Radezolid (RX-1741), a Novel Oxazolidinone, Accumulates Extensively within Human Macrophages and PMNs and Shows Activity towards Intracellular Linezolid-Sensitive and Linezolid-Resistant *Staphylococcus aureus*

Sandrine Lemaire,¹ Klaudia Kosowska-Shick,² Peter C. Appelbaum,² Paul M. Tulkens,¹ and Françoise Van Bambeke¹

¹ Unité de Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain
² Pennsylvania State Hershey Medical Center, Hershey, Pennsylvania
Intracellular S. aureus: is it important?

Intracellular *Staphylococcus aureus*. A mechanism for the indolence of osteomyelitis.

Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.

Phagocytosis of *Staphylococcus aureus* by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.

Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis.
Clement et al., J Infect Dis. (2005) 192:1023-8
S. aureus can also survive and multiply in phagocytic cells

PMN and macrophages: S. aureus found in vesicles
Intracellular vs extracellular activity of antibiotics: PK – PD in action

Activity of new antibiotics requires testing in appropriate models.
From Linezolid to Radezolid

**Linezolid**

**Radezolid**

- **Heteroaryl substituant**
- **Biaryl spacer**
- **2 protonable aminated functions**

**Designed and developed by Rib-X Pharmaceuticals**
Structure-based design of biaryl-oxazolidinones

- derived from observations made using the crystal structure of the 50S ribosomal unit complexed with known drugs and antibiotics
- combines the most important interactions defined by sparsomycin and linezolid into a single molecular design

additional interaction with A2602 and U2585 of the 50S ribosomal binding site

Structure-based design of radezolid

In vitro activity of radezolid

Improved intrinsic activity against several organisms, including those capable of surviving within eukaryotic cells

<table>
<thead>
<tr>
<th>bacteria</th>
<th>MICs in mg/L</th>
<th>Linezolid</th>
<th>Radezolid</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae ErmB</td>
<td>0.5-2</td>
<td>≤ 0.25</td>
<td></td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>2-4</td>
<td>0.03-0.125</td>
<td></td>
</tr>
<tr>
<td>E. faecalis &amp; faecium</td>
<td>1-16</td>
<td>≤ 0.25-4</td>
<td></td>
</tr>
<tr>
<td>H. influenzae</td>
<td>2-64</td>
<td>0.25-2</td>
<td></td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>2-16</td>
<td>≤ 0.25-1</td>
<td></td>
</tr>
<tr>
<td>S. aureus MSSA</td>
<td>2-4</td>
<td>0.5-4</td>
<td></td>
</tr>
<tr>
<td>S. aureus MRSA</td>
<td>2-8</td>
<td>≤ 0.25-8</td>
<td></td>
</tr>
<tr>
<td>L. pneumophila</td>
<td>4-16</td>
<td>1-4</td>
<td></td>
</tr>
<tr>
<td>C. trachomatis</td>
<td>8-16</td>
<td>0.5-1</td>
<td></td>
</tr>
</tbody>
</table>

Aim of the study

- to determine the cellular accumulation/distribution of radezolid in phagocytic cells (macrophages, PMN)

- to compare the intracellular activity of radezolid and linezolid against isogenic LZD-S and LZD-R strains of Staphylococcus aureus
Aim of the study

- pharmacokinetics

- to determine the cellular accumulation/distribution of radezolid in phagocytic cells (macrophages, PMN)
General methodology: cellular accumulation

THP-1 human macrophages

PMNs from human volunteers

• incubated with linezolid or $^{14}$C-radezolid
• washed in PBS and collected by low-speed centrifugation

• resuspended in water

cell prot. (Lowry)
drug

scintillation counting

microbiological assay ($B. subtilis$)

accumulation calculated considering a cell volume of 5 µl/mg prot.
Comparative accumulation level at equilibrium

⇒ THP-1 macrophages

![Bar chart showing cellular accumulation of LZD and RX-1741 at 250 mg/L and 50 mg/L, respectively.]

In contrast to linezolid, radezolid accumulates in eukaryotic cells!
Radezolid accumulation is concentration-independent

- non-saturable accumulation in human phagocytic cells
- no influence of efflux pump inhibitors (verapamil; gemfibrozil) (not shown)
Kinetics of radezolid accumulation and efflux

THP-1 macrophages

accumulation

efflux

T_{1/2}: 6 min

T_{1/2}: 20 min

Radezolid

LNZ

rapid accumulation and slightly slower efflux
Kinetics of radezolid accumulation and efflux

PMNs

Rapid accumulation and slightly slower efflux

T_{1/2}: 5 min

T_{1/2}: 10 min
Subcellular localization of radezolid

J774 mouse macrophages

low speed centrifugation

nuclei / unbroken cells

extract

high speed centrifugation

soluble fraction

sedimentable fraction

lacticodehydrogenase (LDH)

N-acetyl-β-glucosaminidase (NAB)

cytochrome C oxydase (CytOx)

lysosome

mitochondria

N-acetyl-β-glucosaminidase

lacticodehydrogenase

cytochrome C oxydase

lysosome

mitochondria
Subcellular localization of radezolid

**J774 mouse macrophages**

- **Low speed centrifugation**: nuclei / unbroken cells
- **Extract**: soluble fraction
- **High speed centrifugation**: sedimentable fraction

<table>
<thead>
<tr>
<th></th>
<th>Low Speed Centrifugation</th>
<th>High Speed Centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radezolid</td>
<td>58.2 %</td>
<td>41.8 %</td>
</tr>
<tr>
<td>NAB</td>
<td>10.6 %</td>
<td>89.4 %</td>
</tr>
<tr>
<td>CytOx</td>
<td>1.7 %</td>
<td>98.3 %</td>
</tr>
<tr>
<td>LDH</td>
<td>97.0 %</td>
<td>3.0 %</td>
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**Dual subcellular localization**
- Cytosol
- Organelles

**Enzymes**
- Lactic dehydrogenase (LDH)
- N-Acetyl-β-glucosaminidase (NAB)
- Cytochrome C oxidase (CytOx)
Subcellular localization of radezolid

soluble fraction  →  sedimentable fraction

high speed centrifugation on sucrose gradient

NAB  CytOx  RDZ

density

ΔQ/Δρ

1.12  1.14  1.16  1.18  1.20

N-acetyl-β-glucosaminidase (NAB)

lacticodehydrogenase (LDH)

cytochrome C oxidase (CytOx)

mitochondria

lysosome

cytosol

lysosomes

dual subcellular localization

20-06-2009 26th ICC
Aim of the study

- pharmacodynamics

- to compare the intracellular activity of radezolid and linezolid against isogenic LZD-S and LZD-R strains of *Staphylococcus aureus* in THP-1 macrophages
General methodology: intracellular activity

THP-1 human macrophages

- infected by pre-opsonized S. aureus
- extracellular bacteria eliminated by short incubation with gentamicin
- incubation with increasing concentrations of antibiotics for 24 h
- washings

- resuspended in water

- cell prot. (Lowry)
- plating and CFU counting

activity expressed as change from initial inoculum

Bacterial strains under study

<table>
<thead>
<tr>
<th>strain</th>
<th>phenotype</th>
<th>MIC pH 7.4 (mg/L)</th>
<th>MIC pH 5.5 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>linezolid</td>
<td>radezolid</td>
</tr>
<tr>
<td>SA 238</td>
<td>LZD-S</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>SA 238L</td>
<td>LZD-R</td>
<td>16</td>
<td>2</td>
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• at neutral pH, radezolid is ~equipotent against the LZD-R strain
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<td>16</td>
<td>2</td>
</tr>
</tbody>
</table>

- at neutral pH, radezolid is ~equipotent against the LZD-R strain
- at acidic pH, radezolid activity is reduced
Intracellular activity (clinically-relevant comparison)

<table>
<thead>
<tr>
<th>drug</th>
<th>SA 238</th>
<th>SA 238L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{\text{static}}$</td>
<td>$E_{\text{max}}$</td>
</tr>
<tr>
<td>linezolid</td>
<td>5.8</td>
<td>-0.3</td>
</tr>
<tr>
<td>radezolid</td>
<td>0.5</td>
<td>-0.6</td>
</tr>
</tbody>
</table>

Radezolid is
- more potent and effective than LZD
- as potent and effective against both strains
Intracellular activity (equipotent concentrations)

- **Radezolid** and \( \text{LZD} \) ~ equipotent against the \( \text{LZD-S} \) strain
- **Radezolid** ~ equipotent against both strains

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<th>SA 238L</th>
</tr>
</thead>
<tbody>
<tr>
<td>linezolid</td>
<td>2.1</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>radezolid</td>
<td>1.1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

- \( \Delta \log \text{CFU from time 0} \)
- \( \text{Log extracell. conc.} \times \text{MIC [pH 7.4]} \)
Intracellular activity (x MIC pH 5.5)

SA 238

SA 238L

\[
\text{Log extracell. conc. (x MIC [pH 5.5])}
\]

\[
\Delta \log \text{CFU from time 0}
\]

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<td>linezolid</td>
<td>1.1</td>
<td>&gt; 10</td>
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<tr>
<td>radezolid</td>
<td>0.1</td>
<td>0.1</td>
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- Radezolid is active at lower multiples of the assumed MIC in the infected compartment.
Intracellular activity (pharmacological comparison)

<table>
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<tr>
<th>drug</th>
<th>SA 238</th>
<th>SA 238L</th>
</tr>
</thead>
<tbody>
<tr>
<td>linezolid</td>
<td>1.9</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>radezolid</td>
<td>0.7</td>
<td>0.6</td>
</tr>
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- cellular accumulation of Radezolid compensates for the effect of acid pH on activity
Summary

 Hemisphere pharmacokinetics

- \textit{radezolid} accumulates
  - \sim 10\text{-}fold in human macrophages and PMNs
  - quickly, reversibly
  - independently of extracellular concentration
  - in both cytosolic and lysosomal compartments

Hemisphere pharmacodynamics

- as compared to \textit{linezolid}, \textit{radezolid} shows
  - a higher efficacy (improved $E_{\text{max}}$)
  - a higher potency (lower $C_{\text{static}}$)

- \textit{radezolid} proves equipotent against LZD\text{-}S and LZD\text{-}R strains