

## Pharmacokinetics of Cephalosporins in Normal and Septicemic Rabbits

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Received 25 February 1985/Accepted 16 July 1985

The differences in the pharmacokinetics of cefotaxime, moxalactam, and CPW 86-363, a new expanded-spectrum cephalosporin, were studied in healthy rabbits and in rabbits infected intravenously with *Streptococcus pneumoniae*. The pharmacokinetic analysis of concentration-time courses in the sera of infected animals according to a two compartment-model evidenced a clear decrease of drug fractions in the central compartment but enhanced drug fractions in the peripheral compartment. The shift was more pronounced in animals which received CPW 86-363 (60%;  $P < 0.05$ ) than in those which received cefotaxime (20%) or moxalactam (5%). Corresponding increases in drug concentration were observed in soft tissue interstitial fluid; therefore, the areas under the curve and mean residence times in the soft tissue interstitial fluid of infected rabbits were prolonged. The shift of drug fractions from the central compartment to other body fluid compartments during infection was thought to be due to cardiovascular changes associated with fever. No changes in serum binding of the three drugs were found during the course of the infection. The quantitative differences in the extent of altered distribution properties of the drugs might be due to variations in the physicochemical properties of the drugs.

The pharmacokinetics of various antibiotics have been extensively studied in healthy individuals and in patients with various degrees of renal or hepatic impairment. Only limited information, however, on the pharmacokinetics of antibiotics in patients or experimental animals with septicemia is available (2, 13). It might be anticipated that during such a severe pathological condition, the altered blood flow and permeability of blood vessel walls would influence the pharmacokinetics of antibiotics. Recent results indicate that during experimental meningitis in rabbits, the volume of distribution is larger than that in healthy rabbits (10). Hence, the concentrations of cefoperazone, moxalactam, and cefotaxime in the sera of infected rabbits are lower than those in the sera of healthy rabbits. We therefore studied the influence of septicemia on the pharmacokinetics of three expanded-spectrum cephalosporins, cefotaxime, moxalactam, and CPW 86-363, by monitoring their concentrations in serum and in the fluid of subcutaneously implanted diffusion chambers.

### MATERIALS AND METHODS

In each set of experiments, one group of four healthy and one group of four infected male chinchilla rabbits (weight, 3.0 to 3.5 kg each) were used. Infection was induced by the intravenous inoculation of  $5 \times 10^8$  CFU of an overnight culture of *Streptococcus pneumoniae*  $\Delta 179$  15 h before the pharmacokinetic investigation was carried out.

**Drug administration and sampling protocol.** CPW 86-363, a cephalosporin under preclinical and clinical investigation at Sandoz Inc. (9), was dissolved in a small volume of 10% sodium hydrogen phosphate and made up to the required concentration with sterile physiological saline. Moxalactam (Eli Lilly & Co.) and cefotaxime (Hoechst-Roussel Pharmaceuticals) were dissolved in physiological saline. The drugs were administered as single intravenous bolus doses of 30 mg/kg of body weight. Blood samples for antibiotic analysis

were obtained from a marginal ear vein of each rabbit before and at 5, 15, and 30 min and 1, 2, 3, and 4 h after drug administration. In addition, viable bacteria were estimated from blood samples drawn at 1, 2, 5, 6, 8, and 15 h after the inoculation and at the same time as blood was sampled for pharmacokinetic analysis.

In each rabbit, six diffusion chambers filled with physiological saline were implanted subcutaneously in the back 3 days before the study, by the procedure of Laber et al. (8). These chambers consist of filters (Millipore Corp.) with a pore size of 0.22  $\mu\text{m}$  and have a total volume of 0.3 ml. The composition of the interstitial fluid yielded by these devices is described elsewhere (12). After antibiotic administration, the suture clip was opened under surface analgesia and the chambers were individually removed with forceps at 0.5, 1, 2, 4, 6, and 8 h. Samples of serum and soft tissue interstitial fluid (STIF) were immediately stored in liquid nitrogen until the antibiotic assay was carried out.

**Antibiotic assay.** Drug concentrations in serum and STIF were measured by a modified agar diffusion technique described elsewhere (1), with *Providentia stuartii*  $\Delta 2457$  as the indicator strain. Duplicates of each sample were tested. The diameter of each zone of inhibition was semiautomatically measured twice, the two diameters being rectangular. These values were converted to concentrations with a microcomputer by use of calibration curves calculated for each drug on each day of the assay. The low limit of detection was between 0.3 and 0.15 mg/liter for all drugs.

**Pharmacokinetic analysis.** Antibiotic concentration-time curves in serum were analyzed according to an open two-compartment model. The antibiotic concentration after drug administration was determined by use of the exponential equation  $C = Be^{-\beta t} + Ae^{-\alpha t}$ , where  $A$  and  $B$  are the zero time intercepts of the two components of the biexponential curves and  $\alpha$  and  $\beta$  are the hybrid constants for distribution and elimination, respectively. Best-fitting curves for serum and STIF were computed by the method of least-squares analysis, and pharmacokinetic parameters were calculated by standard equations (7). Areas under the concentration-

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time curve and mean residence times were determined with the trapezoidal rule. Since the mean residence time reflects the tendency of a drug to remain in the body, this model-independent parameter describes the drug distribution (5).

Utilizing the pharmacokinetic parameters in serum, we calculated the dose fractions in the central and tissue compartments (14). The fraction of the dose in the central compartment ( $F_C$ ) was determined by the equation:

$$F_C = \frac{(\alpha - k_{21})e^{-\alpha t} + (k_{21} - \beta)e^{-\beta t}}{(k_{10} - \beta)e^{-\alpha t} + (\alpha - k_{10})e^{-\beta t}}$$

Where  $k_{21}$  is the transferrate constant of drug from the peripheral to the central compartment and  $k_{10}$  is the constant in the central compartment. The fraction of the initial dose in the tissue compartment ( $F_T$ ) was determined by the equation:

$$F_T = \frac{k_{12}(e^{-\beta t} - e^{-\alpha t})}{(k_{10} - \beta)e^{-\alpha t} + (\alpha - k_{10})e^{-\beta t}}$$

**Statistical analysis.** Pharmacokinetic parameters in normal and infected animals were compared by the Mann-Whitney U test (3).

**Protein binding.** After samples containing 5 to 20 mg of drug per liter were incubated at 37°C for 30 min, the binding of the three cephalosporins to rabbit serum proteins was determined in duplicate by the ultrafiltration method (Centrifree micropartition system; Amicon Corp.) as described by Craig and Suh (4).

## RESULTS

**Experimental septicemia.** The intravenous inoculation of *S. pneumoniae*  $\Delta 179$  caused systemic infection with bacteremia and elevation of rectal temperature in all animals and sets of experiments. The bacterial growth pattern and changes in body temperature of infected animals are depicted in Fig. 1. Untreated animals died 20 to 30 h after inoculation. Viable bacteria were recoverable only during the first hour after drug administration (detection limit,  $10^1$  CFU/ml); however, the administered dose was too low to cure the animals. The infecting organism was susceptible to all drugs tested. MICs were determined by a standard micromethod technique (MIC 2000; Dynatech Laboratories)

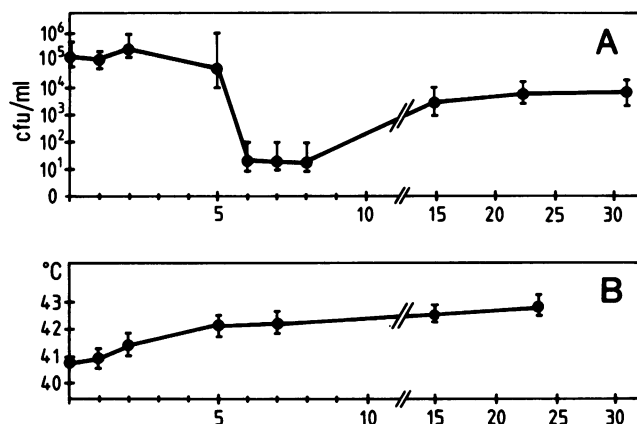


FIG. 1. Time course of CFU (A) and rectal temperature (B) in rabbits after infection with  $5 \times 10^8$  CFU of *S. pneumoniae*  $\Delta 179$ . Pharmacokinetic experiments were performed 15 h after inoculation.

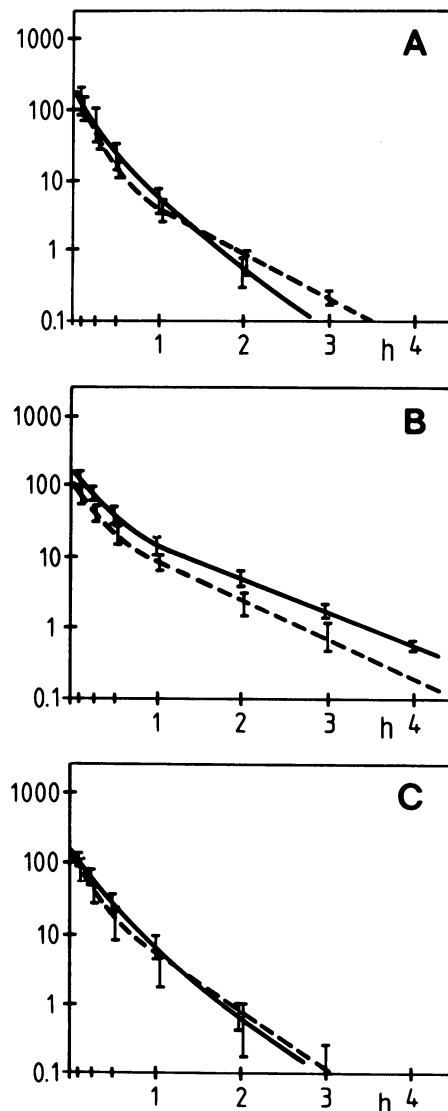


FIG. 2. Concentration-time courses in serum after intravenous injection of 30 mg of CPW 86-363 (A), moxalactam (B), and cefotaxime (C) per kg of body weight in healthy (—) and infected (----) rabbits. Values are plotted as means  $\pm$  standard deviations. Ordinate, Time (hours); abscissa, concentration (milligrams per liter).

in volumes of 0.05 ml of Trypticase soy broth (BBL Microbiology Systems). Overnight cultures in Trypticase soy broth supplemented with 5% fetal calf serum were used as inocula and were adjusted to  $10^6$  CFU/ml. MICs were as follows: CPW 86-363, 0.125 mg/liter; moxalactam, 3.12 mg/liter; cefotaxime, 0.156 mg/liter. After administration of the cephalosporins, body temperatures of the animals continued to increase and reached mean ( $\pm$  standard deviation) maximum values that were  $1.9 \pm 0.4^\circ\text{C}$  higher than those of normal animals.

**Protein binding.** The binding of the three cephalosporins to proteins in sera of healthy rabbits did not differ from that in infected animals and was 80.8% for CPW 86-363, 85.1% for cefotaxime, and 25.1% for moxalactam.

**Drug concentrations in serum and STIF and pharmacokinetic data.** Serum and STIF concentration-time curves of the three cephalosporins in healthy and infected rabbits are

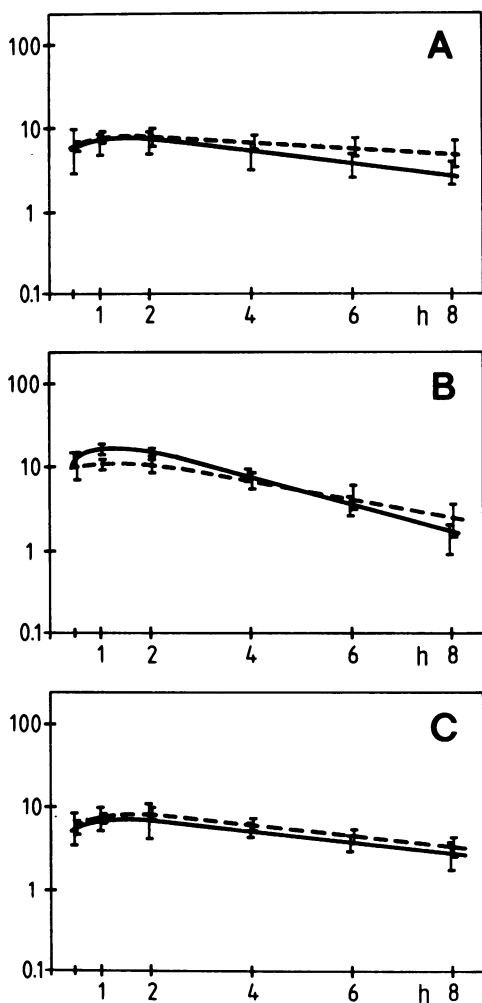


FIG. 3. Concentration-time courses in STIF diffusion chambers after intravenous injection of 30 mg of CPW 86-363 (A), moxalactam (B), and cefotaxime (C) per kg of body weight in healthy (—) and infected (---) rabbits. Values are plotted as means  $\pm$  standard deviations. Ordinate, Time (hours); abscissa, concentration (milligrams per liter).

shown in Fig. 2 and 3. The antibiotic concentrations in serum declined biexponentially for each drug in all experiments. In STIF one invasion and one elimination phase were seen.

All three drugs showed lower levels of drug in the sera of infected animals than in sera of normal animals. For example, 0.5 h after drug administration, the concentrations of the cephalosporins in the sera of infected rabbits decreased to about 50% (CPW 86-363), 40% (moxalactam), and 20% (cefotaxime) of that present in the sera of healthy rabbits. These differences were less marked at 3 to 4 h postadministration, indicating a prolonged elimination of the drugs.

Table 1 summarizes the pharmacokinetic parameters of the three drugs in healthy and infected animals. The most obvious alteration in the pharmacokinetics of the drugs in infected rabbits was a marked decrease in the zero time intercept for the elimination phase ( $P < 0.05$  for CPW 86-363) and of the hybrid constants of the elimination phase ( $P < 0.05$  for cefotaxime and CPW 86-363). The microscopic rate constants (rate of transfer from the central to the peripheral compartment and elimination rate constant from the central compartment) of all three drugs increased in infected animals, whereas the transfer rate constant from the peripheral to the central compartment decreased for CPW 86-363 ( $P < 0.05$ ) and moxalactam but increased for cefotaxime. The reduced drug concentrations in the sera of infected animals led to reduced areas under the concentration-time curve for all three drugs; these reductions were paralleled by enhanced clearance values ( $P < 0.05$  for moxalactam). The mean residence times were unaltered. The volume of distribution at steady state increased in infected rabbits ( $P < 0.05$  for moxalactam and CPW 86-363), indicating that, for all drugs, the distribution in infected rabbits was different from that in healthy animals. The dose fractions in the central and peripheral compartments in healthy and infected rabbits were therefore evaluated (Fig. 4). Under normal conditions, the mean ( $\pm$  standard deviation) dose fraction of moxalactam in the tissue compartment is  $0.60 \pm 0.07$ , which is significantly ( $P < 0.05$ ) higher than those of CPW 86-363 ( $0.45 \pm 0.09$ ) and cefotaxime ( $0.47 \pm 0.06$ ). Under pathological conditions, the dose fraction of moxalactam in the tissue compartment rose slightly, to  $0.62 \pm 0.05$ , whereas the corresponding values for cefotaxime and CPW 86-363 increased to  $0.57 \pm 0.15$  and  $0.72 \pm 0.01$ ,

TABLE 1. Pharmacokinetic data for CPW 86-363, moxalactam, and cefotaxime after intravenous injection of 30 mg/kg of body weight into healthy and infected rabbits

Parameter <sup>a</sup>	Mean $\pm$ SD in rabbits given <sup>b</sup> :					
	CPW 86-363		Moxalactam		Cefotaxime	
	Noninfected	Infected	Noninfected	Infected	Noninfected	Infected
A (mg/liter)	180.6 $\pm$ 67.5	172.6 $\pm$ 37.6	156.8 $\pm$ 27.2	119.8 $\pm$ 15.7	143.2 $\pm$ 25.3	168.2 $\pm$ 88.9
$\alpha$ ( $h^{-1}$ )	6.06 $\pm$ 2.1	6.62 $\pm$ 1.82	5.73 $\pm$ 1.0*	8.05 $\pm$ 1.57*	5.87 $\pm$ 1.5	8.54 $\pm$ 2.9
B (mg/liter)	57.7 $\pm$ 14.2*	16.9 $\pm$ 11.4*	51.9 $\pm$ 9.4	41.8 $\pm$ 3.8	45.6 $\pm$ 19.7	44.9 $\pm$ 31.2
$\beta$ ( $h^{-1}$ )	2.26 $\pm$ 0.32*	1.43 $\pm$ 0.27*	1.11 $\pm$ 0.05	1.32 $\pm$ 0.22	2.13 $\pm$ 0.08*	1.98 $\pm$ 0.09*
$k_{12}$ ( $h^{-1}$ )	0.86 $\pm$ 0.65	1.15 $\pm$ 0.75	1.74 $\pm$ 0.54	2.85 $\pm$ 0.77	0.86 $\pm$ 0.6	1.95 $\pm$ 1.6
$k_{21}$ ( $h^{-1}$ )	3.27 $\pm$ 0.92*	1.90 $\pm$ 0.60*	2.26 $\pm$ 0.33*	3.06 $\pm$ 0.53*	3.10 $\pm$ 0.65	3.55 $\pm$ 1.5
$k_{10}$ ( $h^{-1}$ )	4.17 $\pm$ 1.01	5.00 $\pm$ 0.70	2.84 $\pm$ 0.45	3.46 $\pm$ 0.55	4.06 $\pm$ 0.57	5.03 $\pm$ 1.6
AUC <sub>0-<math>\infty</math></sub> (mg $\cdot$ h/liter)	65.0 $\pm$ 18.0	42.4 $\pm$ 8.0	79.7 $\pm$ 6.5*	50.4 $\pm$ 4.3*	54.3 $\pm$ 7.6	47.0 $\pm$ 14.1
MRT (h)	0.28 $\pm$ 0.03	0.28 $\pm$ 0.02	0.58 $\pm$ 0.03	0.54 $\pm$ 0.1	0.29 $\pm$ 0.03	0.29 $\pm$ 0.08
$V_{ss}$ (liters/kg)	0.16 $\pm$ 0.04*	0.25 $\pm$ 0.03*	0.26 $\pm$ 0.01*	0.36 $\pm$ 0.03*	0.49 $\pm$ 0.19	0.69 $\pm$ 0.21
CL <sub>tot</sub> (liters/h)	0.49 $\pm$ 0.13*	0.72 $\pm$ 0.12*	0.38 $\pm$ 0.03*	0.60 $\pm$ 0.05*	0.56 $\pm$ 0.08	0.69 $\pm$ 0.21

<sup>a</sup> Abbreviations: A, zero time intercept for distribution ( $\alpha$ ) phase; B, zero time intercept for elimination ( $\beta$ ) phase;  $k_{21}$ , rate constant of drug transfer from the central to the peripheral compartment;  $k_{12}$ , rate constant of drug transfer from the peripheral to the central compartment;  $k_{10}$ , elimination rate constant from the central compartment; AUC<sub>0- $\infty$</sub> , area under concentration-time curve from 0 h to infinity; MRT, mean residence time;  $V_{ss}$ , volume of distribution at steady state; CL<sub>tot</sub>, total body clearance.

<sup>b</sup> \*, Level of significance for difference ( $P < 0.05$ ) between groups.

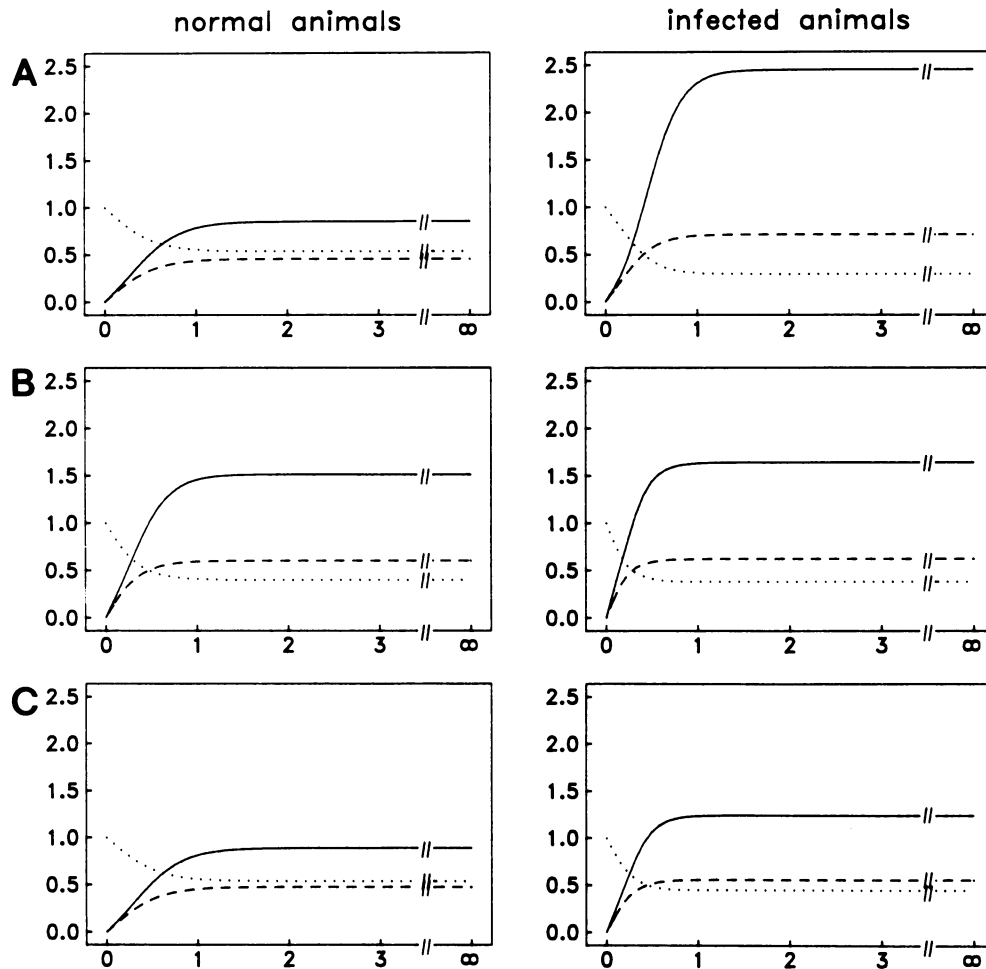


FIG. 4. Dose fractions in the central compartment (.....), in the tissue compartment (-----), and the quotient thereof (—) after intravenous administration of 30 mg of CPW 86-363 per kg (A), moxalactam (B), and cefotaxime (C) in healthy and infected rabbits. Ordinates, Time (hours); abscissae, dose fractions.

respectively. This increase is statistically significant ( $P < 0.05$ ) in comparison with CPW 86-363.

The enhanced drug fraction of CPW 86-363 in the peripheral compartment in infected rabbits correlated with increased ( $P < 0.05$ ) areas under the curve, longer mean residence times, and shorter ( $P < 0.05$ ) elimination rate constants in the STIF of infected animals. Increased maximum concentrations of CPW 86-363 in STIF, though not

statistically significant, were paralleled by faster drug transfer from the central to the peripheral compartment. Similar results were found with cefotaxime. No relevant changes in the area under the curve and mean residence time of moxalactam were found in the STIF of infected animals. Although maximum concentrations, which were reached later in infected rabbits than in normal rabbits, decreased significantly, the rate constant for drug invasion decreased,

TABLE 2. Pharmacokinetic data for CPW 86-363, moxalactam, and cefotaxime in STIF after intravenous injection of 30 mg/kg of body weight into healthy and infected rabbits

Parameter <sup>a</sup>	Mean $\pm$ SD in rabbits given <sup>b</sup> :					
	CPW 86-363		Moxalactam		Cefotaxime	
	Noninfected	Infected	Noninfected	Infected	Noninfected	Infected
$C_{max}$ (mg/liter)	8.16 $\pm$ 1.99	9.24 $\pm$ 0.52	18.96 $\pm$ 2.20*	13.09 $\pm$ 1.63*	7.42 $\pm$ 2.46	8.17 $\pm$ 1.31
$T_{max}$ (h)	1.33 $\pm$ 0.56	1.33 $\pm$ 0.40	1.11 $\pm$ 0.16	1.23 $\pm$ 0.13	1.83 $\pm$ 0.41	1.21 $\pm$ 0.57
$k_{inv}$ ( $h^{-1}$ )	2.11 $\pm$ 0.42*	4.13 $\pm$ 1.93*	1.76 $\pm$ 0.42	1.67 $\pm$ 0.36	1.27 $\pm$ 0.22*	2.95 $\pm$ 1.35*
$k_{el}$ ( $h^{-1}$ )	0.17 $\pm$ 0.04*	0.11 $\pm$ 0.03*	0.40 $\pm$ 0.06	0.33 $\pm$ 0.09	0.17 $\pm$ 0.06	0.15 $\pm$ 0.02
AUC <sub>0-∞</sub> (mg · h/liter)	63.1 $\pm$ 15.3*	103.0 $\pm$ 20.8*	78.4 $\pm$ 6.0	65.2 $\pm$ 16.6	63.3 $\pm$ 16.2	67.7 $\pm$ 13.6
MRT (h)	6.7 $\pm$ 1.5	10.3 $\pm$ 2.9	3.0 $\pm$ 0.3	3.8 $\pm$ 0.9	7.5 $\pm$ 2.5	7.5 $\pm$ 0.9

<sup>a</sup> Abbreviations:  $C_{max}$ , maximum concentration;  $T_{max}$ , time to maximum concentration;  $k_{inv}$ , invasion rate constant;  $k_{el}$ , elimination rate constant; AUC<sub>0-∞</sub>, area under the concentration-time curve from 0 h to infinity; MRT, mean residence time.

<sup>b</sup> \*, Level of significance ( $P < 0.05$ ) for differences between groups.

but that for drug elimination increased. Table 2 summarizes the derived pharmacokinetic parameters of the three drugs in STIF.

### DISCUSSION

Experimentally induced septicemia in rabbits, characterized by a significant elevation of body temperature, caused concentrations of cephalosporins in serum to decrease. All of the pharmacokinetic parameters used to describe distribution processes and the drug concentrations in STIF clearly demonstrated that the drug fractions transferred to the peripheral compartment in infected animals were higher than those transferred in healthy animals. This phenomenon was not due to altered drug binding in the sera of infected animals, but to the fever-induced circulatory changes and vessel wall permeability (6), which shifted the amount of drugs from the central compartment to other body fluid compartments. Quantitative differences in the extent of drug transfer to the peripheral compartment are probably related to physicochemical characteristics such as lipophilicity or degree of ionization, although specific data are lacking. The enhanced drug fractions in the peripheral compartment resulted in a reduced elimination of drug from the central compartment in the terminal phases. The increased concentrations of CPW 86-363 in STIF could therefore be correlated directly to the altered distribution parameters and prolonged elimination and mean residence times. In addition, the enhanced transfer rate constant from the central to the peripheral compartment, especially for CPW 86-363, correlates with the faster invasion of drug concentrations in STIF. It is not clear whether fever also alters renal clearance in infected rabbits as a result of enhanced renal blood flow (11), as only total clearance values were evaluated because of the difficulties in measuring renal clearance in rabbits. The altered pharmacokinetic profile might explain the reported excellent activity of CPW 86-363 in experimental infections (9).

### ACKNOWLEDGMENTS

We are indebted to G. de Jong, G. Kaufmann, H. Reiter, and H. Sigmund for excellent technical assistance. The help of Charles Lam in preparing the manuscript is also gratefully acknowledged.

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