ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2010, p. 2212–2215 Vol. 54, No. 5
0066-4804/10/$12.00 doi:10.1128/AAC.01653-09

Comparative Activities of TR-700 (Torezolid) against Staphylococcal Blood Isolates Collected in Spain[▽]

Carmen Betriu,* Gracia Morales, Icíar Rodríguez-Avial, Esther Culebras, María Gómez, Fátima López-Fabal, and Juan J. Picazo

Servicio de Microbiología Clínica, Hospital Clínico San Carlos, Madrid, Spain

Received 21 November 2009/Returned for modification 14 January 2010/Accepted 11 February 2010

The *in vitro* activity of TR-700 (torezolid) was evaluated against a collection of 660 staphylococcal blood isolates. TR-700 showed excellent activity against all the staphylococci tested. The MIC_{50} and MIC_{90} values of TR-700, linezolid, daptomycin, and vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were 0.25 and 0.5, 2 and 4, 0.5 and 0.5, and 1 and 2 μg/ml, respectively. TR-700 demonstrated greater *in vitro* potency than linezolid against staphylococci, including linezolid-resistant and vancomycin-nonsusceptible strains, and was 32-fold more active than linezolid against the seven *cfr*-positive MRSA strains tested.

The increasing incidence of invasive infections caused by multidrug-resistant staphylococcal isolates has caused significant clinical concern. Newer antibiotics, such as linezolid, are alternatives in the treatment of severe infections caused by multidrug-resistant staphylococci. In the last few years, linezolid-resistant staphylococci, mainly in patients undergoing prolonged therapy, have been reported (4, 5, 13). The most common mechanism of linezolid resistance involves mutations in the 23S rRNA gene. The presence of *cfr*, a plasmid-borne gene that encodes a 23S rRNA methyltransferase in some linezolid-resistant staphylococcal isolates, has been described (14, 19), and an outbreak of *cfr*-positive methicillin-resistant *Staphylococcus aureus* (MRSA) in a Spanish hospital was recently reported (3, 15). Furthermore, mutations in ribosomal proteins L3 and L4 have been associated with resistance to oxazolidinones (11, 12).

TR-700 (torezolid) is the active moiety of the prodrug TR-701 (torezolid phosphate), a new oxazolidinone with potent activity against Gram-positive bacteria. Schaadt et al. (17) demonstrated that TR-700 was more active against staphylococci and enterococci than linezolid. In addition, for MRSA and methicillin-susceptible *S. aureus* (MSSA), the spontaneous mutation frequency was 16-fold lower for TR-700 than for linezolid (12). TR-700 presents a better pharmacokinetic profile than linezolid; its mean half-life of 8 to 11.1 h is approximately 2-fold longer, thus allowing once-daily dosing (16).

The *in vitro* activity of TR-700 was evaluated against a collection of staphylococcal blood isolates. The strains were obtained as part of a multicenter program for surveillance of antimicrobial resistance, the Vigilancia de Resistencias a los Antimicrobianos (VIRA) study.

(This study was presented in part at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 9 to 12 September 2009.)

We tested 660 nonduplicate isolates of staphylococci recovered from bacteremic patients during the period 2004 to 2008 in 40 medical centers throughout Spain. Organisms were identified using Slidex-Staph and a Vitek 2 Gram-positive identification card (bioMérieux, Marcy l'Etoile, France). Included were 202 MSSA isolates, 254 MRSA isolates, and 204 clinically significant methicillin-susceptible and methicillin-resistant coagulase-negative staphylococci (41 MSCoNS and 163 MRCoNS, respectively). *S. aureus* ATCC 29213 was used as a control strain. The agents tested in the study were TR-700, linezolid, vancomycin, teicoplanin, and daptomycin. TR-700 was kindly provided by Trius Therapeutics, Inc., San Diego, CA. Antimicrobial susceptibility tests were performed according to the reference broth microdilution method described by the Clinical Laboratory Standards Institute (1). A calcium supplement (up to 50 mg/liter) was used for testing daptomycin. MICs were interpreted as susceptible, intermediate, or resistant in accordance with CLSI criteria (2). Etest strips (AB Biodisk, Solna, Sweden) were used to confirm linezolid MICs of >4 μg/ml. The presence of the *cfr* gene in the 12 linezolid-nonsusceptible strains was investigated using PCR with specific primers as described elsewhere (9). The presence of the 746-bp amplified *cfr* gene fragment was assessed by agarose gel electrophoresis.

Table 1 summarizes the MIC results for TR-700 and the comparator agents tested against the 660 staphylococcal blood isolates. The MIC_{50} and MIC_{90} values for TR-700 against the linezolid-susceptible MRSA isolates were 0.25 and 0.5 μg/ml, respectively. Against the MSSA and the linezolid-susceptible CoNS isolates, these values were both 0.25 μg/ml. Based on the MIC_{90} values, TR-700 was 4-fold to 8-fold more active than linezolid against linezolid-susceptible staphylococci and 2-fold to 4-fold more active than vancomycin. Against MRSA, the MIC_{90} values of TR-700, daptomycin, vancomycin, and linezolid were 0.5, 0.5, 2, and 4 μg/ml, respectively. Among the MRCoNS, the MIC_{90} values (μg/ml) were 0.25 for TR-700, 0.5 for daptomycin, 2 for both linezolid and vancomycin, and 8 for teicoplanin.

Twelve isolates were not susceptible to linezolid (seven MRSA and five MRCoNS). The seven MRSA isolates with linezolid MICs of 16 μg/ml showed TR-700 MICs of 0.5 μg/ml. These isolates were susceptible to daptomycin, vancomycin,

* Corresponding author. Mailing address: Servicio de Microbiología Clínica, Hospital Clínico San Carlos, Plaza Cristo Rey s/n, 28040 Madrid, Spain. Phone: 34 913303486. Fax: 34 913303478. E-mail: cbetriu.hcsc@salud.madrid.org.

▽ Published ahead of print on 22 February 2010.

TABLE 1. Activities of TR-700 and comparator agents against 660 staphylococcal bloodstream isolates[d]

Organism (no. of isolates tested) and antimicrobial agent	MIC (μg/ml)			% Susceptible strains[a]
	Range	50%	90%	
S. aureus, linezolid susceptible (449)				
TR-700	0.125–0.5	025	0.5	—
Linezolid	≤0.25–4	2	2	100
Daptomycin	≤0.125–1	0.25	0.5	100
Vancomycin	≤0.5–4	1	1	99.6
Teicoplanin	≤0.5–4	≤0.5	1	100
Oxacillin susceptible (202)				
TR-700	0.125–0.5	0.25	0.25	—
Linezolid	≤0.25–4	1	2	100
Daptomycin	≤0.125–1	0.25	0.5	100
Vancomycin	≤0.5–2	1	1	100
Teicoplanin	≤0.5–2	≤0.5	1	100
Oxacillin resistant (247)				
TR-700	0.125–0.5	0.25	0.5	—
Linezolid	≤0.25–4	2	4	100
Daptomycin	≤0.125–1	0.5	0.5	100
Vancomycin	≤0.5–4	1	2	99.2
Teicoplanin	≤0.5–4	≤0.5	1	100
S. aureus, linezolid resistant (7)				
TR-700	0.5	0.5	NA	—
Linezolid	16	16	NA	0
Daptomycin	0.5	0.5	NA	100
Vancomycin	1–2	2	NA	100
Teicoplanin	≤0.5–2	1	NA	100
CoNS, linezolid susceptible (199)[b]				
TR-700	≤0.03–0.5	0.25	0.25	—
Linezolid	≤0.25–4	1	2	100
Daptomycin	≤0.125–1	0.25	0.5	100
Vancomycin	≤0.5–4	2	2	100
Teicoplanin	≤0.5–32	2	8	94.5
Oxacillin susceptible (41)				
TR-700	0.06–0.25	0.25	0.25	—
Linezolid	≤0.25–2	1	2	100
Daptomycin	≤0.125–0.5	0.25	0.5	100
Vancomycin	≤0.5–2	2	2	100
Teicoplanin	≤0.5–8	2	4	100
Oxacillin resistant (158)				
TR-700	≤0.03–0.5	0.125	0.25	—
Linezolid	≤0.25–4	1	2	100
Daptomycin	≤0.125–1	0.25	0.5	100
Vancomycin	≤0.5–4	2	2	100
Teicoplanin	≤0.5–32	2	8	93
CoNS, linezolid resistant (5)[c]				
TR-700	0.25–4	2	NA	—
Linezolid	16–256	16	NA	0
Daptomycin	0.25–0.5	0.5	NA	100
Vancomycin	1–2	2	NA	100
Teicoplanin	1–16	4	NA	80

[a] MICs for susceptible isolates are those described by CLSI.
[b] *Staphylococcus epidermidis*, 135 isolates; *S. hominis*, 40 isolates; *S. haemolyticus*, 19 isolates; *S. lugdunensis*, 3 isolates; *S. intermedius*, 1 isolate; and *S. warneri*, 1 isolate.
[c] *S. epidermidis*, 2 isolates; *S. hominis*, 2 isolates; and S. *haemolyticus*, 1 isolate.
[d] —, breakpoints for TR-700 are not currently provided by CLSI; NA, not applicable.

and teicoplanin. For the five linezolid-resistant (MICs of 16 to 256 μg/ml) MRCoNS (two *S. epidermidis*, two *S. hominis*, and one *S. haemolyticus*) isolates, the MICs of TR-700 were 0.25 to 4 μg/ml. These five isolates were inhibited at daptomycin and vancomycin MICs of 0.5 and 2 μg/ml, respectively. One of the five linezolid-resistant CoNS was nonsusceptible to teicoplanin. The two MRSA isolates with vancomycin MICs of 4 μg/ml and the five teicoplanin-resistant MRCoNS isolates were inhibited by TR-700 at 0.25 to 0.5 μg/ml. Table 2 shows the distribution of the TR-700 and linezolid MICs for the organisms tested. All the *S. aureus* and linezolid-susceptible CoNS tested were inhibited by 0.5 μg/ml of TR-700. Linezolid inhibited these isolates at 4 μg/ml.

Two MRCoNS isolates (one *S. epidermidis* and one *S. homi-*

TABLE 2. Distribution of TR-700 and linezolid MICs

Organism (no. of isolates tested) and antimicrobial agent	Cumulative % inhibited at MIC (μg/ml)										
	≤0.25	0.5	1	2	4	8	16	32	64	128	256
S. aureus, linezolid susceptible (449)											
TR-700	83.1	100									
Linezolid	1.3	2.4	45.2	91.1	100						
Oxacillin susceptible (202)											
TR-700	99.0	100									
Linezolid	0.5	0.5	51.0	99.0	100						
Oxacillin resistant (247)											
TR-700	70.0	100									
Linezolid	2.0	4.0	40.5	84.6	100						
S. aureus, linezolid resistant (7)											
TR-700		100									
Linezolid							100				
CoNS, linezolid susceptible (199)											
TR-700	99.5	100									
Linezolid	3.0	8.0	80.4	99.0	100						
Oxacillin susceptible (41)											
TR-700	100										
Linezolid	4.9	9.8	75.6	100							
Oxacillin resistant (158)											
TR-700	99.4	100									
Linezolid	2.5	7.6	81.6	98.7	100						
CoNS, linezolid resistant (5)											
TR-700	20.0	20.0	20.0	80.0	100						
Linezolid							60.0	60.0	80.0	80.0	100

nis) and seven MRSA isolates carried the *cfr* gene. Both *cfr*-positive MRCoNS isolates were collected from different hospitals in 2006. Five MRSA isolates with the *cfr* gene were collected during 2008 and belonged to a recently reported outbreak in an intensive care unit (15). The remaining two *cfr*-positive MRSA isolates were collected in different hospitals in 2006 and in 2008, respectively.

In the present study, TR-700 exhibited excellent activity against the staphylococcal bloodstream isolates tested. With the exception of 4 of 660 strains (0.6%), all isolates were inhibited by 0.5 μg/ml of TR-700. The detection of *cfr*-mediated resistance among staphylococcal clinical isolates is a cause for concern because of the potential for rapid transmission between species. We detected the *cfr* gene in three different staphylococcal species (*S. aureus*, *S. hominis*, and *S. epidermidis*) collected in 2006 from different Spanish hospitals. In 2008, an outbreak of linezolid-resistant *S. aureus* was reported at Hospital Clínico San Carlos, Madrid, Spain (15). Five *cfr*-positive MRSA isolates obtained from blood during the outbreak were included in this study. A *cfr*-positive *S. hominis* isolate was also collected in 2006 in the same hospital. These data indicate the need for continued monitoring of susceptibility. Global surveillance studies show that linezolid resistance remains rare, particularly among *S. aureus* strains (6, 8). TR-700 was 32-fold more active than linezolid against the seven *cfr*-positive MRSA strains tested. The results of our study agree with those of other authors (7, 10, 17, 18) and confirm the greater *in vitro* potency of TR-700 over linezolid against staphylococci, including linezolid-resistant and vancomycin-nonsusceptible strains. Further clinical evaluations are warranted to confirm the efficacy of this agent.

REFERENCES

1. **CLSI.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 8th ed., vol. 29, no. 2. CLSI document M7-A8. CLSI, Wayne, PA.
2. **CLSI.** 2009. Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement, vol. 29, no. 3. CLSI document M100-S19. CLSI, Wayne, PA.
3. **de la Torre, M., M. Sánchez, G. Morales, E. Baos, A. Arribi, N. García, R. Andrade, B. Peláez, M. P. Pacheco, S. Domingo, J. Conesa, M. Nieto, F. J. Candel, and J. J. Picazo.** 2008. Outbreak of linezolid-resistant *Staphylococcus aureus* in intensive care. Abstr. 48th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1835a.
4. **Gales, A. C., H. S. Sader, S. S. Andrade, L. Lutz, A. Machado, and A. L. Barth.** 2006. Emergence of linezolid-resistant *Staphylococcus aureus* during treatment of pulmonary infection in a patient with cystic fibrosis. Int. J. Antimicrob. Agents **27:**300–302.
5. **Hentschke, M., B. Saager, M. A. Horstkotte, S. Scherpe, M. Wolters, H. Kabisch, R. Grosse, P. Heisig, M. Aepfelbacher, and H. Rohde.** 2008. Emergence of linezolid resistance in a methicillin resistant *Staphylococcus aureus* strain. Infection **36:**85–87.
6. **Jones, R. N., S. Kohno, Y. Ono, J. E. Ross, and K. Yanagihara.** 2009. ZAAPS International Surveillance Program (2007) for linezolid resistance: results from 5591 Gram-positive clinical isolates in 23 countries. Diagn. Microbiol. Infect. Dis. **64:**191–201.
7. **Jones, R. N., G. J. Moet, H. S. Sader, R. E. Mendes, and M. Castanheira.** 2009. TR-700 in vitro activity against and resistance mutation frequencies among Gram-positive pathogens. J. Antimicrob. Chemother. **63:**716–720.
8. **Jones, R. N., J. E. Ross, J. M. Bell, U. Utsuki, I. Fumiaki, I. Kobayashi, and J. D. Turnidge.** 2009. Zyvox annual appraisal of potency and spectrum program: linezolid surveillance program results for 2008. Diagn. Microbiol. Infect. Dis. **65:**404–413.
9. **Kehrenberg, C., and S. Schwarz.** 2006. Distribution of florfenicol resistance genes *fex*A and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. Antimicrob. Agents Chemother. **50:**1156–1163.
10. **Livermore, D. M., S. Mushtaq, M. Warner, and N. Woodford.** 2009. Activity of oxazolidinone TR-700 against linezolid-susceptible and -resistant staphylococci and enterococci. J. Antimicrob. Chemother. **63:**713–715.
11. **Locke, J. B., M. Hilgers, and K. J. Shaw.** 2009. Mutations in ribosomal protein L3 are associated with oxazolidinone resistance in staphylococci of clinical origin. Antimicrob. Agents Chemother. **53:**5275–5278.
12. **Locke, J. B., M. Hilgers, and K. J. Shaw.** 2009. Novel ribosomal mutations in *Staphylococcus aureus* identified through selection with the oxazolidinones

linezolid and torezolid (TR-700). Antimicrob. Agents Chemother. **53:**5265–5274.

13. **Meka, V. G., H. S. Gold, A. Cooke, L. Venkataraman, G. M. Eliopoulos, R. C. Moellering, Jr., and S. G. Jenkins.** 2004. Reversion to susceptibility in a linezolid-resistant clinical isolate of *Staphylococcus aureus*. J. Antimicrob. Chemother. **54:**818–820.
14. **Mendes, R. E., L. M. Deshpande, M. Castanheira, J. DiPersio, M. A. Saubolle, and R. N. Jones.** 2008. First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. Antimicrob. Agents Chemother. **52:**2244–2246.
15. **Morales, G., J. J. Picazo, E. Baos, F. J. Candel, A. Arribi, B. Peláez, R. Andrade, M. A. de la Torre, J. Fereres, and M. Sánchez-García.** 2010. Resistance to linezolid is mediated by the *cfr* gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. Clin. Infect. Dis. **50:** 821–825.
16. **Prokocimer, P.** 2008. Human pharmacokinetics of the prodrug TR-701 and TR700, its active moiety, after multiple oral doses of 200 and 400 mg TR-701, a novel oxazolidinone. Abstr. 48th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F1-2064.
17. **Schaadt, R., D. Sweeney, D. Shinabarger, and G. Zurenko.** 2009. In vitro activity of TR-700, the active ingredient of the antibacterial prodrug TR-701, a novel oxazolidinone antibacterial agent. Antimicrob. Agents Chemother. **53:**3236–3239.
18. **Shaw, K. J., S. Poppe, R. Schaadt, V. Brown-Driver, J. Finn, C. M. Pillar, D. Shinabarger, and G. Zurenko.** 2008. In vitro activity of TR-700, the antibacterial moiety of the prodrug TR-701, against linezolid-resistant strains. Antimicrob. Agents Chemother. **52:**4442–4447.
19. **Toh, S. M., L. Xiong, C. A. Arias, M. V. Villegas, K. Lolans, J. Quinn, and A. S. Mankin.** 2007. Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotic linezolid. Mol. Microbiol. **64:**1506–1514.