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

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Oligopeptidic drugs such as β-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 increased by ca. 30% by coadministration of quinapril (40.1 versus 31.4 μg·h/liter; P = 0.04). The mean elimination clearance of cephalexin was significantly decreased by quinapril (0.249 to 0.177 h⁻¹; 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 β-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 β-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 β-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 β-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 β-lactams. Indeed, β-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 β-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 modeling 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. 30H0307) 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.

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 (intravenous [IV]) administration of the drugs and the collection of blood samples on the day of the experiment. For oral administration via a gastric tube (GT), the drug used for treatment was suspended in 2% methylcellulose solution.

All samples were collected in tubes containing EDTA (200 mM, 10 μl) 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 Microsorb Filtron microconcentrators (Pall Corp., 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 × g for 1 h at 37°C, thus yielding 0.2 ml of ultrafiltrate. The cephalexin concen-
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 non post hoc estimates. Fitting of the population model was made by using the software NONMEM, version IV.2.0.1 (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 (V); (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 t1/2 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 Cmax and Tmax 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 Kc and β were not included in Table 2 because in some cases there was a flat-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 Kc.

Population analysis. Population pharmacokinetic analysis was performed with all data (groups 1 to 5) and confirmed that a bicompartimental model was more adequate than a one-compartment model (data not shown). The results obtained by fitting the “basic” model with parameters CL, V, CLD, VSS, Kp, and F are presented in Table 3. Although the standard errors of Var β(CpCt) and Var β(Vc) 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 β(Kc) and Var β(β) 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 1. Individual parameters for cephalexin when cephalexin was given IA

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC (mg·h/liter)</th>
<th>t1/2 (h)</th>
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<tbody>
<tr>
<td>1</td>
<td>80.9 ± 27.7</td>
<td>40.0–130.4</td>
</tr>
<tr>
<td>2</td>
<td>83.9 ± 71.8</td>
<td>40.0–250.2</td>
</tr>
<tr>
<td>3</td>
<td>65.8 ± 56.8</td>
<td>24.0–197.3</td>
</tr>
</tbody>
</table>

P value

a NS, not significant.

Noncompartmental analysis.

b Mann-Whitney test.

TABLE 2. Individual parameters for cephalexin when cephalexin was administered via a GT

<table>
<thead>
<tr>
<th>Group</th>
<th>Cmax (mg/liter)</th>
<th>Tmax (min)</th>
<th>AUC (mg·h/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8.7 ± 1.4</td>
<td>6.2–10.15</td>
<td>60–120</td>
</tr>
<tr>
<td>5</td>
<td>9.5 ± 2.8</td>
<td>6.5–14.4</td>
<td>60–240</td>
</tr>
</tbody>
</table>

P value

a NS, not significant.

Noncompartmental analysis.

b Mann-Whitney test.
objective function values [OBJs] were less than 2 in all comparisons); i.e., no such interaction was observed when cephalaxin 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 cephalaxin 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 cephalaxin was lowered when quinapril was coadministered with cephalaxin via a GT (0.249 versus 0.177 $h^{-1}$; 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 cephalaxin was significantly lower when quinapril and cephalaxin were administered via a GT (0.810 versus 0.640 liter/h/kg).

Therefore, the final model describing cephalaxin pharmacokinetics in all five groups is $P_j = \bar{P} \exp (\eta_j)$ for CL, $V_e$, $CL_P$, and $V_{ss}$; $CL = \bar{CL}_1$ for group 1 to 4; $CL = \bar{CL}_2$ for group 5; $K_a = \bar{K}_a$ if quinapril is not given; $K_a = \bar{K}_{a2}$ 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 model has been considered to fit the data adequately. Figures 3 through 5 show the medians and nonparametric 90% confidence intervals for the cephalaxin 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 cephalaxin kinetics after IA administration, while Fig. 4 and 5 illustrate cephalaxin 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 cephalaxin was given IA. When cephalaxin was administered via a GT, CL was significantly lowered with coadministration of cephalaxin 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 cephalaxin 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 $fu$ of cephalaxin 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, cephalaxin, was demonstrated in rats. Noncompartmental analysis of the data revealed no significant interaction when cephalaxin 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 cephalaxin AUC could have resulted from an increased bioavailability or a decreased elimination clearance of cephalaxin. However, the interpretation of the

<table>
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<th>TABLE 3. Population pharmacokinetic parameters for cephalaxin in rats: reduced (basic) model</th>
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<tr>
<td>Value</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Population mean</td>
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<td></td>
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<tr>
<td>Interindividual variability</td>
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* Variance of the residual error model (see text).

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<th>TABLE 4. Population pharmacokinetic parameters for cephalaxin in rats: full model</th>
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<td>Population mean</td>
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$^a$ CL of cephalaxin when quinapril and cephalaxin were administered via a GT (group 5).

$^b$ CL of cephalaxin for groups 1 to 4.

$^c$ $K_a$ of cephalaxin given alone.

$^d$ $K_a$ of cephalaxin when quinapril was administered via a GT.

$^e$ Variance of the residual error model (see text).
noncompartmental analysis was complicated by (i) the occurrence of a flip-flop phenomenon and (ii) the inability to perform successive administrations of cephalexin 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 $b$ or $K_u$, 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 cephalexin, 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 cephalexin clearances, the first corresponding to IA administration and oral administration of cephalexin 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 cephalexin 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 cephalexin elimination, probably at the renal site. Indeed, comparison of the clearance of cephalexin from blood (0.81 liter/h/kg, i.e., 13.5 ml/min/kg) to cephalexin glomerular filtration clearance (which is the product of $f_{ue}$ and the glomerular filtration rate, i.e., $0.82 \times 10 = 8.2$ ml/min/kg) (9, 14) indicates that cephalexin 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 cephalexin elimination clearance when both drugs are given orally. Since cephalexin, 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 cephalexin at the carrier level is the most probable mechanism for this pharmacokinetic interaction. The reason why this interaction was not observed when cephalexin

FIG. 1. Final model for cephalexin: scatterplot of predicted versus observed cephalexin concentrations. Predictions 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:

\[-(dC_a/dt) = \left(\frac{V_{\text{max}}}{K_m} + C_a\right) \cdot C_a + (K_d \cdot C_a)\]

where \(C_a\) is the concentration of cephalexin at the absorption site, \(V_{\text{max}}\) is the maximal velocity of the active transport, \(K_m\) is the Michaelis constant, and \(K_d\) is the rate constant of absorb-
and therefore, the rate of absorption becomes:

\[ \frac{dC}{dt} = \left( \frac{V_{\text{max}}}{K_m} \right) \cdot C_s + K_d \cdot C_s = K_m \cdot C_s \]

i.e., the absorption is first order. When cephalexin 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 cephalexin absorption by a first-order process was likely to produce only a little bias in the observation of a reduction in the amount of cephalexin absorbed.

Finally, it can be concluded that quinapril interacted with cephalexin elimination and cephalexin absorption. The active mechanisms of cephalexin 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 cephalexin 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 cephalexin 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.

**ACKNOWLEDGMENTS**

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**REFERENCES**


