Exploratory Translational Modeling Approach in Drug Development to Predict Human Brain Pharmacokinetics and Pharmacologically Relevant Clinical Doses

W. Kielbasa and R. E. Stratford, Jr.1

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana (W.K., R.E.S.)

Received October 31, 2011; accepted January 27, 2012

ABSTRACT:

The central nervous system (CNS) pharmacokinetics (PK) of drugs that have pharmacological targets in the brain are not often understood during drug development, and this gap in knowledge is a limitation in providing a quantitative framework for translating nonclinical pharmacologic data to the clinical patient population. A focus of translational sciences is to improve the efficiency of clinical trial design via a more judicious selection of clinical doses on the basis of nonclinical data. We hypothesize that this can be achieved for CNS-acting drugs based on knowledge of CNS PK and brain target engagement obtained in nonclinical studies. Translating CNS PK models from rat to human can allow for the prediction of human brain PK and the human dose-brain exposure relationship, which can provide insight on the clinical dose(s) having potential brain activity and target engagement. In this study, we explored the potential utility of this translational approach using rat brain microdialysis and PK modeling techniques to predict human brain extracellular fluid PK of atomoxetine and duloxetine. The results show that this translational approach merits consideration as a means to support the clinical development of CNS-mediated drug candidates by enhancing the ability to predict pharmacologically relevant doses in humans in the absence of or in association with other biomarker approaches.

Introduction

Drug development archetypes for transitioning central nervous system (CNS) molecules into clinical testing involve identification of target brain activity in nonclinical pharmacology models, but often the pharmacokinetics (PK) of such drugs in the brain are not understood. The lack of knowledge of brain PK, which influences the centrally mediated effect at the target, is a limitation in providing a quantitative framework for translating nonclinical pharmacologic data to the clinical patient population (Westerhout et al., 2011). Owing to the effectiveness of the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCB) in limiting access of drugs to the CNS, the use of plasma concentrations to predict brain concentrations for drugs can be misleading. Furthermore, nonhomogeneous drug distribution within the brain, as a result of drug binding to brain tissue and the presence of influx and efflux carrier-mediated transport across the BBB and BCB and brain cell membranes, complicates the prediction of drug concentrations in the brain extracellular fluid (bECF), the site of action for CNS drugs whose targets are in the extracellular space. To address these complexities of CNS drug development, approaches such as positron emission tomography (PET) or single photon emission computed tomography imaging to measure receptor occupancy (RO) (Tauscher and Kapur, 2001) and measurement of drug concentrations in cerebrospinal fluid (CSF) (de Lange and Danhof, 2002; Shen et al., 2004; Lin, 2008) have been used; however, limitations exist preventing consistent application of these approaches. Notable among these limitations is the lack of a suitable tracer ligand to conduct imaging studies and the technical complexities associated with CSF sampling in humans. Application of biomarker approaches that measure CNS target engagement, which are based on proteomics or metabonomics, have received much attention; however, for novel targets, development of a suitable biomarker often proceeds in parallel with drug development, thus lessening the degree to which the biomarker can impact development.

Pharmacokinetic modeling plays an essential role in the process of drug development, yet cogent arguments have been made regarding the need to be more intentional about expanding its role to one of a continuous model-based approach applied across the drug development continuum to increase the efficiency and effectiveness of drug development (Zhang et al., 2008). Incorporation of pharmacokinetic-pharmacodynamics approaches, such as translational modeling or translational drug discovery, has the potential to facilitate the development of CNS drugs and CNS-targeting drugs.

This work was sponsored by Eli Lilly and Company.

1 Current affiliation: Xavier University of Louisiana, College of Pharmacy, Division of Basic Pharmaceutical Sciences, New Orleans, Louisiana.

ABBREVIATIONS: CNS, central nervous system; BBB, blood-brain barrier; BCB, blood-cerebrospinal fluid barrier; bECF, brain extracellular fluid; ECF, extracellular fluid; NE, norepinephrine; DHPG, 3,4-dihydroxyphenylglycol; CSF, cerebrospinal fluid; PK, pharmacokinetics; hNET, human norepinephrine transporter inhibition constant; NET, norepinephrine transporter; hSERT, human serotonin transporter inhibition constant; Ks, brain concentration associated with brain cells; Kp, whole-brain concentration after duloxetine infusion; Kd, brain extracellular fluid concentration; VECF, brain extracellular fluid volume; VBCB, brain cell volume; Vt, total brain volume; Cp, unbound duloxetine plasma concentration; Cb, plasma concentration; Cunbound, unbound brain cell concentration; CSF, duloxetine CSF concentration; fup, unbound plasma fraction; fb, unbound brain fraction; PET, positron emission tomography; QD, once daily; RO, receptor occupancy.

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namic principles into animal models of CNS drug action has been advocated as a comprehensive approach to guide the selection of clinical candidates with a high probability of target engagement (de Lange et al., 2005). An additional goal of this translational sciences approach is to improve the efficiency of clinical trial design through a more rational selection of doses based on knowledge of the relationship between dose, PK, and target engagement obtained in nonclinical studies. We are interested in the application of quantitative brain microdialysis as a translational tool to support clinical development of CNS drugs. Brain microdialysis in rats can be used to measure drug concentrations in bECF, and these data can support the development of brain PK models that quantitatively relate effect-site concentrations to plasma concentrations. Translating preclinical brain PK models to humans using allometric principles allows for the prediction of human brain PK and the human dose-brain exposure relationship, which adds perspective on potential brain activity and target engagement when used in context with information on drug-receptor potency. To explore the potential utility of this translational approach, we evaluated duloxetine and atomoxetine because the targets for these molecules are in the brain (centrally acting), and the human plasma PK and clinical effective doses are known. Atomoxetine [(+)-N-methyl-3-phenyl-3-(o-toloylxy)-propylamine] is a selective norepinephrine (NE) transporter inhibitor (Bymaster et al., 2002) approved as a therapeutic agent for the treatment of attention deficit/hyperactivity disorder. Duloxetine [(+)-(S)-N-methyl-3-(naphthalen-1-yl oxy)-3-(thiophen-2-yl)propan-1-amine] is an inhibitor of both serotonin and norepinephrine reuptake transporters (Wong, 1998; Pitkäsa, 2000) approved for the treatment of major depressive disorder, generalized anxiety disorder, fibromyalgia, chronic musculoskeletal pain, and diabetic neuropathy.

We previously reported on a CNS PK model of atomoxetine in rats using brain microdialysis (Kielbasa et al., 2009). In this study, we applied a rat-to-human translational approach to predict human beECF PK of atomoxetine and duloxetine from microdialysis studies and CNS PK models developed from rats. The investigational approaches, results, and applications are discussed herein.

Materials and Methods

Methods for atomoxetine were described previously (Kielbasa et al., 2009). In brief, methods for duloxetine experiments are described below.

Drugs and Chemicals. Duloxetine HCl and 13CD3-duloxetine, the retrodialysis calibrator for PK experiments and stable label of duloxetine, were synthesized at Eli Lilly and Company (Indianapolis, IN). The perfusion solution consisted of a microdialysis buffer (Kielbasa et al., 2009) with bovine serum albumin (0.5%) and 100 ng/ml 13CD3-duloxetine. The intravenous loading dose of 3 mg/kg duloxetine was given over 0.5 h followed by a 4.1 mg·h·kg−1·kg−1 infusion for 7.5 h to achieve a targeted duloxetine plasma concentration of 600 ng/ml. Microdialysis samples (30 μl) were collected every 0.5 h beginning from the start of the loading dose. At 0.25 h, blood sample (0.15 ml) collections were initiated and taken every 0.5 h thereafter. At 8 h, the intravenous infusion and the perfusion solution were stopped, and animals were killed by CO2 asphyxiation.

Duloxetine Pharmacokinetic Study Design. The perfusion solution (containing 13CD3-duloxetine) was initiated through the microdialysis probe at 1 μl/min for 0.5 h before and during the 8-h intravenous administration of duloxetine. An intravenous loading dose of 3 mg/kg duloxetine was given over 0.5 h followed by a 4.1 mg·h·kg−1·kg−1 infusion for 7.5 h to achieve a targeted duloxetine plasma concentration of 600 ng/ml. Microdialysis samples (30 μl) were collected every 0.5 h beginning from the start of the loading dose. At 0.25 h, blood sample (0.15 ml) collections were initiated and taken every 0.5 h thereafter. At 8 h, the intravenous infusion and the perfusion solution were stopped, and animals were killed by CO2 asphyxiation.

Rat Pharmacokinetic Analysis of Duloxetine. The loss (L) of 13CD3-duloxetine across the microdialysis probe at each dialysate collection time was calculated using the following equation:

\[
L = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}}
\]

where \(C_{\text{in}}\) is the concentration of 13CD3-duloxetine in the perfusate (100 ng/ml) and \(C_{\text{out}}\) is the concentration of 13CD3-duloxetine in the dialysate. At each dialysate collection period, the duloxetine beECF concentration was calculated using the following equation:

\[
C_{\text{ECF}} = \frac{C_{\text{in}}}{R}
\]

where, \(C_{\text{in}}\) is the measured concentration of duloxetine in the dialysate at time \(t\) and \(R\) is the recovery of duloxetine, which is assumed to be equal to the loss of the retrodialysis calibrator, 13CD3-duloxetine. Before PK experiments, separate in vitro and in vivo experiments were conducted to confirm similar loss of duloxetine and 13CD3-duloxetine across the microdialysis probe (data not shown).

The duloxetine concentration associated with brain cells (\(C_{\text{BC}}\)) was calculated using the following equation:

\[
C_{\text{BC}} = \frac{C_{\text{f}} \times V_{\text{f}} - C_{\text{ECF}} \times V_{\text{ECF}}}{V_{\text{fBC}}}
\]

where \(C_{\text{f}}\) is the whole-brain concentration after duloxetine infusion, \(C_{\text{ECF}}\) is the brain extracellular fluid concentration, \(V_{\text{f}}\) is the brain extracellular fluid volume, \(V_{\text{BC}}\) is the brain cell volume, and \(V_{\text{fBC}}\) is the total brain volume. Values for \(V_{\text{ECF}}\) and \(V_{\text{fBC}}\) were assumed to be 0.00029 and 0.00099 liters, respectively (Mahar Doan and Boje, 2000). The sum of \(V_{\text{ECF}}\) and \(V_{\text{fBC}}\) was assumed to be equal to \(V_{\text{f}}\) (Scism et al., 2000).

The unbound duloxetine plasma concentration (\(C_{\text{up}}\)) was determined by multiplying the measured duloxetine plasma concentration (\(C_{\text{f}}\)) by \(f_{\text{up}}\). Duloxetine unbound brain cell concentration (\(C_{\text{unBC}}\)) was determined by multiplying \(C_{\text{BC}}\) by \(f_{\text{unBC}}\). The duloxetine CSF concentration (\(C_{\text{CSF}}\)) and \(C_{\text{ECF}}\) were considered to be unbound because duloxetine binding to proteins in CSF and beECF
and brain cell compartments, described using differential equations as follows: The CNS compartment, the latter of which was divided into CSF, bECF, and a compartment, described using differential equations as follows:

\[
\frac{dA_{CB}}{dt} = -\frac{CL_{BC}}{V_{BC}}A_{CB} - \frac{Q_{bECF}}{V_{bECF}}A_{bECF} - \frac{Q_{CSF}}{V_{CSF}}A_{CSF} + \frac{Q_{uPABC}}{V_{uPABC}}A_{uPABC} + \frac{Q_{uPAP}}{V_{uPAP}}A_{uPAP}
\]

\[
\frac{dA_{ECF}}{dt} = \frac{CL_{ECF}}{V_{ECF}}A_{ECF} - \frac{CL_{ECF-CSF}}{V_{ECF}}A_{ECF-CSF} + \frac{Q_{uBC}}{V_{uBC}}A_{uBC}
\]

\[
\frac{dA_{BC}}{dt} = -\frac{CL_{BC}}{V_{BC}}A_{BC} - \frac{CL_{BC-ECF}}{V_{ECF}}A_{BC-ECF}
\]

The unbound duloxetine plasma concentrations were modeled for use in model predictions, therefore, amounts, volumes, and parameters are described as unbound terms. Terms were represented as follows: \(A_{ECF}\) was the amount in ECF, \(A_{bECF}\) was the amount in bECF, \(A_{CSF}\) was the amount in CSF, \(A_{BB}\) was the amount bound to plasma protein, \(A_{BC}\) was the amount associated with the brain cell, \(CL_{BC}\) was the plasma clearance, \(Q_{bECF}\) was the distributional clearance at the BCB, \(Q_{CSF}\) was the distributional clearance at the BBB, \(CL_{ECF-CSF}\) was the clearance from the ECF to the CSF, \(CL_{BC-ECF}\) was the clearance from the ECF to the brain cell, \(CL_{ECF-CSF}\) was the clearance from the brain cell to the ECF, and \(V_{uBC}\) was the plasma volume of distribution. Model terms \(V_{ECF}\) (0.00099 liters), \(V_{bECF}\) (0.00025 liters), and \(V_{CSF}\) (0.00029 liters) represent the volume of the brain cell; CSF and ECF, respectively, and were fixed in the PK model (Segal, 1993; Mahar Doan and Boje, 2000; Shen et al., 2004).

Rat PK Model Development of Duloxetine. Pharmacokinetic modeling was performed using NONMEM software (version V, level 1.1; GloboMax LLC). The PK model (Fig. 1) consisted of a systemic compartment, a CNS compartment, the latter of which was divided into CSF, bECF, and unbound brain cell concentrations, described using differential equations as follows:

\[
\begin{align*}
\frac{dA_{ECF}}{dt} & = \frac{CL_{ECF}}{V_{ECF}}A_{ECF} - \frac{CL_{ECF-CSF}}{V_{ECF}}A_{ECF-CSF} + \frac{Q_{uBC}}{V_{uBC}}A_{uBC} \\
\frac{dA_{CSF}}{dt} & = \frac{Q_{bECF}}{V_{bECF}}A_{bECF} + \frac{Q_{CSF}}{V_{CSF}}A_{CSF} + \frac{Q_{uPABC}}{V_{uPABC}}A_{uPABC} + \frac{Q_{uPAP}}{V_{uPAP}}A_{uPAP} \\
\frac{dA_{bECF}}{dt} & = -\frac{CL_{bECF-CSF}}{V_{bECF}}A_{bECF-CSF} + \frac{CL_{bECF}}{V_{bECF}}A_{bECF} \\
\frac{dA_{BC}}{dt} & = -\frac{CL_{BC}}{V_{BC}}A_{BC} - \frac{CL_{BC-ECF}}{V_{ECF}}A_{BC-ECF} \\
\end{align*}
\]

The residual error, \(\hat{\epsilon}\), was assumed to be negligible. The \(C_{ECF}\) and \(C_{bECF}\) from each rat were determined by averaging the data collected from 4 to 8 h after duloxetine administration, and the \(C_{CSF}\) and \(C_{BC}\) were determined at 8 h after duloxetine administration. The following ratios were calculated in each rat when applicable: \(C_{bECF}/C_{ECF}\), \(C_{ECF}/C_{bECF}\), \(C_{uPABC}/C_{ECF}\), \(C_{ECF}/C_{bECF}\), and \(C_{CSF}/C_{bECF}\).

During model development, \(C_{uPABC}\) was either fixed to the calculated value or estimated. Fixing \(C_{uPABC}\) provided a better fit of the data to the model.

The model incorporated the unidirectional transport from ECF to CSF (\(k_{ECF-CSF}\)). The first-order rate constant for flow from ECF to CSF \(k_{ECF-CSF}\) was estimated to be 0.084 h\(^{-1}\) (Szentisťávnyi et al., 1984). A calculated value for \(CL_{ECF-CSF}\) is 0.000024 l/h (\(CL_{ECF-CSF} = k_{ECF-CSF} \times V_{ECF}\)). During model development, \(CL_{ECF-CSF}\) was either fixed to the calculated value or estimated. Fixing \(CL_{ECF-CSF}\) provided a better fit of the data to the model (data not shown).

Interindividual variability in PK parameters was included in the model, as described by the following equations: \(P_i = P_{ij}(1 + \sigma_{ij})\), where \(P_{ij}\) is the \(i\)th parameter for the \(j\)th individual, \(P_i\) is the typical population parameter estimate for the \(j\)th parameter, and \(\sigma_{ij}\) is the deviation of \(P_i\) from \(P_j\) for the \(i\)th parameter for the \(j\)th individual. For \(\sigma_{ij}\), it is assumed that the parameter is normally distributed with a mean zero and a variance \(\sigma^2\) to be estimated. Residual error was estimated using a proportional error model as described by the following equation: \(C_{ij} = \text{Pred}_{ij}(1 + \sigma_{ij})\), where \(C_{ij}\) and \(\text{Pred}_{ij}\) are the measured and model-predicted concentration at the \(i\)th sampling time in the \(j\)th individual, respectively. The residual error, \(\sigma\), is a random variable normally distributed with mean zero and estimated variance \(\sigma^2\). The residual error describes errors arising from assay errors, sampling inaccuracies, and model misspecification.

The modeling data set consisted of 207, 166, 8, and 13 data points for unbound plasma, bECF, CSF, and unbound brain cell concentrations, respectively, from 13 rats. The criteria used for the model evaluation were the fit between observed and predicted concentrations, the parameter’s percentage S.E. of the estimate, the randomization of weighted residual concentrations versus time between observed and predicted concentrations, and the objective function.

Prediction of Human Atomoxetine and Duloxetine bECF Pharmacokinetics. Simulations were conducted using NONMEM software (version VII, level 1.2; GloboMax LLC) in a population consisting of 1000 humans to generate plasma and bECF PK profiles after oral administration of 40 and 80 mg of atomoxetine once daily (QD) and 5 and 60 mg of duloxetine QD. The human unbound plasma binding for atomoxetine (\(f_{ub} = 0.013\)) (Sauer et al., 2003) and duloxetine was incorporated in the modeling such that only unbound drug in plasma was available to cross the BBB and BCB.

Rat PK parameters were scaled to humans using allometric principles based on the following equation:

\[
P_h = P_r \times \left(\frac{W_h}{W_r}\right)^{0.75}
\]

where \(P_h\) is the scaled human parameter, \(P_r\) is the model-predicted parameter in rat, \(W_h\) is the average human brain weight (1.35 kg), and \(W_r\) is the average rat brain weight (0.0015 kg). On the basis of an adult brain volume of 1.35 liters, the estimated human \(V_{ECF}\) was 0.31 liters and \(V_{BC}\) was 1.04 liters (Segal, 1993). The human \(V_{CSF}\) was fixed in the model to be 0.16 liters (Shen et al., 2004).

Statistical Analysis. Unless otherwise indicated, all data shown are represented as the mean and S.D. For graphical presentation, total (bound + unbound) plasma concentrations are shown.

Results

Plasma and Brain Binding Assessment. At 3 \(\mu\)M, duloxetine was highly bound to plasma and brain with an unbound fraction of 0.047 ± 0.002 (\(n = 3\)) and 0.00283 ± 0.00012 (\(n = 3\)), respectively.

Duloxetine Pharmacokinetics in Rats. Figure 2 shows the duloxetine total plasma, CSF, bECF, and whole-brain concentrations after duloxetine administration (\(n = 13\)). The \(C_p\) and \(C_{ECF}\) were 762 ± 421 ng/ml (\(n = 13\)) and 19 ± 9 ng/ml (\(n = 13\)), respectively. The \(C_{h}\) and \(C_{ECF}\) were determined by averaging the data collected from 4 to 8 h after duloxetine administration, and the \(C_{CSF}\) and \(C_{BC}\) were determined at 8 h after duloxetine administration. The following ratios were calculated in each rat when applicable: \(C_{bECF}/C_{ECF}\), \(C_{ECF}/C_{bECF}\), \(C_{uPABC}/C_{ECF}\), \(C_{ECF}/C_{bECF}\), and \(C_{CSF}/C_{bECF}\).
C_{CSF} were 3467 ± 755 ng/ml (n = 13) and 29 ± 8 ng/ml (n = 9). Duloxetine concentration ratios were C_{PL}/C_{E} = 6 ± 3 (n = 13), C_{ECF}/C_{E} = 0.027 ± 0.012 (n = 13), and C_{CSF}/C_{E} = 0.043 ± 0.017 (n = 9). The C_{E}/C_{ECF} and C_{CSF}/C_{ECF} were 0.6 ± 0.3 (n = 13) and 0.9 ± 0.4 (n = 9), respectively. The C_{BC} was 233 ± 142 (n = 13) and 128 ± 76 (n = 9), respectively. The C_{BC} was 4477 ± 976 ng/ml (n = 13), and the C_{BC}/C_{ECF} and C_{BC}/C_{CSF} were 301 ± 183 (n = 13) and 0.9 ± 0.5 (n = 13), respectively.

The rat PK model incorporates both physiologically based parameters and compartmental PK parameters. We used compartmental PK estimation of clearance at the BBB to inform the nature (passive, active) and extent of drug transport across the BBB, which also accounts for potential transporter expression or activity at the BBB. The CNS PK data obtained for atomoxetine (Kielbasa et al., 2009) or duloxetine fit better to a model parameterized in terms of distribution parameters and compartmental PK parameters. We used compartmental PK parameters determined previously (Kielbasa et al., 2009) are shown to support the translational modeling approach for atomoxetine herein.

Prediction of Atomoxetine and Duloxetine Human bECF Pharmacokinetics. Table 2 shows the atomoxetine and duloxetine human PK parameters used as inputs in the translational models to generate the human simulations (Lobo et al., 2009). Atomoxetine exposure is substantially influenced by cytochrome P450 2D6 (CYP2D6) polymorphism (Farid et al., 1985). The clinical atomoxetine PK parameters shown in Table 2 were estimated from subjects that were extensive metabolizers of the CYP2D6 enzyme.

Figure 3 illustrates the predicted PK profiles after administration of 40 and 80 mg of atomoxetine QD and the in vivo human norepinephrine transporter inhibition constant (hNET K_i = 1.1 ng/ml). At 80 mg of atomoxetine, the median plasma and bECF maximal concentrations were approximately 561 and 5 ng/ml, respectively. The median hNET K_i value is lower than or contained within the 90% prediction interval of the bECF concentrations for both doses of atomoxetine. The t_{1/2} of atomoxetine in plasma and bECF was approximately 4 and 13 h, respectively. The plasma-to-bECF ratios for atomoxetine were approximately 6 and 0.17 ng/ml, respectively. The bECF prediction interval of the bECF concentrations for both doses of atomoxetine at 5 mg and 60 mg of duloxetine QD and the hNET K_i was 2 ng/ml and the in vivo human serotonin transporter inhibition constant (hSERT K_i = 0.07 ng/ml). At 60 mg of duloxetine, the median plasma and bECF C_{max} were approximately 70 and 2 ng/ml, respectively. The bECF concentration was essentially unchanged during the dosing interval and approximately 29 times greater than the hSERT K_i and similar to the hNET K_i. At 5 mg of duloxetine, the plasma and bECF C_{max} were approximately 6 and 0.17 ng/ml, respectively. The bECF

### TABLE 1

Model-estimated rat pharmacokinetic parameters of atomoxetine and duloxetine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atomoxetine</th>
<th>Variability (% SEE)</th>
<th>Duloxetine</th>
<th>Variability (% SEE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL_u, l/h</td>
<td>14.5</td>
<td>(8.69)</td>
<td>35.6</td>
<td>(17.6)</td>
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<td>V_u, liters</td>
<td>6.92</td>
<td>(30.8)</td>
<td>33.2</td>
<td>(15.7)</td>
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<td>Q_{BC}, l/h</td>
<td>0.0009909</td>
<td>(29.9)</td>
<td>0.0000527</td>
<td>(31.7)</td>
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<tr>
<td>Q_{BBM}, l/h</td>
<td>0.00110</td>
<td>(34.5)</td>
<td>0.000160</td>
<td>(39.8)</td>
</tr>
<tr>
<td>CL_{ECF-CSF}, l/h</td>
<td>0.001619</td>
<td>(26.2)</td>
<td>0.000124</td>
<td>(26.2)</td>
</tr>
<tr>
<td>CL_{ECF-BC}, l/h</td>
<td>0.02216</td>
<td>(25.1)</td>
<td>0.00168</td>
<td>(41.8)</td>
</tr>
<tr>
<td>CL_{BC-ECF}, l/h</td>
<td>0.00934</td>
<td>(25.7)</td>
<td>0.00249</td>
<td>(56.0)</td>
</tr>
<tr>
<td>V_{ECF}, liters</td>
<td>0.000990</td>
<td>(fixed)</td>
<td>0.000999</td>
<td>(fixed)</td>
</tr>
<tr>
<td>V_{BC}, liters</td>
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<td>(fixed)</td>
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<td>(fixed)</td>
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<tr>
<td>Q_{BC}, l/h</td>
<td>0.00290</td>
<td>(9.09)</td>
<td>0.00290</td>
<td>(9.09)</td>
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<tr>
<td>σ_{bprop}</td>
<td>34.7</td>
<td>(9.09)</td>
<td>28.8</td>
<td>(14.6)</td>
</tr>
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</table>

% SEE: percentage S.E. of the estimate; σ_{bprop}: Proportional residual error.

### TABLE 2

Pharmacokinetic parameters of atomoxetine and duloxetine used in translational models to predict human brain extracellular pharmacokinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atomoxetine</th>
<th>Clinical, Scaled from Rat</th>
<th>Duloxetine</th>
<th>Clinical, Scaled from Rat</th>
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</thead>
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<tr>
<td>k_i, h^{-1}</td>
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<td>CL/F, l/h</td>
<td>20.6</td>
<td>45.1</td>
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<td>V/F, liters</td>
<td>121</td>
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<tr>
<td>V_{ECF}, liters</td>
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<td>0.16</td>
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<td>V_{BC}, liters</td>
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<tr>
<td>V_{BC}, liters</td>
<td>1.04</td>
<td>1.04</td>
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<tr>
<td>CL_{ECF-CSF}, l/h</td>
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<td>CL_{ECF-BC}, l/h</td>
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<tr>
<td>Q_{BBM}, l/h</td>
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<tr>
<td>CL_{ECF-CSF}, l/h</td>
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<td>0.004</td>
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<td>CL_{ECF-BC}, l/h</td>
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<tr>
<td>CL_{BC-ECF}, l/h</td>
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<td>α-CL/F, %</td>
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<td>α-V/F, %</td>
<td>65.6</td>
<td>96.6</td>
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</table>

α, intersubject variability; NE, not estimated.
concentration was approximately 2 to 3 times greater than the hSERT \( K_i \), but the hNET \( K_i \) was approximately 12 times greater than the bECF concentration over the 24-h dosing interval. The \( t_{1/2} \) of duloxetine in plasma and bECF was approximately 16 and 92 h, respectively. The AUC\(_{\text{pl}}/\text{AUC}_{\text{ECF}} \) and \( C_{\text{max, pl}}/C_{\text{max, ECF}} \) ratios were approximately 28 and 34, respectively, irrespective of the dose. These predictions show that 5 and 60 mg of duloxetine would be expected to have bECF concentrations substantially above the hSERT \( K_i \) and in the target range of the hNET \( K_i \) at 60 mg only. At 5 mg, duloxetine bECF concentrations are substantially below the hNET \( K_i \).

**Discussion**

**Pharmacokinetics of Duloxetine.** On the basis of the \( C_{\text{ECF}}/C_{\text{pl}} = 0.6 \) and \( C_{\text{ECF}}/C_{\text{B}} = 0.9 \) and being within 3 times of unity (Doran et al., 2005; Maurer et al., 2005; Kalvass et al., 2007), we conclude that duloxetine transport across the BBB and BCB is primarily passive. Similar values for duloxetine \( f_{\text{pl}}/f_{\text{B}} \) and \( C_{\text{pl}}/C_{\text{B}} \) of 17 and 23, respectively, supports the same conclusion at the BBB (Maurer et al., 2005). Estimations of \( C_{\text{B}}, C_{\text{BC}}, \) and \( C_{\text{uBC}} \) and their comparison with \( C_{\text{ECF}} \) provide insight regarding the brain distribution of duloxetine. The \( C_{\text{B}}/C_{\text{ECF}} \) and \( C_{\text{BC}}/C_{\text{ECF}} \) ratios of 233 and 301, respectively, indicate that duloxetine does not preferentially reside in bECF, which is suggestive of nonspecific binding to brain tissue. A \( C_{\text{uBC}}/C_{\text{ECF}} = 0.9 \) indicates that duloxetine passively distributes within brain parenchyma.

**Translational Pharmacokinetic Modeling of Duloxetine and Atomoxetine.** The derived translational human PK model was used to simulate human PK in plasma and bECF, the matrix in direct contact with the hNET and hSERT. On the basis of the free drug hypothesis, it is a commonly accepted assumption that unbound drug is the entity available for interaction with drug targets. It is also assumed that unbound drug in the brain is in direct contact or in equilibrium with the site of action (de Lange and Danhof, 2002). Several key assumptions were used for this translational modeling approach. First, both drugs studied do not have an active metabolite(s), or active metabolites in sufficient concentrations that cross the BBB and BCB and interact with the target transporters to elicit pharmacologic activity. Although extensive metabolism occurs for atomoxetine (Sauer et al., 2003) and duloxetine (Lantz et al., 2003), their pharmacologic activity is not believed to be due to target engagement from metabolites. Second, hNET and hSERT \( K_i \) can be used as target concentrations that...
would be expected to have appreciable interaction at the target transporter. Third, the mechanism of transport across the BBB and BCB in rat and human is similar. In our evaluations, we show that duloxetine and atomoxetine (Kielbasa et al., 2009) transport in rats is primarily passive; therefore, we assume that this remains the case for humans. Finally, we assume that rat CNS PK model parameters can be translated accurately to humans using allometric principles based on differences in brain weight between species. To corroborate this assumption, PK parameters were compared from the scaling approach and clinical PK data after 80 mg of atomoxetine QD (Table 2). In theory, bidirectional clearances (CL\textsubscript{PL-CSF} and CL\textsubscript{CSF-PL}) and \( Q_{\text{BCB}} \) are similar if the drug primarily crosses the BCB by passive diffusion. The rat atomoxetine \( Q_{\text{BCB}} \) as shown in Table 1 was scaled to humans using eq. 10, resulting in a human \( Q_{\text{BCB}} \) prediction of 0.015 l/h. This value was similar to the actual human CL\textsubscript{PL-CSF} of 0.00825 l/h and CL\textsubscript{CSF-PL} of 0.0205 l/h shown in Table 2 (the mean of CL\textsubscript{PL-CSF} and CL\textsubscript{CSF-PL} = 0.014 l/h) and suggests that the translational scaling approach has merit for predicting the human CSF PK from rats.

Ideally, such translational CNS PK models could be used to evaluate the influence of the model parameters on the predicted human PK by simulating various scenarios with altered PK parameters (and variability). For example, the impact of efflux clearance at the human BBB (CL\textsubscript{LICT-PL}) clearance of drug from the bECF to plasma) in relation to plasma concentration may be explored if data exist suggesting the drug may be a substrate for \( P_{\text{gp}} \) in human. Simulation can lead to optimized study designs and effective management of clinical plans and strategies.

The translational approach for predicting human bECF PK is not high-throughput methodology and is not well suited for incorporation into a project flow scheme in early drug discovery. Alternatively, it is applicable in supporting candidate drugs selected for clinical investigation or to discriminate between a smaller set of compounds that have been identified as potential clinical candidates. For atomoxetine and duloxetine, a wealth of clinical PK data were available to validate the translational approach. In the context of drug development when human PK have yet to be determined, in vitro or allometric techniques could be used initially to support model development. When human systemic PK data are obtained from a clinical study, the model can then be updated.

At 40 and 80 mg of atomoxetine, the predicted human bECF concentrations are suggestive of target inhibition at the NET (Fig. 3). Putting these results in clinical context, according to the atomoxetine product label in the United States, dosing adults, children, and adolescents over 70-kg b.wt. should be initiated at 40 mg QD and increased after a minimum of 3 days to approximately 80 mg QD. After 2 to 4 additional weeks, the dose may be increased to a maximum of 100 mg QD in patients who have not achieved an optimal response. Biochemical evidence for NE reuptake inhibition can be provided through evaluation of the NE metabolite 3,4-dihydroxyphenylglycol (DHPG), and assessment of DHPG can provide insight of a drug’s effect on NET function. In humans given 80 mg of atomoxetine QD, both plasma and CSF DHPG concentrations were reduced, indicating that atomoxetine has activity at the NET (Kielbasa et al., 2006). Also, children with attention deficit/ hyperactivity disorder given atomoxetine exhibited changes in the urine DHPG/NE ratio (Montoya et al., 2011). The prediction of human bECF PK at 40 and 80 mg inhibiting hNET transport is consistent with clinical dosing recommendations and human atomoxetine biomarker data.

Human bECF concentrations of duloxetine were predicted at 5 mg QD and at a clinically efficacious dose of 60 mg QD. Because the duloxetine mechanism of action is to act at the hNET and hSERT, the human bECF PK predictions relative to in vitro potency at those transporters were examined. The predicted bECF concentrations were below the hNET \( K_i \) during the dosing interval, suggesting that 5 mg of duloxetine QD would be expected to have no or minimal activity at the hNET. Conversely, at 60 mg of duloxetine QD, the predicted bECF concentrations were similar to the hNET \( K_i \) during the entire dosing interval of 24 h. These predictions are corroboritated with published reports where target activity of duloxetine was assessed by measurements of NE and DHPG. At 60 mg of duloxetine QD, reductions of DHPG in CSF, plasma, and urine were observed (Quinlan et al., 2009). It was also demonstrated that the plasma DHPG/NE ratio was reduced significantly at 2 weeks of treatment with 80 mg of duloxetine QD, the lowest dose tested in the study (Vincent et al., 2004). Similar results were obtained when assessing NE metabolites in urine (Chalon et al., 2003). No reports of the effects of duloxetine on NE and DHPG seem to exist at 5 mg of duloxetine. In rat ECF, concentrations of atomoxetine or duloxetine that were comparable to or greater than their respective rat NET \( K_i \) resulted in increased NE concentrations (data not shown).

There is currently no validated PET tracer to measure brain NET occupancy, but brain SERT blockade by duloxetine was measured in humans using PET (Takano et al., 2006). In that study, the SERT RO at 6 h after dose was approximately 44% at 5 mg and 82% at 60 mg when duloxetine was given as a single dose. After 60 mg of duloxetine QD, the SERT RO at 6 h after dose was 84% and estimated to be approximately 78% about 24 h later, indicating little change in SERT RO during the dosing interval at steady state. Serotonin RO at 60 mg of duloxetine is consistent with clinically efficacious doses of selective serotonin reuptake inhibitors providing over 80% occupancy (Meyer et al., 2004). The predicted duloxetine bECF PK agree with the dose-dependent SERT RO findings clinically, including the ability to discriminate a 5-mg dose from the clinically effective 60-mg dose. The data show that duloxetine total plasma and predicted bECF PK profiles are distinct. The plasma \( t_{1/2} \) was approximately 16 h, as expected, and the bECF \( t_{1/2} \) was approximately 92 h, demonstrating a relatively stable profile and lesser peak-to-trough fluctuation in bECF concentrations over the dosing interval. Similar results were obtained for atomoxetine, where the plasma \( t_{1/2} \) was approximately 4 h, as expected, and the predicted bECF \( t_{1/2} \) was approximately 13 h. Interestingly, in the study by Takano et al. (2006), the duloxetine plasma concentrations declined appreciably, whereas SERT RO did not decrease to any reasonable extent during the dosing interval at steady state, which is consistent with the predicted PK profile in bECF.

These data indicate that bECF predictions for drugs with appreciable binding, such as atomoxetine and duloxetine, may be a better surrogate matrix than plasma for estimating the time course of brain target engagement. Furthermore, prediction of bECF PK in humans may be insightful when considering the clinical dose and frequency of administration.

The exploratory translational approach aims to predict the human dose-bECF concentration relationship to identify clinical doses that should engage the brain target and provide insight into early drug development about the dose and regimen to test the clinical hypotheses. Regardless of the mechanism of a centrally acting drug or the indication being investigated, without significant activity at the CNS target, a positive clinical outcome would not be expected. On the basis of the results obtained, we believe this translational approach merits consideration as a robust tool to support the clinical development of CNS-mediated drug candidates. This approach may enhance the ability to predict pharmacologically relevant doses in the absence of, or in association with, other biomarker approaches.
Acknowledgments

We acknowledge Tonya Quinlan, Siak Leng Choi, Karen Ellis Sprague, Jenny Hanes, Maria Pavlakos, and Mary E. Perron for scientific and/or technical contributions and Jennifer Witcher, Lan Ni, Thomas Raub, Stephen Hall, A. J. Allen, and Celine Goldberger for collegial support in preparing this manuscript.

Authorship Contributions

Participated in research design: Kielbasa and Stratford.

Conducted experiments: Kielbasa and Stratford.

Performed data analysis: Kielbasa and Stratford.

Wrote or contributed to the writing of the manuscript: Kielbasa and Stratford.

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Address correspondence to: W. Kielbasa, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285. E-mail: wkielbasa@lilly.com