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Assessment of Serum Bactericidal Activity After Administration of Cefoperazone, Cefotaxime, Ceftizoxime, and Moxalactam to Healthy Subjects

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Bactericidal activity in serum produced after administration of 1-g intravenous doses of cefoperazone, cefotaxime, ceftizoxime, and moxalactam was ascertained in six healthy subjects. The assay organisms were a strain of *Staphylococcus aureus* which was moderately susceptible to the drugs (MBC, 2 to 8 μg/ml) and an isolate of *Escherichia coli* which was highly susceptible (MBC, 0.08 to 0.3 μg/ml). Drug concentrations and bactericidal titers were measured from samples taken for up to 12 h after the dose. No bactericidal activity against the *S. aureus* strain was found at 4 to 6 h and beyond for any of the drugs. Ranking of the in vivo bactericidal activity of the drugs was cefoperazone = cefotaxime > ceftizoxime = moxalactam. Against the *E. coli* isolate, bactericidal activity was present for 8 h for cefotaxime, and for 12 h for the other drugs. Ranking of the drugs in terms of extent and duration of in vivo bactericidal activity versus *E. coli* was moxalactam = ceftizoxime > cefoperazone > cefotaxime. After administration of 1-g doses of these new beta-lactams, bactericidal activity in serum was maintained for 12 h against highly susceptible bacteria. More frequent (6 to 8 h) or higher (≥2 g) dosing appears to be necessary to achieve prolonged serum bactericidal activity against less susceptible isolates (MBC, ≥2 to 8 μg/ml).

The currently available cephalosporins include cefoperazone, cefotaxime, and ceftizoxime and the oxacephem moxalactam. These compounds possess broad-spectrum activity in vitro and are significantly more potent than most other available antimicrobial agents against many of the members of the family *Enterobacteriaceae* (3, 9). Additionally, these newer drugs are generally removed from the body more slowly than older drugs of the same class (1). This slower removal has permitted extension of the dosing intervals of these compounds; for example, generally accepted dosing regimens of ceftizoxime, moxalactam, and cefoperazone range from 1 to 2 g every 12 h to as much as 3 to 4 g every 6 to 8 h. Pharmacokinetic studies have been performed with these drugs documenting the presence of serum concentrations of the drug for as long as 8 to 12 h (7, 13), which supports these extended dosing intervals. In contrast, the relatively short half-life of cefotaxime (1 h) necessitates dosing every 6 to 8 h for most infections, and some clinicians have given this drug as frequently as every 4 h (1, 13).

Sculier and Klatersky have recently demonstrated that peak bactericidal activity in serum in high titers (≥1:16) correlated with improved outcome in infected neutropenic patients being treated with antimicrobial agents (11). These new cephalosporins appear to have a role in the treatment of critically ill, often immunosuppressed, patients. Maintenance of antibacterial activity throughout a dosing interval may be necessary in the management of infection in the neutropenic host, particularly with beta-lactam compounds (4). Therefore, it is important to assess not only the extent but also the duration of bactericidal activity of these long-acting drugs. No studies have assessed and compared the bactericidal activity of these compounds over a time span greater than 6 h.

The purpose of this investigation was to assess and compare bactericidal activity in serum against selected isolates of bacteria after administration of these new cephalosporins to healthy subjects.

MATERIALS AND METHODS

Drugs. Cefoperazone (Cefobid; Roerig), cefotaxime (Claforan; Hoechst-Roussel), ceftizoxime (Cefizox; Smith, Kline, & French), and moxalactam (Moxam; Eli Lilly & Co.) were obtained as 1-g vials of powder for reconstitution. Standard analytical powders were used for determinations of MIC and MBC as well as for the bactericidal titer control sera.

Drug administration. Six healthy adult volunteers (five males and one female; weight, 52 to 84 kg) participated in the study. Each subject received all four drugs in a random cross-over fashion. The subjects were asked to refrain from ingesting alcohol or caffeine for 24 h before and during each study day. On the study day, the subjects received a single 1-g dose of drug reconstituted in 50 ml of 5% glucose in water infused intravenously over 25 min by a constant-rate infusion pump. Blood samples for measurement of bactericidal titers and drug concentrations in sera were obtained before the infusion (blank), just after the end of the infusion, and 4 h (cefotaxime only), 6, 8, and 12 h after the start of the infusion.

Serum bactericidal titer determination. Two organisms were selected for this study. *Escherichia coli* CF 2086 (MBC, ≤0.3 μg/ml) and *Staphylococcus aureus* ATCC 29213 (MBC, 2 to 8 μg/ml). These strains were chosen as representative of bacteria that were highly susceptible (*E. coli*) and moderately susceptible (*S. aureus*) to the drugs tested.

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TABLE 1. Concentrations of cephalosporins and moxalactam (μg/ml) in serum

Drug	Concn. (μg/ml)[a] in serum at:				
	0.5 h	4 h	6 h	8 h	12 h
Cefoperazone	116.0 (27.2)	—[b]	6.7 (1.3)	3.6 (0.8)	ND[c]
Cefotaxime	80.0 (13.0)	1.9 (0.6)	0.6 (0.1)	ND	ND
Ceftizoxime	57.1 (6.3)	—	3.5 (0.3)	1.3 (0.3)	ND
Moxalactam	107.9 (15.6)	—	11.0 (2.9)	6.4 (2.0)	2.2 (0.9)

[a] Values in parentheses are standard deviations.
[b] —, No samples.
[c] ND, Not detectable.

MBCs for these organisms were comparable to 90% MBCs reported in other series (1, 3).

Microtiter trays were used for determinations of bactericidal titer in serum. Serum samples of drug controls were tested in triplicate; blank serum from every subject was run simultaneously as a growth control. The methodology of Reller and Stratton was followed for media, dilutions, inoculum size, and bactericidal endpoint (10).

Drug concentration. Cefotaxime and moxalactam concentrations were determined by specific high-pressure liquid chromatographic techniques developed in our laboratory. Briefly, samples were deproteinated with acetonitrile containing the internal standard (cephalothin for cefotaxime and cefmenoxime for moxalactam). The samples were centrifuged, and the supernatant was evaporated to one-half the original volume and injected directly onto the column. Equipment for each drug assay was as follows. For the cefotaxime assay a Waters model 441 UV detector with a 254-nm wavelength (Waters Associates, Milford, Conn.), an Alltech C_8, 5-μm (4.6 mm by 25 cm) column (Alltech Inc.), and a mobile phase of 0.2% H_3PO_4 buffer–acetonitrile in a ratio of 73:27 pumped at 1 ml/min were used. Desacetylcefotaxime was not detected with this method. For the moxalactam assay a Waters model 450 variable wavelength UV detector with a 270-nm wavelength, an Altex C_{18} ODS 15-cm column (Altex Associates), and a mobile phase of 0.3% H_3PO_4 buffer–methanol–acetonitrile in a ratio of 82:10:8 pumped at 1 ml/min were used. Experiments were carried out at ambient temperature. Peak heights were measured manually, and a standard curve was constructed from the peak height ratios of drug to internal standard versus drug concentration. Variability of the assays was no more than 6%. The lower limit of detection was 0.5 μg/ml for both drugs. Concentrations of desacetylcefotaxime were not quantitated. The reported concentrations of moxalactam are the sum of concentrations of the two active epimers of the drug.

TABLE 2. Bactericidal activity in serum versus *S. aureus* ATCC 29213[a]

Drug	MBC (μg/ml)	Peak titer[b]
Cefoperazone	2	69 (47)
Cefotaxime	2	48 (18)
Ceftizoxime	4	15 (3)
Moxalactam	8	12 (4)

[a] Cefoperazone versus ceftizoxime, $P < 0.025$; cefoperazone versus moxalactam, $P < 0.025$; cefotaxime versus ceftizoxime, $P < 0.005$; cefotaxime versus moxalactam, $P < 0.005$; no significant differences for other comparisons.
[b] Values in parentheses are standard deviations. F ratio for peak titer, 7.43; $P = 0.003$.

TABLE 3. Bactericidal in serum versus *E. coli* CF2086

Drug	MBC (μg/ml)	Peak titer[a] at:				
		0.5 h	4 h	6 h	8 h	12 h
Cefoperazone	0.3	1,877 (1,197)	—[b]	37 (13)	24 (9)	6 (2)
Cefotaxime	0.15	2,560 (1,254)	23 (11)	7 (2)	4 (2)	–[c]
Ceftizoxime	0.08	1,280 (627)	—	85 (33)	43 (17)	12 (4)
Moxalactam	0.15	2,560 (1,254)	—	139 (63)	64 (35)	15 (3)
F ratio		2.9		18	14.7	32.2
P value[d]		0.07		<0.001	<0.001	<0.001

[a] Values in parentheses are standard deviations.
[b] —, No samples.
[c] –, Bactericidal activity (1:2) detected in four of six subjects.
[d] Statistically significant differences at 0.5 h, cefotaxime or moxalactam versus ceftizoxime, $P < 0.025$; 6 h, moxalactam or ceftizoxime versus cefoperazone, $P < 0.01$, cefotaxime versus other three drugs, $P < 0.005$; 8 h, moxalactam or ceftizoxime versus cefoperazone, $P < 0.01$, cefotaxime versus other three drugs, $P < 0.005$; 12 h, ceftizoxime versus cefoperazone, $P < 0.025$, moxalactam versus cefoperazone, $P < 0.005$.

Cefoperazone and ceftizoxime concentrations were measured by a standard bioassay, using *E. coli* CF2086 and *Bacillus subtilis* ATCC 6433, respectively, as the assay organisms (2). Variability of the assay was no more than 10%. The lower limit of detection for both assays was 1 μg/ml.

Statistical analysis. Differences in mean bactericidal titers were analyzed by paired t-test corrected for multiple comparisons (8) and by two-way analysis of variance.

RESULTS

Table 1 illustrates the plasma concentrations obtained at various times for the four drugs.

Table 2 lists the MBCs and bactericidal titers in serum for the four drugs tested against the strain of *S. aureus*. These values are typical for most clinical isolates as reported by others (1, 3). Bactericidal activity in serum was detected only from samples obtained just after the end of the infusion. The peak titers for cefoperazone and cefotaxime were significantly higher than those obtained for either ceftizoxime or moxalactam.

Table 3 lists the MBCs for the four drugs tested against the isolate of *E. coli*. These values are typical of most isolates of this organism which are generally highly susceptible to the newer cephalosporins. There was a great deal of variation in the peak titers obtained from the subjects at the end of the infusion; however, cefotaxime- and moxalactam-containing sera had significantly higher titers at peak than did ceftizoxime. Although titers for cefoperazone were lower than those for cefotaxime or moxalactam, the difference was not statistically significant owing to the variance of the data. The decline in bactericidal activity was most rapid for cefotaxime, although some activity was detected even at 12 h in four subjects. The activity of cefotaxime at 6 h was significantly less than that of the other three drugs. Additionally, the activities from moxalactam and ceftizoxime administration were significantly greater than that found for cefoperazone. At 8 h, again cefotaxime activity was significantly less than the other three drugs, and the activities from ceftizoxime and moxalactam were significantly greater than that from cefoperazone. Bactericidal activity was detected at 12 h in four of six subjects after cefotaxime administration. The activity in serum after administration of both ceftizoxime and moxalactam was significantly greater than that of cefoperazone at 12 h.

DISCUSSION

Bactericidal titers in serum are widely accepted as a measure of in vivo efficacy. The titer generally reflects the relationship between the in vitro activity of the drug and the achievable concentrations in vivo after drug administration (6). Other investigators who have assessed and compared bactericidal activity in serum have generally sampled blood from patients or subjects only at 1 and 6 h after administration of the drug (12). This sampling schedule does not take into account the initial magnitude of activity during and immediately after the infusion. Although this very high titer is short-lived, it is a reflection of the available antimicrobial activity which is then distributed into tissues and other fluids; moreover, the short (6-h) sampling ignores the slower elimination of new compounds from the body. We have demonstrated that all of the available new cephalosporins and moxalactam produce bactericidal activity for as long as 12 h after a 1-g dose, when tested against a highly susceptible organism. The activity found at 12 h after administration of cefotaxime is most likely attributable to the presence of its active metabolite, desacetylcefotaxime or possibly to synergistic activity between low concentrations of cefotaxime and desacetylcefotaxime (5). This metabolite has an elimination half-life of approximately 1.5 to 2.0 h in subjects with normal renal function (1). We have detected concentrations in excess of 0.5 μg/ml of the metabolite for more than 8 h after a dose of 30 mg of cefotaxime per kg in healthy subjects (unpublished observations).

This demonstration of prolonged activity is useful information for designing less costly dosing regimens for the treatment of infection produced by isolates with susceptibility comparable to our test isolate. However, less susceptible organisms such as the *S. aureus* strain may well require more frequent dosing, higher dosing, or both since bactericidal activity was not detected at 6 h for any of the drugs and not even at 4 h for one of the most active drugs, cefotaxime.

Serial sampling of bactericidal titers in this fashion allows assessment of the integration of in vitro bactericidal activity of the drug and the in vivo pharmacokinetic disposition. The highest and most prolonged titers should be found for the drug with the best combination of three factors, antibacterial activity, achievable free drug concentrations, and half-life. The elimination half-lives and serum protein binding for the tested drugs are, respectively, cefoperazone, 2 h and 90%, cefotaxime, 1.1 h and 38%, ceftizoxime, 1.7 h and 31%, moxalactam, 2.5 h and 50% (1).

In our study, although cefoperazone was nearly as active as the other drugs in vitro against the isolate of *E. coli* and its half-life is shorter only than moxalactam the extensive serum protein binding of the drug (90%) appeared to negate these other factors. Ceftizoxime and cefotaxime are not highly protein bound. However, ceftizoxime did not achieve high peak levels in serum, and cefotaxime has a short half-life. Only moxalactam combines a low-to-moderate degree of protein binding (50%) with good in vitro activity and a long half-life to produce high and prolonged bactericidal activity, as noted in our investigation. The four drugs can be ranked with regard to the extent and duration of bactericidal activity. Cefotaxime resulted in reliable activity for only 8 h. The other three drugs produced good activity for 12 h; the highest titers were produced by moxalactam, followed by ceftizoxime and cefoperazone in that order. Moxalactam use is associated with severe coagulopathies when given in doses greater than 4 g per day, (1), and many clinicians have abandoned this drug. Of the remaining three compounds, ceftizoxime produced the most extensive bactericidal activity against our test organism, combining high drug concentrations throughout the sampling period and good antibacterial activity.

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