Chapter 8

PHARMACOKINETICS AND PHARMACODYNAMICS ANALYSIS OF TRANSDERMAL IONTOPHORESIS OF 5-OH DPAT IN RATS: IN VITRO – IN VIVO CORRELATION

Akhmad Kharis Nugroho\textsuperscript{1,2}, Stefan Romeijn\textsuperscript{1}, Raphaël Zwier\textsuperscript{3}, Jan B de Vries\textsuperscript{4}, Durk Dijkstra\textsuperscript{4}, Håkan Wikström\textsuperscript{4}, Oscar Della-Pasqua\textsuperscript{5}, Meindert Danhof\textsuperscript{5} and Joke A. Bouwstra\textsuperscript{1}

\textsuperscript{1}. Division of Drug Delivery Technology Leiden/Amsterdam Center for Drug Research, Einsteinweg 55 2300 RA Leiden The Netherlands, \textsuperscript{2}. Faculty of Pharmacy Gadjah Mada University Sekip Utara Yogyakarta 55281 Indonesia, \textsuperscript{3}. Fine and Mechanical Department Leiden University Einsteinweg 55 2300 RA Leiden The Netherlands, \textsuperscript{4}. Department of Medicinal Chemistry, University Center of Pharmacy, University of Groningen, Antonius Deusinglaan 1 Groningen, NL-9713 AV Groningen, The Netherlands, \textsuperscript{5}. Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Einsteinweg 55, 2300 RA Leiden The Netherlands.

(submitted for publication, 2004)

ABSTRACT

The pharmacokinetics (PK) and the dopaminergic effect (PD) of 5-OH-DPAT in vivo were determined following transdermal iontophoretic delivery in rats on the basis of: 1) drug concentration in plasma ($C_p$), 2) drug concentration in striatum ($C_s$), and 3) the dopamine levels in striatum ($C_{DA}$). Data were analyzed on the basis of an integrated population PK-PD model. The PK analysis was based on the previously proposed compartmental model for transdermal iontophoresis whilst the PD analysis was based on the indirect response model with inhibition of response production, i.e. dopamine release (IDR type I). To determine the correlation of the in vitro transport with the in vivo PK-PD profiles during transdermal iontophoresis, the in vitro transport of 5-OH-DPAT was characterized in the transport in dermatomed rat skin (DRS) and in rat stratum corneum (RSC). A quantitative analysis of the transport was performed on the basis of an in vitro compartmental model for transdermal iontophoresis.

The integrated in vivo PK-PD model and the in vitro model allowed the estimation of PK, PD and in vitro iontophoretic transport parameters as well as successfully described the time course of $C_p$, $C_s$, $C_{DA}$, and the in vitro flux in DRS and in RSC. The population value of the steady state flux ($J_{ss}$) in vivo was comparable to $J_{ss}$ in vitro in DRS (31.8 nmol cm$^{-2}$ h$^{-1}$ versus 39.0 nmol cm$^{-2}$ h$^{-1}$, $p>0.05$). On the other hand, the skin release rate constant ($K_R$) in vivo was
similar to the $K_R$ in RSC ($4.4 \text{ h}^{-1}$ versus $2.58 \text{ h}^{-1}$, $p>0.05$). The kinetic lag time ($t_L$) $in vivo$ was negligible, which is close to the in vitro $t_L$ in RSC (0.04 h, $p>0.05$). Based on non-linear mixed effects modeling, the profiles of $C_p$, $C_s$, and $C_{DA}$ were successfully predicted using $in vitro$ values of $J_{ss}$ in DRS with $K_R$ and $t_L$ in RSC. Transdermal delivery of 5-OH-DPAT by iontophoresis in rats yields a considerable dopaminergic effect. These findings strongly indicate that it is feasible to reach therapeutically effective concentrations of 5-OH-DPAT upon transdermal iontophoretic delivery.

A. INTRODUCTION

Recently, we have proposed a family of compartmental models for characterizing transdermal iontophoretic transport $in vitro$ (1) and $in vivo$ (2) in a strictly quantitative manner. These models constitute the theoretical basis for the evaluation of $in vitro$-$in vivo$ correlations of transdermal iontophoretic transport.

5-OH-DPAT is one of the most potent dopamine agonists from 2-aminotetralin group (3). However, due to its low oral bioavailability (approximately 1% (4)), its utility in Parkinson’s disease therapy is limited. Transdermal iontophoresis is an alternative route of delivery that can overcome these bioavailability issues. The method may offer the possibility to deliver a sufficient amount of the drug transdermally, as well as to optimize dose titration by controlling current intensity (5).

Previously, we reported the feasibility of transdermal iontophoretic delivery of 5-OH-DPAT $in vitro$, as determined by drug transport across human stratum corneum (HSC) and dermatomed human skin (DHS) (6). Assuming that a sufficient amount of 5-OH-DPAT can be delivered to the striatum, the activation of the dopaminergic receptors will result in a negative feedback on the dopamine production under the control of dopamine $D_2$ autoreceptors (7). In an animal model, such a feedback can be measured by the suppression of the dopamine release in the striatum using brain-microdialysis (4,8) The negative feedback mechanism can subsequently be characterized by an indirect response model with the inhibition of the input rate (IDR type I) (9,10).

In the present study we applied non-linear mixed effects modeling: 1) to characterize the transdermal iontophoretic transport of 5-OH-DPAT $in vivo$ in rats; 2) to characterize the transdermal iontophoretic transport of 5-OH-DPAT $in vitro$ in dermatomed rat skin (DRS) and in rat stratum corneum (RSC); 3) to explore the $in vitro$-$in vivo$ correlation of transdermal iontophoretic transport as a predictor of the effect of 5-OH-DPAT on striatal dopamine release.

The use of non-linear mixed effects modeling enables assessment of population and individual pharmacokinetic-pharmacodynamic (PK-PD) parameter estimates using experimental data from separate experiments (11). This approach is therefore essential to optimally combine data from the various
in vitro and in vivo experiments required to establish an in vivo-in vitro correlation.

**B. THEORY**

The assessment of the correlation between in vitro drug transport and in vivo PK and PD profiles requires certain quantitative models. Previously, we have proposed the kinetic models based on compartmental mass transfer to describe the iontophoretic transport in vitro (1) and in vivo (2). In the present investigation these models are combined with the IDR type I into a comprehensive PK-PD model for the effect of 5-OH-DPAT delivered by transdermal iontophoresis on striatal dopamine release.

1. **In vitro models**

The schematic presentations of the in vitro models are depicted in Fig. 1A, in which panel I describes the transport in iontophoretic phase and panel II describes the transport in the post iontophoretic period. The equations derived for the in vitro flux from those models are presented in equation 1 and equation 2, respectively for the iontophoretic phase and for the post iontophoretic period.

\[
J(t) = \frac{I_0}{S} \left( 1 - e^{-K_R(t-t_L)} \right) \]

\[
J(t) = \frac{P_{PI}}{S} \left( 1 - e^{-K_R(t-t_0)} \right) + \frac{I_0}{S} \left( 1 - e^{-K_R(T-t_0)} \right) e^{-K_R(T-t)}
\]

In these equations \(J(t)\) is the flux at time \(t\), \(I_0\) is the zero order drug input due to the iontophoretic driving force, \(P_{PI}\) is the zero order drug input due to the passive driving force in the post iontophoretic period, \(K_R\) is the skin release rate constant, \(S\) is the area of diffusion in DRS and in RSC, \(t_L\) is the kinetic lag time of the drug to enter skin compartment, and \(T\) is duration of current application.

2. **In vivo PK model**

The PK model used in this study differs slightly from the previously proposed in vivo models for transdermal iontophoresis (2). Modifications were introduced to account for the need to simultaneously fit 5-OH-DPAT concentrations in plasma (\(C_p\)), and in striatum (\(C_s\)), as well as dopamine concentration in striatum (\(C_{DA}\)) upon transdermal iontophoresis. Data from 5-OH-DPAT plasma concentrations following an intravenous infusion was also included in the analysis to improve estimation of relevant PK parameters, such as clearances and volumes of distribution. The schematic illustration of the model is presented in Fig. 1B.
**Fig. 1.** The schematic representation of the compartment model of the iontophoretic transport *in vitro* (A), the pharmacokinetics models following intravenous infusion and transdermal iontophoresis (B) and the indirect response model (C).

**Legends:**

$I_0$: the zero order input due to iontophoretic driving force, $P_{PI}$: the zero order input due to passive driving force post iontophoresis, $K_R$: Skin release rate constant, $N$: flag number, i.e. $N=0$ for intravenous infusion and $N=1$ for transdermal iontophoresis, $M$: flag number, i.e. $M=1$ for iontophoretic phase and $M=0$ at post iontophoretic period, $O$: flag number, i.e. $O=1$ during the duration of intravenous infusion and $O=0$ after termination of intravenous infusion, *Rate*: intravenous infusion rate, $k$: Elimination rate constant, $k_{23}$: plasma-tissue distribution rate constant, $k_{32}$: tissue-plasma distribution rate constant, $k_{24}$: plasma-striatum distribution rate constant, $k_{32}$: striatum-plasma distribution rate constant, IDR: Indirect response to inhibition of dopamine production.
The PREDPP subroutine in NONMEM (12) was used to solve the ordinary differential equations (ODE). Data fitting was performed with the derived ODEs as presented in equation 3 to equation 6. In addition, preliminary analysis indicated that the kinetic lag time in vivo \( t_L \) was negligible. Therefore, the value of \( t_L \) was constrained to zero in all cases.

\[
\frac{dX_1(t)}{dt} = N \cdot (I_0 \cdot M - K_R \cdot X_1(t)) 
\]

\[
\frac{dX(t)}{dt} = K_R \cdot X(t) + \text{Rate} \cdot (1-N) \cdot O - k \cdot X(t) - k_{23} \cdot X_2(t) - k_{24} \cdot X_4(t) + k_{32} \cdot X_3(t) + k_{42} \cdot X_4(t) 
\]

\[
\frac{dX_3(t)}{dt} = k_{23} \cdot X_2(t) - k_{32} \cdot X_3(t) 
\]

\[
\frac{dX_4(t)}{dt} = k_{24} \cdot X_2(t) - k_{42} \cdot X_4(t) 
\]

In these equations, \( \frac{dX_1(t)}{dt} \) is the rate of change in the amount of the drug in compartment \( i \). \( X(i) \) is the amount of the drug in compartment \( i \), which refers to a skin compartment \( (i=1) \), plasma compartment \( (i=2) \), tissue compartment \( (i=3) \) and striatum compartment \( (i=4) \). The term \( \text{Rate} \) refers to the zero-order intravenous infusion rate, \( k \) is the first-order elimination rate constant, \( k_{23}, k_{32}, k_{24} \) and \( k_{42} \) are the first-order distribution rate constants, respectively from plasma to tissue, from tissue to plasma, from plasma to striatum, and from striatum to plasma. The terms \( N, M, \) and \( O \) are flags in the model and are defined as follows: \( N=0 \) for intravenous infusion and \( N=1 \) for transdermal iontophoresis; \( M=1 \) for the iontophoretic phase and \( M=0 \) in the post iontophoretic period; \( O=1 \) during the duration of intravenous infusion and \( O=0 \) after termination of intravenous infusion.

3. PD model

The IDR type I model was used to correlate the inhibition of dopamine release to 5-OH-DPAT striatum concentration \( (C_s) \). Since microdialysis enables the assessment of brain drug concentrations, the levels of 5-OH-DPAT in striatum could be linked directly to the dopaminergic effect instead of relying on 5-OH-DPAT concentrations in plasma \( (C_p) \). The IDR model is schematically presented in Fig. 1C. The equation and the ODE of the model is as follows:

\[
\frac{dC_{DA}(t)}{dt} = k^0_{in} \cdot (1 - \frac{I_{max} \cdot C_s(t)^H}{IC_{50}^H + C_s(t)^H}) - k_{out} \cdot C_{DA}(t) 
\]

\[
k^0_{in} = k_{out} \cdot C_{DA0} 
\]

In these equations \( \frac{dC_{DA}(t)}{dt} \) is the rate of the change in the dopamine concen-
tration in striatum, $I_{\text{max}}$ is the maximum inhibition of the dopamine production, $C_s(t)$ is the concentration of 5-OH-DPAT in striatum at time $t$, $IC_{50}$ is the concentration of 5-OH-DPAT in striatum required to produce 50% of $I_{\text{max}}$, $H$ is the Hill-slope coefficient (which is constrained to 1), $k_{\text{out}}$ is the first-order rate constant for the loss of response, $k_{\text{in}0}$ is the zero order rate constant for the production of response, $C_{DA0}$ is the baseline values of dopamine (i.e., dopamine concentration prior to the inhibition effect of 5-OH-DPAT).

In this research two groups of animals were involved, i.e. group A (rats number 1-8, for PK and in vitro iontophoretic transport studies) and group B (rats number 9-12, for PD studies). To implement the PK-PD modeling, two important assumptions are used: 1) the pharmacokinetics of 5-OH-DPAT is identical in group A and group B, and 2) the recovery of the microdialysis probe is 100% for both 5-OH-DPAT and dopamine in all animals.

C. MATERIALS AND METHODS

1. Materials

5-OH-DPAT (HBr salt, purity >98%) was synthesized at the Department of Medicinal Chemistry of the University of Groningen, Groningen, The Netherlands. Silver and silver chloride (purity > 99.99%) were obtained from Aldrich (Borneum, Belgium). Ascorbic acid, trypsin (Type III, from a bovine pancreas) and trypsin inhibitor (Type II-S from soybean) were purchased from Sigma Chemicals (Zwijndrecht, The Netherlands). HPLC grade acetonitrile was obtained from Rathburn (Walkerburn, UK). All other chemicals and solvents were of analytical grade. All solutions were prepared in Millipore water with resistivity of more than 18MΩ.

2. Iontophoretic patches

The iontophoretic patches were prepared from elastic silicon materials (silicon: RTV-400T with the activator Beta19, GE Bayer Silcones, BV, Bergen op Zoom, The Netherlands). The active area of the patches was 2.5 cm² with a volume of 2.3 ml. The circle electrodes (diameter of the contact area was 0.5 cm) were placed in the upper side of the patch with a distance from the skin surface of approximately 1 cm. The anodes were made from the silver plate while the cathodes were made from silver plate after electrochemical coating with silver chloride at the side with contact to the PBS solution. The wound dressing film (Opsite* FLEXIGRID*, Smith&Nephew, Hoofdorp, The Netherlands) was used to attach the patch to the rat skin. The attachment of silicon patch to the back part of the Opsite* FLEXIGRID* was performed by using the medical silicon adhesive (MD7-4602, obtained as a gift from Dow Corning, Belgium). The design of the patch is depicted in Fig. 2.
3. Animals

The PK and the PD studies described below were approved by the ethical committee of Leiden University and by the ethical committee of the University of Groningen respectively.

The PK-PD studies were performed in male albino Wistar WU rats, weight 280-320g (Charless River, The Netherlands). The rats were housed in plexiglas cages, 6 animals in each cage with free access to water and standard laboratory chow. The cages were placed in a room with a controlled environmental conditions (temperature 21°C, humidity 60-65%, the duration of light on and off was 12 hours). The animals were housed at least one week prior to surgery. The surgery consisted of femoral artery and femoral vein cannulations (for PK studies) or implantation of the microdialysis probes in the striatum (PD studies). In the PK studies, rats were used for either intravenous infusion or transdermal iontophoresis studies immediately after the surgery. In PD studies, the rats were given a 10-24 hours recovery period prior to the microdialysis studies. After surgery, the animals were housed individually in cages and were supplied with a standard laboratory chow and a free access to water.

![Fig. 2. The schematic design of the iontophoretic patch.](image)

**Legends:**
1. Wire, 2. Active chamber from elastic silicon (volume 2.3 ml, active area 2.5 cm²), 3. Gold-plated connector, 4. Electrode, 5. Opsite* FLEXIGRID* adhesive layer.
4. PK studies following intravenous infusion and transdermal iontophoresis

The animals were anaesthetized with a combination of Dormicum® (midazolam 5 mg ml\(^{-1}\), Roche Nederland, Mijdrecht, the Netherlands) and Hypnorm® (fentanyl citrate 0.315 mg ml\(^{-1}\) + fluanizone 10 mg ml\(^{-1}\), Janssen Pharmaceutica, Beerse, Belgium) at a dose of 0.5 mg kg\(^{-1}\) rat weight. The permanent cannulation was performed using Polythene tubings (Rubler BV, Hilversum The Netherlands) with the diameter of 0.58 mm (ID) - 0.96mm (OD) and 0.28 mm (ID) - 0.61 mm (OD), respectively for femoral vein cannulation and femoral artery cannulation. The tubings were inserted approximately 3 cm inside the vessels. The cannulas were externalized through the skin at the back of the neck.

Directly after the surgery, the rats received 5-OH-DPAT solution either via an intravenous infusion or via transdermal iontophoretic delivery. During both studies, the rats were maintained in an anaesthetized condition with the same dose of Hypnorm and Dormicum.

The total intravenous dose administered to the rats was 75 \(\mu g\) over 15 minutes, with the exception of one animal, which received a total dose of 16.5 \(\mu g\). The blood samples (0.2 ml) were transferred from the femoral vein cannula into the lithium-heparin containing tubes (microvette CB 200 LH-transparent, Sarstedt BV, Etten-Leur, The Netherlands) at 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, and 180 minutes. Subsequently, the plasma samples were separated from the blood cells by centrifugation at a speed of 1400 rpm for 10 minutes prior to storage at –20\(^{\circ}\)C until analysis.

Prior to transdermal iontophoretic studies, the hairs of the back of the rats were electrically clipped out. The skin surface was gently wiped with a tissue paper containing Millipore water to remove any contaminants from the skin surface. A pair of patches was then attached to the skin surface. At approximately 15 minutes after patch attachment, the solutions of 1.3 mg ml\(^{-1}\) of 5-OH-DPAT and PBS pH 7.4 were filled, respectively into the anodal and cathodal patches using disposable syringes. After 15 minutes of passive diffusion, the current (0.25 mA cm\(^{-2}\)) was switched on for 3 hours. During iontophoresis blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes. After current removal at 3 hours blood samples were collected at 195, 210, 240, 270, 300, and 360 minutes. These samples were treated identical to the protocol described above for the intravenous administration.

5. PD (On-line microdialysis) studies following transdermal iontophoresis

Prior to surgery, the rat was anaesthetized with Isoflurana 2% in \(N_2O/O_2\) (2/1). The rat was then mounted into a stereotaxic frame (Kopf). The incisor bar was placed in a position to allow the skull to be horizontally held. The skull was exposed and was anaesthetized with 10% lidocaine before the holes for probe insertion were drilled. An Y-shaped dialysis probe was used for the
PK-PD of transdermal iontophoresis of 5-OH-DPAT in vivo

...experiments, with an exposed tip length of 3 mm. The dialysis tube (diameter: 0.22 mm (ID) - 0.31 mm (OD)) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis membrane was implanted in the striatum. The dura was cut with a sharp needle. Two anchor screws were positioned in different bone plates nearby. The following coordinates were used according to the atlas of Paxinos and Watson (13): AP ± 0.5, LM ± 3.0 relative to bregma, and Vd - 6.0 below dura. Before insertion into the brain, the dialysis probe was perfused successively with Millipore water, methanol, Millipore water and Ringer solution (1.2 mM Ca^{2+}). The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with dental cement. After the surgery, the rats received fynadine 1 mg kg^{-1} subcutaneously as an analgesic agent. Thereafter the rats were housed individually.

The microdialysis experiments upon transdermal iontophoretic delivery of 5-OH-DPAT were performed in anaesthetized rats 17–48 h after implantation of the probes. The same protocol of transdermal iontophoresis for PK studies was applied. During the experiment, the animals were kept under anesthesia by a subcutaneous injection of 6% chloralhydrat. The striatum was perfused with Ringer solution (147 mmol l^{-1} NaCl, 4 mmol l^{-1} KCl, 1.2 mmol l^{-1} CaCl_{2}, 1.1 mmol l^{-1} MgCl_{2}) at a flow of 2 μl minute^{-1} (CMA/102 microdialysis pump, Sweden). The concentration of dopamine was quantified from the probe implanted at the left striatum by an on-line HPLC with electrochemical detection with the detection limit of 1 fMol/sample. Prior to application of the drug, four measurements of C_{DA} for the baseline values were performed. The HPLC pump (LC10-AD-Shimadzu) was used in conjunction with an electrochemical detector (Coulochem, ESA) working at oxidation + 250mV and reduction -275 mV. The analytical column was Supelco Supelcosil LC-18 (particle size: 3 μm). The mobile phase consisted of a mixture of 4.1 g l^{-1} sodium acetate (Merck), 85 mg l^{-1} octane sulphonic acid (Sigma-Aldrich Chemie B.V, Zwijndrecht, The Netherlands), 50 mg l^{-1} EDTA (Merck B.V, Amsterdam, The Netherlands), 8.5% methanol (Labscan, Hasselt, Belgium) and Millipore water (pH 4.1 with glacial acetic acid). In addition, the dialysate from the probe in the right striatum were collected hourly. The solution was then analyzed using the LC-MS-MS method for the concentration of 5-OH-DPAT (defragmentations are at m/z 248 followed by m/z 147).

6. Preparation of Dermatomed Rat Skin (DRS) and Rat Stratum Corneum (RSC)

At least 1.5 weeks after the last in vivo experiment, the rats were sacrificed by an intravenous injection of an overdose of pentobarbital. The hairs of the rats skin from the back of the rats (the place where the iontophoretic patches were situated during the in vivo studies) were removed by using the electric clipper. The skin was then collected using a surgical blade. After the
removal of the subcutaneous residual, the skin surface was carefully wiped with a tissue paper soaked in Millipore water. Dermatomed rat skin (DRS) was then obtained by dermatoming the skin to a thickness of approximately 300 μm using a Padgett Electro Dermatome Model B (Kansas City, USA). In order to obtain rat stratum corneum (RSC), DRS was treated with the same protocol to isolate the stratum corneum in human skin as described previously (6,14).

7. **In vitro Iontophoretic Studies**

The *in vitro* iontophoretic studies were performed according to the similar protocol for the studies in human stratum corneum (HSC) and dermatomed human skin (DHS) as described previously (6,14) with several exceptions as follows: 1) The donor solution was the solution of 1.3 mg ml⁻¹ of 5-OH-DPAT with the composition identical to the solution used for *in vivo* studies (buffered at pH 5 with NaCl concentration of 0.07M); 2) The protocol of current application was identical to the *in vivo* studies; 3) The acceptor phase was maintained at 32 °C.

8. **HPLC analysis**

Rotigotine solution at a concentration of 500 ng ml⁻¹ (volume of 10 μl), which was used as the internal standard, was vortex-mixed into 100 μl of plasma samples. Thereafter, 100 μl of 10% NaCO₃ was vortex-mixed to the plasma. After addition of 0.5 ml of Millipore water and 2 ml of dichloromethane/cyclohexane (45:55 v/v), the mixtures were shaken for 15 minutes at 1400 rpm (IKA-VIBRAX-VXR, Omnilabo International BV, Breda, The Netherlands). Subsequently, the mixtures were centrifuged at 3000 rpm for 5 minutes. The organic layer was separated from the water phase and was transferred into a clean glass-tube. To the water phase, the extraction using dichloromethane/cyclohexane (45:55) was repeated with the same protocol. The organic layer was then evaporated to dryness under a stream of nitrogen at 40 °C. The residue was then reconstituted in 200 μl of 20% acetonitrile in Millipore water, and then analyzed using HPLC methods. The recoveries of 5-OH-DPAT and the internal standard in this protocol were more than 80%.

Collected samples from plasma extraction and from the *in vitro* transport studies were injected into the HPLC system. For separation, a Superspher® 60, RP-select B, 75 mm - 4 mm column (Merck KGaA, Darmstadt, Germany) was used and eluted with the mobile phase, consisting of acetonitrile/0.1M acetate buffer at pH 3.6 (40/60) v/v at a flow rate of 1.0 ml minute⁻¹ or 0.7 ml minute⁻¹, respectively for plasma samples and samples of the *in vitro* transport studies. The detection was performed by a scanning fluorescence detector (Waters™ 474, Millipore Corporation, Milford, MA, USA) at excitation and emission wavelengths of 275 and 303 nm, respectively. In all cases the attenuation was set to 1, while the gain was set to 10 and 100, respectively for the *in vitro* transport studies analysis and the plasma samples analysis. The calibration
curves were linear \((t>0.999)\) in the concentration range of 0.01 to 10 \(\mu\)g ml\(^{-1}\) \((in\ vitre\ analysis)\) and 0.5 to 200 ng ml\(^{-1}\) (plasma samples analysis). The intra and inter-assay variation was less than 5\% for all concentrations tested. The detection limit under these conditions was 3 and 0.5 ng ml\(^{-1}\), respectively for the samples of the \(in\ vitre\) transport and plasma extraction analysis.

9. Data Analysis

The \(in\ vitre\) data following transdermal iontophoresis and intravenous infusion were combined for analysis. Fitting of data to the integrated PK/PD model according to equation 3-6) was performed using the subroutines ADVANCE6 TRANS1 TOL=5 from PREDPP in NONMEM. The fixed effect parameters \((\theta)\) evaluated in this case were: \(I_0\), \(K_R\), Clearance (\(CL\)), Inter compartmental clearance (\(Q\)), volume of central compartment (\(V_2\)), volume of peripheral compartment (\(V_3\)), \(k_{24}\), \(k_{42}\), \(k_{out}\), \(I_{max}\), and \(IC_{50}\).

Inter-individual variability was modelled by an exponential error model as written in equation 9.

\[
P_i = \theta \cdot \exp(\eta_i)
\]

in which \(\theta\) is the population value for the fixed effect parameter \(P\), \(P_i\) is the individual Bayesian estimate value and \(\eta_i\) is the inter-individual variation, for which the values are assumed to be independently and normally distributed with mean zero and variance \(\omega^2\). The interindividual variability was applied for \(I_0\), \(K_R\), \(CL\), \(V_2\), \(V_3\), \(k_{out}\), \(I_{max}\), and \(IC_{50}\).

The residual error was characterised by the combination of the exponential and the additive error model as follows:

\[
F_{p_{ij}} = F_{o_{ij}} \cdot \exp(\epsilon_i) + \epsilon_2
\]

where \(F_{p_{ij}}\) is the prediction of the \(j^{th}\) evaluated functions (\(C_p\), \(C_s\), or \(C_{DA}\)), \(F_{o_{ij}}\) is the measured value of the evaluated function (\(C_p\), \(C_s\), or \(C_{DA}\)), and \(\epsilon\) represents the residual deviation of the predicted from the observed value and is assumed to be independently and normally distributed with mean zero and variance \(\sigma^2\). The analysis of the population parameters \(\theta\), \(\omega^2\), and \(\sigma^2\) was performed using the conventional first-order estimation method (FO).

The data of the \(in\ vitre\) iontophoretic transport of 5-OH-DPAT was also analyzed by non-linear effects modelling, using the proposed compartmental models. The parameters estimated in this analysis were \(I_0\), \(K_R\), \(I_L\), and \(J_{pas}\). The inter-individual variability was introduced using the exponential equation model as proposed in equation 9. The residual error was determined using the combination of the exponential and the additive error model in equation 10. The estimation of the population parameters was also performed using the conventional first order (FO) method.

To compare the level of transport during transdermal iontophoresis \(in\ vitre\) and \(in\ vitre\), the steady-state flux \((J_{ss})\) values were determined on the basis of \(I_0\) values according to the equation below:
The estimates of the in vivo fixed-effect of $J_{ss}$, $K_R$, and $t_L$ were compared to the values obtained from the in vitro transport studies in DRS and in RSC. Due to the limited information, the statistical significance ($p<0.05$) was estimated by a conservative method on the basis of the overlap of the 95% confidence interval of the fixed-effect parameter estimates (15).

The results of the population modeling were evaluated graphically using plots of correlation between population prediction ($PRED$) and the observed values of the dependent variable ($DV$) and the individual prediction ($IPRE$) versus $DV$. In addition, the bias and the precision of the models were also evaluated by estimating the “mean prediction error” ($mpe$) and “root mean squared prediction error” ($rmse$) (16) provided by Visual-NM software (17).

The ultimate objective of the population data analysis was to establish a link between in vitro and in vivo transport, which allow prediction of the time course of drug effect in vivo on the basis of the estimated transport parameters in vitro. To test this statement, $C_p$, $C_s$, and $C_{DA}$ during iontophoresis of 5-OH-DPAT were simulated on the basis of the optimum in vitro transport parameters. Simulations were performed using the SSIMULATION function provided in NM-TRANS. The Kolmogorov-Smirnov test was applied to evaluate differences in the time course of model predictions, as compared to the observed data. This test is based on the comparison between the cumulative density of the geometric means of the simulated and observed data ($p<0.05$).

D. RESULTS

A. Profiles of $C_p$, $C_s$ and $C_{DA}$ following transdermal iontophoresis in vivo.

In most of the rats, the values of $C_p$ during 15 minutes of passive diffusion prior to iontophoresis were negligible (data are not shown). At the start of iontophoresis, $C_p$ steadily increased to reach a plateau in approximately 2 hours of iontophoresis (see Fig. 3). In principle, by current removal at 3 hours, the level of $C_p$ should have dropped according to a first-order elimination process. Interestingly however, in most animals a short delay (approximately 15 minutes) was observed before concentrations started to decay.

As expected, during the passive diffusion, $C_s$ was negligible (data are not shown). After the start of iontophoresis, there was a time delay before $C_s$ steadily increased. The value of $C_s$ directly decreased with the current removal at 3 hours (see Fig. 4).

The value of $C_{DA}$ during passive diffusion was not different from base line values (data are not shown). Similarly to $C_s$, with the start of iontophoresis, except in one animal a delay for approximately 7.5 minutes was observed before $C_{DA}$ began to decrease as depicted in Fig. 5. The reduction of the $C_{DA}$ in the next 15 minutes was dramatic and reached a maximal inhibition after approximately
37 minutes of iontophoretic period. The maximum inhibition of dopamine release was approximately 80% of the average baseline values in each rat. Interestingly, although the current application has been removed at 3 hours, the level of dopamine did not return to the baseline even after 3 hours of post iontophoretic period. Only two animals showed a slight trend of recovery to the base line after 1 and 2 hours of current removal.

Fig. 3. The observed data of $C_p$ (filled circles) following transdermal iontophoresis of 5-OH-DPAT presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

B. Simultaneous PK-PD analysis of $C_p$, $C_s$, and $C_{DA}$ following transdermal iontophoresis in vivo

The results of the population analysis of the profiles of $C_p$, $C_s$, and $C_{DA}$ following iontophoresis of 5-OH-DPAT are presented in Fig. 3 ($C_p$ profile), Fig 4 ($C_s$ profile) and Fig. 5 ($C_{DA}$ profile). In these figures the observed data are presented together with the population prediction (dashed line) and the individual prediction (solid line). The fitted lines show that the simultaneous
modeling of the data adequately describe $C_p$, $C_s$, and $C_{DA}$. Fig. 6A (panel I and II) depicts two graphs showing the goodness of fit for the data ($PRED$ versus $DV$ and the $IPRE$ versus $DV$). The quality of the fitting and model parameter estimation were also evaluated by bias and imprecision parameters as presented in Table I. Despite a slight bias in the population parameter values, this bias disappeared after interindividual variability was introduced in the post-hoc Bayesian estimates ($IPRE$ versus $DV$, Fig 6A panel II). The estimated population parameters are presented in Table II. Except for the parameters $k_{24}$ and $k_{42}$, estimates of all the fixed effect parameters were obtained with a reliable precision as demonstrated by the low values of %RSE. The inter-individual variability of $J_{ss}$, $K_R$, and $CL$ was relatively high, as indicated by the values of their etas. The estimates for inter-individual variability of $IC_{50}$ and $k_{out}$ were found to be very high. This is likely to be caused by difficulties in parameter estimation, rather than true differences between individuals. As indicated by values of $sigma$, the residual error of this simultaneous fitting was relatively small.

![Graphs](image.png)

**Fig. 4.** The observed data of $C_s$ (filled circles) following transdermal iontophoresis of 5-OH-DPAT presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

**C. Transdermal iontophoretic transport in vitro of 5-OH-DPAT**

To estimate the correlation of the in vitro transport of 5-OH-DPAT to its PK/PD profiles following transdermal iontophoresis, the in vitro iontophoretic transport of 5-OH-DPAT was studied across DRS obtained from rats number 1-8 (see Fig. 7). In this case, a delay to the start of the iontophoretic flux was observed after the current was switched on. The flux then gradually increased, but in most cases no steady state flux was reached during 3 hours of iontophoresis. Subsequently, the flux directly decreased when the current was switched off at 3 hours.
PK-PD of transdermal iontophoresis of 5-OH-DPAT \textit{in vivo}

Fig. 5. The observed data of \(C_{DA}\) (filled circles) following transdermal iontophoresis of 5-OH-DPAT presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

Table I. Calculation of the absolute, \% relative and 95\% confidence interval of the bias and imprecision of the model \textit{in vivo} and \textit{in vitro}

<table>
<thead>
<tr>
<th>model</th>
<th>Bias (mpe)</th>
<th>Imprecision (rmse)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>absolute</td>
<td>%relative</td>
</tr>
<tr>
<td>\textit{PRED-\textit{in vivo}}-All</td>
<td>1.2*</td>
<td>12.0</td>
</tr>
<tr>
<td>\textit{IPRE-\textit{in vivo}}-All</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>\textit{PRED-\textit{in vitro}}-DRS</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>\textit{IPRE-\textit{in vitro}}-DRS</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>\textit{PRED-\textit{in vitro}}-RSC</td>
<td>1.7</td>
<td>3.6</td>
</tr>
<tr>
<td>\textit{IPRE-\textit{in vitro}}-RSC</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>\textit{PRED-\textit{in vitro}}-DHS</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>\textit{IPRE-\textit{in vitro}}-DHS</td>
<td>0.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Legends:
* indicates a significant bias (p<0.05)

Moreover, for several subjects (rats number 1, 3, and 8), the \textit{in vitro} iontophoretic transport of the dopamine agonist was also studied in RSC. As shown in Fig. 8, the increment of the flux was faster than in the transport across DRS and the steady-state flux was reached in all subjects. Similar to the transport in DRS the flux gradually decreased after switching off the current.

To quantitatively describe the \textit{in vitro} iontophoretic transport of 5-OH-DPAT in DRS and RSC, the flux \textit{versus} time profiles were analyzed on the basis of the \textit{in vitro} compartmental model as described in the previous section. The results of the population prediction curves (dashed line) as well as the individual post-hoc Bayesian prediction curves (solid line) are presented.
together with the observed data in Fig. 7 (in vitro transport in DRS) and in Fig. 8 (in vitro transport in RSC). The figures show that the compartmental model properly describes the flux versus time profiles in vitro. As clearly demonstrated in the in vitro transport in DRS, the model also describes the lag period where the flux was not yet observed (by parameter $t_L$).

**Table II.** The population estimates obtained from the simultaneous fitting of the in vivo data, the in vitro transport across DRS and the in vitro transport across RSC upon transdermal iontophoresis of 5-OH-DPAT.

<table>
<thead>
<tr>
<th>Data</th>
<th>Parameter</th>
<th>Unit</th>
<th>Theta mean</th>
<th>%RSE</th>
<th>Eta mean</th>
<th>%RSE</th>
<th>Sigma mean</th>
<th>%RSE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo</strong></td>
<td>$J_{ss}$</td>
<td>nmol cm$^{-2}$ h$^{-1}$</td>
<td>31.80</td>
<td>20</td>
<td>0.22</td>
<td>52</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$K_R$</td>
<td>h$^{-1}$</td>
<td>4.41</td>
<td>31</td>
<td>0.51</td>
<td>68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$CL$</td>
<td>L h$^{-1}$</td>
<td>1.00</td>
<td>18</td>
<td>0.35</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$Q$</td>
<td>L h$^{-1}$</td>
<td>3.70</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$V_2$</td>
<td>L</td>
<td>0.46</td>
<td>15</td>
<td>0.12</td>
<td>51</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$V_3$</td>
<td>L</td>
<td>1.49</td>
<td>12</td>
<td>0.07</td>
<td>51</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$K_{24}$</td>
<td>h$^{-1}$</td>
<td>0.02</td>
<td>178</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$K_{42}$</td>
<td>h$^{-1}$</td>
<td>31.80</td>
<td>180</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$K_{out}$</td>
<td>h$^{-1}$</td>
<td>4.61</td>
<td>16</td>
<td>1.21</td>
<td>37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$IC_{50}$</td>
<td>ng ml$^{-1}$</td>
<td>0.21</td>
<td>26</td>
<td>3.98</td>
<td>81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$I_{max}$</td>
<td>-</td>
<td>0.95</td>
<td>3</td>
<td>0.03</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\sigma_1$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>$\sigma_2$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.06</td>
<td>35</td>
</tr>
<tr>
<td><strong>In vitro</strong> across</td>
<td>$J_{ss}$</td>
<td>nmol cm$^{-2}$ h$^{-1}$</td>
<td>39.00</td>
<td>5</td>
<td>0.02</td>
<td>31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DRS</td>
<td>$K_R$</td>
<td>h$^{-1}$</td>
<td>1.45</td>
<td>6</td>
<td>0.19</td>
<td>32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$t_L$</td>
<td>h</td>
<td>0.24</td>
<td>6</td>
<td>0.02</td>
<td>76</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$J_{pas}$</td>
<td>nmol cm$^{-2}$ h$^{-1}$</td>
<td>3.89</td>
<td>12</td>
<td>0.28</td>
<td>44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\sigma_1$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>$\sigma_2$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.31</td>
<td>42</td>
</tr>
<tr>
<td><strong>In vitro</strong> across</td>
<td>$J_{ss}$</td>
<td>nmol cm$^{-2}$ h$^{-1}$</td>
<td>62.70</td>
<td>10</td>
<td>0.03</td>
<td>52</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RSC</td>
<td>$K_R$</td>
<td>h$^{-1}$</td>
<td>2.58</td>
<td>1</td>
<td>0.03</td>
<td>46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$t_L$</td>
<td>h</td>
<td>0.04</td>
<td>66</td>
<td>0.51</td>
<td>187</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$J_{pas}$</td>
<td>nmol cm$^{-2}$ h$^{-1}$</td>
<td>2.49</td>
<td>5</td>
<td>0.01</td>
<td>34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$\sigma_1$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.004</td>
<td>20</td>
</tr>
</tbody>
</table>

Analogous to the in vivo data, goodness of fit for the in vitro iontophoretic transport was evaluated graphically (Figs. 6B and Fig 6C, respectively for the transport in DRS and in RSC) as well as statistically by calculating bias and precision parameters (Table I). No bias was observed for either population or individual parameter estimates ($p>0.05$), confirming the accuracy of the model for iontophoretic transport in DRS and in RSC. A summary of the population parameters of the models of the in vitro iontophoretic transport across DRS and RSC is presented in Table II. In the
transport across DRS and RSC, all the fixed-effects parameters were estimated with a reliable precision. The residual errors of the \textit{in vitro} transport across DRS and RSC were relatively small as indicated by the values of $\sigma_1$ in both models.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig6}
\caption{Diagnostic of the simultaneous modeling of the $C_p$, $C_s$, and $C_{DA}$ data following transdermal iontophoresis of 5-OH-DPAT in rats (Panel A), the \textit{in vitro} transport in DRS (panel B), and the \textit{in vitro} in RSC (panel C). Panels I represent the plot of the population prediction (PRED) \textit{versus} the observed value (DV) and panels II represent the plot of the individual prediction (IPRE) \textit{versus} DV.}
\end{figure}
D. Correlation of the *in vitro* transport to the PK-PD modeling of 5-OH-DPAT following iontophoresis

Overlap of the population estimates (geometric mean and 95% confidence intervals) showed that the values of three parameters characterizing the iontophoretic transport are similar in *in vivo* and *in vitro* skin preparations. The *in vivo* $J_{ss}$ was not significantly different from $J_{ss}$ of the *in vitro* transport across DRS. The *in vivo* $K_R$ was not significantly different from the *in vitro* $K_R$ in the transport across RSC. Despite the constrained $t_L$ values *in vivo*, the estimated of $t_L$ was close to the *in vitro* drug transport across RSC. These similarities suggest that prediction of *in vivo* PK-PD profiles may be derived from *in vitro* data.
PK-PD of transdermal iontophoresis of 5-OH-DPAT \textit{in vivo}

Fig. 8. The observed data of flux (filled circles) following transdermal iontophoresis \textit{in vitro} of 5-OH-DPAT in RSC presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

E. Prediction of the population PK-PD profiles on the basis of the optimum iontophoretic transport

To explore the predictive value of \textit{in vitro} parameters, model-based simulations of the time course of $C_p$, $C_s$, and $C_{DA}$ were performed following iontophoretic delivery of 5-OH-DPAT. The results of this approach are presented in Fig. 9 in panel A (simulation of $C_p$ profiles), in panel B (simulation of $C_s$) and in panel C (simulation of $C_{DA}$). In these panels, the geometric mean of $C_p$, $C_s$, and $C_{DA}$ are also depicted with 95% confidence intervals. As expected the observed data fall within the prediction intervals. In addition, this was formally compared using the Kolmogorov-Smirnov test. The profiles of the geometric means of the population data of $C_p$, $C_s$, and $C_{DA}$ were not significantly different from the geometric mean of the simulated values (p>0.05).
Fig. 9. The prediction of the $C_p$ (panel A), $C_s$ (panel B), and $C_{DA}$ (panel C) following transdermal iontophoresis of 5-OH-DPAT on the basis of the optimum values of the *in vitro* transport parameters. The dashed lines are the range of 95% confidence interval of the population prediction of $C_p$, $C_s$, or $C_{DA}$. The solid line is the geometric mean of the population prediction of $C_p$, $C_s$, or $C_{DA}$. The filled circles are the observed data of $C_p$, $C_s$, and $C_{DA}$ following transdermal iontophoretic delivery of 5-OH-DPAT.
E. DISCUSSION

In this study we showed the application of an integrated in vitro-in vivo approach to characterize the pharmacokinetics, pharmacodynamics and iontophoretic properties of 5-OH-DPAT in rats.

Despite the overall ability of the compartmental models in describing the time course of drug concentrations in plasma and in tissue compartments, we realize that the model may not be able to describe some features of drug transport, for example the delay in the $C_p$ decay at 15 minutes after current termination as observed in most animals in the PK studies. This delay might be due to a depot effect, which may be present in the skin or in the subcutaneous tissue. This effect is consistent with the lipophilic nature of the drug (log $P$=2.19 (6)). Further extension of the model as well as increased sampling scheme for $C_p$ may be required to account for this phenomenon. Nevertheless, the profiles of $C_s$ and $C_{DA}$ are not affected by the presence of a possible depot effect, suggesting that the amount of drug deposited is minimal.

The recent approach has given us the possibility to correlate the in vitro to the in vivo transport (flux), and consequently to describe the time course of $C_p$, $C_s$, and $C_{DA}$. The characterization of the correlation of in vitro to in vivo transport offers the benefit of predicting the impact of in vitro drug properties in an in vivo experiment. Conversely, it is also possible to optimize the in vitro experimental conditions required to mimic relevant in vivo profiles.

Most importantly, this study demonstrated the fact that transdermal iontophoresis successfully delivers a therapeutic level of 5-OH-DPAT to achieve a very strong dopaminergic effect in rats. Approximately half of the maximum concentration of 5-OH-DPAT that can be prepared in the donor phase as well as half of the maximum current density that can be safely used in human (6), were used in this study. Although requires further study and analysis, by assuming similarity in the iontophoretic flux in human and in rat skin, (18,19) these findings might suggest the feasibility of transdermal iontophoretic delivery of 5-OH-DPAT in patients with Parkinson’s disease.

The assessment of an in vitro - in vivo correlation and PK-PD relationship would not have been possible without nonlinear mixed effect modeling as integration of data from different experiments is not feasible or labour- and resource intensive using the standard data analysis methodology (11). This highlights the need for implementation of model libraries to support further research in transdermal iontophoretic delivery in future.

In summary the simultaneous fitting of the PK-PD data and the fitting of the in vitro iontophoretic transport of 5-OH-DPAT in DRS and RSC adequately characterize transdermal iontophoretic delivery of 5-OH-DPAT in rats. The time course of plasma and brain concentrations of 5-OH DPAT, as well as the inhibition of dopamine release upon transdermal iontophoresis can be predicted from in vitro parameter estimates of $J_{ss}$, $K_R$, and $t_L$. Transdermal iontophoresis
of 5-OH-DPAT successfully delivers sufficient amount of the drug into striatum to achieve a strong dopaminergic effect, which might indicate the promising future of transdermal iontophoresis of 5-OH-DPAT in patients with Parkinson’s disease.

F. ABBREVIATIONS:

DRS : Dermatomed rat skin  
RSC : Rat stratum corneum  
IDR type I: Indirect response model with the inhibition of production of response  
$I_0$ : The zero-order iontophoretic mass transfer from the donor phase/patch into the skin compartment  
$J(t)$ : Flux at time $t$  
$J_{ss}$ : Steady-state flux  
$K_R$ : The first-order rate constant of drug release from the skin into acceptor compartment (in vitro) or to the systemic circulation (in vivo)  
$P_{PI}$ : The zero order post-iontophoretic mass transfer due to PIDF  
$S$ : Diffusion active area or patch area  
$t_L$ : The kinetic lag time of the drug molecules to enter the skin compartment  
$T$ : Duration of current application  
$X_n(t)$ : Drug amount at time $t$ in the compartment $n$, which refers to the skin ($n=1$), acceptor phase (in vitro) or plasma (in vivo) ($n=2$), tissue ($n=3$), and striatum ($n=4$) compartments.  
k : The elimination rate-constant  
k_{ab} : The distribution rate constant from compartment $a$ to compartment $b$  
$C_p$ : Concentration of 5-OH-DPAT in plasma  
$C_s$ : Concentration of 5-OH-DPAT in striatum  
$C_{DA}$ : Concentration of dopamine in striatum  
$I_{max}$ : The maximum inhibition of the dopamine production  
$IC_{50}$ : The concentration of 5-OH-DPAT in striatum required to produce 50% of $I_{max}$  
$H$ : The Hill-slope coefficient (which is constrained to 1)  
k_{out} : The first-order rate constant for the loss of response  
k_{0\,in} : The zero order rate constant for the production of response  
$C_{DA0}$ : The baseline values of $C_{DA}$ prior to the inhibition effect of 5-OH-DPAT.

G. ACKNOWLEDGMENTS

This research was supported by QUE Project Batch III year of 2000 – 2004 Faculty of Pharmacy Gadjah Mada University Yogyakarta Indonesia.
H. REFERENCES
