Activities of Ertapenem, a New Long-Acting Carbapenem, against Penicillin-Sensitive or -Resistant Pneumococci in Experimental Meningitis

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The penetration of ertapenem, a new carbapenem with a long half-life, reached 7.1 and 2.4% into inflamed and noninflamed meninges, respectively. Ertapenem had excellent antibacterial activity in the treatment of experimental meningitis due to penicillin-sensitive and -resistant pneumococci, leading to a decrease of 0.69 ± 0.17 and 0.59 ± 0.22 log10 CFU/ml·h, respectively, in the viable cell counts in the cerebrospinal fluid. The efficacy of ertapenem was comparable to that of standard regimens (ceftriaxone monotherapy against the penicillin-sensitive strain and ceftriaxone combined with vancomycin against the penicillin-resistant strain). In vitro, ertapenem in concentrations above the MIC was highly bactericidal against both strains. Even against a penicillin- and quinolone-resistant mutant, ertapenem had similar bactericidal activity in vitro.

The continuous global increase and spread of resistant pneumococci has jeopardized the treatment of pneumococcal infections (3). Furthermore, additional resistance to cephalosporins has limited the therapeutic options against penicillin-resistant isolates. Nevertheless, β-lactam antibiotics remain the first-line drugs for pneumococcal infections, except when penetration into infected tissues is compromised, as in meningitis. Currently the standard regimen for meningitis due to penicillin-resistant strains is the combination of an extended-spectrum cephalosporin with vancomycin (3, 12).

In addition, pneumococcal strains resistant to quinolones have been isolated, limiting the choice of alternative therapies (4). A regimen based on monotherapy would represent a clear advantage, especially when quinolone-resistant strains are suspected.

Ertapenem is a new carbapenem with a long half-life and activity against the majority of human bacterial pathogens, including penicillin-resistant pneumococci (11, 16, 17). On the other hand, little is known about the penetration and kinetics of ertapenem into the cerebrospinal fluid (CSF) and its efficacy in pneumococcal meningitis. The aim of this study was to investigate the penetration of ertapenem into inflamed and noninflamed meninges and to test its bactericidal properties against a penicillin-sensitive strain and a penicillin-resistant strain in experimental pneumococcal meningitis.

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Measurement of antibiotic levels in CSF. Antibiotic concentrations in serum and CSF were determined by bioassay with the agar diffusion method. For serum levels, standard curves were determined in rabbit serum and in saline with 5% rabbit serum in order to mimic CSF protein concentration (15). Bacillus subtilis (ATCC 6633) was used as test strain for ertapenem (20). The intra- and interday variability of this method was less than 10%. The limit of detection was 0.1 mg/liter for ertapenem.

In vitro assays. The pneumococcal strains (a penicillin-sensitive strain and a penicillin-resistant strain) were grown in C+Y medium (13) to an optical density of 0.3 at 590 nm and then diluted 40-fold to 10^6 CFU/ml, corresponding approximately to the CSF bacterial titer in rabbits before the initiation of therapy. Ertapenem was added in concentrations corresponding to 1, 5, and 10 times the MIC (0.03, 0.15, and 0.3 mg/mliter for the penicillin-sensitive strain and 0.5, 2.5, and 5 mg/mliter for the penicillin-resistant strain). Bacterial titers were determined at 0, 2, 4, 6, and 8 h by serial dilution of samples plated on agar plates containing 5% sheep blood and incubated at 37°C for 24 h. Experiments were performed in triplicate, and results were expressed as means ± standard deviations.

Pharmacokinetic (PK) analysis. A zero-order input (bolus injection), first-order elimination, and expanded three-compartment model is used to describe the time course of serum and CSF drug concentrations. The three-compartment model consists of a central compartment (serum), which is linked to a peripheral compartment, and the CSF compartment (5, 18). Influx and efflux clearance in the CSF compartment are modeled as \( CL_{in,CSF} = CL_{in} = CL_{in} + CL_{out} + CL_{metab} \), where \( CL_{in} \) is the total clearance from the central compartment to the CSF compartment (in milliliters/minute) and is assumed to be equal to \( CL_{in,CSF} \), the passive transcellular diffusion clearance (i.e., the active influx clearance is absent). \( CL_{out} \) is the total clearance from the CSF compartment to the central compartment (in milliliters/minute). \( CL_{metab} \) is the clearance by CSF bulk flow, or active efflux (14). \( CL_{in,CSF} \), the clearance by drug metabolism in the CSF, is assumed to be negligible (14). \( P_{ic} \) equals the CSF-to-serum area under the concentration-time curve ratio and is a measure of CSF penetration.

The population kinetic model was fit to all measured concentrations of ertapenem in serum and CSF from all rabbits by using ADVAN5 TRANS1 and the FOCE method of the computer program NONMEM (nonlinear mixed effects modeling; NONMEM user’s guide, NONMEM Project Group, University of California at San Francisco, San Francisco) (1). The NONMEM output, when the program is used to fit a population model, among other things, consists of the value of the objective function at convergence (approximately minus twice the maximized log likelihood of the data). This can be used to test the merit of a value of the objective function at convergence (approximately minus twice the maximized log likelihood of the data). This can be used to test the merit of a value of the objective function at convergence (approximately minus twice the maximized log likelihood of the data). This can be used to test the merit of a value of the objective function at convergence (approximately minus twice the maximized log likelihood of the data).

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RESULTS

Figure 1 shows the kinetics of ertapenem after one dose of 60 mg/kg of body weight. The peak serum level ranged around 70 mg/liter, declining slowly to 6.5 mg/liter 8 h later. The peak CSF level around 3.4 mg/liter appeared 2 h after intravenous injection, decreasing slowly to 1.5 mg/liter at the end of the treatment period. Ertapenem levels remained above the MIC (0.03 and 0.5 mg/liter for the penicillin-sensitive and -resistant strains, respectively) during the entire treatment period. The CSF/MIC ratios ranged between 6.8 and 50 for the penicillin-sensitive strain and between 113 and 50 for the penicillin-resistant strain.

Table 1 presents the estimates of the population parameters. The population average of passive diffusion clearance was increased by 2.9 in rabbits with meningitis compared to rabbits without meningitis. The population average value for CSF penetration in rabbits with meningitis was 7.1%. Using the NONMEM computer program, the expanded three-compartment model for ertapenem kinetics demonstrates excellent goodness of fit (Fig. 2).

Predicted and measured ertapenem concentrations in serum and CSF agreed reasonably well by using population mean PK parameters \( R^2 = 0.91, P < 0.0001 \) [serum]; \( R^2 = 0.72, P < 0.0001 \) [CSF]]. The agreement between predicted and measured concentrations in serum and CSF was excellent when using individual PK parameters \( R^2 = 0.97, P < 0.0001 \) [serum]; \( R^2 = 0.95, P < 0.0001 \) [CSF]].

The efficacies of the different treatment groups are summarized in Table 2. In untreated controls, the bacterial titers
increased slowly over 8 h (+0.30 ± 0.06 log_{10} CFU/ml). Before the initiation of treatment, the initial bacterial titer did not differ significantly between all treatment groups. Against the penicillin-sensitive strain, ertapenem was highly bactericidal in comparison to the standard regimen (ceftriaxone monotherapy), and it sterilized the CSF of all rabbits. Against the penicillin-resistant strain, ertapenem was also highly efficacious (~0.59 ± 0.22 log_{10} CFU/ml), producing killing rates

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Mean</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central volume of distribution (ml) (V_s)</td>
<td>1,170</td>
<td></td>
</tr>
<tr>
<td>Peripheral volume of distribution (ml) (V_p)</td>
<td>1,630</td>
<td>16%</td>
</tr>
<tr>
<td>Intercompartmental clearance between V_s and V_p (ml/min)</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>Total clearance (ml/min)</td>
<td>12.6</td>
<td>24</td>
</tr>
<tr>
<td>CSF-serum barrier transfer parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive diffusion clearance (CL_{diff}) (ml/min) in rabbits with (without) meningitis</td>
<td>0.00084 (0.00029)</td>
<td>49</td>
</tr>
<tr>
<td>Clearance by bulk flow (CL_{bulk}) (ml/min)</td>
<td>0.0116</td>
<td>58</td>
</tr>
<tr>
<td>CSF penetration (%) in rabbits with (without) meningitis</td>
<td>7.1 (2.4)</td>
<td></td>
</tr>
</tbody>
</table>

* CV, interindividual variability shown as a percentage.
* Fixed CV.
* Clearance by active efflux and or bulk flow.
* CSF penetration, calculated as the ratio CL_{in}/CL_{out}, where CL_{out} = CL_{diff} + CL_{bulk}. 

FIG. 2. Goodness-of-fit plots of the three-compartment PK model for ertapenem. The upper panels show measured serum concentrations (CONC) versus population and individual predictions (PRED and IPRED, respectively). The lower panels show measured CSF concentrations versus PRED and IPRED. Each solid line in the left panels represents one animal. Fine dashed lines are the lines of identity. The heavy dashed line represents the smoothness of measured concentrations versus PRED and IPRED, respectively.
TABLE 2. Activities of single drug and combination therapies against penicillin-sensitive and penicillin-resistant Streptococcus pneumoniae in experimental meningitis

<table>
<thead>
<tr>
<th>Antibiotic (infecting strain susceptibility)*</th>
<th>n</th>
<th>Mean initial titer ± SD (log10 CFU/ml)</th>
<th>Mean killing rate ± SD (Δlog10 CFU/ml/hr)</th>
<th>Mean killing rate/8 h ± SD (log10 CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (controls) (Pen+)</td>
<td>4</td>
<td>6.02 ± 0.49</td>
<td>+0.08 ± 0.15*a</td>
<td>+0.30 ± 0.08*b</td>
</tr>
<tr>
<td>Ertapenem (Pen+)</td>
<td>10</td>
<td>5.12 ± 0.10</td>
<td>−0.69 ± 0.17*</td>
<td>−5.63 ± 0.97*</td>
</tr>
<tr>
<td>Ceftriaxone (Pen+)</td>
<td>4</td>
<td>5.50 ± 0.63</td>
<td>−0.80 ± 0.25*</td>
<td>−5.65 ± 0.72*</td>
</tr>
<tr>
<td>None (controls) (Pen+)</td>
<td>4</td>
<td>6.35 ± 0.61</td>
<td>+0.10 ± 0.06</td>
<td>+0.35 ± 0.32</td>
</tr>
<tr>
<td>Ertapenem (Pen+)</td>
<td>10</td>
<td>5.30 ± 0.48</td>
<td>−0.59 ± 0.22*</td>
<td>−4.60 ± 1.10*</td>
</tr>
<tr>
<td>Ceftriaxone + vancomycin (Pen+)</td>
<td>4</td>
<td>5.80 ± 0.73</td>
<td>−0.50 ± 0.20*</td>
<td>−4.05 ± 0.68*</td>
</tr>
</tbody>
</table>

*a Pen+, penicillin sensitive; Pen−, penicillin resistant.
*b P < 0.05 versus all groups.
*c P not significant.

In conclusion, the sufficient penetration into inflamed meninges in rabbits, the antimicrobial spectrum against common meningeal pathogens, and the efficacy against penicillin-sensitive and -resistant pneumococci might qualify ertapenem in humans; after 8 h, 6.5 mg/liter in rabbits versus 9.5 mg/liter in humans) (Fig. 1B). As the recommended dose in humans is 1 g once a day, the data reported in this model are likely to represent the minimum effectiveness possible.

As expected with a β-lactam agent, inflammation at the serum-CSF barrier affects the CSF PKs of ertapenem. In rabbits with experimental meningitis, passive diffusion clearance of ertapenem is significantly increased. The large variability of passive diffusion clearance in rabbits with inflamed meninges is reflected by the large variability in CSF concentration (Fig. 2A). The average CSF penetration was estimated to be 7.1% in rabbits with inflamed meninges and 2.4% in rabbits with uninflamed meninges.

The difference between influx and efflux clearance was estimated to be 0.0116 ± 0.0088 ml/min, which is close to previously reported values for clearance by CSF bulk flow (19, 21). Although this finding indicates that the difference between influx and efflux clearance can be primarily explained by CSF bulk flow (14), we cannot rule out other transport mechanisms at the serum-CSF barrier such as active efflux transport (e.g., via P-glycoprotein). However, based on our results, these active efflux mechanisms probably play a minor (if any) role in the CSF PKs of ertapenem.

DISCUSSION

The continuous increasing spread of penicillin-resistant pneumococci has endangered the use of β-lactam antibiotics for the treatment of pneumococcal infections, especially in meningitis, for which the therapeutic options are limited. In the search for potential alternatives, it was recently shown that the combination of ceftriaxone with quinolones acts synergistically and was very effective against penicillin-resistant pneumococci in the same experimental model (6), although monotherapy would represent a considerable advantage. Among the potential candidates, new carbapenems could play a central role in the treatment of pneumococcal meningitis due to resistant strains.

Ertapenem, a new carbapenem with a long half-life and resistance to degradation by renal dehydropeptidase I, has a broad antimicrobial spectrum, covering the majority of human bacterial pathogens, including Listeria monocytogenes. In this study, we have investigated the efficacy of ertapenem in pneumococcal meningitis due to penicillin-sensitive and -resistant strains and its penetration into inflamed and noninflamed meninges.

The dose of ertapenem used (60 mg/kg) produced levels in serum corresponding to one intravenous injection of 500 mg in humans (peak level, 70 mg/liter in rabbits versus 70.3 mg/liter in humans; after 8 h, 6.5 mg/liter in rabbits versus 9.5 mg/liter in humans) (Fig. 1B). As the recommended dose in humans is 1 g once a day, the data reported in this model are likely to represent the minimum effectiveness possible.

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In our experimental meningitis model, ertapenem was very efficacious and sterilized the CSF of all rabbits infected with the penicillin-sensitive strain and 8 of 10 rabbits after 8 h. In time-killing assays over 8 h, in vitro ertapenem was highly bactericidal (data not shown). Against the penicillin-sensitive strain, ertapenem in concentrations above the MIC (5 and 10 times the MIC) sterilized the cultures within 4 h. Even with penicillin-resistant strains and its penetration into inflamed meninges in rabbits, the antimicrobial spectrum against common meningeal pathogens, and the efficacy against penicillin-sensitive and -resistant pneumococci might qualify ertapenem...
for the empirical treatment of bacterial meningitis, especially when penicillin-resistant strains are suspected. These data deserve further investigations in humans.

REFERENCES