Torezolid (TR-700), a novel methyltetrazolyl-oxazolidinone, accumulates markedly within human THP-1 macrophages and shows activity towards intraphagocytic *Legionella pneumophila*: comparison with linezolid

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Abstract (revised)

Background. Treatment of intracellular infections requires that antibiotics reach their intracellular target and express activity in the intracellular environment. The aim of the present study was to examine the cellular pharmacokinetic properties and intracellular activity of TR-700 towards *L. pneumophila*, in view of the higher lipophilicity and intrinsic activity of this molecule in comparison with linezolid.

Methods. Human THP-1 macrophages and *L. pneumophila* strain ATCC 33153 were used throughout. Cellular accumulation of antibiotics were measured by microbiological assay. Susceptibility testings were determined in α-ketoglutarate Yeast Extract broth (pH 6.9, 48 h). Dose-effect relationships at 24 h were examined for concentrations from 0.01 to 100 x the MIC. Results, expressed as the change in the intracellular inoculum at 48 h compared to time 0, were used to fit a Hill equation to allow determination of the values of two key pharmacological descriptors of antibiotic activity (relative potency [EC_{50} or 50% effective concentration] and maximal relative efficacy [Emax]; see Barcia-Macay et al, AAC 50(3):841-51).

Results. TR-700 accumulated quickly and extensively within THP-1 macrophages, reaching an apparent cellular to extracellular concentration ratio of about within 15 min vs 1-2 for linezolid. Dose-effect relationships could be modeled using a sigmoidal function (Hill equation, $R^2 > 0.96$), and showed that (i) on a weight concentration basis (mg/L), torezolid was more potent (lower values for EC_{50} and static conc.) than linezolid against phagocytized *L. pneumophila*, with no change in values for maximal efficacy (E_{max}); (ii) on an equipotent concentration basis (multiples of MIC) no difference was seen between both antibiotics (see Figures and Tables in the poster)

Conclusions. Compared to linezolid, TR-700 shows an increased potency (lower static concentrations) towards intraphagocytic *L. pneumophila* probably in relation with its higher intrinsic activity (lower MIC values).

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Background and aim

Legionella pneumophila, the causative agent of the legionnaires's disease, is a facultative intracellular bacterium that easily invade and survive within human phagocytes (1-3). Treatment of such infections remains challenging since the activity of antibiotics may differ markedly between the extracellular and intracellular milieu.

In this context, our aim was to assess the cellular pharmaco-kinetic properties and intracellular activity of torezolid (TR-700, [4]), a novel oxazolidinone antibiotic, in view of its higher lipophilicity and intrinsic activity.

Methods

Cells. Experiments were performed with human THP-1 cells, a myelomonocytic cell line displaying macrophage-like activity.

Assay of cell-associated antibiotics. TR-700 and linezolid were assayed by the disc-plate method, using *S. aureus* ATCC 25923 as test organism.

Bacterial strain and susceptibility testing. *L. pneumophila* strain ATCC 33153 (Manassas, VA) was used thorough. MIC determinations were performed in α-ketoglutarate Buffered Yeast Extract broth (pH 6.9, 48 h).

Cell infection and determination of the intracellular activities of antibiotics. Phagocytosis was initiated at a bacteria per macrophage ratio of 10 (2 h at 37°C), followed by elimination of non-phagocytosed bacteria by exposing the cells to 50 mg/L gentamicin (30-45 min). Cells were then transferred to fresh medium supplemented with increasing concentrations of antibiotics for 48 h. Results, expressed as the change in the intracellular inoculum at 48 h compared to time 0, were used to fit a Hill equation to allow determination of the values of two key pharmacological descriptors of antibiotic activity (relative potency [EC_{50} or 50% effective concentration] and maximal relative efficacy [E_{max}]; see ref. 6 for a detailed description of the method).

References

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(4) Schaadt et al, Antimicrob Agents Chemother., 2009, 53: 3236-3239.
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Results

Cellular accumulation of torezolid vs. linezolid within THP-1 cells

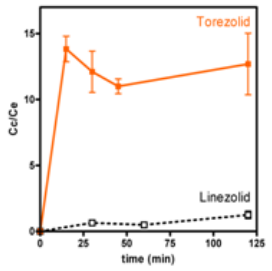

Figure 1. Kinetics of antibiotic uptake (extr. Concentration, 50 mg/L) within THP-1 macrophages. The ordinate (Cc/Ce) shows the apparent cellular to extracellular concentration ratio.

In contrast to linezolid, torezolid accumulates very quickly within THP-1 macrophages reaching Cc/Ce values close to ~ 15 within 15 min

Pertinent regression parameters of the dose-response curves illustrated in figure 2

Dose-effect relationship could be modeled using a sigmoidal function (Hill equation, $R^2 > 0.96$).

Condition	THP-1 macrophages			
	Emin[a]	*Emax*[b]	EC_{50}[c] (mg/L)	Static conc.[d]
Linezolid	0.8 ± 0.1 (a)	-1.2 ± 0.2 (a)	7.9 ± 1.6 (a) (~ 1.1 x MIC (a))	~ 5.5 mg/L (~ 0.7 x MIC)
Torezolid	**1.3 ± 0.2 (b)**	**-1.2 ± 0.1 (a)**	**0.2 ± 1.3 (b)** **(~ 0.7 x MIC (a))**	**~ 0.2 mg/L** **(~ 0.8 x MIC)**

[a] Increase in log CFU compared to time 0 for an infinitely low concentration in antibiotic (bact. growth)
[b] Decrease in log CFU compared to time 0 for an infinitely high concentration in antibiotic (bact. killing)
[c] Concentration (mg/L) causing a reduction of the inoculum halfway between E*0* and E*max*, as obtained by graphical intrapolation using the corresponding Hill equation

Statistical analysis for the differences between torezolid and linezolid: parameters with different letters are significantly different from each other ($p < 0.05$; analysis per column by one-way ANOVA with Tukey test for multiple comparisons)

MICs and intraphagocytic activities

Antibiotics	MIC (mg/L)
Linezolid	4-8
Torezolid	**0.25-0.5**

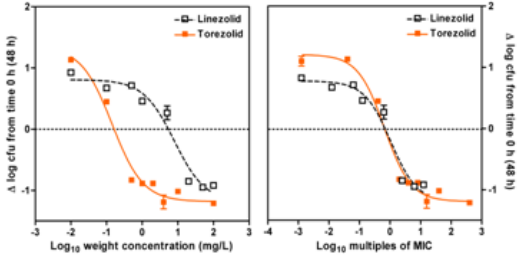

Figure 2. Concentration-killing effects of torezolid vs. linezolid towards L. pneumophila phagocytized by human THP-1 macrophages. The ordinate shows the change in cfu per mg of cell protein after 48 h compared with the original inoculum. Data are plotted against the weight concentration (mg/L; left-hand panel) or equipotent antibiotic concentrations (multiple of the MICs). All values are mean ± SD.

Torezolid is more potent than linezolid (lower EC*50* and static conc.) against phagocytized L. *pneumophila* when drugs are compared on a weight concentration basis (mg/L) but not when drugs are compared on a multiples of MIC basis. Relative efficacies (*Emax*) are similar.

Conclusions

Torezolid shows a higher cellular accumulation and an increased relative potency against intraphagocytic *L. pneumophila* when concentrations are expressed as a weight basis, but not as multiples of MIC. This suggests that the main driver of antibiotic activity for torezolid, in comparison with linezolid, is its higher intrinsic activity, and not its greater cellular accumulation *per se*. Torezolid should demonstrate greater potency against intracellular organisms *in vivo* if used under conditions creating similar serum concentrations than linezolid.

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