Pharmacokinetic-pharmacodynamic model for fantofarone cardiac and brachial haemodynamic effects in healthy volunteers

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Aims To investigate the pharmacokinetics of SR 33671, the main active metabolite of the calcium antagonist fantofarone, and the relationships between its concentrations and pharmacodynamic effects after a single oral administration of two doses (100 and 300 mg) of fantofarone.

Methods A placebo-controlled, randomized, double-blind and crossover study was performed in six healthy volunteers. SR 33671 plasma concentrations (C , ng ml^{-1}) and effects (E) on heart rate (HR, beats min^{-1}), PR interval duration (ms), brachial artery flow (BAF, ml min^{-1}) and brachial vascular resistance (BVR, mmHg s ml^{-1}) were determined repeatedly after drug intake. Haemodynamic effects were expressed as percent changes from initial values. Bi-exponential (pharmacokinetics), and linear [$E = S \cdot C + E_0$, for cardiac effects] or sigmoid [$E = E_{\text{max}} \cdot C^\gamma / (CE_{50}^\gamma + C^\gamma)$, for haemodynamic effects] models were fitted to individual data.

Results Peak plasma concentrations and areas under the curve up to 24 h were (mean $\pm$ s.d.) $16 \pm 10 \text{ ng ml}^{-1}$ and $157.50 \pm 89.13 \text{ ng ml}^{-1} \text{ h}$, and $63 \pm 11 \text{ ng ml}^{-1}$ and $535.50 \pm 135.11 \text{ ng ml}^{-1} \text{ h}$, after 100 and 300 mg, respectively. Terminal half-life was approximately 4 h. For pharmacodynamics, we obtained: $S = -0.201 \pm 0.057 \text{ beats min}^{-1} / \text{ng ml}^{-1}$ for HR, $S = 0.526 \pm 0.114 \text{ ms/ng ml}^{-1}$ for PR interval duration, $E_{\text{max}} = 42 \pm 6\%$, $CE_{50} = 8.8 \pm 7.2 \text{ ng ml}^{-1}$ and $\gamma = 2.2 \pm 1.5$ for BAF, and $E_{\text{max}} = -28 \pm 4\%$, $CE_{50} = 5.8 \pm 5.1 \text{ ng ml}^{-1}$ and $\gamma = 3.4 \pm 1.8$ for BVR. At a SR 33671 concentration of 15 ng ml^{-1} , BVR is decreased by 27% whereas HR is reduced by less than 3 beats min^{-1} and PR interval duration is increased by less than 8 ms.

Conclusions Fantofarone is able to induce submaximal peripheral vasodilating effects at doses that are devoid of any clinically significant cardiac effect.

Keywords: concentration-effect relationship, effect compartment, fantofarone, haemodynamics, healthy volunteers, PK-PD modelling, sigmoid model

Introduction

Pharmacokinetic-pharmacodynamic (PK-PD) modelling affords various information of major interest to determine the optimal dosage and the best mode of administration of a new drug. Indeed, it allows to predict the intensity and duration of drug effects for various dosing regimens and it constitutes an interesting tool to investigate kinetic or dynamic instabilities. Moreover, when a class of drugs clearly induces dose-dependent side-effects, PK-PD

modelling permits to determine the range of concentrations that optimizes the efficacy/tolerance ratio.

Calcium antagonists constitute such a class of drugs and have therefore been subject to several PK-PD studies, not only in healthy volunteers [1–3], but also in patients with paroxysmic supraventricular tachycardia [4], hypertension [5–7], and congestive heart failure [8]. For example, modelling has allowed definition of the concentration and the dosing regimen of nifedipine required to induce optimal haemodynamic effects with limited untoward effects [1], to show acute tolerance to the electrophysiologic effect of diltiazem but that this phenomenon does not result from the antagonistic effect of the metabolites [3], and to demonstrate that the effects of verapamil on PR interval duration depend exclusively on the rate

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of appearance of the drug in plasma [4]. Moreover, since, for arterial blood pressure effects, the values of the pharmacodynamic parameters determined after a single administration proved to be predictive of those determined after repeated administrations [6, 7], and normotensive subjects were found to provide valuable information for predicting the response in hypertensive patients [9, 10], it can be argued that PK-PD studies should be conducted as early as possible during the development of new calcium antagonists.

Fantofarone (SR 33557) is a calcium antagonist which belongs to the sulphone-indolizine chemical family [11]. In a previous study, we have investigated its cardiac, systemic and regional (in the brachial and carotid vascular beds) haemodynamic effects after a single oral administration of two doses in healthy volunteers [12]. Simultaneously, we measured its plasma concentrations and those of its *N*-demethyl metabolite, SR 33671. Since fantofarone is extensively metabolized into SR 33671 as a result of a first pass mechanism and since all experimental studies performed either *in vitro* (isolated rabbit and rat preparations) or *in vivo* (anaesthetized dogs) show that SR 33671 induces qualitatively and quantitatively the same vascular and cardiac effects as fantofarone (unpublished data), it may reasonably be assumed that SR 33671 is responsible for the greatest part of the pharmacological activity of fantofarone. Therefore, the goals of the present study were to analyse the pharmacokinetics of fantofarone and of SR 33671, and to investigate the relationships between SR 33671 plasma concentrations and fantofarone administration-induced effects.

Methods

Experimental protocol

The protocol of the study has been extensively detailed previously [12]. It was a placebo-controlled, randomized, double-blind and crossover study performed in six healthy male volunteers (mean $\pm$ s.d. 23.3 ± 1.4 years, 76.3 ± 2.7 kg, 181 ± 4 cm) designed to investigate the pharmacokinetics and the pharmacodynamics of a single oral administration of two doses (100 and 300 mg) of fantofarone. Each subject had given informed written consent before taking part in the study which was approved by the Ethics Committee (GREBB) of the hospital of Bicêtre.

Pharmacodynamic variables

The following cardiac as well as systemic and regional haemodynamic variables were investigated at rest, in the recumbent position, before and repeatedly after drug intake. Cardiac and systemic haemodynamic variables were measured at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and

24 h whereas regional haemodynamic variables were measured at 0, 2, 4, 8 and 24 h. Heart rate (HR, beats min^{-1}) and PR interval duration (ms) were measured from ECG records. Systolic and diastolic arterial pressures (SAP, DAP, mmHg) were obtained using a Roche Sentron automatic monitor (Bard Medical, Lombard, Ill., USA) connected to a brachial cuff sphygmomanometer. Mean arterial pressure (MAP, mmHg) was calculated as $\text{MAP} = (1/3) \text{SAP} + (2/3) \text{DAP}$. Brachial artery flow (BAF, ml min^{-1}) was measured with a bidimensional pulsed Doppler system (Echovar Doppler pulsé 8 MHz, Alvar Electronics, Montreuil, France) as previously described and validated [13]. Brachial vascular resistance (BVR, mmHg s ml^{-1}) was calculated as $\text{BVR} = \text{MAP} \times 60 / \text{BAF}$.

Fantofarone and SR 33671 plasma concentrations

Fantofarone and SR 33671 plasma concentrations (ng ml^{-1}) were determined from venous blood samples taken before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 24 h after drug intake. The assay was performed by high performance liquid chromatography (h.p.l.c.) after prior extraction. The para-decarboxylated form of fantofarone, SR 33632, was used as the internal standard.

In brief, 0.05 μg of SR 33632 and 0.5 ml of 0.052 M dodecyl sulphate were added to 0.5 ml of plasma and the pH was adjusted to 3.0 by adding 0.2 ml of 0.5 M perchlorate buffer and 0.1 ml of 1 M phosphoric acid. Extraction was performed with 7 ml of 50/50 dichloromethane-hexane mixture. The organic phase was evaporated to dryness at 37 °C under a nitrogen stream and the residue was dissolved in 0.2 ml of the h.p.l.c. mobile phase (a 49/51 mixture of 0.02 M perchlorate buffer at pH 2.9 and acetonitrile). Compounds were separated on a Nucleosil C18 column (25×0.4 cm; 7 μm). The flow rate was 0.9 ml min^{-1} . Compound detection was performed by fluorimetry using an excitation wavelength of 330 nm and an emission wavelength of 400 nm. Retention times for fantofarone, SR 33671 and SR 33632 were 10.5, 9.5 and 13.7 min, respectively. The repeatability (coefficient of variation) of the assay was 12.4 and 8.9% for fantofarone and SR 33671 (at concentrations of 0.0025 mg l^{-1}), respectively. The range of linearity, the limit of detection and the limit of quantification of the method were 0.001–0.100 mg l^{-1} , 0.001 mg l^{-1} and 0.0025 mg l^{-1} , respectively.

Pharmacokinetic study

The pharmacokinetic study was performed, in each individual subject, similarly for fantofarone and SR 33671 with the Siphar pharmacokinetic software (Simed, Créteil, France).

Peak concentration ($C_{\max}$, ng ml⁻¹) and time to peak concentration ($t_{\max}$, h) were determined from the observed data. Area under the concentration *vs* time curve between 0 and 24 h (AUC(0,24 h), ng ml⁻¹ h) and mean residence time (MRT, h) were determined accordingly using the trapezoidal rule.

Pharmacokinetic modelling was performed to predict the kinetics of SR 33671 concentrations. A two exponential pharmacokinetic model with lag-time was fitted to observed concentrations of SR 33671. The general form of the model is $C = -A.e^{-\alpha(t-t_{\text{lag}})} + B.e^{-\beta(t-t_{\text{lag}})}$ where C (ng ml⁻¹) is the predicted concentration, t (h) is the time after administration, t_{lag} (h) is the lag-time, A and B (ng ml⁻¹) are hybrid constants, and α and β (h⁻¹) are hybrid coefficients which characterize the initial and final phases of the kinetics. Since SR 33671 is a metabolite, this model has to be viewed only as an arbitrary function describing the concentration-time profile.

In practice, non-linear regression was performed with a non weighed least squares criterion. After first estimate of the parameters using log-linear transformations (peeling algorithm), more accurate estimates were obtained using a Gauss-Newton minimization algorithm. The goodness of fit was assessed from the visual distribution of residuals. When several sets of parameters allowed to satisfactorily describe experimental data, the choice of the best set was made based upon the values of (a) the objective function and (b) the criteria derived from the log-likelihood function and proposed by Akaike, Schwarz and Leonard (Siphar pharmacokinetic software, Simed, Créteil, France). The estimates of α and β were used to calculate half-lives of initial ($t_{1/2,\alpha}$, h) and final ($t_{1/2,\beta}$, h) phases of the kinetics.

PK-PD modelling

PK-PD modelling was performed, in each individual subject, with a software developed in our laboratory [14]. 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 determination of the concentration-effect relationship for that subject using the data of all doses simultaneously [15]. Secondly, when the concentration-effect relationship displays an anticlockwise hysteresis loop, it allows use of an effect compartment [16], but the estimation of the first order rate constant ke_0 which governs the kinetics of the drug in the effect compartment is performed with non parametric kinetic and dynamic models [17]. Thirdly, the optimal value of ke_0 can be obtained by using either a one-dimensional minimization algorithm [18] (golden section search or parabolic interpolation) implemented in the program [19], 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 [15]. Fourthly, when several doses of a drug are studied in the same subject and when the concentration-effect relationships display anticlockwise hysteresis loops, the software allows to estimate an optimal value of ke_0 either for each dose separately or for all doses simultaneously [15].

The PK-PD modelling included two or three successive steps depending upon the type of the relationship (direct or indirect) between SR 33671 plasma concentrations and effects. When the concentration-effect relationship did not display a hysteresis loop (direct relationship), pharmacodynamic modelling was performed by using the concentrations of SR 33671 computed in plasma (using the pharmacokinetic model) and the observed effects. When the concentration-effect relationship displayed a hysteresis loop (indirect relationship), an effect compartment was used (link model) [16]. After determination of the optimal value of ke_0 [17], pharmacodynamic modelling was performed by using the concentrations of SR 33671 computed in the effect compartment (using both the pharmacokinetic and link models) and the observed effects. Finally, the pharmacokinetic, link (when required) and pharmacodynamic models were combined to predict kinetics of effects [15].

Linear and sigmoid pharmacodynamic models were used to establish the relationships between SR 33671 plasma or effect compartment concentrations and observed cardiac or haemodynamic effects. The following effects were studied: decrease in HR and increase in PR interval duration, expressed in absolute values, and variations in BAF and BVR, expressed in percent changes from their initial values. The general form of linear and sigmoid models are $E = S.C + E_0$ and $E = E_{\max} \cdot C^\gamma / (CE_{50}^\gamma + C^\gamma)$, respectively, where E is the predicted effect and C is the concentration of SR 33671. For the linear model, S and E_0 are the slope and intercept of the relationship, respectively. S represents the responsiveness to the drug in terms of effect per unit of change in drug concentration and E_0 represents the basal effect (i.e. the effect without drug). For the sigmoid model, $E_{\max}$ is the maximum theoretical effect, CE_{50} is the concentration of SR 33671 that induces an effect of 50% of $E_{\max}$ and γ is the Hill coefficient. The sigmoid model was chosen when the experimental data showed, at least after 300 mg of fantofarone, a clear tendency towards a maximum effect. The linear model was chosen in all other cases.

For each cardiac or haemodynamic variable, the pharmacodynamic modelling was performed using the data obtained with the two doses simultaneously. In practice, linear or non linear regression was performed with a non weighed least squares criterion. For the linear model, the correlation coefficient (r) was computed and

its significance was tested. A P value lower than 0.05 allowed to consider the relationship as statistically significant. For the sigmoid model, owing to the small amount of data (5 pairs of concentrations and effects for each dose), only two parameters ($E_{\max}$ and CE_{50}) were estimated, the third one (γ) being constrained to a fixed value (several values of γ , in the range [0.5–5.0] and including 1.0, were tested). After a first estimate of the parameters using, for $E_{\max}$, the maximum observed effect, and for CE_{50} , the mean of the three concentrations that induced the nearest effects to half the maximum observed effect, more accurate estimates were obtained using four multidimensional minimization algorithms (downhill simplex, direction set, conjugate gradient and variable metric) implemented in the program [19]. The goodness of fit was assessed from the visual distribution of residuals. When several sets of parameters allowed to satisfactorily describe experimental data, the choice of the best set was performed based upon the value of the objective function and of the determination coefficient (r^2).

Statistical presentation of results and analyses

For each subject, results of the pharmacokinetic study and of the PK-PD modelling are expressed as point-estimates. For PK-PD modelling, s.d. (for linear model) or asymptotic s.d. (for sigmoid model) of estimates derived from the variance-covariance matrix estimated at the optimum are also reported (these s.d. correspond to standard errors). For the whole population, the distribution of each pharmacokinetic and (estimated or fixed) pharmacodynamic parameter was expressed as the mean with a s.d. computed from individual point-estimates. For ke_0 , due to the variability of the number of subjects in whom a hysteresis was observed, the median was reported instead. Finally, mean estimates of SR 33671 pharmacokinetic parameters obtained with each of the two doses of fantofarone were compared with the BMDP statistical software (BMDP, Los Angeles, Ca, USA) using paired Student's t -test (program 3D).

Results

Pharmacokinetic study

Fantofarone After 100 mg, plasma concentrations of fantofarone were, for each subject, either below the limit of quantification or below the limit of detection. In contrast, after 300 mg, plasma concentrations of fantofarone were detectable between 0.5 or 1 h and at most up to 4 h. $C_{\max}$ was observed before 1 h ($t_{\max}=0.5$ h for 50% of the subjects) and was equal to 4 ± 1 ng ml⁻¹. AUC(0,24 h) and MRT were 5.54 ± 4.31 ng ml⁻¹ h and 1.38 ± 0.87 h, respectively. Finally, three subjects (numbers 2, 3 and 6) had sufficient data during the elimination

phase to allow an estimation of a terminal half-life for fantofarone. This half-life appeared to be approximately 1 h.

SR 33671 Table 1 shows the individual and mean $\pm$ s.d. values of the pharmacokinetic parameters (except the lag-time, hybrid constants and hybrid coefficients of the pharmacokinetic model) of SR 33671 after 100 mg (top panel) and 300 mg (bottom panel) of fantofarone.

After 100 mg, $C_{\max}$ was observed between 2 and 3 h and reached about 16 ng ml⁻¹, AUC(0,24 h) was equal to 157.50 ± 89.13 ng ml⁻¹ h, and MRT and terminal half-life were approximately 9.5 and 4.0 h, respectively. The A and B hybrid constants and α and β coefficients of the pharmacokinetic model were equal to 55.7 ± 33.9 and 53.1 ± 32.7 ng ml⁻¹ and to 0.40 ± 0.09 and 0.18 ± 0.04 h⁻¹, respectively. The lag-time was equal to 0.27 ± 0.15 h. For example, in subject 6, the estimates are $A=32.2$ ng ml⁻¹, $B=30.7$ ng ml⁻¹, $\alpha=0.34$ h⁻¹, $\beta=0.20$ h⁻¹ and $t_{\text{lag}}=0.35$ h and the determination coefficient r^2 is equal to 0.8250.

After 300 mg, $C_{\max}$ was observed between 1 and 3 h and reached about 63 ng ml⁻¹, AUC(0,24 h) was equal to 535.50 ± 135.11 ng ml⁻¹ h, and MRT and terminal half-life were of about 10.0 and 4.5 h, respectively. The A and B hybrid constants and α and β coefficients of the pharmacokinetic model were equal to 104.1 ± 17.5 and 98.2 ± 16.4 ng ml⁻¹ and to 0.93 ± 0.28 and 0.18 ± 0.06 h⁻¹, respectively. A short lag-time, equal to 0.09 ± 0.04 h, was observed. For example, in subject 6, the estimates are $A=82.3$ ng ml⁻¹, $B=78.2$ ng ml⁻¹, $\alpha=1.23$ h⁻¹, $\beta=0.21$ h⁻¹ and $t_{\text{lag}}=0.05$ h and the determination coefficient r^2 is equal to 0.8832. Finally, the values of all the pharmacokinetic parameters obtained after 300 mg, except $t_{\max}$, MRT, β and $t_{1/2,\beta}$ were significantly different from the corresponding ones obtained after 100 mg.

PK-PD modelling

Effects on HR and PR interval duration For each cardiac variable and each subject, a linear model was fitted to the data. Significant correlations (at the 0.01 level) were obtained for each type of effect and for all subjects, except subject 2. Table 2 shows the individual and mean $\pm$ s.d. values of the pharmacodynamic parameters which characterize, in these subjects, the relationships between SR 33671 concentrations and the drug effects on HR (top panel) and PR interval duration (bottom panel). The PK-PD modelling was performed simultaneously on the data obtained with both doses of fantofarone for all subjects, except subject 3 for HR. In this case, the fitting was only performed on the data obtained with the 300 mg dose of fantofarone.

Table 1 Pharmacokinetic parameters of SR 33671 after 100 mg (top panel) and 300 mg (bottom panel) fantofarone.

Subject	C_{max} (ng ml ⁻¹)	t_{max} (h)	$AUC(0,24\ h)$ (ng ml ⁻¹ h)	MRT (h)	$t_{1/2,\alpha}$ (h)	$t_{1/2,\beta}$ (h)	r^2
1	28	2.5	241.25	9.51	1.53	3.13	0.8949
2	15	2.0	171.00	11.55	1.79	4.84	0.7861
3	7	2.0	69.75	6.43	1.49	3.56	0.9048
4	10	3.0	125.50	11.86	2.75	5.64	0.7884
5	27	2.0	277.25	9.45	1.45	3.21	0.8776
6	7	3.0	60.25	6.84	2.05	3.45	0.8250
Mean $\pm$ s.d.	16 $\pm$ 10	2.4 $\pm$ 0.5	157.50 $\pm$ 89.13	9.27 $\pm$ 2.28	1.84 $\pm$ 0.50	3.97 $\pm$ 1.03	
1	73	1.0	587.00	8.41	0.57	3.70	0.9181
2	52	3.0	768.00	17.02	1.38	9.93	0.8947
3	62	1.5	517.25	9.93	0.90	4.33	0.8105
4	78	1.5	459.00	7.67	0.74	3.08	0.7451
5	59	2.0	513.25	9.02	0.77	3.54	0.8696
6	52	1.5	368.50	7.98	0.56	3.36	0.8832
Mean $\pm$ s.d.	63 $\pm$ 11	1.8 $\pm$ 0.7	535.50 $\pm$ 135.11	10.01 $\pm$ 3.53	0.82 $\pm$ 0.30	4.66 $\pm$ 2.62	

r^2 : determination coefficient (bi-exponential model).

Table 2 Pharmacodynamic parameters of the relationship between SR 33671 concentrations and effects on heart rate (top panel) and on PR interval duration (bottom panel). Modelling was mostly performed using the data of both doses of fantofarone simultaneously.

Subject	S (U/ng ml ⁻¹)	$s.d. S$ (U/ng ml ⁻¹)	E_0 (U)	$s.d. E_0$ (U)	keo (100 mg) (h ⁻¹)	keo (300 mg) (h ⁻¹)	r	P value
1	-0.177	0.038	59.7	1.2	NH	1.84	-0.7054	<0.01
3	-0.183	0.038	64.4	1.1	—	0.90	-0.8353	<0.01
4	-0.183	0.054	63.5	1.3	NH	2.66	-0.5878	<0.01
5	-0.161	0.043	60.2	1.3	NH	NH	-0.6187	<0.01
6	-0.302	0.047	66.8	1.0	NH	NH	-0.8100	<0.01
Mean $\pm$ s.d.	-0.201 $\pm$ 0.057		62.9 $\pm$ 3.0					
1	0.400	0.128	166.8	4.2	NH	2.02	0.5562	<0.01
3	0.455	0.138	154.5	3.5	NH	NH	0.5739	<0.01
4	0.669	0.062	165.5	1.7	NH	NH	0.9160	<0.01
5	0.485	0.117	147.0	3.5	NH	NH	0.6612	<0.01
6	0.620	0.127	179.4	2.3	NH	1.02	0.7204	<0.01
Mean $\pm$ s.d.	0.526 $\pm$ 0.114		162.6 $\pm$ 12.4					

r: correlation coefficient (linear model). The unit U stands for beats min⁻¹ for heart rate and ms for PR interval duration. NH: no hysteresis. No significant correlation between SR 33671 concentrations and effects on both HR and PR interval duration for subject 2. For HR: modelling using the data of only the 300 mg dose for subject 3.

Estimated S and E_0 were, respectively, -0.201 ± 0.057 beats min⁻¹/ng ml⁻¹ and 63 ± 3 beats min⁻¹ for HR and 0.526 ± 0.114 ms/ng ml⁻¹ and 163 ± 12 ms for PR interval duration.

Figure 1 displays, in a representative subject (number 6), the main steps of the modelling performed on cardiac effects after 100 and 300 mg of fantofarone. The relationships between SR 33671 plasma concentrations and effects on HR do not display, when the data points of each dose are connected in chronological order, a hysteresis loop (top-left plot). The general shape of these relationships fits a linear model. The optimal values of S and E_0 are -0.302 beats min⁻¹/ng ml⁻¹ and 67 beats min⁻¹, respectively (bottom-left plot). In contrast with

what is observed for HR, one of the relationships between SR 33671 plasma concentrations and effects on PR interval duration (the one obtained after 300 mg of fantofarone) shows a hysteresis loop (top-right plot). This indicates an effect compartment. The optimal value of ke_0 is 1.02 h⁻¹. As for effects on HR, the general shape of the two relationships fits a linear model. The optimal values of S and E_0 are 0.620 ms/ng ml⁻¹ and 179 ms, respectively (bottom-right plot).

Effects on BAF and BVR For each haemodynamic variable and each subject, a sigmoid model was fitted to the data. Table 3 shows the individual and mean $\pm$ s.d. values of the pharmacodynamic parameters which characterize the

Figure 1 PK-PD model for heart rate (HR) and PR interval duration in a representative subject (no. 6) after 100 (circles) and 300 (triangles) mg fantofarone. a) effects on HR as a function of SR 33671 plasma concentrations (points connected in chronological order for each dose). b) effects on HR as a function of SR 33671 plasma concentrations and fitted pharmacodynamic model (solid line). c) effects on PR interval duration as a function of SR 33671 plasma concentrations (points connected in chronological order for each dose). d) effects on PR interval duration as a function of SR 33671 plasma or effect compartment ($ke_0 = 1.02 \text{ h}^{-1}$) concentrations and fitted pharmacodynamic model (solid line).

Table 3 Pharmacodynamic parameters of the relationship between SR 33671 concentrations and effects on brachial artery flow (top panel) and on brachial vascular resistance (bottom panel). Modelling was mostly performed using the data of both doses of fantofarone simultaneously.

Subject	$E_{\max}$ (%)	Asymptotic s.d. $E_{\max}$ (%)	CE_{50} (ng ml^{-1})	Asymptotic s.d. CE_{50} (ng ml^{-1})	γ	keo (100 mg) (h^{-1})	keo (300 mg) (h^{-1})	r^2
1	40	2	10.0	1.4	1.0	0.50	NH	0.9521
2	37	5	19.8	4.4	1.5	NH	NH	0.7206
3	43	12	1.5	1.6	1.0	1.76	—	0.6800
4	49	2	5.3	0.2	5.0	1.46	—	0.9893
5	49	4	14.1	1.4	2.5	0.42	0.21	0.8629
6	35	3	2.4	0.5	2.0	0.46	0.49	0.6335
Mean $\pm$ s.d.	42 ± 6		8.8 ± 7.2		2.2 ± 1.5			
1	—34	1	4.3	0.3	1.0	0.16	NH	0.9805
2	—27	3	15.7	3.0	2.0	NH	NH	0.6618
3	—31	3	1.9	0.4	5.0	1.66	—	0.7003
4	—26	1	4.7	0.3	5.0	1.24	NH	0.9169
5	—23	2	6.0	0.9	5.0	0.26	0.35	0.5725
6	—29	2	2.0	0.4	2.5	NH	NH	0.7963
Mean $\pm$ s.d.	-28 ± 4		5.8 ± 5.1		3.4 ± 1.8			

r^2 : determination coefficient (sigmoid model). NH: no hysteresis. For brachial artery flow: modelling using the data of only the 100 mg dose for subject no. 3 and 4. For brachial vascular resistance: modelling using the data of only the 100 mg dose for subject 3.

relationships between SR 33671 concentrations and the drug effects on BAF (top panel) and BVR (bottom panel). The PK-PD modelling was performed simultaneously on the data obtained with both doses of fantofarone for all subjects, except subject 3 for BAF and BVR and subject 4 for BAF. In these cases, the fitting was only performed on the data obtained with the 100 mg dose of fantofarone. Estimated $E_{\max}$ and CE_{50} were, respectively, $42 \pm 6\%$ and $8.8 \pm 7.2 \text{ ng ml}^{-1}$ for BAF and $-28 \pm 4\%$ and $5.8 \pm 5.1 \text{ ng ml}^{-1}$ for BVR. The value of γ which allowed to obtain the best fittings was 2.2 ± 1.5 for BAF and 3.4 ± 1.8 for BVR.

Figure 2 displays the main steps of the modelling performed on haemodynamic effects in subject 6 after 100 and 300 mg fantofarone. The relationships between SR 33671 plasma concentrations and effects on BAF show, when the data points of each dose are connected in chronological order, major hysteresis loops (top-left plot). This indicates an effect compartment. The optimal values of ke_0 are 0.46 and 0.49 h^{-1} after 100 and 300 mg of fantofarone, respectively. The general shape of the two relationships, considered together, fits a sigmoid

model. The optimal values of $E_{\max}$, CE_{50} and γ are 35%, 2.4 ng ml^{-1} and 2.0, respectively (bottom-left plot). In contrast with that observed for BAF, the relationships between SR 33671 plasma concentrations and effects on BVR do not show hysteresis loops (top-right plot). Nevertheless, as for effects on BAF, the general shape of the two relationships, considered together, fits a sigmoid model. The optimal values of $E_{\max}$, CE_{50} and γ are -29% , 2.0 ng ml^{-1} and 2.5, respectively (bottom-right plot).

Integrated models. Prediction of the kinetics of effects The pharmacokinetic, link (when required) and pharmacodynamic models were used in combination to predict the kinetics of cardiac and haemodynamic effects in each subject. Figure 3 shows such kinetics of effects on HR, PR interval duration and BVR in subject 6 during the 24 h following the administration of 100 and 300 mg fantofarone. The 100 mg dose did not induce any detectable cardiac effect. The 300 mg dose induced a slight and short lasting reduction in HR and a more prolonged increase in PR interval duration. The negative

Figure 2 PK-PD model for brachial artery flow (BAF) and brachial vascular resistance (BVR) in a representative subject (no. 6) after 100 (circles) and 300 (triangles) mg fantofarone. a) effects on BAF as a function of SR 33671 plasma concentrations (points connected in chronological order for each dose). b) effects on BAF as a function of SR 33671 effect compartment concentrations ($ke_0 = 0.46$ and 0.49 h^{-1} after 100 and 300 mg of fantofarone, respectively) and fitted pharmacodynamic model (solid line). c) effects on BVR as a function of SR 33671 plasma concentrations (points connected in chronological order for each dose). d) effects on BVR as a function of SR 33671 plasma concentrations and fitted pharmacodynamic model (solid line).

Figure 3 a) Kinetics of predicted effects on heart rate (HR), b) PR interval duration and c) brachial vascular resistance (BVR) in a representative subject (no. 6) after 100 (solid line) and 300 (dotted line) mg fantofarone.

chronotropic effect appeared, peaked and disappeared more quickly than the positive dromotropic effect. These two effects had almost completely disappeared 10 and 12 h after dosing, respectively. In contrast, both doses of fantofarone induced arteriolar vasodilation. The 100 mg dose was sufficient to induce a submaximum vasodilating effect, but the duration of this effect was, as compared with that obtained with the 300 mg dose, clearly shorter. In fact, increasing the dose to 300 mg allowed an almost immediate and long lasting maximum effect of which the duration ranged between 12 and 16 h.

Reasons for failure of the modelling

For cardiac effects in subject 2, no significant concentration–effect relationship was found because neither 100 nor 300 mg fantofarone induced any detectable effect. For HR in subject 3, the modelling was impossible using the data of both doses simultaneously because of a clear difference in the levels of HR, both before drug intake and afterwards, between the 100 and 300 mg sequences. Nevertheless, it was possible to find a significant correlation ($r = -0.5420$, $P < 0.01$) between SR 33671

plasma concentrations and effects on HR using both doses simultaneously when effects were expressed as absolute changes from initial values. For BAF in subjects 3 and 4, and for BVR in subject 3, the modelling was impossible at the 300 mg dose because the value of BAF measured before drug intake had probably been over-estimated thus resulting in no clear effect throughout the whole period of observation.

Discussion

The purpose of this work was to extend our pharmacodynamic study, performed after a single administration of two doses of fantofarone, with a pharmacokinetic study of the parent drug and of its active metabolite, and a PK–PD investigation of the relationships between the plasma concentrations of the active metabolite and the main cardiac and haemodynamic effects.

In the pharmacokinetic study, our results show that the parent drug and its metabolite display quite different kinetics. The parent drug was quickly absorbed ($t_{\max} < 1$ h) and quickly metabolized (MRT < 1.5 h and elimination half-life of about 1 h). The metabolite appeared in the plasma with a short delay (< 20 min) and reached its peak quickly ($t_{\max} < 2.5$ h), but its disappearance was rather slower (MRT between 9.5 and 10 h and $t_{1/2,\beta}$ of approximately 4 h). The AUC(0,24 h) of the metabolite being clearly greater than that of the parent drug, it is possible to infer that, whichever the fraction metabolized, the clearance of the metabolite is much smaller than that of the parent drug [20]. Moreover, the terminal half-life of the parent drug being very short as compared with that of the metabolite, it is also possible to conclude that the latter half-life represents the elimination half-life of the metabolite and that the initial half-life of the metabolite represents its formation half-life [20]. Finally, the $C_{\max}$ and AUC(0,24 h) of the metabolite were almost proportional to the dose of fantofarone administered, and the $t_{1/2,\beta}$ was not significantly different between doses. These results suggest that the kinetics of the metabolite are linear, at least up to the dose of 300 mg fantofarone.

We found sigmoid relationships between the plasma concentrations of SR 33671 and the effects induced on brachial haemodynamics. The sigmoid model closely fits to the data of both doses and for almost all subjects, as demonstrated by the relatively high values of the determination coefficients (the medians are 0.79 and 0.75 for BAF and BVR, respectively) and by the good precision of the estimates (the medians of the variation coefficients of $E_{\max}$ and CE_{50} are 11.1 and 17.4% for BAF, and 7.8 and 17.1% for BVR).

This good precision results in part from two particular methodological choices [15]. Firstly, to optimize the information brought by the experimental design, the

pharmacodynamic model was fitted simultaneously to the data obtained with both doses. Secondly, to decrease the number of parameters to estimate, only two parameters, $E_{\max}$ and CE_{50} , were estimated simultaneously and γ was determined by testing several values in a step-wise manner. The rationale, advantages and limits of these methodological choices have already been reported and extensively discussed [15, 21]. Quantitatively, the maximum effects determined by the modelling correspond to an increase in BAF of 42% (i.e. $\sim 30 \text{ ml min}^{-1}$) and a decrease in BVR of 28% (i.e. $\sim 20 \text{ mmHg.s ml}^{-1}$). The CE_{50} is lower for BVR than for BAF. In contrast, the value of γ is slightly greater for BVR than for BAF expressing that the relationship is somewhat steeper for BVR. The quantification of these relationships allows to estimate the level of SR 33671 plasma concentrations required to reach a desired effect: for example, a plasma concentration of 15 ng ml^{-1} allows to obtain a 27% decrease in BVR, i.e. more than 95% of $E_{\max}$ on this haemodynamic variable. This level corresponds to that achieved after 100 mg fantofarone and this explains why the effect did not clearly increase when a dose of 300 mg was administered [12]. Finally, the kinetics of these haemodynamic effects were sometimes delayed as compared with those of SR 33671 thus generating hysteresis phenomena. When they were observed, these phenomena were very marked expressing delays of equilibrium between effect compartment and plasma, amounting several hours. In this respect, it must be observed that individual values showed, between subjects, a wide variability. Moreover, a great variability was also observed within subjects, from one administration to another. For example, for BVR, in one subject, there was a hysteresis at each dose (optimal values of ke_0 were similar); in two subjects, there was a hysteresis with only one dose (100 mg); finally in two other subjects, there was no hysteresis at all whichever the dose. This wide heterogeneity in the observation of hysteresis phenomena in case of several single administrations in the same subject has already been reported with the angiotensin I-converting enzyme inhibitor, zalcipril, and extensively discussed [22]. After using an effect compartment to deal separately with each hysteresis phenomenon [15], it was possible to fit the same pharmacodynamic model to the data of both doses simultaneously. Thus, as for zalcipril, we conclude that the magnitude of the delay for the metabolite to reach the effect compartment may vary from one administration to another, but that this does not affect the concentration-effect relationship.

Regarding cardiac effects, the pharmacodynamic model was also fitted to the data obtained with both doses simultaneously. The modelling allowed us to find individual significant relationships between the plasma concentrations of SR 33671 on the one hand, and the

negative chronotropic or the positive dromotropic effects observed in all subjects but one. In these subjects, the determination of the slopes of the relationships allows to compute that a plasma concentration of 15 ng ml^{-1} of SR 33671 induces a slight decrease in HR ($< 3 \text{ beats min}^{-1}$) and a slight increase in PR interval duration ($< 8 \text{ ms}$). In fact, plasma concentrations above 50 ng ml^{-1} would be required to induce clinically significant effects, e.g. a reduction in HR of at least $10 \text{ beats min}^{-1}$ or an increase in PR interval duration of at least 26 ms. Thus, our data indicate that fantofarone can exert peripheral vasodilating effects without simultaneously inducing clinically significant cardiac effects. Finally, the kinetics of the cardiac effects were somewhat different from those of the haemodynamic effects, the effect compartment being required only after 300 mg and for only three subjects for HR and two subjects for PR interval. In fact, the effects induced after 100 mg were generally too weak to emerge from the spontaneous variability of the variable and therefore to induce clear hysteresis loops. In any case, after 300 mg, when the effect compartment was used, the values of ke_0 were clearly higher than for brachial haemodynamic effects, expressing a shorter delay of equilibrium between effect compartment and plasma. These findings suggest that cardiac effects are direct whereas haemodynamic effects result from a balance between direct effects on vascular smooth muscle cells and reflex effects induced by the decrease in arterial pressure.

In conclusion, in healthy volunteers, there are sigmoid relationships between SR 33671 plasma concentrations and brachial haemodynamic effects. Concentrations of about 15 ng ml^{-1} will allow 95% of maximum effects on BVR. There are linear relationships between SR 33671 plasma concentrations and cardiac effects. At concentrations of about 15 ng ml^{-1} , these effects are not clinically relevant. Thus, fantofarone is probably able to induce submaximal peripheral vasodilating effects without any accompanying clinically significant cardiac effect.

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