

Cellular pharmacokinetics and intracellular activity of torezolid (TR-700): studies with human macrophage (THP-1) and endothelial (HUVEC) cell lines

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Received 18 May 2009; returned 18 June 2009; revised 1 July 2009; accepted 2 July 2009

Background and aims: Optimal treatment of infections caused by *Staphylococcus aureus*, *Listeria monocytogenes* and *Legionella pneumophila* requires antibiotics with intracellular activity. Linezolid accumulates poorly within cells. Torezolid (TR-700) is a novel methyltetrazolyl oxazolidinone with potentially different cellular pharmacokinetic properties. Our aim was to examine the accumulation and intracellular activities of torezolid in this context.

Methods: Measurement of torezolid cell content and antibacterial activity in comparison with linezolid using human macrophages (THP-1) and human endothelial cells [human umbilical vein endothelial cells (HUVECs)], applying models allowing for the quantitative evaluation of the pharmacodynamics of antibiotics towards intracellular bacteria.

Results: Torezolid accumulated rapidly in THP-1 macrophages, reaching a stable intracellular to extracellular ratio of ~10 (compared with ~1–2 for linezolid) within 15 min. On a weight concentration basis (mg/L), torezolid was ~5- to 10-fold more potent intracellularly (lower concentration needed to achieve a bacteriostatic effect) than linezolid against phagocytosed *S. aureus*, *L. monocytogenes* and *L. pneumophila*, with no change in maximal efficacy (~1 log₁₀ reduction of the original, post-phagocytosis inoculum). When drugs were compared at equipotent concentrations (multiples of the MIC), no difference was seen between linezolid and torezolid, but the higher potency of torezolid allowed control of intracellular infections caused by linezolid-resistant *S. aureus*.

Conclusions: Torezolid exerts intracellular activity at lower extracellular concentrations than linezolid because of its greater potency independent of its greater intracellular accumulation. This may confer an advantage to torezolid *in vivo* if the drug can be used at dosages creating serum concentrations similar to those achieved with linezolid.

Keywords: linezolid, *Staphylococcus aureus*, *Legionella pneumophila*, *Listeria monocytogenes*, pharmacodynamics

Introduction

The cellular and tissue accumulation of antibiotics has been the subject of a large number of studies, but its importance for activity against intracellular bacteria remains controversial.^{1–6} Studies examining a series of antibiotics belonging to the main pharmacological classes (with several derivatives within each class) have convincingly shown that accumulation *per se* may not be predictive of efficacy.⁷ Thus, antibiotics belonging to

a pharmacological class known for its low accumulation level, such as β -lactams, are not necessarily inactive against intracellular forms of susceptible bacteria as demonstrated for *Listeria monocytogenes* or even *Staphylococcus aureus*.^{8,9} Conversely, macrolides are considerably less potent than fluoroquinolones against the same organisms,^{10–12} even though their cellular accumulation is much higher. There are, however, other situations, such as observed with the lipoglycopeptides telavancin and oritavancin, in which structural changes made in comparison

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with vancomycin result in a higher accumulation and a commensurately higher intracellular activity.^{11,13} It is therefore important to experimentally assess the intracellular accumulation and activity of novel drugs individually, in order to obtain a clear description of their behaviour in this context.

Torezolid (TR-700) is a novel methyltetrazolyl oxazolidinone, with enhanced activity against Gram-positive cocci including linezolid-non-susceptible strains,^{14,15} owing to additional target site interactions.¹⁶ Linezolid accumulates in only modest amounts in eukaryotic cells and displays a low level of activity (mostly static) against phagocytosed *S. aureus* (including strains of clinical origin).^{7,17} The structure of torezolid (see Figure 1) suggests different cellular pharmacokinetic properties compared with linezolid. In the present study, we have examined: (i) the cellular pharmacokinetics of torezolid and linezolid in THP-1 macrophages and their pharmacodynamics in cells infected with *S. aureus* (this model has been designed and validated for this type of study,⁷ and macrophages represent a first line of defence against a variety of staphylococcal infections¹⁸); (ii) pharmacodynamic properties of both oxazolidinones were also studied with human endothelial cells [human umbilical vein endothelial cells (HUVECs)] since adhesion and invasion by *S. aureus* in vascular endothelium is probably an important aspect in the persistent and relapsing character of endocarditis;¹⁹ and (iii) the experiments were then extended to *L. monocytogenes* and *Legionella pneumophila*, as these organisms develop in different subcellular compartments than *S. aureus* and provide us, therefore, with a broader view of the parameters governing the activity of intracellular antibiotics.

Materials and methods

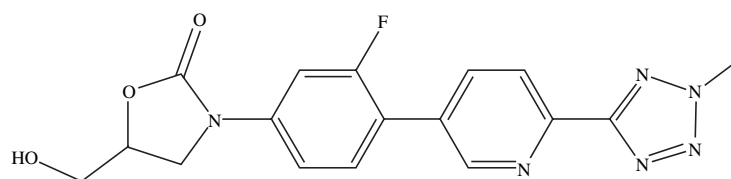
Antibiotics and main reagents

Torezolid was obtained as microbiological standard from Trius Therapeutics (San Diego, CA, USA). The other antibiotics were obtained as the clinical products for intravenous administration to humans and complying with the European Pharmacopoeia [gentamicin as GEOMYCINE[®] (distributed in Belgium by GlaxoSmithKline, Genval); and linezolid as ZYVOXID[®] (distributed in Belgium by Pfizer, Brussels)]. Cell culture media and sera were from Invitrogen (Carlsbad, CA, USA) and Lonza (Basel, Switzerland).

Cell lines

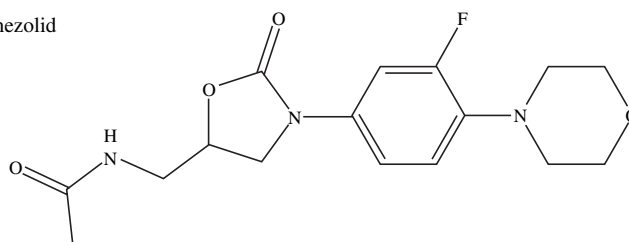
Experiments were performed with: (i) human THP-1 cells (ATCC TIB-202; American Tissue Culture Collection, Manassas, VA, USA), a myelomonocytic cell line displaying macrophage-like activity maintained in our laboratory as previously described;⁷ and (ii) HUVECs (Lonza, Walkersville, MD, USA). The latter were handled as follows. Cells were maintained in endothelial growth medium (EGM) and 25 cm² gelatin-coated flasks as described in the manufacturer's instructions, and used before the eighth passage. For each experiment, cells having reached ~80% confluency were removed from flasks by use of 0.05% trypsin/EDTA, washed in Dulbecco's modified Eagle's medium (DMEM)-glutamax medium supplemented with 10% fetal calf serum (FCS; for trypsin inactivation) and seeded into 12-well gelatin-coated plates in EGM. Monolayers were obtained after ~3–4 days. The day prior to *S. aureus* cell invasion assays, the medium was removed and cells were washed with

Torezolid (TR-700)



3-{3-Fluoro-4-[6-(2-methyl-2H-tetrazol-5-yl)-pyridin-3-yl]-phenyl}-5-hydroxymethyl-oxazolidin-2-one

Linezolid



N-[3-(3-Fluoro-4-morpholin-4-yl-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-acetamide

Figure 1. Structural formulae and full chemical names of torezolid and linezolid. The figure highlights the key structural differences made in torezolid in comparison with linezolid, namely the replacement of the methylacetamide by a hydroxymethyl moiety (left) and of the morpholine by a methyl-tetrazole-pyridine moiety (right), around the central common core (3-fluorophenyl-2-oxo-oxazolidine). The pK_a of the amino function in the methyltetrazole pyridinyl moiety (torezolid) is not expected to be higher than that of the amino function of the morpholine (linezolid) because of the electroattracting properties of the tetrazole.

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phosphate-buffered saline (PBS) to ensure complete removal of the antibiotics present in EGM and transferred to DMEM–glutamax medium (Invitrogen) supplemented with 10% FCS.

Accumulation and assay of cell-associated antibiotics

Uninfected THP-1 macrophages were used for these studies as the lack of radiolabelled drug forced us to use extracellular concentrations (≥ 10 mg/L) that would have prevented the intracellular growth of the bacteria. Cells were incubated with the drugs for the appropriate duration (with or without the inhibitors under study) and collected after thorough washing with pre-warmed PBS and gentle pelleting.⁷ For experiments evaluating the influence of the pH and the temperature of the culture medium, cells were resuspended in RPMI 1640 medium (supplemented with 10% FCS) adjusted to the specific pH (7.4 or 5.5) or temperature (37 or 4°C) values for 2 h before addition of the drug under study, and the corresponding pH or temperature value was maintained throughout the experiment until cell collection. Torezolid and linezolid were assayed by the disc-plate method, using *S. aureus* ATCC 25923 as a test organism as described previously^{7,9} [typical values for the lowest limit of detection, 2 and 8 mg/L; typical linear response between 2 and 500 and 16 and 500 mg/L for torezolid and linezolid, with correlation coefficients (R^2) for standards (5–8 per experiment) >0.987]. The cell-associated content of antibiotic was expressed by reference to the total cell protein content and converted into apparent total cell concentrations using a conversion factor of 5 μ L per mg of cell protein. For determination of antibiotic accumulation in media at different pH values, we ensured that the lysates of cells, collected as described above, had the same pH as cells cultivated under control conditions [note also that, as will be shown in the Results section, acid pH (5.5) did not affect the MICs of torezolid and linezolid for the strain used for the assays].

S. aureus cell invasion and intracellular growth

For both THP-1 macrophages and HUVECs, invasion by *S. aureus* was performed as follows. Bacterial cultures were centrifuged at 14000 rpm for 10 min, and the pelleted bacteria resuspended and incubated for 30 min at 37°C in RPMI 1640 medium supplemented with 10% fresh human serum (Lonza) to allow for opsonization. Opsonized bacteria were then added to the cell culture medium of either THP-1 macrophages or HUVECs at an initial ratio of four bacteria per cell and maintained for 1 h at 37°C, after which extracellular bacteria were removed by washing. Cells were then re-incubated for 24 h in the presence of the antibiotics under study (or in the presence of gentamicin at half its MIC for controls, to minimize growth of extracellular bacteria). Cell viability was tested at the end of each critical experiment by Trypan Blue staining ($<10\%$ stained cells).

Bacterial strains and susceptibility testing

Table 1 shows the strains used in our study. MIC determinations were made in Mueller–Hinton broth (pH 7.3, 24 h) for *S. aureus*, in tryptic soy broth (pH 7.4, 24 h) for *L. monocytogenes*, and in α -ketoglutarate buffered yeast extract broth (pH 6.9, 48 h) for *L. pneumophila*.

Determination of extracellular and intracellular activities

For *S. aureus*, extracellular activities were measured in Mueller–Hinton broth; intracellular activities were determined towards

bacteria phagocytosed by either THP-1 macrophages or HUVECs following the general procedures described in detail in our earlier publication⁷ (typical starting inocula: $\sim 1\text{--}2 \times 10^6$ cfu per mL (broth) or per mg of cell protein (cells)). For other bacteria, infection of THP-1 cells and assessment of the intracellular activity of antibiotics were performed as described previously for *L. monocytogenes*,⁹ with minor adaptations for *L. pneumophila* using a starting inoculum of 10 bacteria per macrophage, and exposing the cells to 50 mg/L gentamicin for 30–45 min after phagocytosis for elimination of adherent, non-internalized bacteria. As discussed previously,²⁰ the large dilutions of the cellular material made during its collection and actual spread on plates ensured absence of interference with cfu counts by the presence of carried-over antibiotics.

Curve fitting and statistical analyses

For curve fitting (GraphPad Prism[®] version 4.03, GraphPad Software, San Diego, CA, USA), data were used to fit sigmoidal functions [Hill's equation (see Barcia-Macay *et al.*⁷ for details)] to obtain, for each condition, numeric values of four key descriptors, namely: (i) the intracellular growth in each condition over the original inoculum for an infinitely low concentration of antibiotic [E_{\min} (in \log_{10} units)]; (ii) the relative efficacy of each drug [E_{\max} (in \log_{10} units), or the decrease in bacterial counts from the original post-phagocytosis inoculum for an infinitely large drug concentration]; (iii) the relative potency; and (iv) the static concentrations [i.e. the drug concentrations yielding a reduction of bacterial counts half way between E_{\min} and E_{\max} (EC_{50}) or causing no apparent change compared with the original inoculum (C_s), respectively]. Statistical analyses were made with GraphPad InStat version 3.06 (GraphPad Software).

Results

Cellular accumulation of torezolid and linezolid by THP-1 cells

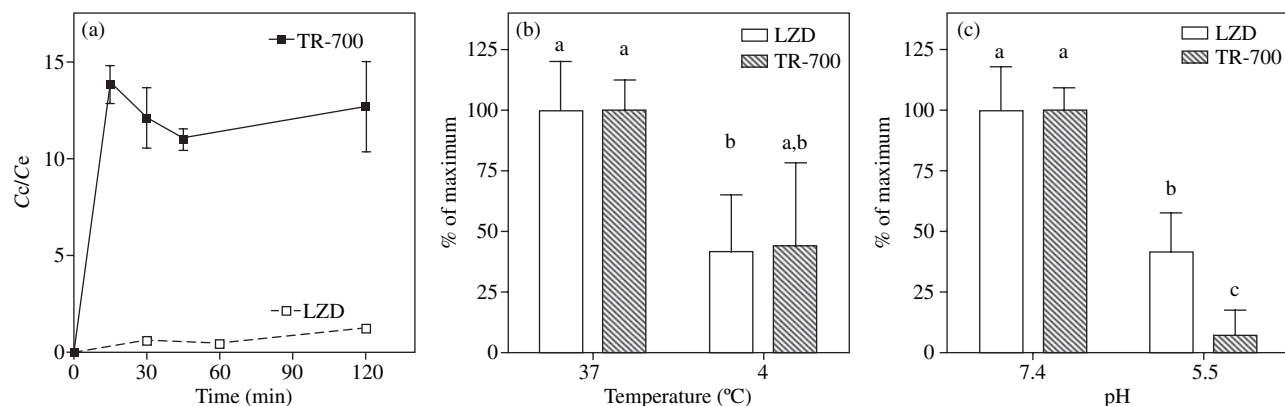
In the first series of experiments we investigated whether torezolid and linezolid could be differentiated with respect to accumulation by macrophages and influence of pH and temperature. Figure 2(a) shows that torezolid accumulated very quickly, reaching cellular concentrations $\sim 10\text{--}15$ -fold the extracellular concentration within 15 min (or less), whereas linezolid reached a cellular concentration similar to the extracellular concentration after ~ 1 h. Both antibiotics, however, showed $\sim 50\%$ impairment in their accumulation when incubated at 4°C (Figure 2b). In contrast, the accumulation of torezolid was drastically reduced ($>90\%$) when cells were incubated at pH 5.5 in comparison with pH 7.4, whereas the accumulation of linezolid was reduced by only 60% (Figure 2c). These changes were not due to alteration of cell viability, as Trypan Blue exclusion assays did not show meaningful differences between control and treated cells ($<10\%$ stained cells for all conditions).

Susceptibility testing

Table 1 shows the MICs of torezolid and linezolid for the strains used in this study. Torezolid showed consistently lower MICs (2–5 dilutions) than linezolid for all linezolid-susceptible strains, and maintained a low MIC (≤ 1 mg/L) against the linezolid-resistant *S. aureus* strains [MIC > 4 mg/L; European

Table 1. Susceptibility of the strains of *S. aureus*, *L. monocytogenes* and *L. pneumophila* used in this study to linezolid and torezolid

Species, phenotype and strain no.	MIC (mg/L) ^a	
	linezolid	torezolid
<i>Staphylococcus aureus</i>		
MSSA ATCC 25923 ^b	2	0.25
HA-MRSA ATCC 33591 ^b	1	0.125–0.25
SA 238 ^c	2	0.25–0.5
CM 05 ^d	8	0.25–0.5
SA 238L (LZD ^R after drug exposure) ^e	16	1
CA-MRSA NRS 192 ^e	2	0.125–0.25
NRS 384 (US300) ^e	2	0.25
VISA NRS 52 ^e	2	0.125
VRSA VRS 1 ^e	1–2	0.125–0.25
VRS 2 ^e	1–2	0.25
animal MRSA N7112046 ^f	2	0.125
<i>Listeria monocytogenes</i>		
EGD ^g	1–2	0.125
<i>Legionella pneumophila</i>		
ATCC 33153 ^b	4–8	0.25–0.5

LZD^R, resistant to linezolid.^aRepresentative values of at least two determinations.^bFrom the American Tissue Culture Collection (Manassas, VA, USA).^cProvided by P. C. Appelbaum.³⁶^dProvided by J. P. Quinn, John H. Stroger Jr. Hospital, Rush University, Chicago, IL, USA.^eFrom the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) programme (operated by Eurofins Medinet, Inc., Hendon, VA, USA; supported under NIAID/NIH contract no. HHSN2722007 00055C); details on each strain are available at <http://www.narsa.net/content/home.jsp>.^fProvided by Y. Glupczynski, Cliniques universitaires UCL de Mont Godinne, Yvoir, Belgium.^gProvided by P. Berche, Hôpital Necker, Paris, France.²⁸**Figure 2.** Accumulation of linezolid (LZD) and of torezolid (TR-700) in THP-1 macrophages (extracellular concentration, 250 mg/L). (a) Uptake kinetics; the ordinate (C_c/C_e) shows the apparent cellular to the extracellular concentration ratio (±SD; n=3); open symbols, linezolid; filled symbols, torezolid. (b) Influence of the temperature (2 h incubation); values are expressed as a percentage of the maximum value of accumulation observed (±SD; n=3) for the antibiotic under study; blocks with different letters have values significantly different from each other (unpaired two-tailed *t*-test with *P*<0.05). (c) Influence of pH (30 min incubation); values are expressed as a percentage of the maximum (±SD; n=3); bars with different letters have values significantly different from each other (unpaired two-tailed *t*-test with *P*<0.05).

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Committee on Antimicrobial Susceptibility Testing (EUCAST)] included in our panel. No difference in MICs for *S. aureus* was seen when tested at acidic (5.5) compared with neutral (7.4) pH for either linezolid or torezolid.

Susceptibility of extracellular (broth) and intracellular (THP-1 macrophages; HUVECs) forms of *S. aureus* ATCC 25923 [methicillin-susceptible *S. aureus* (MSSA)]

Bacteria in broth or infected THP-1 macrophages and HUVECs were exposed for 24 h to a wide range of concentrations of torezolid or linezolid (from ~ 0.01 to $100\times$ the MIC) to determine the overall shape of the response. Data are summarized together in Figure 3 (with the corresponding pertinent regression parameters shown in Table 2). In all cases, antibiotic activity was related to concentration, obeying the classical pharmacological model reported for all other antibiotics examined in this model so far.^{7,9,21} Results showed that: (i) *S. aureus* displayed similar growth for all conditions (broth, THP-1 or HUVECs); (ii) torezolid and linezolid had a similar relative efficacy (~ 0.5 – $1.5 \log_{10}$ cfu decrease) at equivalent multiples of the MIC; (iii) torezolid and linezolid significantly differed with respect to their relative potencies if data are plotted against drug weight concentrations (mg/L) but not if expressed as multiples of the

corresponding MIC; and (iv) both torezolid and linezolid showed a slightly but significantly higher relative efficacy (lower E_{\max}) in HUVECs compared with THP-1 cells.

Susceptibility of intracellular forms (THP-1) of *S. aureus* with different resistance phenotypes

Comparative 24 h concentration–response studies were then performed using the strain ATCC 25923 and four methicillin-resistant *S. aureus* (MRSA) strains chosen for being either susceptible (NRS 384 and SA 238) or resistant (CM 05 and SA 238L) to linezolid. Results are presented in Figure 4 with drug concentrations shown as multiples of the MIC (pharmacological comparison). This shows that all strains behaved similarly when exposed to either torezolid or linezolid over the wide range of concentrations explored. Although statistical analysis showed that the functions describing the concentration–response effects are significantly different (see numeric values in the figure legend), these differences were minor and affected in an opposite way the relative potencies (EC_{50}) and relative efficacies (E_{\max}) of each drug. Of note, a different picture would emerge, however, if data are plotted as a function of the weight concentrations of the antibiotics, in which case the relative potencies (EC_{50}) and the static concentrations (C_s) of the corresponding

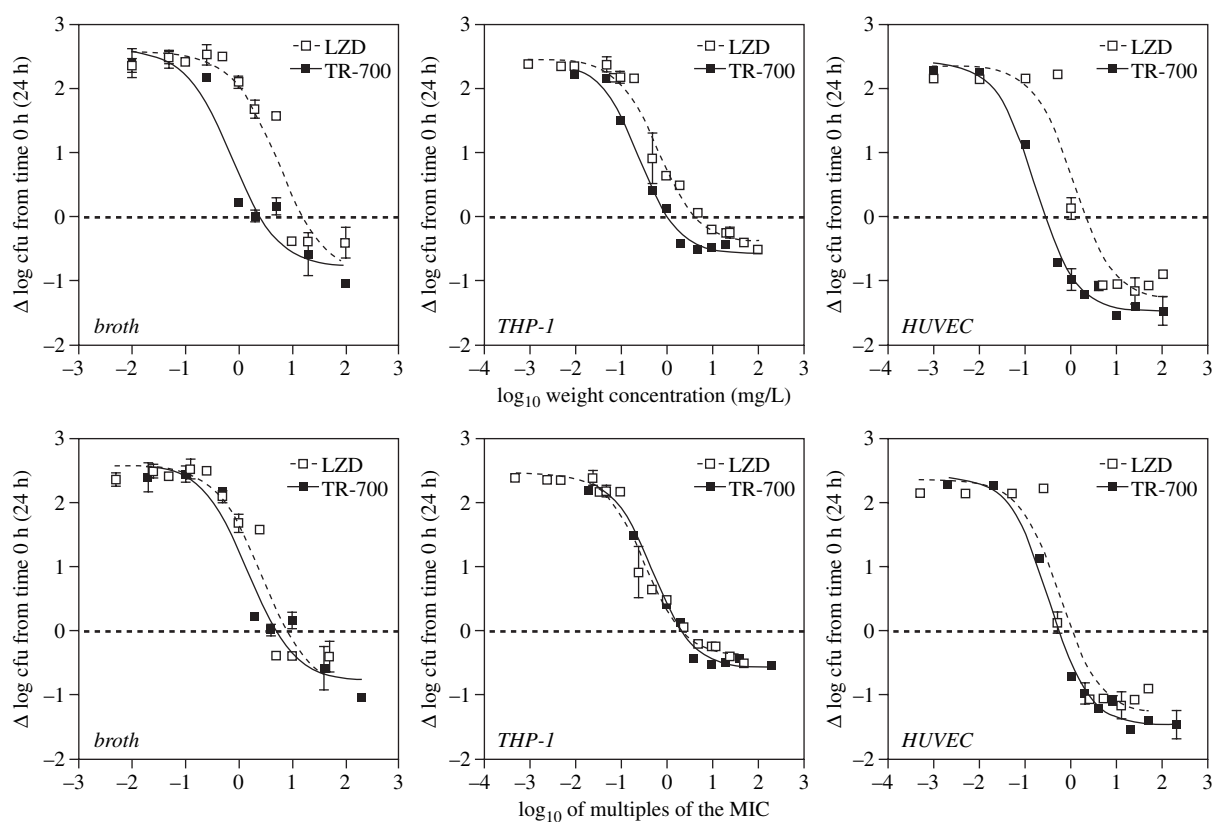


Figure 3. Concentration-dependent effects of linezolid (LZD) and torezolid (TR-700) towards *S. aureus* ATCC 25923 in broth (left panels) or after phagocytosis by THP-1 macrophages (middle panels) or HUVECs (right panels). The ordinate shows the change in cfu ($\Delta \log$ cfu) per mL of broth (left panels) or per mg of cell protein (middle and right panels) after 24 h compared with the initial inoculum [dotted line; $\sim 1 \times 10^6$ bacterial per mL (broth) or per mg of cell protein (THP-1 macrophages and HUVECs)]. Upper row: data are plotted against the weight concentration (mg/L) of the antibiotics. Lower row: data are plotted against equipotent antibiotic concentrations (multiples of the MIC). All values are means \pm SD of three independent experiments (SD bars that are not visible are smaller than the size of the symbols).

Table 2. Pertinent regression parameters of the dose–response curves illustrated in Figure 3

Condition	Torezolid					Linezolid				
	E_{\min}^a (CI)	E_{\max}^b (CI)	EC_{50}^c (CI)	C_s^d	R^2	E_{\min}^a (CI)	E_{\max}^b (CI)	EC_{50}^c (CI)	C_s^d	R^2
Broth	2.6 (1.9 to 3.4)	-0.8 (-1.5 to -0.1)	0.8 mg/L (0.2 to 2.5) 1.5× MIC (0.4 to 4.9)	~ 2.4 mg/L ~4.9× MIC	0.946	2.6 (2.1 to 3.0)	-0.9 (-1.9 to 0.1)	5.6 mg/L (1.9 to 15.6) 2.8× MIC (1.0 to 7.8)	~ 15.7 mg/L ~8.2× MIC	0.923
THP-1	2.4 (2.1 to 2.7)	-0.6 (-0.8 to -0.4)	0.2 mg/L (0.1 to 0.4) 0.5× MIC (0.3 to 0.8)	~ 1.0 mg/L ~2.2× MIC	0.988	2.5 (2.3 to 2.6)	-0.4 (-0.5 to -0.2)	0.7 mg/L (0.5 to 1.0) 0.3× MIC (0.2 to 0.5)	~ 4.2 mg/L ~2.2× MIC	0.984
HUVEC	2.4 (2.1 to 2.7)	-1.5 (-1.6 to -1.3)	0.2 mg/L (0.1 to 0.2) 0.3× MIC (0.2 to 0.5)	~ 0.3 mg/L ~0.5× MIC	0.992	2.3 (1.6 to 3.0)	-1.3 (-1.9 to -0.6)	1.1 mg/L (0.3 to 3.8) 0.6× MIC (0.2 to 1.9)	~ 2.1 mg/L ~1.1× MIC	0.927

Statistical analysis for the differences between torezolid and linezolid: only the figures shown in bold [EC₅₀ values when expressed as a function of weight concentrations (mg/L)] are significantly different from each other between torezolid and linezolid (unpaired two-tailed *t*-test).

^acfu increase (in log₁₀ units) at 24 h from the corresponding original inoculum, as extrapolated for infinitely low concentrations of antibiotics.

^bcfu decrease (in log₁₀ units) at 24 h from the corresponding original inoculum, as extrapolated for antibiotic concentrations at infinitely high concentrations.

^cConcentration [mg/L (total drug) and multiple of the MIC] causing a reduction halfway between the minimal (E_{\min}) and maximal (E_{\max}) values, as obtained from the Hill equation (by using a slope factor of 1).

^dConcentration resulting in no apparent bacterial growth (the number of cfu was identical to that of the original inoculum), as determined by graphical interpolation.

antibiotics would be widely different and in relation to the differences in MIC of the organisms tested.

Susceptibility of intraphagocytic forms of L. monocytogenes and L. pneumophila

In these experiments, we examined the behaviour of torezolid and linezolid towards other organisms that could live and multiply in compartments other than phagolysosomes. Concentration–effect studies were therefore performed with phagocytosed *L. monocytogenes* and *L. pneumophila* (see MICs in Table 1). As seen in Figure 5, torezolid and linezolid again showed concentration-dependent activities against both phagocytosed organisms, with no significant differences in relative potencies (EC₅₀ parameter), static concentrations (C_s) and relative efficacies (E_{\max}) when data were plotted against equipotent drug concentrations (multiples of the MIC). As for *S. aureus*, however, plotting the data as a function of the weight concentration revealed differences in relative potencies and static concentrations that essentially matched the differences in MIC.

Discussion

Intracellular survival of bacteria remains an important cause of bacterial spread, life-threatening therapeutic failures, and persistence of infection in spite of apparently effective antimicrobial therapy as far as *in vitro* activity is concerned.^{22,23} This may be of direct application to linezolid, as this drug shows poor activity against intracellular forms of bacteria in models in which extracellular and intracellular activities have been directly compared.^{7,17}

The present study expands our knowledge of the role played by intracellular accumulation of antibiotics in general, and of oxazolidinones in particular in two main respects. First, we showed that the intracellular accumulation of oxazolidinones may differ, since torezolid concentrates ~10-fold in cells under conditions in which linezolid showed only a modest accumulation. This accumulation is largely reduced by exposure to acid pH, suggesting that it could depend upon the cellular to extracellular pH gradient, as observed with macrolides^{24,25} and weak basic drugs in general.^{26,27} Yet torezolid is probably not a weaker basic drug than linezolid, based on its calculated physicochemical properties (see legend of Figure 1), indicating that properties other than their basic character may be more critical. These may include the ratios of the membrane permeability coefficients of the unionized and ionized forms of the two molecules, or their ability to bind to cellular constituents. While membrane diffusion, cell fractionation and morphological studies would be most useful in this context, these could not be undertaken to date due to lack of sufficiently sensitive and specific methods for differential assay or visualization of intracellular torezolid and linezolid.

Secondly, we showed that torezolid: (i) expresses a higher relative antibiotic potency than linezolid (lower values for the EC₅₀ and C_s pharmacodynamic parameters) as long as concentrations are expressed as a weight basis, but not when they are expressed as multiples of the MIC; and (ii) shows essentially a similar maximal efficacy when compared with linezolid (no significant difference in the E_{\max} pharmacodynamic

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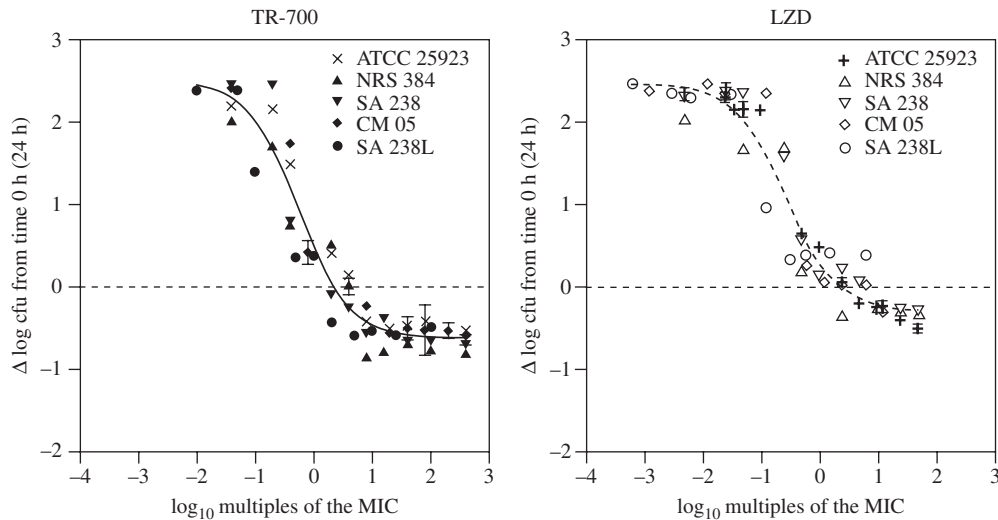


Figure 4. Concentration-dependent effects of torezolid (TR-700; left panel) and linezolid (LZD; right panel) towards intraphagocytic (THP-1) forms of linezolid-susceptible (ATCC 25923, NRS 384 and SA 238) and linezolid-resistant (CM 05 and SA 238L) *S. aureus*. The ordinate shows the change in cfu ($\Delta \log \text{cfu}$) per mg of cell protein at 24 h, compared with the initial post-phagocytosis inoculum as a function of the extracellular concentration (plotted as multiples of the MIC). All values are means \pm SD of three independent experiments (SD bars that are not visible are smaller than the size of the symbols). Statistical analysis (unpaired *t*-test using all data values for each antibiotic): the two Hill's functions fitted to the data are significantly different from each other ($P=0.0035$); however, this difference is minimal and affects in opposite ways the relative efficacies [E_{\max} : -0.63 (CI -0.76 to -0.51) versus -0.29 (CI -0.45 to -0.14)] and the relative potencies [EC_{50} (\log_{10} multiples of the MIC): 0.55 (CI 0.39 to 0.80) versus 0.28 (CI 0.19 to 0.39)] of torezolid and linezolid, respectively [removing the data for strains CM 05 and SA 238L (MICs of 8 and 16 mg/L) does not invalidate this general conclusion ($P=0.03$), even though the value of E_{\max} for linezolid becomes slightly more negative [-0.37 (CI -0.52 to -0.22)]].

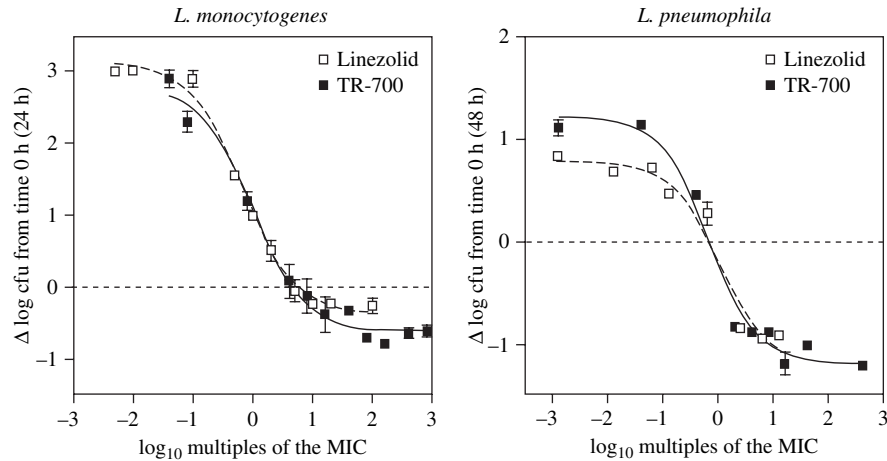


Figure 5. Concentration-dependent effect of torezolid (TR-700) versus linezolid towards intraphagocytic (THP-1) forms of *L. monocytogenes* strain EGD (left panel) and *L. pneumophila* strain ATCC 33153 (right panel). The ordinate shows the change in cfu ($\Delta \log \text{cfu}$) per mg of cell protein at 24 h (*L. monocytogenes*) or 48 h (*L. pneumophila*), compared with the initial inoculum. Data are plotted against equipotent concentrations [multiples of the MIC; *L. monocytogenes*, 0.125 mg/L (torezolid) and 1 mg/L (linezolid); *L. pneumophila*, 0.25 mg/L (torezolid) and 8 mg/L (linezolid)]. All values are means \pm SD of three independent experiments (SD bars that are not visible are smaller than the size of the symbols). Statistical analysis (unpaired *t*-test using all data values for each antibiotic): the Hill's functions fitted to the data are not significantly different between torezolid and linezolid for each of the two organisms examined.

parameter). This indicates that the main if not exclusive driver of intracellular activity for torezolid in comparison with linezolid with respect to its relative potency is its higher intrinsic activity (as denoted by a lower MIC), whereas its greater accumulation plays no detectable role. The data also show that the two drugs remain similar with respect to their level of maximal activity, suggesting a similar mode of action. Thus, torezolid keeps the essentially bacteriostatic character of

linezolid (as the maximal efficacies of both drugs in all conditions never exceed $\sim 1 \log_{10}$ cfu decrease over the original inoculum). It is important to note that the similarity of behaviour between torezolid and linezolid, when data are expressed as a function of multiples of their MIC, is observed over a wide range of concentrations, and is, therefore, likely to correspond to true intrinsic pharmacological properties of the molecules.

The fact that accumulation of torezolid seems not to play a significant role in its activity is probably unrelated to a specific subcellular localization (which, as indicated above, remains to be established) and/or physicochemical environment. Indeed, similar results are observed with the three organisms studied with respect to the key role of MIC as a driver of activity, even though each of them localizes and multiplies in a distinct subcellular compartment, namely the cytosol^{28,29} (where pH is close to neutrality) for *L. monocytogenes*, phagosomes and weakly acidic vacuoles for *L. pneumophila*,³⁰ and phagolysosomes and acidic vacuoles for *S. aureus* in macrophages.^{11,31} Interestingly enough, this is also seen for *S. aureus* in HUVECs, although bacterial localization in these cells involves both the cytosol and the phagolysosomes³² (confirmed in our experimental conditions).³³ This could explain the higher relative intracellular efficacy (lower E_{max}) observed in these cells compared with THP-1 macrophages.

Pending further investigations, the present study strongly suggests that the accumulation of torezolid is quantitatively offset by a commensurate decrease in its activity in the intracellular milieu. This surprising situation is actually not specific to oxazolidinones, as it exactly parallels what we observed earlier for fluoroquinolones, and moxifloxacin in particular (see data and discussions in Carryn *et al.*,¹² and in Seral *et al.*³⁴ and Barcia-Macay *et al.*⁷) in the same models of *L. monocytogenes*- and *S. aureus*-infected THP-1 cells. Thus, whereas moxifloxacin accumulates ~6- to 20-fold in these cells,³⁵ quantitative analysis shows that its relative potency is similar to that exerted against extracellular bacteria, as if the intracellular concentration of the bioavailable drug was limited to that of the extracellular concentration.

At this point, it nevertheless remains obvious that torezolid exerts intracellular antimicrobial activities that are superior to linezolid, in terms of relative potency, when used at the same weight concentration. This may lead to improved therapy of intracellular infections provided that the modifications made to obtain torezolid from the oxazolidinone scaffold do not adversely alter its safety profile and allow its use in patients under conditions creating extracellular concentrations similar to those of linezolid or, at least, maintaining a higher concentration/MIC ratio. Animal studies may be helpful to further substantiate this hypothesis, as they will be able to take into account a series of parameters that cannot be included in our model but are also critical, such as serum protein and tissue binding, inoculum effects, influence of the scheme of administration and host defences.

Acknowledgments

M. C. Cambier and C. Misson provided dedicated technical assistance. S. L. and F. V. B. are *Chargé de Recherches* and *Maître de Recherches* of the Belgian *Fonds de la Recherche Scientifique (F.R.S.-FNRS)*.

Funding

Belgian Fonds de la Recherche Scientifique Médicale (FRSM; grant no. 3.4.597.06) and a grant-in-aid from Trius Pharmaceuticals.

Transparency declarations

None to declare.

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