Introduction

Severe infections caused by *Pseudomonas aeruginosa* require emergency treatment, usually by intermittent infusion of β-lactams. In view of the pharmacodynamic and pharmacokinetic properties of β-lactams, continuous infusion has been advocated as an alternative to intermittent dosing to promote maximal bactericidal effect. β-Lactams show concentration-independent killing and have a poor post-antibiotic effect against Gram-negative bacteria. Thus, it would appear that the major determinant for bactericidal activity is the time for which serum drug concentrations remain above the MIC (t > MIC) and not the magnitude of antibiotic concentrations. Continuous intravenous administration produces a relatively constant concentration of antibiotic that can be maintained above the MIC. The purpose of our study was to compare the *in vivo* bactericidal activity of continuous and intermittent dosing of ceftazidime, alone or in combination with amikacin, in a *P. aeruginosa* model of endocarditis, using a computer-controlled system simulating human kinetics. Using this approach, the experimental model was improved to correspond more closely to the human situation by adjusting the pharmacokinetics of the drugs tested in animals so that they are similar to those observed in humans.

*Corresponding author. Tel/Fax: +33-2-40-41-28-54; E-mail: gpotel@sante.univ-nantes.fr

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Materials and methods

Microorganisms

Four strains of *P. aeruginosa* were studied: a reference strain, *P. aeruginosa* ATCC 27853 (PA1), with no acquired resistance mechanism, and three clinical isolates, OXA-1 (PA2), TEM-2 (PA3) and PA557 (PA4), producing respectively an oxacillinase, a TEM-2 penicillinase and an inducible cephalosporinase (chromosomally encoded type Id β-lactamase). The clinical strains were isolated from protected brush specimens in mechanically ventilated patients presenting with nosocomial pneumonia. The choice of these strains was based on their high frequency in French hospitals (Dr Cavallo, Hopital d’Instruction des Armées, Saint-Mandé, France).

Antibiotics

Ceftazidime and amikacin were supplied by GlaxoWellcome (Marly-le-Roi, France) and Bristol Myers Squibb (Paris, France), respectively.

Susceptibility testing

For the four strains, MICs and minimal bactericidal concentrations (MBCs) were determined in Mueller–Hinton broth (supplemented with Ca²⁺ 25 mg/L and Mg²⁺ 12.5 mg/L) by the microdilution technique. Overnight Mueller–Hinton broth cultures were used to prepare inocula of 10⁵ cfu/mL. The MIC was defined as the lowest concentration of an antimicrobial agent killing 99.9% of organisms within 24 h, as determined by plating broth of overnight Mueller–Hinton agar cultures on Mueller–Hinton plates. Bacterial counts are expressed as log 10 cfu/g of protected brush specimen after 24 h of incubation at 37°C, and the MBC as the lowest concentration of an antimicrobial agent preventing turbidity after 24 h of incubation at 37°C, and the MBC as the lowest concentration of an antimicrobial agent preventing turbidity after 24 h of incubation at 37°C.

Pharmacokinetic studies

Blood samples (taken at 5, 10, 20 and 30 min and 1, 2, 4 and 8 h after iv bolus) were taken from three rabbits receiving 100 mg/kg of ceftazidime every 8 h for determination of spontaneous drug kinetics. These data were then processed in a computer-controlled system to obtain human kinetic profiles for ceftazidime in rabbits, derived from a method previously published for aminoglycosides. The simulation was intended to provide pharmacokinetic parameters identical to those observed in healthy volunteers or patients after a single 2 g bolus (c. 33 mg/kg): mean half-life about 2 h, mean concentration of the peak between 160 and 185 mg/L, and area under the serum drug concentration–time curve between 133 and 175 mg·h/L. A bolus dose of 35 mg/kg was infused and then the infusion was delivered by a pump controlled by a computer, thereby allowing the flow to be adjusted to a profile mathematically defined in time.

The same regimen was repeated every 8 h for 24 h. The total daily dose necessary to simulate the human kinetics of 4, 6 or 8 g/24 h in intermittent dosing was administered as a continuous, constant rate infusion in the groups given a constant intravenous infusion. For amikacin, a previous simulation was used to obtain a mean half-life of 2 h and a mean serum peak concentration of ≈50 mg/L in rabbits after 30 min, following a single bolus of 15 mg/kg human dose. In order to validate the human simulation, the serum concentrations of ceftazidime were determined in two rabbits from each treated group (samples were taken at 5 min and 1, 2, 4 and 8 h after the start of the infusion).

Endocarditis model

Female New Zealand White rabbits weighing approximately 2.5 kg were used for *in vivo* studies. They were kept in individual cages and allowed free access to food and water throughout the experiment. A polyethylene catheter was positioned under general anaesthesia in the left ventricle of each rabbit, with the tip passing through the aortic valve, as described previously. The catheter was left in place throughout the study. Each animal was inoculated intravenously with 10⁸ cfu of *P. aeruginosa* 24 h after catheterization.

Animals were assigned to group PA1, PA2, PA3 or PA4. In each group, animals were randomized for no treatment (control group) or one of the following ceftazidime regimens 24 h after infection, corresponding to various human doses and regimens: intermittent dosing (2 g/8 h) or continuous daily infusion of 4, 6 or 8 g. The other regimens corresponded to the same doses of ceftazidime combined with a dose of amikacin, simulating a single bolus of 15 mg/kg human dose.

Amikacin and ceftazidime were administered separately via two different venous accesses. Continuous constant-rate infusion of ceftazidime was administered as a 24 h infusion by electric syringe pumps. Intermittent dosing of ceftazidime and amikacin was administered using computer-controlled intravenous infusion pumps. All antibiotics were administered by catheters inserted into a marginal ear vein. Animals were killed using an intravenous bolus of thiopental 100 mg before treatment (control group) or 24 h after the beginning of treatment. Thus, a relevant evaluation of the antibiotic regimens was possible, comparing the number of surviving bacteria before and after treatment. A short treatment period was chosen, probably corresponding to a critical end-point for prognosis of severely ill patients with septicaemic *P. aeruginosa* infections. Aortic valve vegetation was excised, immediately put on ice, then weighed, homogenized and quantitatively cultured for 24 h at 37°C on trypticase soy agar plates. Bacterial counts are expressed as log₁₀ cfu/g of vegetation. Dilutions at 10⁻² and 10⁻⁴ were performed, ruling out the possibility of carryover effect, mainly for animals treated by continuous infusion.
Continuous versus intermittent dosing of ceftazidime

The MICs of ceftazidime for surviving bacteria were determined again after regrowth in Mueller–Hinton broth.

Antibiotic concentrations in serum and vegetations

When animals were killed, blood samples were taken from animals on each regimen and immediately centrifuged. Serum was frozen at –80°C until assay. After being weighed and homogenized with 0.5 mL of saline, vegetations were centrifuged and the supernatant fluid was sampled.

The concentrations of ceftazidime and amikacin in serum and cardiac vegetations were determined by high-performance liquid chromatography and fluorescence polarization immunoassay (Abbott, Rungis, France), respectively. Serum concentrations are expressed in mg/L (lower limit of detection: 0.5 and 1 mg/L for ceftazidime and amikacin, respectively) and concentrations in vegetations in μg/g. The between-day reproducibility for an 8 mg/L serum control was 4.6 and 7.5% for ceftazidime and amikacin, respectively.

Statistical methods

All the quantitative results are expressed as mean ± S.D. Analysis of variance (Statview; Abacus Concepts, Berkeley, CA, USA) was used to compare the effects of the different groups for each strain tested, followed by a Sheffe test for comparing treated groups with controls (P < 0.05 was considered significant).

Results

Susceptibility tests

For *P. aeruginosa* strains PA1, PA2, PA3 and PA4, the MICs and MBCs of ceftazidime were 1/1, 8/8, 4/4 and 8/8 mg/L, respectively, and those of amikacin were 2/2, 32/64, 4/4 and 4/4, respectively.

Pharmacokinetic studies

Ceftazidime kinetic parameters obtained with a dose simulating a 2 g bolus dose were as follows: peak serum concentration, 160 ± 22 mg/L; apparent elimination half-life, 1.65 ± 0.15 h (compared with a spontaneous mean half-life of 43 min); area under the curve (AUC) of serum concentrations (0 to 8 h), 369 ± 32 mg·h/L (Figure).

For continuous regimens of 4, 6 or 8 g, the serum concentrations obtained when animals were killed (steady-state concentrations) were respectively 22.7 ± 12.2, 34.8 ± 15.5 and 79.6 ± 23.4 mg/L. For amikacin, the peak serum concentration was 53.2 ± 4.5 mg/L, the elimination half-life 2.0 ± 0.3 h and the AUC at 24 h 195 ± 26 mg·h/L.

Experimental endocarditis

The in vivo results are shown in the Table. The mean weight of the vegetations was relatively homogeneous (35 ± 8 mg), so the number of surviving bacteria was expressed as log cfu/g of vegetation.

For the PA1 strain, which was susceptible to both drugs tested, continuous infusion of ceftazidime 4 g/day, alone or in combination with amikacin, had the same bactericidal effect in vivo as the intermittent regimen in combination with amikacin.

For the PA2 strain, which was resistant to amikacin and intermediate to ceftazidime, continuous infusion at doses equivalent to a treatment of 4 g/day induced no significant reduction in bactericidal effect as compared with controls. A dose of 6 g/day was necessary to obtain a reduction equivalent to that obtained with intermittent treatment. Moreover, with this strain there was no difference in killing effect between single- and two-drug therapies for the same regimen.

For the PA3 strain, standard intermittent treatment (alone or in association with the aminoglycoside) produced no significant activity compared with controls. The continuous infusion regimens at high total doses (6 and 8 g) allowed statistically significant killing to be obtained in vivo at 24 h. The association of amikacin with the continuous infusion regimens provided no additional bactericidal effect.

For the PA4 strain, which produced a cephalosporinase, no treatment was statistically significant compared with controls. However, a treatment equivalent to a dose of 8 g/day, in association with amikacin, seemed to provide relative bactericidal efficiency. The reduction in cfu/g of vegetation was not significant because of the considerable variability in the results for this strain (S.D. of 2.3 log10 cfu/g vegetation).

Figure. Serum concentrations of ceftazidime in eight infected rabbits, using a computer-controlled syringe pump to mimic human pharmacokinetics after a 2 g iv bolus.
The MICs of ceftazidime for surviving bacteria were the same as those for the initial strains. However, the method used can probably detect the emergence of a resistant mutant, but is not appropriate for detection of induced resistance.

**Antibiotic concentrations in vegetations**

The mean steady-state concentrations of ceftazidime in vegetations, expressed in μg/g of vegetation, were 60.4 ± 26, 27.7 ± 15.5 and 21.7 ± 12.2 in the 8, 6 and 4 g/24 h in continuous infusion regimens, respectively. For the intermittent treatment (2 g/8 h), the trough concentration was 15.8 ± 11 μg/g, but 33% of determinations were below the detection limit of the assay (lower limit 8.8 μg/g), so the mean concentration was probably widely overestimated in this therapeutic group. The absence of precise knowledge about the amount of free/bound ceftazidime in the vegetations makes these data very difficult to interpret.

**Discussion**

For ceftazidime, *in vivo* and *in vitro* studies have suggested that continuous infusion, as compared with intermittent treatment, improves the \( t > \text{MIC} \) for the same total daily dose,\(^{16-18} \) and that a serum steady-state concentration of 4–8 × MIC seems to be the optimum for maximal activity.\(^{19-21} \) A recent *in vitro* study suggested that 6.6 was the optimum serum steady-state concentration/MIC ratio.\(^{22} \) Continuous infusion may allow a reduction in the total daily dose without any loss of activity.\(^{19,23} \)

The experimental endocarditis model in the rabbit is an efficient means of determining antibiotic doses and achieving a more effective rate of administration in very serious diseases such as severe septicaemic *P. aeruginosa* infection.\(^{24-27} \) The brief treatment period in our experiment (24 h) allowed assessment of the early antibacterial effect, which is probably the determinant for the treatment of the severest infections in an intensive care setting. The efficacy of new modes of administration for an antibiotic needs to be compared with that of conventional treatment.\(^{28} \) Accordingly, computer-controlled simulation of the human kinetics of ceftazidime and amikacin in the rabbit, mimicking human intermittent regimens, was used in this study.\(^{10} \) Thus, the comparison of continuous regimens with conventional treatment was justified. No loading dose was used for the continuous (constant-rate) regimens in the rabbit since the elimination half-life of ceftazidime in these animals is too short and steady state is reached quite rapidly (in c. 120 min). Nevertheless, the absence of loading dose could affect the results in humans, where the longer half-life (c. 2 h, as compared with 43 min in rabbits) is responsible for it taking longer (c. 6 h) for a steady-state concentration to be reached.\(^{5} \)

For the PA1 strain, which was sensitive to all the antibiotics tested, a continuous dose of 4 g/day (providing a serum steady-state concentration of c. 20 mg/L, i.e. c. 20 × MIC) gave results similar to those obtained with an intermittent dose of 6 g/day associated with amikacin. The association of the 4 g continuous infusion regimen with amikacin gave no additional benefit in terms of the bactericidal effect *in vivo*. To our knowledge, this is the first experimental study simulating human kinetics in which the possible superiority of a continuous over an intermittent daily dose of ceftazidime has been demonstrated against a susceptible *P. aeruginosa* strain.

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**Table. In vivo effects of intermittent dosing (ID; 2 g/8 h) and continuous intravenous infusion (CIV; 4, 6 or 8 g/24 h) of ceftazidime, alone or combined with amikacin (15 mg/kg as a single dose), on the experimental model of *P. aeruginosa* endocarditis**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>strain PA1</th>
<th>strain PA2</th>
<th>strain PA3</th>
<th>strain PA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>8.0 ± 0.5</td>
<td>7.5 ± 0.8</td>
<td>8.4 ± 0.5</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>ID</td>
<td>6.4 ± 1.2</td>
<td>4.8 ± 1.8(^a)</td>
<td>6.7 ± 1.7</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>ID + amikacin</td>
<td>4.3 ± 1.5(^a)</td>
<td>4.8 ± 0.9(^a)</td>
<td>7.4 ± 0.5</td>
<td>6.6 ± 1.7</td>
</tr>
<tr>
<td>CIV 4 g</td>
<td>4.5 ± 1.3(^b)</td>
<td>6.6 ± 0.7</td>
<td>6.3 ± 1.8</td>
<td>ND</td>
</tr>
<tr>
<td>CIV 4 g + amikacin</td>
<td>4.1 ± 1.9(^b)</td>
<td>6.4 ± 1.1</td>
<td>6.9 ± 0.8</td>
<td>ND</td>
</tr>
<tr>
<td>CIV 6 g</td>
<td>ND</td>
<td>4.8 ± 1.2(^a)</td>
<td>6.1 ± 1.5(^a)</td>
<td>ND</td>
</tr>
<tr>
<td>CIV 6 g + amikacin</td>
<td>ND</td>
<td>ND</td>
<td>6.4 ± 0.9</td>
<td>ND</td>
</tr>
<tr>
<td>CIV 8 g</td>
<td>ND</td>
<td>4.7 ± 1.2(^a)</td>
<td>6.1 ± 1.4(^a)</td>
<td>7.1 ± 1.7</td>
</tr>
<tr>
<td>CIV 8 g + amikacin</td>
<td>ND</td>
<td>5.3 ± 1.6(^a)</td>
<td>6.7 ± 1.5</td>
<td>5.4 ± 2.3</td>
</tr>
</tbody>
</table>

ND, not done.

\(^{a}\)Significantly different from controls (\( P < 0.05 \)) (Sheffe’s \( S \)-test after ANOVA).

\(^{b}\)Significantly different from controls (\( P < 0.01 \)) (Sheffe’s \( S \)-test after ANOVA).
For the PA2 strain (MIC of ceftazidime 8 mg/L), the 6 g continuous infusion regimen (providing a serum steady-state concentration of around 34 mg/L, i.e. about 4 × MIC) exhibited a bactericidal activity in vivo equivalent to that of the standard intermittent treatment. It is highly probable that the steady-state concentration of ceftazidime with a 4 g regimen was insufficient to give a significant antibacterial effect because of the high inoculum in the vegetations (7.5 log_{10} cfu/g). The lack of efficacy of the 4 g continuous infusion regimen (despite a concentration in vegetations above the MIC) is probably due to the high rate of oxacillinase production responsible for antibiotic inactivation at the infected site, while efficacious concentrations might be reached for part of the time during intermittent treatment. As this strain is resistant to amikacin (MIC 32 mg/L), no greater bactericidal effect can be achieved in vivo by addition to monotherapy.

For the PA3 strain, which produced a TEM-2 penicillinase (MIC of ceftazidime 4 mg/L), the same total dose of 6 g/day (providing a serum steady-state concentration of c. 8 × MIC) exhibited similar activity with the intermittent and continuous infusion regimens, although the effect was statistically significant only for the continuous infusion. The effect with the continuous dose of 6 g/day was identical to that with 8 g/day, confirming the time-dependent activity of the drug. Once again, the association of the continuous regimen with amikacin did not increase bactericidal activity.

No regimen had a statistically significant effect in vivo (as compared with controls) on the PA4 strain, which produced a hyperinducible cephalosporinase, and only one regimen (8 g continuous infusion regimen + amikacin) led to a decrease in cfu. This 2 log_{10} drop (compared with controls) was not significant because of the large standard deviation.

The continuous infusion was at least as efficacious as the standard intermittent 2 g/8 h dosing regimen, providing that the serum steady-state concentration was ≥4 × MIC of the strain.

Our experiments showed that the association of amikacin with continuous iv regimens of ceftazidime did not improve on the effects of ceftazidime alone, except for the strain that hyperproduced cephalosporinase, although the reduction in cfu/g of vegetation was not significant. This lack of synergy in vivo for the association of ceftazidime (administered as a continuous infusion) with amikacin has also been reported by Xiong et al.,21 who used a similar P. aeruginosa model. Synergy has been described more consistently in in vitro studies19 or in animal models other than endocarditis.30

The shortness of the treatment period in our experiments did not allow us to evaluate properly the emergence of resistant mutants. The combination may be particularly beneficial in preventing emergence of resistance which, according to the simulation performed by Mouton & den Hollander,21 is greater with the continuous than the intermittent regimen.

With the same model as used in our study, Gengo & Schentag32 and McColm & Ryan33 found that the concentrations in plasma and vegetations reached a steady state rapidly, to give a similar elimination half-life in the two compartments. It would be expected, therefore, that the concentration in vegetations would remain relatively stable once a plasma steady state was attained, whereas that obtained with intermittent treatment would be affected by the same variations as for plasma kinetics. Finally, it is likely that continuous infusion would allow a relatively stable concentration to be obtained at the infection site for 24 h, as indicated by the concentration measured when animals were killed. However, the concentration obtained during intermittent treatments is variable, with very low residual concentrations. Thus, continuous infusion allows concentrations much greater than the MIC to be obtained at the infection site throughout treatment, with an equivalent or lower daily dose than that used in intermittent treatment.

In conclusion, in this model simulating different human dosing regimens of ceftazidime alone or associated with amikacin, the continuous, constant-rate intravenous infusion proved at least as efficacious as traditional, intermittent intravenous infusion providing that the serum steady-state concentration reached ≥4 × MIC for the strain studied. The magnitude of the antibacterial activity after 24 h depended more on the type of strain than on the combination with amikacin.

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