In Vitro and In Vivo Assessment of Linezolid Combined with Ertapenem: a Highly Synergistic Combination against Methicillin-Resistant Staphylococcus aureus

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The latest additions to the armory for treating antibacterial infections have included ertapenem, a new carbapenem, and linezolid, an oxazolidinone drug. Ertapenem is a parenteral carbapenem antibiotic with a broad antibacterial spectrum and once-a-day dosing that is supported by clinical studies and an extended half-life (19). Linezolid is particularly effective against gram-positive infections, even among methicillin- and vancomycin-resistant bacteria, and offers a valuable alternative to more standard therapies, such as vancomycin. A synergy of linezolid combined with imipenem was reported, both in vitro and in vivo, for a Staphylococcus aureus endocarditis experimental model (8). The present study used in vitro methods and an animal experimental model to evaluate the antibacterial activity of linezolid combined with ertapenem.

We studied three methicillin-resistant S. aureus (MRSA) strains, two of which were isolated from blood cultures (BCB8 and P9) and one of which was an S. aureus reference strain (COL). The MICs of linezolid and ertapenem were determined in Mueller-Hinton (MH) broth (1, 13). The dynamic checkerboard method was performed in 96-well microtiter plates (Nalge Nunc International, Roskilde, Denmark) containing linezolid combined with ertapenem in twofold dilutions dispensed in a checkerboard fashion, with a final inoculum of about 10^7 CFU/ml. The procedure used was described previously (5, 7). Time-kill studies were performed in MH broth in glass flasks and used an inoculum of 5.10^6 to 10^7 CFU/ml in the presence of either a single antibiotic or a combination of both (14). Surviving bacteria were counted after 0, 6, and 24 h of incubation at 37°C by subculturing 50-μl serial dilutions (in 0.9% sodium chloride) on MH plates, using a spiral plater (Spiral System; Interscience, Saint-Nom-La-Bretèche, France). The detection limit was 20 CFU/ml.

Linezolid pharmacokinetic simulation was performed as previously validated (9). For ertapenem, blood samples were taken from three healthy rabbits after administration of an ertapenem bolus of 60 mg/kg of body weight in order to determine the spontaneous drug kinetics. Simulation was intended to provide apparent pharmacokinetic parameters close to those observed in healthy volunteers after a single 1-g bolus (ca. 16.67 mg/kg), as follows: mean half-life (T1/2), 3.8 to 5.2 h; peak concentration (Cmax), 154.9 ± 22.0 mg/liter; and area under the curve, 572.1 ± 68.6 to 781.5 ± 95.9 mg · h/liter (11, 12). A total dose of 40 mg/kg needed to be infused into the rabbits over a 24-h period in order to simulate human serum kinetics after a 16.67-mg/kg dose (i.e., 1 g once daily) (2).

Experimental endocarditis was induced with an inoculum of 10^8 CFU of S. aureus (6, 16) and was approved by the Committee of Animal Ethics of the University of Nantes. For each MRSA strain, animals were randomly assigned to receive either no treatment (controls), a linezolid regimen mimicking the human dose of 10 mg/kg/12 h, an ertapenem regimen mimicking the human dose of 1 g/day, or a combination of both regimens. Animals were euthanized at the beginning of the treatment period (controls) or at the end of the 4-day regimen. Aortic valve vegetations were excised, placed immediately on ice, and then weighed, homogenized in 0.5 ml of saline buffer, and plated on MH plates using a spiral system. Dilutions were performed to eliminate potential carryover effects. The lower detection limit for this method is 1 CFU per 50 μl of undiluted vegetation homogenate.

High-performance liquid chromatography was used to determine the concentrations of linezolid (15) and ertapenem (12) (lower detection limits, 0.1 and 0.2 mg/liter, respectively, with a coefficient of variation of <10% for both drugs). Statistical analyses were performed with StatView software (Abacus Concepts, Berkeley, CA). For each strain studied, analysis of variance was used to compare the effects between the different groups, followed by Scheffe’s test to compare

TABLE 1. MICs of linezolid and ertapenem for MRSA strains

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>BCBS</th>
<th>P9</th>
<th>COL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>8</td>
<td>256</td>
<td>256</td>
</tr>
</tbody>
</table>
treated groups two by two. *P* values of *H*0.05 were considered significant.

The MICs for *S. aureus* strains are summarized in Table 1. The results of the dynamic checkerboard method obtained for strains BCB8 and COL at 24 h are shown in Fig. 1. Synergy was observed with subinhibitory concentrations of ertapenem and with linezolid concentrations of about two to eight times the MIC for both strains. The results of time-kill studies for the BCB8 and COL strains are shown in Fig. 2. Linezolid at four times the MIC exhibited synergy with an ertapenem concentration of 1/128 the MIC for the BCB8 strain (3-log10 CFU/ml decrease compared to the most active single antibiotic) and the COL strain (2-log10 CFU/ml decrease).

The corresponding mean peak concentration, area under the curve, and half-life after simulating a 1-g/day dose of ertapenem in animals were 166.4 ± 18.4 mg/liter, 1,127.1 ± 318.3 mg · h/liter, and 4.3 ± 1.1 h, respectively. For linezolid, the corresponding mean *C*max area under the curve, and *T*1/2 were 11.9 ± 1.1 mg/liter, 76.3 ± 5.9 mg · h/liter, and 2.7 ± 0.1 h, respectively, at the first dose and 19.6 ± 1.1 mg/liter, 136.9 ± 8.9 mg · h/liter, and 3.3 ± 0.6 h, respectively, on day 4.

The in vivo results for experimental endocarditis are shown in Table 2. For all strains, linezolid exhibited no bactericidal effect versus controls, and the ertapenem regimen showed no activity. However, the combination exhibited a highly bactericidal and synergistic activity against the three MRSA strains, with a 5-log10 CFU/g decrease versus controls.

Experimental animal studies have investigated the activities of linezolid combined with different antibiotics, such as vancomycin (3), rifampin (4), and gentamicin (10). Among these reports, only linezolid plus gentamicin showed bactericidal activity against MRSA strains, but the use of this combination is limited to gentamicin-susceptible strains. We previously reported the in vitro and in vivo synergistic activities of linezolid combined with subinhibitory concentrations of imipenem against MRSA strains (8). The once-daily administration of ertapenem and the possibility of switching from an intravenous to an oral formulation for linezolid strengthened the potential for home

**FIG. 1.** Results of dynamic checkerboard method at 24 h for the BCB8 and COL strains. Concentrations of linezolid were as follows: ○, 0; □, one-eighth the MIC; ◻, one-fourth the MIC; △, one-half the MIC; ●, 1× the MIC; ■, 2× the MIC; ◤, 4× the MIC; ▲, 8× the MIC. Each curve represents a fixed concentration of linezolid combined with different concentrations of ertapenem.

**FIG. 2.** Killing curves for linezolid alone and in combination with ertapenem against BCB8 (solid symbols) and COL (open symbols). Circles, control; diamonds, linezolid at 4× the MIC; squares, ertapenem at 1/128 the MIC; triangles, linezolid at 4× the MIC plus ertapenem at 1/128 the MIC.

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**TABLE 2.** Bacterial titers in vegetations after 4 days of treatment

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Mean log10 CFU/g of vegetation ± SD (no. of sterile vegetations/total no. of vegetations)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCB8</td>
</tr>
<tr>
<td>Control</td>
<td>8.7 ± 0.9 (0/7)</td>
</tr>
<tr>
<td>Linezolid (10 mg/kg every 12 h)</td>
<td>6.9 ± 0.7 (0/6)</td>
</tr>
<tr>
<td>Ertapenem (1 g/day)</td>
<td>8.0 ± 0.2 (0/4)</td>
</tr>
<tr>
<td>Linezolid plus ertapenem (10 mg/kg every 12 h)</td>
<td>2.6 ± 0.3 (0/6)</td>
</tr>
<tr>
<td></td>
<td>P9</td>
</tr>
<tr>
<td>Control</td>
<td>9.1 ± 0.4 (0/6)</td>
</tr>
<tr>
<td>Linezolid (10 mg/kg every 12 h)</td>
<td>7.3 ± 0.8 (0/6)</td>
</tr>
<tr>
<td>Ertapenem (1 g/day)</td>
<td>9.9 ± 0.4 (0/4)</td>
</tr>
<tr>
<td>Linezolid plus ertapenem (10 mg/kg every 12 h)</td>
<td>2.7 ± 0.6 (0/6)</td>
</tr>
<tr>
<td></td>
<td>COL</td>
</tr>
<tr>
<td>Control</td>
<td>9.6 ± 0.5 (0/6)</td>
</tr>
<tr>
<td>Linezolid (10 mg/kg every 12 h)</td>
<td>6.9 ± 0.9 (0/6)</td>
</tr>
<tr>
<td>Ertapenem (1 g/day)</td>
<td>10.1 ± 0.1 (0/4)</td>
</tr>
<tr>
<td>Linezolid plus ertapenem (10 mg/kg every 12 h)</td>
<td>3.6 ± 0.8 (0/6)</td>
</tr>
</tbody>
</table>

*P* < 0.0001 versus controls.

*P* < 0.0001 versus linezolid and ertapenem treatment by Scheffe’s test after analysis of variance.

Simulated dose for humans.
therapy of this combination and reinforced the interest in combining linezolid with this new carbapenem.

As previously observed with imipenem, synergy was achieved in vitro with subinhibitory concentrations of ertapenem. The use of higher concentrations seemed to decrease the antibacterial activity, as observed with the COL strain (Fig. 1), which suggests a slight antagonism between the two drugs under these conditions. The same results were obtained with the P9 strain (data not shown).

The moderate in vivo activity of linezolid alone confirmed the need for a bactericidal combination. No in vivo activity was observed with the use of ertapenem alone, as expected, due to alterations in penicillin-binding proteins of the MRSA strains. The addition of ertapenem to linezolid therapy exhibited highly synergistic and bactericidal activities against the three MRSA strains. Although the combination showed similar in vivo activities against the MRSA strains, linezolid plus ertapenem was able to sterilize about 33, 83, and 100% of vegetative forms for the COL, P9, and BC88 strains, respectively. The low rate of sterilization observed with the COL strain could be explained by the high counts of bacteria observed in the control group. Regarding the MICs of ertapenem for MRSA strains, subinhibitory concentrations are achievable in human clinical practice with standard dosage (i.e., 1 g once daily). The optimal linezolid concentrations (2 to 8× the MIC) are achievable in clinical use for 60 to 70% of the time interval.

The practice of administering intravenous antimicrobial therapy in the home and alternate care settings has grown rapidly since it was first described in 1974 by Rucker and Garrison (17). The increased use of outpatient parenteral antimicrobial therapy can be explained by a variety of factors, including the push for cost containment, the development of antimicrobial agents that can be administered once daily or by the oral route, and technical advances in vascular access and infusion devices (18). According to these data, the linezolid plus ertapenem combination seems to be a reasonable choice with potential interest for use in outpatient parenteral antimicrobial therapy.

In conclusion, confirming the in vitro data, the linezolid plus ertapenem combination exhibited a highly bactericidal and synergistic activity in vivo against three MRSA strains after 4 days of treatment. In addition, this combination could help to open new therapeutic avenues in the field of severe gram-positive bacterial infections, notably as an option for outpatient parenteral antimicrobial therapy.

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REFERENCES


