Synergistic Activity of Carbenicillin and Gentamicin in Experimental Pseudomonas Bacteremia in Neutropenic Rats

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Rats made neutropenic with cyclophosphamide were infected intraperitoneally with Pseudomonas aeruginosa. The challenge organism was killed synergistically in vitro by the combination of gentamicin and carbenicillin. Untreated neutropenic rats infected with $3 \times 10^8$ Pseudomonas died between days 2 and 7, and the overall mortality was 70%. Groups of infected neutropenic rats were treated intramuscularly with 1.5 or 6 mg of gentamicin per kg per dose, 100 or 400 mg of carbenicillin per kg per dose, or 1.5 mg of gentamicin and 100 mg of carbenicillin per kg per dose. Treatment was begun at 2 h postinfection and was continued every 8 h for about 72 h. Cultures of blood and peritoneal washings were performed in control and treated rats at 1, 4, 24, 48, and 72 h postinfection. Gentamicin at either dose level was ineffective in preventing death, but mortality was significantly reduced by high-dose carbenicillin and low-dose combination therapy. In addition, the latter regimens sterilized the peritoneal fluid and blood. Carbenicillin and gentamicin showed in vivo synergy in the treatment of neutropenic Pseudomonas-infected rats.

Infections caused by Pseudomonas aeruginosa constitute a serious problem in patients with underlying neoplastic disease (26). The incidence of bacteremic Pseudomonas (refers throughout the text specifically to P. aeruginosa) infection has increased largely due to the aggressive use of potent antineoplastic chemotherapy that may result in prolonged periods of severe neutropenia. Patients colonized with Pseudomonas are at an increased risk of invasive infection during periods of drug-induced neutropenia (23, 24).

Antimicrobial therapy of Pseudomonas bacteremia has often proved unsuccessful, and few controlled comparative trials of therapy have been performed in humans (10). Although polymyxin B and sodium colistimethate exhibit in vitro activity against almost all strains of Pseudomonas, they are usually ineffective in the therapy of bacteremic infection, particularly in patients with unremitting neutropenia (26). Gentamicin, also active in vitro against most Pseudomonas isolates, may be similarly ineffective in the neutropenic host with bacteremic infection (4, 5, 21, 25). The introduction of carbenicillin was reported by Bodey et al. to have led to the increased survival of patients with Pseudomonas bacteremia; good results were noted even in patients with persisting neutropenia (6, 7). A 71% complete response rate was reported in a group of 38 patients treated with carbenicillin alone for bacteremic Pseudomonas infection (7).

Gentamicin and carbenicillin show antibiotic synergy in vitro against many strains of Pseudomonas (11, 19). This antibiotic combination is frequently employed in the therapy of proven Pseudomonas infection as well as empirically in febrile neutropenic patients prior to the availability of culture results (21). Despite the early institution of combined antibiotic regimens incorporating carbenicillin and gentamicin, the therapy of Pseudomonas bacteremia occurring in a neutropenic host may still fail, particularly if the underlying hematological disease cannot be controlled. In recent studies the successful control of Pseudomonas bacteremia with combination therapy has been reported variously in 33% (23), 60% (27), 62% (22), and 85% of cases (21).

Due to the difficulties involved in performing and interpreting trials of antibiotic efficacy in severely ill patients with varying types and degrees of underlying illness, the potential disparity between in vitro and in vivo results, and the potential emergence of Pseudomonas strains resistant to formerly effective agents (12), studies of therapy in appropriate animal models seem justified. Such animal experiments may generate significant data that may be impossible to obtain from studies in humans.

The present experiments were designed to
evaluate the therapeutic efficacy of gentamicin and carbenicillin alone and in combination in experimental *Pseudomonas* bacteremia in neutropenic rats. A model infection similar to that described by Andriole (1, 2) for nonneutropenic animals was employed; severe neutropenia was induced by the administration of cyclophosphamide. The *Pseudomonas* strain employed was killed synergistically in vitro by the combination of gentamicin and carbenicillin. Animals treated with a low-dose antibiotic combination showed significantly improved survival as compared with those treated with either gentamicin or carbenicillin in equivalent doses.

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**MATERIALS AND METHODS**

**Experimental animals.** Female, pathogen-free, Sprague-Dawley CFE rats (Carworth Farms, Wilmingon, Mass.) weighing 185 to 200 g were used.

**Induction of neutropenia.** Cyclophosphamide (Procytox; Frank W. Horner Ltd., Montreal, Quebec, Canada) was administered intraperitoneally in a dose of 120 mg/kg. The median leukocyte count on days 4 and 5 after cyclophosphamide was approximately 1,000 cells/mm³; on the same days, median neutrophil counts were about 100/mm³. Circulating leukocytes increased by day 6 and reached normal levels by day 10 after injection. No special precautions were taken with the rats during the period of neutropenia, and there were no deaths in neutropenic noninfected rats observed for 15 days. In subsequent experiments neutropenic rats were infected 96 h after the administration of cyclophosphamide.

**Microorganism and in vitro susceptibility studies.** *P. aeruginosa* 407 was provided by V. T. Andriole, New Haven, Conn. This strain is known to be inhibited synergistically by the combination of gentamicin and carbenicillin (1). The microorganism was lyophilized; for each experiment a fresh vial was used.

The synergistic bactericidal activity of the combination of gentamicin and carbenicillin against *Pseudomonas* 407 was determined in Trypticase soy broth. A 1-ml amount of an overnight broth culture of *Pseudomonas* 407 was added to 99 ml of broth to give a concentration of about 10⁶ colony-forming units (CFU)/ml. Appropriate antibiotic solutions were added to a series of flasks to produce the following final concentrations (in micrograms per milliliter): gentamicin, 2 or 10; carbenicillin, 25 or 100; a combination of gentamicin, 2, plus carbenicillin, 25; or a combination of gentamicin, 2, plus carbenicillin, 100.

Flasks were incubated at 37°C without shaking; at various times after incubation 1-ml portions were removed, and the number of CFU of *Pseudomonas* was determined in agar pour plates. Synergistic bactericidal activity was defined as a 100-fold increase in killing by the combination as compared with the most effective single agent after 24 h of incubation.

**Studies of lethality.** Rats were infected intraperitoneally with graded 1.0-ml inocula containing *Pseudomonas* 407; deaths were scored daily and the animals were observed for 15 days. Commercially supplied gentamicin sulfate (Schering Corporation Ltd., Pointe Claire, Quebec, Canada) and disodium carbenicillin (Ayerst Laboratories, Montreal, Quebec, Canada) were dissolved in water to the required concentration immediately prior to injection. The amounts of drug administered are expressed in milligrams per kilogram per dose. Antibiotic doses were administered by the intramuscular route in volumes of 0.2 ml into the hind limb at 2, 10, 20, 28, 34, 44, 52, 56, and 68 h after bacterial challenge. Attempts were made to alternate injection sites. Doses of the antibiotic combination were administered concurrently, but each agent was injected into a different hind limb. The results were analyzed statistically for significance by the fourfold, chi-square contingency table method.

**Quantitative bacteriology.** Blood was obtained by cardiac puncture from groups of three rats in the different treatment groups at 1, 4, 24, 48, and 72 h after infection. The number of CFU per milliliter of *Pseudomonas* was determined by incorporating portions of 10-fold dilutions of blood in pour plates. Immediately after blood had been obtained for quantitative culture, the rats were sacrificed and the enumeration of *Pseudomonas* in the peritoneal cavity was carried out by using the method of Cohn (9).

**RESULTS**

Synergistic bactericidal activity in vitro of gentamicin and carbenicillin against *Pseudomonas* 407. Synergy in vitro was demonstrated for both combinations of gentamicin and carbenicillin against *Pseudomonas* 407. The flask containing the combination of 2 µg of gentamicin per ml and 100 µg of carbenicillin per ml contained no viable organisms at 24- and 48-h incubation periods (Fig. 1).

**Virulence of *Pseudomonas* 407 for normal and neutropenic rats.** A total of 15 of 20 normal rats infected with 3 × 10⁶ bacteria died of infection (Fig. 2A). All deaths occurred within the first 24 h after bacterial challenge. Smaller inocula were nonlethal for normal rats.

Eighteen of twenty neutropenic rats challenged with 3 × 10⁶ organisms died (Fig. 2B). Fourteen of twenty neutropenic rats infected with 3 × 10⁶ *Pseudomonas* died; all deaths occurred between days 2 and 6 postinfection. One-half of the deaths occurred on day 2 postinfection. Only 3 of 20 animals infected with 3 × 10⁶ bacteria died. At autopsy *Pseudomonas* was recovered from heart blood cultures. Pleural effusions were usually noted, and most spleens contained numerous abscesses of 2 to 3 mm in diameter that yielded *Pseudomonas* in pure culture.
The percent mortality of neutropenic rats infected with Pseudomonas was related to the number of organisms employed in the challenge. Neutropenia induced by cyclophosphamide greatly increased the susceptibility of rats to lethal infection. A challenge inoculum of $3 \times 10^6$ Pseudomonas was employed in the subsequent experiments.

Efficacy of antibiotic regimens in neutropenic infected rats. Groups of 30 neutropenic rats received either no therapy, 1.5 or 6 mg of gentamicin per kg, 100 or 400 mg of carbenicillin per kg, or a combination of 1.5 mg of gentamicin plus 100 mg of carbenicillin per kg.

Twenty-two of thirty neutropenic infected control rats (73%) died. Therapy with gentamicin alone was ineffective (Fig. 3A). A total of 24 of 30 animals (80%) treated with 1.5 mg of gentamicin per kg and 21 of 30 (70%) treated with 6 mg/kg died, although the deaths were delayed in the group that received high-dose gentamicin therapy. A total of 15 of 30 rats treated with 100 mg of carbenicillin per kg died (Fig. 3B). None of the animals in this treatment group died during the treatment period in the first 3 days after infection. Although the results suggested a beneficial effect of low-dose carbenicillin therapy, this regimen was not significantly better than the control ($\chi^2 = 3.45; 0.1 > P > 0.05$). A total of 4 of 30 (13%) and 6 of 30 (20%), respectively, animals that received either high-dose carbenicillin or combined low-dose therapy died. Both of these forms of therapy were significantly better than the control ($P < 0.01$) and also were better than 100 mg of carbenicillin per kg ($P < 0.01$). No rat treated with high-dose carbenicillin or combined low-dose therapy died during the first 3 days after infection. The results indicate that the combination of carbenicillin and gentamicin exhibited antibiotic synergy both in vitro against Pseudomonas 407 and in vivo against infection caused by this organism in neutropenic animals.

Effect of antibiotic regimens on bacteremia and peritoneal lavage cultures. Pseudomonas was present in the blood of untreated animals at 1 h after infection, and the degree of bacteremia rose progressively over the first 24 h of infection (Fig. 4). Too few control animals survived past 24 h to allow later cultures. Bacteremia was detected at some time in all other treatment groups. At 48 and 72 h after infection, rats treated with 100 mg of carbenicillin per kg showed persistent bacteremia of $10^2$ to $10^3$ colony-forming units/ml.
10^6 CFU/ml of blood. Blood cultures of rats treated with 400 mg of carbenicillin per kg were sterile at 24 and 48 h, although one of three treated with this dose was bacteremic at 72 h. All blood cultures of rats treated with combined carbenicillin and gentamicin were sterile at 24 h after infection and at later times.

Peritoneal lavage cultures correlated well with the degree and persistence of bacteremia (Fig. 5). The number of Pseudomonas present in the peritoneum at zero time (immediately after infection) has been plotted as a theoretic value, assuming complete recovery and viability of the inoculum in the washout fluid. The mean number of CFU recovered from the peritoneum of control rats rose between 4 and 24 h after infection to greater than 10^9. Peritoneal counts of Pseudomonas diminished progressively in rats treated with 100 mg of carbenicillin per kg, but organisms were recovered at all time intervals. Both 400 mg of carbenicillin per kg and combined carbenicillin-gentamicin regimens sterilized the peritoneal fluid. This effect was observed at 24 h after infection in rats treated with the antibiotic combination and at 48 h in rats treated with high-dose carbenicillin.

**DISCUSSION**

There are some important similarities between this model infection and Pseudomonas infection in a human neutropenic host. Normal rats are highly resistant to infection with Pseudomonas 407. In our hands an intraperitoneal inoculum of 3 x 10^6 organisms caused 70% mortality of control animals within 24 h, but smaller inocula were nonlethal. During the period of severe neutropenia 100-fold fewer organisms produced equivalent mortality. Quantitative bacteriological studies in neutropenic infected control rats showed increasing numbers of Pseudomonas in the peritoneum and blood between 4 and 24 h after infection, manifestations of uncontrolled sepsis. Splenic abscesses were noted, indicating the additional presence of multiple parenchymal foci of bacterial proliferation. Native resistance to Pseudomonas in healthy individuals, increased susceptibility during periods of granulocytopenia, and bacteremic infection with metastatic abscesses are features of the human interaction with this pathogen.

Antibiotic therapy was administered early (2 h) after the onset of infection, since protective effects had been previously shown when treatment was instituted at 2 h, but not when delayed to 6 h after Pseudomonas 407 infection of nonneutropenic rats (1). The critical importance of early antibiotic therapy to a successful outcome in infected neutropenic humans has been well documented (21).

Despite a prompt institution of therapy, gentamicin alone in doses of 1.5 or 6 mg/kg failed to
improve the overall survival of neutropenic infected rats. Rats treated with high doses of gentamicin showed short-term benefit only, with no deaths observed during the treatment period, whereas the course of infection was completely unaffected by the lower dose. The reason for the lack of efficacy of gentamicin in the neutropenic rat is not entirely clear and requires further study; however, preliminary data suggest an important pharmacological reason for drug failure in this model. Infected neutropenic rats showed concentrations of detectable gentamicin in serum for only 1.5 to 2 h after an intramuscular dose of 6 mg/kg (R. E. Scott and H. G. Robson, unpublished data). Similar observations were reported by Luft and Kleit; gentamicin was barely detectable in rat sera at 3 h after a single subcutaneous injection of 10 mg/kg (17). The half-life of gentamicin in rat serum was estimated to be about 30 min (17). The failure of gentamicin to cure some patients with Pseudomonas sepsis has also been attributed to inadequate serum and tissue levels that may occur at various times after intermittent drug administration (13, 18). Shorter intervals between doses, larger doses, or the continuous administration of gentamicin as suggested by Bodey et al. (3) may provide improved therapeutic results; such approaches can be assessed using this model. Other factors may also be implicated, however, including antibiotic inactivation by purulent material (8) and deficient phagocytosis and intracellular killing of gentamicin-damaged microorganisms during neutropenia (13).

In contrast to gentamicin, carbenicillin in the large dose employed (400 mg/kg) was highly effective. Despite its short duration, this therapeutic regimen significantly reduced mortality, eradiated Pseudomonas from the original peritoneal site of infection, and sterilized the blood. Although the lower dose employed (100 mg/kg) did not significantly reduce eventual mortality, deaths were prevented during therapy, the number of viable Pseudomonas present in the peritoneum was reduced, and the magnitude of bacteremia did not increase between 24 and 72 h of infection. The lack of overall benefit of this regimen may be attributable to the eventual multiplication of persisting Pseudomonas when treatment was discontinued, despite survival of the animals to the stage where return of neutrophils to the circulation could be predicted. The effect of more prolonged carbenicillin therapy at the 100-mg/kg dose level remains to be explored. A similar dose response relationship for nonneutropenic rats infected with Pseudomonas 407 has been observed (2).

Although low-dose gentamicin and carbenicillin regimens were largely ineffective when employed singly, the combination was highly active. We interpret this as evidence of in vivo drug synergy; this phenomenon has previously been documented in nonneutropenic rats (1, 2). Our finding is in agreement with the recent work of Lumish and Norden in a similar neutropenic rat model (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 201, 1975). These authors also noted the lack of efficacy of gentamicin employed as the sole antimicrobial agent under similar conditions. In another study Saslaw et al. failed to show in vivo synergy in neutropenic monkeys infected with Pseudomonas (20).

An equivalent reduction in mortality was observed in rats treated with high-dose carbenicillin, but the effect of the antibiotic combination on bacteremia and peritoneal fluid cultures was even more striking; all cultures were sterile at 24 h and subsequent times. Recent studies by Klastersky et al. have shown that an improvement in response rates of gram-negative infection occurring in patients with neoplasia can be obtained with combinations of antibiotics when the causative agents can be shown to be synergistically inhibited in vitro (14). The number of severely neutropenic patients infected with Pseudomonas was small (14–16); improved survival of this subgroup treated with the carbenicillin-gentamicin combination as compared with either drug alone could not be demonstrated (16).

Our studies provide further evidence to support the view that the initial therapy of Pseudomonas sepsis in the neutropenic patient should include both carbenicillin and gentamicin. It will be important to extend these observations to infection in neutropenic rats caused by strains of Pseudomonas that show partial synergy or no synergy in vitro to the carbenicillin-gentamicin combination. The combination has previously been shown to be effective in vivo in nonneutropenic rats infected with a strain of Pseudomonas that showed only partial synergy in vitro (1). This model infection may also prove valuable in assessing the efficacy of agents such as tobramycin, amikacin, and ticarcillin in infections caused by Pseudomonas resistant to carbenicillin or gentamicin.

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ADDENDUM

Since submission of this manuscript, the work of R. M. Lumish and C. W. Norden has been published (J. Infect. Dis. 133:538–547, 1976).

LITERATURE CITED


