Torezolid (TR-700), a novel methyltetrazolyl-oxazolidinone, accumulates extensively within human macrophages and shows activity towards intraphagocytic linezolid-sensitive and linezolid-resistant *S. aureus*

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Abstract

Background. Treatment of intracellular infections requires that antibiotics reach their intracellular target and express activity herein. Linezolid accumulates poorly within cells, and shows only modest intracellular activity against *S. aureus* or *S. epidermidis* (Barcia-Macay et al, AAC, 2006; Pascual et al, AAC, 2002). The aim of the present study was to examine the cellular pharmacokinetic properties and intracellular activity of torezolid (TR-700) towards *S. aureus*, in view of its higher lipophilicity and intrinsic activity of this molecule in comparison with linezolid.

Methods. Human THP-1 macrophages were used throughout this study. Accumulation of both oxazolidinones was measured by microbiological assay, using *S. aureus* ATCC 25923 as test organism. The phenotypes of the strains used are shown in Table. MICs were determined in MHB. Intracellular activity was determined against bacteria phagocytised by human THP-1 macrophages as previously described (Barcia-Macay et al, AAC, 2006) and the results expressed as the change in the intracellular inoculum at 24 h compared to time 0 (post-phagocytosis).

Results. TR-700 accumulated quickly and extensively in macrophages, reaching an apparent cellular to extracellular concentration ratio of about 13 within 15 min vs 1-2 for linezolid. MICs in broth and intracellular activities are shown in the Table.

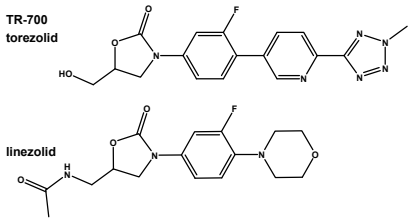
Organisms	Linezolid			TR-700		
	MICs (mg/L)	Static conc. (mg/L)[a]	Emax[b]	MICs (mg/L)	Static conc. (mg/L)[a]	Emax[b]
ATCC 25923	2	~ 4.5	-0.4 ± 0.1	0.25	~ 1.0	-0.6 ± 0.1
SA238[1]	2	~ 5.8	-0.3 ± 0.1	0.25-0.5	~ 0.5	-0.7 ± 0.1
SA238 L[1]	16	N.D.	0.2 ± 0.1	1	~ 1.0	-0.6 ± 0.1
CM-05[2]	8	~ 21.3	-0.5 ± 0.3	0.25-0.5	~ 0.7	-0.6 ± 0.1

[a] Extracellular concentration of antibiotic yielding no apparent change in cfu after 24 h compared to post-phagocytosis inoculum
[b] Maximal decrease in intracellular cfu compared to the post-phagocytosis inoculum (log scale)
N.D., not measurable (bacterial growth in all conditions)
[1] laboratory strains; [2] clinical isolate

Conclusions. Compared to linezolid, TR-700 shows increased potency (lower static concentrations) towards intraphagocytic *S. aureus* (unaffected by resistance of the strain to linezolid), probably in relation with its extensive accumulation within cells and its higher intrinsic activity (lower MIC values).

Background

Selecting an optimal treatment against *S. aureus* infections requires facing two major issues, namely (i) the increasing emergence of resistance to first line antibiotics, and (ii) the difficulty of eradicating intracellular forms.[1,2] Linezolid accumulates poorly within THP-1 cells, showing only modest intraphagocytic activity towards *S. aureus*.[3,4] Torezolid (TR-700) is a novel methyltetrazolyl-oxazolidinone,[5] the structure of which (see Figure) suggests different cellular pharmacokinetic properties compared to linezolid.



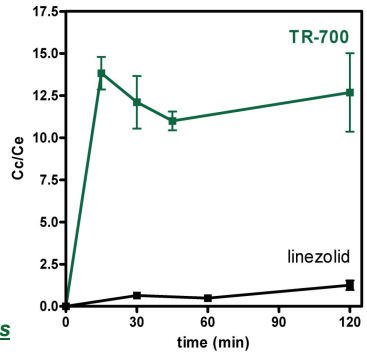
Results

1 Kinetic of TR-700 vs. linezolid uptake within THP-1 cells

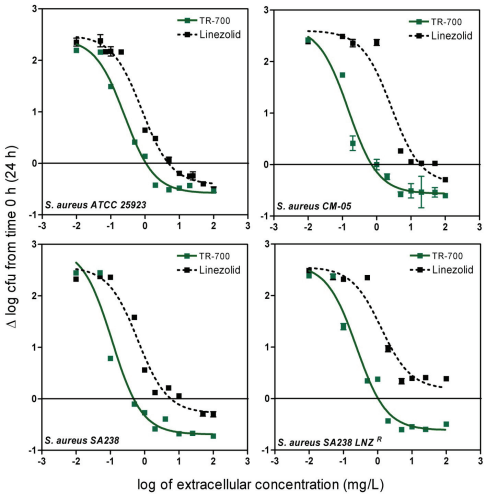
Cellular accumulation of antibiotics (extracellular concentration, 250 mg/L) was determined in uninfected THP-1 macrophages.

The ordinate shows the apparent cellular to extracellular concentration ratio (Cc/Ce).

TR-700 accumulates quickly and extensively within macrophages, reaching within 15 min (or less) intracellular concentrations about 10-15-fold the extracellular ones, while linezolid reach an apparent cellular concentration of 1-2.



2 Activity of TR-700 and LNZ towards intraphagocytic *S. aureus*



Intrinsic activities and pertinent regression parameters of the dose-response curves illustrated in the figure

In all cases, antibiotic activity was related to concentration, obeying the classical pharmacological model described earlier in this model.[3] Data were used to fit sigmoidal functions (Hill's equation) to obtain values of key pharmacological descriptors of antibiotic activity, namely the relative efficacy (E_{max}) and static concentrations (C_s) of each drug.

Bacteria	Linezolid			TR-700		
	MIC (mg/L)	Cs (mg/L)[a]	Emax[b]	MICs (mg/L)	Cs (mg/L)[a]	Emax[b]
ATCC 25923	2	~ 4.5	-0.4 ± 0.1	0.25	~ 1.0	-0.6 ± 0.1
SA238[1]	2	~ 5.8	-0.3 ± 0.1	0.25-0.5	~ 0.5	-0.7 ± 0.1
SA238 LNZ R[1]	16	N.D.	0.2 ± 0.1	1	~ 1.0	-0.6 ± 0.1
CM-05	8	~ 21.3	-0.5 ± 0.3	0.25-0.5	~ 0.7	-0.6 ± 0.1

[a] Extracellular concentration of antibiotic resulting in no apparent bacterial growth (the number of CFU was identical to that of the original inoculum), as determined by graphical interpolation.
[b] CFU decrease (in log10 units) at 24 h from the corresponding original inoculum, as extrapolated for antibiotic concentrations at infinitively high concentrations
N.D., not measurable (bacterial growth in all conditions)
1 Strains obtained by resistance selection studies.[6]

The ordinate shows the change of cfu (log_{10}) per mg of cell protein observed after 24 h of incubation, in comparison with the original inocula (mean ± SD [n=3]).

Methods

Assay of cell-associated antibiotics:

TR-700 and linezolid were assayed by disc-diffusion using *S. aureus* ATCC 25923

MICs and intracellular activities of antibiotics:

MICs was determined in MH broth.

THP-1 macrophages were infected with preopsonized bacteria (1 h; 37°C), washed with phosphate-buffered saline, and incubated for 45 minutes with gentamicin (50 mg/L) to eliminate non-adherent and non-internalized bacteria.

Infected cells were thereafter exposed for 24 h to increasing concentrations of antibiotics (control cells were maintained in the continuous presence of gentamicin [0.5 x MIC] to prevent the extracellular growth of bacteria released from dead cells).

The model and its validation are described in details in ref 3.

Conclusions

In contrast to linezolid, TR-700 accumulates quickly and extensively in macrophages, reaching an apparent cellular concentration ratio of 10-15 at equilibrium.

Intracellularly, TR-700 shows increased potency (lower static concentrations) towards intraphagocytic *S. aureus* (irrespective of the phenotype of linezolid resistance), which may be ascribed to its higher intrinsic activity (lower MIC values) compared to linezolid.

Acknowledgments

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