Systemic and Direct Nose-to-Brain Transport Pharmacokinetic Model for Remoxipride after Intravenous and Intranasal Administration

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ABSTRACT:

Intranasal (IN) administration could be an attractive mode of delivery for drugs targeting the central nervous system, potentially providing a high bioavailability because of avoidance of a hepatic first-pass effect and rapid onset of action. However, controversy remains whether a direct transport route from the nasal cavity into the brain exists. Pharmacokinetic modeling is proposed to identify the existence of direct nose-to-brain transport in a quantitative manner. The selective dopamine-D2 receptor antagonist remoxipride was administered at different dosages, in freely moving rats, by the IN and intravenous (IV) route. Plasma and brain extracellular fluid (ECF) concentration-time profiles were obtained and simultaneously analyzed using nonlinear mixed-effects modeling. Brain ECF/plasma area under the curve ratios were 0.28 and 0.19 after IN and IV administration, respectively. A multicompartment pharmacokinetic model with two absorption compartments (nose-to-systemic and nose-to-brain) was found to best describe the observed pharmacokinetic data. Absorption was described in terms of bioavailability and rate. Total bioavailability after IN administration was 89%, of which 75% was attributed to direct nose-to-brain transport. Direct nose-to-brain absorption rate was slow, explaining prolonged brain ECF exposure after IN compared with IV administration. These studies explicitly provide separation and quantitation of systemic and direct nose-to-brain transport after IN administration of remoxipride in the rat. Describing remoxipride pharmacokinetics at the target site (brain ECF) in a semiphysiological-based manner would allow for better prediction of pharmacodynamic effects.

Introduction

Many diseases, including Parkinson disease, schizophrenia, and depression, are related to dysfunctions of the dopaminergic system in the central nervous system (CNS). The effects of therapeutic agents after oral administration are often limited because of active first-pass clearance by the liver and restricted blood-brain barrier (BBB) transport. In theory, direct injections into the brain, by intracerebroventricular or intraparenchymal injections, are an alternative to the oral route. However, these methods are invasive and risky and are therefore not suitable for application in clinical practice. Moreover, local injection does not always result in sufficient CNS target site distribution because brain diffusion may be slow relative to elimination processes (de Lange et al., 1995; Dhuria et al., 2010).

Intranasal (IN) administration could be an attractive alternative mode of delivery for drugs targeting the CNS, potentially providing high bioavailability because of avoidance of a hepatic first-pass effect and rapid systemic uptake via perivascular spaces in the respiratory epithelium (Chien et al., 1989). Apart from that, the olfactory epithelial pathway may allow therapeutic agents to diffuse into the perineural spaces, crossing the cribriform plate, ending up in the cerebral spinal fluid (CSF) (Baker and Spencer, 1986; Frey et al., 1997). In addition, the olfactory nerve pathway may allow intracellular transport through olfactory sensory neurons, passing the cribriform plate, into the olfactory bulb (Bagger and Bechgaard, 2004). By directly targeting the brain, it has been hypothesized that IN delivery can enhance the CNS target site bioavailability and the efficacy of CNS
drugs (Jansson and Bjork, 2002; Illum, 2004; Graff and Pollack, 2005).

IN administration data are typically compared to various other administration routes on the basis of area under the curve (AUC) of plasma and CSF concentration-time profiles. However, although CSF/plasma AUC ratios reflect differences in exposure after IN administration (van den Berg et al., 2004), they do not allow the distinction between direct nose-to-brain transport and systemic uptake in terms of absorption rate and bioavailability. Consequently, the existence of a direct nose-to-brain route is still a matter of debate (Dhuria et al., 2010). Pharmacokinetic (PK) modeling would provide the opportunity to separately quantify the systemic and direct nose-to-brain absorption, which is not possible on the basis of AUC comparison.

Another important factor is that CSF concentrations do not necessarily reflect target site concentrations (de Lange and Danhof, 2002). This is because of factors related to CSF turnover, intrabrain diffusion, extra-intracellular exchange, and qualitative and quantitative differences in BBB and blood-CSF-barrier transport mechanisms. Because many targets (receptors) face the brain extracellular fluid (ECF), brain ECF concentrations are anticipated to reflect target site concentrations best and will therefore provide a better basis to describe PK and pharmacodynamic (PD) relations in a more mechanistic manner (Del Bigio, 1995; de Lange et al., 1999, 2005; de Lange and Danhof, 2002; Watson et al., 2009; Jeffrey and Summerfield, 2010).

Our interest is to investigate the PK-PD mechanisms that play a role in modulation of the dopaminergic system and the use of IN administration of dopaminergic drugs that often encounter very low bioavailability and/or limited BBB transport. The aim of the study was to quantitatively assess direct transport of remoxipride into the brain after IN administration. Remoxipride is a weak, but selective, dopamine-D2 receptor antagonist (Farde and Von Bahr, 1990; Köhler et al., 1990; Ogren et al., 1993) and was prescribed as an atypical antipsychotic (Roxiam) at the end of the 1980s. Because of a few cases of aplastic anemia, the drug was withdrawn from the market (Philpott et al., 1993). However, remoxipride is still of interest as a paradigm compound in mechanism-based PK-PD studies on the dopaminergic system.

Using our previously reported minimal stress, freely moving rat model for IN and intravenous (IV) drug administration (Stevens et al., 2009), plasma and brain ECF samples were obtained over time, after IV and IN administration of 4, 8, or 16 mg/kg remoxipride. Compartmen- tal PK nonlinear mixed-effects modeling using NONMEM (Beal and Sheiner, 1992) was applied to quantify the direct nose-to-brain distribution in terms of absorption rate and bioavailability.

Materials and Methods

Animals. All animal procedures were performed in accordance with Dutch laws on animal experimentation. The study protocol was approved by the Animal Ethics Committee of Leiden University (UDEC 6132). Male Wistar WU rats (n = 60, 253 ± 20 g; Charles River, Margate, Kent, UK) were housed in groups for 7 to 13 days (Animal Facilities, Gorlaeus Laboratoria, Leiden, The Netherlands) under standard environmental conditions (ambient temperature 21°C, humidity 60%, 12:12 h light/dark cycles, background noise, and daily handling) with ad libitum access to food (laboratory chow, Hope Farms, Woerden, The Netherlands) and acidified water. Between surgery and experiments, the rats were kept individually in Makrolon type 3 cages for 7 days to recover from the surgical procedures.

Surgery. Rat surgery and experiments were performed as previously reported (Stevens et al., 2009). In short, during anesthetized surgery, all animals received cannulas in the femoral artery and vein for serial blood sampling and drug administration, respectively. In addition, an IN probe (anteroposterior 12 mm and lateral −0.5 mm relative to Bregma) for drug administration and a microdialysis guide (caudate putamen; anteroposterior +0.4 mm, lateral 3.2 mm, and ventral −3.5 mm relative to Bregma) were implanted. After 6 days, the microdialysis guide was replaced by a microdialysis probe (CMA/12, 4 mm polycarbonate membrane, cutoff 20 kDa; Aurora Borealis Control, Schoonebeek, The Netherlands) for continuous brain ECF sampling (Chaursaia et al., 2007). At 24 ± 1 h later, the experiments were started.

Experiments. The rats were randomly assigned to three groups (n = 20 per group) to receive 4, 8, or 16 mg of remoxipride (Tocris Bioscience, Bristol, UK) per kilogram body weight. Per group, at time = 0 min (corresponding with the actual time of 10.00 AM ± 1 h), remoxipride in saline (B. Braun Melsungen AG, Melsungen, Germany) was administered via either a 1-min IN infusion (n = 10) or a 30-min IV infusion (n = 10) using an automated pump (Harvard apparatus 22, model 55-2222; Harvard Apparatus Inc., Holliston, MA). For the IN infusions, different remoxipride solutions were used to ensure similar flow rates (± 19 μl/min) and total IN administered volumes.

Before the experiments, perfusion fluid consisting of NaCl (145 mM), KCl (0.6 mM), MgCl₂ (1 mM), CaCl₂ (1.2 mM), and ascorbic acid (0.2 mM) in a potassium phosphate buffer (2 mM, pH 7.4) (Moghaddam and Bunney, 1989) was prepared. From time = −30 to 240 min, the microdialysis probe was continuously flushed with perfusion fluid at a flow rate of 2 μl/min. Microdialysate samples were collected every 10 min for the first 2 h and every 20 min thereafter until the end of the experiments in a cooled fraction collector (Univisor 820 microsampler; Univisor Limited, Zetjum, Malta). The microdialysis samples were weighed to confirm accurate sampling volumes and stored at −80°C, pending analysis. Microdialysate samples were considered accurate and were further used only when their volume was within 95 to 105% of the expected volumes of 20 or 40 μl for 10- and 20-min samples, respectively. Blood samples of 200 μl each were taken from the arterial cannula at time = −5 (blank), 5, 10, 20, 35, 60, 90, 120, 150, 180, and 240 min and collected in EDTA-coated tubes. After centrifuging for 15 min at 5000 rpm, the plasma was stored at −20°C. After the experiments, the animals were decapitated after an overdose of Nembutal (1 ml, IV).

Two animals from the 4 mg/kg IN dosing group were excluded because the nasal cannula was partially blocked. At some instances during plasma sampling, the arterial cannula was blocked, thereby preventing further sampling. Ultimately, 350 plasma and 235 brain ECF samples could be obtained from 58 remoxipride-treated rats and were analyzed for remoxipride. The IV study consisted of 190 plasma and 126 brain ECF data points, and the IN study consisted of 160 plasma and 109 brain ECF data points.

Analytical Methods. Analytical methods for the quantitation of remoxipride in small plasma and brain microdialysate samples have been previously reported (Stevens et al., 2010). In short, for the measurements of remoxipride concentrations in plasma, online solid-phase extraction was followed by high-pressure liquid chromatography-tandem mass spectrometry (Finnigan TSQ Quantum Ultra Mass Spectrometer System; Thermo Fisher Scientific, Breda, The Netherlands). Brain microdialysate samples were measured using high-pressure liquid chromatography-tandem mass spectrometry without sample clean-up. Remoxipride concentrations in microdialysate samples were corrected for in vivo recovery through the microdialysis probe and tubing [on the basis of in vivo loss (20, 100, and 500 ng/ml), with mean ± S.E.M. = 20% ± 0.6] to yield estimates of brain ECF concentration values (Chaurasia et al., 2007). Data acquisition and processing was performed using LC-Quan (Thermo Fisher Scientific). For constructing the calibration curve, linear regression analysis was applied using weight factor 1/I². The lower limits of detection were 0.15 and 0.08 ng/ml remoxipride in plasma and microdialysate samples, respectively. The lower limits of quantification were 0.5 and 0.25 ng/ml for plasma and microdialysate samples, respectively.

Using the obtained individual plasma and brain ECF profiles, mean AUC ± S.E.M. values were calculated per matrix (plasma /brain ECF), dose (4, 8, and 16 mg/kg), and study (IV/ IV) group using the trapezoidal rule (from time = 0 min until the end of experiments).

Pharmacokinetic Model Building and Random Variability. Nonlinear mixed-effect modeling using NONMEM (version VI, level 2.0; Icon Development Solutions, Ellicott City, MD) was used for the structural model building, performed under ADVAN 6, and the first-order conditional method with interaction was used for estimation with a convergence criterion of three significant digits in the parameter estimates. NONMEM reports an objective function value (OFV), which is the −2 log likelihood. Model hypothesis testing was done using the likelihood ratio test under the assumption that the
difference in the \(-2 \cdot \log \text{likelihood} = \chi^2\) distributed with the degrees of freedom determined by the number of additional parameters in the more complex model. Hence, with a decrease in the OFV of at least 3.84 points \((p < 0.05)\), the model with one additional parameter is preferred over its parent model.

Additive, proportional, or combined residual variability models were investigated separately for the remoxipride concentrations in plasma and in brain ECF (measurement compartments). Log-normal distribution of the interindividual variability (IIV) was assumed, and possible covariate correlations were taken into account. Calculation of the coefficient of variation (CV) was used to derive the uncertainty in the parameter estimates of the model and considered acceptable when lower than 50%. By this approach, several compartmental model structures were optimized (Fig. 1).

Typical values for the PK parameters clearance (CL), volume of distribution \((V)\), and intercompartmental clearance \((Q)\; \text{clearance between the compartments}) were estimated based on parameter estimates \((\theta)\). When identifiable, a term expressing IIV \((\eta)\) was included (eq. 1).

\[
\text{Typical value } = \theta \times e^\eta
\]

Transport of remoxipride over time between the compartments and elimination processes were defined by rate constants (eq. 2) on the basis of the typical estimated values for CL (or \(Q\)) and \(V\).

\[
k_{xy} = \frac{\text{CL}_{xy}}{V_x}
\]

In model 1, plasma and brain ECF data after IV administration of remoxipride were simultaneously modeled in a structural model consisting of a central, a brain, and a peripheral compartment (Fig. 1, model 1). For the elimination of remoxipride from plasma, a first-order elimination rate constant was applied \((k_{40})\). Because removal of remoxipride from the brain was underestimated, an additional first-order elimination rate constant \((k_{40})\) was applied in a second structural model approach (Fig. 1, model 2). The value for \(k_{40}\) was assumed to be smaller than \(k_{30}\) and therefore calculated as the estimated fraction of \(k_{30}\) (eq. 3).

\[
k_{40} = (\theta \times e^\eta) \times k_{30}
\]

Because models 1 and 2 are nested (based on identical data sets), their OFVs can be used as comparative means to identify the model that best describes the data, taking into account the number of additional parameters. These two models formed the basis for the identification of more complex model structures that include the IN data set.

For inclusion of the IN data set, an absorption compartment with an absorption rate constant \((ka)\) was added to the model structure, here as \(kl_{13}\). In the first instance, \(ka_{13}\) and bioavailability from the site of absorption to the central plasma compartment \((F_1)\) were estimated, leaving out brain elimination (Fig. 1, model 3). An improvement was made by addition of \(k_{40}\) (Fig. 1, model 4) using the same approach as for model 2.

In the final model (5), a second absorption compartment was added that describes the hypothesized direct nose-to-brain transport. Absorption from the IN site of administration to the brain results from transport of a compound over a longer distance compared with systemic absorption because the latter depends on the nasal vascular system that is closely located to the site of administration. As a result, direct nose-to-brain transport is a relatively slow process compared with systemic absorption (Dhuria et al., 2010). Therefore, typical values for the absorption rate constants were estimated based on the assumption that the absorption rate constant into the central compartment \((ka_{13})\) is higher than the absorption rate constant into the brain compartment \((ka_{12})\). The total bioavailability \((F_{TOT})\) was defined as the sum of the bioavailability to the central compartment \((F_1)\) and that for the brain compartment \((F_3)\). During model optimization, typical values for \(F_1\) and \(F_{TOT}\) were estimated, whereas \(F_3\) was calculated \((F_3 = F_{TOT} - F_1)\). The change in amount of remoxipride \((dA)\) in each compartment over time \((dr)\) was described using differential equations (eqs. 4–8).

**Model 1**

3. Central \(\xrightarrow{k_{34}}\) 5. Peripheral

4. Brain (MD)

**Model 2**

3. Central \(\xrightarrow{k_{34}}\) 5. Peripheral

4. Brain (MD)

**Model 3**

1. ABS 1 \(\xrightarrow{ka_{12}}\) 3. Central \(\xrightarrow{k_{34}}\) 5. Peripheral

4. Brain (MD)

**Model 4**

1. ABS 1 \(\xrightarrow{ka_{12}}\) 3. Central \(\xrightarrow{k_{34}}\) 5. Peripheral

4. Brain (MD)

**Model 5**

1. ABS 1 \(\xrightarrow{ka_{24}}\) 2. ABS 2 \(\xrightarrow{k_{43}}\) 3. Central \(\xrightarrow{k_{34}}\) 5. Peripheral

4. Brain (MD)

*Fig. 1. Compartmental model structures. The models consists of (1) an absorption compartment (ABS 1), (2) a second absorption compartment (ABS 2) describing direct nose-to-brain transport, (3) a central measurement compartment (Central; plasma concentrations), (4) a brain measurement compartment (Brain; brain ECF concentrations), and (5) a peripheral compartment (Peripheral) describing the distribution into other tissues and organs.*
on the basis of OFV.

Central absorption and elimination: 

\[
\frac{dA_{\text{central}}}{dt} = A_{\text{abs1}} \times k_{a13} - A_{\text{central}} \\
\times k_{34} + A_{\text{brain}} \times k_{43} - A_{\text{central}} \times k_{35} + A_{\text{periph}} \times k_{53} - A_{\text{central}} \times k_{50}
\]

Brain absorption and elimination: 

\[
\frac{dA_{\text{brain}}}{dt} = A_{\text{abs2}} \times k_{a24} + A_{\text{central}} \\
\times k_{34} - A_{\text{brain}} \times k_{43} - A_{\text{brain}} \times k_{50}
\]

Peripheral distribution and elimination: 

\[
\frac{dA_{\text{periph}}}{dt} = A_{\text{central}} \times k_{55} - A_{\text{periph}} \times k_{53}
\]

The optimized models 3, 4, and 5 are nested and were therefore compared on the basis of OFV.

Model Evaluation. All optimized models were internally qualified based on goodness of fit for individual concentration-time profiles in plasma and brain ECF. As for the intravenous administration group’s doubling of the dose leading to doubling of the values for plasma and brain ECF AUCs, BBB transport of remoxipride was not considered to be subjected to active influx or efflux processes. Hence, observed remoxipride concentrations were normalized to dose (16 mg/kg) before performance of a visual predictive check (VPC). The VPCs were performed using NONMEM VI by simulating 1000 replications of the PK model and a simulation data set that contained dosing information for one individual rat per dosing regimen and administration group. The median and the 5th and 95th percentiles were calculated for each simulated time point. The predictions at each time point (median and 90% prediction interval) were compared visually with the actual normalized data. Resemblance between simulated and original distributions indicates the accuracy of the model (i.e., 90% of the observed data should fall within the predicted range for 90% of the variability) (Post et al., 2008).

**Results**

Remoxipride Plasma and Brain ECF Data after IV and IN Administration. Plasma concentration-time profiles of remoxipride after IV and IN administration of 4, 8, and 16 mg/kg remoxipride are shown in Fig. 2A. The maximal remoxipride concentration (C_max) in plasma is higher after IV administration compared with the C_max after IN administration of a similar dose. Moreover, the slope of the concentration-time profile during the elimination phase seemed to be slower after IN compared with IV administration and indicates slow absorption processes.

The brain ECF concentration-time profiles are shown in Fig. 2B. The C_max for brain ECF concentrations after IN administration was lower than the C_max after IV administration. Furthermore, after IN administration, remoxipride brain ECF concentrations decreased slower compared with IV administration. As a consequence, the AUC_{brain ECF}/AUC_{plasma} ratio value after IN administration was higher compared with IV administration (Table 1). This indicates direct nose-to-brain transport (van den Berg et al., 2004). Doubling of the dose (4 to 8 to 16 mg/kg) resulted in a doubling of the mean AUC_{plasma} and AUC_{brain ECF} in both IV and IN studies (Table 1), indicating linear BBB distribution.

### TABLE 1

Mean areas under the curve (±S.E.M.) from 0 to 4 h of individual plasma and brain ECF concentration-time profiles per study and matrix

| Dose (mg/kg) | Intravenous Study | | Intravenous Study |
|-------------|-------------------|---|-------------------|---|---|
|              | Plasma AUC μg · min/ml | Brain ECF AUC μg · min/ml | Brain ECF AUC/Plasma AUC % | Plasma AUC μg · min/ml | Brain ECF AUC μg · min/ml | Brain ECF AUC/Plasma AUC % |
| 4           | 44.9 (7.7) | 7.9 (1.7) | 17.5 | 10.8 (2.9) | 4.9 (0.8) | 24.7 |
| 8           | 70.1 (4.7) | 14.0 (0.7) | 19.9 | 20.0 (5.5) | 4.9 (0.8) | 24.7 |
| 16          | 173.0 (9.0) | 34.6 (7.1) | 20.0 | 53.9 (11.4) | 16.7 (2.4) | 31.0 |
| Mean brain/plasma AUC per study | 19 (0.8) | 28 (2.6) | 

* Not determined.
The medians of the VPCs of model 1 until 4 are represented by the FIG. 3. The observed remoxipride concentrations (C) per study and measurement compartment. The medians of the VPCs of model 1 until 4 are represented by the dotted, dash-dotted, dashed, and solid lines, respectively.

Addition of brain elimination in model 2 (Fig. 1) showed improved model predictions for plasma and brain ECF observations (Fig. 3). The brain ECF observations were now randomly distributed around the simulated median because of overprediction of the high concentration range (including C_{max}) and underprediction of the lower concentration range. Significant IIV variability was identified in CL3, V4, k_{13}, and F_{13}, and a proportional error model best described residual error in the central and brain compartment. Because the parameter estimates did not allow good predictions of the brain ECF observations, total bioavailability could not be accurately estimated.

Again, incorporation of brain elimination in the model (model 4) greatly improved the OFV (424 points). In addition, the VPC median for the brain ECF concentration-time profiles after IV administration was better compared with model 3 (Fig. 3), albeit that still a slight underprediction of C_{max} and overprediction of concentrations during the elimination phase was observed. In particular, after IN administration, the C_{max} in brain ECF was underestimated, as were all concentrations during the elimination phase. The uncertainties for the parameter estimates (CV) were slightly higher; IIV variability was estimated parameter.

### TABLE 2

<table>
<thead>
<tr>
<th>Parameter estimate</th>
<th>Model Summary</th>
<th>Model 1</th>
<th>II V</th>
<th>Model 2</th>
<th>II V</th>
<th>Model 3</th>
<th>II V and IN</th>
<th>Model 4</th>
<th>II V and IN</th>
<th>Model 5</th>
<th>II V</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL3 (l \cdot h^{-1} \cdot kg^{-1})</td>
<td>2.22 (12.1)</td>
<td>0.41</td>
<td>0.59 (24.1)</td>
<td>0.32</td>
<td>1.80 (15.2)</td>
<td>0.38</td>
<td>0.36 (36.2)</td>
<td>0.27</td>
<td>1.12 (10.1)</td>
<td>0.04</td>
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<tr>
<td>V3 (l/kg)</td>
<td>0.050 (24.2)</td>
<td>N.E.</td>
<td>0.062 (16.6)</td>
<td>N.E.</td>
<td>0.073 (25.2)</td>
<td>N.E.</td>
<td>0.08 (14.8)</td>
<td>N.E.</td>
<td>0.088 (13.6)</td>
<td>N.E.</td>
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<tr>
<td>Q4 (l \cdot h^{-1} \cdot kg^{-1})</td>
<td>0.98 (9.8)</td>
<td>N.E.</td>
<td>1.38 (13.3)</td>
<td>N.E.</td>
<td>1.66 (27.3)</td>
<td>N.E.</td>
<td>1.59 (20.1)</td>
<td>N.E.</td>
<td>0.70 (19.9)</td>
<td>N.E.</td>
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<tr>
<td>V4 (l/kg)</td>
<td>0.41 (39.3)</td>
<td>N.E.</td>
<td>1.47 (12.0)</td>
<td>N.E.</td>
<td>0.18 (20.7)</td>
<td>0.31</td>
<td>3.02 (40.7)</td>
<td>0.21</td>
<td>0.873 (24.1)</td>
<td>0.06</td>
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<tr>
<td>Q5 (l/h/kg)</td>
<td>1.13 (21.6)</td>
<td>N.E.</td>
<td>1.18 (11.1)</td>
<td>N.E.</td>
<td>0.567 (12.3)</td>
<td>N.E.</td>
<td>1.04 (15.6)</td>
<td>N.E.</td>
<td>1.20 (10.2)</td>
<td>N.E.</td>
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<tr>
<td>V5 (l/kg)</td>
<td>0.13 (22.4)</td>
<td>0.10</td>
<td>0.425 (7.9)</td>
<td>0.05</td>
<td>0.325 (7.14)</td>
<td>N.E.</td>
<td>0.36 (16.5)</td>
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<td>0.417 (8.60)</td>
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<td>FK_{IV} (h)</td>
<td>—</td>
<td>—</td>
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<td>k_{13} (h)</td>
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<tr>
<td>RV plasma</td>
<td>0.0629</td>
<td>0.057</td>
<td>0.12</td>
<td>N.E.</td>
<td>0.11</td>
<td>0.098</td>
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<td>RV microdialysate</td>
<td>0.48</td>
<td>0.203</td>
<td>0.368</td>
<td>N.E.</td>
<td>0.19</td>
<td>0.103</td>
<td></td>
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</table>

**Calculated parameters**

| F_{2} | — | — | — | — | — | — | — | — | — | — |
| k_{12} (h) | 44.4 | 9.5 | 24.7 | N.E. | 4.5 | 12.7 |
| k_{13} (h) | — | 3.45 (8.9) | — | 1.62 | 3.84 |

**Residual variability**; **FK_{IV}, k_{12}, k_{13} as fraction of k_{10}; N.E., not estimated in model.**

**a** Parameter estimates are given with their compartment number, according to Fig. 1, and are presented as technical efficiency (CV%).

**b** Fixed parameter.

**d** Not determined.
The absorption rate constant to the brain (ability parameter estimates can be considered accurate. In the final in plasma and brain ECF, the absorption rate constant and bioavailability compartments.

Because the remoxipride concentrations were accurately predicted in plasma and brain ECF, the absorption rate constant and bioavailability parameter estimates can be considered accurate. In the final model, the low value of the absorption rate constant to the brain (\(k_{at1}\)) represents slow transport mechanisms from the nose to the brain and showed a relatively high CV (43.8%). The bioavailability of direct nose-to-brain transport was found to be 75% (\(F_2/F_{TOT}\)) of the \(F_{TOT}\) (89%). The \(k_{at2}\) was low compared with the \(k_{at1}\), explaining the relatively slow decrease in plasma and brain ECF concentrations over time after IN administration. This indicates fast absorption into plasma and consecutive BBB transport, after which concentrations in brain ECF are maintained by (slower) direct nose-to-brain transport. IV for \(F_{TOT}\) was 0.48. Furthermore, the use of dense, serial blood and microdialysis sampling allowed for a precise estimation of the \(F_{TOT}\) (CV of only 4.6%).

Further data analysis showed a smaller apparent total volume of distribution when comparing models 2 and 5. These factors indicate a more complex distribution from the nasal cavity to the site of measurement. Addition of absorption and/or brain compartments were considered in the early phase of the structural model building [e.g., dual (slow and fast) direct nose-to-brain transport or an extra compartment between the absorption and brain compartment]. However, additional absorption processes and/or compartments could not be identified with the present data set from our animal model.

Discussion

The aim of the study was to quantitatively assess direct transport of remoxipride into the brain after IN administration. To this end, plasma and brain ECF concentration-time profiles were obtained after IV and IN administration of three different dosages, and data were analyzed using a PK modeling approach. A multicompartment, semiphysiologically based PK model with one absorption compartment from the nose into the systemic circulation and one from the nose into the brain compartment was found to best describe the observed data. Total bioavailability after IN administration was 0.89, of which 75% was attributed to direct nose-to-brain transport. The absorption rate constant from the nose to the brain was low compared with the absorption rate constant for systemic uptake, explaining the relatively slow decrease in plasma and brain ECF concentrations over time after IN administration. These studies explicitly provide separation and quantification of systemic and direct nose-to-brain transport after IN administration.

To characterize BBB transport in rats, increasing and decreasing remoxipride concentrations in plasma and brain ECF over time were obtained using 30-min IV infusion. For IN administration, the duration of the intranasal administration was restricted by maximal solubility of remoxipride and the maximal administration volume in freely moving rats (Stevens et al., 2009). At later time points (>150 min), the mean concentration-time profiles of remoxipride in brain ECF after intravenous administration of the 4 mg/kg dose group is higher than the 8 and 16 mg/kg dose groups (Fig. 2B). Using fewer animals in each group, effects of interindividual variability can explain the inconsistency for this single dose group, as is taken into account by population-based modeling. Although highly unlikely, we cannot fully exclude a potential structural difference in brain ECF exposure between the 4 mg/kg and higher dose regimens. In addition, considering the fact that plotting on a semilogarithmic scale puts higher visual emphasis on the lower concentration range, we did not include potential structural differences for the lowest dose group in our modeling approach.

Standard nonparametric analysis of our data showed that BBB transport was linear. Although no preclinical literature could be found to compare these results, in patients with schizophrenic remoxipride readily passes the BBB (Farde and Von Bahr, 1990). Furthermore, because only free drug concentrations can cross the BBB, protein binding needs to be considered. Slight concentration-dependent plasma binding has been reported in patients with tardive dyskinesia (Widerløv et al., 1991); however, we did not find any indication for this in our dose range. Because BBB transport appeared to be linear and measurements were taken in the plasma and brain compartment, \(Q_4\) represents movement of unbound remoxipride concentrations, thereby incorporating protein binding.

Despite an approximate doubling of \(C_{max}\) in plasma and brain ECF for IV compared with IN administration, the elimination is slower for the latter (Fig. 2). The slower elimination for IN administration indicates an absorption-rate-dependent elimination rate (flip-flop kinetics), being most pronounced in the brain ECF (Fig. 3). The resulting increase of \(\text{AUC}_{\text{brain ECF}}/\text{AUC}_{\text{plasma}}\) for IN administration compared with IV administration (Table 1) suggests direct nose-to-brain transport (van den Berg et al., 2004). For remoxipride, it seems that a fast onset of action can be reached by the fast systemic uptake, whereas the slower direct nose-to-brain transport is expected to result in a prolonged duration of effect after intranasal administration.

In model 1, the VPCs revealed misspecification of the brain ECF concentration predictions. Hepatic clearance and urinary excretion are known to result in elimination of remoxipride from plasma (Widerløv et al., 1989; Widman et al., 1993; Nilsson, 1998), as was included in our model approaches by the first-order elimination rate constant \(k_{SO}\). An additional parameter for brain elimination was essential to predict plasma and brain ECF observations adequately (model 2), indicating that more complex processes are responsible for the clearance of remoxipride from the brain ECF. The main metabolic routes in rodents are hydroxylation and O-demethylation at the aromatic moiety of remoxipride. Remoxipride metabolites have been previously identified in rat brain and have been attributed to liver metabolism and consecutive BBB transport (Ahlenius et al., 1997). However, in another study, O-demethylase activity has been identified in rat brain (Jolivalt et al., 1995), which could account for the brain metabolism of remoxipride. To our knowledge, there are no studies that implicate remoxipride as a substrate for brain efflux transporters. In our model,
the clearance processes in the brain were lumped in a single first-order elimination rate constant ($k_{el}$), awaiting more mechanistic data on brain elimination processes (e.g., by in vitro studies) to further develop the model for specific mechanistic understanding.

Simultaneous analysis of the IV and IN data sets using models 3 and 4 yielded the consistent finding that brain elimination is essential to predict brain ECF remoxipride concentrations. However, this approach could not explain the slower decrease in remoxipride concentrations in brain ECF after IN administration. The final model took into account a second, direct transportation route from the nasal cavity to the brain ECF compartment as well as brain elimination. This led to a highly significant improvement in goodness of fit for remoxipride plasma and brain ECF concentration-time profiles as reflected in the reduction of the OFV, CVs, IIV, and improved VPC. Consequently, the bioavailability parameters could be considered precise.

Regarding intradindividual variability after IN administration, one has to take into account that there are many protective barriers in the nasal cavity (e.g., mucociliary clearance, efflux transporter proteins, and metabolizing enzymes) that influence the absorption of compounds (Dhuria et al., 2010), which may all contribute. The low value found for the $ka_{24}$ (0.033/h) represents slow transport mechanisms via the olfactory epithelial and/or olfactory nerve pathway that are both subject to IV. Although IV in $ka_{24}$ as such could not be identified, it may explain the lower precision of $ka_{24}$ compared with $k_{el}$, the latter of which is associated with a smaller absorption distance. It would be of interest to separately quantify the olfactory epithelial and nerve pathways, which is not possible with the current data set. In future investigations, simultaneously measuring remoxipride brain ECF concentrations in several brain regions could allow for the quantification of separate absorption rate constants for the different direct nose-to-brain transport routes.

From our studies, it is clear that brain ECF concentrations are influenced by direct nose-to-brain transport, BBB transport after systemic uptake, and brain elimination processes. To understand the effect of CNS-active drugs, it is pertinent to understand the drug exposure at the target site (brain ECF) because this provides a better basis for the determination of concentration-effect relationships after IN administration. In humans, information on target site distribution is more difficult to obtain compared with animal studies. However, on the basis of preclinical-derived semiphysiologically based PK parameters, translational modeling would allow for simulation of human brain ECF concentrations that can form the basis of PK-PD models that will help toward increased safety and efficacy (Danhof et al., 2008). Small-scale, efficient clinical trials could subsequently provide the accuracy of these preclinical-derived translational models.

In conclusion, by simultaneous modeling of plasma and brain ECF concentration-time profiles obtained after IV and IN routes of administration of remoxipride, we have provided a semiphysiologically based PK model on the target site distribution of remoxipride. The model provides quantitative evidence of direct nose-to-brain transport after IN administration. In addition, pharmacokinetic modeling allowed for the quantification of brain elimination, which contributed significantly to the clearance of remoxipride. Further investigations on the plasma and brain ECF PK of other compounds should lead to a more generalized model for IN administration because direct nose-to-brain transport is compound-dependent. Describing rat brain PK in a semiphysiologically based manner is anticipated to allow for simulation of human brain ECF concentrations by means of translational models. This will aid in the prediction of the efficacy and safety of CNS-active compounds after IN administration in humans.

**Authorship Contributions**

**Participated in research design:** Stevens, van der Graaf, Danhof, and de Lange.

**Conducted experiments:** Stevens.

**Contributed new reagents or analytic tools:** Ploeger.

**Performed data analysis:** Stevens and Ploeger.

**Wrote or contributed to the writing of the manuscript:** Stevens, Ploeger, van der Graaf, Danhof, and de Lange.

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