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Comparative Study of Anti-Pseudomonas Activity of Azlocillin, Mezlocillin, and Ticarcillin

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The anti-pseudomonas activities of azlocillin and mezlocillin were compared with that of ticarcillin. We measured the minimal inhibitory and minimal bactericidal concentrations of the three drugs against 20 different strains of *Pseudomonas aeruginosa* and found significantly lower values for azlocillin than for the other two drugs. We then infused 5 g of each drug into 10 volunteers on three consecutive days and determined the serum levels of the three antibiotics at 1-h intervals from 1 to 6 h after injection. The levels of azlocillin were significantly higher than those of mezlocillin and ticarcillin (at 1 h: 236.55 μg/ml ± 12.9 for azlocillin, 192.45 μg/ml ± 28.8 for mezlocillin, and 131.5 μg/ml ± 10.9 for ticarcillin). The inhibitory and bactericidal activities of the sera obtained 1 and 6 h after the injection against the same 20 strains of *P. aeruginosa* demonstrated a significantly greater anti-pseudomonas activity of azlocillin when compared with mezlocillin and ticarcillin; mezlocillin and ticarcillin had approximately the same activity. The mean values for bactericidal activity against the strains tested were 1/32 for azlocillin, 1/8 for mezlocillin, and 1/8 for ticarcillin. Azlocillin thus appears to be a promising anti-pseudomonas drug and should be tested in clinical trials.

The prognosis of infections caused by *Pseudomonas aeruginosa* has been dramatically improved by the clinical use of carbenicillin, even in patients with impaired defense mechanisms, such as neutropenic patients with leukemia. Nevertheless, carbenicillin given in a high dosage has serious side effects, such as alteration of the ionic balance with hypokalemia and increased anion gap; in addition, large doses of carbenicillin represent an important extra load of sodium, which makes the drug difficult to use in patients with cardiac or renal impairment. Infections caused by *P. aeruginosa* remain an important cause of morbidity and mortality. In a recent series of tests, bacteremia due to this type of organism was associated with a 63% rate of mortality. Those patients with a severe underlying disease and those with respiratory tract infection had a particularly poor prognosis (2). Although the high rate of mortality which results from *P. aeruginosa* is due to the severe underlying disease in most patients, the development of resistance to carbenicillin is also an important factor in explaining clinical failures. Thus, some improvement can be expected from new modalities of antimicrobial therapy. Among these are combinations of carbenicillin or ticarcillin with aminoglycosides (1) and a number of recently developed anti-pseudomonas drugs, including azlocillin and mezlocillin, which have more favorable microbiological and pharmacological characteristics than carbenicillin and ticarcillin.

These new semisynthetic penicillins are highly active against pseudomonas in vitro. In addition, they are easier to manipulate from the ionic point of view. Whereas 1 g of carbenicillin imposes a sodium load of 108 mg, 1 g of either azlocillin or mezlocillin imposes a sodium load of only 45.8 and 39.6 mg, respectively. In the present study, we compared azlocillin and mezlocillin to ticarcillin not only in vitro, but also in human volunteers who received these drugs and whose sera were tested for antibacterial activity against *P. aeruginosa* strains isolated from clinical material.

MATERIALS AND METHODS

Azlocillin and mezlocillin were supplied as sterile powders by Bayer, and ticarcillin was supplied by Beecham Laboratories.

The anti-pseudomonas activity of the three drugs was studied in vitro against 20 strains of *P. aeruginosa* recently isolated from patients hospitalized at the Institut Jules Bordet. The susceptibility of these strains to azlocillin, mezlocillin, and ticarcillin was measured by the Kirby-Bauer method (3). The minimal inhibitory concentrations (MIC) of these drugs for these strains were determined in Trypticase soy broth (Biomérieux) by a twofold dilution technique, using an overnight bacterial suspension in Trypticase soy broth diluted to a final concentration of 10^6 viable

microorganisms per ml. The MIC was defined as the concentration at which no visible growth occurred after 18 h of incubation at 37°C. All tubes which remained clear were subcultured on blood agar plates to determine the minimal bactericidal concentration (MBC), defined as the lowest concentration of the drug yielding less than five colonies on an overnight subculture at 37°C.

Five grams each of azlocillin, mezlocillin, and ticarcillin, diluted in 50 ml of sterile saline, was infused over a 15-min period into 10 volunteers. These were patients hospitalized at the Institut Jules Bordet; none had renal or liver function impairment. Each drug was given on a separate day, and the sequence of administration of the study drugs was assigned randomly for each patient. Serum samples were obtained 1 and 6 h after the onset of the infusion in every patient; in addition, two other samples of blood were obtained from each volunteer at various times between h 1 and 6. The concentrations of azlocillin, mezlocillin, and ticarcillin in these samples were measured by the cup plate method of Bennett et al. (4). Nutrient agar (Difco) and spores of *Bacillus subtilis* were used as the medium and the test microorganism, respectively. The inhibitory activity of the sera obtained 1 and 6 h after the administration of azlocillin, mezlocillin, and ticarcillin against the 20 strains of *P. aeruginosa* was determined by a twofold dilution technique; a pool of serum was used as diluent, and the microorganisms were added to obtain a final concentration of 10^6 colony-forming units of *P. aeruginosa* per ml. The highest dilution of serum that failed to support any growth as indicated by subcultures on blood agar plates was considered to represent the bactericidal activity of the serum.

RESULTS

The calculated MIC and MBC of the three drugs for cumulative percentages of the strains are shown in Table 1. Based on either bacteriostatic or bactericidal end points, azlocillin was the most active of the three drugs. It must be stressed that five strains of *P. aeruginosa* included in this evaluation were resistant to carbenicillin by the Kirby-Bauer method. The MIC of carbenicillin for these strains was 125 μg/ml; in contrast, the MIC for azlocillin was ≤15.6 μg/ml and that for mezlocillin was ≤62.5 μg/ml.

Serum levels measured 1 to 6 h after injection of azlocillin, mezlocillin, or ticarcillin are shown in Fig. 1. The mean levels at h 1 were, respectively, 236.5 μg/ml ± 12.9 for azlocillin, 192.4 μg/ml ± 28.8 for mezlocillin, and 131.5 μg/ml ± 10.9 for ticarcillin. Moreover, the blood levels of azlocillin were significantly higher during the entire 6-h experiment than those of mezlocillin or ticarcillin. The level of azlocillin 6 h after the injection was still 44.3 μg/ml ± 6.4. On the other hand, the levels of mezlocillin and ticarcillin were approximately the same 3 and 6 h after injection. At h 6, the levels of mezlocillin and ticarcillin were, respectively, 17 μg/ml ± 4.6 and 17.8 μg/ml ± 8.

TABLE 1. *Cumulative percentages of strains inhibited and killed by azlocillin, mezlocillin, and ticarcillin*

DRUG	CALCULATED MIC/MBC, μg/ml, for per cent of strains							
	25		50		75		100	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
AZLOCILLIN	4.5	5.6	8.4	10	11.5	15.3	31.2	62.4
MEZLOCILLIN	16.5	22.5	23	39	31.2	56	62.4	125
TICARCILLIN	11.2	20.3	18.4	28	27	44.5	62.4	250

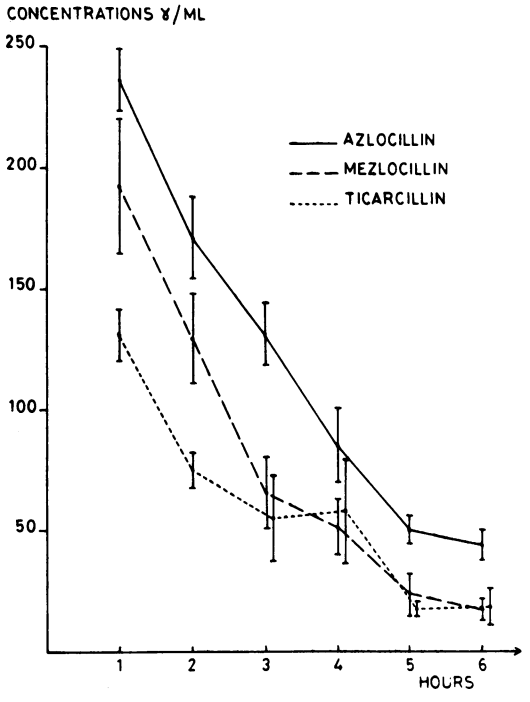

FIG. 1. *Serum levels obtained after the injection of 5 g each of azlocillin, mezlocillin, and ticarcillin.*

The mean inhibitory and bactericidal dilutions of the sera of the 10 volunteers studied against the 20 strains of *P. aeruginosa* are shown in Table 2. These mean values were identical to the corresponding median values. The analysis of variance shows that there are significant differences between azlocillin and the other two drugs ($P < 0.001$). The difference between azlocillin and mezlocillin or ticarcillin is important; the mean inhibitory activity of the serum after the injection of azlocillin was 1/32 at 1 h and 1/8 at 6 h. The mean bactericidal activity after the injection of azlocillin remained 1/32 at 1 h and was 1/4 at 6 h. On the other hand, injection of both mezlocillin and ticarcillin resulted in the

TABLE 2. *Mean values of inhibitory and bactericidal activities of 1- and 6-h sera against 20 strains of P. aeruginosa*

	Inhibitory dilutions		Bactericidal dilutions	
	1 hour	6 hours	1 hour	6 hours
AZLOCILLIN	1/32 (1/16 - 1/64)	1/8 (1/4 - 1/8)	1/32 (1/16 - 1/64)	1/4 (1/4 - 1/8)
MEZLOCILLIN	1/8 (1/8 - 1/32)	1/2 (<1/2 - 1/4)	1/8 (1/4 - 1/16)	<1/2 (<1/2 - 1/4)
TICARCILLIN	1/8 (1/8 - 1/16)	1/2 (1/2 - 1/4)	1/8 (1/8 - 1/16)	1/2 (1/2 - 1/4)

Azlocillin vs {Mezlocillin / Ticarcillin} : $p < 0.001$

same inhibitory activities at 1 (1/8) and 6 (1/2) h. The bactericidal activity of the serum 6 h after the injection of mezlocillin was somewhat lower than that obtained after the administration of ticarcillin, i.e., <1/2 and 1/2, respectively; there was no statistically significant difference between these values.

DISCUSSION

It appears from our study that mezlocillin is quite comparable to ticarcillin. The MICs and MBCs of these agents for 20 strains of *P. aeruginosa,* the mean serum levels after intravenous infusion of 5 g of these drugs, and the inhibitory and bactericidal activities of serum obtained 1 and 6 h after onset of the infusion were similar. These findings are in accordance with those reported by others (5, 8, 9, 12) who found a similar in vitro activity of mezlocillin and ticarcillin against *P. aeruginosa;* the concentration of mezlocillin that inhibited 75% of strains studied by others was quite similar to our findings. We also found a twofold difference between the MICs and MBCs of mezlocillin. Azlocillin, on the other hand, seems to be more active against *P. aeruginosa* than mezlocillin or ticarcillin both in vitro and in vivo, as reported previously by Stewart and Bodey (11). The concentration of azlocillin which killed 75% of their strains (12.5 μg/ml) (11) was similar to the concentration needed to kill 75% of our strains (11.5 μg/ml). Bywater et al. obtained even more favorable results. About 4 μg of azlocillin per ml inhibited 75% of their strains of *P. aeruginosa* (6). It must be stressed here that the MBCs of azlocillin are not different from the MICs by more than one dilution and that this is a striking difference in comparison to mezlocillin or ticarcillin, whose MBCs for 75% of the strains are about twice as high as the MICs. Strains resistant to carbenicillin showed high susceptibility to azlocillin (MICs, <15.6 μg/ml) and were still susceptible to mezlocillin (MICs, ≤62.5 μg/ml). Bodey and Pan found similar data provided the MIC of carbenicillin was not higher than 400 μg/ml (5).

The superiority of azlocillin as compared to mezlocillin and ticarcillin is not only the consequence of its better in vitro activity. The infusion of 5 g of azlocillin resulted in higher serum levels than those obtained after the infusion of the same dose of mezlocillin or ticarcillin, and this advantage of azlocillin remained significant throughout the 6-h period after the injection of the drugs. It is not surprising, therefore, that in our study azlocillin appeared to be at least twice as active as mezlocillin or ticarcillin when the inhibitory and bactericidal activities of the sera of treated patients were compared. The mean inhibitory and bactericidal activities were strikingly different at 1 and 6 h for azlocillin as compared to mezlocillin or ticarcillin. The relatively narrow margin between bacteriostatic and bactericidal end points for azlocillin was confirmed by the study of the antibacterial activity of the serum.

The level of antibacterial activity of sera of patients treated with an antibiotic reflects both the microbiological and the pharmacological properties of the drug tested. The significance of the serum activity for the outcome of clinical sepsis has been well demonstrated in bacterial endocarditis (7) and in other severe infections (10).

In conclusion, azlocillin appears to be an extremely active anti-pseudomonas drug. Its advantage over mezlocillin or ticarcillin results from its higher in vitro activity and from its higher serum levels after administration of identical doses. The promising efficacy of relatively low doses of azlocillin justifies further clinical studies comparing azlocillin and ticarcillin in severe infections caused by *P. aeruginosa,* since our results, and especially the serum activity data, suggest that azlocillin might be more active than mezlocillin or ticarcillin at the same dosage. The major advantage of azlocillin might prove to be an adequate efficacy even with the administration of relatively low doses. Lower doses may be expected to result in a reduction of the serious side effects frequently associated with the high doses of carbenicillin or ticarcillin needed to treat *P. aeruginosa* infections. In addition, azlocillin appears to be active against some carbenicillin-resistant strains of *P. aeruginosa.*

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