

Vancomycin Concentrations in Infected and Noninfected Human Bone

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Concentrations of vancomycin in bones of 14 patients undergoing total hip arthroplasty (group 1) and 5 patients with osteomyelitis (group 2) were studied. Group 1 received vancomycin, 15 mg/kg intravenously, 1 h prior to anesthesia. Group 2 received doses adjusted to achieve peak levels in serum of 20 to 30 µg/ml and trough levels of <12 µg/ml; bone specimens were collected during surgical debridement. The specimens were pulverized and eluted into phosphate buffer, and the supernatants were analyzed for vancomycin content by fluorescence polarization immunoassay. In group 1, vancomycin was detectable in all cancellous specimens with a mean concentration of 2.3 ± 4.0 µg/g (range, 0.5 to 16 µg/g); 10 of 14 cortical specimens had detectable vancomycin; the mean cortical concentration was 1.1 ± 0.8 µg/g (range, not detectable to 2.6 µg/g). In group 2, vancomycin was detectable in only two of five cortical bone specimens (mean concentration, 5.9 ± 3.5 µg/g). Cancellous bone was obtained in one patient; the vancomycin concentration was 3.6 µg/g. In most patients the vancomycin levels in bones were higher than the MIC for susceptible staphylococci following single prophylactic doses. In the few infected patients studied, penetration was variable and deserves further study.

Vancomycin is often recommended for the treatment of osteomyelitis caused by methicillin-resistant staphylococcal species, as well as for all forms of gram-positive staphylococcal and streptococcal osteomyelitis in patients with allergies to beta-lactam antibiotics. Nonetheless, no studies of vancomycin penetration into human bone have been reported. Therefore, we measured concentrations in noninfected bone specimens from humans following a single preoperative intravenous dose of vancomycin and in infected bone specimens following multiple-dose therapy for osteomyelitis.

MATERIALS AND METHODS

Study groups. Nineteen adult patients were studied. Group 1 consisted of 14 patients undergoing total hip arthroplasty for osteoarthritis. Group 2 consisted of five patients with osteomyelitis undergoing surgical bone debridement.

(i) **Group 1.** Vancomycin (15 mg/kg of total body weight, to a maximum dose of 1 g) was administered intravenously over 60 min beginning approximately 1 h prior to induction of anesthesia. A 2- to 3-g sample of cortical and cancellous bone was obtained from the femoral neck intraoperatively. Blood was collected for vancomycin assay 15 min after the end of the infusion and at the time of bone sampling.

(ii) **Group 2.** Patients in group 2 had been receiving vancomycin for at least 48 h for treatment of sternal (four patients) or tibial (one patient) osteomyelitis. Doses were adjusted to achieve peak levels of 20 to 40 µg/ml of serum and trough levels of ≤ 12 µg/ml. Peak levels were obtained 15 min after the end of a 60-min infusion. Bone debrided intraoperatively was collected for analysis. On the day of bone sampling, blood was obtained simultaneously and at the nadir of the dosing interval.

Specimen analysis. Serum was separated by centrifugation and stored at -70°C until the time of analysis. Bone specimens were processed by a method adapted from Wilson and Mader (16). On collection, bone was separated into cortical and cancellous segments, washed in 10 ml of sterile saline for 10 s, blotted dry, and frozen at -70°C . At the time of analysis, specimens were thawed, dissected free of soft tissue, and crushed in a hydraulic press at 24,000 lb/in² for two 30-s intervals. The specimens were then ground in a mortar with 2 to 5 ml of 0.1 M phosphate-buffered saline, transferred to a rotating wheel, and extracted at 4°C for 4 h. The bone homogenates were then centrifuged at $1,400 \times g$ for 20 min. The supernatants were removed, their volumes were recorded, and they were analyzed for vancomycin concentration by fluorescence polarization immunoassay (TDx; Abbott Laboratories, Diagnostics Division, Irving, Tex.). The assay has a sensitivity limit of 0.6 µg/ml, with intrarun and interrun coefficients of variation of less than 5% in the concentration range of 0.6 to 100 µg/ml.

The concentration of vancomycin in the supernatant (in micrograms per milliliter) was converted to the concentration of vancomycin in bone by the following method: $C_b = C_s \times V/Wt_b$, where C_b is the concentration of vancomycin in the bone (in micrograms per gram), C_s is the concentration in the supernatant (in micrograms per milliliter), V is the volume of supernatant (in milliliters), and Wt_b is the weight of the bone sample (in grams). This method assumes that all drug present is extracted into the buffer.

To correct for vancomycin present in blood contaminating the bone specimens, the hemoglobin concentration in the bone supernatant was determined by the hemoglobin cyanide method (5). The amount of vancomycin in bone samples as a result of blood contamination was calculated by the method of Rancoroni (9). The contribution from blood was subtracted from the total supernatant vancomycin concentration. The results reported are net concentrations.

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TABLE 1. Group 1 (total hip arthroplasty) data

Patient no.	Concn ($\mu\text{g/g}$) in bone		Time (min) postdose	Concn ($\mu\text{g/ml}$) in serum		Bone/serum ratio	
	Cancellous	Cortical		Simultaneous	Peak	Cancellous	Cortical
1	1.49	0.83	0	38.6	35.6	0.04	0.02
2	1.32	ND ^a	0	52.9	37.9	0.03	NA ^b
3	0.81	2.26	185	10.5	24.2	0.08	0.21
4	1.53	0.59	95	10.5	21.1	0.15	0.06
5	2.2	1.75	11	37.0	— ^c	0.06	0.05
6	2.65	1.60	50	14.4	23.4	0.17	0.10
7	0.61	0.50	100	18.2	26.8	0.03	0.03
8	0.53	ND	90	17.1	25.4	0.03	NA
9	0.95	ND	106	10.9	24.0	0.09	NA
10	0.58	0.52	138	16.2	30.0	0.04	0.03
11	16.0	2.58	70	17.0	26.0	0.94	0.15
12	1.0	0.57	92	15.7	28.0	0.06	0.04
13	0.71	0.19	98	20.9	37.3	0.03	0.01
14	1.63	ND	45	16.7	28.0	0.10	NA
Mean	2.3	1.14	77.1	22.1	28.3	0.132	0.07
Range	0.5–16.0	ND–2.58	0–185	10.5–52.9	21.1–37.9	0.03–0.94	0.01–0.21
SD	4.0	0.84	52.6	12.6	5.45	0.24	0.066

^a ND, Vancomycin not detectable in bone supernatant.

^b NA, Not applicable.

^c —, Specimen not obtained.

RESULTS

Group 1 (normal bone). The mean age of the 14 subjects was 67 ± 10 years (range, 54 to 83 years); eight were men. The average dose was 940 ± 77 mg (range, 790 to 1,000 mg), and the mean creatinine level in serum was 0.8 ± 0.2 mg/dl (range, 0.4 to 1.1 mg/dl). Vancomycin was detectable in cancellous bone in all 14 subjects, with an average concentration of 2.3 ± 4.0 $\mu\text{g/g}$ (range, 0.5 to 16.0 $\mu\text{g/g}$) (Table 1). Vancomycin was detectable in 10 of 14 cortical bone specimens, with a mean concentration of 1.1 ± 0.8 $\mu\text{g/g}$ (range, not detectable to 2.6 $\mu\text{g/g}$). The bone-to-serum ratio of vancomycin concentrations was 0.13 ± 0.24 (range, 0.03 to 0.94) for cancellous bone and 0.07 ± 0.07 (range, 0.01 to 0.21) for cortical bone.

Group 2 (infected bone). The mean age of the five subjects was 55 ± 12 years (range, 42 to 72 years); all were men. The mean creatinine level in serum was 0.9 ± 0.2 mg/dl (range, 0.7 to 1.2 mg/dl). Vancomycin was detectable in only two of five cortical bone specimens, with a mean concentration of 5.9 ± 3.5 $\mu\text{g/g}$ (range, not detectable to 8.4 $\mu\text{g/g}$); the bone-to-serum ratio of vancomycin concentrations was 0.30

± 0.12 (range, not detectable to 0.38) (Table 2). Cancellous bone was available in only one of the five patients owing to the sclerotic nature of the debrided bone specimens. The vancomycin concentration in that specimen was 3.6 $\mu\text{g/g}$, and the bone-to-serum ratio of vancomycin concentrations was 0.21.

For both groups 1 and 2, the concentrations in bone and the bone-to-serum ratios of vancomycin concentrations presented above and in Tables 1 and 2 have been corrected for contamination by blood. The mean amount of vancomycin attributed to blood contamination was 0.99 ± 0.95 $\mu\text{g/g}$ of bone (range, 0.13 to 3.85 $\mu\text{g/g}$) for group 1 and 1.96 ± 2.77 $\mu\text{g/g}$ of bone (range, 0.48 to 6.11 $\mu\text{g/g}$) for group 2.

DISCUSSION

Our results were similar to those found by Wilson et al. in a study of rabbits with normal and osteomyelitic bone (16). They found mean concentrations of 36.4 ± 4.6 $\mu\text{g/ml}$ in serum, 5.3 ± 0.8 $\mu\text{g/g}$ in infected bone, and 3.0 ± 0.2 $\mu\text{g/g}$ in noninfected bone 1 h after administration of a single dose of 30 mg/kg. In our study, two of the patients with infected

TABLE 2. Group 2 (osteomyelitis) data

Patient no.	Concn ($\mu\text{g/g}$) in bone		Time (min) postdose	Concn ($\mu\text{g/ml}$) in serum		Bone/serum ratio	
	Cancellous	Cortical		Simultaneous	Trough	Cancellous	Cortical
1	3.6	3.48	0	16.8	10.9	0.21	0.21
2	— ^a	8.4	125	22.0	9.1	NA ^b	0.38
3	—	ND ^c	75	26.3	9.1	NA	NA
4	—	ND	210	19.0	10.5	NA	NA
5	—	ND	360	13.6	11.7	NA	NA
Mean	3.6	5.94	154	17.5	10.26	0.21	0.30
Range		ND–8.4	0–360	13.6–26.3	9.1–11.7		ND–0.38
SD		3.48	138	6.7	1.14		0.12

^a —, No specimen obtained.

^b NA, Not applicable.

^c ND, Vancomycin not detectable in bone supernatant.

bone also had higher vancomycin concentrations than patients undergoing elective arthroplasty. This may be due to increased vascular supply to infected bone and hence increased delivery of vancomycin to the sampling site (6). Another possible explanation is that the infected patients received multiple doses of vancomycin prior to bone sampling.

The MIC of vancomycin for *Staphylococcus aureus* is approximately 1.0 µg/ml for methicillin-susceptible organisms, 1.5 µg/ml for methicillin-resistant strains, and 3.1 µg/ml for coagulase-negative staphylococci (2, 7, 15). Most patients achieved vancomycin levels in this range in cancellous bone, although 7 of 19 patients did not have detectable levels in cortical bone. There are several possible reasons for the latter finding. First, the extraction procedure may have failed to elute vancomycin from the specimens. We used a 4-h extraction period to be consistent with the procedure used in previous studies, and we believed that most of the vancomycin would be extracted during that period, taking into account the pulverization process (8, 14, 16). It is possible, however, that a longer extraction would remove more vancomycin. Also, vancomycin may not have been completely extracted owing to binding to the mineral content of bone. Second, the concentrations in bone may have been lower than the lower limit of detection of the assay (0.6 µg/ml). Third, the small size of some of the samples and the relatively large volume of buffer needed for elution may have diluted the vancomycin to below detectable levels. This was perhaps the case for two specimens in group 2, which weighed less than 0.7 g, but it is unlikely for the remaining specimens, which were equivalent in weight to all other specimens processed. Fourth, vancomycin may have been inactivated during the pulverization and extraction process. This is unlikely, because vancomycin is known to be stable at 80°C for up to 8 h (Eli Lilly & Co., unpublished data). Finally, the undetectable levels may have been due to interpatient variability in the penetration of vancomycin into bone, particularly the densely packed sclerotic bone in our osteomyelitis debridement specimens, which did not homogenize well even after being crushed and ground. Our data should therefore be viewed as a conservative estimate of vancomycin penetration into bone, representing minimal achievable concentrations in bone.

How do our data compare with the reported concentrations of other antistaphylococcal antibiotics in bone? Following a 1-g intravenous dose of cefazolin, concentrations in bone range from 5.7 to 43.3 µg/g, representing 2.2 to 31.8% of the average peak concentration in serum (3, 10, 12, 13). The MIC for 90% of susceptible staphylococci is 0.5 to 1 µg/ml (1, 15). Clindamycin concentrations in bone of 40 to 50% of the levels in serum (3.9 µg/g) have been found following three 600-mg intramuscular doses (11). This is 4 to 8 times the MIC for most methicillin-susceptible staphylococci (0.5 to 1.0 µg/ml) (4). Thus, vancomycin does not appear to penetrate bone as well as cefazolin and clindamycin do. However, after single doses, most patients achieved concentrations in bone that equaled or exceeded the MIC for susceptible staphylococci, and in the few infected specimens with detectable vancomycin, the levels were severalfold greater than the MIC.

On the basis of our results and those of Wilson and Mader with rabbits (16), further documentation of the concentration of vancomycin in normal and infected human bone and a study to test the efficacy of vancomycin as prophylaxis in prosthetic joint implantation seem in order.

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LITERATURE CITED

1. Actor, P. 1984. In vitro experience with cefonicid. *Rev. Infect. Dis.* 6(Suppl. 4):S783-S790.
2. Archer, G. L. 1978. Antimicrobial susceptibility and selection of resistance among *Staphylococcus epidermidis* isolates recovered from patients with infections of indwelling foreign devices. *Antimicrob. Agents Chemother.* 14:358-359.
3. Cunha, B. A., H. R. Gossling, H. S. Pasternak, C. H. Nightingale, and R. Quintiliani. 1977. The penetration characteristics of cefazolin, cephalothin, and cephradine into bone in patients undergoing total hip replacement. *J. Bone J. Surg. Am. Vol.* 59:856-859.
4. Garrod, L. P., H. P. Lambert, and F. O'Grady. 1981. *Antibiotic and Chemotherapy*, 5th ed., p. 276-277. Churchill Livingstone, New York.
5. Henry, J. B. 1979. *Clinical diagnosis and management by laboratory methods*, 16th ed., p. 863-868. The W. B. Saunders Co., Philadelphia.
6. Hughes, S. P. F. 1987. The use of antibiotics in orthopaedic surgery, total joint single versus multiple dose prophylaxis. *Orthop. Rev.* 16:27-32.
7. Klustersky, J., J. Beumer, and D. Daneau. 1971. Bacteriophage types and antibiotic susceptibility of *Staphylococcus aureus*. *Appl. Microbiol.* 22:1000-1007.
8. Norden, C. W., and M. A. Shaffer. 1982. Activities of tobramycin and azlocillin alone and in combination against experimental osteomyelitis caused by *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 21:62-65.
9. Roncoroni, A. J., C. Manues, C. Nedjar, J. Bauchet, and D. Mariani. 1981. Cefamandole bone diffusion in patients undergoing total hip replacement. *Chemotherapy (Basel)* 27:166-172.
10. Schurman, D. J., H. P. Hirshman, G. Kajiyama, K. Moser, and D. S. Burton. 1978. Cefazolin concentrations in bone and synovial fluid. *J. Bone J. Surg. Am. Vol.* 60:359-362.
11. Schurman, D. J., J. Johnson, G. Finerman, and H. C. Amstutz. 1975. Antibiotic bone penetration. *Clin. Orthop. Relat. Res.* 111:142-146.
12. Smilack, J. D., W. H. Flithe, and T. W. Williams, Jr. 1976. Bone concentrations of antimicrobial agents after parenteral administration. *Antimicrob. Agents Chemother.* 9:169-171.
13. Sorensen, T. S., H. Colding, E. Schroeder, and V. Thamdrup Rosdahl. 1978. The penetration of cefazolin, erythromycin and methicillin into human bone tissue. *Acta Orthop. Scand.* 49:549-553.
14. Tetzlaff, T. R., J. B. Howard, G. H. McCracken, E. Calderon, and J. Larrondo. 1978. Antibiotic concentrations in pus and bone of children with osteomyelitis. *J. Pediatr.* 92:135-140.
15. Watanakunakorn, C. 1978. Antibiotic-tolerant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 4:561-568.
16. Wilson, K. J., and J. T. Mader. 1984. Concentrations of vancomycin in bone and serum of normal rabbits and those with osteomyelitis. *Antimicrob. Agents Chemother.* 25:140-141.