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Serum bactericidal activity against 20 strains of Pseudomonas aeruginosa was studied in 10 volunteers after administration of imipenem (25 mg/kg), imipenem (25 mg/kg) plus amikacin (7.5 mg/kg), and ceftazidime (25 mg/kg) plus amikacin (7.5 mg/kg). Eight strains were susceptible and 12 were resistant to ticarcillin. Serum levels were measured microbiologically after 30 and 60 min and were, respectively, 97 and 46 μg/ml for imipenem given alone and 79 and 45 μg/ml for imipenem given with amikacin. Despite the very large dose of imipenem used, imipenem and imipenem plus amikacin appeared slightly less active than ceftazidime plus amikacin (P ≤ 0.1; Wilcoxon matched-pairs test), with respective median titers at 30 min of 1:128, 1:128, and 1:256 against ticarcillin-susceptible strains and 1:32, 1:32, and 1:64 against ticarcillin-resistant strains; however, more than 90% of the serum determinations, regardless of the regimen, had a serum bactericidal activity ≥1:8. Amikacin significantly increased the rate of killing in serum of P. aeruginosa by imipenem. Imipenem plus amikacin appeared as effective as ceftazidime plus amikacin in reducing the viable counts of P. aeruginosa after 24 h of incubation.

Gram-negative bacterial infections are still a major cause of morbidity and death in cancer patients, especially in neutropenic and in burned patients (16). Treatment of these infections often consists of the administration of a broad-spectrum β-lactam antibiotic with or without aminoglycoside (6). Imipenem has a broad spectrum of activity against most of the microorganisms responsible for sepsis in granulocytic patients (12, 18) and might be considered as a single-drug empiric therapy for these patients (10). The combination of ceftazidime with amikacin is one of the most active regimens for the empiric treatment of febrile granulocytic-penic patients, based on in vitro studies (17, 22; S. H. Zinner and J. Klastersky, Proc. 12th Int. Congr. Chemother., Curr. Chemother. Immunother., 1:728-730, 1981), animal studies (7), and clinical trials (17; J. Klastersky, Abstr. 14th Int. Congr. Chemother. 1985, WS-36-8, p. 94), and may therefore serve as a reference regimen. The usual dosage of imipenem has been 1 to 2 g per day (23–25); however, several studies have reported clinical and bacteriological failures in Pseudomonas aeruginosa infections, some of which were due to the emergence of resistance to imipenem (24, 25; E. M. Kasworm, and J. A. Jacobson, Abstr. 85th Annu. Meet. Am. Soc. Clin. Microbiol. 1985, A57, p. 10; H. L. T. Mobley, G. L. Drusano, R. A. Salata, and W. M. Scheld, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 990, 1985). This has also been observed in an animal model of endocarditis (26). Blaser et al. (2, 3), using an in vitro pharmacological model, have shown that the emergence of resistance to various antibiotics was more likely to occur when the concentration/MBC ratio was less than 8, whereas it was not observed for ratios above 8. Thus, we were interested in studying higher doses of imipenem.

The purpose of the present investigation was to evaluate the efficacy, as measured by the titration of the serum bactericidal activity (SBA) and the rate of the SBA of imipenem used alone or in combination with amikacin compared with that of ceftazidime plus amikacin against P. aeruginosa. The SBA has been shown to predict the outcome of bacteremic infections in both granulocytic and nongranulocytic cancer patients (15, 19).

MATERIALS AND METHODS

Volunteers. Ten healthy volunteers were included in the study, five males (55 to 90 kg; mean, 75 kg), and five females (45 to 75; mean, 60 kg). Informed consent was obtained. The exclusion criteria were as follows: pregnancy, allergy to β-lactam antibiotics, abnormal renal (serum creatinine, >1.0 mg/dl) or hepatic (serum bilirubin, >1 mg/dl) function, prior antibiotic exposure within the 2 previous weeks, prior exposure to an aminoglycoside within the previous year, history of hearing troubles, and hormonal contraception or therapy for the females.

Administration of antibiotics. Each volunteer received the following on separate days and by random allocation: imipenem (25 mg/kg), imipenem (25 mg/kg) plus amikacin (7.5 mg/kg), and ceftazidime (25 mg/kg) plus amikacin (7.5 mg/kg). Ceftazidime and amikacin were given by short (<15 min) intravenous infusions in 100 ml of 5% dextrose in water; imipenem was infused in 30 min. When combinations were administered, amikacin was infused in the opposite arm and started either 15 min after the beginning of the infusion of imipenem or else simultaneously to the infusion of ceftazidime. Blood samples were taken before and at 30 and 60 min after the start of the infusion of imipenem. For the purpose of comparison, the end of infusion of ceftazidime plus amikacin was considered as time 30 min. The serum was separated from clotted blood and immediately stored (within 1 h after collection) at −80°C until assay. The assays were performed within 3 weeks after collection.

Test strains. Twenty strains of P. aeruginosa isolated from clinical specimens of cancer patients hospitalized at the
Institut Jules Bordet were selected. Eight strains were susceptible (MIC, ≤32 μg/ml) and twelve were resistant (MIC, ≥64 μg/ml) to ticarcillin.

Susceptibility testing. MICs and MBCs were determined for all strains by microtiter serial dilution in Mueller-Hinton broth supplemented with Ca²⁺ and Mg²⁺ (50 and 20 mg/liter, respectively). Each test was done in duplicate. The starting inoculum in each well was 10⁶ CFU/ml obtained from an 8-h broth culture and adjusted turbidimetrically. The actual inoculum was controlled by performing a viable count determination on a sample. The mean actual inoculum was 2.3 × 10⁶ CFU/ml (range, 9 × 10⁵ to 3.5 × 10⁶ CFU/ml). MBC determination was done by subculturing 4 μl of each well on drug-free agar. The criteria for determining the MBC were a 99.9% reduction of the original inoculum (13) based on a theta value of 8 and a 10% error on sampling volume and initial inoculum determination (20). The strains were considered to be susceptible when the MIC was ≤12.5 μg/ml for amikacin, imipenem, and ceftazidime.

SBA. SBA against all test strains was measured for each serum taken at 30 min and at 1 h after the start of infusion. Serum titration was done in a microtiter system, using a 1:1 mixture of Mueller-Hinton broth and normal human serum as the diluent (14). Inoculum concentration and sampling for bactericidal determination were the same as above. Results were expressed as the median SBA for each microbial species at a given time and drug regimen and as the percentage of sera with an SBA of ≥1:8.

Serum assays. Imipenem and ceftazidime serum levels were measured in each sample by the bioassay method of Bennett et al. (1), using Escherichia coli 273 as the test organism for imipenem and Bacillus subtilis 1904E as the test organism for ceftazidime; the ceftazidime assay was done using nutrient agar supplemented with 0.3% sodium citrate. Sodium polyanetholsulfonate (SPS) (1%, wt/vol) (Hoffmann-La Roche, Inc., Brussels, Belgium) was added to the medium to eliminate the activity of amikacin (5) when assaying ceftazidime or imipenem in the presence of amikacin. The influence of SPS on the assay of imipenem and ceftazidime in the presence of amikacin was studied by measuring the recovery of imipenem and ceftazidime in sera spiked with 100, 50, and 25 μg/ml in the presence or absence of amikacin (50 μg/ml). Each combination was tested three times. For the assay of amikacin, fluorescence polarization immunoassay was used (Abbott Laboratories, Brussels, Belgium) (8, 9).

Rate of killing in serum. Each serum obtained 30 min after the start of infusion was tested for serum killing rate by the method of Drake et al. (4). After a 1:2 dilution in supplemented Mueller-Hinton broth (final volume, 2 ml), time-kill curves were started for two strains of P. aeruginosa susceptible to ticarcillin and two strains resistant to ticarcillin. The inoculum concentration was 10⁶ CFU/ml at time zero. All tubes were agitated throughout the experience on a rotator at 37°C. Samples were obtained at time zero and at 1, 2, 4, 6, and 24 h with a 10-μl calibrated loop. Suitable dilutions were made and plated on Mueller-Hinton agar, and colonies were counted after overnight incubation.

Study of the interaction between imipenem, cilastatin, and ceftazidime when combined with amikacin. The concentrations of imipenem (initial concentrations of 50 and 100 μg/ml) with or without cilastatin (50 and 100 μg/ml), with cilastatin alone (50 and 100 μg/ml), or with ceftazidime (50 and 100 μg/ml) alone or in combination with amikacin (25 and 50 μg/ml) were measured in human serum incubated at 37°C and then compared to those measured in phosphate-buffered saline (pH 7.0) at 4°C. Each test was done in triplicate. Samples were taken at time zero and at 30, 60, and 120 min. The concentrations were measured by bioassay. The assay plates were being immediately incubated from the time of sampling. Amikacin was measured by fluorescence polarization immunoassay. Cilastatin was a gift from C. Derouwaux from Merck Sharp & Dohme (Brussels, Belgium).

Statistical test. A Wilcoxon matched-pairs rank test was used to compare the results obtained in volunteers receiving the different regimens.

## RESULTS

Six of the ten volunteers experienced side effects during and after the administration of imipenem with or without amikacin consisting of general malaise, dizziness, perspirations, and moderate hypotension (systolic arterial blood pressures of >80 and <110 mm Hg) occurring during infusion. These clinical manifestations disappeared within 1 h after the end of infusion. None of the volunteers experienced any side effect during or after the administration of ceftazidime with amikacin.

The MICs and MBCs of the tested strains are listed on Table 1. All the strains susceptible to ticarcillin were susceptible to imipenem, ceftazidime, and amikacin. Ticarcillin-resistant strains were one dilution less susceptible to imipenem and ceftazidime than were ticarcillin-susceptible strains. Five strains resistant to ticarcillin had an MIC of 25 μg/ml to amikacin, one of them had also an MIC of 25 μg/ml to ceftazidime. All ticarcillin-resistant strains were susceptible to imipenem.

The assay of imipenem in the presence of amikacin was not influenced by the presence of amikacin or of 1% SPS. The recoveries of imipenem (100, 50, and 25 μg/ml) in the presence of amikacin (50 μg/ml) were, respectively, 98 ± 4 (mean of three assays ± standard deviation), 99 ± 5 and 101 ± 7%. In comparison, the recoveries of imipenem in the absence of amikacin were, respectively, 100 ± 4, 98 ± 4, and 102 ± 7%. Similar results were obtained with ceftazidime.

### TABLE 1. In vitro susceptibility of 20 P. aeruginosa strains

<table>
<thead>
<tr>
<th>Phenotype (no. of strains)</th>
<th>Imipenem (range, μg/ml)</th>
<th>Ceftazidime (range, μg/ml)</th>
<th>Amikacin (range, μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>Ticarcillin susceptible (8)</td>
<td>0.2–0.8</td>
<td>0.2–1.6</td>
<td>3.1–12.5</td>
</tr>
<tr>
<td>Ticarcillin resistant (12)</td>
<td>0.2–6.2</td>
<td>0.2–12.5</td>
<td>0.8–25</td>
</tr>
</tbody>
</table>

### TABLE 2. Serum concentrations of imipenem, ceftazidime, or amikacin in 10 volunteers

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Serum conc (μg/ml, mean ± SD) postinfusion</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem alone</td>
<td>97 ± 27.4</td>
<td>45.7 ± 16.7</td>
<td></td>
</tr>
<tr>
<td>Imipenem with amikacin</td>
<td>78.7 ± 22.3</td>
<td>44.4 ± 8.8</td>
<td></td>
</tr>
<tr>
<td>Amikacin with imipenem</td>
<td>41.8 ± 6.9</td>
<td>26.9 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime with amikacin</td>
<td>75.5 ± 15</td>
<td>55.8 ± 12.4</td>
<td></td>
</tr>
<tr>
<td>Amikacin with ceftazidime</td>
<td>51.3 ± 9.5</td>
<td>27.6 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3. Serum bacteriostatic activity and SBA 30 and 60 min after the start of infusion in volunteers receiving imipenem with or without amikacin and ceftazidime

<table>
<thead>
<tr>
<th>Phenotype (no. of strains)</th>
<th>Regimen</th>
<th>SSA mediator*</th>
<th>% of sera ≥ 1:8</th>
<th>SBA median</th>
<th>% of sera ≥ 1:8</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ticarcillin susceptible (8)</td>
<td>Imipenem</td>
<td>1:256</td>
<td>100</td>
<td>1:128</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>Imipenem + amikacin</td>
<td>1:256</td>
<td>100</td>
<td>1:128</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime + amikacin</td>
<td>1:512</td>
<td>100</td>
<td>1:256</td>
<td>100</td>
</tr>
<tr>
<td>Ticarcillin resistant (12)</td>
<td>Imipenem</td>
<td>1:64</td>
<td>100</td>
<td>1:32</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>Imipenem + amikacin</td>
<td>1:64</td>
<td>100</td>
<td>1:32</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime + amikacin</td>
<td>1:128</td>
<td>100</td>
<td>1:64</td>
<td>100</td>
</tr>
<tr>
<td>60 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ticarcillin susceptible (8)</td>
<td>Imipenem</td>
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<td></td>
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<td>1:128</td>
<td>100</td>
<td>1:64</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime + amikacin</td>
<td>1:128</td>
<td>98.7</td>
<td>1:128</td>
<td>97.5</td>
</tr>
<tr>
<td>Ticarcillin resistant (12)</td>
<td>Imipenem</td>
<td>1:32</td>
<td>99.2</td>
<td>1:16</td>
<td>88.3</td>
</tr>
<tr>
<td></td>
<td>Imipenem + amikacin</td>
<td>1:32</td>
<td>98.3</td>
<td>1:16</td>
<td>90.8</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime + amikacin</td>
<td>1:32</td>
<td>94.2</td>
<td>1:32</td>
<td>92.5</td>
</tr>
</tbody>
</table>

* SSA, Serum bacteriostatic activity.

![Graph A](image1.png)

**Pseudomonas aeruginosa S. ticarcillin**

![Graph B](image2.png)

**Pseudomonas aeruginosa R. ticarcillin**

FIG. 1. Rate of killing of *P. aeruginosa* in the serum of 10 volunteers obtained 30 min after administration of imipenem with or without amikacin or of ceftazidime with amikacin. (A) Two *P. aeruginosa* strains susceptible to ticarcillin. (B) Two *P. aeruginosa* strains resistant to ticarcillin. The results are expressed as the mean assayed in the presence of amikacin. This confirmed that 1% SPS in the agar of bioassay test plates inactivates very efficiently the amikacin even in high concentrations without affecting the β-lactam antibiotic assayed.

The serum levels of the three antibiotics are summarized in Table 2. Significantly lower serum levels of imipenem were obtained 30 min after the start of infusion when the drug was given with amikacin than when imipenem was given alone (Wilcoxon matched-pairs rank test, *P* ≤ 0.01). This effect was not observed at 60 min nor did it occur with ceftazidime plus amikacin.

A slight interaction was observed between imipenem and amikacin when they were mixed together in serum. The decay of imipenem (100 µg/ml) in serum was consistent with a single exponential relation: log concentration = slope × (time + constant), in which the slope was −2.15 ± 0.07 (mean of three experiments ± standard deviation) and the constant was the log of the initial concentration. In the presence of 25 and 50 µg of amikacin per ml the slope was, respectively, −2.88 ± 0.06 (Wilcoxon; *P* ≤ 0.1) and −2.99 ± 0.07 (*P* ≤ 0.06). The concentration of ceftazidime was not affected by the presence of amikacin. The concentration of amikacin was not affected by the presence of ceftazidime, imipenem with or without cilastatin, or cilastatin alone. No interaction was observed between amikacin and imipenem, with or without cilastatin, or for cilastatin alone, at 4°C in phosphate-buffered saline at pH 7.0.

The serum bacteriostatic and bactericidal activities observed 30 and 60 min after the start of infusion are shown in Table 3. As expected from in vitro susceptibilities, lower activities were observed against the strains of *P. aeruginosa* resistant to ticarcillin. Amikacin did not enhance the activity of imipenem. Although more than 90% of the sera had a SBA of ≥1:8, imipenem and imipenem plus amikacin appeared slightly less active than ceftazidime plus amikacin, despite the very high dose of imipenem used here. The highest median dilutions showing bacteriostatic or bactericidal activities were observed for the combination of ceftazidime plus amikacin. The killing rate in serum of two strains each of the viable count (log CFU per millilitre). The vertical bars represent the standard deviation. Symbols: ○, imipenem; ●, imipenem plus amikacin; □, ceftazidime plus amikacin; and ●, control in serum without antibiotic.
of ticarcillin-susceptible and -resistant *P. aeruginosa* is given in Fig. 1. Amikacin significantly increased the rate of killing of *P. aeruginosa* by imipenem (Wilcoxon test; *P* ≤ 0.01). Imipenem plus amikacin appeared as effective as ceftazidime plus amikacin in reducing the viable counts of *P. aeruginosa*.

**DISCUSSION**

The high incidence of side effects was probably due to the high dose of imipenem infused over a short period of time (30 min). The relation between the dose and the incidence of nausea and vomiting has been reported by Wang et al. (23). They also reported that the nausea was related to the speed of infusion and could be ameliorated by slowing the rate of infusion, although this was not true for all patients.

SBAs of ≥1:8 were obtained in at least 97% of the sera with all regimens tested against ticarcillin-susceptible *P. aeruginosa* 30 and 60 min after administration. SBAs against ticarcillin-resistant *P. aeruginosa* of ≥1:8 were obtained in at least 88% of the sera with all regimens 30 and 60 min after administration. The SBAs obtained with ceftazidime plus amikacin were usually one dilution higher than those obtained with imipenem or with imipenem plus amikacin. A higher rate of killing was observed with the combination than with imipenem alone. The slight increase in the spontaneous decay of imipenem concentration when mixed with amikacin in vitro at 37°C has most probably no clinical relevance since there was no significant difference in serum concentrations obtained 1 h after administration of imipenem (25 mg/kg) with amikacin (7.5 mg/kg). The emergence of resistance during killing-curve experiments was not observed here, although it is often observed in single-drug experiments. Amikacin in many similar studies has been shown to prevent the emergence of resistant strains (11, 21, 22).

In conclusion, imipenem alone at a high dose seemed to be promising as single-drug therapy for infections due to *P. aeruginosa* strains that are either susceptible or resistant to ticarcillin. A significant increase in the rate of killing was observed when amikacin was added to imipenem, although the resulting SBAs were not significantly increased compared with those for imipenem alone, despite the very high dose of imipenem used in the present study.

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**LITERATURE CITED**


