Effect of Dose on Pharmacokinetics and Serum Bactericidal Activity of Mezlocillin

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Mezlocillin is subject to dose-dependent pharmacokinetics. Previous studies have examined the pharmacokinetic but not the pharmacodynamic aspects of this effect. The pharmacokinetic disposition of mezlocillin was determined in eight healthy volunteers in a randomized, crossover fashion after single infusions of 50 and 80 mg of mezlocillin per kg of body weight. Plasma and urine were assayed with a specific high-pressure liquid chromatography assay and analyzed by noncompartmental methods. Pharmacodynamic (bactericidal) effects were evaluated from serial serum bactericidal titers obtained after each dose by using the area under the bactericidal activity curve method. The mean mezlocillin total body clearance decreased from 203.6 ± 36.2 ml/min after the 50-mg/kg dose to 171.7 ± 42.1 ml/min after the 80-mg/kg dose (P = 0.01). The decreased clearance was reflected by a decrease in nonrenal clearance only (108.9 ± 20.0 to 77.9 ± 23.5 ml/min, respectively; P = 0.001). Mean areas under the curve for concentration in plasma versus time normalized to the 50-mg/kg dose were 314 ± 73 and 375 ± 64 µg·h/ml for the low and high doses, respectively (P = 0.01). No significant changes were observed in the steady-state volume of distribution or elimination half-life. Mean areas under the bactericidal activity curve were 100 ± 77 and 244 ± 143 for the 50- and 80-mg/kg doses, respectively. The decrease in mezlocillin clearance and the disproportionate increase in the area under the curve for concentration in plasma versus time, coupled with the observed prolonged bactericidal effects of the 80-mg/kg dose, lend support for administration of mezlocillin at a higher dose less frequently (e.g., 5 g every 8 h). Clinical trials with the higher-dose regimen are warranted to validate these observations.

Mezlocillin is an acylureidopenicillin with a broad antimicrobial spectrum and proven clinical efficacy in serious infections (11, 13). Mezlocillin, like other acylureidopenicillins, is subject to dose-dependent pharmacokinetics. As the dose is increased, disproportionate increases in concentrations in plasma and areas under the curve (AUC) for concentration in plasma versus time result (3, 8). Previous studies have evaluated the dose dependency of mezlocillin pharmacokinetics after single 1-, 2-, and 5-g doses (3, 8) and in multiple doses of 4 g every 6 h versus 5 g every 8 h (4). To date, no attempt has been made to directly correlate the saturable elimination properties of mezlocillin with the pharmacodynamic (bactericidal) activity of this agent to assess the feasibility of administering larger doses with longer dosing intervals. Such an approach to dosing mezlocillin would be more convenient to administer and less costly. The purpose of this investigation was to determine the extent of concentration-dependent pharmacokinetics of mezlocillin at clinically used doses and to evaluate the impact of the pharmacokinetics on the serum bactericidal activity of mezlocillin over time.

MATERIALS AND METHODS

Subjects. Eight healthy subjects participated in the study after written informed consent was obtained. The subjects were four males and four females with an average age of 27 years (range, 24 to 36 years) and an average weight of 69 kg (range, 46 to 87 kg). For all of the subjects, a complete medical history, physical examination, and laboratory profile including complete blood count with differential, platelet count, direct Coombs test, serum electrolytes, serum bilirubin, serum glutamyl oxalacetic transaminase, serum glutamic pyruvic transaminase, lactate dehydrogenase, triglycerides, cholesterol, serum creatinine, blood urea nitrogen, urinalysis, and 24-h creatinine clearance were done before the study and upon its completion. Individuals under 21 years of age or older than 60 years of age, females known or thought to be pregnant, individuals taking any medications, or those with a known or postulated allergy to beta-lactam antibiotics were excluded.

Drug administration. Each subject received 50 and 80 mg of mezlocillin per kg of body weight randomly, separated by a 1-week washout period. Mezlocillin was administered in 50 ml of 5% glucose in water over 25 min at a constant rate with an infusion pump. Mezlocillin was supplied by Miles Pharmaceuticals (lot numbers BTL-3 and BTW-3).

Sample collection. Blood samples (5 ml) for pharmacokinetic analysis were obtained from the arm contralateral to that used for the drug infusion at the following times: 0, 0.4, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 h. Samples were immediately placed on ice and centrifuged, and the plasma was removed and frozen at −70°C until assayed. Urine samples were obtained from hourly collections over the first 6 h and from pooled collections over 6 to 8, 8 to 12, and 12 to 24 h. Additional blood samples (2.5 ml) for bactericidal analysis were collected before administration of the drug and at 0.4, 4, 6, 8, 12, and 24 h after infusion. These samples were allowed to clot, placed on ice, and centrifuged, and the serum was removed and frozen at −70°C until bactericidal analysis was performed.

Analytical methods. Plasma and urine were assayed for mezlocillin by using a high-pressure liquid chromatography assay developed in our laboratory. Briefly, the assay was done with an M45 pump (Waters Associates, Inc.), a 481
Lambda Max variable-wavelength detector (Waters), and a C18 5-μm (4.6 mm by 25 cm) column (Alltech Associates, Inc.). The mobile phase consisted of 35% acetonitrile and 0.2% H3PO4 adjusted to a pH of 3.0. The flow rate was 1 ml/min, with a sensitivity of 0.05 absorbance units (full scale) × 5 mV, with a chart speed of 0.25 cm/min, and a detector setting of 220 nm. Concentrations of 0.5 μg/ml were detectable in plasma without extraction. The detection limit for urine is 1.0 μg/ml. The interday variability of mezlocillin concentrations in plasma was 8.5% at 45 μg/ml and 9.5% at 4.5 μg/ml, while the intraday variability was less than 6%.

Bactericidal analysis. The bactericidal activity of mezlocillin was determined in serum against *Staphylococcus aureus* ATCC 29213, which required an MBC of 2 μg of mezlocillin per ml by standard broth macrodilution methods (9).

Serum bactericidal titers were determined by the method of Reller and Stratton (12). Serum bactericidal titers were determined in triplicate with microtiter trays by the following procedure. Cation-supplemented Mueller-Hinton broth (50 μl) was added to all tray wells except for those in column 1. A 50-μl serum sample was then added to columns 1 and 2. Serial dilutions for columns 2 through 12 were done with an eight-pronged 50-μl microdiluter. A few colonies from a 24-h *S. aureus* culture were placed in Trypticase soy broth (BBL Microbiology Systems) and incubated for 2 h at 37°C. This bacterial suspension was diluted to a turbidity equivalent to a 0.5 McFarland standard and then was further diluted 1/100 (vol/vol) with cation-supplemented Mueller-Hinton broth, producing a final inoculum of approximately 5 × 10^7 CFU/ml. Quantitation of the inoculum was accomplished by adding 10 μl of the final inoculum mixture to 9.9 ml of normal saline, plating 10 μl of this mixture onto blood agar plates, incubating for 24 h at 37°C, and then multiplying the number of colonies by the dilution factor. Bacterial inoculum (50 μl) was added to all tray wells, the trays were incubated for 24 h at 37°C, and all wells showing no visible growth were plated onto blood agar plates with a 10-μl multipoint inoculator and incubated for an additional 24 h at 35°C. The serum bactericidal titer was defined as the highest dilution which produced a 99.9% reduction in the inoculum.

Pharmacokinetic analysis. Data on mezlocillin concentra-

tion in plasma were analyzed by noncompartmental methods by using the PROPHET computer resource (6, 7). Total body clearance (CL) was estimated by the formula CL = dose/AUC_0−∞, where AUC_0−∞ is the AUC for concentration in plasma versus time from time zero to infinity. AUC was calculated by using the log trapezoidal rule and extrapolated to infinity by dividing the last value for concentration in plasma by the elimination rate constant (derived from at least the last three values for concentration in plasma).

Volume of distribution at steady state (Vss) was determined by using the formula Vss = [dose (AUMC)/(AUC_0−∞)]^2, where AUMC is the area under the first moment of the curve for concentration in plasma versus time (7). A correction was made for the infusion by subtracting [(t/2)(dose/AUC)] from the values for volume of distribution at steady state, where t is the duration of infusion. Half-life was calculated by dividing the natural logarithm of 2 by the elimination rate constant.

Renal clearance (CLR) was calculated by using the formula CLR = A_t/AUC_0−∞, where A_t is the total amount of mezlocillin recovered in the urine. Nonrenal clearance was estimated by subtracting the renal clearance from the total body clearance.

Pharmacodynamic analysis. Pharmacodynamic analysis was accomplished by using the area under the curve for bactericidal activity (AUBC) (2). The AUBC, a dimensionless value, was determined by plotting the reciprocal of serum bactericidal titers versus time and applying the trapezoidal rule.

Statistical analysis. Statistical significance was determined by using the paired t test on mean data. A P value of 0.05 or less was considered significant.

RESULTS

Pharmacokinetic analysis. Curves for the mean concentration in plasma versus time for the low-dose regimen and for the high-dose regimen normalized to 50 mg/kg were plotted (Fig. 1). For both doses, mezlocillin concentrations were below the detectable limit (0.5 μg/ml) in plasma at 12 and 24 h after the dose. The mean pharmacokinetic parameters (± standard deviation) for both doses were calculated (Table 1). The mean total body clearance of mezlocillin was 203.6 ± 36.2 ml/min with the 50-mg/kg dose and decreased significantly to 171.7 ± 42.1 ml/min with the 80 mg/kg dose (P, 0.01). The decrease in total body clearance was reflected entirely by a decrease in nonrenal clearance from a mean of 108.9 ± 20.0 to 77.9 ± 23.5 ml/min with the low and high doses, respectively (Table 1; P, 0.001). Mean renal clearance was essentially unchanged (Table 1).

The AUC for concentration in plasma versus time also increased disproportionately as a consequence of decreased total body clearance (Table 1). Mean AUC for the 50-mg/kg dose was 314 ± 73 μg · h/ml compared with a mean of 600 ± 102 μg · h/ml with the 80-mg/kg dose. If the kinetics of mezlocillin were linear, the AUC with the higher dose would be expected to increase to roughly 1.6 times that of the low dose. Our results reveal a twofold increase in AUC with the high dose. The AUC with the high dose normalized to 50 mg/kg was 375 ± 64 μg · h/ml and was significantly different from the AUC with the low dose (Table 1; P, 0.01). Elimination half-life increased with the high dose, but the difference did not reach statistical significance (Table 1). Mean steady-state volume of distribution did not change appreciably with the change in mezlocillin doses (Table 1).

Bactericidal analysis. Mean reciprocal serum bactericidal activity of mezlocillin versus time for both doses was plotted...
TABLE 1. Mezlocillin pharmacokinetic parameters (mean ± standard deviation) after single 50- and 80-mg/kg dosesa

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>CL (ml/min)</th>
<th>CLr (ml/min)</th>
<th>CLNR (ml/min)</th>
<th>t1/2 (min)</th>
<th>Vss (liters)</th>
<th>AUC24h (µg · h/ml)</th>
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</thead>
<tbody>
<tr>
<td>50</td>
<td>203.6 (36.2)</td>
<td>94.6 (25.1)</td>
<td>108.9 (20.0)</td>
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<td>13.9 (4.4)</td>
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<td>80</td>
<td>171.7 (42.1)</td>
<td>93.5 (21.5)</td>
<td>77.9 (23.5)</td>
<td>83.6 (15.1)</td>
<td>13.6 (4.7)</td>
<td>375b (64)</td>
</tr>
</tbody>
</table>

P 0.01 0.85 0.001 0.42 0.44 0.01

a CL, Total body clearance; CLr, renal clearance; CLNR, nonrenal clearance; t1/2, elimination half-life; and Vss, steady-state distribution volume.

For 80-mg/kg dose normalized to 50 mg/kg.

(Fig. 2). Mean reciprocal serum bactericidal titers (± standard deviation) were 53 ± 36 and 128 ± 59 at 25 min, 3.25 ± 1 and 5.0 ± 2 at 4 h, and 1.5 ± 0.5 and 2.25 ± 0.7 at 6 h for the low and high doses, respectively (Fig. 2). A mean reciprocal serum bactericidal titer of 1.4 ± 0.5 was observed at 8 h with the 80-mg/kg dose (Fig. 2), but only one subject had a measurable titer (1:2) at 8 h after the 50-mg/kg dose. There was no detectable serum bactericidal activity at 12 and 24 h with either dose. The mean AUBC (± standard deviation) for the low-dose regimen was 100 ± 77 compared with a mean AUBC of 244 ± 143 with the high dose. This difference represents a 2.4-fold increase in bactericidal activity with the high dose (P, 0.03), yet the dose was increased only by a factor of 1.6.

DISCUSSION

Previous investigators have shown that mezlocillin possesses dose-dependent pharmacokinetics (3; 8). The majority of these studies have been carried out by comparing a very low dose (usually 1 or 2 g) with a much higher dose (usually 5 g). Bergan (3) noted a 20% decrease in mezlocillin total body clearance with a 2.5-fold increase in dose. Mangione et al. (8) reported a twofold increase in AUC per dose at a 5-g dose compared with that at a 1-g dose. Significant dose dependence was observed with plasma clearance, renal clearance, and nonrenal clearance (8). With increasing dose, the nonrenal clearance displayed more marked dose dependency than renal clearance (8). In contrast, Aronoff et al. (1) failed to demonstrate significant dose dependency in subjects with normal renal function after single 1-, 3-, and 5-g doses. The reason for the different results of Aronoff et al. are not clear. Each of these investigations evaluated only the pharmacokinetic aspects of mezlocillin dose dependence and made no attempt to correlate this effect with the antibacterial effects of the drug. Our pharmacokinetic results are consistent with those of Bergan (3) and Mangione et al. (8), showing a 16% decrease in total body clearance (entirely reflected in nonrenal clearance) with a 1.6-fold increase in dose. We observed an AUC per dose value of 1.2 for the 80-mg/kg dose compared with that of the 50-mg/kg dose. This result is shown by the lack of congruence of the curves for concentration in plasma versus time when the 80-mg/kg dose values were normalized to 50 mg/kg (Fig. 1).

Colaizzi et al. (4) have recently compared the pharmacokinetics of two multiple-dose regimens of mezlocillin (4 g every 6 h versus 5 g every 8 h) with healthy subjects. A significant difference in AUC per gram was observed for the 5-g regimen compared with the 4-g regimen (72.8 ± 8.8 versus 66.7 ± 10.0 µg · h/ml, respectively). Total body clearance also decreased significantly from 257 ml/min with the 4-g regimen to 232 ml/min with the 5-g regimen. These results are not appreciably different from those obtained in our single-dose investigation. A similar total daily AUC (AUC for a single dose multiplied by the number of doses given in a day) was observed by Colaizzi et al. for both regimens (4). By comparing regimens of 50 mg/kg (approximately 3 g) every 4 h and of 80 mg/kg (approximately 5 g) every 8 h, similar total daily AUC values (1,886 ± 440 and 1,799 ± 306 µg · h/ml, respectively) can be calculated from the results obtained from our investigation. Colaizzi et al. (4) made no attempt to directly determine the pharmacodynamic differences between these two regimens. They did suggest, however, that achievable levels in serum and published MICs of mezlocillin indicate that the two regimens should be similar against more susceptible organisms and that the regimen of 4 g every 6 h might be preferred against relatively resistant organisms.

Comparison of achievable levels of an antibiotic in serum with published MICs, together with consideration of whether the drug possesses a postantibiotic effect, is perhaps the most common means of determining antibiotic dosing regimens. This indirect approach does not take into consideration other important factors that might alter pharmacologic response, such as host defenses, site of infection, and synergy (or antagonism) with other antimicrobial agents that may also be present. By measuring the serum bactericidal titer, investigators have attempted to deal with many of these factors. Previous investigations with serum bactericidal titers have examined the bactericidal activity of antimicrobial agents at peak, trough, or both levels. This approach allows correlation of antibacterial killing at the highest or lowest concentrations in serum, but does not address the changing bactericidal effects occurring over time. The AUBC technique is a relatively new means of

![FIG. 2. Mean reciprocal serum bactericidal titers versus time for mezlocillin doses of 50 (△) and 80 (▲) mg/kg.](http://aac.asm.org/)

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assessing the bactericidal effects of antimicrobial agents over time (2). Measurement of pharmacodynamic activity by methods similar to the AUBC method has been used to assess the immunosuppressive activity of corticosteroids in human plasma (5) and the pharmacodynamic effects of warfarin by measuring AUC for prothrombin activity (10). Using the AUBC approach, we found an enhancement of bactericidal effect beyond what might be predicted if mezlocillin kinetics were linear in nature (Fig. 2). In fact, if the sum of the total daily AUBC for a 50-mg/kg dose given every 4 h is compared with the total daily AUBC for an 80-mg/kg dose given every 8 h, similar total daily AUBCs can be calculated (597 ± 465 versus 732 ± 430, respectively; P, 0.538). Results from this analysis provide support for the regimen of a higher dose less frequently administered.

In summary, the results of this investigation confirm that mezlocillin kinetics are significantly dose dependent with doses that are likely to be used clinically that this effect correlates directly with a prolongation of bactericidal activity with the higher dose. These results taken together lend support for the administration of mezlocillin in doses that are higher than currently recommended but administered less frequently (e.g., 5 g every 8 h). Such a regimen would allow improved convenience with less cost. Clinical trials validating this dosing scheme, compared with standard dosing regimens, are warranted.

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LITERATURE CITED