Pharmacokinetic-pharmacodynamic model relating zabciprilat plasma concentrations to brachial and femoral haemodynamic effects in normotensive volunteers

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Aims To investigate, in healthy volunteers, the relationships between the plasma concentrations (C , ng ml^{-1}) of zabciprilat, the active metabolite of the angiotensin I-converting enzyme inhibitor (ACEI) zabcipril, and the effects (E) induced on plasma converting enzyme activity (PCEA, $\text{nmol ml}^{-1} \text{ min}^{-1}$), brachial and femoral artery flows (BAF, FAF, ml min^{-1}), and brachial and femoral vascular resistances (BVR, FVR, mmHg.s ml^{-1}) after a single oral administration of two doses (0.5 and 2.5 mg) of zabcipril.

Methods The study was placebo-controlled, randomized, double-blind and crossover. E was related to C by the Hill model, $E = E_{\text{max}} \cdot C^\gamma / (CE_{50}^\gamma + C^\gamma)$, fitted to the data of both doses simultaneously.

Results We obtained (mean $\pm$ s.d.) $E_{\text{max}} = -99 \pm 1\%$, $CE_{50} = 2.2 \pm 1.0 \text{ ng ml}^{-1}$ and $\gamma = 1.0 \pm 0.4$ for PCEA, $E_{\text{max}} = 55 \pm 26 \text{ ml min}^{-1}$, $CE_{50} = 5.1 \pm 4.0 \text{ ng ml}^{-1}$ and $\gamma = 2.4 \pm 1.6$ for BAF, and $E_{\text{max}} = -45 \pm 10\%$, $CE_{50} = 2.0 \pm 1.3 \text{ ng ml}^{-1}$ and $\gamma = 2.3 \pm 1.4$ for BVR. The parameters obtained for FAF and FVR were similar to those obtained for BAF and BVR, respectively. The CE_{95} (C required to induce 95% of E_{max}) varies from 7 to 17 ng ml^{-1} for haemodynamic effects.

Conclusions As zabciprilat peak plasma concentrations average 20 ng ml^{-1} after the 2.5 mg dose of zabcipril, this dose of the drug should be sufficient to induce optimal haemodynamic effects.

Keywords: zabcipril, healthy volunteers, haemodynamics, PK-PD modeling, concentration-effect relationship, sigmoid model, effect compartment

Introduction

Pharmacokinetic-pharmacodynamic (PK-PD) modeling is now widely recognized as a meaningful tool to determine the optimal dosage and the best mode of administration of a new drug [1, 2]. To achieve these goals, plasma concentrations and effects must be measured at different times after drug administration, a PK model must be fitted to the observed temporal profile of plasma concentrations and a PD model must be fitted to the observed concentration-effect relationship. Since both PK and PD models are non linear ones, the use of non linear regression and of minimization algorithms is required to estimate the parameters characterizing these models. However, despite this common methodological approach, several major differences explain why PK modeling is generally easier to perform and more successful than PD modeling. First, the information contained in the experimental data is usually of a better quantitative and qualitative value in pharmacokinetics than in pharmacodynamics. Second, in pharmacokinetics, the experimental design defines the times of measurement of concentrations. Since the time is the

independent variable of the regression, it is possible, if the study is properly designed, to obtain a data set that fully covers the kinetics of the drug. In pharmacodynamics also, the experimental design defines the times of measurement of effects, but then the time is not the independent variable of the regression and this induces several difficulties. On the one hand, at the stage of an experiment design, one can never be sure, even if the times of effects measurement are optimized, that the dose administered will generate a data set which will fully cover the concentration-effect relationship. On the other hand, the kinetics of effects are often delayed as compared with those of plasma concentrations which makes it very difficult to determine the times of effects measurement which would maximize the information afforded by the data.

Studies in healthy volunteers could be of particular interest to establish concentration-effect relationships. Indeed, effects can be measured repeatedly more easily than in patients. Moreover, several different doses can be investigated in the same volunteer thus allowing data sets distributed in different parts of the relationship to be obtained and to approach maximum effects. Since concentration-effect relationships are independent of the administered dose, it is theoretically possible to fit the same pharmacodynamic model simultaneously to the data

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obtained with multiple doses. However, until now, this approach does not seem to have been used and therefore its feasibility remains to be assessed.

One field for testing this assessment could be that of the anti-hypertensive drugs since it is now recognized that normotensive subjects provide valuable information for predicting the response of hypertensive patients [3, 4]. In a previous study performed in healthy volunteers [5], we investigated the systemic and regional haemodynamic and the biological effects of a single oral administration of two doses of the angiotensin I-converting enzyme inhibitor (ACEI), zabcipril. Simultaneously, we had measured the plasma concentrations of zabciprilat, the active metabolite of zabcipril. Therefore, the goal of the present study was to assess the feasibility of the above-mentioned PK-PD modeling approach using the data sets of concentrations and effects obtained after the administration of the two different doses of zabcipril. To reach this goal, we investigated the relationships between zabciprilat plasma concentrations on the one hand, and zabciprilat-induced effects on plasma converting enzyme activity and on regional haemodynamics in the brachial and femoral vascular beds on the other.

Methods

Experimental protocol

The protocol of the study has been extensively detailed previously [5]. Therefore, we will only recall its main features. It was a placebo-controlled, randomized, double-blind and crossover study performed in six healthy male volunteers (mean $\pm$ s.d.: 23.2 $\pm$ 1.2 years, 66.0 $\pm$ 3.2 kg, 176 $\pm$ 3 cm) and designed to investigate the pharmacokinetics and the pharmacodynamics of a single oral administration of two doses (0.5 and 2.5 mg) of zabcipril.

Pharmacodynamic parameters The following systemic and regional haemodynamic as well as biological parameters were investigated at rest, in the recumbent position, before and at least 1.5, 3, 4.5, 6, 9 and 24 h after drug intake. Systolic and diastolic arterial pressures (SAP, DAP, mmHg) were obtained using a Roche Sentron automatic monitor connected to a brachial cuff sphygmomanometer. Mean arterial pressure (MAP, mmHg) was calculated as $MAP = (1/3) SAP + (2/3) DAP$. Brachial and femoral artery flows (BAF, FAF, ml min⁻¹) were measured with a bidimensional pulsed Doppler system (Echovar Doppler pulsé 8 MHz, Alvar Electronics, Montreuil, France) as previously described and validated [6]. Brachial and femoral vascular resistances (BVR, FVR, mmHg.s ml⁻¹) were calculated as $BVR = MAP \times 60 / BAF$ and $FVR = MAP \times 60 / FAF$. Plasma converting enzyme activity (PCEA, nmol ml⁻¹ min⁻¹) was measured from venous blood samples by spectrophotometry [7]. For this parameter, two additional determinations were performed 12 and 48 h after drug intake.

Zabciprilat plasma concentrations Zabciprilat plasma concentrations (ng ml⁻¹) were determined from venous blood samples by radioimmunoassay [5], before and 0.5, 1, 1.5, 2, 3, 4.5, 6, 9, 12, 24 and 48 h after drug intake.

Pharmacokinetic-pharmacodynamic modelling

The PK-PD modeling was performed, in each individual subject, with software developed in our laboratory [8]. This software has four important features. Firstly, when the concentrations and effects of a drug are studied in the same subject after a single administration of several doses, it allows the determination of the concentration-effect relationship for that subject using the data of all doses simultaneously. Secondly, when the concentration-effect relationship displays an hysteresis loop, it allows the use of an effect compartment [9], but the estimation of the first order rate constant *keo* which governs the kinetics of the drug in the effect compartment is performed with non parametric kinetic and dynamic models [10]. As compared with the semi-parametric approach [11], this non parametric approach does not require the pre-determination of a pharmacokinetic model to estimate *keo*: plasma concentrations at any given time are calculated by linear interpolation between the concentrations observed at the two measurement times bracketing this time; effect compartment concentrations at any given time are calculated by numerical integration of the differential equation which governs the kinetics of the drug in the effect compartment. Thirdly, the optimal value of *keo* can be obtained by using either a one-dimensional minimization algorithm (golden section search or parabolic interpolation) implemented in the program [12], or, in case of difficulties of convergence, a step by step procedure driven by the user under the graphic control of the reduction of the hysteresis. Fourthly, when several doses of a drug are studied in the same subject and when the concentration-effect relationships display hysteresis loops, the software allows to estimate an optimal value of *keo* either for each dose separately or for all doses simultaneously.

The PK-PD modeling was performed between zabciprilat plasma concentrations and the following drug effects: percent variations from basal values for PCEA, BVR and FVR and absolute values for BAF and FAF. When the concentration-effect relationship did not display a hysteresis loop, the PK-PD modeling was directly performed using zabciprilat plasma concentrations and observed effects. When the concentration-effect relationship displayed a hysteresis loop, the effect compartment was used. After the determination of the optimal value of *keo*, the PK-PD modeling was performed using the concentrations of zabciprilat computed in the effect compartment and the observed effects.

Since in each case (at least for the 2.5 mg dose), the data showed a tendency towards a maximum effect, a sigmoid pharmacodynamic model was used to establish the relationships between zabciprilat concentrations and zabciprilat-induced effects. The general form of the model is $E = [E_{max} \cdot C^\gamma / (CE_{50}^\gamma + C^\gamma)] + E_0$ where *E* is the predicted effect, *C* the concentration of zabciprilat, *E*_{max} the maximum theoretical effect, *CE*₅₀ the concentration of zabciprilat which induces an effect of 50% of *E*_{max}, γ the Hill coefficient, and *E*₀ the basal effect (i.e., the effect without drug). A non weighed least squares criterion was used to fit the model to individual data and the goodness of fit was assessed from the distribution of residuals. For each effect, we tried to fit the model to the data of both doses simultaneously. Owing to the relatively small number of

data (from seven to nine pairs of concentrations and effects for each dose), only two parameters ($E_{\max}$ and CE_{50}) were estimated, the two others (γ and E_0) being constrained to fixed values [13]. For the effects expressed as percent changes from baseline values (PCEA, BVR and FVR), the procedure was only applied to estimate γ , E_0 being set to 0, whereas for the effects expressed as absolute values (BAF and FAF), the procedure was applied to estimate both γ and E_0 . In the first case, the least squares criterion was computed for all the values of γ in the range [0.5, 5.0] with a step of 0.5. In the second case, the least squares criterion was computed for all the possible pairs (γ , E_0), γ being defined in the range [0.5, 5.0] with a step of 0.5 and E_0 being defined as the 10 values bracketing the observed basal value with a step of 1 unit. In each case, $E_{\max}$ and CE_{50} were estimated simultaneously using four multi-dimensional minimization algorithms (downhill simplex, direction set, conjugate gradient and variable metric) implemented in the program [12].

Statistical presentation of results

For each subject, each estimated pharmacodynamic parameter was expressed as a point-estimate with an asymptotic s.d. derived from the variance-covariance matrix estimated at the optimum. For the whole population, the distribution of each (estimated or fixed) pharmacodynamic parameter was expressed as the mean with a s.d. computed from individual point-estimates. For keo , due to the generally small number of subjects in whom a hysteresis was observed, the median was reported instead.

Results

Zabiciprilat-PCEA relationship

Table 1 shows the individual and mean $\pm$ s.d. values of the pharmacodynamic parameters of the relationship between zabiciprilat concentrations and zabiciprilat effects on PCEA. The fitting of a sigmoid pharmacodynamic model was possible using the data of both doses simultaneously for the six subjects. In two subjects (2 and 5), the plots of individual concentration-effect relationships displayed hysteresis loops, whichever the dose. In two others, it was only for one dose (the 0.5 mg dose for subject 1 and the 2.5 mg dose for subject 3). Estimated $E_{\max}$ and CE_{50} were $-99 \pm 1\%$ and $2.2 \pm 1.0 \text{ ng ml}^{-1}$, respectively. The value of γ which allowed to obtain the best fittings was 1.0 ± 0.4 . When using an effect compartment, the median value of keo (1.04 and 1.03 h^{-1} after 0.5 and 2.5 mg, respectively) corresponded to a half-life of about 40 min.

Figures 1 and 2 display the successive steps of the modeling performed on PCEA in subject 1 after 0.5 and 2.5 mg, respectively. The relationship between zabiciprilat plasma concentrations and PCEA percent inhibition shows, when the points of the plots are connected in chronological order, an hysteresis phenomenon after 0.5 mg (top-left plot of Figure 1) but not after 2.5 mg (top plot of Figure 2). This leads to use an effect compartment for the data obtained after 0.5 mg only. When using such a compartment, the hysteresis collapses for an optimal value of keo of 0.36 h^{-1}

(bottom-left plot of Figure 1). The general shape of the so-obtained relationship, considered together with the one observed (in plasma) after 2.5 mg, leads to fit a sigmoid model to both doses. This model closely fits to the data of each dose. The optimal values of $E_{\max}$, CE_{50} and γ are, for this subject, -100% , 1.29 ng ml^{-1} and 0.75, respectively (top-right plot of Figure 1 and middle plot of Figure 2). The kinetics of observed and predicted effects are very close for each dose (bottom-right plot of Figure 1 and bottom plot of Figure 2).

Zabiciprilat-BAF and zabiciprilat-FAF relationships

Table 2 shows the individual and mean $\pm$ s.d. values of the pharmacodynamic parameters of the relationships between zabiciprilat concentrations and zabiciprilat effects on BAF and FAF. For the zabiciprilat-BAF relationship, the fitting of a sigmoid pharmacodynamic model was possible using the data of both doses simultaneously for five subjects (1, 2, 4, 5 and 6). In four subjects (1, 2, 5 and 6), the plots of individual concentration-effect relationships displayed hysteresis loops, whichever the dose. In contrast, in subject 4, there was no hysteresis. Estimated $E_{\max}$ and CE_{50} were $55 \pm 26 \text{ ml min}^{-1}$ and $5.1 \pm 4.0 \text{ ng ml}^{-1}$, respectively. The values of γ and E_0 which allowed to obtain the best fittings were 2.4 ± 1.6 and $73 \pm 8 \text{ ml min}^{-1}$, respectively. When using an effect compartment, the median value of keo (0.31 and 0.33 h^{-1} after 0.5 and 2.5 mg, respectively) corresponded to a half-life of about 130 min.

For the zabiciprilat-FAF relationship, the fitting of a sigmoid pharmacodynamic model was possible using the data of both doses simultaneously for two subjects (1 and 2) and using the data of only one dose for the four others (the 0.5 mg dose for subject 4 and the 2.5 mg dose for subjects 3, 5 and 6). In these cases, the plots of individual concentration-effect relationships generally displayed hysteresis loops. Estimated $E_{\max}$ and CE_{50} were $56 \pm 19 \text{ ml min}^{-1}$ and $7.1 \pm 7.7 \text{ ng ml}^{-1}$. The values of γ and E_0 which allowed to obtain the best fittings were 3.6 ± 0.9 and $107 \pm 21 \text{ ml min}^{-1}$, respectively. When an effect compartment was used, the median value of keo after 0.5 mg (0.32 h^{-1}) was of the same order of magnitude than that found for BAF, whereas after 2.5 mg (0.65 h^{-1}) it was larger, corresponding to a half-life of about 65 min.

Zabiciprilat-BVR and zabiciprilat-FVR relationships

Table 3 shows the individual and mean $\pm$ s.d. values of the pharmacodynamic parameters of the relationships between zabiciprilat concentrations and zabiciprilat effects on BVR and FVR. For the zabiciprilat-BVR relationship, the fitting of a sigmoid pharmacodynamic model was possible using the data of both doses simultaneously for five subjects (2 to 6) and using the data of only one dose for one subject (the 0.5 mg dose for subject 1). In these cases, the plots of individual concentration-effect relationships generally displayed hysteresis loops. Estimated $E_{\max}$ and CE_{50} were $-45 \pm 10\%$ and $2.0 \pm 1.3 \text{ ng ml}^{-1}$, respectively. The value of γ which allowed to obtain the best fittings was 2.3 ± 1.4 . When an effect compartment was used, the median value of keo (0.23 and 0.34 h^{-1} after 0.5 and 2.5 mg, respectively)

Table 1 Pharmacodynamic parameters of the relationship between zabiciprilat concentrations and zabiciprilat effects on plasma converting enzyme activity.

Subject	E_{max} (%)	Asymptotic s.d. E_{max} (%)	CE_{50} (ng ml ⁻¹)	Asymptotic s.d. CE_{50} (ng ml ⁻¹)	γ	keo (0.5 mg) (h ⁻¹)	keo (2.5 mg) (h ⁻¹)	r^2
1	-100	7	1.29	0.33	0.75	0.36	NH	0.8372
2	-99	8	3.12	0.50	1.50	1.04	1.03	0.8229
3	-98	4	1.56	0.14	1.50	NH	0.27	0.8951
4	-98	18	3.62	2.78	0.50	NH	NH	0.6488
5	-100	7	1.88	0.49	0.75	1.37	2.00	0.8425
6	-99	10	1.67	0.63	0.75	NH	NH	0.6445
Mean $\pm$ s.d.	-99 $\pm$ 1		2.19 $\pm$ 0.95		0.96 $\pm$ 0.43			

r^2 : determination coefficient. NH: no hysteresis. E_0 set to 0% for subjects 1 to 6.

Figure 1 PK-PD model for plasma converting enzyme activity (PCEA) on data obtained after 0.5 mg of zabicipril in subject 1. a) effects (▲) on PCEA as a function of zabiciprilat plasma concentrations (points connected in chronological order). b) effects (▲) on PCEA as a function of zabiciprilat effect compartment concentrations for the optimal value of keo (points connected in chronological order). c) effects (▲) on PCEA as a function of zabiciprilat effect compartment concentrations for the optimal value of keo and fitted pharmacodynamic model (solid line). d) kinetics of observed (▲) and predicted (solid line) effects on PCEA.

corresponded to a half-life comprised between 120 and 190 min.

For the zabiciprilat-FVR relationship, the fitting of a sigmoid pharmacodynamic model was possible using the data of both doses simultaneously for four subjects (1, 2, 5 and 6) and using the data of only one dose for two subjects (the 0.5 mg dose for subject 4 and the 2.5 mg dose for subject 3). In these cases, the plots of individual concentration-effect relationships generally displayed hysteresis loops. Estimated $E_{\max}$ and CE_{50} were $-32 \pm 8\%$ and $3.1 \pm 1.5 \text{ ng ml}^{-1}$ respectively. The value of γ which allowed to obtain the best fittings was 3.2 ± 1.6 . When using an effect compartment, the median value of keo (0.25 and 0.28 h^{-1} after 0.5 and 2.5 mg, respectively) corresponded to a half-life comprised between 150 and 165 min.

Figures 3 and 4 display the successive steps of the modeling performed on FVR in subject 2 after 0.5 and 2.5 mg, respectively. The relationship between zabiciprilat plasma concentrations and FVR percent reduction shows, when the points of the plots are connected in chronological order, an hysteresis phenomenon after 0.5 mg (top-left plot of Figure 3) but not after 2.5 mg (top plot of Figure 4). This leads to use an effect compartment for the data obtained after 0.5 mg only. When using such a compartment, the hysteresis collapses for an optimal value of keo of 0.25 h^{-1} (bottom-left plot of Figure 3). The general shape of the

so-obtained relationship, considered together with the one observed (in plasma) after 2.5 mg, leads to fit a sigmoid model to both doses. This model closely fits to the data of each dose. The optimal values of $E_{\max}$, CE_{50} and γ are, for this subject, -32% , 1.46 ng ml^{-1} and 2.50, respectively (top-right plot of Figure 3 and middle plot of Figure 4). The kinetics of observed and predicted effects are superimposed (bottom-right plot of Figure 3 and bottom plot of Figure 4).

Reasons for failure of the modeling

For BAF and for subject 3, the modeling was impossible for both doses because of two very important hysteresis phenomenons in which the maximum effects were observed at the last time of measurement. Once the effect compartment model was applied, the data obtained did not allow fitting to a pharmacodynamic model, whichever the dose. In the other cases, the modeling was performed using the data of only one dose. Generally, it was because of inconsistencies between 2 or 3 consecutive points of the concentration-effect relationship observed at the other dose (e.g., for FAF and for the 0.5 mg dose, subjects 3 and 6). In one case, it was because of a complete lack of effect (for FAF and for the 0.5 mg dose, subject 5).

Figure 2 PK-PD model for plasma converting enzyme activity (PCEA) on data obtained after 2.5 mg of zabciprilat in subject 1. a) effects (▲) on PCEA as a function of zabciprilat plasma concentrations (points connected in chronological order). b) effects (▲) on PCEA as a function of zabciprilat plasma concentrations and fitted pharmacodynamic model (solid line). c) kinetics of observed (▲) and predicted (solid line) effects on PCEA.

Discussion

This PK-PD study emphasizes the importance of studying two doses, which is easy to perform in healthy volunteers in early pharmacodynamic studies. If the doses are adequately chosen, sets of concentrations distributed in different (and, ideally, complementary) parts of the concentration–effect relationships can be obtained. This was the case in our study and we could fit the same pharmacodynamic model to the data obtained with both doses. This allowed us to estimate the parameters of the concentration–effect relationships more accurately than if we had had only one dose. Beside this feature, several other methodological options must be discussed.

Three of the five variables studied were baseline-corrected. This correction was introduced since it corresponds to the way the effects on these variables are generally displayed. Indeed, for converting enzyme activity as for regional resistances, effects are more commonly presented as percent inhibitions (for the former) or as percent reductions (for the latter) than in $\text{nmol ml}^{-1} \text{ min}^{-1}$ and in mmHg.s ml^{-1} , respectively. In contrast, for regional

blood flows, effects are usually described as absolute values and hence absolute values were used for the modelling.

Although the study was placebo-controlled, none of the five investigated variables was placebo-corrected. This results from the fact that, as shown in our previous paper [5], i) the values of the different investigated variables remained stable under placebo throughout the study thus excluding circadian/diurnal variations over 24 h, and ii) the effects of zabciprilat were, as compared with placebo, completely clear-cut. In this context, we can assume that the small variations observed in a given individual during the placebo period were random ones and therefore a placebo correction would just have increased the variability of the data without inducing any beneficial effect.

We estimated simultaneously, using minimization algorithms, $E_{\max}$ and CE_{50} , but we did not estimate γ and E_0 . The justification for this approach, already used and extensively discussed in two previous studies [14, 15], is that we had a relatively small number of data to estimate three and *a fortiori* four parameters. To avoid biases, the procedure was performed very carefully so that, in each case, we were able to detect a minimum which was not obtained for the extreme values of γ or of the (γ, E_0) pair tested. Thus, it is very likely that the results we obtained are those which would have been reached using a minimization algorithm to estimate three or four parameters simultaneously.

Unexpectedly, our data showed, for a given subject and a given effect, quite different hysteresis phenomena depending upon the dose studied. For example, for FVR, in two subjects, there was a hysteresis with each dose, but in one case the values of *keo* were quite similar (subject 1) whereas in the other one they were very different (subject 5); in two other subjects, there was a hysteresis with one dose and not with the other one, but in one case the hysteresis was observed after 0.5 mg (subject 2) whereas in the other one it was observed after 2.5 mg (subject 6). Such a wide intra-individual variability in the observation of hysteresis phenomena has seldom been reported until now, and only for psychotropic drugs [16, 17]. Knowing that, for a given effect, both the number of subjects in whom a hysteresis is observed and the medians of the distribution of *keo* are almost similar for the different doses, it may be suggested that this variability reflects more an ‘inter-administration’ than an ‘interdose’ variability. Another unexpected observation in our study was that this wide intra-individual variability, which made it mandatory to use a dose by dose approach for the modeling of the hysteresis phenomena, did not forbid us to fit, in a second step, the same pharmacodynamic model to the data of both doses simultaneously. This finding allows us to conclude that the magnitude of the time needed by zabciprilat to reach the effect compartment may vary from one administration to another, but that this phenomenon does not affect the concentration–effect relationship. Indeed, another explanation for this variability could be that some hystereses were fortuitous, in relation with the small number of data points we had [14]. However, since most hystereses were major ones, the use of an effect compartment led to very low values of *keo* (i.e., values below 0.5 h^{-1}). These low values generated kinetics in the effect compartment which were very different from those in the plasma, both in their

Table 2 Pharmacodynamic parameters of the relationships between zabiciprilat concentrations and zabiciprilat effects on brachial (top panel) and femoral (bottom panel) artery flows.

Subject	E_{max} (ml min ⁻¹)	Asymptotic s.d. E_{max} (ml min ⁻¹)	CE ₅₀ (ng ml ⁻¹)	Asymptotic s.d. CE ₅₀ (ng ml ⁻¹)	γ	keo (0.5 mg) (h ⁻¹)	keo (2.5 mg) (h ⁻¹)	r^2
1	21	2	1.22	0.19	3.00	0.31	0.27	0.5875
2	89	34	7.36	4.85	1.00	0.24	0.34	0.5785
3	—	—	—	—	—	—	—	—
4	43	3	0.45	0.05	5.00	NH	NH	0.6685
5	70	24	6.89	3.30	1.50	0.60	0.31	0.5934
6	54	11	9.61	3.22	1.50	0.31	0.84	0.7712
Mean $\pm$ s.d.	55 $\pm$ 26		5.11 $\pm$ 4.04		2.40 $\pm$ 1.64			
1	40	5	1.05	0.17	4.00	0.22	0.27	0.4928
2	40	1	1.96	0.09	3.50	0.41	1.63	0.9505
3	38	3	19.30	1.25	2.50	—	0.65	0.9897
4	83	20	0.67	0.19	3.00	NH	—	0.6356
5	67	5	13.59	0.57	3.50	—	1.97	0.9950
6	65	2	5.72	0.14	5.00	—	0.16	0.9940
Mean $\pm$ s.d.	56 $\pm$ 19		7.05 $\pm$ 7.71		3.58 $\pm$ 0.86			

r^2 : determination coefficient. NH: no hysteresis. For brachial artery flow: modeling impossible for subject 3; E_0 set to 70, 61, 78, 80 and 76 ml min⁻¹ for subjects 1, 2, 4, 5 and 6, respectively. For femoral artery flow: modeling using the data of only the 0.5 mg dose for subject 4 and the 2.5 mg dose for subjects 3, 5 and 6; E_0 set to 90, 82, 110, 140, 100 and 121 ml min⁻¹ for subjects 1 to 6, respectively.

Table 3 Pharmacodynamic parameters of the relationships between zabiciprilat concentrations and zabiciprilat effects on brachial (top panel) and femoral (bottom panel) vascular resistances.

Subject	E_{max} (%)	Asymptotic s.d. E_{max} (%)	CE_{50} (ng ml ⁻¹)	Asymptotic s.d. CE_{50} (ng ml ⁻¹)	γ	keo (0.5 mg) (h ⁻¹)	keo (2.5 mg) (h ⁻¹)	r^2
1	-60	15	1.32	0.16	4.00	0.20	—	0.9386
2	-46	6	1.84	0.45	2.00	0.25	0.14	0.6420
3	-33	4	1.25	0.55	1.00	0.20	0.20	0.5385
4	-36	2	0.40	0.04	4.00	NH	0.57	0.7783
5	-43	8	3.28	1.23	1.50	0.53	0.34	0.5522
6	-50	5	3.81	0.91	1.00	NH	0.48	0.8279
Mean ± s.d.	-45 ± 10		1.98 ± 1.31		2.25 ± 1.41			
1	-33	3	0.90	0.10	5.00	0.20	0.29	0.5836
2	-32	1	1.46	0.06	2.50	0.25	NH	0.9645
3	-21	1	9.77	0.59	5.00	—	0.26	0.9633
4	-37	5	0.54	0.09	3.50	NH	—	0.7707
5	-27	5	4.04	1.56	1.50	0.53	2.39	0.5898
6	-44	4	2.15	0.43	1.50	NH	0.16	0.7471
Mean ± s.d.	-32 ± 8		3.14 ± 1.47		3.17 ± 1.60			

r^2 : determination coefficient. NH: no hysteresis. For brachial vascular resistance: modeling using the data of only the 0.5 mg dose for subject 1; E_0 set to 0% for subjects 1 to 6, respectively. For femoral vascular resistance: modeling using the data of only the 0.5 mg dose for subject 4 and the 2.5 mg dose for subject 3; E_0 set to 0% for subjects 1 to 6, respectively.

Figure 3 PK-PD model for femoral vascular resistance (FVR) on data obtained after 0.5 mg zabicipril in subject 2. a) effects (▲) on FVR as a function of zabiciprilat plasma concentrations (points connected in chronological order). b) effects (▲) on FVR as a function of zabiciprilat effect compartment concentrations for the optimal value of ke_0 (points connected in chronological order). c) effects (▲) on FVR as a function of zabiciprilat effect compartment concentrations for the optimal value of ke_0 and fitted pharmacodynamic model (solid line). d) kinetics of observed (▲) and predicted (solid line) effects on FVR.

temporal aspects and in the magnitude of the concentrations obtained, and this induced important consequences on the concentration-effect relationship. Thus, if our hystereses had been fortuitous, it is very likely that, when using an effect compartment to deal with them, we would in some instances have found relationships very different from those observed with the plasma concentrations and, in this case, it would have been impossible to fit the same pharmacodynamic model to some data obtained in the effect compartment and in the plasma.

There was a sigmoid relationship between zabiciprilat plasma concentrations and the magnitude of zabiciprilat-induced PCEA blockade. Our $E_{\max}$, CE_{50} and γ values (-99% , 2.2 ng ml^{-1} and 1.0 , respectively) are a little higher and probably more accurate (since our $E_{\max}$ corresponds to a complete PCEA blockade) than those reported in healthy volunteers (-94% , 1.2 ng ml^{-1} and 0.8 ; [18]). Our CE_{50} appears to be of the same order of magnitude to those reported in healthy volunteers for lisinopril (1.3 ng ml^{-1} [19, 20]), ramiprilat (1.1 ng ml^{-1} [21]), perindoprilat (1.6 ng ml^{-1} [22]) or cilazaprilat (1.0 ng ml ; [23]). Moreover, like for perindoprilat in healthy volunteers [22], the relationship between zabiciprilat plasma concentrations and PCEA blockade displayed, in some cases, a hysteresis loop. This phenomenon is likely to be related to the temporal modification of the fractions of free and bound (to converting

enzyme) zabiciprilat. Indeed, for a given total concentration of zabiciprilat, and as the elimination process concerns exclusively the free form of the drug, the percentage of the free form was probably greater during the ascending phase of the kinetics than during its descending phase, thus explaining that blockade was less potent in the first than in the second phase of the kinetics.

Regarding BAF and BVR, the relationships between zabiciprilat plasma concentrations and the magnitude of zabiciprilat-induced effects were also sigmoid. The CE_{50} values (5.1 ng ml^{-1} for BAF, 2.0 ng ml^{-1} for BVR) were of the same order of magnitude than the corresponding one for PCEA blockade. However, the γ values found in our study were clearly above 1 demonstrating that, as previously reported with lisinopril in healthy volunteers [14], the concentration-effect relationships are steeper for haemodynamic effects than for effects on PCEA. From these results, it is possible to compute that the CE_{95} (concentration of zabiciprilat which induces an effect of 95% of $E_{\max}$) of zabiciprilat varies from 7 to 17 ng ml^{-1} for the haemodynamic effects. This result has a practical interest since concentrations of about 20 ng ml^{-1} correspond to the observed $C_{\max}$ of zabiciprilat after a single oral dose of 2.5 mg zabicipril [5] and it can therefore be suggested that the 2.5 mg dose will be sufficient to induce maximum haemodynamic effects. Finally, like for PCEA blockade, the

Figure 4 PK-PD model for femoral vascular resistance (FVR) on data obtained after 2.5 mg zabicipril in subject 2. a) effects (▲) on FVR as a function of zabiciprilat plasma concentrations (points connected in chronological order). b) effects (▲) on FVR as a function of zabiciprilat plasma concentrations and fitted pharmacodynamic model (solid line). c) kinetics of observed (▲) and predicted (solid line) effects on FVR.

concentration-effect relationship for BAF and BVR generally displayed hysteresis loops. The values of k_{eo} found for these effects were generally smaller than those observed for PCEA, thus probably expressing the additional delay required to reduce angiotensin II concentrations.

Regarding FAF and FVR, the concentration-effect relationships were also sigmoid, but the modeling was more difficult than for BAF and BVR. It appears from our results that there is a wider heterogeneity of the responses and a tendency towards a lower responsiveness than in the brachial territory.

In conclusion, the modeling of concentration-effect relationships from data sets obtained with two different doses, a characteristic of many crossover designs performed in healthy volunteers, constitutes an interesting approach which makes the fitting of non linear pharmacodynamic models possible and easier to deal with, particularly for clinical effects. When there are hysteresis phenomena, this approach must be preceded by a dose by dose search for the optimal value of k_{eo} . From a pharmacological viewpoint, our study shows that there are sigmoid relationships between zabiciprilat plasma concentrations and zabiciprilat-induced PCEA blockade, BAF (or FAF) increase, and BVR (or

FVR) decrease. In practice, a 20 ng ml^{-1} plasma concentration of zabiciprilat, which corresponds to the C_{max} of zabiciprilat achieved after a 2.5 mg oral dose of zabicipril, should be sufficient to induce optimal haemodynamic effects.

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