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Comparative Penetration of Metronidazole, Clindamycin, Chloramphenicol, Cefoxitin, Ticarcillin, and Moxalactam into Bone

JAMES T. SUMMERSGILL, LINDA G. SCHUPP, AND MARTIN J. RAFF*
Department of Medicine, University of Louisville School of Medicine, Louisville, Kentucky 40292

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The concentrations of metronidazole, clindamycin, chloramphenicol, cefoxitin, ticarcillin, and moxalactam in the serum, femurs, and scapulae of normal rats were measured microbiologically 0.5, 1, 3, and 4 h after intravenous injection of 15-, 15-, 20-, 40-, 75-, and 30-mg/kg doses of the respective drugs. By 0.5 h metronidazole reached levels of 3.0 μg/g in compact femoral bone and 2.7 μg/g in cancellous scapular bone. Clindamycin and chloramphenicol reached levels of 8.1 and 6.1 μg/g, respectively, at 0.5 h. Cefoxitin penetrated bone to a level of 2.6 μg/g, whereas ticarcillin and moxalactam failed to reach significant levels in bone after single intravenous doses.

Antibiotics with demonstrated in vitro and clinical effectiveness against anaerobic bacteria may be useful in the management of patients with osteomyelitis due to these organisms. Metronidazole, a compound with demonstrated effectiveness against anaerobic bacteria (4, 7, 8), has been shown to be successful in treating cases of anaerobic osteomyelitis (M. Raff, J. Melo, C. Chun, R. Varghese, and J. Summersgill, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 367, 1978). However, the extent to which this compound penetrates bone remains undetermined. This report compares the penetration of metronidazole, clindamycin, chloramphenicol, cefoxitin, ticarcillin, and moxalactam into the long and flat bones of normal rats.

MATERIALS AND METHODS

Antibiotics. Metronidazole hydrochloride was supplied by G. D. Searle and Co., clindamycin phosphate was supplied by The Upjohn Co., chloramphenicol sodium succinate was supplied by Parke, Davis, & Co., ticarcillin disodium was supplied by Beecham Laboratories, cefoxitin sodium was supplied by Merck Sharp & Dohme, and moxalactam was supplied by Eli Lilly & Co.

Assay organisms. Concentrations of each antibiotic in serum and bone were determined by a modification of the agar-well bioassay method described by Bennett et al. (2). A strain of *Clostridium sporogenes* (Wadsworth no. 2253) was used in the assay of metronidazole, as described by Ralph and Kirby (10). Clindamycin was assayed with *Sarcinia lutea* ATCC 9341, chloramphenicol was assayed with *Benekea naitriegens* ATCC 14048, as described by Bannatyne and Cheung (1), ticarcillin was assayed with a strain of *Bacillus subtilis* ATCC 6633, cefoxitin was assayed with *Escherichia coli* MB3804, and moxalactam was assayed with *E. coli* ATCC 10536.

Experimental procedure. Groups of 15 Sprague-Dawley rats weighing between 250 and 300 g were injected via the lateral tail vein with a bolus dose of either metronidazole hydrochloride (15 mg/kg), clindamycin phosphate (15 mg/kg), chloramphenicol sodium succinate (20 mg/kg), ticarcillin disodium (75 mg/kg), cefoxitin sodium (50 mg/kg), or moxalactam disodium (30 mg/kg). The administered doses were in the range of maximum recommended dosages in milligrams per kilogram for humans. After injection with the appropriate antibiotic, groups of three animals in each series were decapitated with a guillotine at intervals of 0.5, 1, 2, 3, and 4 h. Blood was clotted at room temperature for 20 min, and serum was separated and stored at −40°C until assayed. Standards for determination of serum antibiotic concentration were prepared in pooled rat serum.

The femurs and scapulae of each animal were excised and dissected free of soft tissue by aseptic techniques. Femurs were split lengthwise, and marrow was removed. Each bone specimen was washed for not more than 10 s in sterile saline to remove any remaining antibiotic-containing blood and tissue from the surface and stored at −40°C overnight. After removal from the freezer, bones were air-dried at room temperature for 1 h and fragmented with a presterilized steel mortar and pestle, and specimens from groups of three rats at each time interval were pooled for microbiological assay. Two milliliters of 0.1 M phosphate buffer (pH appropriate for antibiotic being assayed) was added to 1 g of bone powder. The resulting suspensions were assayed for antimicrobial activity. Standards were prepared by adding 2 ml of predetermined concentrations of antibiotic to 1 g of bone powder from untreated rats and assayed in parallel with the unknowns.

Bone/serum ratios were calculated by dividing the bone concentrations (average of femur and scapula) by the average serum level at a particular time interval. Mean bone/serum ratios were then obtained by averaging the resulting bone/serum ratios for each antibiotic over the total experimental time period.

TABLE 1. Concentrations of antibiotics in serum and bone tissues in rats after intravenous injection

Antibiotic	Dose (mg/kg)	Time after injection (h)	Antibiotic Concn			Bone/serum ratio of antibiotic[c]
			Serum (μg/ml)[a]	Femur (μg/g)[b]	Scapula (μg/g)[b]	
Metronidazole	15	0.5	35.3 ± 0.67	3.0	2.7	0.08
		1.0	29.2 ± 0.60	2.2	1.8	0.07
		2.0	23.4 ± 0.60	1.4	1.6	0.06
		3.0	15.2 ± 0.90	ND[d]	ND	
		4.0	10.4 ± 0.60	ND	ND	
Clindamycin		0.5	5.8 ± 0.42	4.8	8.1	1.11
		1.0	4.8 ± 0.59	2.6	5.4	0.83
		2.0	2.5 ± 0.00	2.0	2.5	0.90
		3.0	2.3 ± 0.59	1.7	1.9	0.78
		4.0	1.3 ± 0.10	1.3	2.1	1.30
Chloramphenicol	20	0.5	13.5 ± 0.55	4.5	6.1	0.39
		1.0	9.1 ± 0.25	1.8	2.6	0.24
		2.0	4.5 ± 0.25	0.8	1.1	0.21
		3.0	1.8 ± 0.23	ND	0.6	0.33
		4.0	0.9 ± 0.27	ND	ND	
Ticarcillin	75	0.5	13.0 ± 2.08	ND	ND	
		1.0	11.0 ± 1.20	ND	ND	
		2.0	ND	ND	ND	
		3.0	ND	ND	ND	
		4.0	ND	ND	ND	
Cefoxitin	40	0.5	17.1 ± 0.34	1.9	2.6	0.13
		1.0	2.8 ± 0.23	0.8	1.3	0.38
		2.0	1.2 ± 0.15	ND	ND	
		3.0	ND	ND	ND	
		4.0	ND	ND	ND	
Moxalactam	30	0.5	35.5 ± 0.90	ND	ND	
		1.0	22.8 ± 3.19	ND	ND	
		2.0	6.6 ± 0.56	ND	ND	
		3.0	ND	ND	ND	
		4.0	ND	ND	ND	

[a] Average of three determinations at each time interval ± standard deviation.
[b] Femurs and scapulae excised at each time interval were pooled and one assay performed.
[c] Concentration of antibiotic in bone used in this determination was the average of femur and scapula level.
[d] ND, Not detectable.

RESULTS

Table 1 shows the antibiotic concentrations in serum and bone. Each value represents the mean and standard deviation of measurements from three rats for serum and the results of assays on the pooled bones of three rats.

After a 15-mg/kg intravenous dose, metronidazole reached a maximum level of 3.0 μg/g in long bone and 2.7 μg/g in flat bone by 0.5 h. This decreased to an undetectable level within 3 h. Serum levels of metronidazole reached a maximum of 35.3 μg/ml at 0.5 h and gradually decreased to 10.4 μg/ml at 4 h. The mean ratio of bone/serum metronidazole concentration was 0.07. Despite lower serum levels clindamycin and chloramphenicol, both reached higher levels in bone than did metronidazole. These two compounds also achieved higher concentrations in flat than in long bones. Chloramphenicol concentrations fell to undetectable levels within 2 h, whereas appreciable levels of clindamycin persisted until the end of the testing period (4 h). Mean ratios of bone/serum concentrations for clindamycin and chloramphenicol were 0.98 and 0.29, respectively.

Cefoxitin reached levels of only 1.9 and 2.6 μg/g in femur and scapula, respectively, at the dosage administered. Like clindamycin, cefoxitin had higher concentrations in flat than in long bone. The mean ratio of bone/serum cefoxitin concentration was 0.26.

Moxalactam and ticarcillin failed to show any detectable degree of penetration into bone at the dosages administered, despite appreciable serum levels.

DISCUSSION

Anaerobic bacteria may be etiological agents of human osteomyelitis (6, 9). For those cases of osteomyelitis due to organisms susceptible to penicillin and other beta-lactam compounds, there are several antibiotics capable of reaching

therapeutic levels in bone. However, *Bacteroides fragilis* is the most common organism responsible for human anaerobic osteomyelitis (9) and is resistant to most beta-lactam compounds. Most beta-lactam antibiotics are effective against strains of *B. fragilis* in vitro only at inordinately high concentrations and usually are bacteriostatic. Although *B. fragilis* is often susceptible to clindamycin and chloramphenicol (5), these compounds are also bacteriostatic rather than bactericidal. It is generally accepted that bactericidal activity may be desirable in an agent used in the therapy of osteomyelitis (9). Metronidazole has been shown to be bactericidal against obligate anaerobic bacteria, including *B. fragilis* (2). The data presented here lend support to the clinical evidence of metronidazole's effectiveness in the treatment of osteomyelitis caused by anaerobic bacteria, particularly *B. fragilis* (Raff et al., 18th ICAAC, abstr. no. 367). Metronidazole was shown to penetrate into long and flat bones in amounts sufficient to exceed the minimal inhibitory concentrations for most anaerobic bacteria (11). Unfortunately, this may or may not be relevant to attainable concentrations in osteomyelitic bones. There were essentially equivalent concentrations of metronidazole in the femurs and scapulae. For chloramphenicol, clindamycin and cefoxitin concentrations in the scapulae were higher than in the femurs. The significance of this is uncertain, but it is of interest that the great majority of cases of anaerobic osteomyelitis occur in long bones (9).

The bone/serum ratio for metronidazole was lower than that for clindamycin, chloramphenicol, and cefoxitin. This may of course relate to the relative degrees of protein binding for each of these compounds in rat serum. Measurements of this were not performed in this study. However, the lower minimal inhibitory concentrations for comparable strains of *B. fragilis*, the bactericidal nature of the drug, and its capacity to be given at appreciable doses due to its relatively low toxicity would seem to make metronidazole a potential therapeutic choice in management of osteomyelitis due to obligate anaerobic organisms. However, this remains to be demonstrated in comparative efficacy studies.

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