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THE PENETRATION OF CEFAZOLIN, ERYTHROMYCIN AND METHICILLIN INTO HUMAN BONE TISSUE

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The penetration of cefazolin, erythromycin and methicillin into normal bone was studied in 20 patients undergoing surgery for fracture in the trochanteric region of the femur. The antibiotic concentrations were determined in serum, bone marrow, and cancellous and cortical bone. For all three antibiotics the bone marrow concentrations were of the same order of magnitude as the serum concentrations. In the eight patients receiving erythromycin, detectable concentrations were found in all the cancellous bone specimens (ranging from 1/7 to 1/2 of the serum concentration) and in three cortical bone specimens (ranging from 1/50 to 1/5 of the serum concentration). In the six patients receiving cefazolin, a detectable concentration was found in only one cancellous bone sample. In the six patients receiving methicillin, detectable concentrations were found only in the blood contaminated specimens of one cancellous and two cortical bone samples. However, by the method used, the recoveries of standard solutions of methicillin in cancellous and cortical bone were about 50 per cent and 15 per cent, respectively.

Key words: antibiotic; bone tissue; cefazolin; erythromycin; methicillin

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Several studies of antibiotic concentrations in bone tissue have been performed, but the results are difficult to compare and often conflicting (Beavis et al. 1977, Smilack et al. 1976). This could be due to differences in the bone type examined (Evaskus et al. 1969, Pitkin et al. 1977), whether there are distinctions made between bone marrow, cancellous and cortical bone (Hansen et al. 1975), variations in the amount of blood contamination of the bone specimens, and differences in the homogenization or extraction procedure, and the assay method used (Parsons 1976, Dornbusch et al. 1977).

The present study was concerned with the measurement of cefazolin, erythromycin and methicillin concentrations in bone tissue from the distal femur of patients with fractures in the trochanteric region, using a homogenization procedure already described for other antibiotics (Hansen et al. 1975, Nielsen et al. 1976).

MATERIAL AND METHODS

Twenty patients, 12 females and 8 males, were studied. Their ages ranged from 48 to 92 years, with a mean of 75 years. All patients had a unilateral per- or subtrochanteric fracture of the femur, and they all underwent osteosynthesis *ad modum* Ender, an intramedullary fixation using three or more nails. (Briefly, the nails are introduced just above the medial femoral condyle and passed up the shaft, across the fracture and into the neck and caput of the femur.) None of the patients had received antimicrobial medication for at least 7 days prior to this investigation. Serum creatinine was normal in all patients.

Bone specimens were taken from the medial supracondylic region of the femur as soon as the bone was exposed for the osteosynthesis. In the first nine patients cortical bone was taken with a Lexcelles cutting nippers, whereas a curette was used for the cancellous bone. It was, however, impossible by this method to avoid visible blood contamination of the specimens. Because of this the subsequent bone specimens were taken with a cylindrical drill with an internal diameter of 10 mm, by means of which it was possible to obtain a cylinder consisting of both cancellous and cortical bone, without visible blood contamination. Immediately after the samples were taken, cancellous and cortical bone were separated and stored at—20°C until assayed. Bone marrow was obtained by aspiration through a metal cannula introduced through the biopsy hole and placed 5–10 cm proximally in the marrow canal. The marrow material was centrifuged and the supernatant (except the fat fraction) was used for the measurements.

After weighing, the bone specimens were homogenized according to the method of Hansen et al. (1975), to a particle size of 0.3–3.2 micrometers. Cancellous bone was homogenized for 1 hour and cortical for 4 hours. Bone materials were dispersed in pooled human serum. For technical reasons serum four times the volume of the specimens was added to the cancellous bone prior to the homogenization procedure as described by Hansen et al. whereas serum twice the volume of the specimens was added to the cortical bone after homogenization.

All the antibiotics were given intravenously prior to surgery. Cefazolin (Kefzol®) was given in doses of 1 g. Erythromycin was administered as a continuous infusion of a solution containing 1 g erythromycin-lactobionate (Abbott) in 1000 ml 0.9 per cent sodium chloride. The average infusion rate was 650 mg per hour. The infusion was stopped prior to the operation, and the total amount given ranged from 500 mg to 1000 mg. Methicillin (Lucopenin®) was given as a single 1 g dose. The time interval from the last dose given (or the end of infusion) until the bone and marrow specimens were taken ranged from 15 to 325 minutes with a mean of 106 minutes.

Two samples of venous blood were drawn between 5–90 minutes before, and between 25–120 minutes after the bone specimens were taken. The antibiotic concentrations in serum were determined either on the same day, or the serum was stored at—20°C until assayed. The antibiotic concentration in serum at the time the bone specimens were taken was calculated according to the formula: $y_0 e^{-kt}$, where y is the concentration corresponding to the time t.

A microbiological method for antibiotic assay was employed using an agar cup method, as described by Rosdahl et al. (1969). A laboratory strain of *Bacillus subtilis* was used as the test organism for all three antibiotics. Difco Antibiotic Medium No. 4 was used for cefazolin and methicillin, and Antibiotic Medium No. 5 for erythromycin.

Standard solutions of antibiotics were prepared in pooled human serum for the following reasons. When standard solutions of cefazolin and erythromycin were prepared by adding known amounts to cancellous bone followed by 1 hour homogenization, standard curves identical to the curves obtained in pooled human serum were found. However, when methicillin was added to cancellous bone and homogenized for 1 hour the recovery was only 32 to 74 per cent measured against serum standards. When the three antibiotics were added to cortical bone and homogenized for 4 hours the recovery was only 9 to 56 per cent for cefazolin, 19 to 50 per cent for erythromycin, and 4 to 30 per cent for methicillin against serum standards. It is seen that the recovery for each antibiotic varied considerably from experiment to experiment, and by using these homogenates as standard solutions inaccurate standard curves would be obtained.

Because of the low recovery after the 4 hour homogenization procedure, measurements of antibiotic concentrations in cortical bone specimens were performed only when the corresponding cancellous bone samples showed detectable concentrations.

Minimum serum and marrow concentrations detectable were 0.5 μg/ml for cefazolin, 0.06 μg/ml for erythromycin, and 0.3 μg/ml for methicillin. The corresponding concentrations in cancellous bone homogenates were 2.5 μg/g, 0.3 μg/g and 1.5 μg/g and in cortical bone homogenates 0.15 μg/g, 0.18 μg/g and 0.9 μg/g, respectively. (In three specimens less than the standard 200 mg bone material was available and the minimal measurable concentrations were higher).

RESULTS

The concentrations of cefazolin, erythromycin and methicillin in bone marrow, cancellous and cortical bone, as well as the calculated serum values are shown in Table 1. Furthermore, the dose of antibiotic and the time interval between the last dose (or the end of infusion) and the taking of bone specimens are shown.

It is seen that the bone marrow concentrations were of the same order of magnitude as the serum concentrations for all three antibiotics.

All the bone specimens from the six patients receiving cefazolin were blood contaminated; even so there was a detectable concentration in only one cancellous bone sample (12 μg/g).

In the eight patients receiving erythromycin, the concentrations in the cancellous bone samples ranged from 0.6 to 5.5 μg/g, which was 1/7 to 1/2 of the corresponding serum values. In cortical bone there were detectable concentrations in the two samples with visible blood contamination which gave 0.51 μg/g and 1.8 μg/g, but a detectable concentration of 0.18 μg/g was found in only one of the non-contaminated samples. However, by the method used the recovery of standard erythromycin solutions in cortical bone was only about 35 per cent (see material and methods).

In the six patients receiving methicillin,

Table 1. The concentration of cefazolin, erythromycin and methicillin in serum (calculated) and bone tissue

Antibiotic	Pt. no.	Dose (g)	Time after last dose or the end of infusion (min)	Antibiotic concentrations			
				Serum (μg/ml)	Bone marrow (μg/ml)	Cancellous bone (μg/g)	Cortical bone (a) (μg/g)
Cefazolin	1	1	45	81	43	12+	ND+
	2	1	325	6.8	10	ND+	
	3	1	175	44	44	ND+	
	4	1 × 4	120	90	94	ND+	
	5	1 × 3	265	31	34	ND+	
	6	1 × 3	180	27	35	ND+	
Erythromycin	7	0.5	90	2.1	2.1	0.6+	ND
	8	0.6	45	7.8	5.7	2.3+	0.5+
	9	1	65	9.8	8.7	4.6	1.8+
	10	1	15	11	NT	5.5	NT
	11	1	95	7.6	6.8	1.8	ND
	12	0.9	40	NT	12	2.3	0.18
	13	1	145	11	11	3.4	ND
	14	0.6	85	7.8	NT	1.2	ND
Methicillin	15	1	75	29	6.2	NT	2.9+
	16	1	70	21	17	3.0+	2.5+
	17	1	75	30	22	ND	
	18	1	70	15	10	ND	
	19	1	60	37	19	ND	
	20	1	80	22	13	ND	

+ : Visible blood contamination. ND: Not a detectable concentration NT: Not tested.
a) Measurements of the concentration in cortical bone specimens were performed only when the corresponding cancellous bone samples showed detectable concentration.

detectable concentrations were found in the blood contaminated specimens, which corresponds to one cancellous (3.0 μg/g) and two cortical bone samples (2.5 μg/g and 2.9 μg/g). However, by the method used the recovery of standard methicillin solutions in cancellous bone was only about 50 per cent.

DISCUSSION

Cefazolin is a broad spectrum antibiotic. The minimal inhibitory concentration (MIC) is approximately 0.5 μg/ml for *Staph. aureus*, approximately 3 μg/ml for *E. coli* and *Klebsiella* species, and more than 3 μg/ml for other gram-negative rods (Wick & Preston 1972).

Recently Beavis et al. (1977) reported high bone penetration of cefazolin. In patients undergoing total hip replacement, they found 101 μg/ml in plasma, 10 μg/g in acetabulum and 29 μg/g in cancellous bone after intravenous administration of 4 g cefazolin as a bolus injection. Furthermore Cunha et al. (1977) found a peak bone concentration of cefazolin at 30 μg/g after administration of 1 g intravenously. On the other hand, Smilack et al. (1976) found, after intramuscular administration of 1 g cefazolin to four patients, a detectable concentration in only one cancellous bone sample (distal femur) at 10.4 μg/g. The minimal detectable bone concentration for the assay method used was 3.6 μg/g, and excess blood was washed from the bone samples.

In the present study, we found that the marrow concentration of cefazolin was at the same level as the serum concentration, which means well above the MIC for *Staph. aureus* as well as for many gram-negative rods. With our assay method the minimum measurable cefazolin concentration in cancellous bone was 2.5 μg/g, which means that in only one of the six cancellous bone samples did the concentration of cefazolin exceed the MIC for gram-negative rods. Whether a sufficient concentration of cefazolin for *Staph. aureus* can be obtained in cancellous bone after a dosage of 1 g was not answered by our study.

In animal experiments Grady & Stern (1965) found detectable erythromycin concentrations in bone materials from rats. In the present study we detected erythromycin concentrations in all the marrow and cancellous bone specimens, which exceeded the MIC of 0.25–0.5 μg/ml for sensitive strains of *Staph. aureus*. If it is taken into consideration that the recovery of erythromycin in cortical bone was approximately 35 per cent, then an additional three cortical bone specimens had erythromycin concentrations (before the homogenization procedure) above the MIC for *Staph. aureus*.

After administration of 1 g methicillin to 24 patients undergoing total hip replacement, Schurman et al. (1975) found concentrations in cancellous bone to be about 3 μg/ml, and about 2.5 μg/ml in cortical bone. The recovery of methicillin by the assay method used was in the range of 21–100 per cent and blood, serum and marrow tissue were included in the assay material. Likewise after administration of 2 g methicillin, Smilack et al. (1976) found concentrations of approximately 2 μg/g in cancellous bone samples (femoral neck) from three out of four patients.

In the present study the methicillin concentrations in bone marrow were of the same order of magnitude as the serum values, which means well above the MIC of methicillin for *Staph. aureus* (about 2.5 μg/ml). Only blood contaminated samples of cancellous and cortical bone showed detectable concentrations of methicillin, and all were above MIC for *Staph. aureus*. The negative results in the non-contaminated cancellous bone specimens might have been caused by the assay method used. In cancellous bone homogenates the minimum measurable concentration was 1.5 μg/g; however, by including the 50 per cent recovery of methicillin in cancellous bone specimens it is seen that initial concentrations of less than about 3 μg/g were not detectable.

Of the three antibiotics investigated erythromycin was found to be superior when evaluated on the basis of the serum-bone concentration ratio, and sufficient concentrations

of erythromycin were found in bone tissue. Cefazolin and methicillin showed sufficient marrow concentrations, but detectable concentrations in bone samples were only found infrequently, which as regards cefazolin was in contrast to the high bone concentrations reported by other authors.

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