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RESEARCH PAPER

Pharmacokinetic–pharmacodynamic modelling of fluvoxamine 5-HT transporter occupancy in rat frontal cortex

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Background and purpose: The pharmacokinetic–pharmacodynamic (PK–PD) correlation of fluvoxamine 5-HT transporter (SERT) occupancy was determined in rat frontal cortex *ex vivo*.
Experimental approach: Rats ($n=47$) with permanent arterial and venous cannulas received a 30 min intravenous infusion of fluvoxamine (1 or 7.3 mg kg^{-1}). At various time points after dosing, brains were collected for determination of fluvoxamine concentration and SERT occupancy. In addition, the time course of fluvoxamine concentration in plasma was determined up to the time of brain collection. In a separate study ($n=26$), the time course of fluvoxamine concentration in brain extracellular fluid (ECF) and plasma was determined. The results of the investigations were interpreted by nonlinear mixed effects modeling
Key results: Highest SERT occupancy was reached at the first time point (10 or 15 min) and maintained for 1.5 and 7 h after 1 and 7.3 mg kg^{-1}, respectively. Thereafter, SERT occupancy decreased linearly at a rate of 8% h^{-1}. SERT occupancy could be directly related to plasma, brain ECF and brain tissue concentrations by a hyperbolic function (B_{max} model). Maximal SERT occupancy (B_{max}) was 95%. Estimated concentrations at half-maximal SERT occupancy (EC_{50}) in plasma, ECF and brain tissue were 0.48, 0.22 and 14.8 ng mL^{-1} respectively. The minimum value of the objective function decreased 12 points for ECF and brain tissue concentrations relative to plasma ($P<0.01$), presumably as a result of nonlinear brain distribution.
Conclusions and implications: The proposed PK–PD model constitutes a useful basis for prediction of the time course of *ex vivo* SERT occupancy in behavioural studies with selective serotonin reuptake inhibitors.
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Abbreviations: B_{max}, maximal SERT occupancy; DMSO, dimethylsulphoxide; EC_{50}, fluvoxamine concentration at half of maximal SERT occupancy; ECF, extracellular fluid; MVOF, minimum value of the objective function; PD, pharmacodynamics; PK, pharmacokinetics; QC, quality control; SERT, 5-HT transporter; SSRI, selective serotonin reuptake inhibitor

Introduction

Reduced 5-hydroxytryptaminergic transmission is a well-known characteristic in the pathogenesis of depression (Coppen, 1967; Owens and Nemeroff, 1994). Not surprisingly, selective serotonin reuptake inhibitors (SSRIs) constitute the first line of treatment in depressive disorders (Isaac, 1999; Ables and Baughman, 2003). By blockade of the 5-HT transporter (SERT) extracellular 5-HT concentrations are increased, resulting in enhancement of 5-hydroxytryptaminergic transmission (Bel and Artigas, 1992; Fuller, 1994). The pharmacodynamics (PD) of SSRIs in depressive disorders are complex. Although SSRIs rapidly inhibit the reuptake of 5-HT, maximal antidepressant effects are observed only after weeks of chronic treatment, indicating that long-term adaptive changes are important for therapeutic efficacy. To date, most long-term investigations of the effects of antidepressants have focused on the functionality of 5-HT autoreceptors and postsynaptic receptors, which appear to be sensitive to adaptive changes (Blier and Bouchard, 1994; Auerbach and Hjorth, 1995; Bosker *et al.*, 1995). However,

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the functionality of SERT itself appears to be subjected to regulatory influences as well (Pineyro *et al*., 1994; Benmansour *et al*., 1999; Ramamoorthy and Blakely, 1999; Horschitz *et al*., 2001; Ginovart *et al*., 2003). Specifically, a decreased SERT gene expression (Lesch *et al*., 1993) and a decreased SERT density (Benmansour *et al*., 1999) have been observed after chronic treatment in rats.

Despite the widespread clinical use of SSRIs and numerous preclinical and clinical investigations, very few studies have addressed the pharmacokinetic (PK)–PD correlations of SSRIs. In recent years, the concept of mechanism-based PK–PD modeling has gained considerable interest. The objective of mechanism-based PK–PD modeling is to understand, in a strictly quantitative manner, the mechanisms that determine the time course of the intensity of the drug effect *in vivo*. A pertinent feature of mechanism-based PK–PD models is that they contain specific expressions to describe pertinent processes on the causal path between drug administration and response (Danhof *et al*., 2005). This includes among other factors the distribution of the drug to the target site, the binding to the target site, the activation of the target site and homoeostatic feedback. The development of mechanism-based PK–PD models relies on biomarkers, which characterize quantitatively the processes on the causal path between drug administration and response (Rolan, 1997; Colburn and Lee, 2003; Danhof *et al*., 2005).

Recently, we have initiated a series of investigations on the PK–PD correlations of fluvoxamine as a prototype for SSRIs. As the first step in this development, a population PK model of fluvoxamine in plasma has been proposed, which enables full characterization of the plasma concentration–time profile on the basis of sparse determinations of blood concentrations. This is important as in behavioural pharmacology investigations, blood sampling may interfere with assessment of the effect (Geldof *et al*., 2007a). The next step has been the development of a PK model describing the nonlinear brain distribution of fluvoxamine, which enables prediction of fluvoxamine concentration in brain extracellular fluid (ECF) of the frontal cortex and brain tissue on the basis of plasma concentrations (Geldof *et al*., 2008). In the present investigation, we propose a PK–PD model for fluvoxamine SERT occupancy in rat frontal cortex. This is important as SERT occupancy is an important intermediary step in the PD of SSRIs. Despite wide interest in the assessment of SERT occupancy in clinical studies (Meyer *et al*., 2001; Suhara *et al*., 2003), very few investigations have addressed *in vivo* SERT occupancy in relation to behavioural responses in animal studies (Ginovart *et al*., 2003; Hirano *et al*., 2005), which can presumably be explained by the relative difficulty of quantifying *in vivo* SERT occupancy.

In this contribution, we present a PK–PD model, which enables characterization and prediction of the time course of *ex vivo* SERT occupancy in behavioural studies.

Methods

The nomenclature used conforms to the British Journal of Pharmacology Guide to Receptors and Channels (Alexander *et al*., 2007).

Two studies were performed to describe the PK–PD correlation of fluvoxamine SERT occupancy in rat frontal cortex. In the first study, brain was collected from rats by destructive sampling at various time points after fluvoxamine administration for determination of the fluvoxamine concentration in brain tissue and SERT occupancy. In addition, the time course of fluvoxamine concentration in plasma was determined in these animals until the time of brain collection. In the second microdialysis study, the time course of fluvoxamine concentration in brain ECF of the frontal cortex and plasma was determined in animals by serial sampling. In addition, fluvoxamine concentration in brain tissue was determined in 16 of these animals at the end of the experiment. The results of the latter study have also been reported separately (Geldof *et al*., 2008). Plasma fluvoxamine concentration versus time profiles were predicted in individual rats, by analysis of the plasma concentrations on the basis of a previously proposed PK model. The time course of the fluvoxamine concentrations in brain ECF and brain tissue was predicted on the basis of a previously proposed PK model describing nonlinear brain distribution.

In the present investigation, information obtained in the two studies was integrated for development of a PK–PD model for fluvoxamine SERT occupancy in the rat, enabling prediction of the time course of *ex vivo* SERT occupancy in behavioural pharmacology studies.

Animals

All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the Ethical Committee on Animal Experimentation of Leiden University.

Male Wistar rats (Charles River Wiga GmbH, Sulzfeld, Germany) weighing 226–250 g were housed in groups for 1 week, under standard environmental conditions (ambient temperature 21 °C, humidity 60%, 12-h light–dark cycle). The animals had free access to food (laboratory chow; Hope Farms, Woerden, The Netherlands) and acidified water. For the brain sampling studies, 47 animals were used and for the microdialysis study, 26 animals were used. After surgery, the animals in the brain sampling studies were housed individually for 2 days and the animals in the microdialysis studies for 7 days.

Brain sampling studies

The animals were anaesthetized by a subcutaneous injection of 0.1 mL 100 g^{-1} Ketanest-S ((*S*)-ketaminebase; Parke-Davis, Hoofddorp, The Netherlands) and an intramuscular injection of 0.01 mL 100 g^{-1} Domitor (medetomidine hydrochloride; Pfizer, Capelle a/d IJssel, The Netherlands). Animals were implanted with a permanent cannula in the right jugular vein for administration of fluvoxamine and a permanent cannula in the left femoral artery for collection of blood samples. The cannulas were subcutaneously tunnelled and externalized at the back of the neck. The venous cannula was filled with NaCl (0.9%; B Braun Melsungen AG, Melsungen, Germany) containing heparin (20 IU mL^{-1}; Pharmacy, Leiden University Medical Center, Leiden, The Netherlands)

and the arterial cannula was filled with 25% (w/v) polyvinylpyrrolidone (Brocacef, Maarssen, The Netherlands) solution in NaCl (0.9%) containing heparin (20 IU mL^{-1}) to prevent blockade by blood clotting.

Of the 47 animals used in the brain sampling studies, 24 animals received 1 mg kg^{-1} and 23 animals received 7.3 mg kg^{-1} fluvoxamine via a 30-min intravenous infusion (BAS BeeHive; Bioanalytical Systems Inc., Lafayette, Indiana, USA) in the jugular vein cannula at a flow rate of 20 μL min^{-1} (1 or 7.3 mg kg^{-1}). Solutions of fluvoxamine in physiological saline (0.9%) were prepared on the day of administration. Dosages and observed concentrations of fluvoxamine are expressed as free base.

Depending on the time of brain sampling, between 2 and 15 arterial blood samples (100 μL) were collected from the cannula in the femoral artery between 2.5 and 600 min following fluvoxamine administration. After the collection of each blood sample, an equal volume of heparinized 0.9% NaCl (20 IU mL^{-1}) was administered. Blood samples were collected in heparinized Eppendorf tubes and kept on ice during the experiment. After centrifugation (10 min, 5000 *g*), 50 μL plasma was transferred into a glass tube and stored at −20 °C until sample analysis.

From the 24 animals that received 1 mg kg^{-1} fluvoxamine, the brains were collected at 10, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 500, 550 and 600 min after dosing. From the 23 animals that received 7.3 mg kg^{-1} fluvoxamine, the brains were collected at 15, 30, 45, 60, 80, 90, 110, 120, 140, 200, 250, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100 and 1300 min after dosing. The animals were killed by decapitation and the brains were quickly isolated. The brains were frozen in dry ice-cooled isopentane that was surrounded with cold ethanol and then stored at −20 °C until sectioning and subsequent analysis.

Microdialysis studies

Animals in the microdialysis studies were implanted with a cannula in the right jugular vein for fluvoxamine administration and a cannula in the left femoral artery for collection of blood samples. In addition, the animals were instrumented with a microdialysis guide cannula (CMA/12; Aurora Borealis Control BV, Schoonebeek, The Netherlands) at the same time. The microdialysis guide cannula with a dummy probe was implanted in the frontal cortex (AP: 3.2; L: −3.0; V: −1.5 mm from bregma, in accordance with the atlas of Paxinos and Watson (1982). Two support screws were placed and the guide was secured in place using dental cement (Simplex Rapid liquid and powder; Kemdent Associated Dental Products, Purton, Swindon, Wiltshire, UK).

Of the 26 animals used in the microdialysis studies, 8 animals received 1 mg kg^{-1}, 8 animals received 3.7 mg kg^{-1} and 10 animals received 7.3 mg kg^{-1} fluvoxamine via the 30-min intravenous infusion.

The microdialysis study was performed as described before (Geldof *et al.*, 2008). Briefly, 0.5% (w/v) bovine serum albumin (Sigma-Aldrich, Zwijndrecht, The Netherlands) was added to artificial CSF (containing 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.2 mM ascorbic acid in 2 mM phosphate buffer pH 7.4) (Moghaddam and Bunney, 1989) to prevent adhesion of fluvoxamine to the microdialysate tubing and microdialysis probe. After fluvoxamine administration, dialysate concentrations were measured over a period of 5 h at a flow rate of 2 μL min^{-1}. Microdialysate samples were collected at a temperature of 4 °C in the microdialysate fraction collector (Univentor 820; Antec Leyden BV, Zoeterwoude, The Netherlands) and subsequently stored at −20 °C until analysis. Individual values for the *in vivo* recovery were determined by the method of reverse dialysis or retrodialysis (de Lange *et al.*, 1997; Bouw and Hammarlund-Udenaes, 1998).

A total of 13 arterial blood samples (100 μL) were collected at variable fixed time intervals from the cannula in the femoral artery over a time period between 0 and 5 h after fluvoxamine administration. Blood samples were collected, handled and stored as described for the brain sampling studies.

The brains from the eight animals that received 1 mg kg^{-1} fluvoxamine were collected at the end of the experiment at 290 ($n=2$), 300 ($n=4$), 305 ($n=1$) and 310 ($n=1$) min after fluvoxamine administration. The brains from the eight animals that received 7.3 mg kg^{-1} fluvoxamine were collected at 300 ($n=3$), 310 ($n=3$), 365 ($n=1$) and 380 ($n=1$) min after fluvoxamine administration. Brain samples were collected, handled and stored as described for the brain sampling studies.

Drug analysis

Fluvoxamine samples were analysed using the liquid chromatography with tandem mass spectrometry as described earlier for plasma (Geldof *et al.*, 2007a) and for brain ECF and brain tissue (Geldof *et al.*, 2008). A volume of 50 μL of the calibration standards or independent quality control (QC) samples in dimethylsulphoxide (DMSO) was added to a volume of 50 μL blank plasma. For the plasma samples from the rats, a volume of 50 μL plasma was added to 50 μL DMSO. A volume of 50 μL of 500 ng mL^{-1} clovoxamine in DMSO was added to these samples as an internal standard. Proteins were precipitated by adding 200 μL acetonitrile, the samples were centrifuged (10 min, 5000 *g*) and a volume of 20 μL was injected into the system. For the ECF samples, a volume of 50 μL of the calibration standards or QC samples in DMSO was added to 50 μL artificial CSF. For the ECF samples from the rats, a volume of 50 μL DMSO, 500 μL H_2O and 50 μL of 500 ng mL^{-1} clovoxamine in DMSO was added. Addition of 100 μL NaOH increased the pH value to approximately 12. A volume of 4 mL of heptane-isoamylalcohol (95:5, v/v) was added, the solution was centrifuged (10 min, 3000 *g*) and the organic layer was evaporated to dryness under nitrogen at 65 °C. The residues were dissolved in a mixture of 10 mM ammonium acetate and acetonitrile (50:50, v/v) and a volume of 30 μL was injected into the system. Control brain and brain from fluvoxamine-treated rats were weighed and 9 volumes of H_2O were added before homogenization. A volume of 100 μL of the calibration standards or QC samples in DMSO was added to 1 mL control brain homogenates. For the brain samples of the rats, a volume of 100 μL DMSO and 100 μL of 500 ng mL^{-1} clovoxamine in DMSO was added to

1 mL of brain homogenates. A volume of 2 mL of methanol was added for extraction and the samples were rotated for 15 min and centrifuged (10 min, 5000 *g*). The solutions were transferred in Eppendorf tubes, centrifuged again (10 min, 9727 *g*) and a volume of 20 μL was injected into the system. All fluvoxamine samples were quantified on a reversed-phase LC column (BDS Hypersil C18, 3 μm particle size, 100 mm × 4.6 mm i.d.; Thermo Hypersil-Keystone, Brussels, Belgium). Liquid chromatography with tandem mass spectrometry analysis was performed on an API-4000 mass spectrometry/mass spectrometry (Applied Biosystems, Toronto, ON, Canada), coupled to an HPLC system (Agilent, Palo Alto, CA, USA). The mass spectrometry/mass spectrometry operated in the positive ion mode using the TurboIonSpray interface (electrospray ionization) was optimized for the quantification of fluvoxamine. For analysis of brain tissue samples, an additional guard cartridge (Hypersil ODS 5 μm 10 mm × 4.0 mm drop-in cartridge; Thermo Electron Corporation, Brussels, Belgium) with holder (Uniguard holder; Thermo Electron Corporation) was used. The intra-batch accuracy from independent QC samples was between 80 and 120% over the entire range of the samples. The limit of quantification for fluvoxamine was 1 ng mL^{-1} in plasma, brain ECF and brain tissue.

Ex vivo *SERT occupancy analysis*

Brain sections of 20 μm were cut at the level of the frontal cortex using a Leica CM 3050 cryostat-microtome (van Hopplynus, Brussels, Belgium) and thaw-mounted on microscope slides (SuperFrost Plus Slides, LaboNord, France). Three adjacent brain slices from the same animal were collected per slide. The sections were stored at −20 °C for approximately 48 h until use.

After thawing, the sections were dried under a stream of cold air. Two brain slices were used to analyse total binding and the third brain slice was used to determine nonspecific binding. The slices were not washed before incubation, to prevent dissociation of the complex of SERT with fluvoxamine. Total binding was analysed by incubation (400 μL for two slices) of the sections for 10 min with 1 nM –[^{3}H]citalopram (81 Ci mmol^{-1}) in Tris-HCl buffer (50 mM, pH 7.4) containing 120 mM NaCl. Nonspecific binding was analysed in the adjacent slice by addition of 10 μM fluoxetine (total 150 μL) to the incubation medium. Incubation was terminated by washing the slices in 50 mM Tris-HCl buffer, pH 7.4 at 4 °C (1 × 1 and 2 × 10 min) followed by a rapid dip in cold distilled H_2O and drying under a stream of cold air.

Slides were made conductive by disposing a copper foil tape (3M; Diegem, Belgium) on the free side. The radioligand-binding signal on the slides was evaluated using a β-imager (BioSpace, Paris, France) (Langlois *et al*., 2001). The levels of bound radioactivity in the brain areas were directly determined by counting the number of β-particles emerging from the delineated area by using the Beta vision program (BioSpace). *Ex vivo* labelling by [^{3}H]citalopram in rat frontal cortex was expressed as the percentage of SERT labelling in corresponding brain areas of control animals. Because only unoccupied transporters are available for the radioligand, *ex vivo* [^{3}H]citalopram labelling of SERT is inversely related to SERT occupancy by fluvoxamine.

Data analysis

In the population analysis, the fluvoxamine concentrations in plasma, brain ECF or brain tissue and SERT occupancy from all individual animals were simultaneously analysed. All fitting procedures were performed on a personal computer (Intel Pentium 4 processor) running under Windows XP using the Compaq Visual FORTRAN standard edition 6.1 (Compaq Computer Cooperation, Euston, TX, USA) with the nonlinear mixed effects modeling software NONMEM (Version V, Level 1.1; NONMEM project group, University of California, San Francisco, CA, USA).

The PK data in plasma obtained in the brain sampling and microdialysis studies were analysed on the basis of a previously proposed population PK model for prediction of the plasma concentration versus time profiles in individual rats (Geldof *et al*., 2007a). Individual values of the plasma fluvoxamine concentration at the time point determination of fluvoxamine SERT occupancy were predicted using the *post hoc* estimates of the PK parameters.

The PK data in brain ECF and brain tissue in the brain sampling and the microdialysis studies were analysed on the basis of a PK model for the nonlinear brain distribution of fluvoxamine (Geldof *et al*., 2008). Individual values of the fluvoxamine concentrations in brain ECF and brain tissue at the time of the determination of fluvoxamine SERT occupancy were estimated using the *post hoc* parameter estimates.

The predicted fluvoxamine concentrations in plasma, brain ECF and brain tissue were related to fluvoxamine SERT occupancy in three PK–PD models, using the first-order conditional estimation with interaction method in NONMEM. All steps in the development of the PK–PD models were executed on the basis of the likelihood ratio test (Kuipers *et al*., 2001; Jolling *et al*., 2004), diagnostic plots (observed concentrations versus predicted individual and population concentrations, weighted residuals versus predicted time and concentrations), parameter correlations and precision in parameter estimates. An extra parameter was included in the structural model if the resulting change in the minimum value of the objective function (MVOF) was larger than 6.6 points ($P \leqslant 0.01$).

The relationship between fluvoxamine concentration and SERT occupancy were successfully analysed by the B_{max} model according to:

$$B = \frac{B_{max} \cdot C}{C + EC_{50}} \quad (1)$$

in which B is SERT occupancy, B_{max} is maximum SERT occupancy, C is the fluvoxamine concentration in plasma, brain ECF or brain tissue and EC_{50} is the fluvoxamine concentration in plasma, ECF or brain at half of maximal SERT occupancy.

Although only one observation per animal for SERT occupancy could be determined and consequently a distinction between intra- and inter-individual variability could not be made, two random effects were explored in the model.

Inter-individual variability on the parameters was modelled according to an exponential equation:

$$P_i = \theta \cdot \exp(\eta_i) \quad (2)$$

in which P_i is the estimate for parameter P for the ith individual, θ is the population estimate for parameter P and $\exp(\eta_i)$ is the inter-individual random deviation of P_i from P. The values of η_i are assumed to be normally distributed with mean zero and variance ω^2 that distinguished the PK parameters for the ith individual from the population typical value θ. Inter-individual variabilities were analysed on each parameter and the inter-individual effects that did not significantly improve the model or could not be estimated were fixed to zero. Correlations between the inter-individual variability of the various parameters were explored. Residual variability of SERT occupancy (for example, caused by measurement and experimental errors) was described by a second level of random effects by an additive error:

$$B_{oij} = B_{ij} + \varepsilon_{1ij} \quad (3)$$

in which B_{oij} is observed SERT occupancy, B_{ij} is the jth SERT occupancy for the ith individual predicted by the model and ε_{1ij} accounts for the residual deviance of the predicted concentration from the observed concentration. The values for ε were assumed to be independently normally distributed with mean zero and variance σ^2.

Drugs and chemicals

Fluvoxamine maleate and clovoxamine fumarate were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Acetonitrile, isopentane, ethanol and methanol were obtained from Acros (Geel, Belgium). Heptane was purchased from Sigma-Aldrich Laborchemikalien (Seelze, Germany) and isoamylalcohol, DMSO and sodium hydroxide (NaOH) from Merck (Darmstadt, Germany). Ammonium acetate was obtained from Baker Chemicals (Deventer, The Netherlands) and Millipore water was obtained from a Milli-Q system (Millipore SA, Molsheim, France). The radioligand [^{3}H]citalopram was obtained from Amersham Biosciences Benelux (Roosendaal, The Netherlands).

Results

Figure 1 shows characteristic images of total and nonspecific [^{3}H]citalopram binding on brain sections of the rat frontal cortex at various time points after a fluvoxamine dose of 1 mg kg^{-1}. Because only unoccupied transporters are available for the radioligand, *ex vivo* [^{3}H]citalopram labelling of SERT is inversely related to SERT occupancy by fluvoxamine. Most contrast was obtained in control brains in which no fluvoxamine was administered. Fluvoxamine was potent in occupying SERT in rat frontal cortex, as the contrast of the image decreased indicating high levels of fluvoxamine SERT occupancy, as shown by the maximal SERT occupancy (B_{max}) for the digital image at 30 min after dose administration. In time, a decrease in fluvoxamine SERT occupancy was observed as shown by the increased contrast of the image at 210 and 550 min.

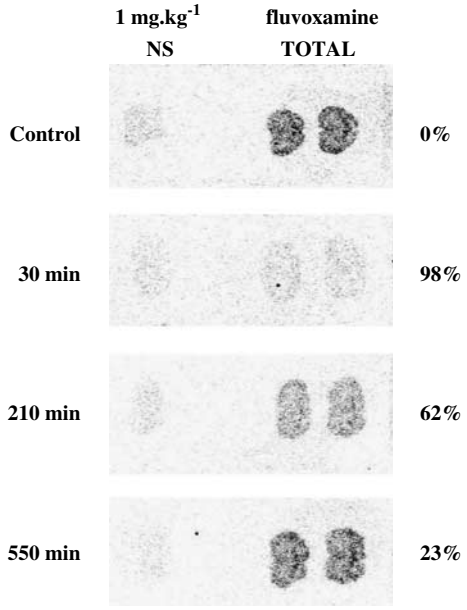

Figure 1 Digital images of fluvoxamine 5-HT transporter (SERT) occupancy in rat frontal cortex after a 30-min infusion of 1 mg kg^{-1}. The images shown are from samples of control or 30, 210 and 550 min after fluvoxamine administration with resulting SERT occupancy obtained after 1.5 h acquisition with the β-imager. (NS: nonspecific binding, TOTAL: total binding).

In Table 1, the mean *post hoc* estimates for the PK parameters of fluvoxamine in plasma obtained by the three-compartment PK model are presented. Included are the *post hoc* estimates for systemic clearance (CL), central volume of distribution (V_1), two peripheral volumes of distribution (V_2, V_3) and inter-compartmental clearances (Q_2, Q_3). On the basis of a covariate analysis, no differences in the PK of the different dose groups or between the rats in the brain sampling studies and the microdialysis studies could be detected. Inter-individual variability could not be identified on the parameters V_3 and Q_3, and therefore the estimates for the total population of 187 animals were used. The individual *post hoc* estimates were used in the current studies for each individual animal to estimate the fluvoxamine concentration in plasma at the time when fluvoxamine SERT occupancy was determined.

In Table 2, the mean *post hoc* estimates for the PK parameters of fluvoxamine in brain ECF obtained by the PK model are shown. Included are the *post hoc* estimates for the influx rate constant in the brain (k_{in}), the efflux rate from the brain (k_{out}) and the fluvoxamine concentration at which 50% of saturation of the active removal flux is reached (C_{50}). Inter-individual variability could not be identified on the parameter C_{50}, and therefore the population estimate of 710 ng mL^{-1} was used for each animal. The individual *post hoc* estimates were used in the current studies for each individual animal to estimate the fluvoxamine concentration in brain ECF and brain tissue at the time when fluvoxamine SERT occupancy was determined.

Table 1 Mean *post hoc* estimates for the pharmacokinetic parameters of fluvoxamine in plasma after i.v. administration in 30 min (1, 3.7 and 7.3 mg kg^{-1}) obtained by the previously proposed population three-compartment pharmacokinetic model for prediction of the plasma concentration versus time profiles in individual rats (Geldof *et al.*, 2007a)

Study	*PK parameter (unit)*					
	CL (mL min^{-1})	*V_1 (mL)*	*V_2 (mL)*	*Q_2 (mL min^{-1})*	*V_3 (mL)*	*Q_3 (mL min^{-1})*
Brain sampling + microdialysis	29.6	294.4	858.1	31.8	136.3	1.0
Brain sampling	27.1	253.8	722.4	29.2	136.3	1.0
Microdialysis	34.2	370.6	1113.3	36.7	136.3	1.0

Abbreviation: PK, pharmacokinetics.
Data shown are the population mean estimates for CL, V_1, V_2, Q_2, V_3 and Q_3. Inter-individual variability could not be identified on the parameters V_3 and Q_3, and therefore the estimates for the total population were used for each animal.

Table 2 Mean *post hoc* estimates for the pharmacokinetic parameters of fluvoxamine in ECF of the frontal cortex after i.v. administration in 30 min (1, 3.7 and 7.3 mg kg^{-1}) obtained by the previously proposed brain distribution pharmacokinetic model for the nonlinear brain distribution of fluvoxamine (Geldof *et al.*, 2008)

Study	*PK parameter (unit)*		
	k_{in} (min^{-1})	*k_{out} (min^{-1})*	*C_{50} (ng mL^{-1})*
Brain sampling + microdialysis	0.2031	0.0183	710
Brain sampling	0.2034	0.0186	710
Microdialysis	0.2026	0.0178	710

Abbreviations: ECF, extracellular fluid; PK, pharmacokinetics.
Data shown are the population mean estimates for k_{in}, k_{out} and C_{50}. Inter-individual variability could not be identified on the parameter C_{50}, and therefore the estimate for the total population was used for each animal.

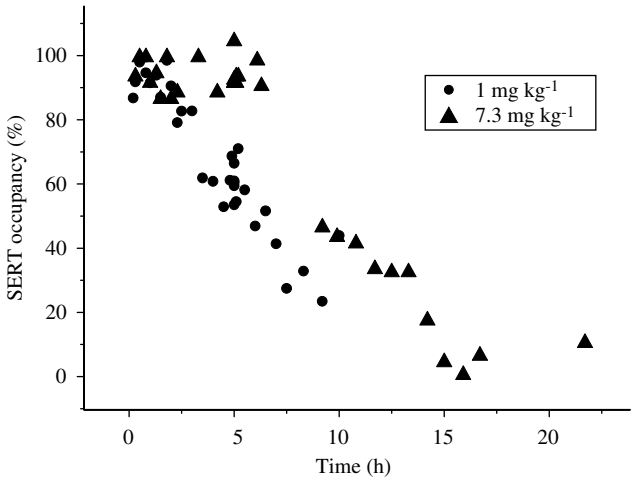

Figure 2 Time course of fluvoxamine 5-HT transporter (SERT) occupancy in rat frontal cortex obtained after a 30-min intravenous infusion of 1 or 7.3 mg kg^{-1}.

In Figure 2, the time courses of fluvoxamine SERT occupancy obtained after a 30-min intravenous infusion of 1 or 7.3 mg kg^{-1} are shown. Highest SERT occupancy was reached instantly at the first time point (10 or 15 min) after a dose of both 1 and 7.3 mg kg^{-1}. Maximal occupancy was observed for about 1.5 h after 1 mg kg^{-1} and for about 7 h after 7.3 mg kg^{-1}. Thereafter, fluvoxamine SERT occupancy linearly decreased in time at a rate of 8% h^{-1}, which was the same after both fluvoxamine dosages, indicated by the same slope of both curves. Fluvoxamine SERT occupancy reached 0% after about 15 h after a dose of 7.3 mg kg^{-1}.

The relationships between the fluvoxamine concentrations in plasma, brain ECF and brain tissue and the degree of SERT occupancy are shown in Figure 3. The concentration–effect relationship could be adequately described by relation of SERT occupancy to PK in plasma (Figure 3a), brain ECF (Figure 3b) and brain tissue (Figure 3c). Figure 3 shows that the fluvoxamine concentrations in plasma and brain ECF were below the limit of quantification (1 ng mL^{-1}) for a significant part of the concentration–effect curves. However, by analysis of these profiles in plasma and brain ECF in relation to the much higher fluvoxamine concentrations in brain tissue in the previously described model for fluvoxamine brain distribution (Geldof *et al.*, 2008), it was possible to predict accurate fluvoxamine concentrations in plasma and brain ECF.

The structural PD parameters for fluvoxamine of the three PK–PD models could be estimated simultaneously with good accuracy and precision (Table 3). No significant correlation was observed between fluvoxamine dose and the PD parameter estimates. Although only one observation per animal for SERT occupancy could be determined, inter-individual variability could be identified for EC_{50}, which significantly improved description of the concentration–effect relationships by the PK–PD models. Improved description was reflected in a reduction in the MVOF of 11.1, 3.8 and 3.8 points for plasma, brain ECF and brain tissue in addition to a reduction of the residual error by 52, 31 and 31, respectively.

When analysing fluvoxamine SERT occupancy in relation to brain ECF and brain tissue concentrations, MVOF was decreased by 12.2 points compared to the model in which SERT occupancy was related to fluvoxamine plasma concentrations. In addition, both the residual error and inter-individual variability in EC_{50} were significantly lower. B_{max} was not significantly different when related to PK in plasma, brain ECF or brain tissue and was estimated as 95% by all models. EC_{50} was 0.48 ng mL^{-1} in plasma, 0.22 ng mL^{-1} in brain ECF and 14.8 ng mL^{-1} in brain tissue.

In Figure 4, the goodness-of-fit plots obtained by the PK–PD model in which SERT occupancy was related to fluvoxamine brain concentrations are shown. Observed SERT occupancies were in close agreement with the predicted individual and population SERT occupancies. Fluvoxamine SERT occupancy could be adequately described, as no substantial or systemic deviation from the identity line was

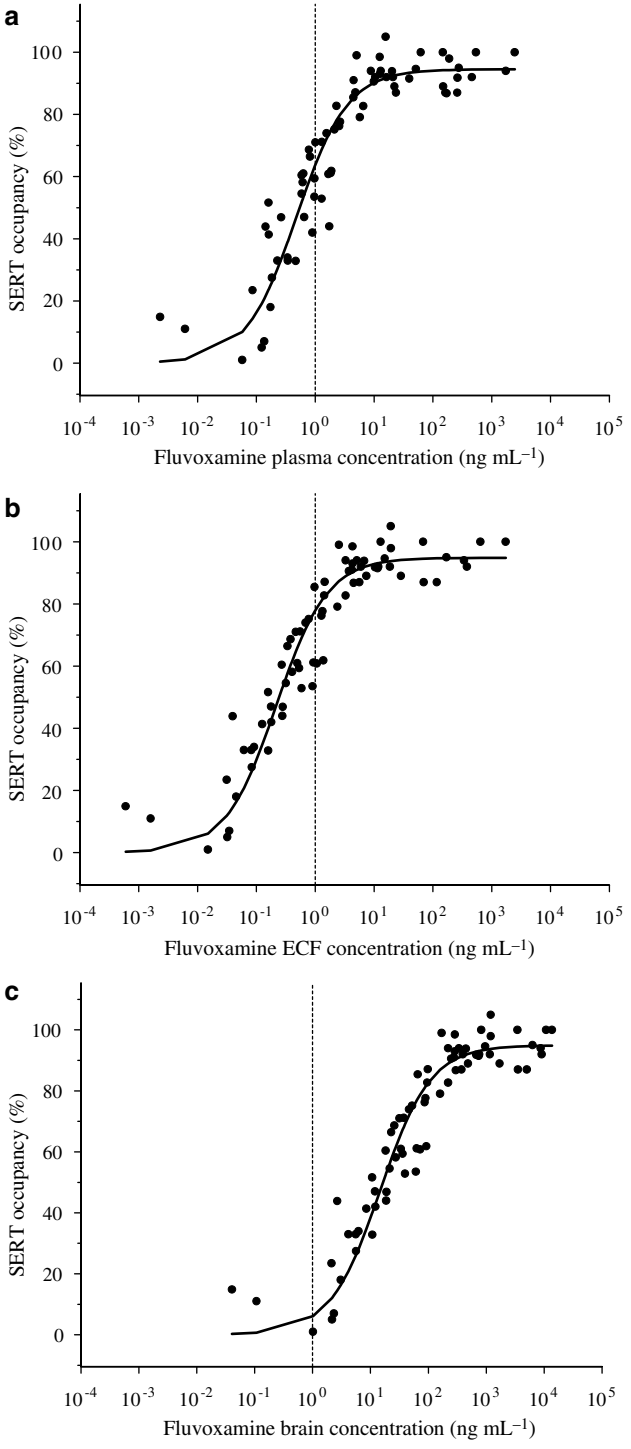

Figure 3 The relationships between fluvoxamine concentrations in plasma (**a**), extracellular fluid (ECF) of the frontal cortex (**b**) and brain tissue (**c**) and the degree of fluvoxamine 5-HT transporter (SERT) occupancy. Dots are the observed SERT occupancies and the solid line represents the predicted SERT occupancies by fluvoxamine. The limit of quantification (1 ng mL^{-1}) is added for clarity (dashed line).

observed in the residual diagnostics. The goodness-of-fit plots for the PK–PD models in which SERT occupancy was related to fluvoxamine concentrations in plasma and brain ECF showed the same satisfactory results (plots not shown).

Table 3 Population PD parameter estimates of fluvoxamine SERT occupancy in rat frontal cortex after i.v. administration in 30 min (1 and 7.3 mg kg^{-1}) obtained by the PK-PD models developed

		Plasma		*ECF*		*Brain*	
Parameter	*Unit*	*Value*	*CV (%)*	*Value*	*CV (%)*	*Value*	*CV (%)*
B_{max}	%	94.5	1.1	94.9	1.1	94.9	1.1
EC_{50}	ng mL^{-1}	0.48	11.6	0.22	10.6	14.8	10.6
$\omega^2\ \eta_{EC50}$	—	0.34	36.2	0.25	38.0	0.25	38.0
$\sigma^2\ \varepsilon_{1\,ij}$	—	33.5	27.2	30.9	27.1	30.8	27.1
MVOF	—	382.9		370.7		370.7	

Abbreviations: CV, coefficient of variation (standard error/value × 100%); ECF, extracellular fluid; PD, pharmacodynamics; MVOF, minimum value of the objective function; PK, pharmacokinetics; SERT, 5-HT transporter.
Fluvoxamine SERT occupancy was related to the simulated PK in plasma, ECF of frontal cortex or brain tissue. Data shown are the population mean estimates for B_{max}, EC_{50}, ω^2, σ^2 with corresponding coefficient of variation (CV) and values for MVOF.

Discussion and conclusions

The objective of this investigation was to develop a PK–PD model for SERT occupancy in rat frontal cortex following intravenous administration of fluvoxamine, which can be used for prediction of the time course of SERT occupancy in behavioural pharmacology studies *ex vivo*. This is important as SERT occupancy is an important intermediate step in the PD of SSRIs. The ultimate goal is to apply this model in behavioural studies with SSRIs to explore the relationships among drug concentration, SERT expression, SERT occupancy and the behavioural effect. An advantage of the present *ex vivo* method is that apart from analysing SERT occupancy by fluvoxamine, the plasma, ECF and brain concentration of fluvoxamine could also be measured within the same animal.

An *ex vivo* technique was used to characterize *ex vivo* fluvoxamine SERT occupancy. A limitation of this approach is that only a single observation of SERT occupancy was obtained. Furthermore, due to the high affinity of fluvoxamine for SERT, drug concentrations in biological fluids are typically below measurable values. In previous investigations, we have proposed population PK models to describe the plasma concentration versus time profile of fluvoxamine on the basis of sparse sampling (Geldof *et al.*, 2007a) as well as a PK model to describe nonlinear brain distribution (Geldof *et al.*, 2008). The latter model showed that fluvoxamine brain distribution is nonlinear, presumably as a result of the effect of an active efflux transporter at the blood–brain barrier. An important feature of these models is that by simultaneous analysis of fluvoxamine concentrations in plasma, brain ECF and brain tissue, fluvoxamine concentrations in plasma and brain ECF can be predicted over a much wider concentration range. In the present investigation, the previously proposed population PK in plasma and brain distribution model were successfully applied to predict fluvoxamine concentrations in plasma and brain ECF in the relevant concentration range with regard to fluvoxamine SERT occupancy. No hysteresis between the fluvoxamine concentration in plasma, brain ECF, brain tissue and SERT occupancy was observed, and therefore these two could be directly related to each

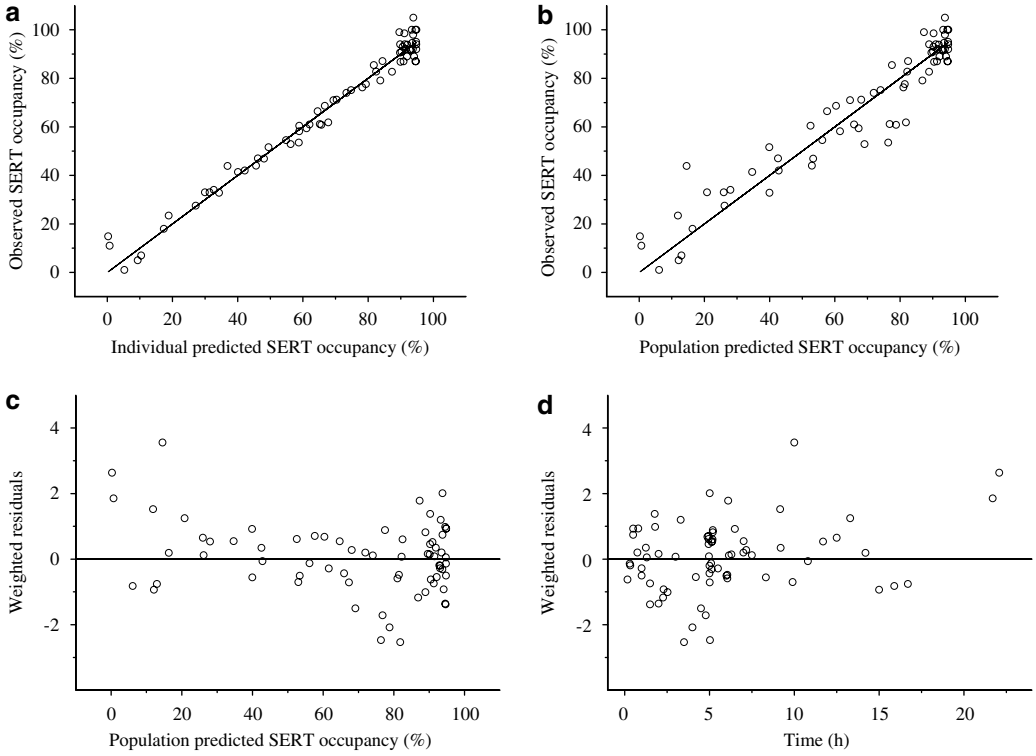

Figure 4 Goodness-of-fit plots obtained by the PK–PD model developed in which 5-HT transporter (SERT) occupancy was related to fluvoxamine concentrations in brain tissue. Data shown are scatter plots of observed fluvoxamine SERT occupancy versus the individual model predictions (**a**), of observed fluvoxamine SERT occupancy versus population model predictions (of the total population of 47 rats) (**b**) and scatter plots of the population-weighted residuals versus population model predictions (of the total population of 47 rats) (**c**), of the population-weighted residuals versus time (**d**).

other. The relation between fluvoxamine concentrations and SERT occupancy was successfully described by a hyperbolic function (simple B_{max} model) allowing estimations of the relevant PD parameters.

It should be taken into account that the experiment could be vulnerable to changes in those experimental conditions, which are known to influence the dissociation process from the receptor. In the present experimental set-up, we have minimized this risk for dissociation of fluvoxamine from the transporter. This was done by minimizing the time of incubation of the sections (10 min) with [^{3}H]citalopram and minimizing the volume of incubation (400 μL for the two brain slices). Furthermore, the results of the present study indicate that fluvoxamine most probably is not significantly dissociating from the transporter during the incubation period, as a very high occupancy (about 95%) was reached after each fluvoxamine dosage. Highest SERT occupancy was reached instantly at the first time point (10 or 15 min) after administration and maintained for 1.5 and 7 h after 1 and 7.3 mg kg^{-1}, respectively. Thereafter, SERT occupancy decreased linearly at a rate of 8% h^{-1} after both fluvoxamine dosages. By relating fluvoxamine SERT occupancy to fluvoxamine levels in brain ECF and brain tissue, the MVOF was decreased by 12 points compared to the model with relation to fluvoxamine plasma levels and both the residual error and inter-individual variability in EC_{50} were significantly decreased, presumably as a result of nonlinear brain distribution. Furthermore, fluvoxamine levels in brain ECF and brain tissue have a small lag time of about 30 min compared with fluvoxamine plasma levels because of the required time for transport of fluvoxamine from plasma to the brain.

EC_{50} was 0.48 ng mL^{-1} in plasma, 0.22 ng mL^{-1} in brain ECF and 14.8 ng mL^{-1} in brain tissue, of which only this latter value was above the quantification limit (1 ng mL^{-1}) of the bioanalytical assay. Estimated EC_{50} values were very low, particularly in plasma and brain ECF, hence only very small levels of fluvoxamine must be present in plasma and brain ECF to be able to occupy SERT in rat frontal cortex. Because fluvoxamine SERT occupancy was determined in frontal cortex of the rat, it would be rational to relate SERT occupancy to the measured fluvoxamine ECF levels, as these were also measured in this brain region.

Because only one SERT occupancy observation could be obtained per animal, no distinction could be made between the inter- and intra-individual variability. However, in the PK–PD models developed, residual variability was described by an additive error in addition to the inter-individual variability identified for EC_{50}. By incorporation of the inter-individual variability for EC_{50}, MVOF was decreased by 11.1, 3.8 and 3.8 points for plasma, brain ECF and brain tissue and additionally, a reduction of the residual error was observed by 52, 31 and 31, respectively. For these reasons, inter-individual variability in EC_{50} was included next to the residual error model, but obviously no distinction between these two random effects could be made.

Only over the recent years, the results of studies on the occupancy of various SSRIs to SERT in healthy volunteers and depressed patients have been described by application of the neuroimaging techniques, positron emission tomography or single photon emission computed tomography (Meyer *et al*., 2001, 2004; Suhara *et al*., 2003; Takano *et al*., 2006). These studies concluded that during acute and chronic treatment with clinical SSRI dosages, about 80% of SERT is occupied in various regions of the brain, implicating that such a high SERT occupancy might be required to increase 5-HT levels to the degree that most therapeutic effects occur. The current acute study showed a high level of SERT occupancy at relatively low fluvoxamine levels in plasma, brain and ECF. In the study of Takano *et al*. (2006), the SERT occupancy by fluvoxamine in various brain regions was related to the plasma concentration, yielding an ED_{50} value of $4.6\,ng\,mL^{-1}$ in human, This value was approximately 10 times higher than the EC_{50} value obtained in the present study ($0.48\,ng\,mL^{-1}$). In the current study, SERT occupancy was analysed after acute fluvoxamine administration. However, similar experimental conditions could be applied to determine the effects on fluvoxamine SERT occupancy after chronic administration to explore the possible subjectivity of SERT to adaptive changes in rat frontal cortex.

In conclusion, three PK–PD models were developed in which SERT occupancy was related to the PK of fluvoxamine in plasma, brain ECF and brain tissue. These relationships were characterized by the B_{max} model, which could adequately describe observed fluvoxamine SERT occupancy in rat frontal cortex. On the basis of a comparison of the MVOF, fluvoxamine SERT occupancy could be best described by the PK of fluvoxamine in brain ECF and brain tissue, which could possibly be explained by description of the previously observed nonlinearity in these PK profiles. The proposed PK–PD model constitutes a useful basis for prediction of the time course of *ex vivo* SERT occupancy in behavioural studies with SSRIs.

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Conflict of interest

The authors state no conflict of interest.

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