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Distribution of Sodium Cefazolin in Serum, Muscle, Bone Marrow, and Bone of Normal Rabbits

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Cefazolin levels were detected in bone and bone marrow of normal rabbits dosed intramuscularly, even in the absence of detectable levels in serum.

Sodium cefazolin, chemically 3-{[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl}-8-oxo-7-[2-(1H-tetrazol-1-yl)acetamido]-5-thio-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium hydrate, has been shown to be effective in vitro and in vivo in both animal and human infections (1-3). Although cefazolin has been reported to be of use in patients with bone and joint infections (1), only limited information is available as to the ability of this antibiotic to penetrate into bone and bone marrow. Consequently, this study was undertaken to determine the cefazolin content of selected tissues, including bone and bone marrow.

Twelve healthy, male New Zealand white rabbits were dosed intramuscularly with 50 mg of sodium cefazolin, dissolved in parenteral water for injection, per kg of body weight. Three rabbits were used in each of four treatment groups. Groups 1 and 2 each received a single dose of cefazolin, whereas groups 3 and 4 each received six loading doses at 9 a.m. and 3 p.m. on days 1, 2, and 3 plus a final dose on day 4. Tissue samples were obtained 1 h after dosing groups 1 and 3 and 3 h after dosing groups 2 and 4. Blood samples were obtained from the central artery of the ear; subsequently, all rabbits were sacrificed with sodium pentobarbital given intracardially. After sacrifice, the fore- and hindlimbs, from the side opposite that used for dosing, were removed surgically, and the muscle tissue was completely removed from each limb. After defleshing, the long bones were cracked and all of the marrow was removed. All tissues were rinsed with water to minimize contamination with blood. Muscle (1.0 g) and marrow (0.1 g) samples were weighed, ground in a Ten-Broeck homogenizer containing about 10 mg of glass beads (100 μm), and extracted for 1 h with pH 6 buffer at 4°C. Bone (1.0 g) samples were weighed, equilibrated to −70°C, pulverized in a mortar and pestle at −20°C, and extracted overnight with pH 6 buffer at 4°C. All samples were stored at −20°C until assayed. The procedure used to prepare bone samples for assay is a modification of the procedure reported by Norden for cephalothin (4).

All samples were assayed by using a disk agar diffusion assay employing *Bacillus subtilis* ATCC 6633 as an internal indicator. Six adsorbent paper disks (Schleicher and Schuell No. 740E; 6.35 mm), containing either a test or reference solution, were alternately placed on each assay plate. Assays were incubated overnight (serum assays at 30°C and other tissues at 23°C) before determining the diameter of the inhibition zone. After correcting for reference variation, a dose-response line was constructed, using data from the four assay plates at each of the five sodium cefazolin concentrations. The resulting regression line was computed by using the method of least squares.

Separate experiments were carried out with cefazolin-spiked tissue extracts to determine the percent recovery of cefazolin as well as the background activity of the tissue extracts (Table 1). The average recovery of cefazolin from these tissue extracts ranged from 73 to 84%. Bone samples were found to be free from nonspecific background activity, whereas muscle and bone marrow showed activities of 0.14 and 0.35 μg/sample, respectively. All final tissue determinations were adjusted to account for average recovery and background activity.

The data obtained in dosed rabbits is presented in Table 2. All samples obtained 1 h after dosing contained higher concentrations of sodium cefazolin as compared with those obtained at 3 h. Tissue loading was not observed; i.e., groups 1 and 3 or 2 and 4 gave equivalent results. Significant concentrations of antibiotic were found in the bone marrow, even in the absence of detectable levels in serum. Sodium cefazolin was detected in all bone samples obtained 1 h after the last dosing and in half of

those obtained 3 h after the last dose. On the average, the tibias contained 1% of the serum content of sodium cefazolin observed at 1 h after dosing normal rabbits.

There appears to be a trend toward higher levels of cefazolin in samples obtained from rabbit forelimb as compared with hindlimb samples. This could reflect a difference in blood supply. Specific tests were not carried out to determine the possible contamination of tissue samples with blood. However, cefazolin tissue levels were obtained in the absence of serum levels, and extreme care was taken in the collection and handling of the samples.

Two additional rabbits were dosed with 50 mg of cephalothin per kg of body weight, using the regimen previously described for group 3. The antibiotic content of the defleshed tibias devoid of marrow was 0.6 μg/g at 1 h after dosing in each rabbit. This concentration is comparable to that reported by Norden (4).

TABLE 1. *Recovery of cefazolin from tissue extracts to which known amounts of cefazolin were added*

Tissue	Sample wt (g)	No. of samples	Avg recovery (%)	Background activity (μg)[a]
Bone	1.0	4	84 (74-95)[b]	0
Bone marrow	0.1	4	73 (58-79)	0.35
Muscle	1.0	4	79 (57-100)	0.14

[a] Average of four bone samples, six marrow samples, and seven muscle samples.
[b] Numbers in parentheses indicate ranges.

The results obtained show that sodium cefazolin penetrates into rabbit bone and can be readily detected in marrow, even in the absence of detectable levels in serum. This finding is in agreement with that presented by Kopta and Lesken (J. A. Kopta and P. Lesken, Abstr. Assoc. Bone Joint Surgeons, 1975), who showed that antibiotic levels in bone remain high in rabbits with experimental osteomyelitis, even after serum levels have decreased. Since osteomyetilis may involve both marrow cavity and cortical bone, these results support the use of cefazolin in the treatment of bone infections. Limited data in humans obtained in eight patients with osteomyelitis show significant cancellus bone concentrations over an 8-h period after a 1-g dose of cefazolin (2). The levels obtained in our study with normal rabbit bone were lower and may not reflect the possible enhanced ability of cefazolin to penetrate into infected tissues.

Parsons, who recently reviewed the subject of antibiotics in bone, concludes that cefazolin seems to be the antibiotic of choice for prophylactic use in subjects undergoing total hip replacement (5). He has obtained cefazolin bone concentrations well above the minimal bactericidal concentration for *Staphylococcus aureus* and those gram-negative organisms causing postoperative prosthetic infections.

TABLE 2. *Concentration of sodium cefazolin in selected rabbit tissues after single or multiple intramuscular dosing with 50 mg/kg*

Group	Rabbit	No. of doses	Time (h) of sacrifice after last dose	Serum (μg/ml)	Tissue concn (μg/ml)					
					Muscle		Marrow		Bone	
					Fore	Hind	Fore	Hind	Fore	Hind
1	1	1	1	64.2	1.6	0.3	12.9	4.2	0.8	0.5
	2	1	1	82.6	6.8	0.7	33.2	13.4	1.1	0.5
	3	1	1	58.8	1.1	0.8	6.0	4.5	0.8	0.6
Avg				68.5	3.2	0.6	17.4	7.4	0.9	0.5
2	4	1	3	0	0	0	0.8	3.4	0	0.4
	5	1	3	0	0	0	1.2	2.7	0	0.2
	6	1	3	2.3	0	0	0.8	1.6	0	0.5
Avg				0.7	0	0	0.9	2.6	0	0.4
3	7	7	1	74.4	2.0	4.4	17.6	7.6	0.8	0.4
	8	7	1	65.4	0.6	0.5	17.0	8.6	0.6	0.4
	9	7	1	24.6	Trace	Trace	2.8	1.6	0.4	0.6
Avg				54.8	0.9	1.6	12.5	5.9	0.6	0.5
4	10	7	3	Trace	0	0	2.7	0.8	0	0
	11	7	3	Trace	0	0	4.0	1.6	0	0
	12	7	3	1.7	0	0	2.0	1.7	0	0
Avg				0.6	0	0	2.9	1.3	0	0

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