Microdialysis Evaluation of Atomoxetine Brain Penetration and Central Nervous System Pharmacokinetics in Rats

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ABSTRACT:
A comprehensive in vivo evaluation of brain penetrability and central nervous system (CNS) pharmacokinetics of atomoxetine in rats was conducted using brain microdialysis. We sought to determine the nature and extent of transport at the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCB) and to characterize brain extracellular and cellular disposition. The steady-state extracellular fluid (ECF) to plasma unbound (uP) concentration ratio ($C_{ECF}/C_uP$ = 0.7) and the cerebrospinal fluid (CSF) to plasma unbound concentration ratio ($C_{CSF}/C_uP$ = 1.7) were both near unity, indicating that atomoxetine transport across the BBB and BCB is primarily passive. On the basis of the ratios of whole brain concentration to $C_{ECF}$ ($C_{BF}/C_{ECF} = 170$), brain cell (BC) concentration to $C_{ECF}$ ($C_{BC}/C_{ECF} = 219$), and unbound brain cell concentration to $C_{ECF}$ ($C_{uB}/C_{ECF} = 2.9$), we conclude that whole brain concentration does not represent the concentration in the biophase and atomoxetine primarily partitions into brain cells. The distributional clearance at the BBB ($Q_{BBB} = 0.00110 \ l/h$) was estimated to be 12 times more rapid than that at the BCB ($Q_{BCB} = 0.000909 \ l/h$) and similar to the clearances across brain parenchyma ($Q_{ECF-BC} = 0.00216 \ l/h$; $Q_{BC-ECF} = 0.000934 \ l/h$). In summary, the first detailed examination using a quantitative microdialysis technique to understand the brain disposition of atomoxetine was conducted. We determined that atomoxetine brain penetration is high, movements across the BBB and BCB occur predominantly by a passive mechanism, and rapid equilibration of ECF and CSF with plasma occurs.

Transport into the central nervous system (CNS) is essential for drugs that have pharmacological targets within the brain. The CNS exposure of a drug is determined by the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCB) transport processes and the kinetics governing distribution and elimination. The CNS pharmacokinetics (PK) of a drug is an important determinant in the time course and intensity of effect. Nonhomogeneous distribution within the brain can occur because of the physicochemical properties of a drug, cellular binding, or the presence of active transporters at the neuronal cell membranes. Understanding brain distribution is important because it can provide valuable insights pertaining to the pharmacological actions of a drug.

It is generally accepted that only unbound drug in plasma crosses the BBB and interacts with receptors. Equilibration across the BBB and BCB can be expressed by the steady-state extracellular fluid to plasma unbound concentration ratio ($C_{ECF}/C_{uP}$) and the cerebrospinal fluid to plasma unbound concentration ratio ($C_{CSF}/C_{uP}$), respectively. When near unity is obtained from such ratios, distribution is consistent with transport by passive diffusion, or the impact of influx and efflux transport is equal. If a ratio below unity is obtained, it is consistent with an active process limiting distribution such as BBB efflux, CSF bulk flow, or metabolism, and a value above unity is consistent with an active influx process enhancing distribution. A ratio of ±3-fold was meaningful to assess the contribution of a passive mechanism at the BBB based on unbound plasma and brain fractions of drugs (Maurer et al., 2005; Kalvass et al., 2007). In addition, the brain/ plasma ratios of P-glycoprotein knockout and wild-type animals were investigated and a ratio of ±3-fold did not seem to have much impact on the success of drugs as CNS-active compounds (Doran et al., 2005).

Atomoxetine HCl [Strattera, (−)-N-methyl-3-phenyl-3-(o-tolyl-oxo)propylamine hydrochloride] has been developed as a therapeutic agent for the treatment of attention deficit/hyperactivity disorder (ADHD), a behavioral disorder characterized by inappropriate levels of motor activity, impulsivity, distractibility, and inattention (Biederman and Faraone, 2002; Biederman, 2005). Atomoxetine inhibited binding of radioligands to clonal cell lines transfected with human norepinephrine (NE), serotonin, and dopamine transporters with dissociation constant ($K_i$) values of 5, 77, and 1451 nM, respectively, demonstrating selectivity for NE transporters. In rat brain microdialysis studies, atomoxetine increased ECF levels of NE and dopamine in the prefrontal cortex similarly but did not alter serotonin levels, leading to the hypothesis that the atomoxetine-induced increase of catecholamines in the prefrontal cortex, a region involved in attention and memory, may play a pivotal role in the therapeutic effects of atomoxetine in ADHD (Bymaster et al., 2002).

The atomoxetine ECF concentration at the NE transporter, the site where atomoxetine inhibits NE reuptake into the neuron, is key to atomoxetine pharmacological activity. The systemic PK of atomox-
etine has been well described in animals and humans (Mattiu, et al., 2003; Sauer et al., 2005); however, limited data exist regarding the disposition in the CNS where the actions of atomoxetine arise. In vitro investigations showed that atomoxetine brain permeability in multi-drug resistance 1-Madin Darby canine kidney cells and in situ brain permeability was relatively moderate compared with that for 49 other marketed drugs for CNS indications (Summerfield et al., 2007). The objective of the present study was to conduct a comprehensive in vivo evaluation of CNS penetrability and disposition of atomoxetine in rats. We sought to determine the nature and extent of transport at the BBB and BCB and to characterize brain extracellular and cellular disposition. After steady-state infusions of atomoxetine, we collected ECF using brain microdialysis and estimated the brain extracellular and cellular concentrations of atomoxetine. Plasma, CSF, and whole brain samples were also collected using conventional PK techniques and assayed for atomoxetine. A neuropharmacokinetic model was developed to characterize the CNS disposition of atomoxetine.

Materials and Methods

Drugs and Chemicals. Atomoxetine HCl and its stable label, [3H]-atomoxetine, were synthesized at Eli Lilly and Company (Indianapolis, IN). The dialysis buffer was prepared using reagent-grade materials purchased from Sigma-Aldrich (St. Louis, MO). The composition of the dialysis medium (pH 7.5) was 1.3 mM CaCl₂, 1 mM MgCl₂, 3 mM KCl, 147 mM NaCl, 1 mM Na₃HPO₄·7H₂O, and 0.2 mM NaH₂PO₄·H₂O; it was prepared in deionized water and filtered through a 0.22-μm filter. For retrodialysis calibration studies, dialysis buffer stock solutions containing 0.1 mg/ml atomoxetine HCl and [3H]-atomoxetine were prepared and stored at −70°C. Stock solutions of dialysis buffer containing 0.1 mg/ml [3H]-atomoxetine were also prepared and stored at −70°C. One day before use, an aliquot of dialysis buffer stock solution was diluted to 100 ng/ml [3H]-atomoxetine with dialysis buffer, and the systemic infusion stock solution was removed from the freezer. Both solutions were refrigerated until use. Solvents used for HPLC analysis were of reagent grade (OmniSolv; EMD Chemicals, Gibbstown, NJ).

Animal Preparation. Male Sprague-Dawley rats weighing between 275 and 325 g were surgically prepared at Charles River Laboratories, Inc. (Wilmington, MA) to support i.v. administration and arterial blood collection via femoral vein/artery cannulations. A BAS guide cannula (BAS Bioanalytical Systems, West Lafayette, IN) was implanted in the medial-prefrontal cortex (coordinates: anteroposterior, +3.2 mm; mediolateral, +0.6 mm; and dorso-ventral, −2.2 mm). Upon receipt, rats were acclimated for 3 days. One day before an experiment, a BAS-4 probe was inserted and perfused with dialysis buffer for 18 to 24 h at a flow rate of 0.2 μl/min. The live phase portion of a retrodialysis probe at a rate of 1 l/min in PK studies. Perfusion of [3H]-atomoxetine was initiated 4 h before atomoxetine administration in the PK studies in which atomoxetine was systemically infused for 4 h; the total perfusion time of [3H]-atomoxetine was 8 h. In the PK studies in which atomoxetine was systemically infused for 8 h, perfusion of [3H]-atomoxetine started at time 0 (along with atomoxetine). For additional study design details, see Pharmacokinetic Study Designs. The loss (L) of [3H]-atomoxetine across the microdialysis probe at each collection time was estimated by the loss from the perfusate during the retrodialysis period using eq. 2:

\[ L = \frac{C_m - C_{soe}}{C_m} \]  

where D represents the fold dilution of brain tissue, and \( f_{u \text{ measured}} \) is the ratio of concentrations determined in buffer versus brain homogenate samples.

Sample Collection, Preparation, and Analysis. Blood and dialysate samples were collected in refrigerated fraction collectors during the live phase portion of the study. Microdialysis samples were collected in a Univentor 820 microsamper (Scintco Inc., Sanborn, NY), and blood samples were collected using the Culex system (BAS Bioanalytical Systems). Blood was collected in disodium EDTA tubes and centrifuged to generate plasma within 30 min of collection. Whole brain and CSF were collected from each animal at the conclusion of the live phase. Before collection and storage at −70°C, atomoxetine-containing blood was removed from brains via carotid arterial perfusion with cold saline. The CSF was collected from the cisterna magna. Plasma, dialysate, and CSF samples were stored in a 96-well plate at −70°C before analysis.

Whole brains were homogenized in acetonitrile at a ratio of 2 ml/g of brain and centrifuged at 3000 rpm for 10 min at ambient temperature using a Beckman GPR centrifuge with a GH 3.7 swinging bucket rotor. Aliquots of 50 μl of supernatant were pipetted into a 96-well plate in duplicate for each brain. These supernatants as well as dialysate and CSF samples were analyzed for atomoxetine using standard LC-MS/MS methodology (Shimadzu high-performance liquid chromatograph with a Sciex API 4000 mass spectrometer; Shimadzu, Kyoto, Japan).

Retrodialysis Calibrator Validation and Loss. [3H]-Atomoxetine and atomoxetine are structurally similar molecules (Fig. 1). Therefore, the dialysis properties of [3H]-atomoxetine and atomoxetine should be similar across the microdialysis probe, and [3H]-atomoxetine should suffice as a retrodialysis calibrator for atomoxetine. To confirm, [3H]-atomoxetine and atomoxetine were prepared in dialysis buffer (100 ng/ml) and coperfused through the microdialysis probe at a rate of 1 μl/min over 8 h. Dialysate was collected at 0.5-h intervals, and the midpoint of the dialysate collection period served as the dialysate collection time. The ratio of the dialysate concentrations of atomoxetine to [3H]-atomoxetine at each collection period was assessed over time. Dialysis buffer containing [3H]-atomoxetine was perfused through the microdialysis probe at a rate of 1 μl/min in PK studies. Perfusion of [3H]-atomoxetine was initiated 4 h before atomoxetine administration in the PK studies in which atomoxetine was systemically infused for 4 h; the total perfusion time of [3H]-atomoxetine was 8 h. In the PK studies in which atomoxetine was systemically infused for 8 h, perfusion of [3H]-atomoxetine started at time 0 (along with atomoxetine). For additional study design details, see Pharmacokinetic Study Designs. The loss (L) of [3H]-atomoxetine across the microdialysis probe at each collection time was estimated by the loss from the perfusate during the retrodialysis period using eq. 2:
where \( C_b \) is the concentration of \(^{3}H\text{-}j\text{atomoxetine}in the incoming perfusate (100 ng/ml) and \( C_{\text{out}} \) is the concentration of \(^{3}H\text{-}j\text{atomoxetine} in the outgoing dialysate.

**Pharmacokinetic Study Designs. Dosing regimens.** Three PK studies having different infusion dosing regimens were conducted. In study 1 (\( n = 7 \) rats), an infusion loading dose was initially given at a rate of 10 mg/kg/h for 0.03 h followed by an infusion maintenance dose given at a rate of 1.25 mg/kg/h for 3.97 h. In studies 2 and 3 (\( n = 8 \) rats each), an infusion loading dose was initially given at rates of 14 and 2.3 mg/kg/h, respectively, for 0.25 h followed by an infusion maintenance dose given at a rate of 1.25 mg/kg/h for 7.75 h. In total, atomoxetine was infused for 4 h in study 1 and for 8 h in studies 2 and 3. The maintenance infusion rate was designed to target a steady-state plasma concentration of 250 ng/ml. In studies 1, 2, and 3 the loading infusion rates were designed to approximate 0.3, 0.6, and 3 times, respectively, the targeted steady-state plasma concentration. Time equal to zero was considered as the start time of the infusion loading dose for pharmacokinetic purposes.

**Sample collections.** In all three studies, dialysate was collected at 0.5-h intervals starting from the time of \(^{3}H\text{-}j\text{atomoxetine} perfusion through the microdialysis probe until the end of the atomoxetine maintenance infusion. The midpoint of the dialysate collection period was used as the dialysate collection time for subsequent PK analyses and for estimation of atomoxetine microdialysis recovery. Blood samples were collected at the midpoint of the dialysate collection period. The CSF and whole brain samples were collected immediately after termination of the atomoxetine maintenance infusion and after euthanasia via CO\(_2\) asphyxiation.

At each dialysate collection period, the atomoxetine ECF concentration was calculated according to the following equation (eq. 3):

\[
ECF = \frac{C_{\text{ATX,i}}}{R}
\]  

(3)

where \( C_{\text{ATX,i}} \) is the measured concentration of atomoxetine in the dialysate at time \( t \) and \( R \) is the recovery of atomoxetine, which is assumed to be equal to the loss of the retrodialysis calibrator, \(^{3}H\text{-}j\text{atomoxetine.}

**Brain Cell Concentration.** The atomoxetine concentration associated with the brain cell was calculated in each rat when appropriate according to the following equation:

\[
C_{\text{BC}} = \frac{C_b \times V_b - C_{\text{ECF}} \times V_{\text{ECF}}}{V_{\text{BC}}}
\]  

(4)

where \( C_b \) is the whole brain concentration after atomoxetine infusion, \( C_{\text{ECF}} \) is the steady-state brain extracellular fluid concentration, \( V_{\text{ECF}} \) is the brain extracellular fluid volume, \( V_b \) is the brain cell volume, and \( V_{\text{BC}} \) is the total brain volume. Values for \( V_{\text{ECF}} \) and \( V_{\text{BC}} \) were assumed to be 0.00029 and 0.00099 liters, respectively (Mahar Doan and Boje, 2000). The sum of \( V_{\text{ECF}} \) and \( V_{\text{BC}} \) was assumed to be equal to \( V_b \) (Scism et al., 2000). Details about the determination of \( C_b \) and \( C_{\text{ECF}} \) are found below under **Pharmacokinetic Analysis.**

From eq. 4, \( C_{\text{BC}} \) represents the total atomoxetine concentration associated with the brain cell. To estimate the unbound atomoxetine concentration associated with the brain cell (\( C_{\text{abc}} \)), the total atomoxetine concentration associated with the brain cell was multiplied by the unbound atomoxetine brain fraction as shown in eq. 5:

\[
C_{\text{abc}} = C_{\text{BC}} \times f_{\text{ab}}
\]  

(5)

**Pharmacokinetic Analysis.** At each blood sampling time, the unbound atomoxetine plasma concentration was determined by multiplying the measured atomoxetine plasma concentration by \( f_{\text{ab}} \). In study 1, the steady-state unbound atomoxetine plasma concentration (\( C_{\text{up}} \)) and \( C_{\text{ECF}} \) in each rat was determined by averaging the data collected from 2 to 4 h after atomoxetine administration, and the \( C_{\text{CSF}} \) and \( C_{\text{B}} \) in each rat was determined at 4 h after atomoxetine administration. In studies 2 and 3, the \( C_{\text{up}} \) and \( C_{\text{ECF}} \) in each rat was determined by averaging the data collected from 4 to 8 h after atomoxetine administration, and the \( C_{\text{CSF}} \) and \( C_{\text{B}} \) in each rat were determined at 8 h after atomoxetine administration. After these parameter values were obtained, the following ratios were calculated in each rat when data were available: \( C_{\text{ECF}}/C_{\text{up}}, C_{\text{CSF}}/C_{\text{ECF}}, C_{\text{BC}}/C_{\text{ECF}}, C_{\text{abc}}/C_{\text{ECF}}, C_{\text{abc}}/C_{\text{up}}, \text{and } C_{\text{CSF}}/C_{\text{up}}.\)

**Model development.** Pharmacokinetic modeling was performed using NONMEM software (version V, level 1.1; GloboMax LLC, Hanover, MD) with first-order condition estimation with interaction. The PK model describing the disposition of unbound atomoxetine is illustrated in Fig. 2. We developed a four-compartment model consisting of a systemic compartment and a CNS compartment, which was further divided into the CSF, ECF, and brain cell. The model can be described using a system of differential equations as follows (see eqs. 6–9):

\[
\frac{dA_p}{dt} = -\frac{CL}{V_{\text{p}}} A_p - \frac{Q_{\text{BCB}}}{V_{\text{p}}} A_p + \frac{Q_{\text{BBB}}}{V_{\text{CSF}}} A_{\text{CSF}} + \frac{Q_{\text{BBB}}}{V_{\text{ECF}}} A_{\text{ECF}}
\]  

(6)

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

(7)

\[
\frac{dA_{\text{ECF}}}{dt} = -\frac{Q_{\text{BBB}}}{V_{\text{ECF}}} A_{\text{ECF}} - \frac{CL_{\text{ECF}}-\text{BC}}{V_{\text{ECF}}} A_{\text{ECF}} + \frac{Q_{\text{BBB}}}{V_{\text{ECF}}} A_{\text{B}} + \frac{CL_{\text{BC}}-\text{ECF}}{V_{\text{ECF}}} A_{\text{ECF}}
\]  

(8)

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

(9)

where \( t \) represents time, \( A_p \) represents the amount of unbound atomoxetine in the plasma, \( A_{\text{CSF}} \) represents the amount of atomoxetine in CSF, \( A_{\text{ECF}} \) represents the amount of atomoxetine in the ECF, and \( A_{\text{BC}} \) represents the amount of unbound atomoxetine associated with the brain cell. For unbound atomoxetine, \( CL \) represents the plasma clearance, \( Q_{\text{BCB}} \) represents the distributional clearance at the BCB, \( Q_{\text{BBB}} \) represents the distributional clearance at the BBB,
CL_{ECF-CSF} represents the clearance from the ECF to the CSF, CL_{ECF-BC} represents the clearance from the ECF to the brain cell, CL_{BC-ECF} represents the clearance from the brain cell to the ECF, and V represents the plasma volume of distribution. Model terms \( V_{BC}, V_{CSF}, \) and \( V_{ECF} \) represented the volume of the brain cell, CSF, and ECF, respectively.

Interindividual variability in PK parameters were included in the model as described by the following equation (eq. 10):

\[
P_{ij} = P_j \times \exp(\eta_i)
\]

where \( P_{ij} \) is the \( j \)th parameter for the \( i \)th individual, \( P_j \) is the typical population parameter estimate for the \( j \)th parameter, and \( \eta_i \) is the deviation of \( P_{ij} \) from \( P_j \) in the \( j \)th parameter for the \( i \)th individual. For \( \eta_i \), 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 (eq. 11):

\[
C_{ik} = \text{Pred}_{ik} \times (1 + \sigma_{res})
\]

where \( C_{ik} \) and \( \text{Pred}_{ik} \) are the measured and model-predicted concentration at the \( k \)th sampling time in the \( i \)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 PK parameters estimated were CL, V, \( Q_{BBB}, Q_{CCB}, CL_{ECF-CSF}, CL_{ECF-BC}, CL_{BC-ECF}, \) and inter-rat variability of CL (\( \omega \)-CL) and V (\( \omega \)-V). Parameters fixed in the model were \( V_{CSF} \) (0.000250 liter), \( V_{ECF} \) (0.000290 liter), which consisted of 303, 23, 151, and 18 data points for plasma, CSF, ECF, and unbound brain cell, respectively. 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.

**Statistical Analysis.** Unless otherwise indicated, all data shown are represented as the mean \( \pm \) S.D.

**Results**

**Plasma and Brain Binding Assessment.** At 3 \( \mu \)M, atomoxetine was highly bound to plasma and brain with an unbound fraction of 0.13 \( \pm \) 0.01 (\( n = 3 \)) and 0.013 \( \pm \) 0.0007 (\( n = 3 \)).

**Retrodialysis Calibrator Validation and Loss.** In in vivo brain microdialysis experiments [\( ^2H_7 \)]atomoxetine and atomoxetine were coperfused through the microdialysis probe and the dialysate concentrations of atomoxetine and [\( ^2H_7 \)]atomoxetine were determined. Figure 3A illustrates the time course of the ratio of atomoxetine to [\( ^2H_7 \)]atomoxetine dialysate concentrations. These data demonstrate that both molecules have similar loss across the microdialysis probe because their concentration ratio is near unity. Figure 3B shows that the [\( ^2H_7 \)]atomoxetine loss was reproducible and from approximately 4 to 8 h after perfusion no discernible decrease in [\( ^2H_7 \)]atomoxetine loss.
was noted. Under analogous conditions in vitro, a similar loss profile was observed for $[^{2}H_7]$atomoxetine; furthermore, $[^{2}H_7]$atomoxetine loss and gain were similar from 4 to 8 h (data not shown). It is possible that the decline in $[^{2}H_7]$atomoxetine loss through the initial 4 h, either in vitro or in vivo, reflects slow equilibration of $[^{2}H_7]$atomoxetine binding to the probe. Taken together, these data indicate that $[^{2}H_7]$-atomoxetine is a suitable retrodialysis calibrator for atomoxetine.

**Pharmacokinetics.** Figure 4 is a scatter plot of the atomoxetine plasma, CSF, ECF, and whole brain concentrations across all dosing regimens. The steady-state plasma, CSF, ECF, and whole brain concentrations were $268 \pm 98$ ($n = 25$), $43 \pm 14$ ($n = 26$), $17 \pm 11$ ($n = 18$), and $2790 \pm 809$ ($n = 26$), respectively. The atomoxetine steady-state concentration ratios were calculated. The $C_{uP}/C_{BP}$ and $C_{uBC}/C_{ECF}$ values were determined to be $11 \pm 3$ ($n = 25$) and $170 \pm 60$ ($n = 18$), respectively, and the $C_{ECF}/C_{AP}$ and $C_{CSF}/C_{AP}$ values were $0.7 \pm 0.4$ ($n = 18$) and $1.7 \pm 0.8$ ($n = 25$), respectively. The $C_{BC}/C_{ECF}$ and $C_{BBB}/C_{ECF}$ values were $219 \pm 77$ ($n = 18$) and $2.9 \pm 1.0$ ($n = 22$), respectively. A four-compartment semiphysiological model comprising a systemic compartment and a CNS compartment, which was further divided into the CSF, ECF, and brain cell, was used to obtain PK parameter estimates for unbound atomoxetine (Fig. 2). The PK parameters estimated from the model are shown in Table 1. The data supported estimation of all model parameters with acceptable precision. The model population and individual predicted concentrations versus measured concentrations were close to the line of unity, indicating an acceptable fit of the data to the model (Fig. 5). Taken together, these data indicate that the model adequately characterizes the disposition of unbound atomoxetine.

**Discussion**

The ability to cross the BBB and BCB is important for drugs with targets in the CNS. It is a commonly accepted assumption that unbound drug is the entity available for interaction with drug targets and is referred to as the free drug hypothesis. It is also assumed that unbound drug in brain is in direct contact or in equilibrium with the site of action (de Lange and Danhof, 2002). For a compound that distributes solely by passive diffusion, at distribution equilibrium the unbound concentration in brain ($C_{ub}$) will equal the unbound concentration in plasma ($C_{ub}$) and the expected steady-state brain partition coefficient ($K_{p,s}$) can be described using these estimates ($K_{p,s} = f_{ub}/f_{ub}$). We determined $f_{ub}$ and $f_{ub}$ to be 0.131 and 0.013, respectively ($K_{p,s} = 10$), consistent with previous literature estimates (Mattiiu, et al., 2003; Summerfield et al., 2007). This value of $K_{p,s}$ was similar to the observed steady-state brain partition coefficient ($K_{p,s}$) after i.v. infusion of atomoxetine obtained in this study, in which $K_{p,s} = C_{uB}/C_{BP} = 11$. These data further support the work of Kalvass and Maurer (2002) and the utility of predicting $K_{p,s}$ from $K_{p,s}$ for drugs in which BBB transport is primarily passive ($K_{p,s}/K_{p,s} \sim 1$). On the basis of brain microdialysis, we determined the steady-state $C_{ECF}/C_{AP}$ to be $0.7$ and near unity. In total, these two sets of data obtained by distinct methodologies are in accordance and are consistent with the transport of atomoxetine across the BBB being primarily passive.

On the basis of $C_{uP}/C_{ECF}$ and $C_{BC}/C_{ECF}$ ratios of 170 and 219, respectively, we conclude that whole brain concentrations of atomoxetine do not represent the concentration of drug in the biophase and partitioning of atomoxetine within brain cells is substantial. We acknowledge that the calculation of the steady-state $C_{BC}$ (eq. 4) does not differentiate between bound or unbound atomoxetine in the cell, and, therefore, limits an understanding of the mechanism of the partitioning. We used our estimate of $f_{ub}$ to calculate the steady-state $C_{ub}$. To do this, we assumed that the brain tissue homogenization process and disruption of the tissue membranes did not compromise the value of $f_{ub}$ and that this value accurately reflects in vivo binding. We also

<table>
<thead>
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<th>Parameter</th>
<th>Estimate (% SEE)</th>
<th>Inter-Rat Variability (% SEE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL$ (liter/h)</td>
<td>14.5 (8.69)</td>
<td>38.6 (23.7)</td>
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<tr>
<td>$V$ (liter)</td>
<td>6.92 (30.8)</td>
<td>65.4 (80.1)</td>
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<tr>
<td>$Q_{BCF}$ (l/h)</td>
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<td>$Q_{BBB}$ (l/h)</td>
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<tr>
<td>$CL_{ECF-CSF}$ (l/h)</td>
<td>0.000129 (20.2)</td>
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<tr>
<td>$Q_{ECF-BCF}$ (l/h)</td>
<td>0.00216 (25.1)</td>
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<td>$V_{ECF}$ (liters)</td>
<td>0.0009934 (25.7)</td>
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<td>$V_{ECF}$ (liters)</td>
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<tr>
<td>$\sigma_{exp}$</td>
<td>34.7 (9.09)</td>
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</table>

% SEE, percentage S.E. of the estimate; $\sigma_{exp}$ proportional residual error.
make the distinction that \( f_{ab} \) is a function of intracellular and/or cell membrane binding only. The \( C_{\text{abc}}/C_{\text{ECF}} \) was determined to be 2.9 and substantially less than \( C_{\text{abc}}/C_{\text{ECF}} \sim 219 \), showing that atomoxetine does not preferentially reside in the extracellular space. We adopted the concept of others (Maurer et al., 2005; Kalvass et al., 2007) based on \( C_{\text{abc}}/C_{\text{ECF}} \) within 3-fold of unity to suggest that atomoxetine passively distributes within brain parenchyma. Fridén et al. (2006) demonstrated an inherent inability of the brain homogenization technique to predict \( K_{\text{uBC}} \) for drugs that preferentially reside in the extracellular space in vivo. Consistent with our results for atomoxetine, the brain homogenization technique and brain microdialysis were in accordance.

We determined \( C_{\text{CSF}}/C_{\text{up}} \) to be 1.7 and, like the BBB, conclude that the transport of atomoxetine is primarily passive across the BCB. The concept of a sink action of CSF suggests that the \( C_{\text{CSF}}/C_{\text{ECF}} \) value of a drug that undergoes only passive diffusion across the BBB and BCB should be below unity. The atomoxetine \( C_{\text{CSF}}/C_{\text{ECF}} \) ratio was determined to be 3, which is not considered to be meaningfully different from unity. Given the extensive partitioning of atomoxetine in brain tissue, this finding indicates that CSF bulk flow is not a significant determinant of atomoxetine residence time in the CSF.

We developed a neuropharmacokinetic model accordingly by incorporating a distributional clearance parameter at the BBB (\( Q_{\text{BBB}} \)) and at the BCB (\( Q_{\text{BCB}} \)). We explored estimation of bidirectional clearances at both the BBB and BCB but found that model parameters were estimated more precisely when distributional clearances at the BBB and BCB were contained in the model. The values of \( Q_{\text{BBB}} \) and \( Q_{\text{BCB}} \) were estimated to be 0.00110 and 0.0000090 l/h. The \( Q_{\text{BBB}}/Q_{\text{BCB}} \) ratio was approximately 12, indicating that the distributional clearance across the BBB was more rapid than the distributional clearance across the BCB, consistent with the larger surface area of the BBB relative to the BCB. Although definitive data are lacking, there seems to be a favored movement of drug from ECF to CSF, rather than the reverse (Rosenberg et al., 1980; Shen et al., 2004). Therefore, in our model, CSF concentrations of atomoxetine are due to direct availability from passage across the BCB or indirectly by passage across the BBB followed by diffusion or convective flow from the ECF. Our model characterized the unidirectional transport from ECF to CSF (\( CL_{\text{ECF-CSF}} \)). In rats, the first-order rate constant for flow from ECF to CSF (\( k_{\text{ECF-CSF}} \)) and \( V_{\text{ECF}} \) were estimated to be 0.084 h\(^{-1}\) and 0.00029 l, respectively (Szentiványi et al., 1984). Taken together, a theoretical value for \( CL_{\text{ECF-CSF}} \) is proposed to be 0.00024 l/h (\( CL_{\text{ECF-CSF}} = k_{\text{ECF-CSF}} \times V_{\text{ECF}} \)). During model development, \( CL_{\text{ECF-CSF}} \) was either fixed to the theoretical value or estimated. When estimated, \( CL_{\text{ECF-CSF}} \) was 0.000129 l/h and approximately 5 times larger than the theoretical value. The cause for this difference is not known. An investigation of the metabolism of atomoxetine in brain may provide insight for this result. Importantly, estimating \( CL_{\text{ECF-CSF}} \) provided a better fit of the data to the model (data not shown).

The transport across brain parenchyma was modeled using bidirectional clearances, where \( CL_{\text{ECF-BC}} \) represented the clearance from ECF to brain cell and \( CL_{\text{BC-ECF}} \) represented the clearance from brain cell to ECF. We attempted to estimate a distributional clearance at this site but found that the model parameters were not estimable when doing so. The values of \( CL_{\text{ECF-BC}} \) and \( CL_{\text{BC-ECF}} \) were estimated to be 0.00216 and 0.000934 l/h, respectively. The \( CL_{\text{ECF-BC}}/CL_{\text{BC-ECF}} \) ratio was 2.3 and, as anticipated, was consistent with our calculation of \( C_{\text{abc}}/C_{\text{ECF}} \) (~2.9).

In summary, we have performed the first detailed examination using a quantitative microdialysis technique to understand the brain disposition of atomoxetine, a centrally acting NE transporter inhibitor used for the clinical treatment of ADHD. We found that atomoxetine is highly bound to brain tissue and that the total brain atomoxetine concentration is not reflective of the concentration in brain ECF, the site where atomoxetine acts to inhibit NE reuptake into the neuron. Data obtained from this study suggest that atomoxetine brain penetration is high, movements of atomoxetine across the BBB and BCB occur predominantly by a passive mechanism, and rapid equilibration of ECF and CSF with plasma occurs. In addition, we developed a model to describe the neuropharmacokinetic behavior of atomoxetine, which enhances our understanding of this molecule as a therapeutic entity.

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