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# MICs of TR-700 – the Active Moiety of the Novel Oxazolidinone Prodrug TR-701 – for Linezolid-Susceptible and -Resistant Enterococci and Staphylococci


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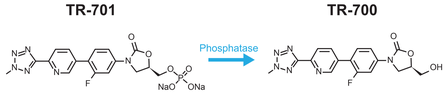
## ABSTRACT


**Background** Linezolid is an effective antibiotic but: (i) its modal MICs for susceptible staphylococci and enterococci are only 1 to 2 doubling dilutions below CLSI and EUCAST breakpoints, and (ii) resistance can emerge via mutation (most often G2576T) and recombination among 23S rRNA gene copies. We determined MICs of TR-700 (the microbiologically active moiety of TR-701) *vs.* linezolid-susceptible and –resistant bacteria. **Methods** MICs of linezolid and TR-700 were determined by CLSI agar dilution for (i) linezolid-susceptible enterococci and staphylococci; (ii) linezolid-resistant clinical isolates, mostly with G2576T and (iii) linezolid-resistant laboratory mutants with mutations conferring linezolid resistance, including G2447T, A2503G and T2504C. Proportions of mutated gene copies were estimated by pyrosequencing. **Results** For 50 linezolid-susceptible MRSA, including hospital and community strains, MICs of TR-700 were 0.25-0.5 mg/L, *vs.* 2 mg/L linezolid; for 50 linezolid-susceptible enterococci, MICs were 0.25-0.5 mg/L TR-700 and 1-2 mg/L linezolid, irrespective of species and vancomycin resistance. For 16 linezolid-resistant staphylococci (MRSA or coagulase negative), heterozygous (where tested) for G2576T, MICs of TR-700 were 1-4 mg/L *vs.* 8-64 mg/L linezolid. For 36 linezolid-resistant enterococci, MICs of TR-700 were 1-16 mg/L (geom. mean 2.2) *vs.* 8-64 mg/L (geom. mean 18.3) linezolid. MICs of TR-700 exceeded 4 mg/L only for 2 *E. faecium*, both of which were homozygous for G2576T (MICs 8 mg/L) and for one without known 23S rRNA changes and with an uncertain mode of linezolid resistance (MIC 16 mg/L). MICs of TR-700 for MRSA with other linezolid-compromising mutations besides G2576T were 1-2 mg/L, *vs.* 8-32 mg/L linezolid. **Conclusions** TR-700 is ca. 8-16 fold more active than linezolid *vs.* staphylococci and enterococci and has the potential to overcome linezolid resistance, particularly in strains heterozygous for G2576T.


## BACKGROUND

- Linezolid –currently the only licensed oxazolidinone– retains good activity against most gram-positive cocci, **BUT**:
  - Its modal MICs for staphylococci and enterococci are only one doubling dilution below CLSI and EUCAST breakpoints of 2-4 mg/L
  - Resistant mutants are occasionally selected, particularly if therapy is prolonged, or if the infection is at a sequestered site [1]
  - Linezolid-resistant clones have caused outbreaks [2] and spread between hospitals (HPA data on file)
- Most linezolid resistance arises via mutations that affect the binding site on the 23S rRNA, [3] though plasmid-mediated resistance was recently described, involving a rRNA methylase encoded by *cfr* [4]
  - G2576T (G2576U in rRNA) is the most common linezolid-resistance mutation in clinical isolates [3]
  - Clinically-relevant gram-positive cocci have 4-7 copies of the 23S rRNA gene; ≥ 2 copies must be altered for resistance [3,5]
  - MICs are highest -up to 64 mg/L- for homozygous mutants, with all their 23S rRNA copies altered [3,5]
- We compared MICs of TR-700, (DA-7157; Trius, San Diego, CA, USA) with linezolid
  - TR-700 is the active moiety of a novel oxazolidinone phosphate monoester pro-drug TR-701 (DA-7218), [6] rapidly generated *in vivo* following oral administration of TR-701 (Figure 1) [7]

**Figure 1:** TR-700 and its prodrug TR-701




## METHODS

- The organisms tested comprised:
  - Linezolid-susceptible *Staphylococcus aureus* and enterococci
  - Clinical and laboratory strains with G2576T and other mutations.
- Clinical isolates were from the UK (mostly) or Austria
- MICs were determined by the CLSI agar dilution method.
- Proportions of native and mutated gene copies were estimated by pyrosequencing [3]

## RESULTS

### *Linezolid-susceptible strains*

- MIC distributions for different groups of isolates are shown in Table 1.
  - For 50 linezolid-susceptible MRSA, geom. mean MICs were TR-700, 0.47 mg/L; linezolid 2.03 mg/L
  - For 50 linezolid-susceptible enterococci, geom. mean MICs were: TR-700, 0.47 mg/L; linezolid 1.95 mg/L
    - MICs for enterococci were independent of species and vancomycin status

### *Linezolid-resistant strains*

- For 10 clinical linezolid-resistant MRSA and 6 coagulase-negative staphylococci -heterozygous where tested, for G2576T- MICs of TR-700 were from 1-4 mg/L *vs.* 8-64 mg/L linezolid
- For 36 linezolid-resistant enterococci MICs of TR-700 were 1-16 (geom. mean 2.2) mg/L *vs.* 8-64 (geom. mean 18.3) mg/L for linezolid
  - MICs of TR-700 were >4 mg/L for only 3 organisms, all enterococci:
    - 2 were *E. faecium* isolates homozygous for G2576T
    - 1 was *E. faecium*, with an MIC of 16 mg/L, lacking any known 23S rRNA lesion, also lacking *cfr*.
- Among isolates with G2576T there was a relationship between the number of gene copies altered and MICs of both linezolid and TR-700 (Table 2)
- MICs of TR-700 for laboratory- MRSA with other linezolid-compromising mutations besides G2576T were 1-2 mg/L *vs.* 8-32 mg/L linezolid (Table 3).

**Table 1.** MIC distributions of TR-700 and linezolid for groups of staphylococci and enterococci (modes in red)

| | MIC (mg/L) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 |
| **Linezolid-susceptible MRSA (n=50)** | | | | | | | | | | |
| TR-700 | | 4 | 46 | | | | | | | |
| Linezolid | | | | | 49 | 1 | | | | |
| **Linezolid-resistant MRSA clinical isolates (n =10): 6 tested and found heterozygous for G2576T** | | | | | | | | | | |
| TR-700 | | | | 1 | 7 | 2 | | | | |
| Linezolid | | | | | | | 2 | 5 | 3 | |
| **Linezolid-resistant coagulase-negative staphylococci, clinical isolates (n=6); mechanisms not determined** | | | | | | | | | | |
| TR-700 | | | | | 1 | 5 | | | | |
| Linezolid | | | | | | | | | 3 | 3 |
| **Linezolid-susceptible enterococci (25 *E. faecalis* and 25 *E. faecium*)** | | | | | | | | | | |
| TR-700 | | 4 | 46 | | | | | | | |
| Linezolid | | | | 2 | 48 | | | | | |
| **Linezolid-resistant enterococci (9 *E. faecalis* and 27 *E. faecium*) ; 12 heterozygous for G2576T, 2 homozygous, 1 with no known 23S rRNA mutation and 23 not determined** | | | | | | | | | | |
| TR-700 | | | | 14 | 8 | 11 | 2[a] | 1[b] | | |
| Linezolid | | | | | | | 12 | 8 | 13 | 3[a,b] |

[a] Two strains homozygous for G2576T in this group
[b] Strain with unknown mechanism in this group

**Table 2.** Oxazolidinone MICs for linezolid-resistant clinical isolates with different copy numbers of the G2576T mutation

| Identifier | Geno-type | # Copies G2576T | LZD MIC (mg/L) | TR-700 MIC (mg/L) | Fold gain: TR-700 vs. linezolid |
|---|---|---|---|---|---|
| ***S. aureus*** | | | | | |
| 080 | rr | 0 | 2 | 0.5 | 4 |
| 040 | rr | 0 | 2 | 0.5 | 4 |
| 367 | Rr | 2 | 8 | 2 | 4 |
| 256 | Rr | 2 | 16 | 2 | 8 |
| B2 | Rr | 2 | 16 | 2 | 8 |
| 258 | Rr | 3 | 16 | 2 | 8 |
| 257 | Rr | 3 | 32 | 2 | 16 |
| 368 | Rr | 3 | 32 | 4 | 8 |
| ***E. faecalis*** | | | | | |
| 587 | rr | 0 | 2 | 0.5 | 4 |
| 485 | Rr | 2 | 16 | 2 | 8 |
| 886 | Rr | 2 to 3 | 32 | 4 | 8 |
| 964 | Rr | 3 | 32 | 4 | 8 |
| 084 | Rr | 3 | 32 | 4 | 8 |
| 875 | Rr | 3 | 32 | 4 | 8 |
| ***E. faecium*** | | | | | |
| 461 | rr | 0 | 2 | 0.5 | 4 |
| 162 | Rr | 0 to 1 | 32 | 4 | 8 |
| 777 | Rr | 2 to 3 | 8 | 1 | 8 |
| 919 | Rr | 2 to 3 | 16 | 2 | 8 |
| 078 | Rr | 4 | 32 | 4 | 8 |
| SA5 | RR | 5 | 32 | 4 | 8 |
| 166 | RR | 5 to 6 | 64 | 8 | 8 |
| 077 | RR | 5 to 6 | 64 | 8 | 8 |

**Table 3.** MICs of TR-700 and linezolid for laboratory mutants of MRSA with various 23S rRNA changes conferring linezolid resistance

| Strain | Mutation | MIC (mg/L) TR-700 | MIC (mg/L) Linezolid |
|---|---|---|---|
| RN4220 | wild-type parent | 0.25 | 2 |
| | G2447T | 1 | 16 |
| | G2576T | 1 | 16 |
| | G2576T | 2 | 16 |
| | G2576T | 1 | 8 |
| | G2576T, A2503G | 2 | 32 |
| ST/02/2121 | wild-type parent | 0.5 | 2 |
| | T2504C | 2 | 16 |
| | G2447T | 1 | 16 |

## CONCLUSIONS

- TR-700 was 4-16 fold more active than linezolid *vs.* staphylococci and enterococci.
- Like linezolid, TR-700 has tightly clustered MICs for susceptible isolates, spread only over 2 dilutions
- TR-700 overcomes most linezolid resistance, particularly for organisms that remain heterozygous for G2576T

## REFERENCES


1 Livermore DM, *J Antimicrob Chemother* 2003; **51 Suppl 2**:ii9-16.

2 Dobbs TE *et al.*, *J Clin Microbiol* 2006; **44:** 3368-3370

3 Sinclair A *et al.*, *Antimicrob Agents Chemother* 2003; **47:** 3620-2

4 Toh SM *et al. Mol Microbiol*. 2007 **64:**1506-14.

5 Meka VG *et al.*, *J Antimicrob Chemother* 2004, **54:** 818-20

6 Shaw KJ *et al.*, ICAAC 2007, Abstract F1-1688

7 Kim E *et al.*, ICAAC 2007, Abstract F1-1692



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