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In Vitro Activity of TR-700, the Active Ingredient of the Antibacterial Prodrug TR-701, a Novel Oxazolidinone Antibacterial Agent[▽]

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TR-701 is the prodrug of the microbiologically active molecule TR-700, a novel orally and intravenously administered oxazolidinone antibacterial agent. The in vitro activity of TR-700 was evaluated against 1,063 bacterial clinical isolates including staphylococci, enterococci, streptococci, *Moraxella catarrhalis*, *Haemophilus influenzae*, and a variety of anaerobic bacterial species. The test strains were recent (2005 to 2008) clinical isolates from diverse U.S. (80%) and non-U.S. (20%) sites. MIC assays were conducted using reference broth microdilution and agar dilution methods with the principal comparators linezolid and vancomycin. TR-700 was four- to eightfold more potent than linezolid against staphylococci and generally fourfold more potent against enterococci and streptococci. TR-700 was less active against *M. catarrhalis* and *H. influenzae* but was twofold more active than linezolid. Against anaerobic species, the activity of TR-700 was equivalent to or up to fourfold higher than that of linezolid. These results indicate that TR-700 is a promising new oxazolidinone antibacterial agent with greater in vitro potency than linezolid against clinically important gram-positive bacteria.

Until the mid-1990s, methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) incidence was confined to the hospital setting (hospital-acquired MRSA [HA-MRSA]). However, in many medical centers throughout the United States, community-acquired strains of MRSA (CA-MRSA) have increased in frequency of isolation and have spread at an alarming rate, now reaching >60% incidence, primarily in skin and skin structure infections (8). This has led to an important change in the choice of antibiotics in the management of community-acquired infections. Initially, CA-MRSA isolates were more susceptible than HA-MRSA to classes of antimicrobial agents such as tetracyclines, trimethoprim-sulfamethoxazole, clindamycin, or quinolones (12). This difference in susceptibility profile is now diminishing as CA-MRSA strains tend to present a profile closer to that of HA-MRSA isolates, as reported for tetracycline and trimethoprim-sulfamethoxazole (15). Furthermore, it has been shown that clinical and epidemiological characteristics are not reliable for distinguishing between methicillin-susceptible *S. aureus* (MSSA) and MRSA skin infections (11). As a result, there is a need for new intravenous (i.v.) and oral (p.o.) agents active against gram-positive bacterial infections that can be used for both inpatient and outpatient therapy.

For treating multidrug-resistant gram-positive bacteria, only linezolid currently offers the versatility of both i.v. and p.o. administration, enabling an i.v.-to-p.o. step-down approach. Linezolid displays high bioavailability, an acceptable safety profile, and excellent rates of clinical efficacy. However, linezolid is administered twice daily, and although the effect is reversible after discontinuation, mild myelosuppression is commonly observed after 10 to 14 days of therapy, requiring regular blood cell monitoring. In addition, enterococcal and staphylococcal strains resistant to linezolid have surfaced (6), including some staphylococcal isolates containing transposon-associated or plasmid-borne resistance (5, 7, 10). As a result, there is a need for a new agent with the potential to (i) be administered in a once-daily regimen; (ii) provide a broader safety margin, particularly with a lower myelosuppression potential at the therapeutic dose; and (iii) treat infections caused by organisms that have developed resistance to linezolid.

TR-701 (formerly DA-70218 and DA-7218) is a prodrug of the active oxazolidinone antibiotic TR-700 (formerly DA-7157 and 70157) that has completed phase 2 clinical development for complicated skin and skin structure infections. Data from phase 1 studies showed that TR-701 is rapidly absorbed and converted to TR-700, with a mean half-life ranging from 8 to 11 h, approximately twofold longer than that of linezolid and consistent with once-a-day dosing (1). Examination of hematologic parameters over 21 days demonstrated that the 200-mg once-a-day projected therapeutic human dose of TR-701 did not demonstrate any hematological effects and was comparable to placebo (13).

The microbiologically inactive prodrug TR-701 can be administered i.v. or p.o. and is readily converted to the microbiologically active form TR-700 by phosphatases. In previous studies, the activity of TR-700 was examined against a collection of Korean clinical isolates (2002 to 2004) (2, 9). TR-700 was four- to eightfold more potent than linezolid against both *S. aureus* and coagulase-negative staphylococci, including methicillin-resistant strains. Similarly, TR-700 was two- to fourfold more potent than linezolid against both vancomycin-susceptible and -resistant enterococci. In a recent study evaluating the activity of TR-700 against linezolid-resistant isolates, TR-700 demonstrated 8- to 16-fold-greater potency than linezolid against all linezolid-resistant strains tested, including MRSA, strains of MRSA carrying the mobile *cfr* methyltransferase gene, and vancomycin-resistant enterococci (14). The purpose of our study was to assess the activity of TR-700 against recent

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TABLE 1. MIC ranges, MIC_{50}s, and MIC_{90}s of TR-700, linezolid, vancomycin, and oxacillin for staphylococci

Group (no. of isolates tested)[a]	Antimicrobial agent	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
MSSA (95)	TR-700	0.25–1	0.5	0.5
	Linezolid	1–4	2	4
	Vancomycin	0.25–2	1	1
	Oxacillin	0.12–0.5	0.25	0.5
MRSA (103)	TR-700	0.25–1	0.5	0.5
	Linezolid	1–4	2	4
	Vancomycin	0.5–2	1	1
	Oxacillin	4–>32	32	32
CA-MRSA (100)	TR-700	0.25–1	0.5	0.5
	Linezolid	1–4	2	4
	Vancomycin	1–4	4	4
	Oxacillin	4–>32	32	32
MSSE (48)	TR-700	0.12–1	0.25	0.5
	Linezolid	0.5–4	1	2
	Vancomycin	1–4	2	2
	Oxacillin	0.06–0.25	0.12	0.25
MRSE (72)	TR-700	0.12–1	0.25	0.5
	Linezolid	0.5–4	1	2
	Vancomycin	0.25–4	2	2
	Oxacillin	0.5–>32	16	>32

[a] MSSE, methicillin-susceptible *Staphylococcus epidermidis*; MRSE, methicillin-resistant *S. epidermidis*.

clinical isolates of predominantly gram-positive bacteria isolated from non-Korean sites.

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MATERIALS AND METHODS

Test agents. TR-700 was provided by Trius Therapeutics (San Diego, CA). The sources of the other agents were Pfizer Inc., New York, NY (linezolid); Sigma-Aldrich, St. Louis, MO (vancomycin HCl, oxacillin sodium, penicillin G sodium, and metronidazole); and U.S. Pharmacopeia, Rockville, MD (imipenem).

Organisms. The test organisms were recent (2005 to 2008) clinical isolates from diverse geographical locations. Approximately 80% of the total number of test isolates originated in the United States, and 20% were from non-U.S. sources (Great Britain, France, Germany, and Australia). All test groups except CA-MRSA, *Haemophilus influenzae*, *Moraxella catarrhalis*, and anaerobic bacteria contained U.S. and non-U.S. isolates; test isolates for these organisms were from U.S. sources only. Aerobic test organisms included the following susceptibility groups: MSSA, MRSA, CA-MRSA, vancomycin-susceptible (VS) *Enterococcus faecalis*, vancomycin-resistant (VR) *Enterococcus faecalis*, VS *Enterococcus faecium*, VR *Enterococcus faecium*, penicillin-susceptible *Streptococcus pneumoniae* (PSSP), penicillin-intermediate *Streptococcus pneumoniae* (PISP), and penicillin-resistant *Streptococcus pneumoniae* (PRSP). Other aerobic test organisms were *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Haemophilus influenzae*, and *Moraxella catarrhalis.* Anaerobic species included *Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, *Clostridium perfringens*, *Peptostreptococcus anaerobius*, *Peptostreptococcus micros*, *Porphyromonas asaccharolytica*, and *Prevotella* species.

Susceptibility testing. MICs for aerobic bacteria were determined using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) (4). MICs for anaerobic bacteria were determined using the reference agar dilution method as described by CLSI (3). For anaerobic assays, the cultures were grown for 48 h in the Bactron II anaerobic glove box, and cell suspensions for the inoculum were prepared in prereduced broth within the glove box. Inoculum was transferred to the wells of a Steers replicator (Melrose Machine Shop, Woodlyn, PA), and the drug-supplemented agar plates were stamped with inoculum. The plates were allowed to stand until the inoculum was absorbed into the agar and incubated for 48 h at 37°C. The plates were then removed from the glove box, and the MICs were read and recorded.

TABLE 2. MIC ranges, MIC_{50}s, and MIC_{90}s of TR-700, linezolid, and vancomycin for enterococci

Group (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
VS *E. faecalis* (73)	TR-700	0.25–1	0.5	1
	Linezolid	1–4	2	2
	Vancomycin	0.5–4	1	2
VR *E. faecalis* (49)	TR-700	0.25–1	0.5	0.5
	Linezolid	1–4	2	2
	Vancomycin	4–>32	>32	>32
VS *E. faecium* (53)	TR-700	0.25–2	0.5	1
	Linezolid	1–4	2	4
	Vancomycin	0.25–4	1	1
VR *E. faecium* (51)	TR-700	0.12–1	0.5	0.5
	Linezolid	1–4	2	2
	Vancomycin	8–>32	>32	>32

RESULTS

The MIC data are summarized in Tables 1 to 5. MICs for all quality control strains tested fell within the published CLSI quality control ranges (data not shown). TR-700 was very active against gram-positive organisms, exhibiting a MIC at which 90% of the isolates tested were inhibited (MIC_{90}) of 0.5 μg/ml for MSSA, MRSA, and CA-MRSA (Table 1). TR-700 was eightfold more active than linezolid against these strains and was two- to eightfold fold more active than vancomycin. Similar activities were seen for methicillin-susceptible *Staphylococcus epidermidis* and methicillin-resistant *S. epidermidis*. TR-700 was very active against enterococci, exhibiting a MIC_{90} of 1 μg/ml for vancomycin-susceptible enterococci and 0.5 μg/ml for vancomycin-resistant enterococci (Table 2), which was two- to fourfold lower than that of linezolid. For all *S. pneumoniae* strains analyzed (PSSP, PISP, and PRSP), the MIC_{90} was 0.25 μg/ml (Table 3). TR-700 was also very active against *S. pyogenes* and *S. agalactiae*, where the MIC_{90} was 0.5 μg/ml. Overall, with the exception of VS *E. faecalis* (where TR-700 was twofold more active than linezolid), TR-700 demonstrated potency that was at least fourfold greater than that of linezolid for the gram-positive species tested. There were no significant differences between U.S. and non-U.S. isolates in susceptibility to TR-700 or linezolid (data not shown).

TR-700 was less active against the fastidious gram-negative aerobes *H. influenzae* and *M. catarrhalis* (Table 4). Against *H. influenzae*, TR-700 yielded a MIC_{90} of 16 μg/ml, while slightly better activity was seen against *M. catarrhalis* (MIC_{90} = 4 μg/ml). MIC_{90}s of TR-700 for both organisms were twofold lower than those of linezolid.

TR-700 was also a potent inhibitor of some anaerobic organisms, as shown in Table 5. For *Peptostreptococcus anaerobius*, *Peptostreptococcus micros*, and *Porphyromonas asaccharolytica*, TR-700 exhibited a MIC_{90} of 0.5 μg/ml. Against *Bacteroides thetaiotaomicron* and *Clostridium perfringens*, TR-

TABLE 3. MIC ranges, MIC_{50}s, and MIC_{90}s of TR-700, linezolid, and vancomycin for streptococci

Group or species (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
PSSP[a] (38)	TR-700	0.06–0.5	0.25	0.25
	Linezolid	0.25–1	1	1
	Vancomycin	0.12–1	0.25	0.5
PISP[a] (37)	TR-700	0.06–0.5	0.25	0.25
	Linezolid	0.5–1	1	1
	Vancomycin	0.25–0.5	0.25	0.5
PRSP[a] (35)	TR-700	0.06–0.5	0.25	0.25
	Linezolid	0.25–2	1	1
	Vancomycin	0.12–1	0.25	0.5
S. pyogenes (102)	TR-700	0.06–0.5	0.25	0.5
	Linezolid	0.06–2	1	2
	Vancomycin	0.25–1	0.5	0.5
S. agalactiae (52)	TR-700	0.06–1	0.25	0.5
	Linezolid	1–2	2	2
	Vancomycin	0.25–1	0.5	0.5

[a] Susceptibility category based upon oral penicillin breakpoints for nonmeningitis isolates (3).

700 exhibited a MIC_{90} of 2 μg/ml. TR-700 exhibited a MIC_{90} of 4 μg/ml against *Bacteroides fragilis*, *Bacteroides vulgatus*, and *Prevotella* species. TR-700 was slightly less active against *Bacteroides ovatus*, with a MIC_{90} of 8 μg/ml. Overall, the activity of TR-700 versus anaerobic species was similar to or up to fourfold better than that of linezolid.

DISCUSSION

TR-700 demonstrated potent activity against gram-positive bacteria, including strains resistant to a variety of medically important antibacterial agents. The MIC data for this set of U.S. and non-U.S. isolates were consistent with those previously reported for South Korean isolates (9). The structural differences between TR-700 and linezolid suggest a rationale for the greater potency of TR-700 (14).

Recent studies have shown that the four- to eightfold-greater potency of TR-700 is maintained against emerging linezolid-nonsusceptible and -resistant strains, such as *S. aureus* with a variety of 23S rRNA mutations including G2576U and G2500A (14). In addition, TR-700 was found to be 16-fold more potent than linezolid against three clinical isolates containing the transposon-associated *cfr* (methyltransferase) gene (5, 14).

TABLE 4. MIC ranges, MIC_{50}s, and MIC_{90}s for TR-700 and linezolid versus *Haemophilus influenzae* and *Moraxella catarrhalis*

Group (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
H. influenzae (19)	TR-700	1–32	8	16
	Linezolid	8–32	16	32
M. catarrhalis (36)	TR-700	0.5–4	2	4
	Linezolid	2–8	8	8

TABLE 5. MIC ranges, MIC_{50}s, and MIC_{90}s for anaerobic bacteria

Species (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
Bacteroides fragilis (10)	TR-700	2–4	4	4
	Linezolid	4	4	4
	Metronidazole	0.12–>32	0.12	1
	Imipenem	0.12–1	0.12	0.5
Bacteroides vulgatus (10)	TR-700	1–8	2	4
	Linezolid	2–4	2	4
	Metronidazole	0.12–0.5	0.25	0.25
	Imipenem	0.25–0.5	0.25	0.5
Bacteroides thetaiotaomicron (10)	TR-700	2–4	2	2
	Linezolid	4–8	4	8
	Metronidazole	0.5–>32	1	1
	Imipenem	0.12–4	0.5	1
Bacteroides ovatus (10)	TR-700	0.06–8	2	8
	Linezolid	0.5–8	8	8
	Metronidazole	0.5–>32	1	1
	Imipenem	0.06–0.5	0.25	0.5
Clostridium perfringens (10)	TR-700	0.25–2	0.5	2
	Linezolid	2–0	2	4
	Metronidazole	1–>32	1	>32
	Imipenem	0.06–1	0.12	0.5
Peptostreptococcus anaerobius (10)	TR-700	0.12–0.5	0.25	0.5
	Linezolid	0.5–8	1	2
	Metronidazole	≤0.06–1	0.5	1
	Imipenem	≤0.03–1	0.06	1
Peptostreptococcus micros (10)	TR-700	0.12–1	0.25	0.5
	Linezolid	0.5–2	1	2
	Metronidazole	≤0.06–>32	≤0.06	>32
	Imipenem	≤0.03–0.06	≤0.03	≤0.03
Porphyromonas asaccharolytica (10)	TR-700	0.25–0.5	0.25	0.5
	Linezolid	0.5–2	1	2
	Metronidazole	0.5–2	1	1
	Imipenem	≤0.03–0.06	<0.03	0.06
Prevotella spp. (20)	TR-700	≤0.06–16	1	4
	Linezolid	0.25–16	2	4
	Metronidazole	≤0.06–>32	0.5	>32
	Imipenem	≤0.03–16	≤0.06	1

In conclusion, TR-700 demonstrated potent activity against gram-positive aerobic bacteria and anaerobic bacteria, including strains resistant to a variety of medically important antibacterial agents. The potent in vitro activity of TR-700 against this collection of U.S. and non-U.S. isolates confirms the previous data published for South Korean isolates and highlights the enhanced potency of the agent relative to linezolid for the species evaluated.

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