ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1988, p. 33–36
0066-4804/88/010033-04$02.00/0

Vol. 32, No. 1

Penetration of Cefpiramide and Cefazolin into Peritoneal Capsular Fluid in Rabbits

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Received 26 May 1987/Accepted 14 October 1987

Penetration of cefpiramide and cefazolin into a specific extravascular fluid was measured with rabbits bearing capsules in the peritoneal cavity. A general feature of slow accumulation and elimination of drugs from extravascular sites having low surface area/volume ratios has also been observed in this study. The capsular concentration-time profiles were well expressed by the following equation: $C(\mathrm{CF}) = A(\mathrm{CF})[e^{-k_{el}(\mathrm{CF})(t - t_0)} - e^{-k_p(\mathrm{CF})(t - t_0)}]$, where $C(\mathrm{CF})$, $A(\mathrm{CF})$, $k_p(\mathrm{CF})$, $k_{el}(\mathrm{CF})$, and t_0 indicate capsular concentration at time t, constant for the dimension of concentration, capsule penetration rate constant, capsule elimination rate constant, and lag time before penetration occurs, respectively. The $k_p(\mathrm{CF})$, $k_{el}(\mathrm{CF})$, and t_0 were 0.139 h^{-1}, 0.0509 h^{-1}, and 0.45 h, respectively, for cefpiramide, and 0.448 h^{-1}, 0.0145 h^{-1}, and 0.14 h, respectively, for cefazolin. $A(\mathrm{CF})$ was 22.7 μg/ml for cefpiramide and 4.53 μg/ml for cefazolin, being parallel to the area under the plasma concentration-time curve for free drug from t_0 to infinity (20.1 μg · h/ml for cefpiramide and 3.43 μg · h/ml for cefazolin). In conclusion, it is suggested that as well as $k_p(\mathrm{CF})$ and $k_{el}(\mathrm{CF})$, the area under the plasma concentration-time curve for free drug from t_0 to infinity may play an important role regarding the circulating reservoir of drugs in determining capsular concentration-time profiles in experimental models for particular extravascular sites of infection, like abscesses into which drugs cannot easily penetrate.

Since antibiotics must usually manifest their antimicrobial activity at extravascular sites of infection, their concentration there must reach sufficiently high levels. Therefore, it seems important to obtain sufficient pharmacokinetic profiles of drug penetration of and elimination from extravascular sites of infection, especially in humans. Obviously we would meet ethical and practical objections if we tried to do pharmacokinetic studies on many infected and noninfected tissues in humans. Thus, several experimental models have been developed to facilitate the determination of the extravascular concentrations of antibiotics in animals, e.g., implantation of different types of diffusion chambers or tissue cages at various sites of the body, subcutaneous installation of fibrin clots, and induction of granuloma pouches.

As has been reported previously, cefpiramide (SM-1652) is a cephalosporin derivative that is long acting in animals (7) and humans (8). The present report is concerned with the penetration and elimination pharmacokinetics of cefpiramide and cefazolin in the fluids of tissue cages implanted in the peritoneal cavity of rabbits.

MATERIALS AND METHODS

Capsule. Ordinary table tennis balls were drilled with 250 holes (1.0 mm) and implanted in the peritoneal cavity by the methods of Gerding and co-workers (5).

Implantation. Male albino rabbits (3.0 to 3.7 kg) were anesthetized with 60 to 74 mg of intravenous sodium pentobarbital. The abdomen was shaved, and the skin was swabbed with iodine and alcohol. A 5-cm midline incision was made just below the xiphoid, and the peritoneum was opened with scissors. Two ethylene oxide-sterilized capsules were inserted and manually maneuvered under the abdominal wall into well-separated positions. No attempt was made to fix the capsules in place. The abdominal wall and the skin was closed with silk sutures.

Cephalosporins. Cefpiramide (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan) and cefazolin (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) were used.

Pharmacokinetics. Six rabbits bearing capsules in the peritoneal cavity were divided into two groups of three each for a crossover study (8-day interval) of intramuscular doses (50 mg/kg of body weight) of cefpiramide and cefazolin. The first medication was given 18 days after implantation of the capsules.

Blood samples and capsular fluid (two capsules in each rabbit) for antibiotic assay were obtained from the marginal ear vein and by percutaneous puncture with a 22-gauge needle through one of capsule perforations, respectively, at 0.5, 1, 2, 4, 6, 8, and 24 h after the drug administration.

Antibiotic assays were performed by using an agar well diffusion method on sensitivity test agar (Eiken, Tokyo, Japan) inoculated with *Escherichia coli* NIHJ as the test organism for cefpiramide (8) and a disk-plate diffusion method on antibiotic medium 1 (Difco Laboratories, Detroit, Mich.) inoculated with *Bacillus subtilis* ATCC 6633 for cefazolin. Standard curves were constructed by spiked rabbit plasma and spiked capsular fluid. The assay sensitivity for cefpiramide was 0.39 μg/ml both in plasma and in capsular fluid and for cefazolin was 1.56 μg/ml in plasma and 0.78 μg/ml in capsular fluid. For calculation of mean values and standard errors of the mean, the concentrations lower than the assay sensitivity were treated as 0. The coefficients of variation in triplicate determinations of spiked rabbit plasma at a concentration of 10 μg/ml were 15.1% for cefpiramide and 13.8% for cefazolin; those coefficients for spiked capsular fluid at a concentration of 2 μg/ml were 16.2% for cefpiramide and 17.8% for cefazolin.

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TABLE 1. Capsular fluid characteristics

Determination	Mean (range)
Leukocyte (count/mm^3)	2,080 (600–6,300)
Polymorphonuclear leukocyte (%)	59 (31–92)
Erythrocyte (count/mm^3)	43,800 (2,200–158,500)
pH	7.34 (7.00–7.57)
Total protein (g/100 ml)	4.0 (2.9–7.2)
Albumin (g/100 ml)	2.4 (0.8–5.2)

Pharmacokinetic analyses of both the mean plasma and the mean capsular concentration-time curves were performed on the basis of a one-compartment open model and model-independent pharmacokinetics (model 18 in NONLIN 84), respectively. The nonlinear least-squares program NONLIN 84, in which a factor of 1 was used for weighting, was employed for regression analysis of the curves. The areas under plasma concentration-time curves [$AUC_{0-\infty}$(PL)] and under capsular concentration-time curves [$AUC_{0-\infty}$(CF)] were calculated by integrating pharmacokinetic equations from 0 h to infinity. In addition, AUC(PL) from 0 to 8 h [AUC_{0-8}(PL)] and AUC(CF) from 0 to 24 h [AUC_{0-24}(CF)] were calculated for each rabbit and capsule by the trapezoidal rule.

Plasma protein binding. Aliquots (200 μl) of an aqueous solution of cefpiramide or cefazolin were added to 1.8 ml of fresh plasma of rabbits to obtain the cephalosporin concentrations of 25, 50, 100, 200, and 400 μg/ml. The reaction mixtures with cefpiramide were supplemented with 50 μl of an aqueous solution containing 0.58 μCi of [^{14}C]cefpiramide. The reaction mixtures were incubated at 37°C for 30 min with gentle shaking and then subjected to ultracentrifugation (156,500 × *g* at 16°C for 18 h) with a model 80P ultracentrifuge (Hitachi Koki Co., Ltd., Tokyo, Japan). The floating lipoproteins were removed. For measuring unbound cefpiramide concentrations, aliquots (200 μl) of supernatants were transferred to vials containing 20 ml of dioxane scintillator {7 g of PPO (2,5-diphenyloxazole) and 0.3 g of POPOP [1, 4-bis(5-phenyloxazolyl)benzene] in 1 liter} and subjected to the radioactivity counting described below. Unbound cefazolin concentrations in supernatants were determined by the high-pressure liquid chromatography method described below. Parallel experiments were run for plasma-free reaction mixtures, in which plasma was displaced by phosphate buffer (0.01 M, pH 7.4). Each binding percentages was calculated as 100 $(N - M)/N$, where M and N represent the cephalosporin concentrations in supernatants of the plasma-containing and plasma-free reaction mixtures, respectively.

Radioactivity was measured on a liquid scintillation counter (model 3255; Packard Instrument Co., Inc., Downers Grove, Ill.). Quenching was corrected by the external standardization method, and the data were processed on a computer (Canola SX-300; Canon Co., Ltd., Tokyo, Japan).

After ultracentrifugation, 200 μl of cefazolin-containing supernatants was transferred into another series of tubes, and then 5 to 20 μl was directly subjected to a high-pressure liquid chromatography assay. Standard solutions were prepared with distilled water. The assay system consisted of a pump (635A; Hitachi Ltd., Tokyo, Japan), a column (4 mm [inside diameter] by 150 mm) packed with Nucleosil C_{18} (5 μm; Macherey-Nagel, Düren, Federal Republic of Germany), and a multiwavelength UV detector (UVIDEC-100-II; Japan Spectroscopic Co., Ltd., Tokyo, Japan). The column temperature was kept at 40°C, and the detector wavelength was set at 280 nm. Degassed mobile phase, consisting of phosphate buffer (0.067 M; pH 7.0)–acetonitrile (89/11 [vol/vol]) was pumped at a flow rate of 1.1 ml/min. The assay sensitivity was 0.3 μg/ml, and the coefficients of variation in triplicate determinations of spiked aqueous samples at concentrations of 1.5 and 15 μg/ml were 2.9 and 5.6%, respectively.

Statistics. In this report, all values with fiducial limits indicate mean values ± standard errors of the mean. An analysis of variance for crossover study was conducted. A probability value of less than 0.05 was used for statistical significance. Period (8-day interval) differences in the crossover study were not significant for maximum plasma level [C_{max}(PL)], AUC_{0-8}(PL), maximum capsular level [C_{max}(CF)],and AUC_{0-24}(CF).

RESULTS

Capsular fluid characteristics. Biological data from capsular fluid were determined by using specimens obtained from 11 capsules 5 days after the first medication, i. e., 23 days after implantation, and are shown in Table 1. No fluid was obtained from one capsule of one rabbit among six rabbits used.

Pharmacokinetics. The antibiotic concentrations of cefpiramide and cefazolin in plasma samples and capsular fluids after intramuscular administration of 50 mg/kg are given in Fig. 1 (plasma) and Fig. 2 (capsular fluid). As for both cefpiramide and cefazolin, the observed levels in plasma fitted well to the calculated curves. The observed C_{max}(PL) achieved at 0.5 h was 47.7 ± 5.6 μg/ml for cefpiramide and 37.4 ± 2.5 μg/ml for cefazolin. Then, the levels in plasma declined down to the 8-h levels (5.3 ± 0.9 μg/ml for cefpiramide and 0.9 ± 0.6 μg/ml for cefazolin, with half-lives in plasma of 2.20 h (cefpiramide) and 1.12 h (cefazolin) (Table 2). All cefpiramide and cefazolin levels in plasma at 24 h were lower than the assay sensitivity. For both drugs, AUC_{0-8}(PL) accounted for about 90% of $AUC_{0-\infty}$(PL), and the difference of cefpiramide and cefazolin in AUC_{0-8}(PL) was statistically significant ($P < 0.01$).

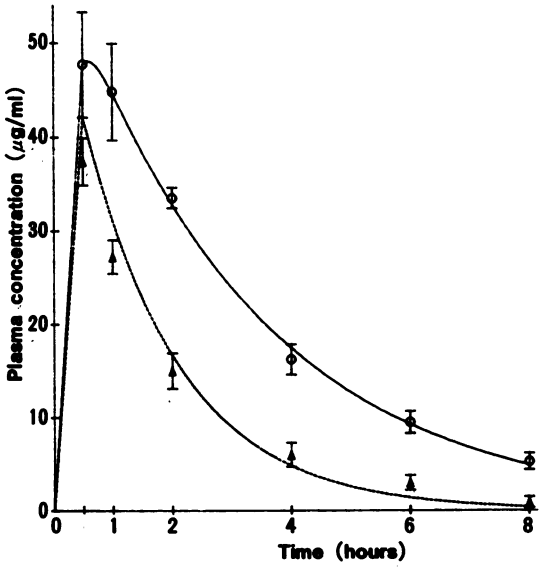

FIG. 1. Plasma concentration-time profiles for cefpiramide and cefazolin administered intramuscularly in doses of 50 mg/kg to rabbits. Shown are mean observed values and standard errors of the mean along calculated curves for cefpiramide (circles) and cefazolin (triangles).

Capsular levels showed considerable variability, even for two capsules implanted in the same rabbit. However, the mean observed capsular levels fitted well to the calculated curves expressed by the following equation: $C(\mathrm{CF}) = A(\mathrm{CF})[e^{-k_{el}(\mathrm{CF})(t - t_0)} - e^{-k_p(\mathrm{CF})(t - t_0)}]$, where C(CF) indicates a capsular level at time t, A(CF) is a constant for the dimension of concentration, k_{el} (CF) is the capsule elimination rate constant, k_p(CF) is the capsule penetration rate constant, and t_0 is the lag time before penetration occurs. The calculated values of these parameters for cefpiramide and cefazolin are listed in Table 2.

Unlike the concentration-time profiles for plasma, the observed C_{max}(CF) was attained at 8 h for cefpiramide (7.5 ± 0.9 μg/ml) and at 6 h for cefazolin (3.9 ± 0.9 μg/ml), whereas the calculated C_{max}(CF) was at 11.8 h for cefpiramide (8.1 μg/ml) and at 8.1 h for cefazolin (3.9 μg/ml). The difference of the two drugs for the observed C_{max}(CF) was significant ($P < 0.01$).

Plasma protein binding. At total drug concentrations of 25, 50, 100, 200, and 400 μg/ml, the plasma protein bindings were 89.5, 88.6, 89.0, 84.9, and 80.8%, respectively, for cefpiramide and 97.0, 96.4, 95.0, 89.2, and 69.2%, respectively, for cefazolin. The mean binding percentages at a total drug concentration range lower than 100 μg/ml, at which the levels of both drugs in plasma in the pharmacokinetic study existed, were 89.0 ± 0.3% for cefpiramide and 96.1 ± 0.6% for cefazolin. The $AUC_{0-\infty}$ (PL) for free drug [free $AUC_{0-\infty}$(PL)] obtained by multiplying the percentages that were drug-free (11% for cefpiramide and 3.9% for cefazolin) times $AUC_{0-\infty}$(PL) was 20.1 μg · h/ml for cefpiramide and 3.43 μg · h/ml for cefazolin.

DISCUSSION

Pharmacokinetics for penetration and elimination of cefpiramide and cefazolin in capsular fluid were studied with rabbits bearing capsules in the peritoneal cavity, a technique developed by Gerding et al. (5). With the exception of erythrocyte counts, the gross and microscopic characteristics of capsules and the capsular fluid characteristics were somewhat similar to those reported by these researchers.

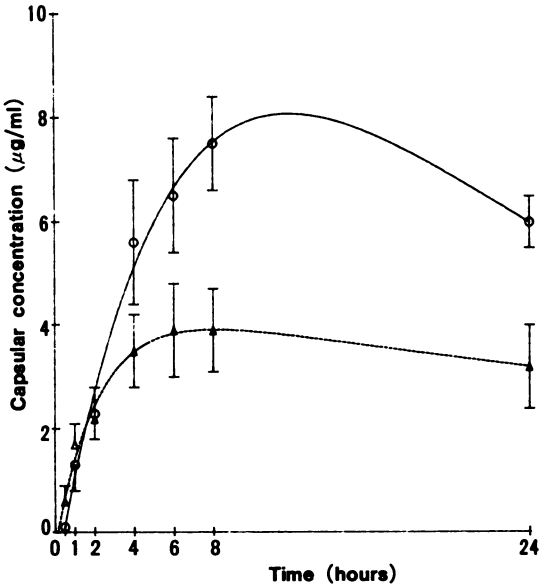

FIG. 2. Capsular concentration-time profiles for cefpiramide (circles) and cefazolin (triangles) administered intramuscularly at doses of 50 mg/kg to rabbits.

TABLE 2. Pharmacokinetic parameters for drug concentrations in plasma and capsules

Sample	Parameter[a]	Unit	Cefpiramide	Cefazolin
Plasma	k_a(PL)	h^{-1}	5.04	11.1
	k_{el}(PL)	h^{-1}	0.316	0.619
	$t_{1/2}$(PL)	h	2.20	1.12
	V(PL)	ml/kg	864	918
	$AUC_{0-\infty}$(PL)	μg · h/ml	183	88.0
	AUC_{0-8}(PL)	μg · h/ml	165 ± 9	76.0 ± 6.4
Capsule	A(CF)	μg/ml	22.7	4.53
	t_0	h	0.45	0.14
	k_p(CF)	h^{-1}	0.139	0.448
	k_{el}(CF)	h^{-1}	0.0509	0.0145
	T_{max}(CF)	h	11.8	8.1
	C_{max}(CF)	μg/ml	8.1	3.9
	$AUC_{0-\infty}$(CF)	μg · h/ml	283	302
	AUC_{0-24}(CF)	μg · h/ml	145 ± 15	81.2 ± 14.6

[a] V(PL), k_a(PL), $t_{1/2}$ and T_{max}(CF) indicate volume of distribution, constant for transfer rate from muscle into circulating blood, drug half-lives in plasma, and time to C_{max}(CF), respectively. The explanations for the other parameters are given in the text.

As often seen in the studies involving peritoneal and subcutaneous tissue capsule models (2, 4–6, 9), the general feature of slow accumulation and elimination of drugs from capsular fluid has also been observed in this study. Since the concentrations of total protein (5.8 g/100 ml) and albumin (2.2 g/100 ml) in the plasma specimens of normal rabbits tested in our laboratories were slightly higher than the total protein concentration and comparable to the albumin concentration in the capsular fluid, capsular protein bindings were nearly equal or slightly less frequent than the plasma protein bindings. Provided that capsular protein bindings are equal to the plasma protein bindings and that the equilibrium is defined as occurring when k_p(CF)C(PL) is equal to k_{el}(CF)C(CF), the time periods required for equilibration, which were calculated by using the equations for the plasma concentration-time curves and capsular concentration-time curves as C(PL) and C(CF), respectively, were 9.6 h for cefpiramide and 9.9 h for cefazolin.

As in the review by Barza and Cuchural (1), these slow equilibrations may be characteristic of drug distribution into particular extravascular sites having low surface area/volume ratios. The inner diameter of a table tennis ball used in this study was 3.72 cm, and the surface area/volume ratio was 1.61/cm when the whole surface area of the sphere, not the total area of 250 holes, was used, because vascularization occurred through perforations in the capsule wall. If it is recognized that the permeability coefficient is an empiric constant which represents a summation of penetration through capillary pores (diffusion), capillary membrane (permeation), and possibly bulk flow, the permeability coefficient values calculated by this surface area/volume ratio and k_p(CF) were 2.40×10^{-5} cm/s for cefpiramide and 7.71×10^{-5} cm/s for cefazolin. These permeability coefficient values were similar in magnitude to those (5.45×10^{-5} cm/s) calculated by Barza and Cuchural (1) for methicillin penetration into the perforated plastic chambers inplanted in the subcutaneous tissue of rabbits (3).

It seems unreasonable to analyze the plasma concentration-time profiles with a one-compartment model, because

the capsule compartments actually exist apart from the plasma compartment. Indeed, there were not great differences between the plasma elimination constant [k_{el}(PL)] and k_p(CF) for both drugs. As described below, however, since only the free drug molecules seem to penetrate into the capsular fluid, there must be great differences between the rate of drug elimination from plasma and the rate of drug penetration into the capsular fluid. Therefore, the plasma concentration-time profiles for both drugs were well expressed by the equations of the one-compartment model by neglecting the existence of capsule compartments.

The capsular concentration-time profiles of both drugs were well expressed by the equations of model-independent pharmacokinetics. Cefpiramide was lower for k_p(CF), higher for k_{el}(CF), and five times higher for A(CF) than was cefazolin, resulting in a C_{max}(CF) of cefpiramide that was twice higher than that of cefazolin. Although A(CF) does not have distinct pharmacokinetic definition, a parameter like dose in compartment model pharmacokinetics must be included in A(CF). In this experimental model with slow equilibration between blood and capsular fluid, AUC(PL) is expected to provide an understanding of the circulating reservoir of the drugs for the extravascular site. As for $AUC_{0-\infty}$(PL) and free $AUC_{0-\infty}$(pL), values for cefpiramide were two and six times larger, respectively, than those for cefazolin. Assuming that only the free drug molecules can penetrate into the capsular fluid, free $AUC_{0-\infty}$(PL) may be a more relevant reservoir parameter. Indeed, the cefpiramide/cefazolin ratios of free $AUC_{0-\infty}$(PL) and A(CF) were nearly equal.

In conclusion, it is suggested that free $AUC_{0-\infty}$(PL), as well as k_p(CF) and k_{el}(CF), may play an important role in determining capsular concentration-time profiles in experimental models for particular extravascular sites of infection, like abscess into which drugs cannot easily penetrate.

ACKNOWLEDGMENT

We thank Dr. Tatsuhisa Yamashita, Department of Physiological Chemistry, School of Medicine, Juntendo University, for helpful discussions.

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