltransferase (17, 35). More studies are needed to determine the contribution of the A ring and D ring substitutions in TR-700 to the 16-fold increase in potency over LZD against *cfr* strains.

The published model of LZD-23S rRNA binding (18), as well as the proposed model of TR-700-23S rRNA binding, indicate potential points of contact with the ribosome. It could be anticipated that mutation of any of these sites of interaction would lead to decreased susceptibility or resistance to oxazolidinones. However, results from clinical surveys of resistance as

well as serial passage laboratory-derived mutation studies show that only a limited number of 23S rRNA domain V changes are selected and that many of these changes do not involve the direct contact points between oxazolidinones and the ribosome. The phylogenetically conserved essential structural features of the peptidyl transferase site are likely responsible for precluding the selection of these mutants. The invariant P site nucleotides U2584, U2585, and U2506 have been implicated in the binding of the donor substrate (25). Mutation of U2506 has been shown to be dominant lethal or to severely reduce the activity of 70S ribosomes (26), with similar effects observed for mutation of U2584 and U2585 (12, 25). The invariant P site residue A2451 has been hypothesized to play a catalytic role, and all mutations also confer a dominant lethal phenotype (34). Interestingly, the proposed model of TR-700 suggests that the additional two hydrogen bonds between TR-700 and the backbone ribose sugars of A2451 and U2584 would serve to stabilize the interaction of the drug and the peptidyl transferase region. These data suggest that mutations which disrupt these unique hydrogen binding sites of TR-700 at U2584 and A2451 would not occur clinically. Thus, the additional TR-700 interactions predict that the binding of TR-700 is less reliant on target residues associated with resistance to LZD.

Mutation to G2447U has been observed only in laboratory-selected LZD resistance experiments (33). Previous studies have shown that G2447 is phylogenetically less conserved and that ribosomes containing either G2447A or G2447C support growth (34). However, G2447U cannot maintain cell viability in the absence of wild-type ribosomes, suggesting that this particular mutation is associated with decreased function. G2505A is another rare mutation observed in *E. faecium* (27). However, G2505X mutants show reduced growth in *E. coli*, with G2505A and G2505C retaining approximately 15% of the activity of wild-type 70S ribosomes (26).

Several copies of the 23S rRNA gene are present in clinically relevant species, including five to six copies in *S. aureus* and *S. epidermidis*, six copies in *E. faecalis*, and four copies in *E. faecium*. Thus, selection of resistance through mutation of a gene encoding 23S rRNA implies that resistance must have a dominant or incomplete dominant phenotype and must be a survivable event. The level of oxazolidinone resistance has been shown to correlate with the number of copies of mutated 23S rRNA genes present in a strain (21, 32, 33, 41). Specifi-

cally, an increase in the copy number of G2576U, the most common 23S rRNA mutation observed both clinically and from serial passage experiments, is correlated with higher MICs for both *S. aureus* and enterococci (2, 21, 32, 40). In a serial passage experiment selecting for increasing LZD resistance, Besier et al. (2) reported that while the first mutation to G2576U was slow to occur, mutations in the other four copies of the 23S rRNA occurred more rapidly. Thus, the rate-limiting step for the generation of a single G2576U event gives rise to a low level of LZD resistance (4 µg/ml) (2), but homologous recombination and gene conversion of the other alleles can then readily arise, resulting in higher MICs (128 µg/ml) (2) which would translate to the clinical failure of LZD therapy. The greater potency of TR-700 against these strains may offer a significant therapeutic advantage, especially in the important complicated skin and skin structure infection-causing pathogens *S. aureus* and *E. faecalis*. Treatment of the higher copy number/homozygous G2576U *S. epidermidis* and *E. faecium* strains will depend on the clinically achievable exposure levels.

The fitness of LZD-resistant mutants has been examined, with somewhat contradictory findings. In one study, accumulation of multiple G2576U mutations in *S. aureus* resulted in a corresponding decline in the growth rate (2). However, in *E. faecalis*, the homozygous (four copies) G2576U mutant conferred a growth advantage over the wild type, whereas a single-copy mutant conferred a disadvantage for growth and fitness (3). The persistence of a G2576U mutation after 40 serial passages of *S. aureus* on antibiotic-free medium suggested that the mutation has only a minor impact on fitness (36). The differences observed in these experiments may be due to different genetic backgrounds and the presence of suppressors or altered gene products that impact fitness. Thus, the likelihood of selecting for oxazolidinone resistance in the clinic during therapy may depend on several factors, including the particular infecting strain, maintenance of appropriate drug levels at the site of infection, length of therapy, potency of the drug against the isolate, and frequency of resistance.

The frequency of spontaneous resistance to LZD has been shown to be $<10^{-9}$ in both *S. aureus* and *Enterococcus* spp. (15, 42). These data correlate with the relatively low level of resistance observed in the clinic. A comparison of the frequency of resistance to TR-700 (DA-7157) and LZD at a concentration of $2\times$ MIC showed that LZD-resistant mutants of enterococci and *S. aureus* were selected at a frequency of approximately 10^{-9} , whereas the frequency of TR-700 resistance was $<10^{-10}$ to $<10^{-11}$ (6). The four- to eightfold-greater activity of TR-700 versus staphylococci and enterococci and the lower frequency of spontaneous resistance may prevent or reduce the selection of first-step mutants in the clinic. Thus, the increased potency and reduced frequency of resistance due to the unique structural features of TR-700, in combination with significant activity against LZD-resistant strains, support the continued clinical development of the TR-701 prodrug.

ACKNOWLEDGMENTS

We are grateful to J. Quinn for strain CM/05 and R. N. Jones for strains 004-737X and 131-6952X. We thank M. Hilgers for graphical assistance in the model TR-700 in the peptidyl transferase site.

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