Poster A1-1937

49th ICAAC
San Francisco, CA, USA
September 12-15, 2009

Radezolid, a New Oxazolidinone, is Active Against Intraphagocytic *Staphylococcus aureus* with Various Resistance Phenotypes in a Model of THP-1 Human Macrophages.

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ABSTRACT (revised)

Background. Antibiotic resistance and intracellular survival are associated with recurrent or persistent *S. aureus* infections. Antibiotics acting on intracellular forms of resistant strains are therefore desirable. Radezolid (RDZ) is an investigational oxazolidinone, which, in contrast with linezolid (LZD), shows a large acid-pH driven cellular accumulation in THP-1 macrophages (Lemaire et al, ECCMID 2009, O29). We have now examined the intracellular activity of RDZ against a series of *S. aureus* strains harbouring clinically-relevant resistance mechanisms.

Methods. The phenotypes of the strains used are shown in the Table. Susceptibility was evaluated in Mueller Hinton broth, and intracellular activity determined against bacteria phagocytised by human THP-1 macrophages as previously described (Barcia-Macay et al, AAC, 2006). Dose-effect relationships at 24 h were examined for concentrations spanning from 0.01 to 100 x the MIC, and the results were expressed as change in the intracellular inoculum compared to time zero.

Results. MICs and intracellular activities at 24 h are shown in the table/figures. In time- dependent studies, RDZ reached its maximal effect against intracellular ATCC 25923 after only a 5 h drug exposure while LZD remained static during this period. In concentration-dependent studies, RDZ shows 5-20 fold higher relative potency, with the most marked difference with LZD being observed for LZD-resistant strains.

Conclusions. Compared to linezolid, radezolid shows lower MICs and higher intracellular relative potency (5-20 fold lower static concentrations) towards all tested *S. aureus* isolates, including LZD-resistant strains.

BACKGROUND AND AIM

Difficulty in eradicating staphylococcal infections has been ascribed to antibiotic resistance and intracellular survival. Antibiotics acting on intracellular forms of resistant strains may offer an advantage in this context.

Radezolid is a novel biaryloxazolidinone in Phase II of clinical development, characterized by:

- an improved interaction with the 50S ribosomal subunit, and, therefore, an enhanced activity, including against linezolid-resistant strains (1-2)
- an increased capacity to accumulate in eucaryotic cells (3 and poster A1-1936).

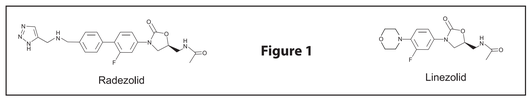

Figure 1

The aim of this study was to compare the intracellular activity of radezolid (RDZ) to that linezolid (LDZ) against a series of *S. aureus* strains displaying clinically-relevant resistance phenotypes.

RESULTS

Susceptibility testing

Strains	Phenotype	MICs (mg/L)	
		LZD	RDZ
ATCC 25923	MSSA	2	**0.25**
ATCC 33591	HA-MRSA	1-2	**0.5**
NRS 384	CA-MRSA	2	**0.5**
NRS 18	HA-MRSA & VISA	2	**0.25**
SA040	MSSA, LZD^S	2	**0.5-1**
SA040L	MSSA, LZD^R	16	**2**
SA238	MRSA, LZD^S	2	**0.5-1**
SA238L	MRSA, LZD^R	16	**2**

RDZ shows MIC values lower than LZD against all *S. aureus* tested, including linezolid-resistant organisms.

METHODS

Susceptibility testings. MICs were measured by microdilution in Mueller Hinton Broth.

Cell lines. Experiments were conducted with THP-1 cells (ATCC TIB-202, Manassas, VA), a human myelomonocytic cell line displaying macrophage-like activity (1).

Cell infection. Infection of THP-1 cells was performed exactly as described earlier for *S. aureus* strain ATCC 25923 (2-3). Typical post-phagocytosis inoculum was about 1-2 x 10^6 CFU/mg prot.

Assessment of intracellular activity of antibiotics. Activity was determined by measuring CFU per mg of cell lysates obtained from cells exposed over time to fixed concentrations of antibiotics or for fixed time (24 h) to increasing concentrations of antibiotic (0.01- to 100 mg/L). Results were expressed as the change in the inoculum at 24 h compared to time 0.

Data were analyzed by non-linear regression using Hill's equation to calculate pharmacological descriptors:

- E_{max}, maximal reduction of the intracellular inoculum [in $\log_{10}$ units] for an infinitely large antibiotic concentration;
- EC_{50}, antibiotic concentration [in mg/L] yielding a response half-way between E_0 and E_{max}, as obtained using the corresponding Hill equation, together with an estimation of the static concentration (C_{static}) calculated by graphical interpolation.

This poster will be made available for download after the meeting: http://www.facm.ucl.ac.be/posters.htm

ACKNOWLEDGEMENTS: We thank M.C. Cambier and C. Misson for technical assistance. S.L. and F.V.B. are Post-doctoral and Senior Research Associate of the Belgian Fonds de la Recherche Scientifique (FSR-FNRS). This work was supported by the Belgian Fonds de la Recherche Scientifique Médicale (FRSM) and by a grant-in-aid from Rib-X Pharmaceuticals Inc.

Intracellular activity of antibiotics

A) Influence of time

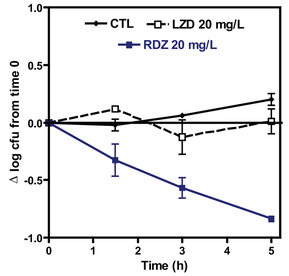

Fig. 2. Time-kill curve for linezolid and radezolid against a fully susceptible *S. aureus* strain (ATCC 25923) phagocytosed by human THP-1 macrophages. Cells were incubated with antibiotics for up to 5 h in the presence of a fixed extracellular concentration of oxazolidinone (20 mg/L) or 0.15 mg/L gentamicin (controls cells, to avoid extracellular bacterial growth and ensuing acidification of the culture medium).

Intracellularly, radezolid reached its maximal effect at 5 h while linezolid remained almost static towards *S. aureus* strain ATCC 25923

B) Influence of concentration

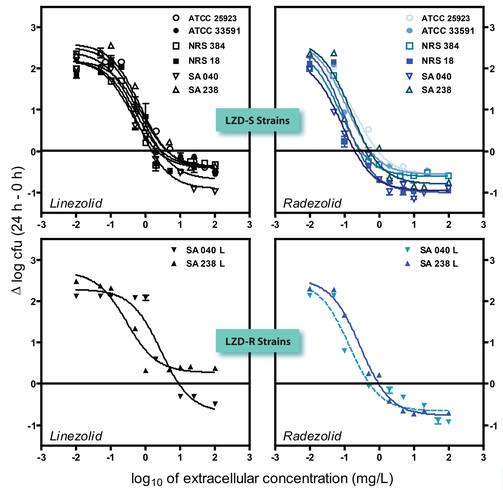

Fig. 3. Concentration-killing effect of linezolid vs. radezolid against linezolid-susceptible (upper panel) and linezolid-resistant (lower panel) *S. aureus* isolates phagocytosed by human THP-1 macrophages. Cells were incubated with antibiotics for 24 h in the presence of increasing concentrations of antibiotics.

C) Pertinent pharmacological descriptors as calculated by non-linear (sigmoid) regression of the dose-response curves illustrated in Fig. 3.

Strains	Linezolid		Radezolid		C_{static}
	E_{max} [a]	Static conc. [b] (mg/L)	E_{max} [a]	Static conc. [b] (mg/L)	Ratio LZD/RDZ
ATTC 25923	-0.4 ± 0.1	~ 4.3	**-0.6 ± 0.1**	**~ 1.0**	4.3
ATCC 33591	-0.4 ± 0.1	~ 3.0	**-0.6 ± 0.1**	**~ 0.6**	5.0
NRS 384	-0.4 ± 0.2	~ 2.3	**-0.6 ± 0.1**	**~ 0.3**	7.7
NRS 18	-0.7 ± 0.1	~ 2.8	**-1.0 ± 0.1**	**~ 0.3**	9.3
SA 040	-0.9 ± 0.1	~ 1.6	**-1.0 ± 0.1**	**~ 0.2**	8.0
SA 040 L	-0.7 ± 0.3	~ 9.6	**-0.7 ± 0.2**	**~ 0.5**	19.2
SA 238	-0.4 ± 0.2	~ 4.3	**-0.8 ± 0.2**	**~ 0.6**	5.3
SA 238 L	0.3 ± 0.1	N.A.	**-0.8 ± 0.1**	**~ 1.0**	> 100

[a] Maximal decrease in cfu compared to time 0 h (log scale)
[b] Extracellular concentration of antibiotic yielding no apparent change in cfu after 48 h compared to the post-phagocytosis inoculum, as determined by graphical interpolation
N.A., not applicable

Compared to LZD, radezolid shows higher intracellular relative potency, with static concentrations 5-10-fold lower than linezolid for linezolid-susceptible strains and at least 20-fold lower for linezolid-resistant strains .

CONCLUSIONS

- **Radezolid shows lower MICs than linezolid against *S. aureus* with clinically-relevant resistance mechanisms, including linezolid-resistant strains**
- **Radezolid shows significant greater antibacterial effect than linezolid towards intracellular *S. aureus*, as it faster reaches its maximal effect and shows increased intracellular relative potency towards both linezolid-susceptible and linezolid-resistant isolates.**

REFERENCES

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