Comparison of in vivo intrinsic activity of cefepime and imipenem in a Pseudomonas aeruginosa rabbit endocarditis model: effect of combination with tobramycin simulating human serum pharmacokinetics

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Objectives: The purpose of this experimental study was first to compare the in vivo intrinsic activity of imipenem and cefepime administered as a continuous infusion and to determine their lowest effective serum steady-state concentration (LESSC). Secondly, we studied the effect of combining therapy with tobramycin.

Methods: In a Pseudomonas aeruginosa (ATCC 27853) rabbit endocarditis model, β-lactam antibiotics were administered by continuous infusion over a 24 h treatment period at different doses until the LESSC was reached, i.e. able to achieve a 2-log drop of cfu/g of vegetations versus untreated animals. The effect of adding tobramycin (3 mg/kg once daily) was then studied.

Results: The LESSC was between 3× and 4× MIC of cefepime for P. aeruginosa and about 0.25× MIC of imipenem. Combination of tobramycin with each of the two β-lactams did not result in any further significant killing.

Conclusion: The optimal C_{ss}/MIC ratio might differ from one molecule to another. The LESSC of imipenem is lower than that of cefepime, giving a better intrinsic activity in vivo, despite a higher MIC in vitro.

Keywords: continuous infusion, lowest effective serum concentration, animal models

Introduction

Severe infections due to Pseudomonas aeruginosa are a major problem in hospitalized patients who often do not respond to traditional medical therapy.1–3 Successful treatment critically depends upon giving the appropriate regimen at the optimal dose, as soon as possible, and generally requires combined therapy of a β-lactam antibiotic plus an aminoglycoside.4 Continuous infusion of β-lactams has been advocated for years as an alternative method of administration based on both pharmacodynamic and pharmacokinetic properties.5–7 This regimen is mentioned in the French Summary of Product Characteristics beginning in 2000 only for ceftazidime. Today, continuous infusion has not supplanted intermittent infusion as the standard method of administration for antipseudomonal β-lactam antibiotics especially in severe infections. One important reason may lie in the fact that the active concentrations in vivo have not been clearly determined for all β-lactams. While comparative trials in humans are rare because of the severity and high mortality of such infections, experimental models can provide information relating to the in vivo efficacy of different antibiotic regimens or assessment of new therapeutic schedules.8,9

Experimental endocarditis due to P. aeruginosa, considered to be a discriminative model for severe bacteraemic infections, seems to be appropriate to study the best therapeutic regimen for these critical clinical settings. Although MICs are commonly used to compare the in vitro intrinsic activity of antibiotics, the conditions of MIC determination may differ greatly from the in vivo conditions at the site of natural infection.10,11

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Thus, our purpose was first to compare the in vivo intrinsic activity of two antipseudomonal agents (imipenem and cefepime) administered as a continuous infusion in a P. aeruginosa rabbit endocarditis model. Indeed, continuous infusion of different antibiotic doses results in stationary in vivo concentrations, similar to the determination of MICs with its scale of constant antibiotic levels. Secondly, we studied the effect of combining therapy with an aminoglycoside (tobramycin). Each regimen was evaluated for its ability to reach a 2 log cfu drop in vegetations following a short 24 h therapy, reflecting the critical end-point for prognosis in severe septicemic P. aeruginosa infections.

Materials and methods

Microorganisms

A reference strain of P. aeruginosa (ATCC 27853) lacking any acquired resistance mechanisms was studied.

Antibiotics

Clinical forms of antibiotics were used, and were supplied by: MSD Laboratories (Paris, France) for imipenem–cilastatin; Bristol-Myers Squibb Laboratories (Paris, France) for cefepime; and Lilly Laboratories (Paris, France) for tobramycin.

In vitro susceptibility testing

MICs and MBCs were determined in Mueller–Hinton (MH) broth and in rabbit serum (50%) by the microdilution method. Overnight MH broth cultures were used to prepare inocula of 10^5 cfu/mL. The MIC was defined as the lowest concentration of an antimicrobial agent preventing turbidity after 24 h of incubation at 37°C. The MBC was defined as the lowest concentration of an antimicrobial agent killing at least 99.9% of organisms within 24 h as determined by plating of MH broth cultures used to prepare inocula of 10^5 cfu/mL. The MBC as the lowest concentration of an antimicrobial agent killing at least 99.9% of organisms within 24 h as determined by plating of MIC dilutions on MH agar.

Endocarditis model

In vivo studies were performed on New Zealand white female rabbits (Cegav, St Mars-d’Egremnies, France) weighing ~2.5 kg, and were approved by the animal study committee of the University of Nantes. The animals were kept in individual cages and allowed free access to food and water throughout the experiment. Aortic valve endocarditis was induced as described previously. A polyethylene catheter was positioned in the left ventricle via the carotid artery under general anaesthesia (intramuscular ketamine 25 mg/kg) and was left in place throughout the study. After 24 h catheterization, each animal was inoculated intravenously with 10^8 cfu of P. aeruginosa.

Animals were randomly assigned to a control group (i.e. no antibiotic) or treatment groups. Therapy was started 30 h after bacterial inoculation, and antibiotics were given by catheter inserted into a marginal ear vein.

Each monotherapy group received a continuous 24 h constant rate infusion of imipenem or cefepime, administered by electric syringe pumps. Taking into account recent investigations into the stability of the drugs, the use of a single 48 mL syringe maintained at room temperature was allowed for cefepime. Imipenem solutions were renewed every 4 h. No loading dose was used since the native elimination half-life of β-lactams in the rabbit is very short, and steady state is reached quite rapidly. For each drug, several dosages were tested until the lowest effective steady-state concentration (LESSC) was reached. This concentration was defined as the lowest serum steady-state concentration able to achieve a 2-log drop in cfu/g of vegetations compared with untreated animals.

The other groups received a combination of a β-lactam antibiotic, administered at its LESSC, and a computer-controlled infusion of tobramycin simulating a 3 mg/kg once-daily human dose of tobramycin. The tobramycin dose was consistent with that indicated in the French Summary Product Characteristics for this drug.

Animals were killed using a 100 mg intravenous (iv) bolus of thiopental before the treatment period (control group) or 24 h after the onset of treatment. The heart was aseptically removed. Aortic valve vegetations were excised, gently blotted with sterile absorbent compresses to remove blood, placed immediately on ice and weighed. The vegetations were then homogenized in 500 μL of sterile saline solution. Dilutions were made at 10^-2 and 10^-4, to prevent the possibility of carry-over. Fifty microlitres of undiluted homogenate and of each dilution were then spread on trypticase soya agar plates using a spiral system plater (Interscience). After a 24 h incubation at 37°C, viable bacteria were counted, and results were expressed as log_{10} cfu/g of vegetations. The lower detection limit for this method was 1 cfu per 50 μL of undiluted vegetation homogenate.

Antibiotic concentrations in sera

Blood samples were taken from animals receiving β-lactam antibiotics, before death (at the end of the 24 h continuous infusion), and samples were immediately centrifuged at 4°C. The sera containing imipenem were immediately mixed with an equal volume of a pH 6 stabilizing MES buffer [2-[N-morpholino]ethanesulfonic acid] before freezing. All sera were then stored at –80°C before analysis.

β-Lactam concentrations were determined in serum by HPLC with a sensitivity limit of 0.2 mg/L for biological samples of imipenem or cefepime. The between-days coefficient of variation was 4.6% for imipenem and 7.6% for cefepime.

Tobramycin serum samples were taken 30 min after beginning the perfusion (peak level), at 4 h and at 24 h. Sera were immediately centrifuged and frozen at –80°C before immunoenzymic assay [TDx/ TDx FLx (Abbott)], which had a detection threshold of 0.3 mg/L in biological samples. The between- and within-day coefficients of variation were 0.1% and 3%, respectively. Results are expressed in mg/L.

Statistical analysis

Statistical analysis was performed with StatView software (Abacus Concepts, Berkeley, CA, USA). The main judgement criterion was the number of surviving bacteria in vegetations, expressed in log_{10} cfu/g. The efficacies of the different groups were compared by analysis of variance (ANOVA) followed by a Scheffe’s test for inter-group comparison. A P value of ≤0.05 was considered significant.

Results

In vitro studies

For the P. aeruginosa strain ATCC 27853, the MIC/MBC ratio for cefepime, imipenem and tobramycin was 1/1, 2/2 and 0.5/0.5, respectively. Rabbit serum had no bactericidal activity against this strain when used alone. Similar results were obtained on MH agar or in rabbit serum (50%).
In vivo activity of cefepime and imipenem against P. aeruginosa

Table 1. Determination of the LESSC of imipenem (IPM) or cefepime (FEP) in a P. aeruginosa ATCC 27853 rabbit endocarditis model: effect of combination with tobramycin (TOB)

<table>
<thead>
<tr>
<th>Regimen (mg/kg/24 h)</th>
<th>n (no. of rabbits)</th>
<th>$\log_{10}$ cfu/g of vegetations (mean ± S.D.)</th>
<th>$C_{ss}$ (mg/L, mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>22</td>
<td>$7.1 ± 1.1$</td>
<td>–</td>
</tr>
<tr>
<td>IPM (10)</td>
<td>11</td>
<td>$6.4 ± 1.5$</td>
<td>ND*</td>
</tr>
<tr>
<td>IPM (15)</td>
<td>19</td>
<td>$5.1 ± 1.7*$</td>
<td>$0.5 ± 0.2^c$</td>
</tr>
<tr>
<td>IPM (25)</td>
<td>5</td>
<td>$4.5 ± 1.4*$</td>
<td>$0.8 ± 0.3$</td>
</tr>
<tr>
<td>IPM (100)</td>
<td>7</td>
<td>$4.1 ± 1.5*$</td>
<td>$2.3 ± 0.9$</td>
</tr>
<tr>
<td>IPM (15) + TOB (3’f)</td>
<td>8</td>
<td>$4.3 ± 1.7*$</td>
<td>$0.3 ± 0.1$</td>
</tr>
<tr>
<td>FEP (10)</td>
<td>7</td>
<td>$5.8 ± 1.4$</td>
<td>$1.6 ± 0.4$</td>
</tr>
<tr>
<td>FEP (25)</td>
<td>11</td>
<td>$4.6 ± 1.5*$</td>
<td>$3.8 ± 1.1^d$</td>
</tr>
<tr>
<td>FEP (40)</td>
<td>11</td>
<td>$4.4 ± 1.8*$</td>
<td>$5.7 ± 1.5$</td>
</tr>
<tr>
<td>FEP (100)</td>
<td>8</td>
<td>$4.4 ± 1.8*$</td>
<td>$15.1 ± 12.5$</td>
</tr>
<tr>
<td>FEP (25) + TOB (3’f)</td>
<td>12</td>
<td>$5.4 ± 1.6*$</td>
<td>$5.1 ± 1.5$</td>
</tr>
<tr>
<td>TOB (3’f) alone</td>
<td>5</td>
<td>$7.5 ± 0.9$</td>
<td>–</td>
</tr>
</tbody>
</table>

MIC/MBC ratio of FEP, IPM and TOB was 1/1, 2/2 and 0.5/0.5, respectively.

*aND. 8/11 assays were under the sensitivity limit (<0.2 mg/L).

**LESC.

*Tobramycin: peak level, 11 mg/L; t1/2, 2 h.

*P<0.01 versus control group.

Animal studies and antibiotic assays

Results are shown in Table 1. In vivo efficacy of each regimen was assessed by measuring the number of surviving bacteria per gram of vegetations. Interestingly, a significant antibacterial effect was obtained with a dose of 15 mg/kg/day of imipenem ($5.1 \log_{10}$ cfu/g of vegetations versus $7.1 \log_{10}$ cfu/g in the control group). The serum steady-state concentration ($C_{ss}$), corresponding to the LESSC, was 0.5 mg/L, which was 0.25 × MIC. With 25 mg/kg/day of cefepime, a significant antibacterial effect was observed ($4.6 \log_{10}$ cfu/g of vegetations versus $7.1 \log_{10}$ cfu/g in the control group). The LESSC was between 3 and 4 mg/L, representing 3 × or 4 × MIC.

The two β-lactam antibiotics demonstrated increased bacterial killing until a threshold serum concentration was reached. After that, increasing the concentration further had a minimal effect, supporting the in vivo concentration-independent antibacterial activity of these β-lactam antibiotics.

The computer-controlled infusion of tobramycin allowed the drug to reach a peak level, defined in humans as 30 min after the end of a 30 min infusion, of ~20 × MIC (mean 11.0 mg/L). After 4 h, serum concentrations of tobramycin were ~2.5 mg/L. The half-life of tobramycin was 2 h, as in humans. After 24 h, tobramycin was not detectable in serum. Tobramycin was ineffective alone. Combination of tobramycin with the β-lactam, administered at the target concentration, did not significantly change the antibacterial effect. We noted a non-significant rise of $1 \log_{10}$ cfu/g of vegetations with cefepime, whereas a drop of $1 \log_{10}$ was found with imipenem.

Discussion

At present, there is a wealth of evidence supporting the continuous infusion of β-lactams,16,21–26 but questions regarding the amount by which the MIC should be exceeded are not yet answered for all β-lactams. Although dose-ranging studies have not been (and will never be) performed in humans, the results from in vitro27–29 and in vivo studies30–31 indicate that increasing drug concentrations above 4 × to 6 × MIC, corresponding to an AUIC of >125,34 provide no added benefit. While ceftazidime is one of the β-lactams that have been studied extensively for continuous infusion, data about cefepime and imipenem are lacking. Thus, we conducted this study to compare the antibacterial activity of cefepime and imipenem, antibiotics widely prescribed in severely ill patients with P. aeruginosa infections. This work was not designed to improve the treatment of the very frequent P. aeruginosa endocarditis, which usually combines prolonged antibiotic treatment and surgery.

Although there are few studies of cefepime use in continuous infusion, variable and low trough plasma drug concentrations have been reported in critically ill patients treated with 2 g twice daily.35 Similar to an in vitro model of P. aeruginosa infection that simulates human drug kinetics,36 our in vivo experimental study supports the suggestion that 4 to 6 times the MIC may also be the target concentration for cefepime administered as a continuous infusion for severe infections.

In comparison with cefepime, imipenem appears to be more potent against P. aeruginosa. The ceiling-effect of antimicrobial activity was observed at $C_{ss}$ < MIC. In an in vitro dynamic model, maximum kill was observed with simulations of $C_{ss}$ ≥ 2 mg/L, corresponding to 2 × MIC for P. aeruginosa.37 Earlier studies reported a maximal antibacterial effect in vivo for imipenem at concentrations close to the MIC.38 In a human-adapted mouse model, testing P. aeruginosa strains (including strain ATCC 27853 used in the present study), $T >$MIC was shown to be important in determining the outcome with imipenem. However, the percentage of the dosage interval during which the serum concentrations should exceed the MIC to produce a bacteriostatic effect was smaller with imipenem than with cephalosporins like ceftazidime.39 The presence of a post-antibiotic effect for imipenem in contrast to ceftazidime against P. aeruginosa, as suggested by the authors, could not explain our results, in which such an effect was not explored. However, imipenem has been found to be active against slowly growing bacteria, as in
the vegetations, whereas β-lactam antibiotics are generally more active against rapidly growing bacteria. To explain the in vivo activity of sub-MIC serum concentrations of imipenem, the synergic contribution of host factors (e.g. platelet microbical proteins) cannot be excluded. If the optimal \( C_{ss}/MIC \) ratio was lower for imipenem than for ceftazidime or cefepime, we would not recommend the use of a \( C_{ss} \) below the MIC in clinical settings.

The association of once-daily tobramycin with a continuous iv regimen of each β-lactam antibiotic tested did not significantly improve the antibacterial effect of β-lactams alone. This lack of early in vivo synergy with amikacin has been reported previously in the same experimental model. Synergy has been described more consistently in vitro or in animal models other than endocarditis. Nevertheless, the shortness of the treatment period in our experiments (24 h) does not allow us to exclude a beneficial effect of the combination beyond the delay of 24 h, in terms of antibacterial activity as well as of resistant mutant production.

Few data related to the stability of the aminoglycosides in the presence of third-generation cephalosporins have been reported. Although studies were performed in vitro for imipenem and in vivo for ceftazidime with tobramycin, no significant interaction was found. Cefepime and ceftazidime compatibility with tobramycin under conditions mimicking their co-administration through the same infusion line were recently realized, and no evidence of tobramycin degradation was noted.

Although few clinical trials show continuous infusion to be superior to intermittent infusion, there are strong theoretical arguments, results from animal studies and case reports, supporting the efficacy of β-lactam continuous infusion. This regimen constitutes an optimization of the therapeutic schedule, especially in specific populations for which the monitoring of plasma drug concentrations is essential. Because serum concentrations are easily accessible in clinical settings, the determination of target concentrations seems to be an interesting therapeutic tool for continuous infusion of β-lactam antibiotics. If the optimal \( C_{ss}/MIC \) ratio to maximize β-lactam activity is within 4–6, at least for ceftazidime and cefepime, our investigations indicate that imipenem could be active at lower serum concentrations.

References

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