Comparative Activities of Piperacillin, Ceftazidime, and Amikacin, Alone and in All Possible Combinations, against Experimental Pseudomonas aeruginosa Infections in Neutropenic Rats

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This study compared the efficacy of therapy with the double beta-lactam combination of ceftazidime plus piperacillin with that of single-agent therapy with ceftazidime, piperacillin, or amikacin alone and with that of two aminoglycoside-beta-lactam combinations against Pseudomonas aeruginosa peritonitis and bacteremia in neutropenic rats. Rats made severely granulocytopenic with cyclophosphamide became bacteremic secondary to peritonitis which was induced by intraperitoneal challenge with P. aeruginosa. Antibiotic therapy with single agents (amikacin, 20 mg/kg of body weight, intramuscularly; ceftazidime, 20 mg/kg of body weight, subcutaneously; piperacillin, 200 mg/kg of body weight, intramuscularly) or with the various combinations of agents was begun 2 h after bacterial challenge and was continued every 6 to 8 h for 62 h. Therapeutic efficacy was judged on the basis of survival 72 h after bacterial challenge, rate of mortality, incidence of bacteremia, and the emergence of resistant organisms. Based on these criteria, therapy with the double beta-lactam combination had no advantage over single-agent therapy and was in all cases clearly inferior to beta-lactam-aminoglycoside combinations.

Synergistic antimicrobial combinations for empiric therapy of febrile, granulocytopenic patients frequently include an aminoglycoside for enhanced activity against gram-negative aerobic bacilli (6). Although effective in treating infections caused by those organisms, aminoglycosides are associated with the development of ototoxicity and nephrotoxicity (11, 13). To avoid these side effects, double beta-lactam combinations have been investigated as alternatives to aminoglycoside-containing combinations and have been used in prospective clinical trials for empiric therapy of suspected sepsis in febrile granulocytopenic patients (12; C. de Jongh, J. Joshi, K. Newman, F. Danhaver, R. Finley, M. Moody, P. Wiernik, and S. Schimpff, Program Abstr. 22nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 943, 1982; R. Feld, T. Louie, L. Mandell, H. Robson, A. Chow, E. Bow, A. Belch, G. Goldsand, and J. Pater, Proc. 13th Int. Congr. Chemother., SS8.2/2-7:44/24-44/28, 1983; J. H. Joshi, Proc. 14th Int. Congr. Chemother. SS 8.2/2-9:44/34-44/36, 1983; M. O. Loveless, D. N. Gilbert, and J. Jackson, Proc. 14th Int. Congr. Chemother. SS 8.2/2-8:44/29-44/33, 1983). Most of these double beta-lactam combinations contained moxalactam or ticarcillin. Ceftazidime (Glaxo Inc., Research Triangle Park, N.C.), a new extended-spectrum cephalosporin, has been shown to be more active in vitro against Pseudomonas aeruginosa than is moxalactam or ticarcillin (8). Since P. aeruginosa is an important pathogen in granulocytopenic patients (2), the enhanced in vitro activity of ceftazidime against this organism suggests that it may be a better candidate for inclusion in a double beta-lactam combination than moxalactam or ticarcillin.

Normal and granulocytopenic rats infected with gram-negative organisms have been used in several assessments of the activities of antimicrobial drugs and their combinations (1, 4, 7, 10). Applications of this model have made it possible to assess antimicrobial efficacy by means of rat survival, delay in the appearance of death, and the emergence of resistant organisms. Resemblances of these experimental infections to serious P. aeruginosa infections in granulocytopenic patients include the greater susceptibility of the granulocytopenic host to P. aeruginosa infection and the critical importance of early antibiotic therapy to a successful outcome (10).

Clinical trials are useful in evaluating overall efficacy of antimicrobial therapy in granulocytopenic patients, but evaluation of efficacy against specific organisms is frequently not possible because of small numbers of patients. The rat model is particularly well suited to these evaluations and may offer the additional advantage of systematic exploration of dose and dosage regimen-response relationships. Therefore, the purpose of this study was to compare the therapeutic efficacies of the combination of piperacillin (Lederle Laboratories, Pearl River, N.Y.) and ceftazidime with those of piperacillin, ceftazidime, or amikacin (Bristol Laboratories, Syracuse, N.Y.) alone or with combinations of piperacillin plus amikacin or ceftazidime plus amikacin against infections with P. aeruginosa in granulocytopenic rats.

MATERIALS AND METHODS

Bacteria. Several blood culture isolates of P. aeruginosa from patients at the University of Maryland Cancer Center were screened in vitro and in rats. P. aeruginosa no. 25 was selected for study because of the following characteristics: antibiotic susceptibility (MIC and MBC in micrograms per milliliter) to piperacillin (4 and 8), ceftazidime (2 and 4), and amikacin (8 and 8); 50% lethal dose (LD$_{50}$) for intraperitoneally (i.p.) challenged neutropenic rats, 2.6 $	imes$ 10$^6$ organisms; and antibiotic synergy, as determined by isobologram.
plotted from checkerboard antibiotic susceptibility test results.

**Animals.** Female Sprague-Dawley rats weighing 180 to 200 g (Harlan Sprague-Dawley Inc., Walkersville, Md.) were conditioned in our laboratory for 1 week after receipt from the breeder. During the conditioning period, stool cultures were examined to ensure that animals were not colonized with *P. aeruginosa*. During conditioning and throughout the experiment, rats had free access to rat chow and fresh drinking water which was acidified to prevent contamination with *P. aeruginosa*. Noninfected neutropenic control rats remained free of *P. aeruginosa* throughout the experiment.

**Induction of neutropenia.** Cyclophosphamide (Mead Johnson, Evansville, Ind.) was administered i.p. in doses of 100 mg/kg of body weight on day 0 and 75 mg/kg of body weight on day 4. Leukocyte (WBC) counts were taken daily on noninfected rats to assess the effect of cyclophosphamide. Differential counts were taken on Wright-stained smears. As demonstrated previously (4), cyclophosphamide therapy effected a reduction in the mean WBC count from 12,700 WBCs per μl on day 0 to 470 WBCs per μl on day 5 and maintenance of the count at the latter level throughout the therapeutic period (days 5 to 8). Reduction in total WBC count was accompanied by a reduction in granulocytes. On day 0, the mean granulocyte count was 1,905 granulocytes per μl, and on days 5 through 8 it was <50 granulocytes per μl.

**LD₉₀ determinations.** Neutropenic rats were inoculated i.p. on day 5 with 1 ml of serial 10-fold dilutions (10 rats per dilution) of an overnight culture of *P. aeruginosa* no. 25 grown in Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy broth. The LD₉₀ of 2.6 × 10⁶ organisms was calculated by the method of Reed and Meunich (9) from results 72 h after bacterial challenge (day 8). At a challenge inoculum level of 10⁶ organisms, 10 of 10 rats died; at 10⁵ organisms, 8 of 10 died; at 10⁴ organisms, 7 of 10 died; at 10³ organisms, 5 of 10 died; and at 10² organisms, only 1 of 10 died.

**Antimicrobial agents.** Piperacillin, ceftazidime, and amikacin were studied. All of the antibiotics used in rats were from vials prepared for clinical use. Reference solutions of each agent for pharmacokinetic studies were prepared from standardized antibiotic powders supplied by the manufacturers.

**In vitro susceptibility studies.** MIC, MBC, and tests of synergy for several *P. aeruginosa* clinical blood culture isolates were determined by the microtiter variation of the checkerboard technique (5). For studies of beta-lactamaminoglycoside synergy, serial twofold dilutions of piperacillin or ceftazidime, prepared in Mueller-Hinton broth (BBL) containing 30 μg of Ca²⁺ per ml and 25 μg of Mg²⁺ per ml (0.05 ml per well), were made in one direction with an automatic diluter (Dynatech Laboratories, Inc., Alexandria, Va.). Twofold dilutions of amikacin were added manually in the perpendicular direction (0.05 ml per well). For double beta-lactam synergy studies, serial twofold dilutions of piperacillin were made in one direction, and serial twofold dilutions of ceftazidime were made in the perpendicular direction. For each strain of *P. aeruginosa* tested, a dilution of an overnight culture was added to each well (0.0015 ml per well) to yield a final concentration of approximately 5 × 10⁵ CFU/ml. The contents of each microtiter tray were mixed, and the trays were covered and incubated at 37°C overnight. The MIC was defined as the lowest antibiotic concentration that prevented visible growth. A 0.0015-ml sample from each MIC microtiter well was inoculated onto a Trypticase soy agar plate which was incubated at 37°C overnight. The MBC was defined as the lowest antibiotic concentration producing ≥99.9% reduction in viable bacteria, the endpoint being no growth. Synergy was defined as a fourfold or greater reduction in the MBC of both antibiotics in the combination when compared with the MBC of the individual antibiotics.

**Concentrations of antimicrobials in rat serum.** Antimicrobial rat serum concentration studies were performed in normal, noninfected rats so that equivalent antibiotic doses could be identified for use in therapeutic trials. Criteria for equivalent doses included: (i) rat serum levels at 30 min after administration similar to attainable serum levels in humans, and (ii) rat serum antibiotic levels approximately equal to the MBC of the challenge organism 2 h after administration of antibiotic. Piperacillin and amikacin were given intramuscularly, and ceftazidime was given subcutaneously. Rats were injected with various doses of these three antibiotics (three rats per dose) and then were bled retro-orbitally at 0.5, 1, 1.5, 2, 2.5, and 3 h. Concentrations in serum were determined by modified cylinder plate procedures supplied by the manufacturers. The assay organism was *Bacillus subtilis* ATCC 6633 for piperacillin and amikacin and *Proteus morganii* NCTC 235 for ceftazidime. The assay organism was added to molten agar (nutrient agar for amikacin, antibiotic medium no. 2 for ceftazidime, and antibiotic medium no. 1 for piperacillin) before distribution of the latter into assay plates. Agar wells (5 mm in diameter) were filled with reference antibiotic solutions or rat serum and were diluted with pooled normal rat serum (Pel-Freeze Biologicals, Rogers, Ark.). Zones of inhibition of bacterial growth were measured after incubation of plates at 30°C overnight, and serum antibiotic concentrations were calculated from the curves of the inhibition zone sizes from reference antibiotic concentrations.

**Therapeutic trials.** Neutropenic rats (20 rats per group) were challenged i.p. on day 5 (counting from the initial cyclophosphamide injection) with 1 ml of an overnight culture of *P. aeruginosa* diluted to yield multiples of LD₉₀. Antibiotics were administered 2, 8, 14, 22, 30, 38, 46, 54, and 62 h after bacterial challenge. In combination regimens, each antibiotic was administered at a different site. The following combinations were tested: 1) rats receiving cephalosporin only, rats receiving cephalosporin and piperacillin; 2) rats receiving cephalosporin, piperacillin, and amikacin; 3) rats receiving cephalosporin, piperacillin, and ceftazidime; 4) rats receiving cephalosporin, piperacillin, and amikacin, and rats receiving cephalosporin, piperacillin, and ceftazidime. Mortality was recorded immediately before each antibiotic dose, and the final mortality was recorded 72 h after bacterial challenge (day 8). Cultures of cardiac blood were prepared from rats that died of infection (usually within 1 h of death) and from all survivors (10 h after the terminal dose). The MICs and MBCs for *P. aeruginosa* recovered from these cultures were compared with the MICs and MBCs for the challenge organism to determine whether resistance had developed. Resistance was defined as a fourfold or greater increase in MIC or MBC for an isolate from rat blood culture over the MIC or MBC for the challenge organism tested at the same time.

**Statistical analysis.** Death rates recorded throughout the experiment were compared by the Kaplan and Meier life table and the proportional-hazard models of Cox (3). Anti-biotic-treated rats were compared with infection control rats, and rats that received single-agent therapy were compared with rats that received combination therapy, by analysis of differences in final mortality rates by using the chi-square test with the Yates correction. Rates of resistant organisms recovered after each therapeutic regimen were compared by the Fisher exact test.
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PIPERACILLIN-CEFTAZIDIME FOR P. AERUGINOSA INFECTION

FIG. 1. Rat serum antibiotic concentrations after subcutaneous (ceftazidime, 20 mg/kg of body weight) or intramuscular (piperacillin, 200 mg/kg of body weight; amikacin, 20 mg/kg of body weight) administration. Each data point is the mean ± standard deviation from three rats. MICs and MBCs are for P. aeruginosa 25.

RESULTS

Concentrations of antibiotics in rat serum. Doses of 200 mg of piperacillin per kg of body weight, 20 mg of ceftazidime per kg of body weight, or 20 mg of amikacin per kg of body weight produced serum levels in rats 30 min after injection similar to those attained in humans, and at approximately 2 h after injection, produced concentrations similar to the MBC for P. aeruginosa 25 (Fig. 1). These doses were used in the therapeutic trials.

Therapeutic trials. Antibiotic efficacy against each of two different-sized bacterial challenges was evaluated. At the

5-LD50 challenge level, all of the therapeutic regimens provided some protection. The survival rate with amikacin alone was clearly greater than that with either piperacillin or ceftazidime ($P \leq 0.005$) and was similar to that attained with piperacillin plus ceftazidime ($P \geq 0.18$). Differences between survival rates after therapy with amikacin plus either piperacillin or ceftazidime and therapy with piperacillin plus ceftazidime approached significance ($P = 0.056$).

At the 50-LD50 challenge level, survival in the groups treated with piperacillin, ceftazidime, or amikacin alone, and in the groups treated with piperacillin combined with cefta-

TABLE 1. Comparative efficacies of therapy with piperacillin, amikacin, and ceftazidime alone, combination therapy of amikacin with piperacillin or ceftazidime, and piperacillin with ceftazidime for infections induced by 5 LD50s (1.3 $\times 10^7$ CFU) and 50 LD50s (1.3 $\times 10^6$

<table>
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<th>Inoculum (LD50)</th>
<th>Therapeutic agent</th>
<th>Rats surviving/20</th>
<th>Positive blood cultures/20</th>
<th>Resistant isolates/20</th>
<th>Median survival time (h) of rats that died (range)</th>
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<tbody>
<tr>
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<td>0</td>
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<td>8</td>
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<td>3</td>
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<td>2</td>
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<td></td>
<td>Piperacillin + ceftazidime</td>
<td>15</td>
<td>11</td>
<td>5 (P), 7 (C)</td>
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*Doses of agents: piperacillin, 200 mg/kg of body weight; ceftazidime, 20 mg/kg of body weight; amikacin, 20 mg/kg of body weight.

*Letters in parentheses indicate organisms resistant for the following individual agents used in the combination: P, piperacillin; C, ceftazidime; A, amikacin.

*From data recorded throughout the 72-h observation period.
zidime and saline, was similar \((P \geq 0.11)\) and clearly inferior to survival accorded by combination therapy of amikacin plus piperacillin or ceftazidime \((P \leq 0.001)\).

Proportional-hazard model analysis (3) of survival curves of rats challenged with 5 and 50 LD\(_{50}\)s of \(P\). \textit{aeruginosa} 25 (Table 1) was used to describe interactions between antimicrobial agents used in combination therapy. At both challenge levels, results of the analysis for the aminoglycoside-containing combinations were consistent with the hypothesis that the antimicrobial agents interacted in a multiplicative fashion which may be analogous to synergistic interaction in vitro. At the 50-LD\(_{50}\) challenge level, results of analysis of the piperacillin-ceftazidime combination suggested a less-than-multiplicative interaction \((P = 0.07)\) that is not significant at the \(\alpha = 0.05\) level.

**Rate of bacteremia and emergence of resistant organisms.** Within each therapeutic group, the number of bacteremic animals was similar to the number of animals that died (Table 1). Dead animals were usually bacteremic, but the challenge organism was recovered infrequently from blood cultures of rats that were alive 72 h after challenge.

\(P\). \textit{aeruginosa} isolates from rat blood cultures were tested for susceptibility to the antibiotics used in therapy. The MICs and MBCs of resistant organisms (defined as fourfold or greater change over the challenge organism) were usually four or eightfold greater than those of the challenge organism. Highly resistant organisms were not recovered. Differences in the rates of recovery of resistant \(P\). \textit{aeruginosa} between challenge groups (i.e., 5 and 50 LD\(_{50}\)s), regardless of the therapeutic regimen, were not significant \((P \geq 0.13)\). For animals receiving single-agent therapy, differences in the emergence of resistant organisms were also not significant \((P \geq 0.13)\). Differences in the rates of emergence of resistant organisms between single-agent therapy and therapy with the double beta-lactam combination were not significant \((P \geq 0.17)\). In contrast, differences in the rates of emergence of resistant organisms were greater with single-agent therapy than with the aminoglycoside-beta-lactam combinations \((P \leq 0.05)\).

**DISCUSSION**

The overall efficacies of double beta-lactam combinations and aminoglycoside-containing combinations in the treatment of febrile episodes among granulocytopenic cancer patients have been similar in randomized prospective trials. However, therapeutic efficacy of double beta-lactam combinations against \(P\). \textit{aeruginosa} infections frequently could not be analyzed because of the small number of patients with such infections. As an example, in prospective evaluation of the efficacy of ceftazidime plus either tobramycin or piperacillin in 198 febrile, granulocytopenic cancer patients (J. Joshi, R. Ruxer, K. Newman, R. Finley, M. R. Moody, G. Drusano, J. Tenney, and S. Schimpff, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 383, 1984), \(P\). \textit{aeruginosa} was recovered from only seven patients, and of these only four were bacteremic. This number was too small for a valid statistical comparison of different regimens. Based on the patient distribution found in this study, large, multicenter clinical trials would most likely be required to obtain enough patients to permit an adequate evaluation of efficacy of those drug combinations against infections caused by gram-negative bacilli. Animal model studies are particularly useful for such evaluations in that large numbers of animals can be tested under controlled conditions.

Winston et al. (12) conducted a prospective randomized trial comparing the efficacy of moxalactam plus piperacillin or amikacin for empiric therapy of suspected sepsis in febrile, granulocytopenic patients. Although the overall efficacies of moxalactam plus piperacillin and of moxalactam plus amikacin were similar, the response rate following moxalactam-plus-amikacin therapy was better \((P = 0.06)\) for patients with \(P\). \textit{aeruginosa} infections.

The studies reported here, conducted in severely granulocytopenic (≤50 granulocytes per μl) rats, compared the efficacy of a double beta-lactam combination with that of single agents and of aminoglycoside-beta-lactam combinations against \(P\). \textit{aeruginosa} bacteremia secondary to induced \(P\). \textit{aeruginosa} peritonitis. Ceftazidime was chosen for study because it is more active in vitro against \(P\). \textit{aeruginosa} than is moxalactam. Results of this study agree with the findings reported by Winston et al. (12) in that therapeutic efficacies of aminoglycoside-containing combinations were superior to those of the double beta-lactam combination. They further indicate that double beta-lactam therapy was no better than single-agent therapy (Table 1) in preventing death from \(P\). \textit{aeruginosa} peritonitis and bacteremia in rats, even though the ceftazidime-piperacillin combination was synergistic in vitro (Fig. 1). Lastly, the numbers of bacteremic rats and the emergence of resistant organisms following therapy were similar for animals treated with single agents or with the double-beta-lactam combination. Each of these regimens was inferior to the aminoglycoside-beta-lactam combinations.

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**LITERATURE CITED**


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