Intracellular Activity of Antibiotics against Methicillin-Sensitive, Hospital-Acquired Methicillin-Resistant, and Vancomycin-Intermediate Staphylococcus aureus (MSSA, HA-MRSA, and VISA) in Human Macrophages (THP-1)

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ABSTRACT

Background: Relapsing and chronic S. aureus infections have been ascribed to intraphagocytic bacterial persistence (resulting also in emergence of resistance). The activity of antibiotics against S. aureus markedly differs between the extracellular and intracellular milieu (Barcia-Macay et al. Antimicrob Agents Chemother. 2006;50:841-851). Intracellular activity, therefore, needs to be assessed in specific models. We have examined the activity of antibiotics commonly used for the treatment of S. aureus infections (oxacillin, vancomycin, linezolid, and rifampicin) in comparison with quinupristin-dalfopristin (30:70; [Synercid]), moxifloxacin, daptomycin (both recently approved for skin and soft-tissue infections), and telavancin (a lipoglycopeptide with multiple modes of action, under clinical development) in macrophages.

Methods: MSSA (ATCC 25923), HA-MRSA (ATCC 35391), and VISA (NRS23) were used. MICs were determined by broth microdilution method. Infection of THP-1 macrophages was obtained as described by Barcia-Macay and colleagues (Antimicrob Agents Chemother. 2006;50:841-851), and the intracellular localization of the bacteria was assessed by confocal and electron microscopy. Activity was measured after 24 hours of exposure to a drug concentration corresponding to the Cmax (control cells: gentamicin [0.5 x MIC] to prevent extracellular growth).

Results: Phagocytosed bacteria were unambiguously seen in membrane-bounded vacuoles (phagolysosomes) in controls. MICs and intracellular activities are shown in the table.

Conclusions: With the exception of rifampicin, the antibiotics commonly recommended for the management of S. aureus infections only poorly eradicate intracellular forms, but this can be improved with new molecules.

METHODS

1. SUSCEPTIBILITY TESTING

Susceptibility testing was performed by broth microdilution method in Mueller-Hinton broth.

2. INTRACELLULAR INFECTION

Cells were infected with preopsonized bacteria (1 hour; 37°C), washed with phosphate-buffered saline, and incubated for 45 minutes with gentamicin (50 mg/L) to eliminate noninternalized bacteria. Electron microscopy was performed as described by Ouadhiri and colleagues (Antimicrob Agents Chemother. 1999;43:1242-1251).

3. DETERMINATION OF INTRACELLULAR ANTIBIOTIC ACTIVITY


Infected cells were exposed for 24 hours to antibiotics at a concentration corresponding to the Cmax reached in patients treated with conventional dosages (control cells were maintained in the continuous presence of gentamicin [0.5 x MIC] to prevent extracellular growth of bacteria released from dead cells).

RESULTS

1 Susceptibility Testing

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Abbreviation</th>
<th>MSSA (µg/L)</th>
<th>MRSA (µg/L)</th>
<th>VISA (µg/L)</th>
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</thead>
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<tr>
<td>Oxacillin</td>
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<td>Vancomycin</td>
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<tr>
<td>Linezolid</td>
<td>LZD</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>RIF</td>
<td>0.03</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>DAP</td>
<td>0.12</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>Quinupristin</td>
<td></td>
<td></td>
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<tr>
<td>Dallopristin</td>
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</tr>
<tr>
<td>Moxifloxacin</td>
<td>MXF</td>
<td>0.06</td>
<td>0.125</td>
<td>2</td>
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<tr>
<td>Telavancin</td>
<td>TLV</td>
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</tr>
</tbody>
</table>

‡ ATCC 25923.

* ATCC 35391 (HA-MRSA).

** NRS23.

2 Electron Microscopy

After 24 hours cell-associated S. aureus appears clearly confined within membrane-bounded vacuoles, regardless of resistance phenotype.

3 Comparative Intracellular Activity Of Antibiotics

The ordinate shows the change in cfu (log10) per mg of cell protein observed after 24 hours of incubation, in comparison with the original inocula (mean ± SEM [n=3]), in cells incubated with a concentration of drug equivalent to their human Cmax (in µg/L: OXA, 8; VAN, 50; LZD, 20; RIF, 4; DAP, 77; Q-D, 10; MXF, 4; and TLV, 90).

CONCLUSIONS

• OXA, VAN, and LZD (commonly recommended for the management of S. aureus infections) are only poorly active against intracellular S. aureus (−1 log cfu decrease).

• In contrast, RIF, DAP, Q-D, MXF, and TLV display more intense intracellular activity against intracellular MSSA, with lower but still highly significant activity against intracellular forms of resistant bacteria (MRSA and VISA).

BACKGROUND

S. aureus can cause chronic and relapsing infections, probably because of its ability to survive and multiply within eukaryotic cells (Lowy. Trends Microbiol. 2000;8:341-343). Appropriate treatment should, therefore, aim at eradicating intracellular forms of infection. However, antibiotics are routinely evaluated under clinical development) in macrophages.

AIMS OF THE STUDY

• To develop a 24-hour model of intracellular infection with S. aureus using various susceptibility phenotypes (MSSA, MRSA, and VISA) in human THP-1 macrophages.

• To compare the intracellular activities of commonly recommended antibiotics and newly developed anti–gram-positive intracellular molecules at an extracellular concentration corresponding to the level observed in patients receiving conventional dosages.