The Penetration of Cephalosporin Antibiotics into Bone (35414)

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The current interest in the treatment of osteomyelitis with broad-spectrum antimicrobial agents has resulted in a number of studies on the ability of various antibiotics to penetrate bone *in vivo* (1–5). Of these studies, only those of Grady and Stern (4) and Evaskus *et al.* (5) were performed on a quantitative scale. These investigators studied the penetration of tetracycline, lincomycin, penicillin, and erythromycin into rat bone.

As a further extention of these studies, this laboratory investigated the bone penetration of three cephalosporin antibiotics, cephalothin, cephaloridine and cephalexin in rats. The widespread clinical use of these compounds has shown their effectiveness as broad-spectrum antibiotics (6–8).

Materials and Methods. Six female Harlan rats that weighed 85 to 125 g were given single subcutaneous doses of 100 mg/kg of ^{14}C-cephalothin[2] (sp act = 0.902 μCi/mg) as a 1.0% solution in physiological saline. At each time interval, a blood sample was taken by cardiac puncture and the rat was sacrificed. The femur and tibial bones were removed, scraped free of muscle and periosteum, split lengthwise, and allowed to air-dry for 24 hr. The dried marrow was removed, the bones were crushed and 20–50-mg aliquots of the crushed bone were prepared for assay by the Kelly *et al.* (9) modification of the Schöniger combustion technique. The radioactivity was assayed by liquid scintillation counting.

Female Harlan rats (nonfasted) that weighed 138 to 230 g were given single subcutaneous doses of cephaloridine or oral doses of cephalexin. Cephaloridine was prepared as a 4.0% solution in physiological saline and cephalexin was prepared as a 1.0% solution in 0.9% saline (100 mg/kg dose) or as a 2.0% suspension in acacia (200 mg/kg dose). Doses of 100 mg/kg of each antibiotic were administered to 2 groups of 15 rats each; doses of 200 mg/kg were administered to 2 groups of 10 rats each. At selected time intervals 1/5 of the rats from each group were bled by cardiac puncture and sacrificed. The bones were prepared as described above except that 500-mg aliquots of the crushed bone samples were extracted with 0.9% saline for 60 min on an automatic shaker. The supernatant portion was assayed for microbiological activity by a disc-plate method. The assays were performed against *Sarcina lutea* using the appropriate antibiotic as a standard.

An estimate of the extraction efficiency was made by mixing crushed bone samples from untreated rats with solutions of known concentrations of cephaloridine or cephalexin. The samples were air-dried, extracted, and assayed as described above for the treated animals. From these results, the recovery of cephaloridine and cephalexin from rat bone was estimated to be 96 and 94%, respectively.

The calculations of the half-life, $T_{1/2}$, values (10) in bone and serum were based on the assays of the samples taken at 0.5, 1, and 2 hr (and at 4 hr if the data were definitive).

Results. The concentrations of cephalothin and cephaloridine in rat bone and blood serum after subcutaneous administration of 100 mg/kg, and of cephalexin after oral administration of 100 mg/kg are shown in Table I and Figs. 1 and 2. Cephalothin produced the highest initial concentrations in

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[2] 7-[Thienyl (1-^{14}C)-acetamido] cephalosporanic acid.

TABLE I. Cephalosporin Concentrations in Bone (μg/g) and Blood Serum (μg/ml) of Rats.

	Cephalothin (radiocarbon assay)		Cephaloridine (microbiological assay)				Cephalexin (microbiological assay)			
	100 mg/kg sc[a]		100 mg/kg sc[b]		200 mg/kg sc[c]		100 mg/kg po[b]		200 mg/kg po[c]	
(hr)	Bone	Serum	Bone	Serum	Bone	Serum	Bone	Serum	Bone	Serum
.25	34.9	115.3	6.1	19.9	5.5	39.8	0.9	14.2	2.2	29.0
.5	18.5	97.4	7.1	47.3	17.3	121.0	5.2	38.7	7.0	64.5
1	7.9	38.7	4.2	34.7	7.6	95.0	2.8	31.0	7.9	52.0
2	1.8[c]	6.9[c]	2.7	19.7	9.3	75.0	2.0	18.9	4.2	54.0
4	1.0	4.5	0.3	1.5	3.2	18.9	0.6	9.5	2.2	15.9
$T_{1/2}$ (min)	27.0	23.6	67.2	71.5	101.5	78.8	69.3	103.8	113.4	106.4

[abc] Each value represents the mean value derived from the following numbers of rats: [a] 1; [b] 3; [c] 2. All tissue samples were assayed in triplicate.

bone and serum, but because of the short half-life of the drug, the concentations fell below those of cephaloridine and cephalexin at the 2-hr period. The maximum concentration of cephalothin in bone was detected at the initial sampling time, 15 min, whereas the peak concentrations of cephaloridine and cephalexin were found at 30 min. Within 4 hr after administration of each of the 3 cephalosporins, the antibiotic concentrations in the bone were $\leqq 1.0$ μg/g.

The half-life values of cephaloridine and cephalexin were greater than 1 hr in both the bone and blood serum. Cephalothin, however, had a half-life of less than 30 min (Table I).

The data from the 200 mg/kg doses of cephaloridine and cephalexin are shown in Table I. The resulting levels of antibiotic activity at this dose were approximately double those of the 100 mg/kg doses and the half-life of each drug in bone was significantly longer.

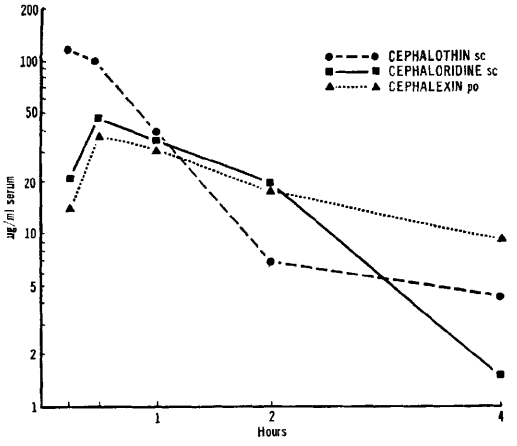

FIG. 1. Concentration of cephalosporins in rat serum following single doses of 100 mg/kg.

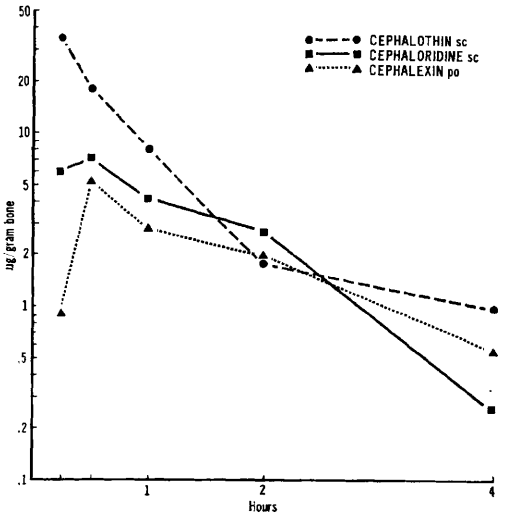

FIG. 2. Concentration of cephalosporins in rat bone following single doses of 100 mg/kg.

Discussion. Initially, the method of extraction of the bone from cephaloridine-treated rats (200 mg/kg sc) involved the homogenization of stripped bone with 2 *N* HCl. The slurries were centrifuged, the supernatants were neutralized and assayed for antimicrobial activity. Under these conditions, the concentrations of cephaloridine detected were almost identical to the results obtained by

saline extraction of bone from rats treated with 200 mg/kg of cephaloridine sc. Subsequently, both cephaloridine and cephalexin were extracted from bone with saline and were assayed microbiologically.

The saline extraction of cephalothin from bone, however, resulted in the loss of antimicrobial activity of the compound (determined from previous studies); consequently, radiocarbon (acetamido)-labeled cephalothin was used and extraction was not required. The position of the ^{14}C label on the cephalosporin molecule did not allow differentiation between the parent compound and the microbiologically active metabolite, desacetyl cephalothin (12, 13). Therefore, the cephalothin concentrations reported in this study may have included any of the active metabolite present. The inclusion of microbiologically inactive metabolites possibly introduced error in the 4 hr data but this error would be minimal in the earlier results (12, 14).

It should be noted that the antibiotic activity in the saline extracts of bone would be more representative of the amount of free antibiotic present, whereas, combustion and radioactive assay of the bone samples would be more indicative of the amount of free cephalothin plus any that might be bound to protein matrix.

The peak concentrations of cephalothin in the bone and serum were greater, but the half-life was shorter, than those of cephaloridine following subcutaneous administration of the 2 antibiotics to rats. The concentration and half-life of cephalexin in both bone and serum after oral administration were similar to those of the parenterally administered cephaloridine.

The low cephalosporin concentrations found in bone at 4 hr, the similar $T_{1/2}$ in serum and bone and the high recovery efficiency from bone, *in vivo* and *in vitro,* indicated there was little or no bone retention of these antibiotics as was reported with the tetracyclines (4, 11).

A doubling of the dose of cephaloridine or cephalexin resulted in an approximate 2-fold increase in the antibiotic concentrations in both the serum and bone. (This dose comparison was not made with cephalothin because of the lack of an adequate amount of the radiocarbon-labeled material.) These results plus the similarity of the serum and bone half-life data of the 3 cephalosporins indicated that the concentrations in bone were dependent on the coexisting antibiotic concentrations in the serum.

Summary. Subcutaneous doses of cephalothin and cephaloridine and oral doses of cephalexin were administered to rats. The 3 antibiotics rapidly entered the blood serum and bone: peak concentrations were reached 15–30 min after administration. The antibiotic activity in the bone was dependent on the coexisting activity in the serum for each of the compounds.

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