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Population pharmacokinetic–pharmacodynamic modelling of S 15535, a 5-HT_{1A} receptor agonist, using a behavioural model in rats ☆

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Abstract

The pharmacokinetic–pharmacodynamic relationship of S 15535 (1-(benzodioxan-5-yl) 4-(indan-2-yl)piperazine) and its active 5-hydroxy metabolite S 32784 (1-(benzodioxan-5-yl) 4-(5-hydroxyindan-2-yl)piperazine), and buspirone as a reference, were studied in male Wistar rats using a behavioural model of anxiety by determining the reduction in the number of fear-induced ultrasonic vocalisations. S 15535 and buspirone were administered p.o. and i.v. S 32784, present in man but not in rat, was administered i.v. The pharmacokinetics and pharmacokinetic–pharmacodynamic relationships were described using non-linear mixed effects modelling. The no-drug effect was constant and all compounds were active in the model, reducing ultrasonic vocalisations immediately after administration. The sigmoid E_{max} model was used to describe the pharmacokinetic–pharmacodynamic relationships, with E_{max} values of a 90% decrease in baseline ultrasonic vocalisations. Corrected for plasma protein binding, all compounds showed similar potency. The study shows that ultrasonic vocalisations can be considered a suitable endpoint for the anxiolytic effect when used in conjunction with non-linear mixed effects modelling to overcome the limited sampling and effect measurements. © 2001 Published by Elsevier Science B.V.

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1. Introduction

Pre-clinical and clinical pharmacokinetic–pharmacodynamic modelling using biomarkers is widely regarded as potentially beneficial in all phases of drug development (Peck et al., 1992; Breimer and Danhof, 1997; Derendorf and Meibohm, 1999). Such biomarkers are particularly required in the development of drugs acting on cortical or cognitive functions.

The use of mechanism-based pharmacokinetic–pharmacodynamic models has been shown to provide understanding in the in vivo pharmacology of central nervous system active drugs, including receptor expression and modulation. In addition, this approach enables the prediction of the pharmacological response in humans based on pre-clinical data. Examples from the literature include the electro-encephalographic (EEG) effects of a series of synthetic opiates. Cox (1997) demonstrated that in vivo potencies of synthetic opioids for EEG effects are related to the in vitro receptor affinity for the μ-opioid receptor. These results support the findings showing that EC_{50} values obtained in rats (Cox et al., 1998, 1999) are closely related to those obtained in man (Scott et al., 1985, 1991; Egan et al., 1996).

Anxiety is often identified in the diagnosis of several forms of depression. Assessment of the therapeutic dose range related to the anxiolytic effect is therefore an impor-

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tant, though often unmet milestone in the development plan of antidepressant compounds because validated biomarkers are not available by the time compounds reach Phase I clinical studies. The choice of a clinically relevant pharmacological endpoint in pre-clinical research, in conjunction with an integrated pharmacokinetic–pharmacodynamic analysis to characterise the concentration–effect relationship of anxiolytic drugs, has important potential practical constraints. Numerous reports on conditioned anxiety have demonstrated the role of serotonergic receptors in the emission of ultrasound vocalisations in rats (Sanchez, 1993; Klint and Andersson 1994; Olivier et al., 1994; Beckett et al., 1996; Schreiber et al., 1998). At present, the use of ultrasonic vocalisations in pre-clinical research has been restricted to pharmacological screening procedures, during which a qualitative rather than a quantitative evaluation is performed. The lack of adequate methodological tools to cope with baseline fluctuation, intra-individual variability in the response and the limited number of pharmacodynamic assessments per animal have been some of the limiting factors for modelling approaches (Meneses and Hong, 1993; Nielsen and Sanchez, 1995; Olivier et al., 1998).

In this paper, we show that fear-induced ultrasonic vocalisations can be used in conjunction with population pharmacokinetic–pharmacodynamic modelling to characterise the time course of the anxiolytic effect and the concentration–effect relationship of anxiolytic drugs in rats. We assessed the effects of the potential anxiolytic compound S 15535 (1-(benzodioxan-5-yl) 4-(indan-2-yl)piperazine) and its active 5-hydroxy metabolite S 32784 (1-(benzodioxan-5-yl) 4-(5-hydroxyindan-2-yl)piperazine) using this integrated approach. Since S 15535, Fig. 1, is an agonist and antagonist (weak partial agonist) at pre- and post-synaptic 5-HT_{1A} receptors, respectively (Gobert et al., 1995; Newman-Tancredi et al., 1996), it seemed appropriate to characterise the effects of buspirone, as a positive control. Thereby, information about the relative drug potency, intrinsic activity and the role of active metabolites can be obtained on the basis of concentration rather than dose. Taking into account pharmacokinetic factors, such as protein binding and clearance, this approach may facilitate dose selection for Phase II studies.

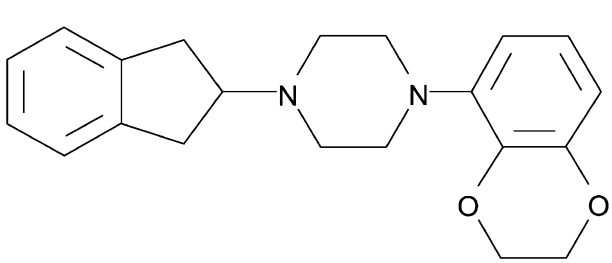

Fig. 1. Structure of S 15535.

2. Materials and methods

2.1. Animals

Male Wistar-derived rats (175–250 g, Harlan, Someren, The Netherlands) were used throughout the study. Animals were housed individually, at constant temperature (21°C) and in controlled lighting (12L/12D). Laboratory chow (Standard Laboratory Rat, Mouse and Hamster Diets, RMH-TM, Hope Farms, Woerden, The Netherlands) and water were available ad libitum, except during the experiments. One day before the actual start of the experiments, indwelling cannulas were implanted into the femoral artery for blood sampling. Animals receiving i.v. infusion had the right jugular vein cannulated for drug administration. Surgical procedures were carried out according to validated internal standard operating procedures. The study protocol was approved by the Ethical Committee for Animal Experimentation of the University of Leiden.

2.2. Drug administration and blood sampling

S 15535 was administered as the mono methanesulphonate salt, buspirone as the hydrochloride salt and S 32784 as the base. Quantities and concentrations for all compounds are expressed as base.

The study consisted of three experimental sets. All experiments were performed according to a randomised crossover design, with 12 rats per treatment group. Vehicle (5% glucose in water) was administered via the same route of administration as the active compounds. The washout period was at least 3 days. In the first set, animals received a single dose of S 15535, buspirone or vehicle. Based on the initial parameter estimates, a second set of experiments was carried out using a computer-assisted i.v. infusion of S 15535. Aim of the computer-assisted infusion was to reduce pharmacokinetic variability by fixing concentration around the EC_{50} values. The contribution of the metabolite to the overall pharmacological response was investigated in the third set. Racemic S 32784 was given i.v. at three consecutive, increasing doses. A sampling matrix was used for blood sampling so that a full pharmacokinetic profile could be assessed for each compound with limited number of samples per animal (Fig. 2).

In the first set of experiments, S 15535 (Servier, Orleans, France) was administered i.v. at 2 mg/kg and p.o. at 7.5 mg/kg. Buspirone (Duphar, Weesp, The Netherlands) was administered at 5 mg/kg i.v. and 10 mg/kg p.o. Intravenous administration was over 10 min at a rate of 90 μl/min. Oral administration was carried out using a gastric catheter. S 15535-3 and buspirone were dissolved in vehicle (5% glucose solution). Another group of animals received vehicle i.v. in both treatment periods to control for treatment sequence variability. Three arterial blood samples (500 μl) were collected into heparinised tubes at

Matrix 1: Pharmacokinetic sampling

Time (h)	Rat 13	Rat 14	Rat 15	Rat 16	Rat 17	Rat 18	Rat 19	Rat 20	Rat 21	Rat 22	Rat 23	Rat 24
0.25				X		X					X	
0.5		X		X					X	X		
0.75	X		X		X			X				
1			X					X		X		X
2		X				X	X			X		
3					X			X	X			X
4	X	X				X					X	
6							X				X	X
8	X		X						X			
10				X	X		X					

Matrix 2: Effect measurements

Time (h)	Rat 13	Rat 14	Rat 15	Rat 16	Rat 17	Rat 18	Rat 19	Rat 20	Rat 21	Rat 22	Rat 23	Rat 24
0.25		X			X			X		X		X
0.5	X		X				X				X	
0.75				X		X			X			X
1		X			X		X	X			X	
1.5	X		X			X			X	X		
2		X	X	X			X					X
3	X					X		X		X	X	
4				X	X		X		X			X
5	X		X					X		X	X	
6		X		X	X	X			X			

Fig. 2. Examples of sampling and effect matrices, in this case for p.o. administration of S 15535. Rats had three blood samples and four effect measurements taken.

different time intervals up to 10 h after drug administration. Upon completion of the experiments, total blood was collected for the determination of plasma protein binding. Plasma was separated by centrifugation for 10 min at 3500 rpm at +4°C and stored until analysis.

In the second set of experiments, S 15535 was administered i.v. in three consecutive 10-min steps using a computer-controlled infusion pump (Stanpump, PAVAMC, Palo Alto, USA), so that the target steady-state concentrations would be approximately 30, 60 and 100 ng/ml. The steady-state concentrations and infusion rates were based on initial pharmacokinetic estimates for S 15535 from the bolus i.v. administration. There was a 30-min interval between the first and second infusion, and a 60 min interval between the second and third infusion. Between each infusion step, effect measurements and blood sampling were performed. Three arterial blood samples (500 μl) were collected into heparinised tubes up to 3 h after the start of the infusion, according to a predefined sampling matrix. Upon completion of the experiments, total blood was collected for the determination of plasma protein binding. Plasma was separated by centrifugation for 10 min at 3500 rpm at +4°C and stored until analysis.

In the third set of experiments, racemic S 32784 was administered i.v. at doses of 450, 850 and 1200 μg/kg/h in three consecutive 10-min steps using a computer-controlled infusion. There was a 50-min interval between the first two steps and an 80-min interval between the second and third steps. Between the infusion steps, effect measurements and blood sampling were performed. Arterial blood samples were taken as described above.

2.3. Fear-induced ultrasonic vocalisations

The time course of fear-induced ultrasonic vocalisations was based on ultrasound emissions in the 20–30 kHz frequency band, as described by Della Paschoa et al.

(1998). On days 1 and 2 (pre-treatment), rats were placed in the test cage and distress calls were induced by random stimulation with electrical footshocks (10 volleys of 0.8 mA rectangular pulses, 5 s) during a 10-min session using a computer-controlled shock generator and a scrambler. On day 3, rats were initially subjected to a 2-min session and returned to their home cages. Thirty minutes later, animals were placed again in the test cage and ultrasound vocalisations were registered upon exposure to a random reminder shock. Only animals vocalising more than 100 calls/10 min were selected for the experiment. On day 4, ultrasonic vocalisations were recorded at four different intervals up to 6 h after administration, as defined in the sampling matrix. Each recording started immediately after stimulation with a reminder shock.

Recordings were carried out using a condenser microphone system (Type 4135, Brüel and Kjaer, Naerum, Denmark) consisting of a measuring amplifier and a programmable dual filter (Type PDF3700B, Difa Benelux, The Netherlands). The ultrasound emissions were quantified by the software program REGUS2.1 (LACDR, Division of Medical Pharmacology, Leiden, The Netherlands). The analysed data were stored on magnetic floppy disks as the frequency in kHz, duration and time of occurrence. From these data, the number of calls (calls/10 min) in the 20–30 kHz frequency band was used as measure of drug effect.

For sets 2 and 3, the screening procedure for responders was slightly modified to stabilise interindividual variability in baseline response. The animals were housed individually from 2 weeks before the start of the experiment, in order to disrupt social interaction. Only animals expressing more than 150 calls/10 min and presenting a rapid reaction to the reminder shock, i.e. vocalisations started within 2 min after the stimulation, were selected for the experiment.

After drug or vehicle administration, up to four effect measurements were performed per animal, according to a predefined sampling matrix (Fig. 2).

2.4. Drug analysis and plasma protein binding

2.4.1. Plasma analysis

For S 15535, plasma (200 μl) was analysed using High Performance Liquid Chromatography (HPLC) with ultraviolet (UV) detection at 220 nm after solid phase extraction (Isolute Phenyl, endcapped). Separation was achieved using reverse-phase HPLC (5 μm Du Pont de Nemours Zorbax Rx C18 column, 150 × 4.6 mm). The calibration curve ranged from 5 to 3000 ng/ml. Quality control standards at three levels in duplicate were included randomly in each analytical run. Intra-assay inaccuracy and precision were 2.0% and 5.9%, respectively.

For buspirone, plasma (100 μl) was analysed using HPLC and amperometric detection (120 mV) following solid phase extraction (phenyl column). Separation was achieved using reverse phase HPLC (Intersil ODS-3V 5 μ column, 150 × 4.5 mm). The calibration curves ranged from 32 to 6000 ng/ml for the samples after i.v. administration and 16 to 2000 ng/ml for the samples after p.o. administration. Quality control standards at five levels in duplicate were included randomly in each analytical run. Intra-assay inaccuracy and precision were 2.0% and 8.7%, respectively.

For S 32784, plasma (100 μl) was analysed using HPLC with tandem mass spectrometric (MS-MS) detection with positive ion spray after solid phase extraction (Isolute SCX). Separation of the enantiomers was achieved using reverse phase HPLC (chiral AGP column, 100 × 4.0 mm). The calibration curve ranged from 1 to 300 ng/ml. Quality control standards at three levels in duplicate for each enantiomer were included randomly in each analytical run. Intra-assay inaccuracy and precision were 0.66% and 7.5%, respectively, for the l(−) isomer and 7.6% and 14% for the d(+) isomer.

2.4.2. Radio receptor assay

Selected samples (250 μl) were analysed by a combination of HPLC and Radio Receptor Assay to check for the presence of metabolites that have affinity for the 5-HT_{1A} receptor. This involved an initial solid phase extraction (Extrelut 1 cartridges). The compounds of interest were separated and collected by reverse phase HPLC, and a fraction between 5 and 11 min was collected.

The binding assays of the extracts were carried out in 24-well picoplates with 8-hydroxy-2-(*N*,*N*,-di[2,3(*n*)-3*H*]propylamino)-1,2,3,4-tetrahydronaphtalene (600,000 dpm/100 μl in incubation buffer) and 500 μl hippocampal membranes from Wistar rat brain (0.2 mg/ml in incubation buffer). The non-specific and control binding were determined using spiked and blank rat plasma extract, respectively. After 2-h incubation at room temperature, separation was achieved using a cell harvester using a 24-well Unifilter-plate. The radioactivity on the filters was counted using a microplate scintillation counter.

2.4.3. Protein binding

The in vitro plasma protein binding was determined by ultrafiltration in heparinised rat and citrated human plasma in triplicate at 37°C (Amicon Centrifree micropartition devices, Millipore, Herts, UK). The binding of S 15535 was assessed at 10 and 250 ng/ml, whereas the binding of S 32784 and its enantiomers (S 33097 and S 33218) was determined at 250 ng/ml only. The binding of both compounds was also determined in a combination of 10 ng/ml S 15535 and 250 ng/ml racemic S 32784.

Analysis was by reverse phase chromatography with MS-MS detection with positive ion electrospray ionisation. Calibration and quality control standards were prepared in plasma water. The calibration curve ranged from 0.03 to 5 ng/ml for S 15535 and 0.15 to 25 ng/ml for S 32784 and its enantiomers. Quality control standards at four levels in

duplicate were included randomly in each analytical run. The non-specific binding for S 15535 was high at 54% and 57% for rat and human plasma, respectively, and lower for S 32784 at 0% in human and 35% in rat plasma. Protein retention was determined using the Bradford Micro-Protein Assay for the filtrate (Bio-rad Laboratories, Hemel Hempstead, UK). Complete retention was obtained in human plasma and 99.9% retention was obtained in rat plasma. Buspirone protein binding data were obtained from the literature.

2.5. Data analysis

All pharmacokinetic and pharmacokinetic–pharmacodynamic data analysis was performed using NONMEM IV (Beal and Sheiner, 1992). During model building, the goodness of fit was based on the log likelihood criterion at $p < 0.05$ and visual inspection of the plots of the weighted residuals vs. time. Hierarchical model building was performed based on the difference in -2 times the log of the likelihood (-2LL) between the postulated model and the new model. The -2LL approximates a χ^2 distribution. The critical value for the χ^2 distribution at $p = 0.05$ with 1 *df* is 3.84, compared to the decrease in -2LL for an additional parameter.

Interindividual variability (IIV) on a parameter was modelled either as:

$P_i = \hat{P}e^{\eta_i}$, for the error associated with an exponential distribution, or
$P_i = \hat{P}(1 + \eta_i)$, for the error associated with a normal distribution,

where P_i is the individual parameter estimate for the ith individual, $\hat{P}$ is the typical value of the parameter estimate for the population and η_i is the interindividual error for the ith individual. The η_i are assumed to be normally distributed, with a zero mean and a variance ω^2.

Residual variability was modelled as:

$Y_{ij} = C_{ij}(1 + \varepsilon_{ij,1})$ for a multiplicative model,
$Y_{ij} = C_{ij} + \varepsilon_{ij,2}$ for an additive model or
$Y_{ij} = C_{ij}(1 + \varepsilon_{ij,1}) + \varepsilon_{ij,2}$ for a combined additive and multiplicative model,

where C_{ij} is the jth plasma concentration predicted for the ith individual by the model, Y_{ij} is the measured concentration. ε_{ij} represents the residual deviation of the model from the jth observation of the ith individual. ε_{ij} is assumed to be normally distributed, with a zero mean and variance σ^2.

2.5.1. Pharmacokinetics

The pharmacokinetics of S 15535 and buspirone was modelled by fitting plasma concentrations from oral and i.v. administration simultaneously. The pharmacokinetic model for S 15535 was developed initially by fitting plasma concentrations from i.v. administration. In order to stabilise parameter estimation, structural pharmacokinetic model parameters (CL, Q, V1 and V2) were fixed before data from oral administration were included in the database. Subsequently, deconvolution of the oral data revealed that absorption consisted of two distinct processes, namely a rapid and a slow phase. An explanation for these two processes could be a partial precipitation of the compound in the stomach. Absorption of the remainder in the solution is rapid, whereas the slow process could be due to dissolution rate limitation of the kinetics. The model was adjusted to include these processes. The first order estimation method with a multiplicative residual error model was applied. Bioavailability (F) was defined as the product of the availability from the fast (F1) and the slow (F2) absorption routes. Rate constants were determined and expressed as volume (V1 and V2) and clearance (CL and Q) parameters The final pharmacokinetic model for S 15535 is shown in Fig. 3. Individual concentration vs. time course profiles were obtained by post-hoc Bayesian estimation using model parameters described above.

The pharmacokinetics of buspirone was fitted according to a one-compartment model with first order absorption, where K_a was fixed. The first order method with conditional estimation with a multiplicative residual error model was used. Furthermore, an additive error term was set at the square of the lowest detected buspirone concentration.

The total concentration of S 32784 was calculated as sum of the concentrations of each enantiomer. The dataset included plasma concentrations from a single i.v. administration of 200 and 400 μg/kg. The pharmacokinetics of S 32784 was fitted according to a two-compartment model. The first order method with conditional estimation with multiplicative residual error was used.

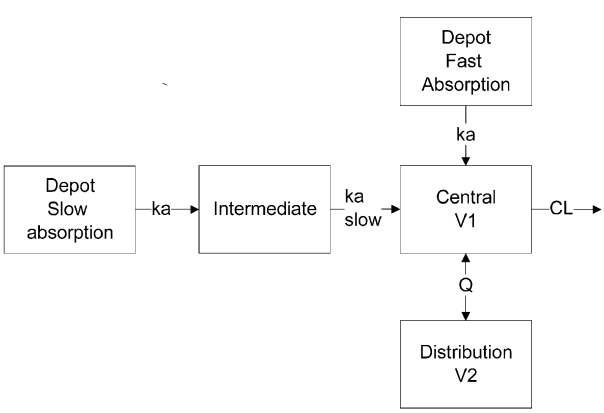

Fig. 3. Pharmacokinetic model for S 15535 after p.o. and i.v. administration. The dose is introduced either in the central compartment after i.v. administration, or in both depot compartments, for p.o. administration. CL is systemic clearance, Q intercompartmental clearance, k_a fast absorption rate constant and $k_{a,\,slow}$ the slow absorption rate constant. V1 is the volume of the central compartment and V2 the volume of the peripheral compartment.

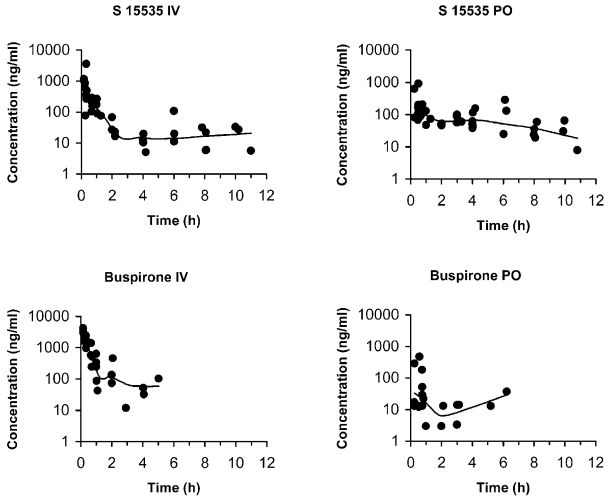

Fig. 4. Plasma concentrations of S 15535 after i.v. administration at 2 mg/kg (top left panel) and p.o. administration at 7.5 mg/kg (top right), and of buspirone after i.v. administration at 5 mg/kg (bottom left) and p.o. administration at 10 mg/kg (bottom right). The concentrations are fitted with a spline for representation.

Goodness of fit and performance of the models was verified using simulated data and by comparing the results to the 95% confidence intervals of the actual parameter estimates. For all compounds, individual post-hoc parameters were obtained for subsequent pharmacodynamic analysis.

2.5.2. Pharmacokinetic–pharmacodynamic analysis

The time course profile of ultrasonic vocalisations emission and the effect treatment sequence were assessed after administration of vehicle in both treatment periods to a control group. These initial analyses showed that induced ultrasonic vocalisations remain constant over time with interindividual variability on the no-drug effect (baseline effect). No effect of treatment sequence was observed.

The relationship between drug concentration and anxiolytic effect was assessed by including the total number of ultrasonic vocalisation calls emitted after administration of vehicle and active treatment into one database for each compound. Data from i.v. and p.o. administration were pooled for the analysis. The sigmoid E_{max} model was used to fit the concentration–effect relationship:

$$E = E_0\left(1 + \frac{E_{max}C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}\right)$$

where E is the observed effect, E_0 is the baseline effect, E_{max} is the maximum effect, EC_{50} the concentration at half the maximum effect, and γ a parameter expressing the slope of the sigmoid curve. C is the plasma concentration derived from the pharmacokinetic model. Data from sets 1 and 2 were fitted separately for S 15535. Interindividual variability was modelled in the same way as described for the pharmacokinetic model. Residual error was modelled

Table 1
Population parameter estimates for S 15535

Pharmacokinetic estimates after bolus administration and mean feedback values after successive infusions

Parameter	Bolus administration				Infusions	
	Population estimate	SE of estimate (%)	Variability (η or σ)	SE of variability (%)	Mean	CV (%)
CL (l/h)	0.53	(fixed)	39%	47	0.89	48
V1 (l)	0.37	(fixed)			0.37	
Q (l/h)	0.40	(fixed)			0.40	
V2 (l)	1.6	(fixed)			1.6	
$K_{a,\,fast}$ (l/h)	10	(fixed)				
$K_{a,\,slow}$ (l/h)	0.12	36				
F_{tot}	0.38	28	68%	82		
F_{slow}	0.58	12	40%	45		
Residual variability			30%	22		

Pharmacokinetic–pharmacodynamic parameters after bolus administration and after successive infusions

Parameter	Bolus administration				Infusions			
	Population estimate	SE of estimate (%)	Variability (η or σ)	SE of variability (%)	Population estimate	SE of estimate (%)	Variability (η or σ)	SE of variability (%)
E_0 (USV)	270	11	48%	40	339	7.0	21%	32
E_{max}	−0.88	7.2			−0.82	5.7		
EC_{50} (ng/ml)	42	15			22	7.6	34%	53
Hill factor	4.5	124			5.4	26		
Residual variability			99 USV	44			73 USV	44

Table 2
Population parameter estimates for buspirone after bolus administration

Pharmacokinetic parameters					Pharmacokinetic–pharmacodynamic parameters				
Parameter	Population estimate	SE of estimate (%)	Variability (η or σ)	SE of variability (%)	Parameter	Population estimate	SE of estimate (%)	Variability (η or σ)	SE of variability (%)
CL (l/h)	0.63	13	38%	35	E_0 (USV)	194	6.9	28%	41
V (l)	0.35	13			E_{max}	−0.92	1.8	6%	60
K_a (l/h)	0.80	(fixed)			EC_{50} (ng/ml)	13	31	164%	40
F_{tot}	0.031	57	135%	36	Hill factor	3.9	32		
Residual variability (σ_1)			40%	30					
Residual variability (σ_2)			9 ng/ml	(fixed)	Residual Variability			47%	13

according to an additive model for S 15535, whereas the multiplicative model was applied for buspirone.

3. Results

3.1. Pharmacokinetics

Fig. 4 shows the observed plasma concentrations together with the fitted curve for some individual rats. S 15535 was detectable in plasma up to 10 h after dosing. Maximum concentrations were 3.5 and 0.91 μg/ml after i.v. and p.o. administration, respectively. Plasma concentrations dropped sharply immediately after the end of the infusion and after peak concentrations were reached following p.o. administration. Model parameters could be estimated with relatively high precision and enabled accurate prediction of individual plasma concentrations. Initially, interindividual variability (η) was identifiable only in CL. Upon p.o. administration, interindividual variability could be estimated for all absorption parameters ($K_{a,\ slow}$, F_{tot} and F_{slow}) (Table 1). The pharmacokinetic model also yielded a good fit of the plasma concentrations after the consecutive stepwise infusions (Fig. 6). However, the mean CL estimate was considerably higher than the values observed in the first set of experiments (0.88 vs. 0.53 l/h).

Buspirone could be detected in plasma up to 6 h, with maximum concentrations reaching 4.1 and 0.47 μg/ml after i.v. and p.o. administration, respectively. The one-compartment model allowed accurate estimation of the individual plasma concentrations. Fig. 4 illustrates the very rapid decrease in concentrations after i.v. administration and immediate absorption after p.o. administration. Buspirone pharmacokinetics showed considerably more variability than S 15535, in particular after p.o. administration. This is reflected in the population parameter estimates (Table 2), which demonstrate low bioavailability (3.1%) associated with an interindividual variability of 135%.

The concentration vs. time course profile of S 32784 was accurately described by a two-compartment model. S 32784 plasma concentrations were detectable, however, only up to 3 h after the start of the infusion. Population parameters are summarised in Table 3. The clearance of S 32784 was approximately three-fold higher than the parent compound S 15535. Estimation of structural and residual parameters showed high precision. Interindividual variability could be identified for structural parameters, but estimates were relatively imprecise. In contrast, residual variability was relatively low (25%). Typical individual curve fits for S 32784 are given in Fig. 7.

Table 4 presents the protein binding values for S 15535, buspirone and S 32784 in rats and in man. Apparently, the protein binding of S 15535 was not influenced by the presence of its metabolite. All three compounds showed higher bound fraction in man, as compared to rats. The in vitro radio receptor assay receptor results were equivalent to the plasma concentrations, whereas in human plasma samples, the affinity for these membranes were about 40

Table 3
Population parameter estimates for S 32784 after successive infusions

Pharmacokinetic parameters					Pharmacokinetic–pharmacodynamic parameters				
Parameter	Population estimate	SE of estimate (%)	Variability (η or σ)	SE of variability (%)	Parameter	Population estimate	SE of estimate (%)	Variability (η or σ)	SE of variability (%)
CL (l/h)	1.5	6.2	11%	88	E_0 (USV)	324	7.3	25%	42
V1 (l)	0.47	10	11%	88	E_{max}	−0.76	8.0		
Q (l/h)	1.1	23	65%	41	EC_{50} (ng/ml)	17	14	32%	96
V2 (l)	0.93	25	65%	41	Hill factor	4.1	36		
Residual variability			25%	28	Residual Variability			56 USV	44

Table 4
Protein binding in rat and human plasma

Compound	Bound fraction (%)	
	Rat	Man
S 15535	92	98
S 15535 in presence of S 32784	89	97
S 32784	69	81
S 32784 in presence of S 15535	67	87
Buspirone	75[a]	95[b]

[a] Data from Caccia et al. (1985).
[b] Data from Mahmood and Sahajwalla (1999).

times higher (unpublished results), showing that in the rat S 15535 does not present circulating metabolites active at the 5-HT_{1A} receptor.

3.2. Pharmacodynamics

3.2.1. Anxiolytic effect after a single bolus infusion and oral administration

The overall time course of the inhibitory effect of S 15535 and buspirone on fear-induced ultrasonic vocalisations is depicted in Fig. 5 by a smoothing spline function together with the individual observations (first experimental set). Ultrasonic vocalisation emission after administration of the vehicle was best described as constant over time, but there was considerable variability in the observed data. Reduction and even total suppression of ultrasonic vocalisations was observed immediately after the i.v. infusion and within 15 min after p.o. administration. In both groups, ultrasonic vocalisation emission progressively returned to baseline levels as plasma concentrations decreased. Interestingly, a trend towards higher baseline values was observed for the last ultrasonic vocalisations measurements after treatment with buspirone. This profile could suggest the occurrence of a rebound effect, previously reported for buspirone. In Fig. 5, one can also notice that baseline effect values in rats treated with buspirone were different from those obtained in rats treated with S 15535. These interindividual differences were confirmed by mixed effects modelling. For S 15535, interindividual variability could be estimated only for E_0.

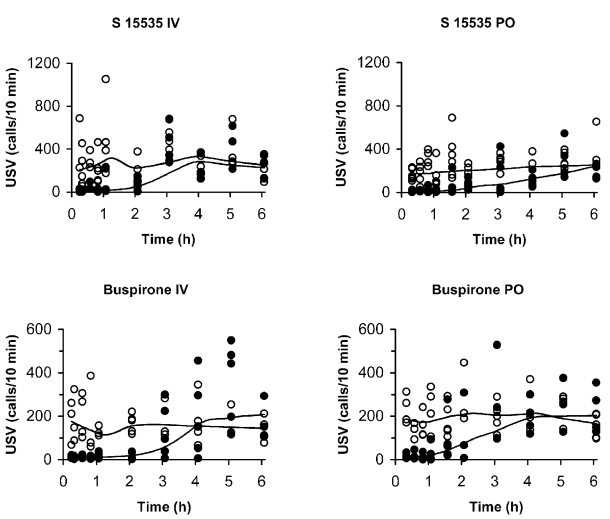

Fig. 5. Time course of the anxiolytic effect of S 15535 and buspirone. Emission of ultrasonic vocalisations after i.v. administration of S 15535 (top left panel) and p.o. administration (top right), and of buspirone after i.v. administration (bottom left) and p.o. administration (bottom right). The open circles are after vehicle administration and the closed circles after drug administration. The effects are fitted with a spline for representation.

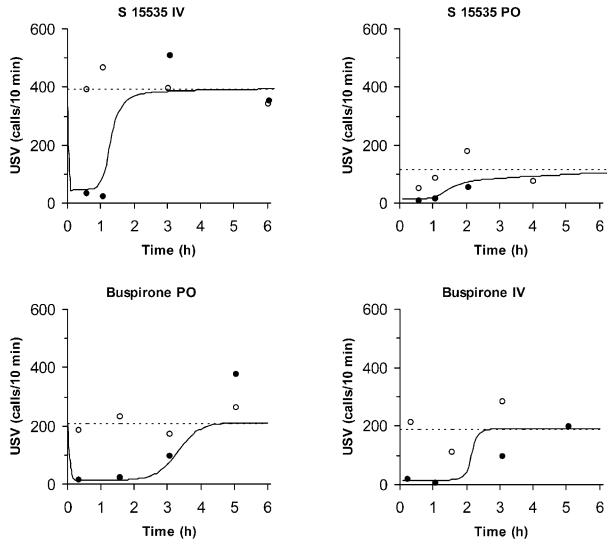

Fig. 6. Anxiolytic effects fitted by Bayesian feedback of S 15535 and buspirone after i.v. (left panel) and p.o. (right panel). The open circles and broken line indicate the effect after vehicle administration. The closed circles and the continuous line indicate the effect after drug administration.

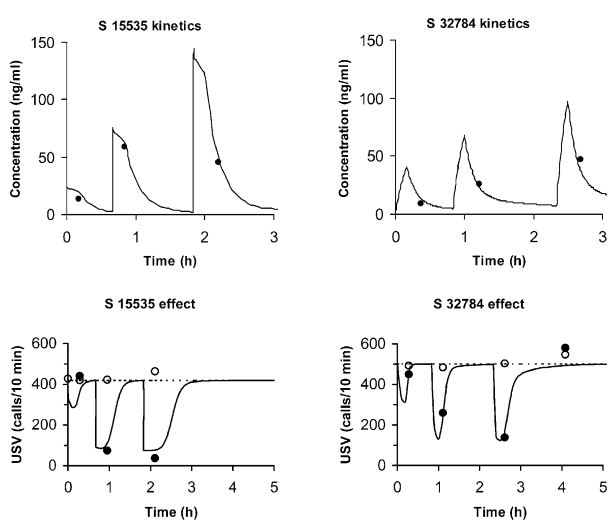

Fig. 7. Plasma concentrations and anxiolytic effects fitted by Bayesian feedback of S 15535 and S 32784 after repeated i.v. infusions. The open circles and broken line indicate the effect after vehicle administration. The closed circles and the continuous line indicate the effect after drug administration.

The concentration–effect relationship could be characterised by the sigmoid E_{max} model. The abrupt shift in the response, which was observed for both drugs irrespective of route of administration, seemed inherent to the nature of the fear-induced ultrasonic vocalisations. E_{max} values, equivalent to a reduction of 90% relative to baseline ultrasonic vocalisations, were obtained for S 15535 (Table 1) and buspirone (Table 2). Model parameters E_0 and E_{max} could be estimated with relatively high precision, but interindividual variability could be identified only for buspirone. Variability was particularly high for EC_{50} (164%), with a residual error of ca. 47%.

Based on total plasma concentration, model estimates showed that buspirone is apparently more potent than S 15535. However, differences in EC_{50} disappeared when potency was corrected for free drug concentrations, with $EC_{50,u}$ values of 8.4 and 10 nM for buspirone and S 15535, respectively. Fig. 6 illustrates the time course of the effect of S 15535 and buspirone in individual animals. Data fitting was based on the post-hoc estimates for the individual animals together with fitted baseline ultrasonic vocalisations following administration of the vehicle.

The meaning of the pharmacodynamic estimates in vivo was complemented by the results obtained for 5-HT_{1A} receptor affinity in plasma extracts. The values for receptor binding indicate that circulating active metabolites were not detected in the rat.

3.2.2. Anxiolytic effect of S 15535 and S 32784 after stepwise infusions

The effect time course profiles of S 15535 and S 32784 following the successive i.v. infusion is depicted in Fig. 7. USV emission decreased progressively in a similar manner with increasing concentrations of either parent drug or metabolite. In addition, the disruption of social interaction prior to the experiments decreased the interindividual variability in baseline response, which halved compared to the initial experiments. Despite higher baseline values, nearly complete ultrasonic vocalisation suppression was observed at maximum effect.

Similar to S 15535 (Table 1), the concentration–effect relationship of S 32784 (Table 3) could be characterised by the sigmoid E_{max} model. The intrinsic activity of both compounds was comparable, and not significantly different from the initial estimates obtained for S 15535 and buspirone. Furthermore, the sequential computer-controlled administration allowed interindividual variability to be identified for E_0 and EC_{50}.

Model parameters and variability for S 15535, S 32784 and buspirone are given in Table 5.

Table 5
Comparison of pharmacokinetic–pharmacodynamic parameters of S 15535, buspirone and S 32784

Parameter	S 15535		Buspirone bolus	S 32784 infusions
	Bolus	Infusions		
Structural				
E_0 (USV)	270	339	194	324
EC_{50} (ng/ml)	42	22	13	17
$EC_{50,u}$ (ng/ml)	3.4	1.8	3.3	5.3
E_{max}	−0.88	−0.82	−0.92	−0.76
Hill factor	4.5	5.4	3.9	4.1
Interindividual variability (%) in				
E_0	48	21	28	25
EC_{50}	–	35	164	32
E_{max}	–	–	6	–

4. Discussion

In the present study, we used a behavioural model of anxiety in conjunction with non-linear mixed effects modelling to characterise the relationship between concentration and anxiolytic effect of S 15535, its 5-hydroxy metabolite S 32784 and buspirone. Neurophysiology data show that enhancement of serotonergic activity in different brain regions, including hippocampus and prefrontal cortex, is the major underlying mechanism for ultrasound emission in rats (Wedzony et al., 1996; Olivier et al., 1994; Millan et al., 1997). Fear-induced ultrasonic vocalisation is sensitive to pharmacological manipulation and its inhibition by serotonergic drugs can be considered a direct measure of drug effect. In addition, it is suggested that this type of aversive behaviour may be associated with human panic and correlated with anticipatory anxiety (Beckett et al., 1996).

Appropriate modifications in the standard behavioural model were, however, necessary to fulfil methodological requirements, such as information on intra-individual variability and time course of the vocalisations following administration of vehicle. Since various design factors can affect the accuracy and precision with which population model parameters are estimated (Ette et al., 1995), balanced sampling matrices were used to account for the limited number of samples for pharmacokinetics and effect measurements that could be taken from each individual rat. Non-linear mixed effects modelling helped to overcome the sampling restrictions for pharmacokinetics and pharmacodynamics, enhancing the informative power of sparse individual data. It also allowed the effects to be analysed for each individual rat, thereby obtaining information on interindividual variability (Thomson and Whiting, 1992).

The suppression of fear-induced ultrasonic vocalisations was directly related to plasma concentrations for all three compounds. Like buspirone, S 15535 suppressed the emission of ultrasonic vocalisations immediately after administration. The effect decreased progressively back to baseline levels within 2 to 3 h following single administration. The maximum effect observed was a 90% reduction in ultrasonic vocalisations. All three compounds showed similar

potency and intrinsic activity in the unbound form. The concentration–effect curves were steep in all cases. Such an "on–off" type of response is reflected by the high values obtained for the Hill coefficient. Interestingly, no sign of sensitisation or tolerance development was observed for S 15535 or S 32784 after consecutive stepwise infusion with increasing concentrations.

The pharmacodynamic parameter estimates obtained after p.o. and i.v. administration also provided relevant information on drug metabolism in vivo. It appears that neither S 15535 nor buspirone present circulating metabolites active at the 5-HT_{1A} receptor in rats, although they are both extensively metabolised. This was confirmed with the radio receptor assay. 1-(2-Pyrimidinyl)-piperazine, a metabolite of buspirone present in rat and man (Caccia et al., 1985), may contribute to the psychostimulatory effect of buspirone via its antagonistic properties on the α_2-adrenoceptor (Berlin et al., 1995). Thus far, the role of α_2-adrenergic activity in the emission of ultrasound vocalisations is not clear (Beckett et al., 1996; Molewijk et al., 1995). In our paradigm, the concentration–anxiolytic effect relationship of buspirone did not include the eventual contribution of its metabolite to the overall anxiolytic response. On the other hand, in vitro data showed that S 32784, a major metabolite obtained after p.o. administration of S 15535 to man, has considerable serotonergic activity. Synthesis of the metabolite at such early phase of development of S 15535 allowed us to assess its relevance for the pharmacological effect. From a modelling point of view, this was an extremely interesting situation. Since S 32784 is not present in rodents, one can identify and make a distinction on the effect of the parent compound from that of the metabolite, by simply administering the compounds separately. The input rate of S 32784 required to characterise the full concentration–effect curve in vivo was derived from the results from the in vitro radio receptor assay. This makes the rat a suitable model to test the relative activity of the metabolite found in humans.

In addition to the assessment of relevant factors such as protein binding and circulating metabolites, the use of buspirone as a reference compound in our paradigm facilitated the extrapolation of the current findings to the clinical scenario. In clinical studies, the maximum recommended daily dose of 15 mg t.i.d. results in average plasma concentrations of 1.3 ng/ml (Mahmood and Sahajwalla, 1999). If one assumes this concentration equivalent to the EC_{50} in man, the potency ratio between rat and man can be determined after correction for protein binding. Taking into account the activity of S 32784, a daily dose for S 15535 between 5 and 35 mg was selected for a phase II study. According to current practice, dose selection is defined by toxicity and exposure data. Our rationale for dose selection contrasts with that defined on the basis of toxicity vs. exposure data alone, in that it clearly takes into account the underlying concentration–effect relationship. Such information allows the assessment of a therapeutically relevant dose range at an early stage of drug development.

The current study illustrates the value of population pharmacokinetic–pharmacodynamic modelling in early drug development. Rather than determining a dose–effect relationship, a concentration–effect relationship can give insight as to the potency and intrinsic activity in vivo (Jochemsen et al., 1999). Pharmacokinetic interspecies differences that must be accounted for are differences in protein binding and metabolic profile. The assessment of in vivo potency based on unbound compound is then a meaningful tool to guide dose selection in subsequent clinical studies. Here, the active metabolite, absent in the rat but present in man, was shown to account for a considerable part of the overall pharmacological response.

In conclusion, our results show that the pharmacokinetic–pharmacodynamic relationship of 5-HT_{1A} receptor agonists can be successfully characterised using ultrasonic vocalisation suppression as a measure of drug effect. Moreover, this study reveals that ultrasonic vocalisations can be considered a suitable endpoint for the anxiolytic effect when used in conjunction with non-linear mixed effects modelling to overcome the limited sampling and effect measurements.

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