Translational pharmacology of dopamine receptor agonists and antagonists : prolactin and oxytocin as biomarkers

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Mechanism-based PK-PD model for the prolactin biological system response following a dopamine inhibition challenge - quantitative extrapolation to humans

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ABSTRACT

The aim of this investigation was to develop a mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) model for the biological system prolactin response following a dopamine inhibition challenge using remoxipride as a paradigm compound.

After assessment of baseline variation in prolactin concentrations, the prolactin response of remoxipride was measured following 1) single intravenous doses of 4, 8 and 16 mg/kg and 2) following double dosing of 3.8 mg/kg with different time intervals. The mechanistic PK-PD model consisted of; i) a PK model for remoxipride concentrations in brain extracellular fluid; ii) a pool model incorporating prolactin synthesis, storage in lactotrophs, release into- and elimination from plasma; iii) a positive feedback component interconnecting prolactin plasma concentrations and prolactin synthesis; and iv) a dopamine antagonism component interconnecting remoxipride brain extracellular fluid concentrations and stimulation of prolactin release. The most important finding was the positive feedback on prolactin synthesis in the lactotrophs, in contrast to the negative feedback in the previous models on the PK-PD correlation of remoxipride.

An external validation was performed using a dataset obtained in rats following intranasal administration of 4, 8, or 16 mg/kg remoxipride. Following simulation of human remoxipride brain extracellular fluid concentrations, pharmacodynamic extrapolation from rat to humans was performed, using allometric scaling in combination with independent information on the values of biological system

specific parameters as prior knowledge. The PK-PD model successfully predicted the system prolactin response in humans, indicating that positive feedback on prolactin synthesis and allometric scaling thereof could be a new feature in describing complex homeostatic mechanisms.

INTRODUCTION

Prolactin release is a common side-effect of antipsychotic drugs (Petty, 1999) and several clinical pharmacokinetic-pharmacodynamic (PK–PD) models have been published on the relationships between dopaminergic drug- and prolactin concentrations. In 1995, a PK-PD model was proposed to describe the effects of double intravenous dosing of the dopamine D2/D3-receptor antagonist remoxipride on the prolactin release (Movin-Osswald and Hammarlund-Udenaes, 1995). The basis was a pool model describing prolactin synthesis, storage in lactotrophs, release into- and elimination from plasma. This model was found to be less suitable to describe the data of subsequent clinical studies following chlorprothixene treatment (D1/D2/D3-receptor antagonist). Next, as regulation of prolactin synthesis and secretion involves both negative (e.g. dopamine) and positive (e.g. oxytocin, estrogen) regulators (Freeman, et al., 2000), putative dopamine concentrations were used to describe mechanistic (biological system) feedback in an agonist-antagonist interaction model (Bagli, et al., 1999). However, actual dopamine concentrations could not be obtained, hence limiting quantitation of system-specific parameters. A next improvement included a circadian prolactin release model, following risperidone and paliperidone (D2-receptor antagonists) treatment (Friberg, et al., 2008). Most recently, the pool- and agonist-antagonist interaction models were compared after remoxipride administration. The agonist-antagonist interaction model proofed superior, based on better descriptive properties for the baseline prolactin release, although the data following remoxipride treatment were slightly better described by the pool model (Ma, et al., 2010).

An important restriction of these models may lie in the fact that its constituents –e.g. drug effect, (negative) system feedback and circadian rhythmicity– drive a single parameter for prolactin release into plasma, which complicates the separation of these constituents of the model. Taken together, these studies indicate that a general model for prolactin release following administration of dopaminergic drugs has yet to be developed. In order to do so, drug- and biological-system specific parameters should be separated in a quantitative, mechanistic manner (De Lange, et al., 2005; Danhof, et al., 2007; Ploeger, et al., 2009; Gabrielsson and Green, 2009), including expressions for target site distribution.

For dopaminergic compounds, the target site is the brain extracellular fluid (ECF) surrounding the dopamine receptors. As obtainment of this data in humans is highly restricted and expensive, information should be derived from *in vivo* animal studies using translational pharmacology approaches (Danhof, et al., 2008). Quantitative measurement of unbound drug concentrations in brain ECF over time is available *in vivo* in animals by intracerebral microdialysis (De Lange, et al., 2000). By using animal experimentation techniques, models can be challenged by administering larger dose ranges than allowed in human studies, resulting in a broader concentration range and thus more precise description of concentration–effect relationships. In animals, mechanism-based PK-PD models describing prolactin release have not yet been explored, but could provide a more mechanistic basis.
Earlier translational investigations to predict drug effects in humans have already shown that preclinical derived drug- and biological-system specific parameters in mechanism-based PK-PD models can be used with reasonable degree of success (Yassen, et al., 2007; Zuideveld, et al., 2007). Such investigations were based on allometric scaling of PK parameters (Boxenbaum, 1982) and independent information on the values of system specific parameters (e.g. target binding). Subsequent simulation studies can provide insight on the clinical applicability of a drug, at an early stage in drug development. Also, clinical studies suffice with fewer individuals and less samples per individual, for proof of concept in man.

In this study we aim to develop a mechanism-based PK-PD model for prolactin response following dopamine inhibition challenge in rats, using remoxipride as a paradigm compound. To that end, remoxipride concentration-time profiles were obtained in plasma and brain ECF, following a single dose of 4, 8, or 16 mg/kg and double dosing of 3.8 mg/kg remoxipride by 30 min intravenous infusions. After assessing the baseline variation in prolactin plasma concentrations, the effects of remoxipride on prolactin plasma concentrations were determined. Using nonlinear mixed effects modeling, we developed a population-based mechanism-based PK-PD model consisting of; i) a previously proposed pharmacokinetic model (chapter 5), ii) a pool model, iii) a positive feedback component interconnecting prolactin plasma concentrations and the synthesis of prolactin and iv) a dopamine antagonism component interconnecting remoxipride brain ECF concentrations and prolactin release.
As external validation, prolactin plasma concentrations from an intranasal remoxipride administration study were compared to model predictions. By translational modeling approaches, the PK and PD were then simulated in humans and compared to a clinical dataset (Movin-Osswald and Hammarlund-Udenaes, 1995).

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 nr. 6023 and 6132). Male Wistar WU rats (n = 92, mean weight ± standard deviation = 245 ± 18 gram, Charles River, The Netherlands), were housed in groups for 7-13 days (Animal Facilities, Gorlaeus Laboratoria, Leiden, The Netherlands), under standard environmental conditions (Ambient temperature 21°C; humidity 60%; 12/12 hour light, 8AM lights on (circadian time 0), background noise, daily handled), with *ad libitum* access to food (Laboratory chow, Hope Farms, Woerden, The Netherlands) and acidified water. Between surgery and experiments, the animals were kept individually in Makrolon type 3 cages for 7 days to recover from surgical procedures.

Surgery

Rat surgery and experiments were performed as previously reported (Stevens, et al., 2009). In short, during anaesthetized surgery, all animals received cannulas in the femoral artery and vein, for serial blood sampling and drug administration respectively. Also, an intranasal probe (AP 12 mm, L –0.5 mm) for drug administration, and a microdialysis guide (CMA/12, Schoonebeek, The Netherlands, AP +0.4, L 3.2, V -3.5) for continuous brain ECF sampling were implanted. After 6 days, 24 ± 1 hour before the experiments, the microdialysis guide was replaced by a probe (CMA/12, 4 mm polycarbonate membrane, cut-off 20 kDa, Aurora Borealis Control, Schoonebeek, The Netherlands). For the natural 24 hour circadian rhythmicity study, the animals only received a chronically implanted cannula in the femoral artery for blood sampling.

Experiments

The control study investigated the natural 24 hour rhythmicity of prolactin in 8 rats by obtaining 20 µl blood samples from the arterial cannula every 30 minutes for a period of 25 hour.

In the placebo study, the effects of handling and experimental techniques on prolactin release were investigated by 30 minute intravenous infusion of 500 µl saline (n = 7) and 30 second intranasal infusion of 10, 20, or 40 µl saline (n = 8, 10, and 9 respectively) by an automated pump (B. Braun Mel-

sungen AG, Melsungen, Germany). Blood samples of 200 µl were taken from the arterial cannula at t = 0, 5, 10, 20, 35, 60, 90, 120, 150, and 180 min.

In the separate remoxipride administration study, prolactin data were acquired following two intravenous remoxipride administration protocols; a single dose and a double dosing study. The experimental procedures for single dose remoxipride administration have been previously reported (Stevens, et al., 2009). In short, 3 experimental groups (n = 10 per group) received a single dose of 4, 8 or 16 mg/kg remoxipride in during a 30 minute intravenous infusion. Blood samples of 200 µl were taken from the arterial cannula at t = -5 (blank), 2, 10, 22, 30, 40, 60, 100, 180, and 240 min. Consecutive remoxipride dosing experiments were performed under similar conditions, but with twice dosing of 3.8 mg/kg of remoxipride during 30 minute intravenous infusions, at different time intervals; 0–1, 0–2, 0–4, 0–6, 2–6, and 4–6 hours (n = 3, 4, 3, 2, 2, and 3 respectively). A number of 20 blood samples of 100 µl were taken at different time points per group.

For the external validation data set, remoxipride experiments were performed under identical experimental techniques as the single dose studies, but with 1 minute intranasal infusion of 4, 8 or 16 mg/kg remoxipride (n = 10 per group).
For all animal experiments, blood samples were collected in EDTA–coated vials and, after centrifuging for 15 minutes at 5000 rpm, plasma was stored at -20˚C pending analysis. After the experiments the animals were sacrificed with an overdose of Nembutal (1 ml, intravenously).

For comparison of rat–to–human prediction, a clinical dataset was obtained from a previously published study (Movin-Osswald and Hammarlund-Udenaes, 1995). In short, eight healthy volunteers (mean weight ± standard deviation = 76 ± 10 kg) received two 30 minute intravenous infusions of 50 mg remoxipride in different time intervals in a randomized, six period cross-over design. The time intervals between the first dose at t = 0 and the second dose were 2, 8, 12, 24, and 48 hours. Remoxipride- and total prolactin plasma concentrations were obtained in 13 or more plasma samples per individual.

■ Analytical Methods

For all single dose animal experiments, remoxipride concentrations were measured in plasma and brain ECF using online solid phase extraction with liquid chromatography–tandem mass spectrometry (Stevens, et al., 2010).

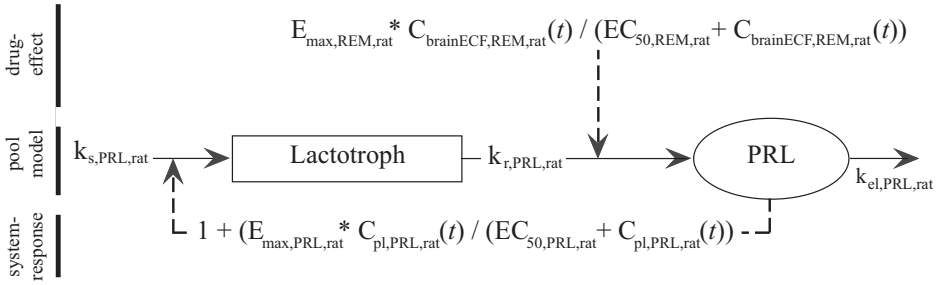
For all plasma samples takes in all remoxipride administration studies, prolactin concentrations were measured with a commercially available enzyme-linked immunosorbent assay (Rat prolactin ELISA (AER011), Biocode-Hycel, Belgium), according to the instructions of the manufacturer. Data acquisition was performed on a uQuant Universal Microplate Spectophotometer (BioTek, Germany). Concentrations were calculated using R 2.5.0 (The R Foundation for Statistical Computing, Vienna, Austria). Individual prolactin concentration-time plots were generated and apparent outliers were excluded from the dataset if exceeding 1.5 times the interquartile range of the median concentration at that time point. Also, prolactin data below the limit of quantification (0.36 ng/ml) were excluded from the dataset.

■ PK-PD model building and random variability

Nonlinear mixed effect modeling using NONMEM (version VI, level 2.0 Icon Development Solutions, Ellicott City, Maryland, USA) was used for modeling of the single- and double intravenous remoxipride administration studies. A sequential PK-PD modeling approach was applied, in which the individual PK parameters were fixed to the posthoc parameter estimates from the previously published population PK model for remoxipride in rat plasma and brain ECF (chapter 5). The structural model building was performed under ADVAN 9, and the first order conditional method with interaction was used for estimation with a convergence criterion of 3 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 -2*log likelihood is Chi–square distributed with degrees of freedom determined by the number of additional parameters in the more complex model. Hence, with 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 for the prolactin concentrations in plasma. Log normal distribution of the inter-individual variability was assumed and possible covariate correlations were taken into account.

The structural model is depicted in figure 1. The general structure of the PD model consists of a pool model which was based on the original pool model (Movin-Osswald and Hammarlund-Udenaes, 1995). Briefly, this model consists of a lactotroph- and a plasma prolactin compartment.
The change in prolactin concentration in the lactotrophs ($dC_{la,PRL,rat}$) over time (dt) is determined by a zero-order rate constant for the synthesis of prolactin ($k_{s,PRL,rat}$) and a first-order rate constant for the release of prolactin from the lactotrophs ($k_{r,PRL,rat}$).

Figure1 Compartmental structure of the final model, including the E_{max} concentration-effect relations of the drug-effect- and biological system effect models on the turnover model. $K_{s,PRL,rat}$, synthesis rate constant of prolactin in lactotrophs; $k_{r,PRL,rat}$, release rate constant of prolactin into plasma; $k_{el,PRL,rat}$, elimination rate constant of prolactin from plasma; $C_{brainECF,REM,rat}(t)$, rat brain extracellular fluid concentrations of remoxipride over time; $C_{pl,PRL,rat}(t)$, rat plasma prolactin concentrations over time; E_{max}, maximal effect of prolactin (PRL) or remoxipride (REM); EC_{50}, concentration PRL/REM inducing 50% of the E_{max}.



The change in prolactin concentration over time in the plasma compartment ($dC_{pl,PRL,rat}$) is described by a turnover model, which consists of the $k_{r,PRL,rat}$ and a first-order rate constant for the elimination of prolactin from plasma ($k_{el,PRL,rat}$). At t = 0, in absence of remoxipride, prolactin concentration in the plasma compartment ($C_{pl,PRL,rat}$) equals the baseline plasma prolactin concentration (BSL_{rat}), and total prolactin synthesis, release, and elimination are in equilibrium, according to principles of mass-balance ($k_{s,PRL,rat} = BSL_{rat} * k_{el,PRL,rat}$) (Ma, et al., 2010). In this pool model, the relatively low value for $k_{s,PRL,rat}$ causes lactotroph depletion after remoxipride administration, and drives therefore the tachyphylaxis component of the model. Positive homeostatic feedback on the $k_{s,PRL,rat}$ is anticipated to correct for this.

The physiological basis for the positive feedback (PF) model lies in the fact that synthesis and release of neurotransmitters are two separate processes that can be independently regulated in a biological system. Prolactin receptor activation in the cell membrane of lactotrophs (Morel, et al., 1994) activates a wide range of transcription factors and immediate early genes in the nucleus via JAK/STAT (Watson and Burdon, 1996) and MAPK pathways (Piccoletti, et al., 1994). Endogenous compounds that increase prolactin

synthesis (like e.g. estrogen) also use MAPK activation (Singh, et al., 1999, Ben-Jonathan, et al., 2008), suggesting that prolactin receptor activation can increase prolactin synthesis. In short, the PF model describes the physiological process by which release of prolactin by lactotrophs (depletion) increases the prolactin synthesis to "refill" the lactotrophs. The PF component was described by applying linear-, log-linear-, E_{max}- and sigmoidal E_{max} concentration-response relationships (equations 1-4) between plasma prolactin concentrations ($C_{pl,PRL,rat}$), normalized to BSL_{rat}, and the value of $k_{s,PRL,rat}$. The terms $E_{max,PRL,rat}$ and $EC_{50,PRL,rat}$ correspond to the values of maximal prolactinergic feedback and the plasma prolactin concentration that induces 50% of the $E_{max,PRL,rat}$, respectively, and c is the slope of the plasma prolactin concentration feedback relationship.

Equation 1; $PF_{linear} = c * (C_{pl,PRL,rat} - BSL_{rat})$

Equation 2; $PF_{log\text{-}linear} = c * \log(C_{pl,PRL,rat} - BSL_{rat})$

Equation 3; $PF_{Emax} = E_{max,PRL,rat} * (C_{pl,PRL,rat} - BSL_{rat}) / (EC_{50,PRL,rat} + (C_{pl,PRL,rat} - BSL_{rat}))$

Equation 4; $PF_{Sigmoidal\ Emax} = E_{max,PRL,rat} * (C_{pl,PRL,rat} - BSL_{rat})^{y} / (EC_{50,PRL,rat}^{y} + (C_{pl,PRL,rat} - BSL_{rat})^{y})$

Consequently, for equations 1, 3 and 4, PF = 0 when $C_{pl,PRL,rat}$ returns to BSL_{rat}. While investigating on the log-linear concentration response relationships, $PF_{log\text{-}linear}$ was reset to 0 when $C_{pl,PRL,rat}$ returned to BSL_{rat}. During the modeling process, the parameter estimates for BSL_{rat} were estimated separately for the single- and double remoxipride dosing studies.

A dopamine antagonist component of the drug effect (DE) describes the drug–effect relationship between unbound remoxipride concentrations in brain ECF ($C_{brainECF,REM,rat}$) and the $k_{r,PRL,rat}$. In the early phase of structural model building, an E_{max}-model (equation 5) described the DE relation, in terms of maximal remoxipride induced prolactin response ($E_{max,REM,rat}$), and the remoxipride concentration that induces half the $E_{max,REM,rat}$ ($EC_{50,REM,rat}$).

Equation 5; $DE = E_{max,REM,rat} * C_{brainECF,REM,rat} / (EC_{50,REM,rat} + C_{brainECF,REM,rat})$

Incorporating the components for PF and DE results in differential equations 6 and 7 for $dC_{la,PRL,rat}/dt$ and for $dC_{pl,PRL,rat}/dt$, respectively.
The terms $C_{la,PRL,rat}$ and $C_{pl,PRL,rat}$ represent the prolactin concentration in the lactotroph- and plasma compartment at time = t, respectively.

Equation 6; $dC_{la,PRL,rat}/dt = k_{s,PRL,rat} * (1+PF) - C_{la,PRL,rat} * k_{r,PRL,rat} * (1+DE)$

Equation 7; $dC_{pl,PRL,rat}/dt = C_{la,PRL,rat} * k_{r,PRL,rat} * (1+DE) - C_{pl,PRL,rat} * k_{el,PRL,rat}$

Based on decrease in OFV and individual goodness-of-fit, the best model for the PF component was selected. Then, the DE component was re-evaluated by applying log-linear and sigmoidal-E_{max} concentration–effect relationships.

Prior information for parameter estimation was used for the values of $EC_{50,REM,rat}$ of the dopamine D2-receptor and for the $k_{el,PRL,rat}$. The equilibrium binding dissociation constant of remoxipride for the D2-receptor is reported to be 41.9 ng/ml, with a standard deviation of 50 ng/ml (Mohell, et al., 1993). Our microdialysis experiments allowed measurement of brain ECF remoxipride concentrations being close to, or equal to the target site (D2-receptor) concentrations. As remoxipride is an antagonist, the EC_{50} can be assumed to be similar to the equilibrium binding dissociation constant of remoxipride binding to the D2-receptor.
Secondly, based on the first-order elimination rate of endogenous prolactin from plasma, the $t_{1/2}$ of prolactin in rats is 6.9 minutes, with a confidence of 6.3 – 7.7 minutes (Chi and Shin, 1978). This allows calculation of the $k_{el,PRL,rat}$ by $\ln 2/t_{1/2}$. The NWPRI subroutine in NONMEM was used, which allowed a penalty function based on a frequency prior to be specified and added to the -2log likelihood function (Gisleskog, et al., 2002), to estimate the $EC_{50,REM,rat}$ and $k_{el,PRL,rat}$.

■ PK–PD model evaluation

A bootstrap procedure, in which the final model was optimized on at least 1000 datasets obtained by random sampling with replacement from the original dataset, was used to derive the uncertainty in the parameter estimates of the final model. From the bootstrap estimation the median and 2.5^{th} and 97.5^{th} percentiles were obtained to represent the non-parametric 95% confidence limits.

The bootstrapped parameter estimates were then used in an internal qualification of the model by means of a visual predictive check (VPC). The VPCs were performed using NONMEM, by simulating 1000 replications of the model and a simulation dataset that contained intravenous dosing information for one individual per dosing regimen. The median, 5^{th} and 95^{th} 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 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).

The model was also externally validated. To this end, a VPC was performed using an external dataset obtained following intranasal administration of remoxipride. 1000 replications of the model and a simulation dataset containing intranasal dosing information for one individual per dosing regimen were generated. Again, the median, 5^{th} and 95^{th} percentiles were calculated for each simulated time-point and visually compared at each time point with the actual data. Resemblance between simulated and original distributions indicates the predictive value of the model for the intranasal administration route.

■ Translation from rat to human

Our ultimate goal was to predict the prolactin response in humans on the basis of our preclinical PK-PD model. Berkeley-Madonna software (Berkeley Madonna 8.3.9, Berkeley Madonna Inc., University of California (REGENTS), Berkeley, California, USA) was used for the simulation of the time course of the prolactin plasma concentrations in humans, which was then compared to the plasma data from the clinical dataset.

To translate the preclinical pool model to that for the human situation, allometric scaling (Boxenbaum, 1982) was applied for estimation of the human prolactin release- and elimination rate constants (equation 8).

Equation 8; $k_{hum} = k_{rat} * (bodyweight_{hum}/bodyweight_{rat})^{-0.25}$

As $k_{s,PRL,rat}$ is initially defined by $k_{el,PRL,rat} * BSL_{rat}$ and the same structural model is applied for the human situation, the human prolactin synthesis rate constant is automatically scaled as well. The clinical value for BSL_{hum} (9.4 ng/ml) was obtained from literature (Movin-Osswald and Hammarlund-Udenaes, 1995).

Concerning the extrapolation of the PF, to our knowledge, no clinical data are available on the $E_{max,PRL,hum}$ and $EC_{50,PRL,hum}$. However, from a physiological perspective, interspecies differences in the these parameters are expected (Ben Jonathan, et al., 2008) and an empiric approach on translation of these parameters was necessary. As prolactin is synthesized in lactotrophs, the interspecies difference in number of lactotrophs forms a basis for extrapolation of the $E_{max,PRL,hum}$. The average lactotroph density in the young adult male rat pituitary (LD_{rat}) is reported to be 37.9 % (Dada, et al., 1984; Phelps, 1986) and in the human pituitary (LD_{hum}) 16.9 % (Asa, et al., 1982). Consequently, the $E_{max,PRL,hum}$ was extrapolated according to equation 9.

Equation 9; $E_{max,PRL,hum} = E_{max,PRL,rat} * (LD_{hum}/LD_{rat})$

Little information is available on the physiological activity of prolactin on lactotrophs in man. In cell proliferation studies, the EC_{50} of human prolactin on the human prolactin receptor was determined (8.74 mg/l: Utama, et al., 2009). Although this does not represent the prolactin elevation of the $k_{s,PRL,hum}$ at a nucleus level, this pharmacological activity of prolactin forms a basis for extrapolating the interspecies difference in our simulation approach and this value was used for the $EC_{50,PRL,hum}$.

In the present investigations, to translate the DE model, the PK of remoxipride of the clinical dataset were reanalyzed using a 2-compartmental approach in NONMEM VI, to obtain PK parameter estimates for volume of distribution (V), clearance (CL) and intercompartmental clearance (Q) of remoxipride in humans. Based on the mean parameter estimates and their standard deviation, calculation of the coefficient of variation (CV) was used to derive the uncertainty in the parameter estimates of the model, which is considered acceptable when lower than 50%. For visual comparison of the model and the actual data, remoxipride concentration-time profiles were plotted for all dosing regimens.

In the preclinical PK-PD model, the DE model is related to the unbound brain ECF concentrations of remoxipride, which are described in a third (brain) compartment. Such information is not available for humans. Our approach was therefore to simulate the human brain ECF concentrations, by assuming that the ratio of concentrations in the brain- and peripheral compartment is equal in rats and man (equation 10 and 11). The human PK parameters for the brain- ($PK_{brain,hum}$) and peripheral compartment ($PK_{periph,hum}$) are calculated based on the human parameter estimate from

the clinical model (peripheral compartment, $\theta_{periph,hum}$) and the preclinical parameter estimates (θ_{rat}) for the brain- and peripheral compartment.

Equation 10; $PK_{brain,hum} = \theta_{periph,hum} * (\theta_{brain,rat}/(\theta_{brain,rat} + \theta_{periph,rat}))$

Equation 11; $PK_{periph,hum} = \theta_{periph,hum} * (\theta_{periph,rat}/(\theta_{brain,rat} + \theta_{periph,rat}))$

For extrapolation of the DE component, the values for $E_{max,REM}$ and $EC_{50,REM}$ in humans had to be acquired. In the clinical dataset, the observed maximum prolactin concentrations after remoxipride administrationa are the combined result of the pool-, DE- and PF model components. The observed maximal prolactin concentration 70 ng/ml (Movin-Osswald and Hammarlund-Udenaes, 1995) minus BSL_{hum} therefore overestimates the required value of $E_{max,REM,hum}$, as the PF is not taken into account. However, for our simulation purposes this approximation on the value of $E_{max,REM,hum}$ was considered acceptable. As remoxipride is a dopamine antagonist and the DE is based on simulated brain ECF concentrations that are close to, or at the target site, the $EC_{50,REM,hum}$ was considered similar to the remoxipride equilibrium binding dissociation constant. In literature, the equilibrium binding dissociation constant of remoxipride for the human D2-receptor is reported to be 5.936 mg/l (Burstein, et al., 2005), and thus incorporated in the model.

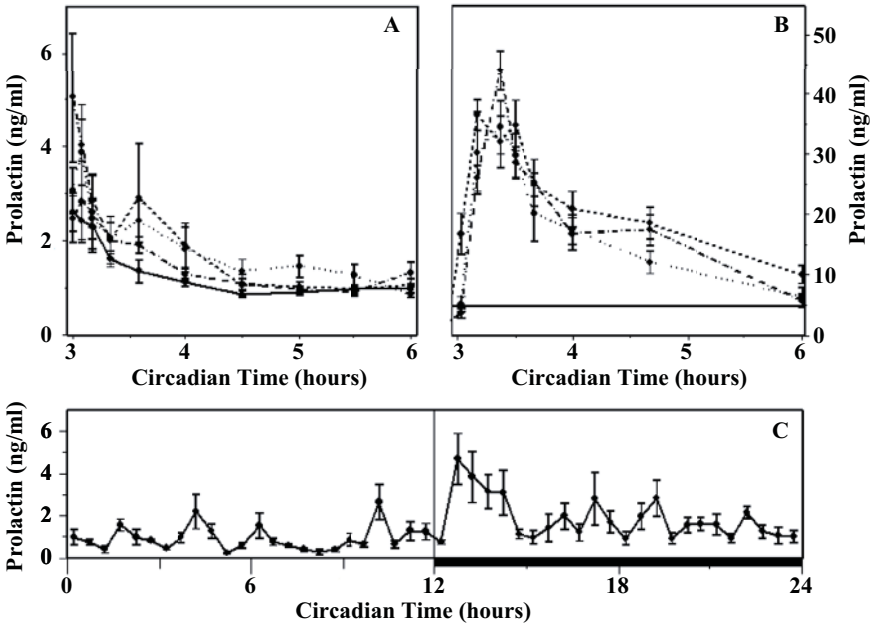
RESULTS

The control and placebo studies show low mean prolactin concentration when compared to the prolactin response after intravenous remoxipride administration (Figure 2). Also, the variations in baseline prolactin concentrations were low, and no handling- or experimental influences on prolactin release were found.

The baseline concentrations of prolactin proved similar to previous findings in individually housed animals (Perello, et al., 2006). As a result, $k_{r,PRL,rat}$ can be considered a rate constant at the baseline, in absence of remoxipride. Following remoxipride administration, a maximal prolactin peak was observed, which is equal for all dosing regimens. Upon visual assessment, the area under the curve of the prolactin concentration time curve seemed to increase with increase of remoxipride dose. After performing the second, double dosing study of remoxipride, no full second response could be generated at short dosing intervals, as expected based on the previously reported tachyphylaxic properties of prolactin release. The intravenous single- and

double remoxipride dosing studies provided 190- and 295 prolactin concentrations, respectively.

Figure 2 Average prolactin concentrations (± SEM) under different conditions over circadian time; A) 500 µl intravenous-, and 20, 30, and 40 µl intranasal saline administration (solid-, dash-dotted-, dotted-, and dashed lines respectively), B) 30 min intravenous infusion of 4, 8 and 16 mg/kg remoxipride (dash- dotted-, dotted- and dashed lines respectively), the solid line represents the highest average prolactin concentration after saline administration. C) 24 hour sampling (solid line), the dark phase (12-24 hours) is represented by the dark bar.



■ PK-PD modeling

Applying the E_{max}-model for the remoxipride brain ECF concentration–effect relationship allowed reasonable description of the prolactin concentrations in plasma for the groups that received a single remoxipride dose and for groups that received double dosing of remoxipride at longer intervals. However, without a PF component, the model underestimated a second plasma prolactin peak in plasma following double remoxipride dosing at short intervals. Next, PF of plasma prolactin concentrations on the $k_{s,PRL,rat}$ was described by linear- and log-linear concentration–effect relationships,

with OFV of 2148 and 2074 respectively. The lowest OFV (2057) was achieved by an E_{max} relationship, while a sigmoidal-E_{max} model caused over-parameterization. As a result of the E_{max}-model for PF, all individual plasma prolactin concentration-time profiles were now adequately described for all dosing intervals.

Next, the drug–effect relationships between unbound remoxipride brain ECF concentrations and plasma prolactin concentrations were re-evaluated. Log-linear relationships and sigmoidal-E_{max} relationships increased the OFV approximately 60 points, so optimization of the model was continued with an E_{max}-model for both the PF- and DE components.
Based on a decrease in OFV, a combined proportional- and additive error model best described the residual variability in the prolactin response compartment for all model structures. Inter-individual variability was identified for BSL_{rat}.

Single inclusion of prior information on the value $k_{el,PRL,rat}$ resulted in more realistic estimations for $E_{max,REM,rat}$ and, as expected, the estimation for $EC_{50,REM,rat}$ was closer to the equilibration binding association constant (OFV 1975). Adding prior information only to the parameter for $EC_{50,REM,rat}$ in the drug–effect relationship, decreased the OFV to 1966. However, the resulting estimation of $E_{max,REM,rat}$ now increased three-fold from earlier model expectations, which is probably caused by the large predefined standard deviation in the penalty function.
When adding prior information for both parameter estimates, the OFV dropped to 1953 points and the parameter estimation for $E_{max,REM,rat}$ was now in similar range of previous model expectations, although the covariance step was now aborted by NONMEM. Table 1 summarizes the parameter estimates, IIV and residual error. Describing the PF- and DE components by E_{max}-relationships resulted in the best predictions for all doses and dosing regiments in all individual rats, as depicted for a typical individual in every double dosing regimen (Figure 3).

■ PK-PD model evaluation

The parameter estimates after 1096 bootstrap replications of the dataset were very close to the NONMEM estimates of the final model (Table 1). Also, the estimates for IIV and residual error were similar, confirming the stability of the model.

Figure 3 Individual predicted (solid line) and observed (open circles) plasma prolactin concentration over time in hours (h) for the repeated (3.8 mg/kg) remoxipride dosing study. Per dosing regimen (dose, with time interval in hours after the start of the experiments), a typical single individual is plotted.

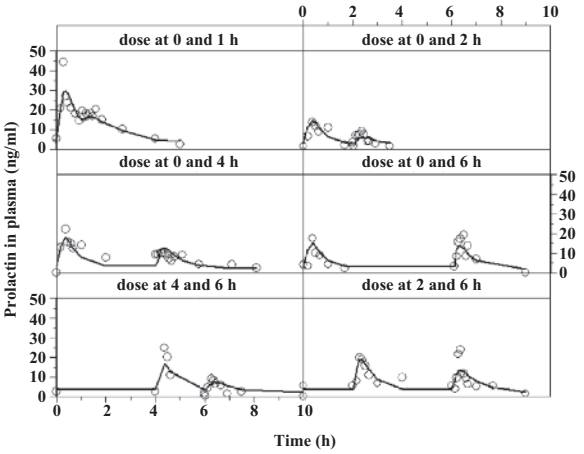


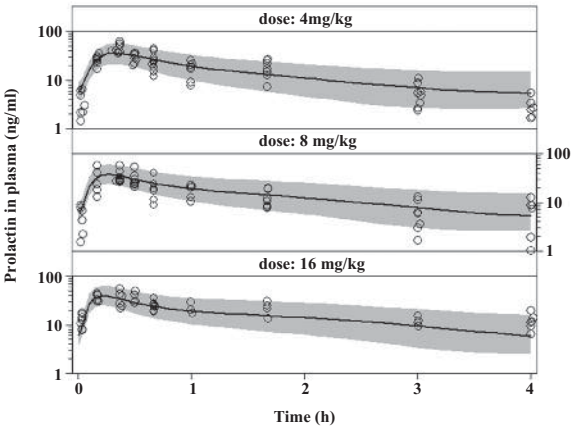
Table 1 NONMEM parameter estimates and bootstrap results.

parameter	Final model estimates	Bootstrap (n = 1096) median	95% CL	CV (%)
Pool model				
$BSL_{sd,rat}$ (ng/ml)	6.2	6.1	4.4-7.2	12.1
$BSL_{dd,rat}$ (ng/ml)	3.9	3.9	2.4-5.6	17.8
$k_{r,PRL,rat}$ (h^{-1})	0.6	0.6	0.4-0.7	12.6
$k_{el,PRL,rat}$ (h^{-1})	5.7	5.8	5.1-6.3	5.4
Positive feedback model				
$E_{max,PRL,rat}$ (ng/ml)	3.5	3.7	3.0-6.8	25.1
$EC_{50,PRL,rat}$ (mg/L)	12.4	12.0	8.5-16.8	17.2
Drug–effect model				
$E_{max,REM,rat}$ (ng/ml)	25.0	24.6	18.8-44.6	24.0
$EC_{50,REM,rat}$ (ng/ml)	0.08	0.08	0.04-0.12	22.30
Error				
σ additive	0.06	0.06	0.04-0.10	23.81
σ proportional	6.73	6.54	2.91-11.70	35.47
η BSL	0.07	0.07	0.04-0.10	21.39

95% CL, confidence limits calculated as 2.5th and 97.5th percentiles;
CV, coefficient of variation calculated from mean bootstrap parameter estimates;
BSL, baseline prolactin concentration in the single- (sd) and double (dd) remoxipride dosing study; parameters as defined in figure 1;
σ, residual error;
η, interindividual error calculated as single estimate for both $BSL_{sd,rat}$ and $BSL_{dd,rat}$.

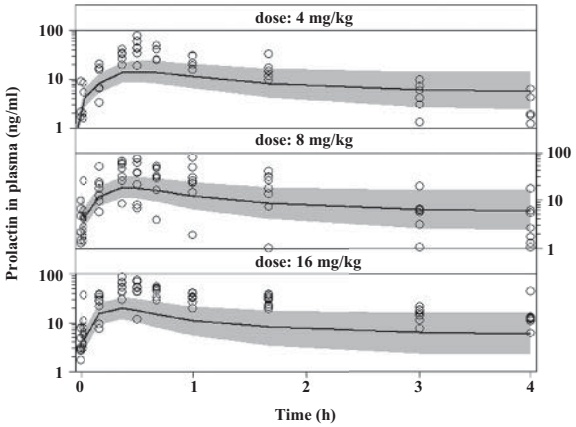
In an internal evaluation of the model, the VPC visualized accurate prediction of observed prolactin concentrations following single dose intravenous administration of remoxipride (Figure 4).
In general, for all single dose groups, the maximal observed prolactin concentrations were well described by the median of the VPC. Although negligible, the observed concentrations at t = 2 min were slightly overestimated by the median of the model for the 4- and 8 mg/kg dose groups and in the 16 mg/kg dose group a slight underestimation of the lower concentration range was observed. Most observations lie within the 90% confidence interval, indicating that the variability was well estimated.

Figure 4 Visual predictive checks of preclinical PK-PD model. The plots represent the simulated median of the plasma prolactin concentration predictions (solid line), and the 90% prediction interval (grey area) over time per dose, following intravenous remoxipride administration. The open circles represent plasma prolactin concentrations measured in the intravenous single dose remoxipride experiments.



The placebo study proved that intranasal administration as such does not influence baseline prolactin concentrations (Figure 2A). As a result, the biological-system parameters were considered equal for intravenous- and intranasal administration of remoxipride. Consequently, the data from the intranasal remoxipride administration study could be used for external validation of the final model. When a VPC was performed (Figure 5), the maximum prolactin plasma concentrations were underestimated (~10%), as was the time to maximal prolactin concentration. Also, a higher percentage of the observations were outside the 90% confidence interval. As the PD variability of the PK-PD model is based on the intravenous administration datasets, this indicates different variability after intranasal administration.

Figure 5 Visual predictive checks of the external validation dataset. The plots represent the simulated median of the plasma prolactin concentration predictions (solid line), and the 90% prediction interval (grey area) over time per dose, following intranasal remoxipride administration. The open circles represent plasma prolactin concentrations measured in the intranasal single dose remoxipride experiments.



Translation from rat to human

The pharmacokinetics of remoxipride in human plasma could be well described by a two-compartmental approach. The parameter estimates for in the human PK model were close to the values estimated earlier (Movin-Osswald and Hammarlund-Udenaes, 1995) and the low CVs indicates accurate parameter estimation (Table 2).

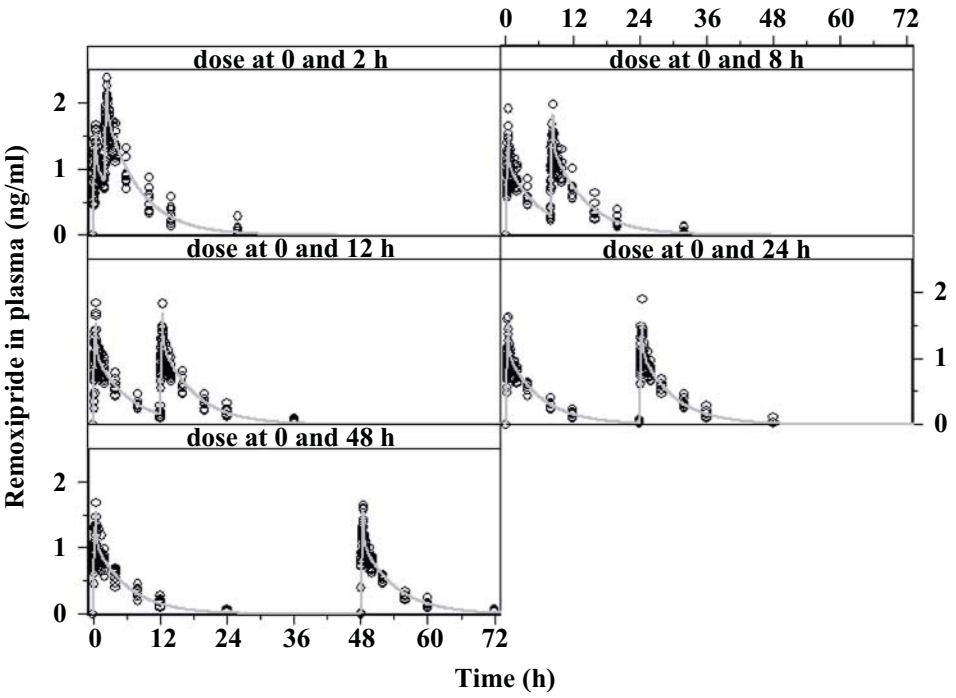
Table 2 NONMEM pharmacokinetic parameter estimates for remoxipride in humans.

	parameter	human PK model estimate (CV in %)	η	Movin-Osswald and Hammarlund-Udenaes, 1995 estimate	η
plasma compartment					
	CL (l/h)	7.4 (5.2)	0.03	6.84	0.14
	V (l)	12.2 (3.1)	-		
peripheral compartment					
	Q (l/h)	137 (11.3)	0.1		
	V (l)	34.7 (6)	0.03		
Error					
	σ additive	0.03			
	σ proportional	0.14			
	Vtotal			40	0.09

η, inter-individual variability; σ, residual variability; Cl, clearance; V, volume of distribution; Q, intercompartmental clearance; Vtotal, total volume of distribution in steady state.

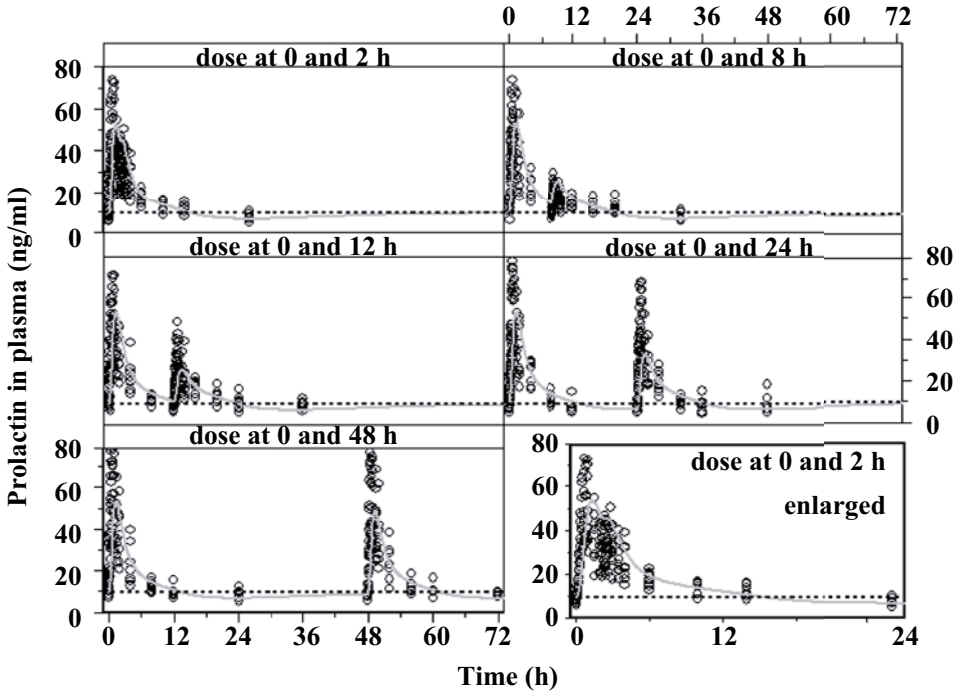
In the preclinical model, the drug–effects are related to brain ECF concentrations in a brain-PK compartment. To allow extrapolation of drug–effect in rats to humans, human brain ECF concentrations were estimated by converting the human two-compartmental- into a three-compartmental PK model. The hence acquired PK model showed accurate description of the remoxipride concentration time profiles in human plasma (Figure 6), for all dosing regimens. Compared to remoxipride plasma concentrations after intravenous administration of remoxipride, brain ECF concentration-time profiles generally showed lower maximal concentrations and a longer time to reach the maximal concentration, due to nose-to-brain distribution characteristics (chapter 5). As no human data are available on remoxipride brain ECF concentrations, no comparison can be made. The predicted human remoxipride brain ECF profiles showed lower maximal concentrations and a short delay in time to maximal concentration when compared to the plasma concentration-time profiles, as expected.

Figure 6 Translation of preclinical to clinical PK (three-compartment PK model). The solid, grey lines represent the prediction of remoxipride concentrations in plasma over time for different dosing regiments. The open circles represent measured remoxipride concentrations, obtained in the clinical dataset (Movin-Osswald and Hammarlund-Udenaes, 1995).



In general, the translational mechanism-based PK-PD simulation described the human prolactin plasma concentrations following the repeated intravenous administration of remoxipride adequately (Figure 7). The time to full equilibrium of the pool model (return to baseline prolactin concentration), is concurrent with the time at which a full second response can be generated in humans (dosing interval 0–48).

Figure 7 Translation of preclinical to clinical PK-PD. The solid, grey lines represent the prediction of plasma prolactin concentrations over time for different dosing regiments. The open circles represent measured prolactin concentrations, obtained in the clinical dataset (Movin-Osswald and Hammarlund-Udenaes, 1995).



DISCUSSION

Our aim was to develop a mechanistic PK-PD model for the prolactin response following administration of dopamine receptor antagonists. To this end, the interrelationships between the time course of remoxipride concentrations in brain ECF and the time course of the plasma prolactin concentrations were assessed under different conditions. A previously proposed pool

model, which accounted for tachyphylaxis upon repeated administration of remoxipride in humans, constituted the backbone of the novel mechanism-based PK-PD model (Movin-Osswald and Hammarlund-Udenaes, 1995).

Prolactin is released into plasma by lactotrophs in the anterior pituitary and is tonically suppressed by three types of hypothalamic dopaminergic neurons. Tuberoinfundibular neurons release dopamine into long portal veins that empty in pituitary sinusoids. Periventricular hypothalamic- and tuberohypophysial dopaminergic neurons project directly to the pituitary (Freeman, et al., 2000). Thus, dopamine antagonists increase prolactin release into plasma, limited by depletion of the prolactin content. Less is know about regulation of the synthesis of prolactin after such depletion. Interestingly, we found a positive feedback of prolactin plasma concentrations on its synthesis. In physiological terms this can be regarded as a homeostatic mechanism, restoring basal conditions of the biological system.

In the original pool model (Movin-Osswald and Hammarlund-Udenaes, 1995), lactotroph depletion and the value of $k_{s,PRL,rat}$ have been identified as rate limiting steps in prolactin release following remoxipride administration, but could not adequately describe the plasma prolactin concentrations at short dosing intervals in subsequent studies. Likewise, in our animal studies, sole use of a pool model underestimated the prolactin response following remoxipride administration at a short interval. To correct for this, previously proposed (clinical) PK-PD model approaches included baseline circadian rhythmicity, linear DE and (negative) linear system feedback, that all drive the single parameter estimate for $k_{r,PRL}$ and thereby complicate separation of these model constituents. By strict separation of model constituents in an animal experimental setup, we provided a basis to quantitate the biological system parameters of prolactin release and the drug–effects thereon.

First, circadian rhythmicity in baseline prolactin plasma concentrations was low, if not neglectible, compared to the magnitude of the prolactin response following administration of remoxipride (Figure 2). Therefore, $k_{r,PRL,rat}$ could be considered constant.

Secondly, to identify drug-specific parameters in a quantitative, semi-mechanistic manner, we obtained remoxipride plasma- and target site (brain ECF) concentration-time profiles. A broader remoxipride dosing regimen was applied than would be allowed in humans, to increase the remoxipride concentration range and thereby the descriptive properties of the DE model. In

our model approach, we included previous literature data on the pharmacology of remoxipride ($EC_{50,REM,rat}$) and prolactin ($k_{el,PRL,rat}$) in a penalty function. The resulting DE model allowed accurate description of a maximal drug–effect relationship between remoxipride brain ECF concentrations and $k_{r,PRL,rat}$, and can be considered more mechanistic compared to previously proposed clinical models that identified (linear) drug–effect relationships between remoxipride plasma drug concentrations and $k_{r,PRL}$.

Thirdly, the use of a positive feedback mechanism between plasma prolactin concentrations and $k_{s,PRL,rat}$ allowed strict distinction between the drug–effect (on $k_{r,PRL,rat}$) and system-effect. Addition of a positive feedback model allowed accurate description of the time course of prolactin concentrations in plasma following a second remoxipride dose, for all dosing regimens (Figure 3). The increase in prolactin synthesis includes a physiological limit, represented by the $E_{max,PRL,rat}$-parameter estimate. Consequently, both lactotroph depletion and the $E_{max,PRL,rat}$ are the rate limiting steps for prolactin release in plasma.

Bootstrapping proved high stability and VPCs showed good predictive properties of the preclinical PK-PD model in the mechanistic description of the drug–effect and biological system response when using prolactin as a PD endpoint following remoxipride administration.

In the external validation, the simulations showed underestimation of the C_{max} and time–to–C_{max} following intranasal administration of remoxipride to rats. In the previously PK-model (chapter 5), maximum observed remoxipride brain ECF concentrations (~0.1 mg/l) are close to the estimated $EC_{50,REM,rat}$ (0.08 mg/l). We also reported slight underestimation of the maximum remoxipride concentration in brain ECF and higher variability on pharmacokinetic parameters following intranasal administration. Both factors contribute to the underestimation of prolactin release and consequently delayed lactotroph depletion as displayed in the VPC (Figure 4). This leads to believe that the value of $EC_{50,REM}$ is slightly overestimated in the final PK-PD model and/or that the prediction bias may come from the PK model. The lowest value for the $EC_{50,REM,rat}$, within the confidence limits of the bootstrap, is 0.04 mg/l. The expectation is that such a low value will cause the observed maximal brain ECF concentrations to induce lactotroph depletion and therefore correct the C_{max} and time-to-C_{max}. Optimization of study design (e.g. dose regimen and sampling times) in subsequent studies would allow for identification of a sigmoidal E_{max} drug-effect relationship (Hill-coefficient) to verify this assumption. However, taking these factors into account, the evaluation shows that the model predicts the prolactin-

ergic effects relatively well after intranasal administration of remoxipride, implying that the mechanism-based PK-PD model has predictive power towards this other routes of administration, rather than only describing the intravenous dataset.

In the extrapolation from rat to human PK–PD, the simulation of human brain ECF concentrations is a critical factor. As remoxipride brain ECF concentrations depend on unbound plasma drug concentrations, interspecies differences in plasma binding (Widman, et al., 1993) should be considered when scaling between species. As data from the clinical study were available, an easier approach was to construct a clinical two-compartment PK model in NONMEM that describes total remoxipride concentrations in plasma and unbound remoxipride concentrations in the peripheral compartment. In this model, the PK parameter estimates proved to match previously published data (Table 2). As remoxipride is reported to rapidly cross the blood-brain barrier in both rat and man (Farde and Von Bahr, 1990; Kohler, et al., 1992), we assumed that the ratio of unbound remoxipride in brain and periphery would be comparable in rat and human (equations 10 and 11). By this approach, we were able to simulate remoxipride brain-ECF concentrations in humans in the translational PK model.

Prolactin synthesis, release pathways, homeostatic feedback, and plasma elimination half-life are well understood and have been found to be structurally similar in rats and man (Ben Jonathan, et al., 2008). For that reason, as well as because prolactin concentrations can be measured relatively easily in plasma samples in both rat and man, prolactin comprises all prerequisites for a translational biomarker (Danhof, et al., 2005) for dopaminergic activity in the brain. We used the simulated brain ECF concentrations in the clinical PK model as the basis for the rat–to–human simulation of the PD effect. The rate constants in the pool model were allometrically scaled, leading to a $k_{el,PRL,hum}$ of 1.4 h^{-1}, which is in agreement with previous studies, that report $k_{el,PRL,hum}$ to range between 1 and 2.09 h^{-1} (Movin-Osswald and Hammarlund-Udenaes, 1995; Bagli, et al., 1999; Ma, et al., 2010). The human BSL values were obtained from literature. As limited information is available on the remaining E_{max} and EC_{50} parameter values, further research (e.g. by *in vitro* bioassays) should validate actual values and thus improve the translational properties of the model, as may the previously proposed inclusion of circadian rhythmicity in prolactin release. The PK-PD model was successful in describing both the PK and PD in humans.

An important question is how to further validate the proposed translational PK-PD model for prediction of the prolactin biological system response, as reflected by prolactin plasma concentrations. An interesting approach is to challenge the model with a training set of different compounds as has previously been successfully applied in the development of mechanistic PK-PD models for adenosine A1-receptor agonists (Van der Graaf, et al., 1997), GABA-ergic compounds (Visser, et al., 2002) and 5-HT1a-receptor agonists (Zuideveld, et al., 2004). Administration of other dopaminergic antagonists in rats will be subject to different drug–effect relationships, but the values describing the biological system response should essentially remain identical. Also, unique physicochemical properties of new chemical entities that are not (yet) approved for use in man can be studied, alternative routes of administration can be explored more easily, as can *in vitro-in vivo* correlations. This provides additional data for the validation of the pool- and positive feedback models. Finally, small scale clinical studies on (intranasal) administration of dopaminergic compounds allows validation on this new structural approach on prolactinergic turnover and homeostatic feedback in humans.

Summarizing, we accomplished the development of a mechanism-based PK-PD model describing prolactin release in plasma in rats following remoxipride administration in different dosages and dosing regimens. The most important finding, relative to previous investigations on the PK-PD correlation of remoxipride, was the identification of a positive feedback of prolactin plasma concentrations on the zero-order rate constant for synthesis of prolactin in the lactotrophs. The use of allometric scaling, literature values of clinical drug-specific- and biological system specific parameters, and simulation of brain ECF concentration-time-profiles allowed extrapolation to humans with reasonable degree of success. This indicates that the structure of the model adequately describes prolactin release in both rats and humans, and that positive feedback of prolactin plasma concentrations on its own synthesis in the lactotrophs and allometric scaling thereof could be a new feature in describing complex homeostatic mechanisms.

REFERENCES

- Asa SL, Penz G, Kovacs K and Ezrin C (1982) Prolactin cells in the human pituitary. A quantitative immunocytochemical analysis. *Arch Pathol Lab Med* **106**:360-363.
- Bagli M, Suverkrup R, Quadflieg R, Hoflich G, Kasper S, Moller HJ, Langer M, Barlage U and Rao ML (1999) Pharmacokinetic-pharmacodynamic modeling of tolerance to the prolactin-secreting effect of chlorprothixene after different modes of drug administration. *J Pharmacol Exp Ther* **291**:547-554.
- Ben Jonathan N, LaPensee CR and LaPensee EW (2008) What Can We Learn from Rodents about Prolactin in Humans? *Endocr Rev* **29**:1-41.
- Boxenbaum H (1982) Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. *J Pharmacokinet Biopharm* **10**:201-227.
- Burstein ES, Ma J, Wong S, Gao Y, Pham E, Knapp AE, Nash NR, Olsson R, Davis RE, Hack sell U, Weiner DM and Brann MR (2005) Intrinsic Efficacy of Antipsychotics at Human D2, D3, and D4 Dopamine Receptors: Identification of the Clozapine Metabolite N-Desmethylclozapine as a D2/D3 Partial Agonist. *J Pharmacol Exp Ther* **315**:1278-1287.
- Chi HJ and Shin SH (1978) The effect of exposure to ether on prolactin secretion and the half-life of endogenous prolactin in normal and castrated male rats. *Neuroendocrinology* **26**:193-201.
- Dada MO, Campbell GT and Blake CA (1984) Pars distalis cell quantification in normal adult male and female rats. *J Endocrinol* **101**:87-94.
- Danhof M, De Jongh J, De Lange EC, Della Pasqua O, Ploeger BA and Voskuyl RA (2007) Mechanism-based pharmacokinetic-pharmacodynamic modeling: biophase distribution, receptor theory, and dynamical systems analysis. *Annu Rev Pharmacol Toxicol* **47**:357-400.
- Danhof M, De Lange EC, Della Pasqua OE, Ploeger BA and Voskuyl RA (2008) Mechanism-based pharmacokinetic-pharmacodynamic (PK–PD) modeling in translational drug research. *Trends Pharmacol Sci* **29**:186-191.
- De Lange EC, De Boer AG and Breimer DD (2000) Methodological issues in microdialysis sampling for pharmacokinetic studies. *Adv Drug Deliv Rev* **45**:125-148.
- De Lange EC, Ravenstijn PG, Groenendaal D and Van Steeg TJ (2005) Toward the prediction of CNS drug–effect profiles in physiological and pathological conditions using micro dialysis and mechanism-based pharmacokinetic-pharmacodynamic modeling. *AAPS J* 7:E532-E543.
- Farde L and Von Bahr C (1990) Distribution of remoxipride to the human brain and central D2-dopamine receptor binding examined in vivo by PET. *Acta Psychiatr Scand Suppl* **358**:67-71.
- Freeman ME, Kanyicska B, Lerant A and Nagy G (2000) Prolactin: structure, function, and regulation of secretion. *Physiol Rev* **80**:1523-1631.
- Friberg LE, Vermeulen AM, Petersson KJF and Karlsson MO (2008) An Agonist-Antagonist Interaction Model for Prolactin Release Following Risperidone and Paliperidone Treatment. *Clin Pharmacol Ther* **85**:409-417.
- Gabrielsson J and Green AR (2009) Quantitative Pharmacology or Pharmacokinetic Pharmacodynamic Integration Should Be a Vital Component in Integrative Pharmacology. *J Pharmacol Exp Ther* **331**:767-774.
- Gisleskog PO, Karlsson MO and Beal SL (2002) Use of prior information to stabilize a population data analysis. *J Pharmacokinet Pharmacodyn* **29**:473-505.
- Kohler C, Radesater AC, Karlsson-Boethius G, Bryske B and Widman M (1992) Regional distribution and in vivo binding of the atypical antipsychotic drug remoxipride. A biochemical and autoradiographic analysis in the rat brain. *J Neural Transm Gen Sect* **87**:49-62.
- Ma G, Friberg LE, Movin-Osswald G and Karlsson MO (2010) Comparison of the agonist-antagonist interaction model and the pool model for the effect of remoxipride on prolactin. *British Journal of Clinical Pharmacology* **70**:815-824.

- Mohell N, Sallemark M, Rosqvist S, Malmberg A, Hogberg T and Jackson DM (1993) Binding characteristics of remoxipride and its metabolites to dopamine D2 and D3 receptors. *European Journal of Pharmacology* **238**:121-125.
- Morel G, Ouhtit A, Kelly PA (1994) Prolactin Receptor Immunoreactivity in Rat Anterior Pituitary. *Neuroendocrinology* **59**:78-84.
- Movin-Osswald G and Hammarlund-Udenaes M (1995) Prolactin release after remoxipride by an integrated pharmacokinetic-pharmacodynamic model with intra- and interindividual aspects. *J Pharmacol Exp Ther* **274**:921-927.
- Perello M, Chacon F, Cardinali DP, Esquifino AI and Spinedi E (2006) Effect of social isolation on 24-h pattern of stress hormones and leptin in rats. *Life Sciences* **78**:1857-1862.
- Petty RG (1999) Prolactin and antipsychotic medications: mechanism of action. *Schizophr Res* **35 Suppl**:S67-S73.
- Phelps CJ (1986) Immunocytochemical analysis of prolactin cells in the adult rat adenohypophysis: distribution and quantitation relative to sex and strain. *Am J Anat* **176**:233-242.
- Piccoletti R, Maroni P, Bendinelli P, and Bernelli-Zazzera (1994) Rapid stimulation of mitogen-activated protein kinase of rat liver by prolactin. *Biochem J* **303**: 429–433
- Ploeger BA, Van der Graaf PH and Danhof M (2009) Incorporating receptor theory in mechanism-based pharmacokinetic-pharmacodynamic (PK–PD) modeling. *Drug Metab Pharmacokinet* **24**:3-15.
- Post TM, Freijer JI, Ploeger BA and Danhof M (2008) Extensions to the visual predictive check to facilitate model performance evaluation. *J Pharmacokinet Pharmacodyn* **35**:185-202.
- Prado MAM, Reis RAM, Prado VF, De Mello MC, Gomez MV, de Mello FG (2002) Regulation of acetylcholine synthesis and storage. *Neurochemistry International* **41**: 291-299
- Singh M, Se´ta´lo´ Jr G, Guan X, Warren M, Toran-Allerand CD (1999) Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. *J Neurosci* **19**:1179 –1188
- Stevens J, Suidgeest E, Van der Graaf PH, Danhof M and De Lange EC (2009) A new minimal-stress freely-moving rat model for preclinical studies on intranasal administration of CNS drugs. *Pharm Res* **26**:1911-1917.
- Stevens J, Van den Berg D-J, De Ridder S, Niederlander HAG, Van der Graaf PH, Danhof M and De Lange ECM (2010) Online solid phase extraction with liquid chromatography-tandem mass spectrometry to analyze remoxipride in small plasma-, brain homogenate-, and brain microdialysate samples. *Journal of Chromatography B* **878**:969-975.
- Utama FE, Tran TH, Ryder A, LeBaron MJ, Parlow AF and Rui H (2009) Insensitivity of Human Prolactin Receptors to Nonhuman Prolactins: Relevance for Experimental Modeling of Prolactin Receptor-Expressing Human Cells. *Endocrinology* **150**:1782-1790.
- Van der Graaf PH, Schaick EA, Mathot RAA, Ijzerman AP and Danhof M (1997) Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling of the Effects of N6-Cyclopentyladenosine Analogs on Heart Rate in Rat: Estimation of in Vivo Operational Affinity and Efficacy at Adenosine A1 Receptors. *J Pharmacol Exp Ther* **283**:809-816.
- Visser SAG, Gladdines WWFT, Van der Graaf PH, Peletier LA and Danhof M (2002) Neuroactive Steroids Differ in Potency but Not in Intrinsic Efficacy at the GABAA Receptor in Vivo. *J Pharmacol Exp Ther* **303**:616-626.
- Watson CJ and Burdon TG (1996) Prolactin signal transduction mechanisms in the mammary gland: the role of the Jak/Stat pathway.y *Reviews of Reproduction* **1**; 1–5.
- Widman M, Nilsson LB, Bryske B and Lundstrom J (1993) Disposition of remoxipride in different species. Species differences in metabolism. *Arzneimittelforschung* **43**:287-297.
- Yassen A, Olofsen E, Kan J, Dahan A and Danhof M (2007) Animal-to-human extrapolation of the pharmacokinetic and pharmacodynamic properties of buprenorphine. *Clin Pharmacokinet* **46**:433-447.

- Zuideveld KP, Van der Graaf PH, Peletier LA and Danhof M (2007) Allometric scaling of pharmacodynamic responses: application to 5-Ht1A receptor mediated responses from rat to man. *Pharm Res* **24**:2031-2039.
- Zuideveld KP, Van der Graaf PH, Newgreen D, Thurlow R, Petty N, Jordan P, Peletier LA and Danhof M (2004) Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling of 5-HT1A Receptor Agonists: Estimation of in Vivo Affinity and Intrinsic Efficacy on Body Temperature in Rats. *J Pharmacol Exp Ther* **308**:1012-1020.