Bone Concentrations of Antimicrobial Agents After Parenteral Administration

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Bone concentrations of seven antimicrobial agents were determined after parenteral administration. Antibiotics were administered in large doses at customary intervals for 12 to 20 h before total hip or knee replacement; anticipated levels of each drug were achieved in the serum. Methicillin, carbenicillin, and clindamycin were detected in bone with greatest frequency. Cefazolin and gentamicin were each detected in bone specimens from only one of four patients. Neither penicillin G nor cephalothin was present in bone in sufficient quantity to be measurable. These data suggest that a number of factors, in addition to serum concentration, affect concentration of antimicrobial agents in bone. The clinical significance of the relationship between bone concentrations of antibiotics and therapeutic outcome is not certain.

Isolated attempts to measure antimicrobial agents in bone after their parenteral administration have been reported (1, 2, 4-6, 8-10, 12, 13, 15-18, 20). These have suggested moderate concentrations of lincomycin, clindamycin, penicillin, oxacillin, carbenicillin, erythromycin, tetracycline, and rifampin are achieved in bone, whereas cephalothin and gentamicin cannot be detected in bone in more than negligible amounts. Data such as these can be interpreted only with great caution. In most cases, each investigator examined only one, or at most two or three, antimicrobial agent in his system. Variations in the species and ages of the subjects studied, types of bone examined, and whether or not bone was infected are all factors which make comparison difficult. Moreover, assay methods were of several types.

The present study was conducted to circumvent several of these problems. A relatively homogeneous group of patients was investigated, antimicrobial agents were administered in a manner analogous to the clinical situation of treatment of serious bone infection, and assay was performed in a uniform manner.

MATERIALS AND METHODS

Patients. Informed consent was obtained from patients undergoing elective total hip or knee replacement. Eighteen patients underwent hip replacement; seven received knee replacement. Median age was 66; ages ranged from 22 to 76 years. Degenerative joint disease was the usual indication for surgery; three patients had severe rheumatoid

disease. In no patient was an active inflammatory process evident. No patient had received an antimicrobial medication for at least 7 days before surgery. Serum urea nitrogen and creatinine were normal in all patients.

Administration of antibiotics. Seven antibiotics were studied. The dosage and route of administration were as follows: (i) aqueous penicillin G, 2 million units intravenously (i.v.) every 4 h; (ii) methicillin, 2 g i.v. every 4 h; (iii) cephalothin, 2 g i.v. every 4 h; (iv) cefazolin, 1 g intramuscularly every 8 h; (v) carbenicillin, 5 g i.v. every 4 h; (vi) clindamycin, 600 mg intramuscularly every 8 h; and (vii) gentamicin, 1.7 mg intramuscularly per kg of body weight every 8 h. Patients receiving penicillin or carbenicillin also received clindamycin, 600 mg i.v., every 8 h. Antibiotics were begun approximately 12 to 20 h before surgery; each patient received at least three doses of medication.

Processing of blood and bone. Venous blood was obtained prior to institution of antibiotics and at the time of removal of the surgical specimen. Blood was allowed to clot at 4 C; serum was separated and saved at -5 C until assayed.

Cancellous bone was removed as small fragments from the excised femoral neck and head, or femoral epicondyles. Excess blood was washed from the bone fragments for 2 min with phosphate buffer in saline (pH 7.4) with constant agitation. Bone specimens from patients receiving penicillin were washed with buffer (pH 6.8). After blood removal, specimens were dried overnight at 4 C in a sterile petri dish. After drying, fragments were pulverized to a granular material in a Plattner mortar (Curtin Matheson Scientific) and weighed. After addition of appropriate buffer (approximately 1 ml/0.5 g of tissue) to pulverized bone, agitation for 5 to 10 min was performed; the suspension was then held at 4 C for 12 to 16 h. Supernatant fluid was removed and frozen for subsequent assay. Gross inspection usually

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revealed the washed fragments to be free of blood. Although diligent effort to elute all blood from bone fragments was made, quantification of blood remaining with the bone fragments was not attempted.

Antibiotic assay. A modification of the disk diffusion method of Sabath et al. (14) was employed. For all antibiotics except penicillin and carbenicillin, *Bacillus subtilis* (ATCC 6633) was seeded in antibiotic medium no. 5 (BBL). *Proteus mirabilis* was the test organism for the carbenicillin assay. Penicillin was assayed using *Neisseria meningitidis* (Houston City Health Department Laboratory no. 403) in brain heart infusion agar. Standard curves were constructed from the sizes of zones of inhibition obtained with antibiotic dilutions in normal human serum. Specimens of bone from patients who had not received antimicrobial agents before surgery were pulverized, mixed with antibiotic dilutions in buffer, and held in suspension at 4 C for 12 to 16 h. Supernatant was removed and frozen for later use in deriving standard curves for the assay of antibiotics in bone. Assays were conducted in duplicate.

Minimal serum concentrations detectable were: penicillin G, 1 µg/ml; methicillin, 1 µg/ml; cephalothin, <1 µg/ml; cefazolin, 5 µg/ml; carbenicillin, <5 µg/ml; clindamycin, 5 µg/ml; and gentamicin, <1 µg/ml. Corresponding concentrations in bone were: <0.5, 0.8, <3.6, 4.1, 3.6, 1.2, and 2.1 µg/g (dry weight) of bone.

Serum containing clindamycin, 25 µg/ml, produced no zone of inhibition in the penicillin or carbenicillin assay. No zone was obtained in any media using serum and bone prior to administration of antibiotic.

RESULTS

Measurement of antibiotics in serum and bone. Table 1 shows concentrations of antimicrobial agents determined in serum obtained at the time of bone removal and the time interval between the last dose of medication and sample acquisition. Serum concentrations were generally within the range of expected values.

No detectable drug was encountered in bone specimens from recipients of penicillin or cephalothin. Three of four bone specimens in methicillin recipients contained from 1.05 to 2.55 µg/g of tissue. Levels of 16.7 and 32.3 µg of carbenicillin/g of bone tissue were present in two of three carbenicillin recipients. Two of three patients treated with clindamycin had detectable antibiotic in resected bone; cefazolin and gentamicin were each detectable in only one of four recipients' bone tissue. Presence of detectable antimicrobial agent could not be correlated with the type of bone removed or the underlying disease process.

DISCUSSION

Previous investigators have studied antibiotic concentrations in bone of man and a num-

TABLE 1. Serum and bone concentrations of antibiotic agents

Patient no.	Bone specimen		Antibiotic	Time after last dose (h)	Antibiotic concn	
	Femoral neck	Distal femur			Bone (µg/g)	Serum (µg/ml)
1	X		Penicillin	0.5	ND ^a	5.0
2		X	Penicillin	1.0	ND	0.5
3	X		Penicillin	0.5	ND	9.4
4	X		Methicillin	2.5	2.55	13.2
5	X		Methicillin	2.0	ND	14.5
6	X		Methicillin	1.5	1.05	15.5
7	X		Methicillin	0.5	2.25	>50
8		X	Cephalothin	6.0	ND	ND
9		X	Cephalothin	0.5	ND	NT ^b
10	X		Cephalothin	3.0	ND	5.4
11	X		Cephalothin	1.5	ND	13.2
12		X	Cefazolin	0.5	10.45	>50
13	X		Cefazolin	2.0	ND	46.0
14		X	Cefazolin	1.0	ND	NT
15		X	Cefazolin	1.2	ND	31.0
16	X		Carbenicillin	1.5	32.3	218
17	X		Carbenicillin	1.5	16.7	190
18	X		Carbenicillin	0.5	ND	155
19	X		Clindamycin	2.5	9.26	12.5
20		X	Clindamycin	0.5	7.33	10.0
21	X		Clindamycin	1.7	ND	3.4
22	X		Gentamicin	2.0	ND	3.7
23	X		Gentamicin	1.5	ND	NT
24	X		Gentamicin	1.0	3.66	4.8
25	X		Gentamicin	1.0	ND	7.1

^a ND, Not detected.

^b NT, Not tested.

ber of experimental animals under a variety of conditions of infection, dosage of medication, route of delivery of medication, and specific bone studied (1, 2, 4-6, 8-10, 12, 13, 15-18, 20). With occasional exception, these studies have demonstrated poor uptake of most antibiotic agents in noninflamed bone, and perhaps increased uptake in infected bone. Nevertheless, because of the variety of experimental subjects and techniques, comparison of data is subject to criticism.

In the present study, we have studied seven antimicrobial agents in a clinically relevant model, utilizing a relatively homogeneous group of patients. Variation of experimental design and techniques have been kept to a minimum. Although the number of specimens studied was small, we have shown that there is significant uptake of methicillin, carbenicillin, and clindamycin in noninflamed human bone. Cefazolin and gentamicin penetrate bone to a variable degree; antibiotic was not detectable in bone of recipients of penicillin G and cephalothin.

The failure to detect measurable amounts of

cephalothin is disconcerting, but in accord with the observations of Norden (12). He found the concentration in noninflamed bone to be only 2% of the serum level and could find no detectable drug 2 h after injection. In the present study three of the four specimens from cephalothin recipients were obtained from 1.5 to 6 h after injection.

Concentrations of methicillin, carbenicillin, and clindamycin in bone approach or exceed the *in vitro* inhibitory concentration against the organisms for which these agents are frequently used (3, 11). An exception is the high concentration of carbenicillin required for inhibition of pseudomonads (19).

One might criticize the inability of our assay system to detect small concentrations of antibiotic. Kaplan et al. (7) have emphasized the importance of the type of tissue inoculum employed in diffusion assays. Since the supernatant of the bone suspensions was often viscous, it is possible that impaired diffusibility in the agar medium resulted in failure to detect minute quantities of antibiotic.

A large number of variables might determine the uptake and measurement of antibiotic in bone tissue. Vascularity, presence of concomitant disease, and histo-anatomy of bone undoubtedly interplay in the distribution of antimicrobial agent. Moreover, specifics of the drug, volume of distribution, protein binding, route of administration, metabolism, and excretion, are likely determinants of delivery to bone tissue. That protein binding alone is the major factor is doubtful since no correlation of protein binding to bone content could be made. How the other factors interrelate is conjectural.

A measure of caution in interpretation of these types of data is warranted. We are unaware of data regarding bioavailability of antibiotics in bone. Moreover, it is not clear that *in vitro* inhibitory concentrations are necessary for successful prophylaxis or eradication of bone infection; whether pulse or constant exposure to adequate concentrations of antibiotic is important is not certain. Additionally, it is probable that a great number of factors other than drug concentration determine the ultimate interaction between drug and organism at the tissue site.

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