

Pharmacokinetics of Cefamandole in Osseous Tissue

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The ability of cefamandole to cross osseous capillary membranes and its concentrations in interstitial fluid spaces were studied in canine cortical bone. Extraction studies were performed by using indicator dilution techniques and demonstrated that cefamandole readily traversed osseous capillary membranes. Volume-of-distribution studies demonstrated that cefamandole was distributed in the plasma and interstitial fluid spaces of cortical bone. There was a direct correlation between the calculated concentrations of cefamandole in the interstitial fluid spaces of bone and the simultaneous serum levels determined by bioassay in animals in which a steady-state equilibrium had been achieved. This suggests that the less expensive and more readily available serum bioassay technique is more useful than bone bioassays in monitoring the osseous concentrations of cefamandole.

The administration of antimicrobial agents for the prevention and treatment of osseous infections necessitates the presence of therapeutic levels of these agents at the site of the infection, the interstitial fluid space (26). Parameters influencing the concentration of an antimicrobial agent in the interstitial fluid space of osseous tissue include the ability of the agent to traverse the capillary membrane, its distribution in the fluid spaces of bone, and its degree of protein binding, which affects both transport and activity (14, 25, 27).

Peak and trough levels in serum are used by most clinicians to monitor antimicrobial therapy. The relationship between serum and interstitial fluid space concentrations in osseous tissue is assumed but has not been proved. Some investigators have suggested that osseous concentrations determined by bioassays should be used to evaluate the ability of an agent to be effective in the prevention and treatment of osseous infections.

Verwey and co-workers (26) explained the resistance of osseous infections to modern antimicrobial therapy by the presence of a capillary barrier to antimicrobial agents in osseous tissue. Rosin and co-workers (22) presented experimental data to substantiate this concept. In a study of the osseous concentrations of gentamicin, these authors demonstrated that the reported osseous levels of gentamicin were equivalent to the concentrations of gentamicin in the retained or "trapped" sera within the osseous specimens.

Bioassays of antimicrobial concentrations in osseous tissue cannot determine the manner or

degree of the ability of an agent to cross the vascular endothelium.

However, the capillary permeability and the distribution of an antimicrobial agent in the interstitial fluid space can be examined by using indicator dilution and volume-of-distribution techniques (2-4, 6, 7, 12). In addition, isotope techniques can be used to quantitate the osseous interstitial fluid concentration of an antimicrobial agent (3). When the concentrations determined by an isotope assay are compared with the concentrations determined by bioassay techniques, the degree of protein binding can be estimated. We describe the application of these techniques to a pharmacokinetic study of [¹⁴C]-cefamandole in normal cortical bone.

MATERIALS AND METHODS

Capillary transport of cefamandole. The first aim of this study was to define the capillary dynamics of cefamandole. Indicator dilution techniques were used to calculate the amount of cefamandole extracted after a single passage through the capillaries of bone. We measured the levels of [¹⁴C]cefamandole in the venous outflows of the ipsilateral femoral veins after arterial injection directly into the nutrient arteries of the tibias in five adult mongrel dogs. The resulting data permitted the calculation of the maximal and net extractions of [¹⁴C]cefamandole. By comparing the maximal extraction of [¹⁴C]cefamandole with the maximal extraction of another isotope of known extraction properties, it was possible to define the transport mechanism of cefamandole.

Serum and osseous concentrations of cefamandole. The second aim of this study was to compare cefamandole concentrations in serum and osseous tissues at different times after intravenous administra-

tion. We used both a bioassay and an isotope assay which permitted an evaluation of the relationship between these two techniques.

Volume of distribution of cefamandole. The third aim of this study was to determine the distribution of cefamandole within the exchangeable water space of osseous tissue. This was accomplished by using the volume-of-distribution techniques described by Morris et al. (M. A. Morris, P. J. Kelly, and J. B. Bassingthwaite, *Fed. Proc.* 37:315, 1978) in four adult dogs. These volume-of-distribution techniques (bone) which would have had the same concentration of the tracer as the reference sample (plasma) but in which the actual concentration may have been more or less. In essence, the volume of distribution of a substance was determined by adding intravascularly a known quantity of the substance containing a radioactive label and allowing the substance to achieve equilibrium (a steady state between plasma and tissue). A comparison of the final concentrations in a known volume of bone and plasma permitted calculation of the volume of distribution.

Interstitial fluid concentration of cefamandole. The final aim of this study was to combine the data obtained from the second and third portions of this study to calculate the interstitial fluid concentration of cefamandole. A comparison of the interstitial fluid and serum concentrations provided data to evaluate the usefulness of serum concentrations to monitor cefamandole therapy.

Techniques. Serum specimens to be analyzed for [^{14}C]cefamandole levels were handled in the following fashion. A serum sample (200 μl) was mixed with 0.5 ml of distilled water in 10 ml of Insta-Gel (Packard). This preparation was then counted with a Packard Tri-Carb liquid scintillation counter. Quench curves were constructed to determine true disintegrations per minute. All specimens were analyzed in triplicate.

Osseous specimens to be analyzed for [^{14}C]cefamandole were oxidized (13). Triplicate samples of filed cortical bone weighing between 50 and 75 mg were placed in combustion bags (constructed from Spectrapor membrane tubing) with equal amounts of sucrose in order to enhance combustion; 1 ml of distilled water was added to each envelope, which was allowed to dry overnight. Combustion was performed in an oxygen atmosphere in a heavy-walled 2-liter flask. The combustion process converted the ^{14}C label into carbon dioxide, which subsequently was converted to bicarbonate with a ^{14}C label after the addition of the scintillation fluid, which was a solvent mixture containing, per liter, 270 ml of phenethylamine, 270 ml of absolute methyl alcohol, 460 ml of toluene, 5 g of PPO (2,5-diphenyloxazole), and 100 mg of POPOP [1,4-bis-[2]-(5-phenyloxazolyl)benzene]. The samples were then counted with a Packard Tri-Carb liquid scintillation counter. Counts were corrected for quenching to obtain true disintegrations per minute.

The concentration of cefamandole in each specimen was calculated by comparing the counts in the serum and osseous samples with the counts in pilot solutions of the clinical dose and [^{14}C]cefamandole injected.

Serum samples for analysis of ^{85}Sr and ^{51}Cr -labeled albumin were counted with a Beckman Gamma 310 automatic well counter. Separation of ^{51}Cr and ^{85}Sr

was accomplished by using different windows and spillover ratios (7).

Bioassays of serum samples were performed by a disk plate method, using *Sarcina lutea* ATCC 9341 as the test organism. Cefamandole concentrations were determined by measuring the zone diameters of inhibition of growth and by comparing these diameters with the zone diameters of inhibition produced by known concentrations of cefamandole in pooled serum. To measure the extent of protein binding of cefamandole in pooled serum, we also compared the zone diameters of inhibition produced by known concentrations in pooled serum and phosphate buffer (pH 6).

Bioassays were also performed on cancellous and cortical specimens. The cancellous specimens (1- to 2-g samples) were washed with saline to remove blood and marrow. They were then dried and crushed. The cortical specimens (samples of 1-g filings) and cancellous specimens were suspended in phosphate buffer (pH 6) and mechanically agitated for 24 h. The homogenates were assayed by using the cylinder plate method and *S. lutea* as the test organism. Cefamandole levels were determined by measuring zone diameters of inhibition of growth and comparing these diameters with the diameters produced by known concentrations of cefamandole.

RESULTS

Capillary transport of cefamandole. We performed indicator dilution studies on five anesthetized dogs (30 mg of sodium pentobarbital per kg of body weight). One tibia was approached by the transfibular route to cannulate the nutrient artery with fine polyethylene tubing (diameter, 0.58 mm). The ipsilateral femoral vein was identified and cannulated. We injected a mixture of two test tracers (20 μCi of [^{14}C]cefamandole [specific activity, 7.24 $\mu\text{Ci}/\text{mg}$; Eli Lilly & Co.] and 5 μCi of ^{85}Sr [administered as SrCl_2 ; specific activity, 6.4 mCi/mg ; New England Nuclear Corp.]) and a reference tracer (15 μCi of ^{51}Cr -labeled albumin [1 mCi/ml ; New England Nuclear Corp.]) into the nutrient artery over a period of 1 min. Samples of blood were collected sequentially over 5-s intervals for 3 min. Each sample was centrifuged, 1 ml of plasma was obtained, and 200 μl of the plasma was counted with a Beckman Gamma 310 automatic well counter. Separation of ^{51}Cr and ^{85}Sr was accomplished by using different windows and spillover ratios. Additional portions of these plasma samples (0.2 ml) were mixed with 0.5 ml of distilled water in 10 ml of Insta-Gel (Packard) and counted with a Packard Tri-Carb liquid scintillation counter. Maximal and net extractions of [^{14}C]cefamandole were determined from these data by using the following calculations.

The concentrations of each of the three tracers at the different times were normalized by dividing each concentration by the dose injected. This yielded the fraction of the injected dose appear-

ing in the venous outflow per second [the $h(t)$ value]. Instantaneous extraction was computed from the following formula:

$$E(t) = \frac{h_R(t) - h_D(t)}{h_R(t)} \quad (1)$$

where $E(t)$ is the instantaneous fractional extraction, $h_D(t)$ is the fraction of the diffusible test tracer (^{85}Sr or $[^{14}\text{C}]$ cefamandole) injected per second that is collected after a single transcappillary passage, and $h_R(t)$ is the fraction of the reference tracer ^{51}Cr -labeled albumin injected per second.

Maximal extraction (E_{\max}), the highest point on the upslope of the $E(t)$ curve before the peak of the reference tracer, was assumed to be the most reliable indicator of the unidirectional transcappillary flux of the tracer across the capillary (2). The maximal extraction of cefamandole ($E_{\max_{\text{cef}}}$) could be related to the product of the permeability (P) and surface area (S), as described by Sangren and Sheppard (23), Renkin (21), and Crone (6):

$$PS = F_s \log_e(1 - E_{\max}) \quad (2)$$

where F_s is the flow rate (in milliliters per gram per minute).

Permeability ratios. Because the tracers were injected simultaneously, the two unknown quantities, S and F_s , canceled out of the ratio of PS values and left the following permeability ratio:

$$\frac{P_{^{85}\text{Sr}}}{P_{[^{14}\text{C}]_{\text{cef}}}} = \frac{\log_e 1 - E_{\max}^{^{85}\text{Sr}}}{\log_e 1 - E_{\max}^{[^{14}\text{C}]_{\text{cef}}}} \quad (3)$$

The permeability of the capillary walls to cefamandole, as calculated from the maximal extraction values, was compared with the diffusion coefficient of cefamandole, which was determined by the methods of Longworth (15) and Stein (24).

When the extraction of $[^{14}\text{C}]$ cefamandole was compared with the extraction of ^{85}Sr (a bone-seeking ion having a known extraction value) by using ^{51}Cr -labeled albumin as the intravascular reference tracer, more $[^{14}\text{C}]$ cefamandole than ^{85}Sr appeared in venous outflow per unit (Fig. 1). When the impulse curve was converted into an extraction curve (equation 1), strontium (the ion which concentrates in bone) was extracted to a greater degree than cefamandole. The mean extraction of cefamandole was 0.48 ± 0.10 , in contrast to 0.61 ± 0.08 for strontium (Table 1). The permeability ratio of strontium to cefamandole (equation 3) was 1.62 ± 0.42 (Table 1). The ratio of the free diffusion coefficients was 3.11. The difference between the permeability and free diffusion coefficient ratios was statistically

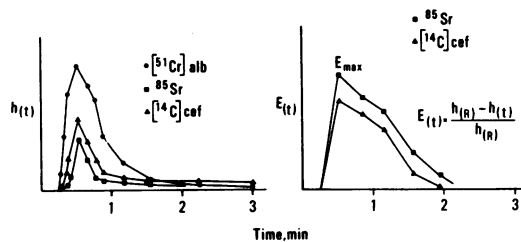


FIG. 1. Impulse and extraction curves for cefamandole (cef) and strontium in canine tibia. alb, Albumin.

TABLE 1. Cefamandole and strontium indicator dilution studies in tibia

E_{\max} of $^{85}\text{Sr}^a$	E_{\max} of $[^{14}\text{C}]$ cefamandole ^b	Permeability ratio ^c
0.68	0.52	1.70
0.70	0.52	1.75
0.64	0.53	1.44
0.52	0.51	1.04
0.53	0.30	2.20

^a Mean \pm standard deviation, 0.61 ± 0.08 .

^b Mean \pm standard deviation, 0.48 ± 0.10 .

^c Mean \pm standard deviation, 1.62 ± 0.42 .

significant ($P < 0.0001$). Since the permeability ratio was less than the ratio of the diffusion coefficients, the permeability of cefamandole across the capillary membrane of cortical bone was greater than expected with passive free diffusion.

The weakly lipophilic character of cefamandole may allow it to pass through the intracytoplasmic vesicular system in addition to its passage through the intercellular pores or clefts, which are the primary sites of passage for hydrophilic molecules that cross the capillary membrane by passive diffusion.

Serum and osseous concentrations of cefamandole. In 10 adult mongrel dogs of similar size, the left carotid artery and the right jugular vein were cannulated after pentobarbital anesthesia (30 mg/kg) was administered. A mixture containing $50 \mu\text{Ci}$ of $[^{14}\text{C}]$ cefamandole and 15 mg of bioactive cefamandole per kg was administered as an intravenous bolus. Blood samples were withdrawn from the carotid artery at 1-min intervals for 10 min and at 10- to 20-min intervals until the conclusion of each experiment. Two dogs were sacrificed at each of five intervals (30, 60, 120, 180, and 240 min) by using overdoses of pentobarbital. Both tibias were removed from each dog and cleaned of soft tissues; one tibia was split lengthwise and curetted to obtain cancellous bone, and the other was used to obtain cortical filings.

Serum and osseous samples from 10 dogs sacrificed at the times described above were subjected to both isotopic and biological assays for cefamandole. Serum isotope levels ranged from $30.50 \pm 4.50 \mu\text{g/ml}$ at 30 min to $10.07 \pm 0.14 \mu\text{g/ml}$ at 4 h. Levels in serum samples assayed by the cylinder plate method ranged from $16.00 \pm 2.12 \mu\text{g/ml}$ at 30 min to $1.15 \pm 0.49 \mu\text{g/ml}$ at 4 h (Fig. 2). Cefamandole concentrations in cortical and cancellous specimens were lower than serum specimen concentrations at all times tested (Fig. 3).

The percentage of protein binding of cefamandole was calculated from the dose-response curves obtained in serum and in phosphate buffer by using the agar diffusion and assay method, according to the following formula described by Craig and Suh (5):

Percent bound =

$$\frac{\text{(concentration required to yield a given zone diameter in serum)} - \text{(concentration required to yield the same zone diameter in buffer)}}{\text{concentration required to yield a given zone diameter in serum}} \times 100 \quad (4)$$

As determined by this method of calculation, the protein binding of cefamandole was 40% (that is, the concentration of drug required in serum was 40% greater than the concentration required in buffer to produce identical zone diameters).

Cefamandole concentrations measured by

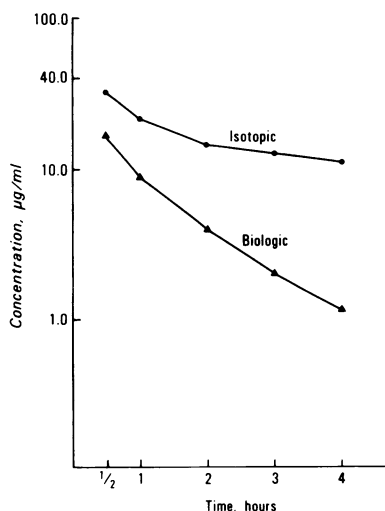


FIG. 2. Concentrations of cefamandole in serum, as measured by the bioassay and the isotope assay. The cefamandole concentrations measured by the isotope assay were consistently higher than those measured by the bioassay.

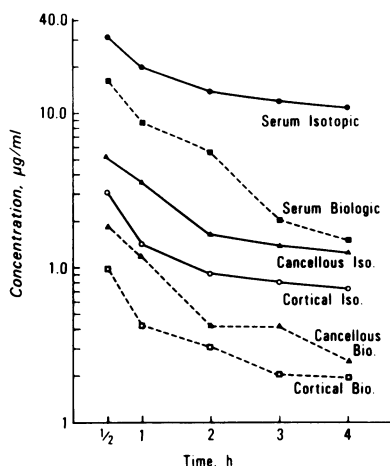


FIG. 3. Cefamandole concentrations in serum, cortical bone, and cancellous bone specimens, as measured by the isotope assay (Iso.) and the bioassay (Bio.).

bioassays were lower than those measured by the isotope technique, reflecting the fact that the isotope assay measured both the protein-bound and tissue-inactivated fractions, as well as the biologically active fractions. In contrast, bioassays of serum identified only the biologically active concentrations. A comparison of bioassay and isotope assay concentrations provided the following different method to determine the degree of binding and inactivation of cefamandole:

Percent bound

$$= \frac{\text{concentration by isotope assay} - \text{concentration by bioassay}}{\text{concentration by isotope assay}} \times 100 \quad (5)$$

When the difference between the isotope assay and the bioassay was used to determine the degree of protein binding, the level of protein binding appeared to increase with passage of time (30 to 240 min) (Table 2). Since cefamandole degradation was a time-related phenomenon, the isotope assay was measuring increasing amounts of inactive metabolites, which falsely elevated the cefamandole concentrations determined by the isotope assay. If the serum values at 30 and 60 min were used to calculate protein binding, the degree of binding was 45%. This compared favorably with the 40% level of protein binding in canine serum reported by Waterman and co-workers (27) and Peterson and co-workers (19).

The concentrations of cefamandole in cortical and cancellous specimens determined by bioassays were lower than those determined by isotope assays at all times tested (Table 3). Since

TABLE 2. Serum concentrations of cefamandole

Time (min)	Cefamandole serum concn ($\mu\text{g/ml}$)		Percent protein binding
	Isotope assay	Bioassay	
30	26.6	14.5	47
	34.4	17.5	
60	19.1	11.0	43
	20.6	5.9	
120	16.3	8.6	
	11.5	2.7	
180	12.8	1.6	
	11.7	2.4	
240	10.6	1.5	
	10.8	0.8	

TABLE 3. Osseous concentrations of cefamandole

Time (min)	Cefamandole cortical bone concn ($\mu\text{g/g}$)		Cefamandole cancellous bone concn ($\mu\text{g/g}$)	
	Isotope assay	Bioassay	Isotope assay	Bioassay
30	1.85	0.35	3.25	0.98
	3.35	0.98	6.92	2.63
60	1.23	0.36	5.43	1.96
	1.31	0.10	1.61	0.44
120	0.98	0.10	1.30	0.18
	0.86	0.52	1.89	0.30
180	0.62	0.25	1.50	0.74
	0.79	0.10	2.54	0.10
240	1.19	0.23	1.42	0.28
	0.79	0.19	1.21	0.18

bioassays of cortical and cancellous specimens were performed with standards diluted with phosphate buffer, they reflected total cefamandole concentrations, which should have been comparable to the concentrations determined by the isotope assays. However, the processing of cortical and cancellous specimens (filing, washing, etc.) may have destroyed the molecular structure which was necessary to measure biological activity, and this may account for the low bone concentrations determined by the bioassays.

Volume of distribution of cefamandole. Volume-of-distribution experiments were performed with four dogs which were similar in size. The dogs were anesthetized with sodium pentobarbital (30 mg/kg), and an endotracheal tube was inserted. The left jugular vein and the right carotid artery of each dog were cannulated, and the vascular pedicles of the kidneys were exposed through a midline incision and were ligated to minimize the loss of tracers, as suggested by Polimeni (20). Although Morris et al. (Fed. Proc. 37:315, 1978) suggested that 3 to 4 h were necessary to achieve a steady state for cations such as calcium and strontium, our pre-

vious experience with antimicrobial agents indicated that these compounds rapidly diffuse and achieve steady states more quickly than ions and molecules that are barrier limited. Thus, two dogs were sacrificed 2 h after ligation of the renal pedicles, and two were sacrificed 3 h after ligation.

Sequential blood samples were collected at 5, 10, and 30 min and thereafter every 30 min until termination of the experiment. Two bone specimens were obtained from each dog at 2 and 3 h by the following procedure. At 10 min before sampling, one tibia was exposed through a medial incision, with the periosteum left intact. At the time of sampling, a segment of diaphyseal bone was removed with a Stryker saw, split lengthwise into two equal fragments, and cleaned of marrow and periosteum. One fragment was weighed in air and water to calculate its specific gravity.

The other cortical specimen and a plasma sample were analyzed for cefamandole concentrations by the isotope assay, as described above.

Because any cefamandole entering the erythrocyte space would not be able to cross the capillary endothelium and be therapeutically active, we also determined the volume of distribution of cefamandole within the erythrocyte space. Thus, 200 μl of erythrocytes was oxidized and then counted with a Packard Tri-Carb liquid scintillation counter.

Plasma samples were also assayed by the disk plate method, using *S. lutea* ATCC 9341 as the test organism (11).

The volume of distribution (V_D) of cefamandole in cortical bone was calculated from the following formulas:

$$\text{Volume} = \frac{\text{mass (weight in air)}}{\text{specific gravity}} \quad (6)$$

to correct the units of cortical bone, and

$$V_D = \frac{\text{counts per minute of } [^{14}\text{C}]\text{cefamandole in 1 ml of cortical bone}}{\text{counts per minute of } [^{14}\text{C}]\text{cefamandole in 1 ml of plasma}} \quad (7)$$

Using equation 7, we obtained the volume of distribution of $[^{14}\text{C}]\text{cefamandole}$ in cortical bone at each sampling time.

The volume of distribution of cefamandole in the erythrocyte fractions (V_{RBC}) of the vascular space was calculated in similar fashion:

$$V_{\text{RBC}} = \frac{\text{counts per minute of } [^{14}\text{C}]\text{cefamandole in 1 ml of erythrocytes}}{\text{counts per minute of } [^{14}\text{C}]\text{cefamandole in 1 ml of plasma}} \quad (8)$$

The mean volume of distribution (V_D) of [^{14}C]cefamandole was 0.0510 ± 0.0079 ml/ml of cortical bone (Table 4). There was no statistically significant difference between the values obtained at 2 and 3 h.

The volume of distribution of cefamandole in the erythrocytes was 0.0462 ± 0.0310 ml/ml of erythrocytes. The volume of distribution was greater during the 3-h experiments (0.0793 ml/ml of erythrocytes) than during the 2-h experiments (0.0204 ml/ml of erythrocytes).

Interstitial fluid concentrations of cefamandole. Combining the data obtained in the volume-of-distribution studies in our experiments with the results of Morris et al. (M. A. Morris, J. B. Day, J. B. Bassingthwaite, K.-N. An, and P. J. Kelly, *Physiologist* 22:90, 1979), who defined the size of the various fluid spaces of cortical bone, made it possible to determine the compartmental distribution of cefamandole in cortical bone. If the volume of the vascular space per milliliter of cortical bone was subtracted from the volume of distribution of cefamandole in cortical bone ($V_{D_{\text{cef}}}$) determined after the establishment of equilibrium, the extravascular distribution could be determined. By using 0.0078 ml/ml of bone for the plasma space (V_p) and 0.0053 ml/ml of bone for the erythrocyte space (V_{RBC}), as determined by Morris et al. (*Physiologist* 22:90, 1979), the extravascular volume of distribution of cefamandole ($V_{EV_{\text{cef}}}$) could be computerized as follows:

$$\begin{aligned} V_{EV_{\text{cef}}} &= V_{D_{\text{cef}}} - V_p + (V_{\text{RBC}_{\text{cef}}}) (V_{\text{RBC}}) \\ &= 0.051 - 0.008 \\ &= 0.043 \text{ ml/ml of bone} \end{aligned} \quad (9)$$

where $V_{\text{RBC}_{\text{cef}}}$ is the volume of distribution of cefamandole in 1 ml of erythrocytes. The resulting value, 0.043 ml/ml of cortical bone, corresponded closely to the value of the interstitial fluid space (0.0346 ml/ml of cortical bone) determined by Morris et al. (*Fed. Proc.* 37:315, 1978). The difference between these two values may be explained on the basis of the lipophilic property of cefamandole, which allows cefamandole to enter the plasmalemma membranes of bone cells.

TABLE 4. Volume of distribution of cefamandole

Dog	Time (h)	V_D of cefamandole	
		Bone ^a	Erythrocytes ^b
H417	2	0.0445	0.0746
H210	2	0.0476	0.0840
H622	3	0.0625	0.0207
H410	3	0.0496	0.0200

^a Mean \pm standard deviation, 0.0510 ± 0.0079 .

^b Mean \pm standard deviation, 0.0462 ± 0.0310 .

Once the extravascular space of cefamandole per milliliter of cortical bone was defined, it was possible to calculate the concentrations of cefamandole in micrograms per milliliter of extravascular space in those animals in which the kidneys were ligated to maintain a steady state (Table 5). The cefamandole concentration in cortical bone in micrograms per gram (measured by the isotope assay) was corrected to the concentration in micrograms per milliliter by using the specific gravity ρ of bone: concentration of cefamandole in micrograms per gram $\times \rho$ in grams per milliliter = cefamandole concentration in micrograms per milliliter.

The concentration of cefamandole in the vascular space ($\text{cef}_{\text{vas.space}}$) of 1 ml of cortical bone was determined by multiplying the serum concentration of cefamandole ($\text{cef}_{\text{serum}}$) (isotope assay) by the vascular space in 1 ml of cortical bone:

Concentration of cefamandole

$$= \text{cef}_{\text{serum}} [V_p + V_{\text{RBC}}(V_{\text{RBC}_{\text{cef}}})] \quad (10)$$

The concentration of cefamandole in the extravascular space of cortical bone ($\text{cef}_{EV_{\text{total}}}$) was determined by subtracting the cefamandole concentration in the vascular space ($\text{cef}_{\text{vas.space}}$) from the total cortical bone cefamandole concentration ($\text{cef}_{\text{cort.bone}}$) and dividing by the extravascular space ($V_{EV_{\text{cef}}}$) (determined by equation 9).

$$\text{cef}_{EV_{\text{total}}} = \frac{\text{cef}_{\text{cort.bone}} - \text{cef}_{\text{vas.space}}}{V_{EV_{\text{cef}}}} \quad (11)$$

Since these values were determined by the isotope assay, the correction for protein binding determined by the tissue experiments permitted calculation of the biologically active concentration of cefamandole ($\text{cef}_{EV_{\text{active}}}$) in the interstitial fluid space:

$$\begin{aligned} \text{cef}_{EV_{\text{active}}} &= \text{cef}_{EV_{\text{total}}} (100 - \text{percent protein bound}) \end{aligned} \quad (12)$$

When these computations were performed with the data obtained from the four dogs in which steady-state conditions were achieved, the serum concentration of cefamandole determined by the bioassay accurately reflected the biologically active concentration in the interstitial fluid space of the cortical bone (Table 5).

DISCUSSION

An antimicrobial agent must achieve and maintain an effective concentration within a tissue in a predictable manner to eradicate an established infection. The mode of administration, degree of serum protein binding, capillary membrane dynamics, and rate of excretion all

TABLE 5. Cefamandole concentrations in the interstitial fluid space of bone^{a,d}

Dog	$V_{EV,cef}$ (ml/ml)	Specific gravity (g/ml)	Cefamandole concn in:						
			Cortical bone ($\mu\text{g}/\text{g}$) ^a	Total se- rum ($\mu\text{g}/\text{ml}$) ^a	Vascular space of cor- tical bone ($\mu\text{g}/\text{ml}$) ^{a,b}	Extravascu- lar space ($\mu\text{g}/\text{ml}$) ^{a,c}	Total intersti- tial fluid space ($\mu\text{g}/\text{ml}$) ^{a,d}	Free intersti- tial fluid space ($\mu\text{g}/\text{ml}$) ^e	Biologically active se- rum ($\mu\text{g}/\text{ml}$) ^f
H410	0.0409	2.26	0.96	44.0	0.3653	1.8147	44.4	24.4	29.0
H417	0.0361	1.98	0.90	38.59	0.3206	1.4634	40.5	22.3	19.8
H210	0.0392	1.89	0.77	30.5	0.2536	1.1988	30.5	16.8	23.5
H622	0.0538	2.01	0.97	31.2	0.2591	1.6949	31.5	17.3	14.1

^a As determined by the isotope assay.

^b Cefamandole concentration in vascular space of cortical bone = $\text{cef}_{\text{serum}} \times [V_p + (V_{\text{RBC}})(V_{\text{RBC},\text{cef}})]$, where V_p is the vascular space in milliliters per milliliter of cortical bone, V_{RBC} is the erythrocyte space in milliliters per milliliter of cortical bone, $V_{\text{RBC},\text{cef}}$ is the portion of the erythrocyte space of cortical bone into which cefamandole diffuses, and $\text{cef}_{\text{serum}}$ is the serum cefamandole concentration in micrograms per milliliter.

^c Extravascular concentration of cefamandole = $(\text{cef}_{\text{cort.bone}} \times \rho) - \text{cef}_{\text{vas.space}}$, where $\text{cef}_{\text{cort.bone}}$ is the concentration of cefamandole in cortical bone in micrograms per gram, ρ is the specific gravity in grams per milliliter, and $\text{cef}_{\text{vas.space}}$ is the cefamandole concentration in the vascular space in micrograms per milliliter.

^d Total interstitial fluid space cefamandole concentration = $\text{cef}_{\text{extravas.}}/V_{\text{EV,cef}}$ where $\text{cef}_{\text{extravas.}}$ is the extravascular concentration of cefamandole in micrograms per milliliter and $V_{\text{EV,cef}}$ is the extravascular volume of distribution of cefamandole.

^e Free interstitial fluid space cefamandole concentration = $\text{cef}_{\text{ISF,total}} \times (1.0-0.45)$, where $\text{cef}_{\text{ISF,total}}$ is the total interstitial fluid space cefamandole concentration in micrograms per milliliter and 0.45 represents the degree of protein binding determined in the tissue experiments.

^f As determined by the bioassay.

affect the concentrations of active antibiotics available in tissues. This study was designed to examine these factors (except for the rate of excretion of cefamandole in cortical bone).

The use of the combustion technique for the isotope assay was a very accurate and reproducible method for determining cefamandole concentrations in the serum and osseous tissue specimens. The errors introduced by chemoluminescence after acid solubilization were eliminated. This method permitted the detection of small quantities of cefamandole in relatively small osseous samples. When this technique was used, the recovery efficiency of standards was 97%. Thus, the values reported slightly underestimated the cefamandole concentrations.

Our extraction studies indicate that cefamandole (molecular weight, 512.5) traverses osseous capillary membranes to a greater extent than can be explained on the basis of passive diffusion through intercellular slits or pores. If free diffusion were the only mechanism involved in the capillary passage of cefamandole, the ratio of the diffusion coefficient of strontium to the diffusion coefficient of cefamandole (3.10) would be equal to the permeability ratio of these two compounds (1.62 ± 0.42). Instead, the weak lipophilic properties of cefamandole at pH 7.4 probably allow it to pass through the intracellular vesicular system. This capability results in a higher maximal extraction and, therefore, a higher computed permeability than expected.

Because we injected cefamandole as a bolus rather than by the infusion technique, the serum

concentrations determined by the bioassay were lower than those reported previously (1, 8-10, 16, 17).

The differences in zone diameters of inhibition observed when cefamandole standards were tested in phosphate buffer and pooled serum suggest that 40% of the cefamandole was protein bound. When serum concentrations from specimens obtained within 60 min of parenteral administration were measured by the isotope assay and the bioassay and compared, 45% of the cefamandole was protein bound. These figures are in close agreement with figures reported previously (18, 19, 27). The relatively sluggish blood flow in cortical bone (2.5 ml/100 g per min, compared with 38 ml/100 g per min in cancellous bone, according to Morris and Kelly [Orthop. Trans. 3:137, 1979]) explains the 2-h lag time between the injection and the peak cortical concentrations. In contrast, cancellous bone achieved peak concentrations within 30 min.

When the volume-of-distribution techniques were combined with the isotope assays of serum and cortical bone specimens, it was possible to evaluate the interstitial fluid concentrations of cefamandole. The close correlation between the active, interstitial fluid concentration which was calculated by the isotope assay and the serum concentration determined by the bioassay suggests that serum peak and trough measurements can provide clinicians with an accurate monitoring technique.

Conclusions. (i) The osseous capillary wall

does not present a significant barrier to the passage of cefamandole into the extravascular fluid space of cortical bone. (ii) The volume of distribution of cefamandole in cortical bone during steady-state conditions is approximately equal to the volumes of distribution in the plasma and the interstitial fluid space combined. (iii) A comparison of the bioassay and the isotope assay results permits estimation of *in vivo* protein binding. (iv) Knowledge of the osseous and serum concentrations of cefamandole and the extravascular distribution of cefamandole in milliliters per milliliter of bone permits calculation of the osseous concentrations of cefamandole in terms of micrograms per milliliter of interstitial fluid. (v) A comparison of the cefamandole concentration in the interstitial fluid space (determined by the isotope assay) with the serum cefamandole concentration (determined by the bioassay) suggests that the serum concentration accurately reflects the interstitial fluid concentration.

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