Quantitative Investigation of the Brain-to-Cerebrospinal Fluid Unbound Drug Concentration Ratio under Steady-State Conditions in Rats Using a Pharmacokinetic Model and Scaling Factors for Active Efflux Transporters

Hiroshi Kodaira, Hiroyuki Kusuhara, Eiichi Fuse, Junko Ushiki, and Yuichi Sugiyama

ABSTRACT

A pharmacokinetic model was constructed to explain the difference in brain- and cerebrospinal fluid (CSF)-to-plasma and brain-to-CSF unbound drug concentration ratios ($K_{puu,brain}$ and $K_{puu,CSF}$, respectively) of drugs under steady-state conditions in rats. The passive permeability across the blood-brain barrier (BBB), $P_{S}$, was predicted by two methods using log(D/molecular weight)$^{0.5}$ for $P_{S,1}$ or the partition coefficient in octanol/water at pH 7.4 ($LogD$), the topologic van der Waals polar surface area, and van der Waals surface area of the basic atoms for $P_{S,2}$. The coefficients of each parameter were determined using previously reported in situ rat BBB permeability. Active transport of drugs by P-glycoprotein (P-gp) and breast cancer resistance protein (Bcrp) measured in P-gp- and Bcrp-overexpressing cells was extrapolated in vivo by introducing scaling factors. Brain- and CSF-to-plasma unbound concentration ratios ($K_{puu,brain}$ and $K_{puu,CSF}$, respectively) of 19 compounds, including P-gp and Bcrp substrates (daidzein, dantrolene, flavopiridol, genistein, loperamide, quinidine, and verapamil), were simultaneously fitted to the equations in a three-compartment model comprising blood, brain, and CSF compartments. The calculated $K_{puu,brain}$ and $K_{puu,CSF}$ of 17 compounds were within a factor of three of experimental values. $K_{puu,CSF}$ values of genistein and loperamide were outliers of the prediction, and $K_{puu,brain}$ of dantrolene also became an outlier when $P_{S,2}$ was used. $K_{puu,CSF}$ of the 19 compounds was within a factor of three of experimental values. In conclusion, the $K_{puu,CSF}$ of drugs, including P-gp and Bcrp substrates, could be successfully explained by a kinetic model using scaling factors combined with in vitro evaluation of P-gp and Bcrp activities.

Introduction

For drugs acting in the central nervous system (CNS), it is assumed that an unbound drug in the brain ($C_{u,brain}$) is available to interact with the target site in the CNS. Estimating or measuring the $C_{u,brain}$ of new chemical entities is a critical issue in drug discovery and development to allow understanding of the relationship between drug exposure and CNS effects. However, direct measurement of $C_{u,brain}$ is not practicable, particularly in nonhuman primates and humans, because of its invasiveness, and thus there are great efforts to identify a surrogate. It is accepted that the unbound drug concentration in plasma ($C_{u,plasma}$) is not necessarily a surrogate of $C_{u,brain}$ (Kalvass and Maurer, 2002; Maurer et al., 2005; Liu et al., 2009; Watson et al., 2009) because of the blood-brain barrier (BBB). The BBB, which is formed by endothelial cells, limits the rapid and free exchange of drugs between the CNS and blood because of its highly developed tight junctions between adjacent endothelial cells (de Lange and Danhof, 2002; Abbott, 2004) and also of the active efflux mediated by P-glycoprotein (P-gp/MDR1/ABC1), breast cancer resistance protein (Bcrp/ABCG2), and multidrug resistance-associated protein 4 (MRP4/ABCC4) (Schinkel, 1999; Leggas et al., 2004; Belinsky et al., 2007; Enokizono et al., 2007, 2008; Ose et al., 2009). In fact, the $C_{u,brain}$ of drugs, and consequently the $C_{u,brain}$, is inversely correlated with the activity of P-gp (Kikuchi et al., 2013).

Drug concentration in cerebrospinal fluid (CSF) ($C_{u,CSF}$) has been considered as a surrogate of $C_{u,brain}$ because the ependymal layer between CSF and CNS has been considered to allow the free exchange of drugs (Lin, 2008; Liu X et al., 2009). CSF is produced by the choroid plexus in the ventricles, slowly turns over by bulk flow with poor mixing, and is finally absorbed into the venous blood. The choroid plexus is referred to as the blood-CSF barrier (BCSFB) because of its highly developed tight junctions between epithelial cells

ABBREVIATIONS: AFE, average fold error; CNS, central nervous system; BBB, blood-brain barrier; Bcrp, breast cancer resistance protein; BCSFB, blood-cerebrospinal fluid barrier; CFR, corrected flux ratio; CSF, cerebrospinal fluid; $C_{CSF}$, total drug concentration in cerebrospinal fluid; $C_{brain}$, total drug concentration in brain; $C_{u,brain}$, unbound drug concentration in plasma; $C_{u,CSF}$, unbound drug concentration in cerebrospinal fluid; $K_{puu,brain}$, brain-to-plasma unbound drug concentration ratio; $K_{puu,CSF}$, cerebrospinal fluid-to-plasma unbound drug concentration ratio; $K_{puu,CSF,brain}$, cerebrospinal fluid-to-brain unbound drug concentration ratio; LogD, the partition coefficient in octanol/water at pH 7.4; Mdr, multidrug resistance protein; MW, molecular weight; P-gp, P-glycoprotein; TPSA, topologic van der Waals surface area; vsa_base, van der Waals surface area of the basic atoms.

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Expression levels of P-gp and Bcrp were also detected in the choroid epithelial cells; however, their localization in these cells is suggested to be cytoplasmic or subapical for P-gp and in the brush-border membrane for Bcrp (Rao et al., 1999; Zhuang et al., 2006; Gazzin et al., 2008). Because of the lack of P-gp and Bcrp in the blood-facing plasma membrane of the choroid epithelial cells, their substrates should easily penetrate into the CSF. Indeed, we reported that the $C_{u,CSF}$ of P-gp and Bcrp substrates exceeds the $C_{u,brain}$ in rodents, and defects in P-gp and Bcrp expression diminished this concentration difference for P-gp and Bcrp substrates, respectively (Kodaira et al., 2011). Recently, a group of drugs were found to undergo active efflux at the BBB mediated by both P-gp and Bcrp (Oostendorp et al., 2009; Kusuhara and Sugiyama, 2009; Kodaira et al., 2010). Simultaneous defects in P-gp and Bcrp expression are necessary to completely eliminate the concentration difference of such dual substrates (Kodaira et al., 2011). Thus, active efflux at the BBB affects the estimation of $C_{u,CSF}$. In addition, the $C_{u,CSF}$ of drugs is determined by multiple parameters, passive permeability across the BBB and BCSFB, CSF bulk flow rate, and diffusion across the ependymal layer. For quantitative interpretation of the relationship between $C_{u,brain}$ and $C_{u,CSF}$, these factors must also be considered.

The purpose of the present study was to develop a pharmacokinetic model to describe the CSF- and brain-to-plasma and CSF-to-brain unbound concentration ratios ($K_{P,uu,brain}$, $K_{P,uu,CSF}$, and $K_{P,uu,CSF/brain}$, respectively) in rats of 19 drugs, including P-gp and Bcrp substrates, which we had determined previously under steady-state conditions (Kodaira et al., 2011). A nonlinear least-squares method was used to simultaneously fit the equations representing $K_{P,uu,brain}$ and $K_{P,uu,CSF}$ of drugs, including P-gp and Bcrp substrates to the observed data. We could reasonably well explain the observed $K_{P,uu,brain}$ and $K_{P,uu,CSF}$ for 17 compounds and the observed $K_{P,uu,CSF/brain}$ for 19 compounds in rats using the fitted parameters.

**Materials and Methods**

**Drug Selection and Animal Data.** The unbound concentrations in the brain, CSF, and plasma were calculated as the product of unbound fraction and total concentration. $K_{P,uu,brain}$ and $K_{P,uu,CSF}$ represent the $C_{u,brain}$ and $C_{u,CSF}$ divided by the $C_{ubp}$, whereas $K_{P,uu,CSF/brain}$ represents the $C_{u,brain}$ divided by the $C_{u,CSF}$. All these values were based on our previous report (Kodaira et al., 2011). Of 25 compounds, 19 were selected for further analysis: benzylpenicillin, cephalexin, and pefloxacin, which are Bcrp-specific substrates and a dual substrate of P-gp and Bcrp, respectively, were excluded because of the uncertain involvement of Bcrp on their brain distribution in vivo.

**Prediction of the BBB Passive Permeability by In Silico Structure Descriptors.** BBB passive permeabilities of 19 compounds were calculated using in silico models. Based on reports by Murakami et al. (2000) and Liu et al. (2004), a linear regression of reported logPS$_1$ against log(D/MW$^{0.5}$) or three-structure descriptors [logD, topologic van der Waals polar surface area (TPSA), and van der Waals surface area of the basic atoms (vsa_base)] for non-P-gp and non-Bcrp substrates was used in this study. PS$_1$ is the passive permeability clearance at the BBB (ml/s per gram), MW is the molecular weight, LogD is the partition coefficient in octanol/water at pH 7.4, TPSA is the topologic van der Waals polar surface area, and vsa_base is the van der Waals surface area of the basic atoms (Ertl et al., 2000). The coefficients of equations in two linear regression models were calculated by regression analysis of the reported BBB permeability of non-P-gp and non-Bcrp substrate compounds (Table 1). The PS values observed at the BBB (observed PS$_1$) in rats were obtained by Liu et al. (2004) and Summerfield et al. (2007). Physicochemical parameters, logD, and TPSA for the test compounds were obtained using ACD/PhysChem Suite (version 12; ACD/Laboratories, Toronto, Canada), and vsa_base was obtained using MOE 2010 (Chemical Computing Group, Montreal, QC, Canada). The PS$_1$ calculated by using log (D/MW$^{0.5}$) or three-structure descriptors was expressed as PS$_1$(1) and PS$_1$(2), which were corrected for brain weight per body weight in rats (1.8 g/0.25 kg) (Davies and Morris, 1993).

**Mass-Balance Differential Equations.** A three-compartment model comprising the blood, brain interstitial, and CSF compartments is shown in Fig. 1. The mass-balance equations for each compartment are shown in eq. 1, eq. 2, and eq. 3:

**Blood:**

$$V_1 \cdot \frac{df_{blood}}{dt} = (PS_1 + PS_2) \cdot fblood \cdot C_{blood} - CL \cdot fblood \cdot C_{blood} + \left( \frac{PS_1}{PS_1 + PS_2} \cdot fu_{brain} \cdot C_{brain} + \frac{PS_2 + CL_{bulkflow}}{PS_1 + PS_2} \cdot fu_{CSF} \cdot C_{CSF} \right)$$

**Brain:**

$$V_2 \cdot \frac{df_{brain}}{dt} = PS_1 \cdot fblood \cdot C_{brain} - (PS_1 + PS_2 + PS_3) \cdot fu_{brain} \cdot C_{brain} + PS_2 \cdot fu_{CSF} \cdot C_{CSF}$$

**CSF:**

$$V_3 \cdot \frac{df_{CSF}}{dt} = PS_1 \cdot fblood \cdot C_{CSF} + PS_2 \cdot fu_{brain} \cdot C_{brain} - (PS_2 + PS_3 + CL_{bulkflow}) \cdot fu_{CSF} \cdot C_{CSF}$$

The parameters are summarized in Table 2. $CL$ and $k_{inf}$ represent the total body clearance and infusion rate, respectively. Under steady-state conditions, $K_{P,uu,brain}$ and $K_{P,uu,CSF}$ are given by eq. 4 and eq. 5:

The drug concentration in arterial blood could be assumed to be the same as that in brain capillary blood because of the negligible extraction rate in the...
Physicochemical parameters to predict passive blood-brain barrier (BBB) permeability and in vitro transport activities of (breast cancer resistance protein (Bcrp) and P-glycoprotein (P-gp) for 19 compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>cLogD pH 7.4</th>
<th>TPSA</th>
<th>vsa_base</th>
<th>Clearance (mL/min per kg)</th>
<th>CFR</th>
<th>Substrate of P-gp and Bcrp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipyrine</td>
<td>188</td>
<td>0.441</td>
<td>23.55</td>
<td>0.00</td>
<td>4.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.45 × 10&lt;sup&gt;-2&lt;/sup&gt; 0.00276 0.00253 0.00 5.91 0.0274 0.00558 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
<tr>
<td>Buspirone</td>
<td>386</td>
<td>1.19</td>
<td>69.64</td>
<td>0.00</td>
<td>—</td>
<td>5.70 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00324 0.00 3.64 0.0191 0.00343 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>194</td>
<td>0.628</td>
<td>58.44</td>
<td>0.00</td>
<td>4.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.84 × 10&lt;sup&gt;-2&lt;/sup&gt; 0.00272 0.00161 0.00 2.58 0.0270 0.00243 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
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<tr>
<td>Carbamazepine</td>
<td>236</td>
<td>1.90</td>
<td>46.33</td>
<td>0.00</td>
<td>6.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.02 × 10&lt;sup&gt;-2&lt;/sup&gt; 0.00456 0.00 6.32 0.0245 0.00596 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
<tr>
<td>Citalopram</td>
<td>324</td>
<td>1.35</td>
<td>36.26</td>
<td>0.00</td>
<td>4.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.21 × 10&lt;sup&gt;-2&lt;/sup&gt; 0.00353 0.00 6.30 0.0209 0.00594 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td>254</td>
<td>2.11</td>
<td>66.76</td>
<td>0.00</td>
<td>—</td>
<td>8.72 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00495 89.3 4.93 0.0236 0.00465 50.5 1.8 1.0 Bcrp</td>
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<tr>
<td>Dantrolene</td>
<td>314</td>
<td>1.43</td>
<td>120.73</td>
<td>0.00</td>
<td>—</td>
<td>6.43 × 10&lt;sup&gt;-2&lt;/sup&gt; 0.00365 65.8 1.80 0.0212 0.00170 18.4 1.8 1.0 Bcrp</td>
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<tr>
<td>Diazepam</td>
<td>285</td>
<td>2.80</td>
<td>32.67</td>
<td>0.00</td>
<td>14.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.5 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00653 0.00 10.0 0.0223 0.00943 0.0 1.0 1.0 Nonsubstrate</td>
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<tr>
<td>Flavopiridol</td>
<td>402</td>
<td>0.678</td>
<td>90.23</td>
<td>0.00</td>
<td>—</td>
<td>4.59 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00261 157.2 2.31 0.0188 0.00218 78.8 1.5 4.7 P-gp, Bcrp</td>
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<tr>
<td>Genistein</td>
<td>270</td>
<td>1.93</td>
<td>86.99</td>
<td>0.00</td>
<td>—</td>
<td>8.05 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00457 113.3 3.45 0.0229 0.00325 48.6 2.1 1.0 Bcrp</td>
<td></td>
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<tr>
<td>Loperamide</td>
<td>477</td>
<td>3.63</td>
<td>43.78</td>
<td>0.00</td>
<td>—</td>
<td>15.6 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00886 619.7 10.7 0.0172 0.01011 425.0 1.0 6.3 P-gp</td>
<td></td>
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<tr>
<td>Midazolam</td>
<td>326</td>
<td>3.78</td>
<td>30.18</td>
<td>0.00</td>
<td>19.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.2 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00977 0.0 13.7 0.0208 0.0129 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
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<tr>
<td>Phenytoin</td>
<td>252</td>
<td>2.52</td>
<td>58.20</td>
<td>0.00</td>
<td>3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.4 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00591 0.0 6.30 0.0237 0.00594 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>324</td>
<td>0.947</td>
<td>45.59</td>
<td>0.00</td>
<td>0.172&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.24 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00211 0.00298 204.4 4.89 0.0209 0.00461 190.0 1.0 6.2 P-gp</td>
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<tr>
<td>Risperidone</td>
<td>410</td>
<td>1.86</td>
<td>61.94</td>
<td>0.00</td>
<td>6.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.53 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00428 95.4 4.94 0.0186 0.00466 62.9 1.0 2.7 P-gp</td>
<td></td>
</tr>
<tr>
<td>Sertraline</td>
<td>306</td>
<td>3.04</td>
<td>12.03</td>
<td>5.68</td>
<td>31.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.7 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00722 0.0 31.6 0.0215 0.00298 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
<tr>
<td>Thiopental</td>
<td>242</td>
<td>2.99</td>
<td>90.29</td>
<td>0.00</td>
<td>—</td>
<td>12.7 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00722 0.0 4.43 0.0242 0.00418 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>455</td>
<td>2.46</td>
<td>63.95</td>
<td>0.00</td>
<td>—</td>
<td>9.58 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00544 172.5 5.68 0.0176 0.00536 102.0 1.0 3.4 P-gp</td>
<td></td>
</tr>
<tr>
<td>Zolpidem</td>
<td>307</td>
<td>2.97</td>
<td>37.61</td>
<td>0.00</td>
<td>—</td>
<td>12.3 × 10&lt;sup&gt;-3&lt;/sup&gt; 0.00699 0.0 9.77 0.0215 0.00922 0.0 1.0 1.0 Nonsubstrate</td>
<td></td>
</tr>
</tbody>
</table>

Bcrp, breast cancer resistance protein; P-gp, P-glycoprotein.

*These PS values observed at the BBB (observed PS<sub>2</sub>) were obtained by Liu et al. (2004).

These PS values observed at the BBB (observed PS<sub>2</sub>) were obtained by Summerfield et al. (2007).

where B and C represent the scaling factor for extrapolation of in vitro P-gp- and Bcrp-mediated efflux activities to the corresponding in vivo values. Corrected flux ratio (CFR) represents the ratio of the basal-to-apical and apical-to-basal transport across the monolayers of epithelial cells expressing either P-gp or Bcrp divided by the corresponding ratio in mock-vector transfected cells, as determined previously (Kodaira et al., 2011). CFR values that showed statistical significance were included in the calculation; otherwise, CFR was regarded as 1. By substituting eq. 6, eq. 7, and eq. 8 into eqs. 4 and 5, we obtained the following:

Where A−D are fitting parameters. Equations 9 and 10 were simultaneously fitted to the observed data (K<sub>p,<sub>u,brain</sub></sub> and K<sub>p,<sub>u,CSF</sub></sub>) of 19 compounds in rats using a nonlinear least-squares method (WinNonlin, version 5.2.1; Pharsight Corporation, Mountain View, CA) to obtain parameters A−D. K<sub>p,<sub>u,CSF/brain</sub></sub>

### TABLE 1

Physiochemical parameters and clearance values for 19 compounds.
values of 19 compounds were calculated by dividing calculated $K_{p_{\text{uu}}}$ by calculated $K_{p_{\text{uu}}}$.

**Evaluation of Predictive Performance.** Predictive accuracy was assessed by comparing the predicted versus observed values of $K_{\text{p_{uu,brain}}}$, $K_{\text{p_{uu,CSF}}}$, and $K_{\text{p_{uu,CSF}/brain}}$. Therefore, the predictive accuracy of each passive permeability was characterized as shown in eq. 11 by using the average fold error ($AFE$), which gives a measure of the extent to which a particular method underpredicts or overpredicts the observed values:

$$AFE = \frac{1}{n} \sum \left( \frac{\log (\text{predicted } K_{p_{uu,i}})}{\log (\text{observed } K_{p_{uu,i}})} \right),$$

(11)

where $n$ represents the size of the data set. In addition, the resulting absolute average fold error, which takes the absolute of plus and minus data and quantifies the magnitude of difference from the true value, was also assessed, as shown in eq. 12:

$$AAFE = \frac{1}{n} \sum \left( \frac{\log (\text{predicted } K_{p_{uu,i}})}{\log (\text{observed } K_{p_{uu,i}})} \right).$$

(12)

The specific fold error of the deviation between the predicted and observed values was also calculated. Therefore, the percentage and number of drugs with a deviation less than 2-fold error and 3-fold error are presented for each method.

Precision was assessed based on the root mean squared error, shown in eq. 13, as follows:

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum \left( \frac{\text{predicted } K_{p_{uu,i}} - \text{observed } K_{p_{uu,i}}}{\text{observed } K_{p_{uu,i}}} \right)^2},$$

(13)

To measure the degree to which the predicted and observed values are correlated, the correlation coefficient ($r$) was determined. The corresponding plots of predicted versus observed values are presented for each prediction method.

**Statistical Analysis.** Values are all presented as mean ± S.E.M. All statistical calculations were performed using SAS software (version 9; SAS Institute, Cary, NC).

### Results

**In Vitro and In Vivo Parameters of 19 Compounds.** The $K_{p_{uu,brain}}$, $K_{p_{uu,CSF}}$, and $K_{p_{uu,CSF}/brain}$ of the 19 compounds ranged from 0.00886 to 2.19, from 0.0376 to 1.35, and from 0.139 to 4.16, respectively. The $K_{p_{uu,CSF}}$ of P-gp substrates (loperamide, quinidine, and verapamil), Bcrp substrates (genistein, dantrolene, and daidzein), and a P-gp and Bcrp dual substrate (flavopiridol) were 2-fold greater than their $K_{p_{uu,brain}}$ (i.e., $K_{p_{uu,CSF}/brain} > 2$). A linear regression analysis of the logPS$_1$ values of the non-P-gp and non-BCRP substrates in present study yielded two linear equations (eq. 14 and eq. 15) that contain the descriptors described in Materials and Methods.

$$\log \text{PS}_1(1) = 0.182 \times \log (D / MW^{0.5}) - 1.86 \quad (R^2 = 0.50)$$

(14)

$$\log \text{PS}_1(2) = 0.123 \times \log D - 0.00656 \times TPSA + 0.0588 \times \text{vas}_\text{base} - 1.76 \quad (R^2 = 0.77)$$

(15)

The passive permeability clearances at the BBB [PS$_1$(1) and PS$_1$(2)] for 19 compounds, which were calculated by two equations, ranged from 2.84 to 17.2 ml/min per kilogram and from 1.80 to 31.6 ml/min per kilogram, respectively (Table 1). PS$_1$(1) showed a positive correlation with $K_{p_{uu,brain}}$ and $K_{p_{uu,CSF}}$.

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration</th>
<th>Volume</th>
<th>Clearance (ml/min per rat)</th>
<th>Unbound fraction</th>
<th>Others</th>
<th>Free parameters for fitting</th>
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<tbody>
<tr>
<td></td>
<td>$C_{\text{blood}}$</td>
<td>$V_1$</td>
<td>$PS_1$</td>
<td>$f_{\text{blood}}$</td>
<td>$\text{Infusion rate}$</td>
<td>$A$</td>
</tr>
<tr>
<td></td>
<td>$C_{\text{brain}}$</td>
<td>$V_2$</td>
<td>$PS_2$</td>
<td>$f_{\text{brain}}$</td>
<td></td>
<td>$B$</td>
</tr>
<tr>
<td></td>
<td>$C_{\text{CSF}}$</td>
<td>$V_3$</td>
<td>$PS_3$</td>
<td>$f_{\text{CSF}}$</td>
<td></td>
<td>$C$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{fick}}$</td>
<td>$CL_{\text{bulk flow}}$</td>
<td>$PS_4$</td>
<td>$f_{\text{brain}}$</td>
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<td>$D$</td>
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</table>

BBB, blood-brain barrier; BCSFB, blood-cerebrospinal fluid barrier; CL, clearance; CSF, cerebrospinal fluid; $V$, volume.
correlation with PS1(2), although the absolute value of PS1(1) was somewhat greater than that of PS1(2). With the exception of some outliers, both PS1(1) and PS1(2) were comparable with the observed BBB permeability; PS1(1) overestimated the BBB permeability of phenytoin and quinidine and underestimated that of sertraline, whereas PS1(2) overestimated the BBB permeability of quinidine.

**Determination of the Parameters A-D to Describe \( K_{p,uu,brain} \) and \( K_{p,uu,CSF} \) of the Test Compounds.** Simultaneous fitting of \( K_{p,uu,brain} \) and \( K_{p,uu,CSF} \) of the 19 compounds with PS1 obtained by two methods was performed to obtain the parameters A–D. The resulting fitted parameters A, B, C, and D were 1760 ± 630 (CV: 35.6%), 7.49 ± 1.68 (CV: 22.4%), 12.8 ± 3.6 (CV: 28.1%), and 0.379 ± 0.200 (CV: 52.7%), respectively, when PS1(1) was used and 1060 ± 310 (CV: 37.9%), 7.48 ± 1.73 (CV: 23.1%), 11.7 ± 3.3 (CV: 28.4%), and 0.376 ± 0.218 (CV: 58.1%), respectively, when PS1(2) was used. The method of calculation of PS1 had no marked effect on the fitted parameters B–D. Parameter A determined by PS1(1) was 1.7 times higher than that determined by PS1(2).

The correlations between the predicted and observed \( K_{p,uu} \) values are shown in Fig. 2. Comparative assessment was conducted based on the number of compounds that fell within 2- to 3-fold error and on statistical indexes (Table 3). The numbers of compounds exhibiting an error less than 2-fold for \( K_{p,uu,brain} \) and \( K_{p,uu,CSF} \) were comparable regardless of PS1, whereas for \( K_{p,uu,brain}/CSF \) with PS1(1) was higher than that with PS1(2). There were outliers: PS1(1) \( K_{p,uu,CSF} \) of genistein and loperamide (the observed and predicted values were 0.589 and 0.155 for genistein and 0.038 and 0.245 for loperamide, respectively); PS1(2) \( K_{p,uu,brain} \) of dantrolene (the observed and predicted values were 0.030 and 0.095, respectively), \( K_{p,uu,CSF} \) of genistein and loperamide (the observed and predicted values are 0.589 and 0.130 for genistein, and 0.038 and 0.271 for loperamide, respectively). Absolute average fold error and root mean squared error were slightly lower when PS1(1) was used for fitting (Table 3).

**Discussion.**

In the present study, we aimed to demonstrate that a pharmacokinetic model comprising the three compartments—brain, CSF, and plasma—is able to explain the \( K_{p,uu,brain} \) and \( K_{p,uu,CSF} \) of drugs and their ratio, including those of P-gp and Bcrp substrates that undergo significant active efflux by P-gp and Bcrp at the BBB, by using scaling factors for P-gp and Bcrp.

PS1 was calculated for 19 compounds using in silico structure descriptors (Table 1). Of the two methods tested, PS1(2) provided a better prediction than PS1(1). This was identical to the result of Liu et al. (2004), and PS1(2) could reproduce the actual BBB passive permeability for all tested drugs except quinidine. Because P-gp has limited penetration to the CNS across the BBB (Kusuhara et al., 1997), it is reasonable that quinidine was an outlier. In contrast to quinidine, both methods predicted similar or slightly lower passive permeability of risperidone, another P-gp substrate, compared with that observed. This finding is inconsistent with the observations that the brain penetration of risperidone at the BBB is also limited by P-gp (Doran et al., 2005) and that P-gp impacts on BBB permeability. Further studies are necessary to explain this discrepancy.

\( K_{p,uu,brain} \) and \( K_{p,uu,CSF} \), and consequently \( K_{p,uu,brain}/CSF \), could be explained well using the fitted parameters. Only a small difference was found in the predictive performance of PS1(1) and PS1(2) (Table 3). This result may be partly because the \( K_{p,uu,brain} \) and \( K_{p,uu,CSF} \) determined under steady-state conditions were used for the calculations. When transcellular transport across the BBB occurs by passive diffusion, the \( K_{p,uu,brain} \) should be 1, irrespective of PS1 value. For \( K_{p,uu,CSF} \), the difference between...
TABLE 3
Accuracy and precision of the prediction of $K_{p,uu,brain}$, $K_{p,uu,CSF}$, and $K_{p,uu,CSF/brain}$ for 19 compounds in rats

<table>
<thead>
<tr>
<th>Permeability</th>
<th>% ≤ 2-fold</th>
<th>% ≤ 3-fold</th>
<th>$\bar{r}^2$</th>
<th>Accuracy</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{p,uu,brain}$</td>
<td>$K_{p,uu,CSF}$</td>
<td>$K_{p,uu,brain}$</td>
<td>$K_{p,uu,CSF}$</td>
<td>$K_{p,uu,brain}$</td>
<td>$K_{p,uu,CSF}$</td>
</tr>
<tr>
<td>PS1 (1)</td>
<td>63%</td>
<td>84%</td>
<td>84%</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>(12/19)</td>
<td>(16/19)</td>
<td>(16/19)</td>
<td>(19/19)</td>
<td>(17/19)</td>
<td>(19/19)</td>
</tr>
<tr>
<td>PS1 (2)</td>
<td>63%</td>
<td>84%</td>
<td>79%</td>
<td>95%</td>
<td>90%</td>
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<td>(12/19)</td>
<td>(16/19)</td>
<td>(15/19)</td>
<td>(18/19)</td>
<td>(17/19)</td>
<td>(19/19)</td>
</tr>
</tbody>
</table>

PS1(1) and PS1(2) could be compensated by parameter A, which represents the difference between the BBB and BCSFB in the clearance for passive transport. Assuming an equal passive permeability per unit surface area across the BBB and BCSFB, parameter A corresponds to the difference in the surface area for drug penetration from the blood circulation into the brain and CSF. The surface area of capillaries at the BBB is 5000-fold larger than that at the BCSFB (Pardridge et al., 1981; De Lange, 2004; Reichel, 2006). The method used for estimating parameter A was the same order of magnitude as the reported value (Pardridge et al., 1981; De Lange, 2004; Reichel, 2006). The method used for estimating PS1 barely fitted the parameters of BBB and BCSFB in humans, indicating that active efflux by Bcrp at the BBB is the predominant mechanism producing the difference in the $K_{p,uu,brain}$ and $K_{p,uu,CSF}$ (Kodaira et al., 2011). Bcrp-mediated efflux of genistein at the BBB may be overestimated for an unknown reason.

The $K_{p,uu,CSF/brain}$ values of the test compounds varied from 0.24 to 2.2, showing a 10-fold difference in the mouse (Kodaira et al., 2011), which is mainly the result of the active efflux at the BBB. The present study demonstrated that in vitro to in vivo extrapolation of the active efflux at the BBB successfully predicted this parameter, even for a P-gp and Bcrp dual substrate, using a simple pharmacokinetic model and in silico prediction of PS1. Once we have determined the in vitro transport activities of investigational substrates of P-gp and Bcrp, our approach will help in the estimation of the $K_{p,uu,brain}$ of these investigational drugs and inform the decision to use CSF concentration as a surrogate for drug development. It should be noted that the scaling factors determined in this study obviously depend on the cell lines that were used for the evaluation of P-gp and Bcrp activities. To ensure the predictability, in addition to the compounds of interest, it is strongly recommended that some P-gp- and Bcrp-specific substrates be measured as positive controls in the cell lines used in the analysis for correction of the CFR.

The our approach will elucidate the relationship between $C_{uu,brain}$ and $C_{uu,CSF}$ in nonhuman primates and humans once we are able to determine the scaling factors for P-gp and Bcrp in humans by accumulating positron emission tomography data or by using quantitative proteomics, an emerging technique for quantifying protein expression. This will in the future improve the predictability of $K_{p,uu,brain}$ and $K_{p,uu,CSF}$ even for P-gp and Bcrp substrates, although our approach will be needed to validate $K_{uu,brain}$ values calculated by the model in humans by comparing the calculated data with in vivo data determined by PET.

In conclusion, a simple pharmacokinetic model that includes active efflux transport at the BBB can reasonably describe the $K_{p,uu,CSF/brain}$ of drugs, even including P-gp and Bcrp substrates, by introducing in vitro and in vivo scaling factors for P-gp and Bcrp.

Authorship Contributions

Participated in research design: Kodaira, Kusuhara, Sugiyama.
Conducted experiments: Kodaira.
Performed data analysis: Kodaira, Kusuhara, Sugiyama.
Wrote or contributed to the writing of the manuscript: Kodaira, Kusuhara, Fuse, Ushiki, Sugiyama.

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