Physiologically Based Modeling Approach to Predict Dopamine D2 Receptor Occupancy of Antipsychotics in Brain: Translation From Rat to Human

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Yin Cheong Wong, PhD [iD], Maddalena Centanni, MS, and Elizabeth C. M. de Lange, PhD

Abstract

Receptor occupancy (RO) is a translational biomarker for assessing drug efficacy and safety. We aimed to apply a physiologically based pharmacokinetic (PBPK) modeling approach to predict the brain dopamine D2 RO time profiles of antipsychotics. Clozapine and risperidone were modeled together with their active metabolites, norclozapine and paliperidone, First, in PK-Sim a rat PBPK model was developed and optimized using literature plasma PK data. Then, blood-brain barrier parameters including the expression and efflux transport kinetics of P-glycoprotein were optimized using literature microdialysis data on brain extracellular fluid (brainECF), which were further adapted when translating the rat PBPK model into the human PBPK model. Based on the simulated drug and metabolite concentrations in brainECF, drug-D2 receptor binding kinetics (association and dissociation rates) were incorporated in MoBi to predict RO. From an extensive literature search, 32 plasma PK data sets (16 from rat and 16 from human studies) and 23 striatum RO data sets (13 from rat and 10 from human studies) were prepared and compared with the model predictions. The rat PBPK-RO model adequately predicted the plasma concentrations of the parent drugs and metabolites and the RO levels. The human PBPK-RO model also captured the plasma PK and RO levels despite the large interindividual and interstudy variability, although it tended to underestimate the plasma concentrations and RO measured at late time points after risperidone dosing. The developed human PBPK-RO model was successfully applied to predict the plasma PK and RO changes observed after risperidone dose reduction in a clinical trial in schizophrenic patients.

From lead generation to clinical trials, the assessment of in vivo target engagement plays an important role in drug discovery and development.[1,2] It serves as a biomarker that reflects the portion of an endogenous target that is bound to the drug at a specific drug dose, which is determined together by the pharmacokinetics (PK) at the target site and drug-target binding kinetics.[3] More importantly, it allows the translation of preclinical dose-efficacy findings to clinical settings. Its translational value has been demonstrated for different drug targets, ranging from G protein–coupled receptors to ligand-gated ion channels and transporters.[2] A well-known example is the dopamine D2 receptor occupancy (RO) of antipsychotics. A therapeutic window of 60% to 80% D2 RO in striatum is preserved across species, from rodents[4,5] to human schizophrenic patients, in which the desired antipsychotic effects can be achieved without excessive extrapyramidal side effects.[6,7] Therefore, knowledge on RO can provide guidance on achieving optimal outcomes in the pharmacotherapy of antipsychotics.

Direct assessment of RO for receptors in the human central nervous system (CNS) is challenging. Despite the advancement of positron emission tomography and single photon emission computed tomography, measuring RO with imaging techniques remain resource

and logistically demanding. Another approach is to indirectly estimate RO based on the drug concentration at brain extracellular fluid (brainECF), which is the driving force for RO for membrane receptors such as D2 receptors, along with the drug's affinity to the receptor. Microdialysis is currently the only method

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that is able to measure PK in brainECF,[8] but for obvious ethical reasons it is seldom used in clinical trials or in the routine clinical setting. Drug concentration in the cerebrospinal fluid (CSF) can provide an indication of brainECF concentrations, but it is not always reliable due to the differences between blood-brain barrier (BBB) and blood-CSF barrier, as well as intrabrain distribution kinetics.[9] Many drugs, including some antipsychotics, are substrates of active transporters such as P-glycoprotein (P-gp) that modulate drug transport across the BBB and blood-CSF barrier.[8,10] Consequently, the PK profile in brainECF can be very different from that in plasma or CSF, and prediction of CNS RO based on plasma PK might not be accurate.

Human CNS PK data are not available in most cases, which makes a direct assessment of the relationship between plasma PK and RO time profiles challenging. Often, rats and mice are used for measuring in vivo CNS RO during preclinical drug development. Since both CNS PK and RO data can be collected from rats, mathematical PK-RO models can first be constructed and validated in rats, and subsequently be extrapolated from rats to humans, particularly with the physiologically based PK (PBPK) approach. PBPK analysis uses models that combine physiology, population and drug characteristics to mechanistically describe the PK and/or pharmacodynamic behaviors of a drug. PBPK models are different from the empirical PK models, primarily because they use more accurate representations of the various body compartments. By adapting the species-specific physiological parameters, the animal PBPK model can be translated to a human PBPK model. The aim of the present study is to establish a PBPK-RO model that can predict D2 RO levels of antipsychotics. Recently, an example of applying PBPK-RO modeling to predict human CNS D2 RO of antipsychotics is reported.[11] Using the commercial PBPK software GastroPlus, the predicted RO level of quetiapine was comparable with data observed in a clinical trial on healthy subjects.[12] However, there is much room for improvement. First, active drug transport across the BBB was not incorporated, although in vivo evidence from rat studies did indicate the involvement of active transports for quetiapine.[13,14] In fact, the histamine H1 RO values in the cortex did not correlate with the plasma quetiapine concentrations in humans,[15] indicating the limitation of predicting CNS RO based solely on plasma PK. Second, the contribution of active metabolites to the RO level was not considered. Third, translation from the animal PBPK model to the human PBPK model was not reported. Fourth, the maximal response and half maximal effective concentration values for RO prediction had to be estimated by the PBPK model using prior RO data from another clinical trial.

Fifth, for validation of the predictive power of this model only a single data set was used.

In the present study, to establish a PBPK-RO model that could predict D2 RO of antipsychotics, 2 antipsychotics, clozapine and risperidone, were selected because (1) they are efficacious[16] and are commonly used in patients with schizophrenic disorders,[17] (2) they have different binding kinetics toward the D2 receptor,[18] and (3) literature data on the plasma PK and D2 RO are available in both rats and humans. Furthermore, the parent drugs and their active metabolites are all P-gp substrates.[19] Our translational approach was to first develop a rat PBPK-RO model using multiple rat data sets and to subsequently develop the human PBPK-RO model based on the rat model with the use of multiple clinical trial data sets.

Methods

Software

Models and simulations were created with the aid of the Computational Systems Biology Software Suite that comprises the software tools PK-Sim (Version 6.2.2, Bayer Technology Services, Leverskusen, Germany) and MoBi (Version 6.2.2, Bayer Technology Services, Leverskusen, Germany). Moreover, the PK-Sim Express Gene Expression Database (Version 5.6.3, Bayer Technology Services, Leverskusen, Germany) was used for the quantification of cytochrome P450 (CYP) expression in the human PBPK model. Data extraction from graphic information was performed with WebPlotDigitizer (Version 3.8, http://www.arohatgi.info/WebPlotDigitizer/app/). Data analysis and visualization were performed with R Studio (Integrated Development for R. RStudio, Inc., Boston, Massachusetts).

PK-Sim is a PBPK software tool that allows whole-body PBPK modeling for humans and the most common laboratory animals. MoBi is a systems biology software tool that enables the user to explore and modify the model structure, mathematical equations, and parameters underlying the PBPK models in PK-Sim. Unless otherwise described, the default anatomic and physiological parameters for rats and humans defined in the software were used.

Workflow for Developing the PBPK Models and Model Structure

The workflow for development of the rat and human PBPK and PBPK-RO models is shown in Figure 1, which will be elaborated in the following section. In brief, PBPK models for the drugs and their metabolites were first developed for rats and then translated to humans. Subsequently, in these models the D2 receptors in the brain were incorporated. These PBPK-RO models were used to simulate the D2 RO levels after
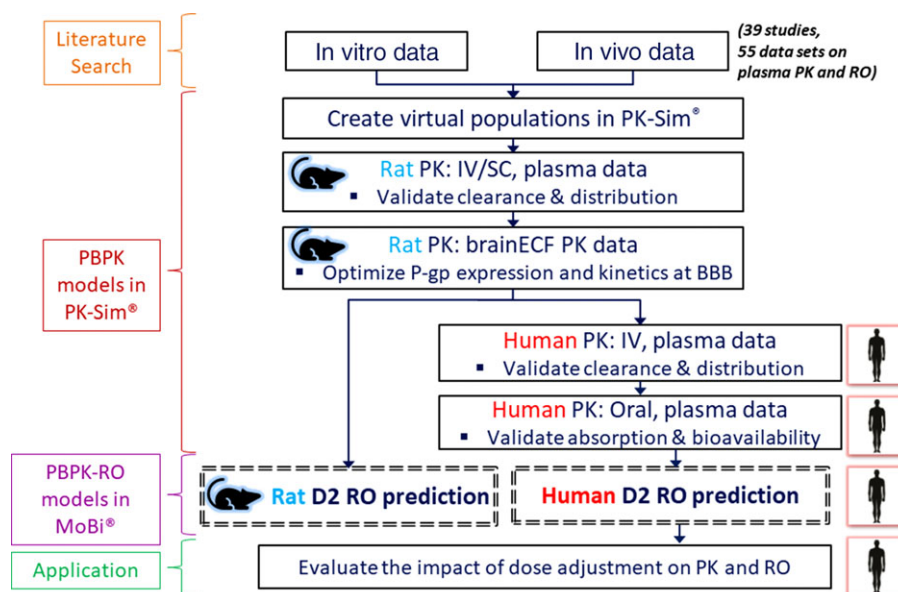
Figure 1. Workflow for developing the PBPK-RO models. IV, intravenous; RO, receptor occupancy; SC, subcutaneous.

clozapine or risperidone dosing. The physicochemical, absorption, distribution, metabolism, elimination, and D2 receptor–binding parameters used for the PBPK-RO models are summarized in Table 1.

Schematic diagrams showing the model structures in relation to the input parameters and the data sets used to estimate these parameters for the parent drugs and the metabolites are provided in Figures S1 (for clozapine) and S2 (for risperidone).

Collection of Plasma PK and RO Datasets From Literature

A digital database search was conducted in PubMed and Google Scholar on literature and patents from 1950 to February 2017. Forward and backward citation searching were performed to identify additional literature. Abstracts were primarily scanned for relevance and, if appropriate, the full content was evaluated further. Rat and human data regarding the 2 antipsychotics risperidone and clozapine and the 2 active metabolites paliperidone (also known as 9-hydroxyrisperidone) and norclozapine (also known as N-desmethylclozapine) were summarized, including (1) in vivo plasma PK data, (2) in vivo D2 RO data in striatum (caudate/putamen); (3) in vitro drug dissolution profiles; and (4) in vitro drug-protein interaction parameters such as plasma protein binding, CYP metabolism kinetics, P-gp transport kinetics, and drug-D2 receptor–binding kinetics. PK and RO data from subjects administered with the metabolite (paliperidone or norclozapine) alone were also collected. The main exclusion criteria were (1) formulations other than the conventional intravenous (IV) and subcutaneous dosage forms for rats and conventional IV and oral dosage forms for humans; (2) data from anesthetized

rats, since anesthesia can cause dramatic changes in the PK[20,21] and RO time profile[22]; (3) data from special patient populations (eg, pediatrics and elderly); (4) publications that were not fully accessible; (5) studies that did not report drug formulation, administration route, or dose; and (6) studies providing RO data without specifying the time at which the measurements were performed with respect to the drug administration. The details of each included study are summarized in Tables S1 to S6.

Development of the PBPK Model for Rats

The rat PBPK models were developed using the Rat Population module in PK-Sim. Experimentally determined physicochemical properties (such as log P and pKa) of the drugs and the metabolites were used as input. If such information was not available, then values estimated from software programs were used.

Regarding the absorption, since subcutaneous administration was not available in PK-Sim, together with the fact that in most literature the exact site of subcutaneous injection was not reported, drug administration into the muscle plasma compartment with a first-order absorption rate into plasma was used as a surrogate, and the corresponding absorption half-life was estimated based on the in vivo data. All IV and subcutaneous formulations were assumed to be fully dissolved before administration.

Regarding the tissue distribution, the 5 methods available in PK-Sim for the calculation of tissue-to-plasma partition coefficient were compared in terms of their accuracy in describing the observed plasma PK after an IV bolus dose. The most accurate methods were utilized for further simulations in both the rat and

Table 1. Parameters Used for the Rat and Human PBPK Models

Parameters	Units	Clozapine	Norclozapine	Risperidone	Paliperidone
Parameter shared by both the rat and human models[a]					
Molecular weight	g/mol	326.8	312.8	410.5	426.5
Log P		3.44[100]	3.07[100]	2.5[101]	2.4[102]
pKa1		3.69[103]	NA	3.11[104]	8.2[102]
pKa2		7.57[103]	NA	8.24[104]	NA
$V_{max,pgp}$	nmol/min/nmol	2.11[19]	27[b]	2.86[19]	15[b] (10[c])
$K_{m,pgp}$	μM	58[19]	0.5[b]	12.4[19]	12.4[b]
$K_{d,D2}$	nM	82[105] (218.78[18])[d]	165.96[18]	7.24[18]	6.17[18]
$k_{off,D2}$	1/s	0.023[105] (0.042[18])[d]	0.048[18]	0.005[18]	0.005[18]
Rat model-specific parameters					
$f_{u,plasma}$		0.061[26]	0.11[26]	0.118[102]	0.285[102]
$t_{1/2,absorption}$	min	40 (87[e])[b]	160[b]	15 (23[e])[b]	15[b]
$CL_{Hepatic}$	ml/min/kg	35 (100%[f])[b]	30[b]	25 (4%[f])[b]	10[b]
CL_{Renal}	ml/min/kg	10[b]	10[b]	15[b]	10[b]
Human model-specific parameters					
$f_{u,plasma}$		0.05[40]	0.097[40]	0.1[106]	0.226[106]
Dissolution time/shape	Min	100/0.92[b,g]	NA	10/0.92[104,g]	NA
$CL_{Hepatic}$	mL/min/kg	NA	4.6[b]	NA	1.04[b]
CL_{Renal}	mL/min/kg	0	0	0	1.04[b]
CYP1A2,V_{max}	pmol/min/pmol	4.4[107]	NA	NA	NA
CYP1A2,K_{m}	μM	18[107]	NA	NA	NA
CYP2D6,V_{max}	pmol/min/pmol	NA	NA	2.3[108]	NA
CYP2D6,K_{m}	μM	NA	NA	1.1[108]	NA
CYP3A4,V_{max}	pmol/min/pmol	5.4[107]	NA	15[108]	NA
CYP3A4,K_{m}	μM	304[107]	NA	61[108]	NA
CYP3A5,V_{max}	pmol/min/pmol	NA	NA	15[108]	NA
CYP3A5,K_{m}	μM	NA	NA	200[108]	NA

$CL_{Hepatic}$, total plasma clearance in liver; CL_{Renal}, total plasma clearance in kidney; CYP, cytochrome P450; $f_{u,plasma}$, fraction unbound in plasma; $K_{d,D2}$, affinity (dissociation constant) at D2 receptor; $k_{off,D2}$, dissociation rate at D2 receptor; $K_{m,pgp}$, substrate concentration at which the transport rate is half of $V_{max,pgp}$; NA, not applicable; $t_{1/2,absorption}$: absorption half-life after subcutaneous injection; $V_{max,pgp}$: maximum rate of the P-gp mediated efflux transport at blood-brain barrier administration.

[a] The rat model and the human model used the same parameter values unless specified.

[b] Values were estimated in PK-Sim.

[c] $V_{max,pgp}$ in the human model (10 nmol/min/nmol) was reduced to two-third of those in the rat model (15 nmol/min/nmol). Further explanations on the $V_{max,pgp}$ and $K_{m,pgp}$ values are provided in Table S8.

[d] The $K_{d,D2R}$ and $k_{off,D2R}$ values, used in both the rat and human models, were provided by Sahlholm et al.[18] The only exception was that for clozapine in the rat model the values were provided by Kapur and Seeman[105] instead, which had improved the model prediction.

[e] The estimated $t_{1/2,absorption}$ values could adequately capture the subcutaneous PK profiles in all rat studies except for those in the Cremers et al[26] and Culot et al.[27] For these two studies the absorption rate from the subcutaneous injection site was slower than the others and a higher $t_{1/2,absorption}$ value was needed to adequately capture the observed plasma and brainECF PK profiles. Possibly, a distinct formulation and/or injection site was applied in these studies. Specifically, 20% cyclodextrin was used for the clozapine injection in Culot's study, and the strong complexation between the drug and cyclodextrin could have slowed down the absorption rate.[109]

[f] The percentage indicates the portion of total plasma clearance in liver ($CL_{Hepatic}$) of the parent drug that generates the metabolite norclozapine and paliperidone.

[g] Weibull function, with dissolution time (50% dissolved) and a shape parameter as input, was used to simulate the dissolution of the oral tablet. The in vitro dissolution profile from Saibi et al[104] was used as the input for risperidone tablet. For clozapine, in vitro tablet dissolution rate was not reported and thus it was estimated in PK-Sim.

human PBPK models, which were the Berezhkovskiy method (a modification of the method proposed by Poulin and Theil) for risperidone, paliperidone, and norclozapine, and the Schmitt method (proposed by Schmitt) for clozapine.[23,24]

Drug distribution from plasma to the brainECF is determined by passive diffusion in conjunction with active efflux transport from brain to plasma by P-gp at the BBB. Passive diffusion parameters were calculated by PK-Sim based on the compound's physicochemical properties, which were adopted without manual modification. There was one identified citation on the P-gp transport kinetics for clozapine, risperidone, and verapamil (a well-characterized substrate for P-gp), which were calculated from an in vitro adenosine triphosphatase assay using Michaelis-Menten kinetics (substrate concentration at which the transport rate is half of maximum velocity [K_m] and maximum velocity [V_{max}]).[19] The concentration of P-gp at BBB was estimated to be 4 μM in the rat PBPK model using verapamil brainECF data from the rat microdialysis study of Nagaya et al[25] in combination with verapamil's P-gp transport kinetics. The certainty of this P-gp concentration value was further confirmed

by validation with additional rat brainECF data from Nagaya et al[25] (risperidone and paliperidone), Cremers et al[26] (clozapine, norclozpaine, risperidone), and Culot et al[27] (clozpaine and risperidone). For the human PBPK model, this value was reduced from 4 μM to 1 μM, based on the approximately 4-fold difference in P-gp expression at isolated brain microvessels between rat (19.1 fmol/μg protein) and human (3.98-6.06 fmol/μg protein).[28–30] This value was comparable to the value calculated based on the P-gp concentration measured in isolated human brain microvessels[29,30] (which is 0.94-1.43 μM, and the calculation is explained in Table S7). Such approach of translating lower P-gp level to lower BBB efflux is further supported by the observation that while the P-gp level at the BBB of cynomolgus monkeys is similar to that in humans,[31] the in vivo brainECF-to-unbound plasma ratios of risperidone and paliperidone in cynomolgus monkeys were 3.9- and 4.8-fold higher than those in rats.[32] For the 2 metabolites norclozapine and paliperidone, since information regarding P-gp transport kinetics was not available, these parameters were optimized based on the values of the parent compounds[19] and rat brainECF data.[25,26] Details on the optimization of the K_m and V_{max} values of the metabolites are provided in Table S8.

The eliminations the parent drugs and metabolites were modeled by linear hepatic and renal clearance, which were estimated by fitting the plasma PK data observed after a single IV dose to the PBPK model. The hepatic conversion of the parent drugs clozapine and risperidone into the specific metabolites, norclozapine and paliperidone respectively, was also estimated using the observed IV data. Simulations of plasma PK time profile by the rat PBPK model were performed individually for each study (Tables S1, S3, and S4), and the hepatic and renal clearance values were automatically adjusted within PK-Sim according to rat body weight reported in that study.

Development of the PBPK-RO Model for Rats
After developing a PBPK model in PK-Sim that could adequately predict the plasma PK profiles observed in multiple data sets, the model was then extended by adding D2 receptors to the brain and incorporating drug-receptor binding in MoBi. The RO-time profile was simulated using the following equation:

$$dN/dt = k_{off}/K_d * A_{D2R\text{-}unbound} * C_{drug}$$

$$* K_{water/brainECF} - k_{off} * A_{D2R\text{-}drug}$$

in which *dN/dt* represents the change in the amount of drug-receptor complex over time, k_{off} is the first-order dissociation rate constant of the drug-receptor complex, K_d is the affinity of the drug to the receptor,

k_{off}/K_d gives the second-order association rate constant (k_{on}) of the drug to the receptor (k_{off} and K_d were fixed at values obtained from in vitro binding kinetics studies, as listed in Table 1), $A_{D2R\text{-}unbound}$ is remaining amount of unbound D2 receptor (not bound to the parent drug or the metabolite) in the brainECF that is still available for drug binding, $A_{D2R\text{-}drug}$ is the amount of drug-receptor complex in the brainECF, C_{drug} is the brainECF drug concentration, and $K_{water/brain\text{-}ECF}$ is the partition coefficient that corrects for the partition of the drug between water and protein within the brainECF ($K_{water/brainECF}$ was calculated by PK-Sim based on the physicochemical properties of the drug). The density of D2 receptors in both rat and human models was fixed to 25 nM based on the receptor density measured in striatum.[33] RO was calculated as the percentage of the receptor that was binding to the drug at a particular time point. After the dosing of the parent drug, RO of the parent drug and the active metabolite were simulated in the same manner simultaneously, and the sum of the drug RO and metabolite RO gave the total RO.

In PK-Sim, the rat model represents an average adult animal of the species and is not strain specific. Wistar and Sprague Dawley were the only strains that had been used in the rat PK (Table S1) and RO (Tables S3 and S4) studies involved in the present PBPK-RO analysis. A recent quantitative proteomic analysis found no difference between these 2 strains in the protein levels of P-gp and also claudin-5 (a major component of tight junction proteins at the rodent BBB) at the BBB.[28] Moreover, in vitro radioligand binding studies identified similar D2 receptor expression in dopaminergic regions including striatum and also similar binding affinity toward radiolabeled D2 antagonists for these 2 strains.[34,35] It thus could be reasonably assumed that the BBB transport and subsequent drug-receptor binding processes are comparable between these 2 strains.

Development of the PBPK and PBPK-RO Models for Humans
After the development of the rat PBPK and PBPK-RO models, the human PBPK and PBPK-RO models were developed in a similar fashion. A major characteristic of the human model was the application of an in vitro to in vivo extrapolation approach for the biotransformation of the parent drugs risperidone and clozapine to their metabolites paliperidone and norclozapine, respectively. In the rat model, the generation of these metabolites was parameterized by an empirical nonspecific hepatic clearance process, while in the human model the Michaelis-Menten kinetics based on the in vitro kinetics data (K_m and V_{max}) of specific CYP isoenzymes was implemented. The PK-Sim library includes 3 human CYP gene-expression

databases that describe the expression of CYP enzymes in different body tissues: reverse transcription polymerase chain reaction–derived gene expression estimates from literature, whole genome expression arrays from ArrayExpress (European Informatics Institute), and expressed sequence tags from UniGene (National Center for Biotechnology Information).[36] Preliminary simulations suggested that the reverse transcription polymerase chain reaction database gave the most accurate human plasma PK profiles of the parent drug and the metabolite. Therefore, this database was used in all the subsequent simulations. Information is limited regarding the elimination pathways for the metabolites. Following administration of a single 1-mg dose of oral solution ^{14}C-paliperidone (a dosage form that had approximately 100% oral bioavailability) to healthy volunteers, renal excretion of the unchanged paliperidone accounted for around 50% of the total clearance of paliperidone. Other than renal excretion, 4 metabolic pathways were identified as being involved in the elimination of paliperidone, each of which accounted for up to a maximum of 6.5% of the administered dose.[37] In vitro studies suggested a role for CYP2D6 and CYP3A4 in the metabolism of paliperidone.[38] As enzyme kinetics data are not available, in the human PBPK model the total plasma clearance of paliperidone was modeled by linear liver clearance and kidney clearance, each accounting for 50% of the total clearance. In contrast, renal elimination of risperidone and clozapine accounts for only <4% of the total clearance of the corresponding drugs,[39–41] and therefore in the human PBPK model we assumed zero renal clearance. For norclozapine, the relative contribution of renal elimination to total clearance has not been reported, and we assumed that norclozapine was eliminated by linear hepatic clearance.

Simulations with the human PBPK model were performed for each individual study (Tables S2, S5, and S6). Human populations were recreated with the aid of the ethnicity-specific models in PK-Sim. If the exact population option was not available, the topographically closest ethnic group was chosen instead. Demographic characteristics (age, weight, height, and body mass index) were defined in the population toolbox. For subjects receiving chronic dosing, the simulated RO time profile was obtained on the 15th day of dosing, based on the fact that steady-state plasma concentrations would be achieved within 6 days for risperidone and 10 days for clozapine after repeated administrations.[42] In the simulations, the time of dosing and RO time profile predictions were set according to the chronological scheme stated in the corresponding publications (Tables S5 and S6), and the total daily dose was administered as a once-daily dosing unless otherwise specified.

Evaluation of Model Performance

The model-predicted drug concentration in plasma and RO levels were compared with those observed in the literature, and the bias was evaluated by calculating the median absolute percentage error.[43] The absolute percentage error (APE) was calculated for every time point as follows:

$$\text{APE} = \frac{\left|\text{Value}_{\text{IPRED,t}} - \text{Value}_{\text{OBS,t}}\right|}{\text{Value}_{\text{OBS,t}}} \times 100\%$$

where $Value_{IPRED,t}$ and $Value_{OBS,t}$ represent the individual predicted and observed value for drug concentration or RO at time t, respectively. Goodness-of-fit plots were also used for the graphic analysis of model results.

Application of the Human PBPK-RO Model and Extension to Non-D2 Receptors

To demonstrate the predictive value of the developed human PBPK-RO model, the model was used to simulate the human plasma PK and RO for D2 and 5-hydroxytryptamine 2A (5-HT$_{2A}$) according to the settings of the clinical trial by Nyberg et al[44] in 1999, which investigated a reduction of the risperidone dose from 6 mg/day to 3 mg/day in schizophrenic patients. The simulations were then compared with the observed data. In addition, the RO time profiles of other non-D2 receptors including alpha-1A, alpha-2 and histamine H1 receptors were also simulated to explore the receptor selectivity toward D2 receptor versus non-D2 receptors during chronic risperidone treatment.

Results

Characteristics of the PK and RO Data Collected From Literature

Altogether, 55 data sets (29 rat data sets, 26 human data sets) were collected from 39 studies that met the inclusion criteria. Thirty-one data sets were collected for plasma PK and 23 data sets were collected for striatum D2 RO. The details of each study are summarized in Tables S1 to S6. The settings of these studies varied considerably in terms of, for example, the rat species/human ethnic populations studied, healthy versus diseased subjects, dose amount and single dose vs chronic doses, which contributed to the variability in the observed plasma PK and D2 RO data. While all the rat data were collected from nondiseased male rats receiving a single dose, human data were collected from a more heterogeneous group of subjects (involving healthy volunteers and schizophrenic patients from both sexes) receiving either a single dose or repeated doses. Nevertheless, even among rat studies variations in the experimental design were considerable. For instance, difference in the recovery period from anesthesia and surgery for cannulation (or vs rats that did not
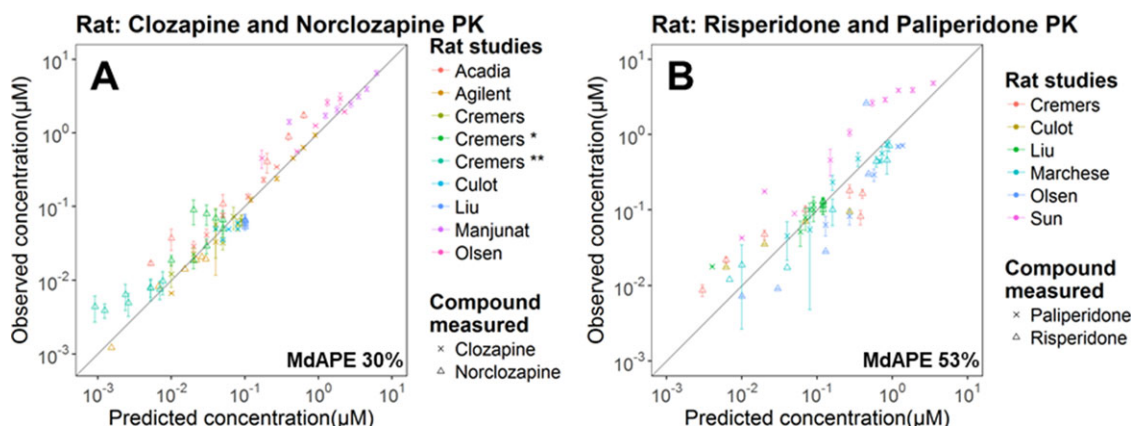
Figure 2. Prediction performance of the rat PBPK model on plasma concentrations of clozapine and norclozapine (A) and risperidone and paliperidone (B) in multiple studies. The gray diagonal line is the unity line. The standard deviation of the observed data, if available, is shown as the error bar. Details of each study are summarized in Table S1.

receive any surgery) could have led to deviation in plasma PK of the parent drugs and the metabolites.[21]

Variations in assay sensitivity is a particular issue of concern. As shown in Table S2, in human PK studies the lower limit of quantification (LLOQ) for clozapine and norclozapine in plasma (or serum) ranged from 0.1 ng/mL[45] to 15 ng/mL, [46] which represented a 150-fold difference in assay sensitivity. For risperidone and paliperidone, the LLOQ was in the range of 0.1 to 1 ng/mL (which is around 0.24-2.4 nM). If a high proportion of samples had concentrations below LLOQ and if samples below LLOQ were simply discarded from the study report, the overall observed concentrations and terminal half-life could be artificially inflated. This could be particularly relevant for samples collected at the later time points during which the drug concentrations became very low, and might indeed be a possible cause of the deviation between model prediction and observation for the plasma concentrations of risperidone and paliperidone (refer to the subsection Prediction Accuracy of Plasma PK in the Human PBPK Model).

Variations in the conditions of the subjects were also found to be prominent. While all the collected risperidone PK studies were performed on healthy volunteers (Table S2), most of the risperidone RO studies were performed on patients with schizophrenic disorders (Table S5). Both CNS and non-CNS conditions could alter the drug PK. For instance, an increase in CNS dopaminergic activity and stress are suggested to modify multiple hormone systems that regulate the expression of CYPs in liver.[47,48] Systemic inflammation and infection have been shown to increase the plasma concentrations of risperidone and the metabolite paliperidone by >20% and the plasma concentration of clozapine by up to 300%, probably via modification of plasma binding to alpha-1 acid glycoprotein (an acute-phase protein elevated by several-fold during inflammation)

and suppression of CYP activity.[49,50] Therefore, higher variability was expected in the observed RO data than the PK data.

Prediction Accuracy of Plasma PK in the Rat PBPK Model

A total of 16 data sets (9 for clozapine/norclozapine and 7 for risperidone/paliperidone) from 9 studies were processed and compared to the simulated rat plasma PK. The model adequately predicted the plasma PK of clozapine and norclozapine (following norclozapine administration as well as metabolite formed following clozapine administration) (Figure 2A) and risperidone and its metabolite paliperidone (following paliperidone administration as well as metabolite formed following risperidone administration) (Figure 2B). Nevertheless, the model underestimated the relatively high paliperidone plasma concentrations observed in the study of Sun et al[51] in which a high IV bolus dose of paliperidone was administered. Noteworthy is that in the rat PBPK model linear, nonsaturable clearance in liver and kidney was applied to all compounds due to a lack of information about the enzyme kinetics in rats. The actual in vivo clearance of paliperidone, however, can be nonlinear (eg, Michaelis-Menten kinetics) and saturable at high concentrations.

Prediction Accuracy of D2 RO in the Rat PBPK-RO Model

A total of 13 data sets (4 for clozapine/norclozapine and 9 for risperidone/paliperidone) from 7 studies were processed and compared to the simulated rat D2 RO levels. The rat model reasonably predicted the total D2 RO (the sum of RO contributed by the parent drug and the metabolite) after administration of clozapine (Figure 3A), risperidone, and paliperidone (Figure 3B). Nevertheless, for both risperidone and clozapine, the model underestimated the relatively high RO observed in Barth.[52] Barth's study was the only RO study in which IV administration rather than subcutaneous
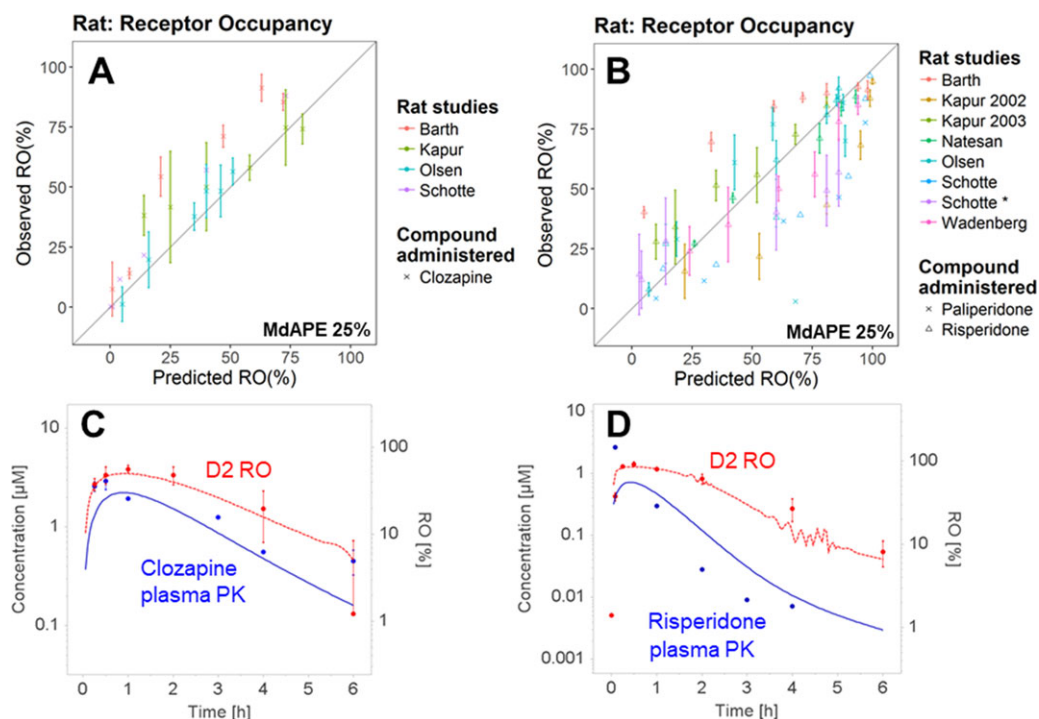
Figure 3. Prediction performance of the rat PBPK model on D2 receptor occupancy (RO) after administration of clozapine (A) and risperidone and paliperidone (B) in multiple studies. Two examples are shown in (C) and (D). Observed (red dots) and predicted (red broken lines) D2 RO time profiles were obtained after subcutaneous administration of clozapine (C) and risperidone (D) according to the study of Olsen et al.[53] The observed (blue dots) and predicted (blue solid lines) plasma concentration time profiles are also presented. Details of each study are summarized in Table S3 (risperidone) and Table S4 (clozapine). In (D) the oscillation of the simulated RO values at around Time = 4 hours could be caused by a numerical instability, which is further explained and with suggestion provided in Figure S3.

administration was applied (Tables S3 and S4), and the sudden surge in plasma concentration might have saturated the in vivo drug clearance, thus possibly inflating the observed plasma concentration and RO.

The performance of the rat PBPK-RO model was demonstrated with the representative examples in the study by Olsen et al,[53] which reported the time profiles of both the plasma PK and D2 RO time profiles after clozapine (Figure 3C) and risperidone (Figure 3D) administration. A MoBi file that contains the PBPK-RO model is provided in the supplemental materials.

Prediction Accuracy of Plasma PK in the Human PBPK Model

A total of 16 data sets (8 for clozapine/norclozapine and 8 for risperidone/paliperidone) from 16 studies were processed and compared to the simulated human plasma PK.

For clozapine (Figure 4A) and the metabolite norclozapine (Figure 4C), the human PBPK model adequately predicted their plasma PK. Nevertheless, the model overestimated the clozapine concentration and underestimated the norclozapine plasma PK observed in Fadiran et al,[46] which contained only 1 subject. A possible explanation is that the subject had a relatively fast metabolism of clozapine to norclozapine.

For risperidone (Figure 4B) and the metabolite paliperidone (Figure 4D), the human PBPK model reasonably predicted the plasma concentration. However, at the very low concentrations (especially when <3 nM), there were underestimations for both risperidone and paliperidone. These plasma concentrations were mostly measured at a late time point (>10 hours after the single-dose administration). In addition, the model underestimated the concentrations of both the parent risperidone and its metabolite paliperidone in Belotto's study in Brazil.[54] A possible explanation is that the absorption of the orally administered risperidone and the paliperidone formed in intestine was enhanced due to a suppressed P-gp efflux activity at the intestine. Concentration of P-gp in intestinal epithelial cells is linked to a C3435T polymorphism of the human MDR1 gene and is substantially lower in people with the T/T genotype (higher frequency in whites) than those with the C/C genotype (higher frequency in Africans).[55] The Brazil population is mainly composed of whites (52%), followed by browns (ie, mixed-race, 36%) and blacks (11%).[56] Unfortunately, the exact ethnic background of the subjects was not mentioned in Belotto's publication. In addition to P-gp, CYP2D6 is also highly polymorphic. Poor metabolizers of CYP2D6
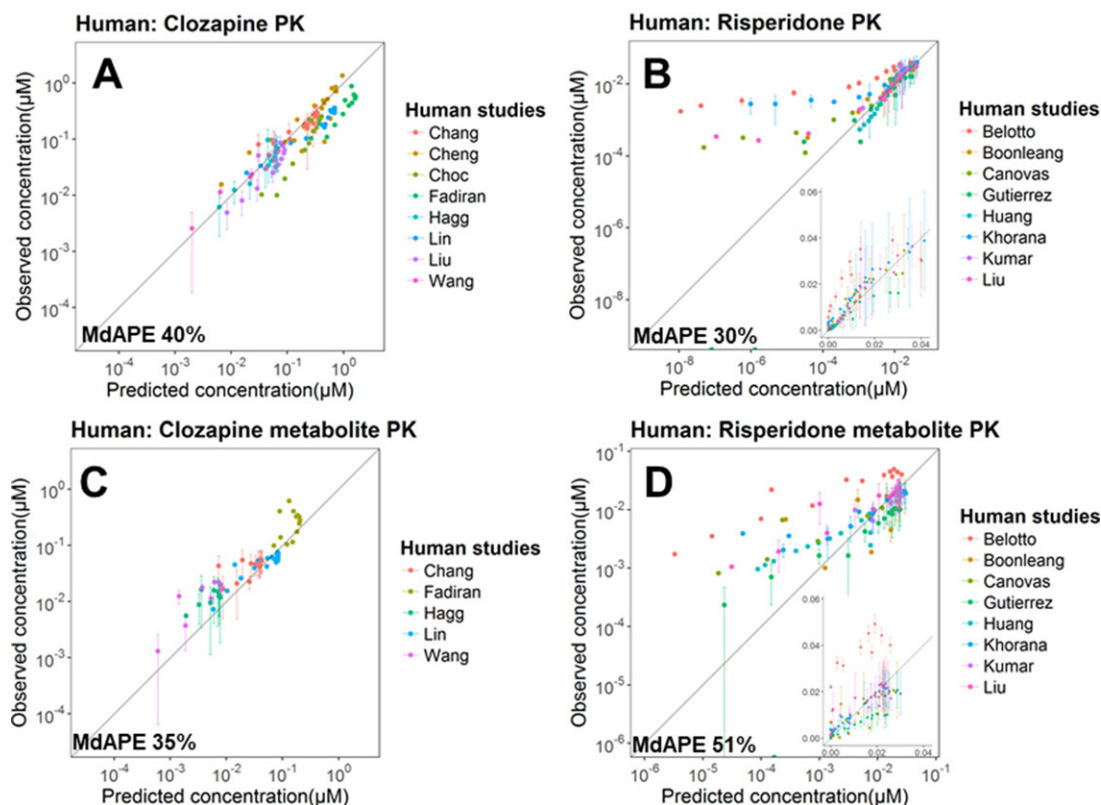
Figure 4. Prediction performance of the human PBPK model on plasma concentrations (in log scale) of clozapine (A), risperidone (B) and the active metabolites norclozapine (C) and paliperidone (D) in multiple studies. In (B) and (D) the data are also shown in normal scale in the inserts. Details of each study are summarized in Table S2.

(frequency ranges from 5.4% in Europeans, 2.5% in Africans/blacks to 0.4% in East Asians[57]) were found to have increased plasma risperidone levels,[58] which might also contribute to the higher-than-expected plasma concentrations observed in some subjects/studies. Nevertheless, the CYP2D6 genotype or phenotype was not reported in most of the clinical studies.

The performance of the model was demonstrated with the representative examples of 2 clinical trials, which reported the plasma PK profiles of both the parent drug and the metabolite after clozapine (Hägg et al[41]; Figure 5A) and risperidone (Cánovas et al[59]; Figure 5B) administration. For clozapine/norclozapine, the mean predicted time to maximum plasma concentration (T_{max}) and maximum plasma concentration (C_{max}) (normalized by clozapine dose) were 2.4/4.7 hours and 5.7/1.0 nM, which were in good agreement with those observed in the 8 collected PK studies: the observed T_{max} and C_{max} (mean, range) were (2.3, 1.0-3.0)/(3.2, 2.0-4.7) hours and (5.6, 2.9-8.1)/(1.6, 1.0-2.2) nM, respectively. For risperidone/paliperidone, the predicted PK parameters were also mostly within the range reported from the clinical trials. After oral administration of 2 mg risperidone, the mean predicted T_{max} and C_{max} were 1.1/1.9 hours and 32.5/17.6 nM, and the observed T_{max} and C_{max} (mean, range) were (1.1,

0.9-1.5)/(4.8, 4.0-5.9) hours and (37.3, 29.8-40.1)/(29.7, 22.7-49.2) nM, respectively. Although the prediction slightly underestimated the plasma concentrations of risperidone/paliperidone as discussed above, the predicted half-life of 8.9/18.1 hours were close to the observed half-life (mean, range) of (6.4, 4.7-8.6)/(18.5, 11.5-29.4) hours. The observed PK parameters are summarized in Table S2.

Prediction Accuracy of D2 RO in the Human PBPK-RO Model

A total of 10 data sets (5 for clozapine/norclozapine and 5 for risperidone/paliperidone) from 7 studies were processed and compared to the simulated human D2 RO.

For clozapine (Figure 6A), the model reasonably predicted the RO observed in all studies except the one by Catafau et al[60] in which the subjects were allowed to smoke. The systemic clearance of clozapine and norclozapine in smokers is around 50% higher than that in nonsmokers,[61] which is at least partly due to the induction of CYP1A2 activity by smoking.[62] This would have lowered the observed plasma concentration and D2 RO.

For risperidone (Figure 6B), the model could capture the general trend of the RO levels; however, a certain
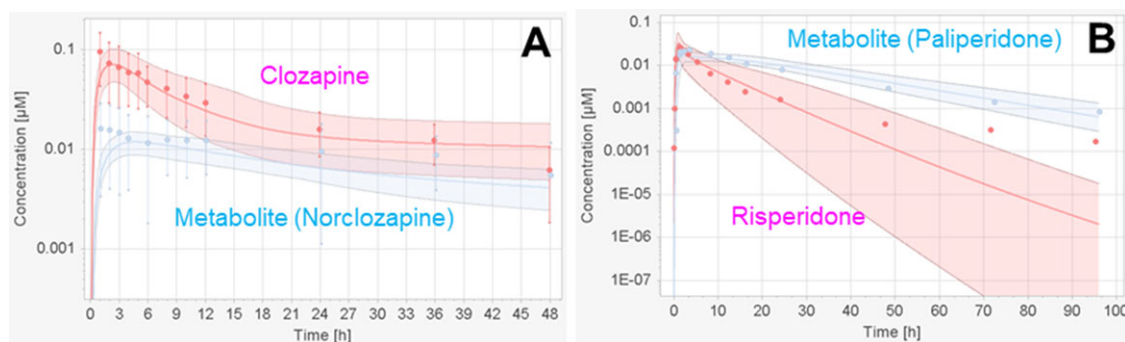
Figure 5. Two examples to show the prediction performance of the human PBPK model on plasma concentrations. Observed (red dots for parent drugs, blue dots for the generated metabolites) and predicted (arithmetic mean as solid lines with 95% confidence interval) plasma concentration time profiles were obtained after oral administration of clozapine (A) and risperidone (B) according to the studies of Hägg et al[41] and Cánovas et al,[59] respectively.
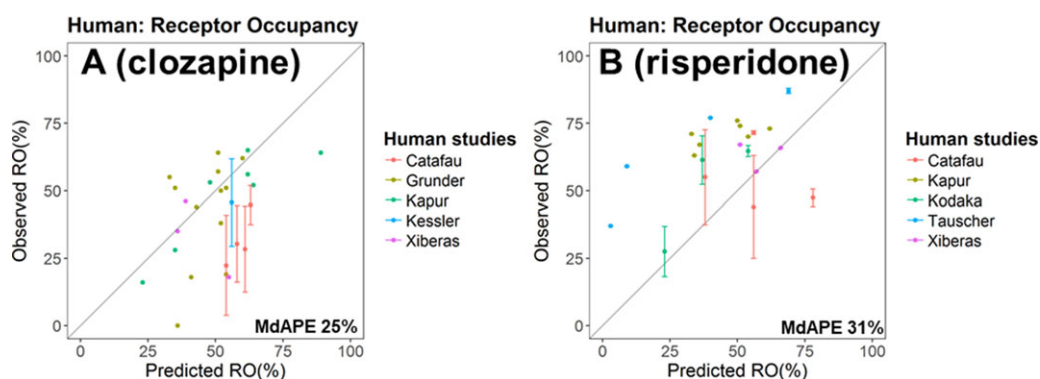

Figure 6. Prediction performance of the human PBPK model on D2 receptor occupancy (RO) after administration of clozapine (A) and risperidone (B) in multiple studies. Details of each study are summarized in Table S5 (risperidone) and Table S6 (clozapine).

degree of underestimation was apparent. Most of the RO was measured between 12 to 72 hours after the last dose (Table S5). As mentioned above, the human PBPK model might have underestimated the plasma concentrations of risperidone and paliperidone for time points later than 10 hours after the dose, which might explain the underestimation of the RO.

In the human PBPK-RO model, we assumed that the same P-gp efflux kinetics at BBB and drug-receptor–binding kinetics values were shared by all the ethnic populations because we could not identify any reports providing data on the quantitative differences in these 2 kinetic processes among different populations. Certain genetic variations (such as polymorphism in ANKK1) are associated with the D2 receptor availability in brain, and the frequency of these variants is population dependent.[63,64] However, there is no report on how such genetic variations affect the D2 RO level of an antipsychotic drug. Moreover, in most of the PK and RO clinical studies involved in this PBPK work, the genotypes of the subjects were not reported. Because the clinical trial settings differed markedly in terms of, for example, the duration of the chronic drug dosing and disease status at the time of RO evaluation, any

interstudy difference in the observed RO values could be attributed not only to the population but also to genetic variations, disease, and RO assay methods.

Application of the Human PBPK-RO Model and Extension to Non-D2 Receptors

The clinical trial by Nyberg et al[44] reported the changes in human plasma PK and RO time profile after reducing the daily risperidone dose by half from 6 mg/day to 3 mg/day in schizophrenic patients. The simulations generated by the human PBPK-RO model adequately predicted the plasma PK (Figure 7A) and D2 RO levels (Figure 7B), but again with a slight underestimation in D2 RO, as mentioned in the previous section. Our simulations are in good agreement with the observations from another clinical trial on schizophrenic patients dosed with risperidone also at 6 mg/day, of which the trough plasma concentration and D2 RO at steady state were 26 ng/mL (risperidone+paliperidone) and 64%, respectively.[65] The simulation was extended to the serotonin 5-HT_{2A} receptor by replacing the D2-k_{on} and D2-k_{off} values with those measured with 5-HT_{2A} receptors (Table S9). The simulation captured the high 5-HT_{2A} RO, which persisted even after the risperidone
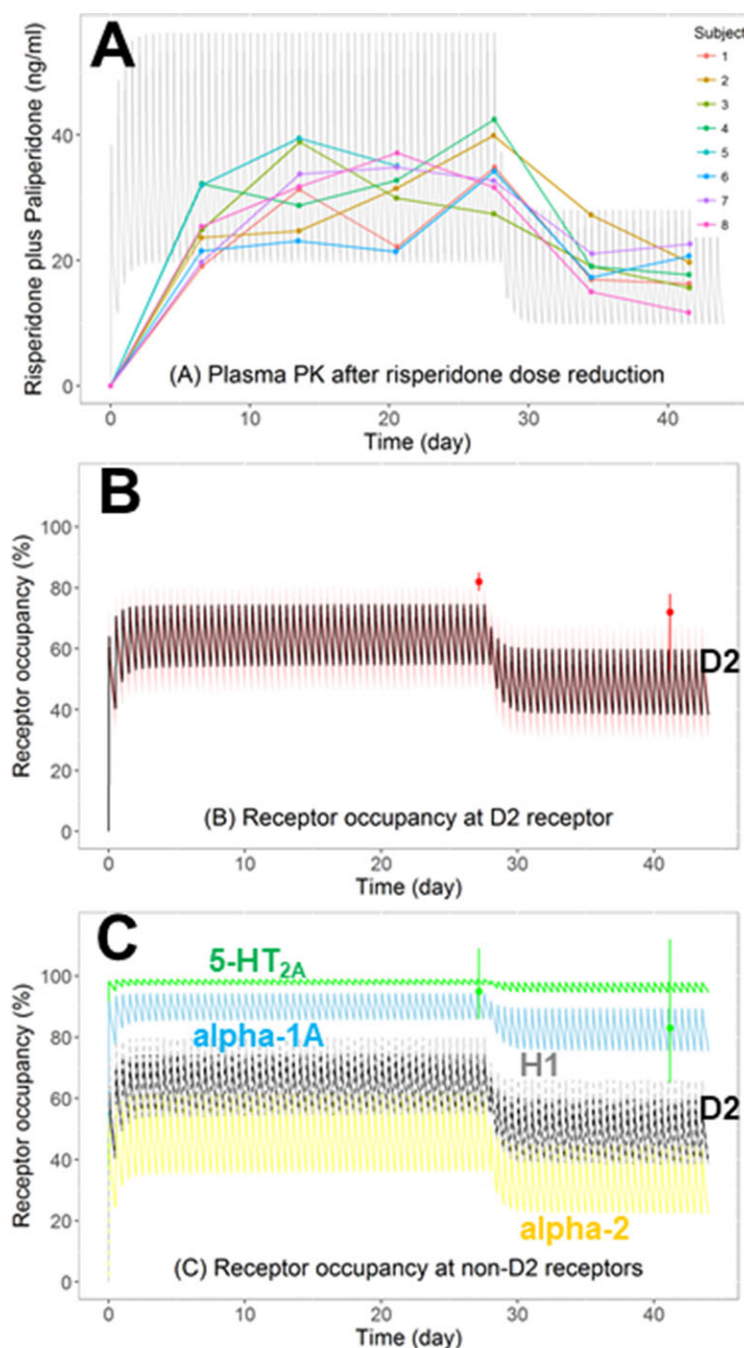
Figure 7. Predicting the impact of dose adjustment on plasma PK and receptor occupancy (RO) according to the clinical trial of Nyberg et al. Eight schizophrenic patients first received risperidone oral dose at 6 mg/day (2 times daily) for 28 days, which was then reduced to 3 mg/day (2 times daily) for 14 days. (A) Observed (dots) and predicted (arithmetic mean as gray lines) plasma concentration-time profiles of the total active moiety (risperidone plus paliperidone). (B) Observed (dots, mean ± range) and predicted (arithmetic mean as black line with 95% confidence interval) D2 RO. (C) Predicted RO-time profiles on D2 (black line) and non-D2 receptors (serotonin 5-HT$_{2A}$, green; adrenergic alpha-1A, blue; alpha-2, yellow; histamine H1, gray) in the brain. The observed RO of 5-HT$_{2A}$ (green dots, mean ± range) was also shown.

dose was halved to 3 mg/day (Figure 7C), consistent with the observations from other clinical trials showing that 5-HT$_{2A}$ RO is >90% when the risperidone dose is ≥3 mg/day.[66] The RO of other non-D2 receptors including alpha-1A, alpha-2, and histamine H1 receptors were also simulated (Figure 7C). Such simula-tion provided insights on the in vivo receptor selectivity of the drug after chronic dosing. For instance, it can be observed that the histamine H1 RO (gray line) is comparable to D2 RO (black line). The considerable binding to H1 receptor has been suggested to be responsible for the sedative effects of antipsychotics.[15]

Discussion

In this work, we developed predictive whole-body PBPK-RO models for the 2 commonly used antipsychotics risperidone and clozapine for both rats and humans, which represented an important step toward model-based prediction of RO of antipsychotics in the management of CNS disorders. Knowledge from rats on the systemic and CNS disposition of these drugs allowed the construction of a rat PBPK-RO model. By adopting a cross-species translational approach, with species differences in physiological and pharmacological parameters taken into account, the rat model was then successfully scaled to a human model, which enabled the prediction of the in vivo D2 RO level during antipsychotic treatment.

The PBPK approach allows the incorporation of the active metabolites, which is crucial in the assessment of total RO and pharmacodynamic outcomes of risperidone and clozapine. In our PBPK-RO models, we explicitly incorporated the active metabolites paliperidone and norclozapine and their characteristic pharmacological properties (such as the systemic elimination rate, P-gp transport kinetics at BBB, and the binding kinetics to D2). Using the developed PBPK models, we simulated the plasma unbound and brainECF drug concentrations (Figure S4). In rats, after risperidone and clozapine administration, the simulated brainECF concentrations of the parent drugs were at least several-fold higher than that of the metabolites, which is consistent with the in vivo observations in microdialysis studies.[26,67] In contrast, the simulated human brain unbound concentrations of the parent drug risperidone were several-fold lower than that of the metabolite paliperidone. Our simulation is in line with the observations from a PK-RO study in monkeys that after repeated dosing of risperidone only paliperidone but not risperidone was detected in the CSF, while the striatum D2 RO measured by positron emission tomography was as high as 80%.[68] In fact, while the simulated total D2 RO in Nyberg's clinical trial was about 60% on average (Figure 7B), about 50% was contributed by the metabolite paliperidone and only 10% was contributed by the parent risperidone (Figure S5). This illustrates the importance of taking into account the contribution of active metabolites in in silico prediction of brain RO levels, which is particularly relevant to the marketed antipsychotics since many of them generate active metabolites whose affinity to D2 (and other neurotransmitter receptors) and antipsychotic potency are comparable to or even higher than that of the parent drugs.[69,70] In fact, paliperidone has been marketed as an antipsychotic drug in its own right, and norclozapine has demonstrated antipsychotic effects in animal studies.[71]

The PBPK approach facilitates the translation of animal findings to humans. The clear separation of drug-specific and system-specific (species-specific) parameters allows cross-species extrapolation of the PK model according to species difference in, for example, physiological parameters and transporter expression, which is mechanistically more realistic than allometric scaling. Model validation first by (the more abundant) rat data can enhance confidence in the later human model. Recently, Johnson et al[72] reported a 5-compartment PK-RO model first in rats and then applied it to predict the human D2 RO of antipsychotics. The model underestimated the observed human D2 RO of haloperidol. The authors suggested that this could be due to in vivo metabolic conversion of haloperidol metabolites back to haloperidol in brain tissue and also active influx of haloperidol across the BBB, which had not been considered in their model. If sufficient in vitro or in vivo information about these pharmacological processes are available, they can be incorporated into the PBPK-RO model relatively easily.

New antipsychotic drugs are needed to address the unmet clinical needs, especially in the areas like improvement in negative symptoms, cognition, and safety.[73] While there are more than 20 US Food and Drug Administration–approved first- and second-generation antipsychotics, the management of schizophrenic disorders with antipsychotics is far from satisfactory. A recent meta-analysis on 167 double-blind randomized controlled trials conducted in the past 6 decades in patients with acute exacerbations of schizophrenia indicated that while approximately twice as many patients improved with antipsychotics as with placebo, only a minority experienced a good response.[74] The value of the model-informed approach at different stages of drug discovery and development for improving efficiency and decision making has been demonstrated in recent years.[75] When implementing model-informed drug development for CNS disorders[76] such as schizophrenia,[77] RO is an indispensable component of the quantitative pharmacology models (population PK-pharmacodynamic models, PBPK models, and systems pharmacology models). Our PBPK-RO model can be further extended to incorporate clinical outcomes such as the responses in Positive and Negative Syndrome Scale or Brief Psychiatric Rating Scale[7,78] (therapeutic outcome), Simpson-Angus Scale or Extrapyramidal Symptom Rating Scale[7,78] and hyperprolactinemia[79] (side effect outcomes), in which quantitative relationships between RO level and the pharmacodynamic outcome have been established. A PBPK-RO-pharmacodynamic model that connects antipsychotic dose, PK, RO, and clinical outcomes can serve as a dose selection tool to aid the development of new antipsychotics.

The application of the PBPK-RO model can be extended from D2 to other CNS receptors. All of the marketed antipsychotics have appreciable affinity to non-D2 CNS receptors,[80] and drug candidates that interact with different subtypes of serotonin receptors and acetylcholine receptors in addition to dopamine receptors are now in phase 2 and phase 3 trials.[81] It is clinically relevant to assess the receptor selectivity. For instance, clozapine is one of the antipsychotics that is most prone to induce metabolic disturbance such as weight gain and diabetes,[16] which could be attributed to its high affinity to both serotonin 5-HT$_{2C}$ and hypothalamic histamine H1 receptors.[82,83] Also, RO at cortical H1 receptors contributes to the prominent sedative effect of clozapine.[15,16] Clozapine's high affinity to both muscarinic M1 and 5-HT$_{2A}$ receptors could have contributed to its low propensity to induce motor side effects.[84] By incorporating the drug-receptor binding kinetics values (k_{on} and k_{off}), the PBPK-RO model would allow simultaneous estimation of the RO of the drug candidate on different receptors, as demonstrated by our simulation of the RO time profiles of serotonin 5-HT$_{2A}$, alpha-1A, alpha-2, and histamine H1 after risperidone administration (Figure 7C). It is worth noting that our simulation captured the persistently high RO at 5-HT$_{2A}$ (>90%) when the RO at D2 was decreasing after dose reduction,[44,85] which further demonstrated the utility of the model in the evaluation of receptor selectivity.

To accurately predict the drug concentration at the brain target site, which is the driving force for drug-receptor binding, the present PBPK model for the brain needs to be further developed. In PK-Sim, the brain is represented by the brain vessel plasma, brainECF, and brain intracellular compartments and the BBB. In the past decade, several PBPK and semi-PBPK models that incorporate additional brain distribution compartments and physiological parameters have been reported.[8] Particularly, drug in the brainECF distributes into the ventricle CSF and is carried by CSF to spinal cord and then returns to systemic circulation.[9] Therefore, compartments related to the ventricular-CSF system (lateral ventricle, third and fourth ventricles, cisterna magna, and subarachnoid space) and the blood-CSF barrier (where both passive and active drug transports present) are necessary for accurate prediction of drug concentrations within the brain. After the development of our models, PBPK CNS drug distribution models that incorporate these compartments have been published, and they adequately predict the drug concentration in brainECF, brain tissue, and CSF in both rats and humans.[86,87] Because the focus of this work was on predicting the D2 RO level, which is driven by the drug concentration at the brainECF, creating additional brain compartments relating to CSF in PK-

Sim and validating them with CSF PK data were outside the scope of this work. Nevertheless, future PBPK models should consider incorporating these CSF compartments and the blood-CSF barrier.

A prominent hurdle of applying a pure bottom-up PBPK approach, that is, to rely solely on in vitro data to predict the in vivo drug transport across the BBB and blood-CSF barrier, is the lack of reliable quantitative information on the amount of active transporters present in the CNS. P-gp expression and localization at BBB, blood-CSF barrier, and brain parenchymal cells (neurons and glial cells) all affect drug distribution to and within the brain, and they need to be explored with better proteomic studies, taking into account species differences and the effects of diseases.[88] Currently, intra- and interlaboratory variations in transporter quantification by the proteomics methods remain large.[89] Moreover, heterogenous P-gp expression among different brain regions have been reported in both rats and humans,[90,91] and this should be taken into account to allow region-specific prediction of drug concentrations in brainECF and subsequent RO levels. Furthermore, maturation of P-gp expression with postnatal age had been reported for some regions of the rat brain and was shown to be region dependent.[92] However, this was not yet considered in the present rat PBPK model because data on striatum region are not available. In spite of that, after risperidone administration, the D2 RO levels in striatum and extrastriatal regions were found to be similar in well-controlled imaging studies in humans and monkeys.[68,93] Besides P-gp expression, transport kinetics of P-gp also need to be explored with better in vitro cellular transport studies and mathematical models.[94] Standardization of the aforementioned expression and transport assays is needed to provide reliable input to the PBPK model.

In our model, P-gp is expressed only at the BBB but not at other body tissues (such as kidney, liver, intestine, and bile duct), which might have impaired the accuracy in PK prediction, especially for risperidone and paliperidone, which are strong P-gp substrates. For instance, enterohepatic circulation could prolong the drug residence time in systemic circulation, as observed in other P-gp substrates such as digoxin.[95] P-gp at proximal renal tubules, which facilitates drug excretion into urine, are also suggested to be contributing to the renal clearance of P-gp substrates.[96] Human ABCB1 (the gene encoding P-gp) polymorphism is associated with altered oral bioavailability and renal clearance of digoxin,[97] although clinical data regarding its impact on the PK of risperidone and paliperidone remain inconclusive.[98] These factors might, at least in part, explain the underestimation of plasma PK of risperidone and paliperidone of our human PBPK model. If the P-gp expression values in these tissues become

available, they could be easily incorporated into the PBPK model.[99]

Conclusions

The present study demonstrated that PBPK modeling is a useful approach to predict RO time profiles, particularly for CNS drugs since the PBPK-RO model could incorporate the active transport processes at the BBB and also the multiple CNS receptors that the drugs interact with. The PBPK approach also allows the translation of knowledge from preclinical studies to the clinical setting and enhance the confidence in the final human PBPK-RO model. The rat and human PBPK-RO models developed could be applied to estimate the RO at D2 receptors and non-D2 receptors of current antipsychotics at different doses. The models can also provide guidance to dose selection, efficacy, and safety assessments and decision making during the preclinical and clinical development of new antipsychotics.

Acknowledgments

Declaration of Conflicting Interests

Data Accessibility Statement

The data used in this manuscript are not accessible.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.