Comparison of the Chemotherapeutic and Pharmacodynamic Activities of Cephadrine, Cephalothin, and Cephaloridine in Mice

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Cephadrine, a new semisynthetic cephalosporin derivative, is 7[D(-)-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hydrate. The compound has a broad spectrum of antimicrobial activity in vitro. When given subcutaneously to mice, cephadrine was appreciably more effective than cephalothin against infections induced by penicillinase-producing *Staphylococcus*, *Escherichia coli*, *Klebsiella pneumoniae*, or *Enterobacter cloacae* strains. Cephadrine and cephaloridine possessed equivalent activity in treating infections caused by these same three gram-negative bacteria. The mean total bioactivity of cephadrine in the serum of mice peaked within 30 min (59 µg/ml) after parenteral administration and was approximately threefold that of cephalothin (20 µg/ml), but less than that of cephaloridine (83 µg/ml). Nearly all of the administered cephadrine (84%) and cephaloridine (70%) were excreted in the urine as the parent compounds. In contrast, only 47% (total bioactivity) of administered cephalothin was recovered, an amount that represented only 15 to 20% of the parent substance.

The semisynthetic cephalosporins have become of considerable interest to clinicians, who are prescribing these antibiotics widely. The broad spectrum of antibacterial activity demonstrated by these antibiotics and their resistance to degradation by penicillinases have made them particularly well accepted. A new member of this group, cephadrine, is [7-D(-)-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hydrate (1).

Previous studies (H. H. Gadebusch et al., *Progr. Antimicrob. Anticancer Chemother.*, *in press*) had shown that cephadrine possessed a broad spectrum of antibacterial activity, was bactericidal to susceptible organisms, and was resistant to degradation over a wide pH range. In model bacterial infections in mice, cephadrine was effective when given either orally or parenterally.

The present studies sought to compare concentrations of cephadrine in serum, its renal excretion, its metabolism, and its chemotherapeutic efficacy with those of two other cephalosporin antibiotics.

MATERIALS AND METHODS

Antibiotics. Antibiotics were supplied by Eli Lilly & Co. (cephaloridine, lot 4RA61, 975 µg/mg;

sodium cephalothin, lot 4UU71, 945 µg/mg) and by E. R. Squibb & Sons (cephadrine monohydrate, lot NN01OND, 929 µg/mg, and lot AAP-000-O/B-14, PGS, 720 µg/mg).

Animals. Female, white Swiss mice of the CF-1-S strain (Carworth Farms, New City, N.Y.) weighing 18 to 20 g were used in all experiments.

Bacterial cultures. The preparation of the challenge inocula was carried out by a modification of a procedure reported earlier (3).

Efficacy tests. Experimental infections were produced by the intraperitoneal injection of suitably diluted cultures containing approximately 1,000 LD₅₀ (streptococci) or 100 LD₅₀ (staphylococcus and all gram-negative bacteria). All infected mice were given the test drugs in water as subcutaneous injections, half of the dose 1 hr before and half 4 hr after infection, except those infected with streptococci (1 and 5 hr after infection). In duplicate tests, at least three different doses of each antibiotic were administered in two-fold increments to groups consisting of 10 mice each. Mice were observed for 6 days, and the ED₅₀ (median effective dose) was determined by the method of Reed and Muench (6).

Pharmacodynamic studies. Mice used in these studies were housed in metabolism cages without food or water (maximal period 6 hr). They were given doses of 25 or 100 mg/kg of the antibiotics as single subcutaneous injections, and each experiment was conducted in quadruplicate to minimize individual variation among mice.

To determine the level of bioactivity in the serum, mice were bled from the retroorbital sinus at 0.5, 1, and 3 hr after dosing. The serum, representing a pool from three mice for each dose level of antibiotic injected, was immediately separated from the whole blood by centrifugation and was stored at 4 C.

To determine the level of bioactivity in the urine, the total urinary output for six mice in the 6-hr period after dosing was collected in tubes packed in Dry Ice. Both sera and urine were tested for bioactivity on the day of collection by a disc diffusion assay, with *Sarcina lutea* SC 2495 as the test organism. Appropriate samples of urine were retained for metabolic studies.

Metabolic studies. Urine samples from mice given cephradine, cephalothin, or cephaloridine, as well as control samples of cephradine, cephalothin, desacetylcephalothin (a known metabolite of cephalothin), and cephaloridine dissolved in urine, were applied to Whatman no. 1 paper that had been impregnated with potassium citrate buffer at pH 4.5. Samples containing cephalothin, desacetylcephalothin, or cephaloridine were developed in acetone-water (9:1); samples containing cephradine were developed in acetone-water (3:1). After development, the paper chromatograms were scanned under ultraviolet light and bioautographed on agar plates that had been seeded with *S. lutea* SC 2495.

Minimal inhibitory concentration (MIC). Twofold broth dilution tests were used to determine the susceptibility of the selected test organisms to the title compounds.

Statistics. The statistical formula for estimation of the 95% confidence limits of the geometric means was adapted from J. Ciminera, as reported by Miller et al. (5).

RESULTS

Based on the chemotherapeutic activities, cephradine, in addition to being more effective

than cephalothin against both a penicillin-susceptible and a penicillin-resistant *Staphylococcus* strain, was also more active than cephalothin in the treatment of infections caused by gram-negative bacteria, as represented by *Escherichia coli* SC 8294, *Klebsiella pneumoniae* SC 8340, and *Enterobacter cloacae* SC 8236 (Table 1).

The MIC of cephradine against *S. aureus* SC 2399, for example, was 3.1 $\mu\text{g/ml}$. The activity of cephalothin in vitro was approximately 30 times greater than this, yet cephradine was substantially more active in vivo than was cephalothin. The same general trend obtains for *S. aureus* SC 2400, for *E. coli* SC 8294, and for *K. pneumoniae* SC 8340.

In contrast, cephradine and cephaloridine showed equivalent activities in mice challenged with the three gram-negative bacteria tested (Table 1). Against gram-positive organisms, however, cephaloridine was the most active of the three cephalosporins examined.

Measurement of the total bioactivity in the serum of mice given a dose of 100 mg of cephradine per kg subcutaneously showed a mean peak concentration of 59 $\mu\text{g/ml}$ in 30 min, a level three times higher than that in mice given a comparable dose of cephalothin (Fig. 1). One hour after dosing, the concentration of cephradine in the serum was 9.7 $\mu\text{g/ml}$, compared with a concentration of 1.3 $\mu\text{g/ml}$ for total bioactivity in mice treated with cephalothin. Bioactivity in the serum of mice treated with cephaloridine (83 $\mu\text{g/ml}$) was slightly higher than that in mice treated with cephradine. By the third hour after dosing, however, all test compounds yielded serum levels less than 1 $\mu\text{g/ml}$.

In the urine, the total bioactivities of the three cephalosporins were dose-related and were

TABLE 1. Activity of selected antibiotics administered subcutaneously to infected mice

Infecting organism	Cephradine		Cephalothin		Cephaloridine	
	MIC ($\mu\text{g/ml}$)	ED ₅₀ ^a (mg/kg)	MIC ($\mu\text{g/ml}$)	ED ₅₀ (mg/kg)	MIC ($\mu\text{g/ml}$)	ED ₅₀ (mg/kg)
<i>S. pyogenes</i> SC 3862	0.04	5.0 (2.9-8.6)	0.02	1.9 (1.1-3.4)	0.01	0.64 (0.44-0.94)
<i>S. aureus</i> SC 2399 ^b	3.1	18 (12-25)	0.12	115 (58-227)	0.04	3.0 (1.2-7.1)
<i>S. aureus</i> SC 2400 ^c	18.7	91 (73-114)	1.2	176 (109-284)	10	2.3 (1.6-3.4)
<i>E. coli</i> SC 8294	9.4	37 (31-45)	2.4	229 (170-309)	2.4	20 (10-42)
<i>K. pneumoniae</i> SC 8340	9.4	122 (71-211)	1.6	261 (211-324)	2.4	95 (62-145)
<i>E. cloacae</i> SC 8236	6.3	50 (35-72)	9.4	>400	18.7	31 (12-83)

^a Each figure represents the geometric mean calculated from at least two experiments, with the 95% confidence limits shown in parentheses.

^b Penicillin-susceptible.

^c Penicillin-resistant.

highest for cephradine. When bioactivity in the urine was expressed in micrograms per milliliter, the value for cephaloridine was higher than that for cephradine. This difference, however, was more apparent than real, for the mean volume of urine excreted by the mice given cephaloridine (2.0 ml) was significantly less than that excreted by mice given cephradine (2.9 ml).

As judged by chromatographic analysis and bioactivity in the urine (Table 2), virtually all of the cephradine administered (84%) was excreted unchanged within the first 6 hr after dosing, confirming earlier observations (Gadebusch et al.,

in press). Cephaloridine was also nonmetabolized, with approximately 70% of the administered dose excreted in the urine. In contrast, only about one-half of the original bioactivity was found in the urine of mice treated with cephalothin. This activity was present mainly (>75%) as *O*-desacetylcephalothin, and only 15 to 20% occurred as the parent substance.

DISCUSSION

Cephradine is active against both gram-positive and gram-negative bacteria *in vitro* and *in vivo* and should be regarded as a broad-spectrum antibiotic.

Against infections considered difficult to treat, such as those caused by penicillinase-producing staphylococci (*S. aureus* SC 2400), and against infections caused by gram-negative bacteria (*E. coli* SC 8294, *K. pneumoniae* SC 8340, and *E. cloacae* SC 8236), cephradine was consistently more effective than cephalothin, generally by a wide margin. Cephradine and cephaloridine showed comparable activities against gram-negative bacteria.

Only in animals infected with gram-positive organisms was cephaloridine appreciably more effective than cephradine, but the nephrotoxicity of cephaloridine reported in man (2), and suspected here because of the low urinary output, has discouraged its use in the clinic, especially since less toxic alternative therapy is available.

It is noteworthy that, in this study, cephradine was decidedly more effective than cephalothin in

FIG. 1. Concentration of cephradine, cephalothin, or cephaloridine in the serum of mice at various intervals after subcutaneous administration.

TABLE 2. Bioactivity^a in the urine of mice in the 6-hr interval after subcutaneous administration of cephradine, cephalothin, or cephaloridine

Drug	Dosage (mg/kg)	Mean total (µg/mouse)	Mean (µg/ml)	Percentage of administered dose excreted
Cephradine	100	1,687 (1,325-2,193)	3,489 (2,596-5,049)	84 (67-108)
	25	420 (368-481)	816 (669-1,000)	84 (74-96)
Cephalothin	100	1,145 (722-1,839)	2,784 (1,726-4,761)	57 (36-91)
	25	186 (118-295)	321 (193-532)	37 (24-59)
Cephaloridine	100	1,434 (1,369-1,525)	4,516 (271-8,485)	72 (68-75)
	25	340 (177-653)	981 (733-1,322)	68 (35-131)

^a Based on total bioactivity, all values are geometric means of at least two determinations, with the 95% confidence limits shown in parentheses.

vivo, even though the data for activity in vitro favored cephalothin over cephradine. The disparities between the biological activities of cephalothin revealed by its MIC and ED₅₀ values can probably be attributed to its extensive metabolic conversion, which leads to relatively low serum concentrations of this compound.

The activity of cephradine in vivo appears to be considerably greater than had been suggested by the MIC values for this compound. Clinical results have already confirmed the efficacy of cephradine in treating infections due to organisms known to be resistant to this antibiotic in vitro (4).

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