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Penetration of Cefamandole, Cephalothin, and Desacetylcephalothin into Fibrin Clots

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The conversion of cephalothin into a less active metabolite (desacetylcephalothin) might influence its distribution in tissues. An experimental rabbit model devised to determine concentrations of antibiotics in subcutaneous fibrin clots was used in this study. Groups of five to six animals received 100-mg/kg intravenous injections of either cefamandole or cephalothin. One hour after the injection, the concentration of cefamandole in serum was 20 times higher than that of cephalothin. Whereas cephalothin was undetectable at 4 h, cefamandole was still detectable at the end of the experiment. The half-lives of cephalothin and cefamandole in serum were 16 and 27 min, respectively. The concentration of cefamandole found in fibrin clots was severalfold higher than that of cephalothin. The half-life of cefamandole in clots (81 min) was superior to that of cephalothin (38 min). Although concentrations of both antibiotics were higher in serum than in clots at 1 h, the concentrations of these drugs in the clots persisted at higher levels throughout the next 5 h of the experiment. The extent of binding of cefamandole (87%) to rabbit serum was greater than that of cephalothin (50%). At least 55% of cephalothin was metabolized in vivo into its less active metabolite desacetylcephalothin. This metabolite was found in higher proportion in the serum (75%) than in the clots (55%). Whereas only 12% of the free (unbound) cephalothin reached the clots, 78% of the free cefamandole was found in the clots. This lower level of penetration of unbound cephalothin might be explained by the short half-life of this antibiotic, not permitting equilibrium to occur.

Although serum antibiotic levels are often used as the guidelines for therapeutic effectiveness, the concentrations of these drugs at the site of infection are often found to be different than those in serum. Several factors, including the pK_a of the drug, its lipophilic and hydrophilic nature, protein binding, mode of administration, peak serum value, or even its metabolic transformation into less active metabolites, might influence the penetration of antibiotics in tissues. The purposes of this study were to compare the degree of penetration of cephalothin and cefamandole into fibrin clots by use of a previously described model in rabbits (4) and to study the influence of the metabolic conversion of cephalothin to desacetylcephalothin on the penetration of the former in the fibrin loci.

MATERIALS AND METHODS

Fibrin clots. Two milliliters of a 2% solution of human fibrinogen (Merck Sharp & Dohme, West Point, Pa.) were distributed in sterile siliconized test tubes (13 by 100 mm). One-tenth milliliter of bovine thrombine (Park, Davis and Co., Detroit, Mich.) was added to the tubes, which were incubated for 0.5 h at 37°C. The resultant clots were gently removed and placed in sterile petri dishes at 20°C for no more than 15 min until use.

Antibiotics. Sodium cephalothin, cefamandole nafate, cefamandole lithium, and desacetylcephalothin were supplied by Eli Lilly Laboratories (Indianapolis, Ind.).

In vivo experiments. Healthy young albino rabbits (2 to 4 kg) were given an intramuscular injection of 20 mg of chlorpromazine per kg. Both flanks were shaved and swabbed with iodine and alcohol. The skin was anesthetized with 2% lidocaine, and a 4-cm incision was made. After blunt dissection of the subcutaneous tissue, no more than three to four clots were placed in each subcutaneous pocket to avoid clustering. Autoclips (18 mm) were applied to close the incision. Most animals were used for experiment only once and never more than twice. Blood counts, urea nitrogen, and creatinine were measured before each experiment. A scalp-vein needle (23 gauge) was inserted in each ear. One served for the infusion of normal saline in between intravenous injections of antibiotics; the other ear vein

was used to collect blood and was kept clear in between sampling with heparin (20 U/ml). Each group of six animals received an intravenous injection of 100 mg of either cefamandole nafate or sodium cephalothin per kg administered over 30 min with an infusion pump. Blood, interstitial fluid, and fibrin clots were removed before the administration of the antibiotic and at 0.5, 1, 2, 3, 4, 5, and 6 h after the beginning of the infusion. The interstitial fluid content of cefamandole and cephalothin was analyzed by impregnating three paper disks with interstitial fluid located in the subcutaneous pockets. Occasionally, disks had to be discarded because of lack of interstitial fluid. At each time interval, clots were removed aseptically and the wound was closed. All samples, except the interstitial fluid, were also assayed for desacetylcephalothin.

Assay procedures. Clots were weighed, cut, and then dissolved with a 2.5% sterile solution of trypsin (Sigma Chemical Co., St. Louis, Mo.). The trypsin was added in a volume equal to the final weight of the clot. A 1-h incubation was necessary to obtain complete dissolution of the clots. Cephalothin and cefamandole levels were determined by a paper disk-agar diffusion method, using *Bacillus subtilis* (Difco spore suspension) as the test organism and Trypticase soy agar as the medium. Standard solutions were prepared by diluting known amounts of antibiotics in rabbit serum for serum, in normal saline for interstitial fluid, and in trypsinized fibrin clots for clot levels. Specimens of plasma, interstitial fluid, clots, and their standards were plated in triplicate and incubated overnight at 37°C. The zones of inhibition of bacterial growth were measured and plotted in the usual manner on semilogarithmic paper.

Concentrations of desacetylcephalothin in serum and clots were determined by descending paper chromatography and bioautography (10). Whatman no. 4 paper strips (19 by 46.5 cm) were impregnated with a 0.1 M sodium acetate buffer at pH 4.6, adjusted with glacial acetic acid, and air dried. Serum and trypsinized clots were deproteinized in an equal volume of acetone and centrifuged. A volume of these samples containing approximately 0.3 μg of cephalothin was placed at 9 cm from the edge of the paper. The volume of the unknown sample varied from 10 to 100 μl. The standard solutions contained fixed amounts of cephalothin (0.3 μg) and variable amounts of desacetylcephalothin (0.2 to 2.7 μg). The volume used for standards was 10 μl. The unknown and the standards were deposited on the same paper strip. Four chromatograms could be placed in the chamber saturated with 500 ml of 60% methyl ethyl ketone in water. The chromatograms were developed by a 3-h migration in a solvent made of methyl ethyl ketone and water (92:8, vol/vol). After chromatography the chromatograms were air dried for 20 min. The paper sheets were then placed over large agar plates seeded with a 1% spore suspension of *B. subtilis* ATCC 6633, to permit antibiotics to diffuse in the agar. The chromatograms were removed after 15 min, and the agar plates were incubated at 37°C for 18 h. The percentage of desacetylcephalothin was determined by comparing the zone sizes induced by the unknown samples and the standards. The degree of protein binding of cephalothin, desacetylcephalothin, and cefamandole to rabbit serum was determined by an equilibrium dialysis technique (13). The initial concentrations of antibiotic either in the serum or the protein-free side of the dialysis chamber were 50 μg/ml for cephalothin and cefamandole and 30 μg/ml for desacetylcephalothin. Eight experiments were performed for each of the three antibiotics.

Statistical analysis. All results were submitted to Student's *t* test for statistical analysis.

RESULTS

The serum concentrations of cefamandole, cephalothin, and total cephalothin (cephalothin plus desacetylcephalothin) are presented in Fig. 1. Peak concentrations were achieved in the serum at the end of the 30-min infusion of the antibiotics in all animals. The peak level of cefamandole (398 μg/ml) was 2.4 times higher than that of active cephalothin (166 μg/ml). The maximum concentration of total cephalothin was 664 μg/ml. The level of total cephalothin decreased markedly in the first hour and reached lower levels than cefamandole. Whereas cephalothin was undetectable at 4 h, cefamandole was still present at the end of the experiment at a concentration of 1 μg/ml. Figure 2 depicts the concentrations of antibiotics in the clots. The mean levels of cefamandole at the different time intervals studied were up to 17-fold higher than those of cephalothin and total cephalothin ($P < 0.001$). It is to be noted that the antibiotic concentration in the clot was always higher than that in the serum 2 h after the infusion. These higher concentrations persisted until the end of the experiment. The mean desacetylcephalothin/cephalothin ratio was 3.0 in the serum and 1.2 in the clots. These ratios were constant throughout the experiment. These data indicate that desacetylcephalothin contributed to 75% of total cephalothin in serum, whereas it constituted 55% of total cephalothin in clots.

Table 1 shows the cefamandole and cephalothin concentrations in the interstitial fluid. The concentration of both antibiotic agents in the interstitial fluid was initially higher than the respective value found in the clot. The levels of cefamandole were always superior to those of cephalothin.

The half-lives of the antibiotics studied are presented in Table 2. Cephalothin and its metabolite desacetylcephalothin have identical half-lives of 15.8 min in serum and 38.2 min in clots. The half-life of cefamandole was significantly longer than that of cephalothin in serum and clots ($P < 0.001$), but no difference was noted in the interstitial fluid. The areas under

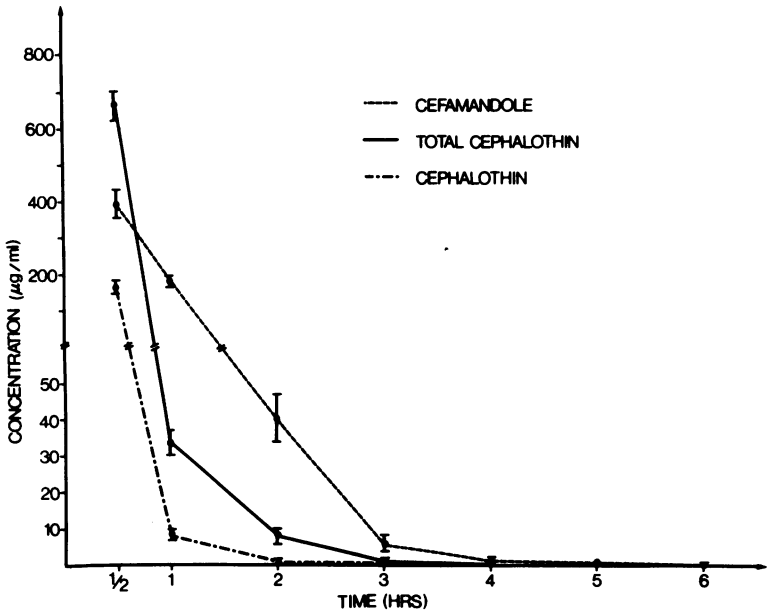

FIG. 1. *Concentration of cefamandole, cephalothin, and total cephalothin (cephalothin plus desacetylcephalothin) in serum. Vertical bars represent standard error of the mean.*

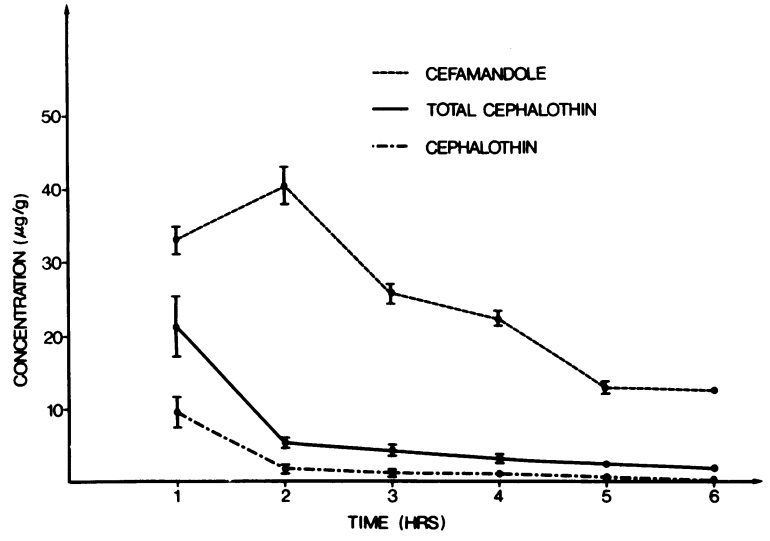

FIG. 2. *Concentration of cefamandole, cephalothin, and total cephalothin (cephalothin plus desacetylcephalothin) in clots. Vertical bars represent standard error of the mean.*

the curves (AUCs), measured by a standard method (9), are shown in Table 3. The intervals used for serum were 0.5, 1, 2, 3, 4, 5, and 6 h; for clots and interstitial fluid, they were 1, 2, 3, 4, 5, and 6 h. The AUCs in serum, interstitial fluid, and clots were significantly greater for cefamandole than for cephalothin or desacetylcephalothin ($P < 0.001$). In fibrin clots there was no significant difference between the AUCs of cephalothin and desacetylcephalothin. In contrast, the AUC in serum was 3.5 times as great for desacetylcephalothin as for cephalothin ($P < 0.001$).

The degree of binding of cefamandole to rabbit serum (87%) was the highest of the three congeners examined, cephalothin and desace-

TABLE 1. *Mean concentrations of cefamandole and cephalothin in interstitial fluid*

Time (h)	Concn (μg/ml)	
	Cefamandole	Cephalothin
1	44.6 (5.7)[a]	22.0 (4.1)
2	16.7 (2.2)	4.7 (0.8)
3	9.2 (0.7)	1.2 (0.2)
4	6.6 (0.7)	1.0 (0.04)
5	3.5 (0.7)	0.5 (0.06)
6	1.6 (0.1)	0.2 (0.2)

[a] Number in parentheses is standard error of the mean.

TABLE 2. *Half-life of cefamandole, cephalothin, and desacetylcephalothin*

Antibiotic	Half-life (min) in:		
	Serum	Interstitial fluid	Clots
Cefamandole	26.3 (1.0)[a]	47.9 (1.2)	81.1 (3.2)
Cephalothin	15.8 (0.1)	39.8 (2.5)	38.2 (1.2)
Desacetylcephalothin	15.8 (0.1)	ND[b]	38.2 (1.2)

[a] Number in parentheses is standard error of the mean.
[b] ND, No data.

TABLE 3. *AUCs of cefamandole, cephalothin, and desacetylcephalothin*

Antibiotic	AUC		
	Serum	Interstitial fluid	Clots
Cefamandole	359.6 (13.3)[a]	75.2 (7.9)	138.0 (4.8)
Cephalothin	77.5 (2.8)	24.8 (3.3)	16.2 (1.6)
Desacetylcephalothin	271.0 (10.9)	ND[b]	19.5 (2.2)

[a] Number in parentheses is standard error of the mean.
[b] ND, No data.

tylcephalothin being 50 and 61% bound, respectively. Protein electrophoresis was performed on clots as they were removed from the subcutaneous pockets. No albumin was present during the first 3 h of the experiment. The albumin content was 0.2, 0.21, and 0.22 g/100 ml, 4, 5, and 6 h, respectively, after the beginning of the experiment. Even though the serum protein binding of cefamandole was higher than that of the other drugs, the mean levels of free cefamandole, with the exception of the 0.5-h value, were higher at most time intervals (Table 4). The percentage of penetration of antibiotics into clots was calculated by expressing the peak concentration in clots as a percentage of the peak antibiotic level found in serum. A total of 79.2% of the free cefamandole penetrated the fibrin clots, whereas only 11.7 and 3.9% of free cephalothin and free desacetylcephalothin penetrated these loci (Table 5). When the data were not corrected for protein binding, only 10.6, 6.0, and 2.4% of cefamandole, cephalothin, and desacetylcephalothin, respectively, penetrated the clots. The penetration of these antibiotics into interstitial fluid was slightly higher than the respective values in clots. These results suggest that the three cephalosporins have different rates of penetration as they go from the vascular space to the interstitial space and from this latter area into the fibrin loci.

TABLE 4. *Mean serum levels of free antibiotic*

Time (h)	Mean serum level (μg/ml)		
	Cefamandole	Cephalothin	Desacetylcephalothin
0.5	52.0	83.0	194.0
1	24.0	4.0	10.0
2	5.0	1.0	2.0
3	1.0	0.3	0.6
4	0.3	<0.1	<0.1
5	0.2	<0.1	<0.1
6	0.1	<0.1	<0.1

DISCUSSION

The biological transformation of cephalothin into desacetylcephalothin has been suspected of being responsible for therapeutic failures in the treatment of severe infections such as enterococcal bacterial endocarditis, where the demonstration of in vitro synergism between cephalothin and aminoglycosides against the enterococcus does not seem to correlate with in vivo efficacy (20). The rapid in vivo transformation into desacetylcephalothin and the poor penetration of the active form of the antibiotic into cerebrospinal fluid (17) have been thought to be responsible for the poor clinical response obtained with cephalothin therapy in bacterial meningitis (7). In contrast to cephalothin, cefamandole is not metabolized into a less active metabolite. This new cephalosporin, which has a wider spectrum of activity than cephalothin and can neutralize ampicillin-susceptible and -resistant *Haemophilus influenzae*, has been shown recently to be as effective as ampicillin in the treatment of experimental *H. influenzae* meningitis (15).

The data presented here indicate that desacetylcephalothin contributed to 75 and 55% of total cephalothin in the serum and clots, respectively. Similar ratios of desacetylcephalothin to cephalothin have been found in the sera of mice (21). Barza et al. suggested that

TABLE 5. *Percentage of penetration of antibiotics (total or free) into clots and interstitial fluid and their protein binding*

Antibiotic	Penetration (%) in:				Protein binding (%)
	Clots		Interstitial fluid		
	Total[a]	Free[b]	Total	Free	
Cefamandole	10.6	79.2	11.2	86.2	87 (0.7)[c]
Cephalothin	6.0	11.7	13.2	26.5	50 (1.0)
Desacetylcephalothin	2.4	3.9			61 (0.8)

[a] Total = Bound and unbound drug.
[b] Free = Unbound drug.
[c] Number in parentheses is standard error of the mean.

levels of desacetylcephalothin were responsible for 5 to 15% of the concentration of the parent substance in human serum (3). Cabana et al. have also shown that the metabolite of cephapirin (desacetylcephapirin) contributes to 75% of the plasma concentrations in rats (6). These authors have also suggested that the biotransformation of this antibiotic is similar in humans, dogs, and rodents. Desacetylcephalothin has also been noted to have 2 to 16 times less antimicrobial activity than cephalothin against microorganisms. Wick (21) has shown that the weak in vitro activity of desacetylcephalothin is more striking with gram-negative organisms than with gram-positive bacteria. This poor killing might jeopardize the therapeutic effectiveness of this agent at the site of infection (21).

In the present investigation, cefamandole was found in significantly higher concentrations than cephalothin and desacetylcephalothin in fibrin loci. The high degree of penetration seen with cefamandole was rather surprising in view of the fact that cefamandole was much more highly bound to serum protein than the other two congeners. It is generally believed that the binding of antibiotics to serum proteins will limit their penetration into fibrin loci (2) and interstitial fluid (5, 8, 16, 18, 19). In the present experiments, the extent of cefamandole (87%), cephalothin (50%), and desacetylcephalothin (61%) binding to rabbit serum protein, most likely albumin, did not appear to correlate with the interstitial fluid and clot concentrations observed. Our data revealed that 10, 6, and 2%, respectively, of the peak serum values of cefamandole, cephalothin, and desacetylcephalothin (Table 5) reached the clot. It is also of interest to note that no albumin could be detected in the clots during the first 3 h of the experiment. The mimimum amount of protein detected in the clots during the last 3 h should have, at least theoretically, reduced even more the active concentrations of the drug with the highest degree of binding. It is also of considerable importance to note that the rate of diffusion of free cefamandole (79.2%) into these loci was much more impressive than those of cephalothin (11.7%) and desacetylcephalothin (3.9%).

These results demonstrate that in contrast to penicillins, where the unbound portion appeared to pass with equal facility from the serum to the interstitial fluid and clots (2), the free portion of the cephalosporins studied showed different degrees of diffusion. These data suggest that other factors besides protein binding could influence the concentrations of cephalothin, desacetylcephalothin, and cefamandole obtained in the clots and interstitial fluid.

The dosage, the mode of administration, or the slight difference between the pK_a values of cephalothin (2.3), desacetylcephalothin (2.5), and cefamandole (2.9) can hardly explain the differences in penetrability. Even though the antibiotics studied are hydrosoluble, investigators have noted that, with most penicillins or cephalosporins, there seems to be a good correlation between the degree of binding to albumin and their relative lipophilia (2). Having found no data on the partition coefficient of cefamandole, it is impossible to evaluate its impact on the diffusion of cefamandole across membrane.

The remarkable discrepancy between the higher degree of penetration of cefamandole in either the clots or interstitial fluid in the presence of a lower peak level of unbound drug (Table 4) can probably be explained by the persistence of this antibiotic at higher levels for several hours in the blood. This could have allowed a better equilibrium among serum, interstitial fluid, and clots. In contrast to cefamandole, cephalothin and desacetylcephalothin had significantly shorter half-lives and lower AUCs. The serum levels of cephalothin and its metabolites fell so rapidly that there was practically no opportunity for equilibrium to occur.

These results are consistent with the notion that central and peripheral compartments tend to equilibrate as serum fluctuates slowly (1, 2, 14).

The clinical importance of the biotransformation of cephalothin into a less active metabolite is very hard to evaluate. The apparent superiority of cefamandole over cephalothin seen in rabbits might not be applicable to humans, since the extent of conversion of cephalothin to desacetylcephalothin in humans can vary considerably from one individual to the other. Lee et al. (12) have reported that the metabolic transformation could be as high as 90% in some individuals. Some authors (11) have also reported that in patients suffering from renal failure the metabolic breakdown product of cephalothin could accumulate in serum and reach much higher concentrations than cephalothin.

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