

## Analysis of the Pharmacokinetic Interaction between Cephalexin and Quinapril by a Nonlinear Mixed-Effect Model

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Oligopeptidic drugs such as  $\beta$ -lactams and angiotensin-converting enzyme inhibitors share the same carriers in humans and animals, which results in possible pharmacokinetic interactions. To model such interactions, the effects of quinapril on cephalexin pharmacokinetics were investigated in rats. Blood cephalexin concentrations were measured by liquid chromatography, and the data were analyzed by a noncompartmental method and by fitting a bicompartamental model by a nonlinear mixed-effect modeling approach. Five groups of eight rats were examined. In the first three groups, cephalexin elimination kinetics after intra-arterial administration alone or in combination with quinapril given by the parenteral or the oral route were studied, and the occurrence of a pharmacokinetic interaction was not revealed. The absence of an effect of quinapril on cephalexin elimination after parenteral administration might be explained either by the higher affinity of cephalexin for the renal anionic transport system than that of quinapril or by the much higher concentrations of cephalexin than those of quinapril. In the last two groups, cephalexin was administered by the oral route alone or in combination with quinapril. The mean area under the concentration-time curve (AUC) for cephalexin was increased by ca. 30% by coadministration of quinapril (40.1 versus 31.4 mg · h/liter;  $P = 0.04$ ). The mean elimination clearance of cephalexin was significantly decreased by quinapril, from 0.81 to 0.64 liter/h/kg of body weight ( $P < 0.05$ ), probably by competitive inhibition of cephalexin secretion at the tubular level. The mean absorption rate constant of cephalexin was significantly lowered by quinapril (from 0.249 to 0.177 h<sup>-1</sup>;  $P < 0.01$ ), without modification of the extent of absorption (89%). This pharmacokinetic interaction could be explained by competitive inhibition of cephalexin active transport by quinapril at the intestinal level.

Intestinal absorption of  $\beta$ -lactams occurs at least in part by an active mechanism involving a dipeptide carrier. This mechanism has been demonstrated in rats (12, 22, 23), rabbits (20), humans (20), and human intestinal cell lines (5). The binding protein has been partly characterized (13). In humans, this active transport results in nonlinearity in the absorption kinetics of several  $\beta$ -lactams including, e.g., amoxicillin (15), bacampicillin (19), and cefatrizine (16). Active transport also results in pharmacokinetic interactions with dipeptides or tripeptides (18, 21), which lower the rate of absorption of  $\beta$ -lactams. In particular, angiotensin-converting enzyme (ACE) inhibitors, which have an oligopeptidic structure, have been shown to be absorbed by the same carrier (6) and to interact with  $\beta$ -lactams in isolated rat intestine (9). The first goal of our study was to evaluate the effect of one ACE inhibitor, quinapril, on the rate and extent of absorption of cephalexin in rats. However, there is a second putative site of interaction between ACE inhibitors and  $\beta$ -lactams. Indeed,  $\beta$ -lactams (2, 7) and ACE inhibitors (14) have been shown to be excreted by the renal anionic transport system, and concomitant administration of both drugs sometimes results in a pronounced inhibition of  $\beta$ -lactam elimination (10). The second goal of our study was therefore to characterize cephalexin elimination kinetics when cephalexin was associated with quinapril. Since a crossover study design could not be used with rats and the absorption of cephalexin was slow, only incomplete kinetic data were obtained for each animal. To allow the estimation of all the kinetic parameters of interest, a nonlinear mixed-effect mod-

eling approach was used to analyze data from parallel groups (11).

### MATERIALS AND METHODS

**Animals.** Male Wistar rats (weight, 250 to 280 g; IFACREDO, L'Arbresle, France) were housed at three rats per cage and were fed standard laboratory rat chow (AO4 entretien; UAR, Epinay sur Orge, France). The rats were fasted for 18 h before the experiment, with water given freely. The environment was maintained at a temperature of 22 to 23°C with a 12-h light and a 12-h dark cycle.

**Chemicals.** Cephalexin (lot no. 30HO307) was purchased from Sigma Chemical Co. Quinapril (lot no. AO-50000) was kindly supplied by Parke-Davis Laboratories. All other chemicals were of reagent grade and were used without further purification. An aqueous stock solution of cephalexin (10 mg/ml) containing quinapril (0.16 mg/ml) or not containing quinapril was prepared fresh for experiment. For drug administration via a gastric tube (GT), the drug used for treatment was suspended in 2% methylcellulose solution.

**Experimental protocol.** Twenty-four hours prior to the experiment, anesthesia was induced by intraperitoneal injection of thiopental (50 mg/kg of body weight). A catheter was installed into the carotid artery to allow the parenteral (intra-arterial [IA]) administration of the drugs and the collection of blood samples on the day of the experiment. For oral administration, the drugs were administered via a GT. In all cases, the administered dose was 50 mg/kg for cephalexin (1) and 0.8 mg/kg for quinapril. Five groups of eight rats were treated. Group 1 received cephalexin IA only; group 2 received cephalexin IA plus quinapril IA; group 3 received cephalexin IA plus quinapril via a GT; group 4 received cephalexin via a GT; group 5 received cephalexin via a GT plus quinapril via a GT.

When quinapril was given per os and cephalexin was given IA, quinapril was administered via a GT 15 min before cephalexin administration. Arterial blood samples (0.15 ml) were taken at time zero and at 5, 15, 30, 45, 60, and 90 min and 2, 3, 4, 5, and 6 h after cephalexin administration. After 30 min, the lost blood was compensated for with a double volume of isotonic 0.9% sodium chloride solution.

All samples were collected in tubes containing EDTA (200 mM, 10  $\mu$ l) and were stored at -80°C until they were assayed.

**Protein-binding studies.** Five rats were treated with cephalexin (50 mg/kg) IA as described above. Sampling times were 5, 30, and 120 min after cephalexin injection. The level of protein binding of cephalexin was determined by the ultrafiltration method (3) with 3-kDa-cutoff Microsep Filtron microconcentrators (Polylabo, Strasbourg, France). Aliquots (0.5 ml) of plasma containing the drug were pipetted into the filter cup, and the cups were centrifuged at 4,000  $\times$  g for 1 h at 37°C, thus yielding 0.2 ml of ultrafiltrate. The cephalexin concentra-

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TABLE 1. Individual parameters for cephalexin when cephalexin was given IA<sup>a</sup>

Group	AUC (mg · h/liter)		<i>t</i> <sub>1/2</sub> (h)	
	Mean ± SD	Range	Mean ± SD	Range
1	80.9 ± 27.7	40.0–130.4	1.4 ± 0.81	0.36–2.98
2	83.9 ± 71.8	40.0–250.2	1.04 ± 0.43	0.21–1.57
3	65.8 ± 56.8	24.0–197.3	1.32 ± 0.85	0.37–3.04
<i>P</i> value <sup>b</sup>	NS <sup>c</sup>		NS	

<sup>a</sup> Noncompartmental analysis.<sup>b</sup> Kruskal-Wallis test.<sup>c</sup> NS, not significant.

tions in the ultrafiltrate and retentate were determined by liquid chromatography. The coefficient of variation for a control sample containing 25 mg/liter was 4%. Nonspecific binding of cephalexin to the ultrafiltration device was estimated by filtering a solution of the antibiotic into the plasma ultrafiltrate, and it was found to be 3.2% ± 1.4% (*n* = 3).

The unbound cephalexin fraction in plasma (*f*<sub>u</sub>) was calculated as *C*<sub>u</sub>/*C*<sub>t</sub>, where *C*<sub>u</sub> is the concentration in the ultrafiltrate and *C*<sub>t</sub> is that in the retentate.

**Analytical methods.** Cephalexin was analyzed by liquid chromatography as described by Tamai et al. (22), with slight modifications. The analytical column was a Spherisorb C<sub>18</sub> (250 by 4.6 mm) column (SFCC). The mobile phase was methanol–0.1 M ammonium acetate (25/75; vol/vol). The flow rate of the mobile phase was 1 ml · min<sup>-1</sup>, and the absorbance was monitored at 262 nm.

Prior to injection, the proteins contained in blood samples were precipitated with 1 volume of acetonitrile. Lipids and acetonitrile were extracted with 3.5 volumes of dichloromethane. The aqueous phase was injected into the chromatographic system (20 μl). The calibration was linear in the range of 2 to 100 mg/liter. The limit of quantification was 2.0 mg/liter. The interassay precision ranged from 10% (coefficient of variation) at 5 mg/liter to 7% at 100 mg/liter. Quality controls were stored with blood samples in order to ensure the stability of cephalexin; no significant decrease in the cephalexin concentration was observed.

**Data analysis.** Cephalexin concentration-versus time data were analyzed by a noncompartmental method with SIPHAR software, version 4.0 (Simed, Creteil, France). The area under the plasma concentration-time curve (AUC) was determined by using the trapezoidal rule and was extrapolated to infinity by adding *C*<sub>t</sub>/*β*, where *C*<sub>t</sub> is the last quantifiable concentration and *β* is the slope of the elimination phase. The maximum concentration in plasma (*C*<sub>max</sub>) and the time to *C*<sub>max</sub> (*T*<sub>max</sub>) were the experimental values. The half-life (*t*<sub>1/2</sub>) was calculated as (log2)/*β*, where *β* was estimated by using weighted least squares with inverse concentration weighting.

Cephalexin data were also analyzed by a nonlinear mixed-effect modeling approach, i.e., the inter- and intra-individual variabilities were explicitly taken into account (17). Cephalexin kinetics were described by a two-compartment model. The pharmacokinetic parameters were the volume of the central compartment (*V*<sub>c</sub>), the volume at steady state (*V*<sub>ss</sub>), the elimination clearance (CL), the distribution clearance describing the exchange of cephalexin between the central and the peripheral compartments (CL<sub>D</sub>), the absorption rate constant (*K*<sub>a</sub>), and the fraction of the dose absorbed (*F*).

Two levels of variability were considered. Interindividual variability was taken into account by assuming that individual pharmacokinetic parameters arise from a log-normal distribution. The value of a given parameter in subject *j*, *P*<sub>*j*</sub>, is related to the typical value of that parameter in the population, *P*, by the equation *P*<sub>*j*</sub> = *P* exp (*η*<sub>*j*</sub>), where *η*<sub>*j*</sub> is a random effect normally distributed with a mean of zero and variance to be estimated in the analysis.

The second level of random variability implemented in the model was residual variability. This variability is a normally distributed random effect (*ε*) with a mean of zero and variance to be estimated, which accounts for the deviation of the observed cephalexin concentration (*C*<sub>*ij*</sub>) from the predicted concentration at time *t*<sub>*i*</sub> (*C*<sub>*ij*</sub>) by the equation *C*<sub>*ij*</sub> = *C*<sub>*ij*</sub> + *ε*<sub>*i*</sub> *C*<sub>*ij*</sub>, where *C*<sub>*ij*</sub> is calculated given *P*<sub>*j*</sub>.

**Model building.** The structural model was fitted to the data to obtain the population parameters (mean and variance of each parameter). Individual pharmacokinetic parameters were obtained as Bayesian post hoc estimates. Fitting of the population model was made by using the software NONMEM, version IV.2.0 (1). The first-order conditional estimation method was used (keyword, METHOD=COND).

Three categorical covariates were used to describe the mode of administration of cephalexin (IA or per os), the association with quinapril (yes or no), and the mode of administration of quinapril (IA or per os) in order to assess a difference in cephalexin pharmacokinetic parameters between the different groups.

**Goodness-of-fit criteria.** The population model was validated according to several criteria (1): (i) visual examination of the goodness of fit of each individual concentration-versus-time curve compared to that of the experimental data; (ii) visual comparison of the distribution of the standardized residuals to the normal

distribution (*N*); (iii) visual examination of the weighted residual-versus-time plot; and (iv) visual examination of the scatterplot of the observed versus the predicted cephalexin concentrations.

**Statistical analysis.** The proposed test is the likelihood ratio test which uses the difference in the log-likelihood statistics for the full and reduced models (25). The null hypothesis is that the goodness of fit of both models do not differ. The critical region for this test is derived from the assumption that twice the difference between the log-likelihood (objective function) asymptotically follows a chi-square distribution.

The likelihood ratio test has been performed with a theoretical level of significance of 0.05 by comparing the difference in the objective function values to the chi-square critical value, 3.84, for 1 degree of freedom. The null hypothesis is rejected if the difference is larger.

The post hoc estimates of individual parameters across the groups were compared by the Mann-Whitney or the Kruskal-Wallis test. Differences were considered statistically significant at a *P* value of ≤0.05.

## RESULTS

**Noncompartmental analysis.** The values of the individual parameters for cephalexin estimated by noncompartmental analysis are given in Tables 1 and 2. The Kruskal-Wallis test indicated that the mean AUC and *t*<sub>1/2</sub> were not different between groups 1, 2, and 3, i.e., in the groups to which cephalexin was given IA (Table 1). The Mann-Whitney test revealed no significant difference for *C*<sub>max</sub> and *T*<sub>max</sub> between group 4 and group 5, i.e., in the groups to which cephalexin was administered via a GT (Table 2). By contrast, the cephalexin AUC was significantly greater when cephalexin was combined with quinapril (40.1 mg · h/liter for group 5 versus 31.4 mg · h/liter for group 4). Data for the rate constants *K*<sub>a</sub> and *β* were not included in Table 2 because in some cases there was a flip-flop; i.e., the rate constant of the terminal phase after oral administration (group 4) was lower than the rate constant of elimination after IA administration (group 1). Elimination appeared to be faster than absorption, at least in some rats, so that the slope of the terminal phase could be either *β* or *K*<sub>a</sub>.

**Population analysis.** Population pharmacokinetic analysis was performed with all data (groups 1 to 5) and confirmed that a bicompartmental model was more adequate than a one-compartment model (data not shown). The results obtained by fitting the “basic” model with parameters CL, *V*<sub>c</sub>, CL<sub>D</sub>, *V*<sub>ss</sub>, *K*<sub>a</sub>, and *F* are presented in Table 3. Although the standard errors of Var(*η*<sub>CL</sub>) and Var(*η*<sub>*V*<sub>ss</sub></sub>) were quite large and their confidence intervals included zero, fixing them to zero resulted in a worse fit according to the likelihood ratio test; by contrast, Var(*η*<sub>*K*<sub>a</sub></sub>) and Var(*η*<sub>*F*</sub>) were not significantly different from zero, and fixing them to zero resulted in a similar fit.

Then, the likelihood ratio test was performed to compare the mean pharmacokinetic parameters for cephalexin in group 1 and group 2 and in group 1 and group 3 in order to assess the occurrence of a pharmacokinetic interaction of quinapril and/or its metabolites on the cephalexin distribution and/or elimination. No difference was found (the differences in the

TABLE 2. Individual parameters for cephalexin when cephalexin was administered via a GT<sup>a</sup>

Group	<i>C</i> <sub>max</sub> (mg/liter)		<i>T</i> <sub>max</sub> (min)		AUC (mg · h/liter)	
	Mean ± SD	Range	Median	Range	Mean ± SD	Range
4	8.7 ± 1.4	6.2–10.15	90	60–120	31.4 ± 8.6	20.7–44.8
5	9.5 ± 2.8	6.5–14.4	90	60–240	40.1 ± 9.6	27.9–53.8
<i>P</i> value <sup>b</sup>	NS <sup>c</sup>		NS		0.04	

<sup>a</sup> Noncompartmental analysis.<sup>b</sup> Mann-Whitney test.<sup>c</sup> NS, not significant.

TABLE 3. Population pharmacokinetic parameters for cephalexin in rats: reduced (basic) model

Value	CL (liter/h/kg)	$V_c$ (liter/kg)	CL <sub>D</sub> (liter/h/kg)	$V_{ss}$ (liter/kg)	$K_a$ (h <sup>-1</sup> )	$F$	$\sigma_e^{2a}$	OBJ
Population mean								
Estimate	0.694	0.386	0.347	1.4	0.200	0.90	0.027	1,141.7
SE	0.125	0.070	0.197	0.407	0.173	0.44	0.014	
Interindividual variability								
Estimate	0.416	0.872	2.14	2.41				
SE	0.194	0.230	1.05	1.29				

<sup>a</sup> Variance of the residual error model (see text).

objective function values [OBJs] were less than 2 in all comparisons; i.e., no such interaction was observed when cephalexin was given IA, thus confirming the noncompartmental analysis (data not shown).

Next, data for all five groups were analyzed together to assess the effects of the interaction on cephalexin absorption parameters. The likelihood ratio test demonstrated that allowing for two different values of  $K_a$  or  $F$  according to the value of the covariate "association with quinapril" significantly improved the fit only in the case of the parameter  $K_a$ . The mean  $K_a$  for cephalexin was lowered when quinapril was coadministered with cephalexin via a GT (0.249 versus 0.177 h<sup>-1</sup>; difference in OBJ, 1,141.7 - 1,133.1 = 8.6;  $P < 0.01$ ) without modification of the extent of absorption (89%).

Allowing for two different values of CL in the case of oral administration according to the value of the covariate "association with quinapril" significantly improved the fit (difference in OBJ, 1,133.1 - 1,129 = 4.1;  $P < 0.05$ ). The mean CL of cephalexin was significantly lower when quinapril and cephalexin were administered via a GT (0.810 versus 0.640 liter/h/kg).

Therefore, the final model describing cephalexin pharmacokinetics in all five groups is  $P_j = \bar{P} \exp(\eta_j)$  for CL,  $V_c$ , CL<sub>D</sub>, and  $V_{ss}$ ;  $CL_j = \bar{CL}_1$  for groups 1 to 4;  $CL_j = \bar{CL}_2$  for group 5;  $K_{a_j} = \bar{K}_{a1}$  if quinapril is not given;  $K_{a_j} = \bar{K}_{a2}$  if quinapril is given via a GT; and  $F_j = \bar{F}$ .

With this model, the population parameters have been obtained with reasonable precision, as shown by the standard errors of the estimates (Table 4).

A graph of the predicted concentrations (more precisely, the individual predictions based on the posterior estimates of cephalexin pharmacokinetic parameters according to the final model) versus observed concentrations is presented in Fig. 1. In the plot in Fig. 1, the residuals are randomly distributed around the identity line. The plot of weighted residuals versus time (Fig. 2) does not invalidate the model. Therefore, the

model has been considered to fit the data adequately. Figures 3 through 5 show the medians and nonparametric 90% confidence intervals for the cephalexin concentration-versus-time curves obtained by simulations based on 200 fictitious individuals with pharmacokinetic parameters arising from the distribution described in Table 4. Figure 3 illustrates cephalexin kinetics after IA administration, while Fig. 4 and 5 illustrate cephalexin kinetics after oral administration alone and combined with quinapril, respectively.

**Analysis of post hoc estimates.** The means and standard deviations of post hoc estimates of individual parameters are given in Table 5. CL and  $V_{ss}$  were not different across groups 1 to 3, i.e., when cephalexin was given IA. When cephalexin was administered via a GT, CL was significantly lowered with coadministration of cephalexin and quinapril (0.93 liter/h/kg for group 4 versus 0.54 ± 0.15 liter/h/kg for group 5), confirming the results of the analysis mentioned above. The new insight brought by this analysis was the tendency for a lower cephalexin CL after IA administration compared to that after oral administration (0.93 liter/h/kg for group 1 versus 0.69 liter/h/kg for group 4;  $P = 0.06$ ).

**Protein binding.** The  $f_u$  of cephalexin determined ex vivo was not different at 5, 30, and 120 min and amounted to 0.82 ± 0.08.

## DISCUSSION

In this study, an effect of an ACE inhibitor, quinapril, on the kinetics of a cephalosporin, cephalexin, was demonstrated in rats. Noncompartmental analysis of the data revealed no significant interaction when cephalexin was administered by the parenteral route, while an interaction was found when both compounds were given by the oral route. The quinapril-induced increase in the oral cephalexin AUC could have resulted from an increased bioavailability or a decreased elimination clearance of cephalexin. However, the interpretation of the

TABLE 4. Population pharmacokinetic parameters for cephalexin in rats: full model

Value	CL (liter/h/kg) <sup>a</sup>	CL (liter/h/kg) <sup>b</sup>	$V_c$ (liter/kg)	CL <sub>D</sub> (liter/h/kg)	$V_{ss}$ (liter/kg)	$K_a$ (h <sup>-1</sup> ) <sup>c</sup>	$K_a$ (h <sup>-1</sup> ) <sup>d</sup>	$F$	$\sigma_e^{2e}$	OBJ
Population mean										
Estimate	0.640	0.810	0.416	0.363	1.23	0.249	0.177	0.89	0.033	1,129.0
SE	0.164	0.110	0.057	0.071	0.238	0.056	0.036	0.25	0.015	
Interindividual variability										
Estimate		0.382	0.783	2.34	2.38					
SE		0.109	0.173	0.997	1.12					

<sup>a</sup> CL of cephalexin when quinapril and cephalexin were administered via a GT (group 5).

<sup>b</sup> CL of cephalexin for groups 1 to 4.

<sup>c</sup>  $K_a$  of cephalexin given alone.

<sup>d</sup>  $K_a$  of cephalexin when quinapril was administered via a GT.

<sup>e</sup> Variance of the residual error model (see text).

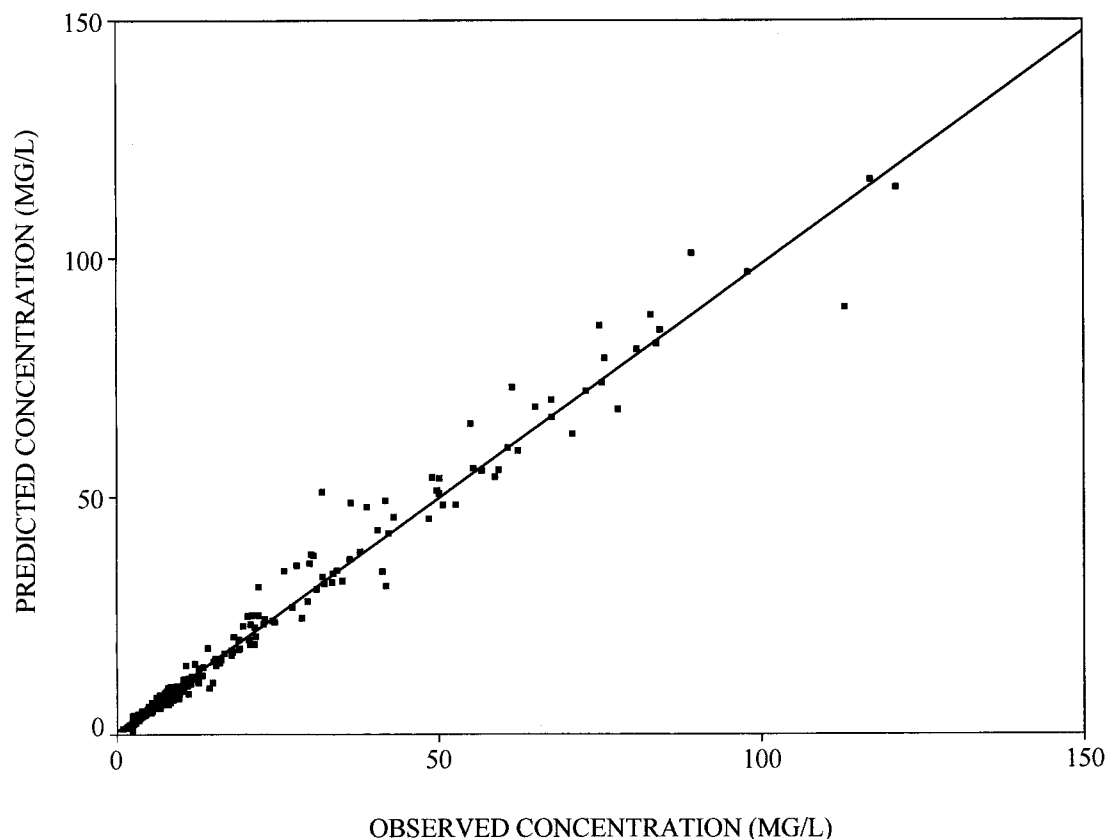


FIG. 1. Final model for cephalixin: scatterplot of predicted versus observed cephalixin concentrations. Predictions are based on the final population model in Table 4.

noncompartmental analysis was complicated by (i) the occurrence of a flip-flop phenomenon and (ii) the inability to perform successive administrations of cephalixin by the parenteral and oral routes in the same animals. The flip-flop phenomenon rendered difficult attribution of the terminal slope of the kinetic curve to  $\beta$  or  $K_e$ , while the inability to perform a crossover study precluded the estimation of individual  $F$  values.

The population approach at least in part alleviated these problems. (i) Combining data for rats receiving the drugs by the IA and oral routes in the analysis was likely to constrain the estimation of the pharmacokinetic parameters so that they were consistent across the groups, because minimization of the objective function forces the individual estimates of the parameters toward the mean value in the population. Since the data for IA administration brought the information about the "true" values of the distribution and elimination parameters, these parameters could be estimated properly from the oral data, even though the flip-flop phenomenon occurred in some rats. (ii) Treating the data for all groups all together allowed the estimation of the bioavailability of cephalixin, provided that the differences in elimination clearance among individuals were adequately accounted for. In this respect, the population analysis led to the individualization of two cephalixin clearances, the first corresponding to IA administration and oral administration of cephalixin alone (groups 1 to 4) and the second corresponding to the combined oral administration of both drugs. The analysis of mean post hoc estimates for each group confirmed the decreased cephalixin clearance induced by quinapril when both drugs were given by the oral route, but

it also showed that there was a tendency for the elimination clearance to be lower after IA administration than after oral administration ( $P = 0.06$ ). The latter phenomenon could be explained by a saturation of cephalixin elimination, probably at the renal site. Indeed, comparison of the clearance of cephalixin from blood (0.81 liter/h/kg, i.e., 13.5 ml/min/kg) to cephalixin glomerular filtration clearance (which is the product of  $f_{iu}$  and the glomerular filtration rate, i.e.,  $0.82 \times 10 = 8.2$  ml/min/kg) (9, 14) indicates that cephalixin is eliminated not only by glomerular filtration but also by tubular secretion in rats as well as humans (8). Therefore, we should in fact have introduced a third clearance in the population analysis to characterize the elimination in groups 1 to 3, group 4, and group 5 separately. However, the resulting model would not have been identifiable, because the data contained no information indicating whether the differences between the groups was related to differences in CL or  $F$ . This situation is similar to that for the estimation of  $F$  by traditional pharmacokinetic methods as the ratio of AUCs, which is based on the assumption that CL is the same after oral and parenteral administration. Therefore, a population model with three clearances could not be evaluated, so there might be a certain degree of misspecification in the model. Nevertheless, all these analyses indicated that quinapril reduces cephalixin elimination clearance when both drugs are given orally. Since cephalixin, like many other  $\beta$ -lactams, and ACE inhibitors are expected to be secreted by a renal anionic transport system (2, 7, 14, 24), inhibition of tubular secretion of cephalixin at the carrier level is the most probable mechanism for this pharmacokinetic interaction. The reason why this interaction was not observed when cephalixin

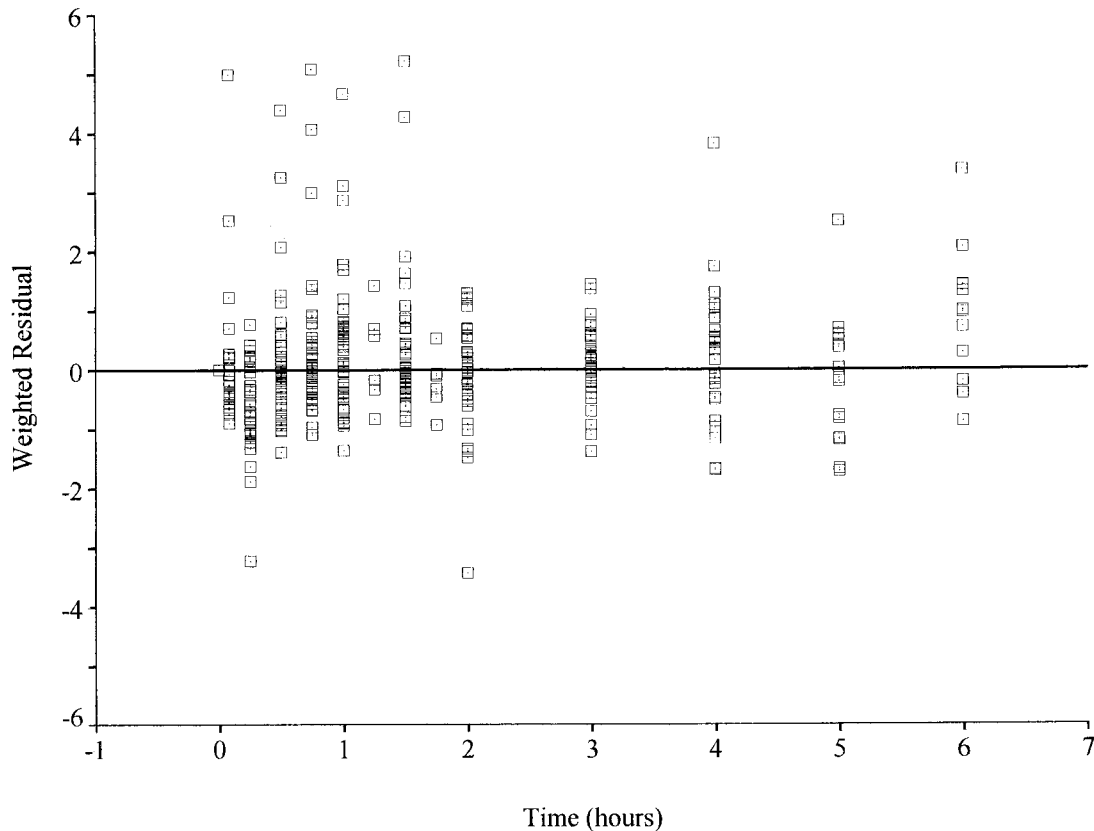


FIG. 2. Adequacy of cephalexin population model: weighted residual-versus-time plot. Weighted residuals are based on the final population model in Table 4.

was given IA could be that cephalexin concentrations were much higher than those of quinapril and/or its metabolites. Since the interaction was expected to be competitive, the high concentrations of cephalexin prevented the binding of quinapril and/or its metabolites to the carrier.

Regarding the interaction at the absorption level, the choice of a linear absorption model deserves to be addressed. If carrier-mediated transport was the only absorption process, a saturable Michaelis-Menten absorption model should be used

(16). However, cephalexin is absorbed both by saturable active transport and by passive diffusion (5). The rate of absorption is therefore given by the following equation:

$$-(dC_a/dt) = (V_{max}/K_m + C_a) \cdot C_a + (K_d \cdot C_a)$$

where  $C_a$  is the concentration of cephalexin at the absorption site,  $V_{max}$  is the maximal velocity of the active transport,  $K_m$  is the Michaelis constant, and  $K_d$  is the rate constant of absorp-

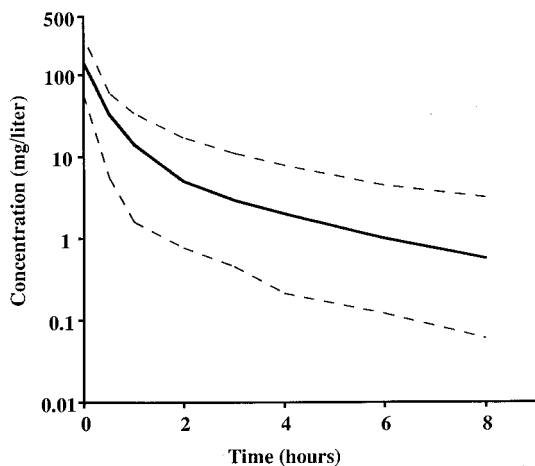


FIG. 3. Median (solid line) and 5th and 95th percentiles (dashed lines) of the distribution of cephalexin concentration versus time after IA administration to rats obtained by simulation based on the final population model in Table 4.

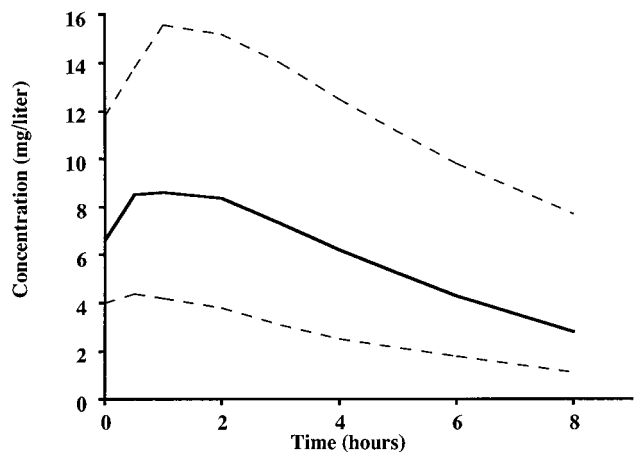


FIG. 4. Median (solid line) and 5th and 95th percentiles (dashed lines) of the distribution of cephalexin concentration versus time after administration of cephalexin alone via a GT to rats obtained by simulation based on the final population model in Table 4.

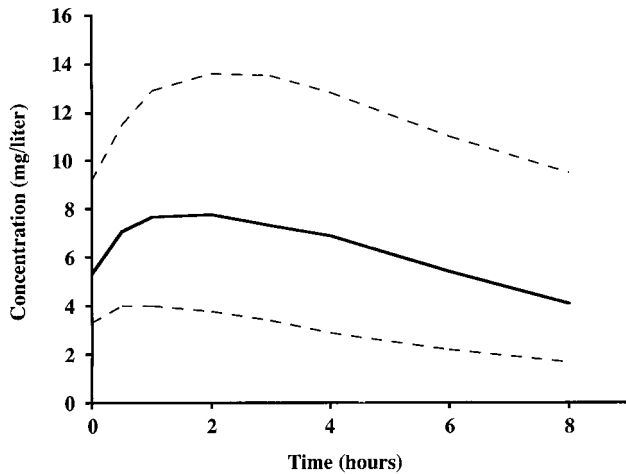


FIG. 5. Median (solid line) and 5th and 95th percentiles (dashed lines) of the distribution of cephalaxin concentration versus time after administration of cephalaxin and quinapril via a GT to rats obtained by simulation based on the final population model in Table 4.

tion by the passive diffusion mechanism. The relative contribution of each absorption mechanism depends on the dose and the respective values of  $V_{\max}$ ,  $K_m$ , and  $K_d$ . In case of a competitive interaction between cephalaxin and an ACE inhibitor,  $K_m$  becomes  $K_m [1 + (I/K_i)]$ , where  $I$  is the concentration of the inhibitor and  $K_i$  has its own  $K_m$ . Given that for cephalaxin,  $K_m$  is 7.5 mM,  $V_{\max}$  is 6.5, and  $K_d$  is 0.18 (4), approximate calculations show that the active component of cephalaxin absorption is zero order and represents only 40% of the overall absorption rate just after administration of the dose and 80% at the end of the absorption phase. At the end of the absorption, however, the cephalaxin concentration is lower than  $K_m$ , and therefore, the rate of absorption becomes:

$$-(dC_a/dt) = (V_{\max}/K_m) \cdot C_a + K_d \cdot C_a = K_a \cdot C_a$$

i.e., the absorption is first order. When cephalaxin is coadministered with an ACE inhibitor, a competitive interaction results in a higher  $K_m$ , and therefore, the active component represents a lower contribution to the overall rate while it becomes more rapidly first order. Thus, describing cephalaxin absorption by a first-order process was likely to produce only a little bias in the modeling. Conversely, the existence of passive absorption of

cephalexin, which is a priori not subject to kinetic interaction with ACE inhibitors, hindered in part the demonstration of an interaction at the carrier level, the contribution of which to the overall absorption might be moderate. This might explain the only modest decrease in  $K_a$  when cephalaxin was associated with quinapril. An alternative explanation is that the  $K_i$  of the ACE inhibitor was too high to promote a significant interaction and/or its concentration was too low. It should be recalled that the dose of the inhibitors was chosen on a clinically relevant basis and was 62.5 times lower than that of cephalaxin. The  $K_m$  of quinapril is not known, but the  $K_m$  of captopril is ca. 6 mM (9), i.e., the same order as that of cephalaxin. Other ACE inhibitors have very low  $K_m$  values, such as lisinopril (0.082 mM) and SQ29852 (0.08 mM) (6), and therefore, a strong interaction could eventually have been observed with quinapril.

The decreased rate of absorption of cephalaxin when it was combined with quinapril was not associated with a reduced bioavailability. Theoretically, a decreased bioavailability could have been observed if the absorption took place in a limited portion of the intestine, as suggested earlier (4). In our study, the reduction of absorption rate (-28%) was too small and/or the length of the absorption zone was too large to allow the observation of a reduction in the amount of cephalaxin absorbed.

Finally, it can be concluded that quinapril interacted with cephalaxin elimination and cephalaxin absorption. The active mechanisms of cephalaxin transport in humans and rats are largely similar, and therefore this pharmacokinetic interaction could also occur in humans. Since the overall effect of the interaction is an increase in the cephalaxin AUC, no decrease in the efficacy of the antibiotic is expected, while toxicity should not be increased because it is not concentration dependent. Hence, this interaction should not be relevant in clinical practice. Moreover, the effect on the absorption could easily be avoided by simply displacing the doses of cephalaxin and quinapril. However, the present study should be regarded as an experimental model for assessing such interactions. Depending on the respective values of  $K_m$  and doses of  $\beta$ -lactamines and ACE inhibitors, interactions between the members of each class could be more or less relevant. The methods developed in the investigation described here provide an example of a way that the difficulties in the analysis of such interactions in small animals can be overcome.

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#### REFERENCES

1. Beal, S. L., A. Boeckman, and L. B. Sheiner. 1992. NONMEM user's guide, version IV. NONMEM Project Group, University of California, San Francisco.
2. Bins, J. W., and H. Mattie. 1988. Saturation of the tubular excretion of  $\beta$ -lactam antibiotics. *Br. J. Clin. Pharmacol.* **25**:41-50.
3. Bowers, W. F., S. Fulton, and J. Thompson. 1984. Ultrafiltration versus equilibrium dialysis for determination of free fraction. *Clin. Pharmacokinet.* **9**(Suppl. 1):49-60.
4. Couet, W., B. Reigner, J. P. Guedes, and T. Tozer. 1991. Theoretical model for both saturable rate and extent of absorption: simulation of cefatrizine data. *J. Pharmacokinet. Biopharm.* **19**:271-285.
5. Dantzig, A., and L. Bergin. 1990. Uptake of cephalosporin, cephalaxin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. *Biochim. Biophys. Acta* **1027**:211-217.
6. Friedman, D. I., and G. L. Amidon. 1989. Intestinal absorption mechanism of dipeptide angiotensin converting enzyme inhibitors of the lysyl-proline type: lisinopril and SQ 29, 852. *J. Pharm. Sci.* **78**:995-998.
7. Granero, L., M. J. Gimeno, F. Torres-Molina, J. Chesajimenez, and J. E. Peris. 1994. Studies on the renal excretion mechanisms of cefadroxil. *Drug Metab. Dispos.* **22**:447-450.

TABLE 5. Individual parameter (post hoc) estimates for of cephalaxin

Group	Adminis- tration route	CL (liter/h/kg)		$V_{SS}$ (liter/kg)	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
1	IA	0.69 $\pm$ 0.23	0.39-1.15	1.32 $\pm$ 0.69	0.29-2.32
2	IA	0.74 $\pm$ 0.29	0.22-1.05	1.15 $\pm$ 0.59	0.07-1.67
3	IA	1.02 $\pm$ 0.50	0.21-1.56	1.40 $\pm$ 0.44	0.84-2.17
<i>P</i> value <sup>a</sup>		NS <sup>b</sup>		NS	
4	GT	0.93 $\pm$ 0.22	0.69-1.24	1.29 $\pm$ 0.55	0.70-2.1
5	GT	0.54 $\pm$ 0.15	0.29-0.72	1.37 $\pm$ 1.59	0.96-1.59
<i>P</i> value <sup>c</sup>		0.002		NS	

<sup>a</sup> Kruskal-Wallis test.

<sup>b</sup> NS, not significant.

<sup>c</sup> Mann-Whitney test.

8. Hori, R., K. Okumura, A. Kamiya, H. Nihira, and H. Nakano. 1983. Ampicillin and cephalexin in renal insufficiency. *Clin. Pharmacol. Ther.* **34**:792-798.
9. Hu, M., and G. Amidon. 1988. Passive and carrier-mediated intestinal absorption components of captopril. *J. Pharm. Sci.* **77**:1007-1011.
10. Jacolot, A., M. Tod, and O. Petitjean. 1996. Pharmacokinetic interaction between cefdinir and two angiotensin-converting enzyme inhibitors in rats. *Antimicrob. Agents Chemother.* **40**:979-982.
11. Karlsson, M., and L. Sheiner. 1994. Estimation of bioavailability when clearance varies with time. *Clin. Pharmacol. Ther.* **55**:623-637.
12. Kimura, T., T. Yamamoto, M. Mizuno, Y. Suga, S. Kitade, and H. Sezaki. 1983. Characterization of aminocephalosporin transport across rat small intestine. *J. Pharmacobio-Dyn.* **6**:246-253.
13. Kramer, W., F. Girbig, U. Gutjahr, S. Kowalewski, F. Adam, and W. Schiebler. 1992. Intestinal absorption of  $\beta$ -lactam antibiotics and oligopeptides: functional and stereospecific reconstitution of the oligopeptide transport system from rabbit small intestine. *Eur. J. Biochem.* **204**:923-930.
14. Lin, J., I. Chen, E. Ulm, and E. Duggan. 1988. Differential renal handling of angiotensin-converting enzyme inhibitors enalaprilat and lisinopril in rats. *Drug. Metab. Dispos.* **16**:392-396.
15. Paintaud, G., G. Aluan, M. L. Dahl, A. Grahnén, J. Sjövall, and J. O. Svensson. 1992. Nonlinearity of amoxicillin absorption kinetics in human. *Eur. J. Clin. Pharmacol.* **43**:283-288.
16. Reigner, B., W. Couet, J. P. Guedes, J. B. Fourtillan, and T. Tozer. 1990. Saturable rate of cefatrizine absorption after oral administration to humans. *J. Pharmacokinet. Biopharm.* **18**:17-34.
17. Sheiner, L. B., and T. M. Ludden. 1992. Population pharmacokinetics/dynamics. *Annu. Rev. Pharmacol. Toxicol.* **32**:185-209.
18. Sinko, P., and G. Amidon. 1989. Characterization of the oral absorption of  $\beta$ -lactam antibiotics. II. Competitive absorption and peptide carrier specificity. *J. Pharm. Sci.* **78**:723-727.
19. Sjövall, J., G. Aluan, and D. Westerlund. 1985. Dose-dependent absorption of amoxycillin and bacampicillin. *Clin. Pharmacol. Ther.* **38**:241-250.
20. Sugawara, M., T. Toda, K. Iseki, K. Miyazaki, H. Shiroto, Y. Kondo, and J. I. Uchino. 1992. Transport characteristics of cephalosporin antibiotics across intestinal brush-border membrane in man, rat and rabbit. *J. Pharm. Pharmacol.* **44**:968-972.
21. Sugawara, M., T. Toda, M. Kobayashi, K. Iseki, K. Miyazaki, H. Shiroto, J. I. Uchino, and Y. Kondo. 1994. The inhibitory effects of cephalosporin and dipeptides on cefitibuten uptake by human and rat intestinal brush-border membrane vesicles. *J. Pharm. Pharmacol.* **46**:680-684.
22. Tamai, I., H. Y. Ling, S. M. Timbul, J. Nishikido, and A. Tsuji. 1988. Stereospecific absorption and degradation of cephalexin. *J. Pharm. Pharmacol.* **40**:320-324.
23. Tsuji, A., T. Terasaki, I. Tamai, and H. Hirooka. 1987.  $H^+$  gradient-dependent and carrier-mediated transport of cefixime, a new cephalosporin antibiotic, across brush-border membrane vesicles from rat small intestine. *J. Pharmacol. Exp. Ther.* **241**:594-601.
24. Van Ginneken, C. A. M., and F. G. M. Russel. 1989. Saturable pharmacokinetics in the renal excretion of drugs. *Clin. Pharmacokinet.* **16**:38-58.
25. White, D. B., C. A. Walawander, D. Y. Liu, and T. H. Grasela. 1992. Evaluation of hypothesis testing for comparing two populations using NONMEM analysis. *J. Pharmacokinet. Biopharm.* **20**:295-313.