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Insights From an Integrated Physiologically Based Pharmacokinetic Model for Brain Penetration

Patrick E. Trapa*, Elena Belova, Jenny L. Liras, Dennis O. Scott, Stefan J. Steyn

Department of Pharmacokinetics, Dynamics, and Metabolism, Worldwide Research and Development, Pfizer, Inc., 610 Main Street, Cambridge, Massachusetts 02139

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ABSTRACT

Central-nervous-system, physiologically based pharmacokinetic (PBPK) models predict exposure profiles in the brain, that is, the rate and extent of distribution. The current work develops one such model and presents improved methods for determining key input parameters. A simple linear regression statistical model estimates the passive permeability at the blood-brain barrier from brain uptake index data and descriptors, and a novel analysis extracts the relative active transport parameter from *in vitro* assays taking into consideration both paracellular transport and unstirred water layers. The integrated PBPK model captures the concentration profiles of both rate-restricted and effluxed compounds with high passive permeability. In many cases, compounds distribute rapidly into the brain and are, therefore, not rate limited. The PBPK model is then simplified to a straightforward equation to describe brain-to-plasma ratios at steady state. The equation can estimate brain penetration either from *in vitro* efflux data or from *in vivo* results from another species and, therefore, is a valuable tool in the discovery setting.

Introduction

The blood-brain barrier (BBB) plays a key role in drug discovery, offering the possibility of reducing central toxicity for peripheral targets and being an additional hurdle to circumvent for neuroscience programs. To date, several strategies have been used to predict brain concentrations.[1-4] *In vitro* approaches include transporter assays to assess active efflux[5] and cell systems to mimic tight junctions.[6] Although these tools are useful for exploring features of the BBB, the Achilles' heel of any *in vitro* experiment is the inability to recapitulate completely the complexities of the physiology[4] including tight junctions, limited unstirred water layer, and transporters. They, therefore, cannot be fully predictive. Statistical[7] and quantitative structure–activity relationship[8] models have also been developed. These efforts again struggle to capture accurately the intricacies of the physiology and often only project the extent (not rate) of brain distribution.

Physiologically based pharmacokinetic (PBPK) models attempt to distill detailed physiology into a simplified mathematical framework. Such models incorporate both compound and species-specific inputs. Because the physiological and drug attributes are defined explicitly, PBPK approaches are capable of describing even complicated systems and can aid in translation between species. The proposed model for distribution to the brain is similar to that proposed by Ball et al.[9] and includes well-stirred compartments associated with the plasma, cerebrospinal fluid (CSF), BBB, blood–CSF barrier (BCSFB), brain extracellular fluid (ECF), and brain intracellular fluid (ICF). Physiological parameters, such as surface areas, volumes, fluid flow rates, and transporter expression levels for each species of interest, are assigned from the literature. Compound-specific parameters including brain and plasma binding, active efflux clearance, and passive permeability are also inputs. Taken together, the PBPK model can estimate for a wide range of chemical matter exposure as a function of time, that is, both the rate and extent of brain penetration. Translation between species can be achieved by adjusting the physiological constants.

Although literature contains many examples of central nervous system PBPK models,[1,9-15] to date, the full utility of the approach has yet to be realized. The model structure is typically simple to construct; the challenge arises when attempting to define critical inputs: passive permeability, active clearance, brain–CSF connectivity, and relative level of transporter activity. Past efforts have relied on fitting *in vivo* data to extract parameters *post hoc* or were theoretical exercises that lacked a comprehensive strategy for efficiently obtaining compound-specific inputs. The current work presents a brain penetration model together with improved *in silico* methodologies to generate passive and active permeability parameters. The

* *Correspondence to:* Patrick E. Trapa (Telephone: 617-395-0793; Fax: 845-474-3676).
E-mail address: patrick.trapa@pfizer.com (P.E. Trapa).

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model is used to illustrate the various sources of brain disequilibrium and simplified to address scaling across species for effluxed compounds with high passive transcellular permeability.

Experimental

Figure 1 illustrates the standard workflow. Brain penetration predictions from *in vitro* data reduce the number of *in vivo* experiments performed. When *in vivo* studies are run, the model presented can be used to translate the results to higher order species (including human) using proteomics data. Concordance of predictions provides additional confidence in projections.

In Vivo *Data*

In vivo experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) using protocols reviewed and approved by the WRD Institutional Animal Care and Use Committee (Groton, CT). The studies and bioanalytical liquid chromatography–mass spectrometry/mass spectrometry analyses followed standard procedures.[16]

Compound Properties

Rodent brain and plasma binding values listed in Table 1 were obtained through equilibrium dialysis as described previously.[17] Permeability assays were performed using a well-established transwell format.[5] Molecular properties (TPSA, logP, etc.) were calculated using ACD/Labs, v12.0 (ACD/Labs, Toronto, Ontario, Canada).

In Silico *Model of Brain Uptake Passive Permeability*

Tsinman et al.[18] developed a quantitative structure–activity relationship model utilizing among other descriptors permeability data obtained from a porcine brain lipid extract artificial membrane *in vitro* permeability assay to predict passive brain permeability. The present work developed a statistical linear regression model based on published *in situ* brain permeability data[18] using

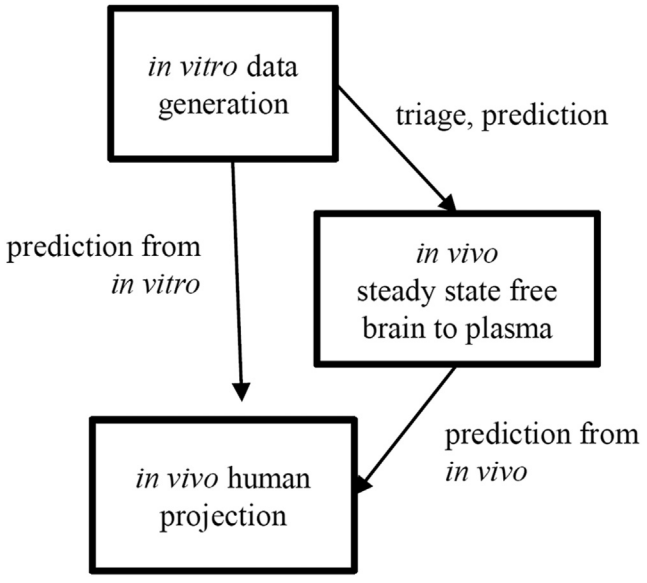

Figure 1. Schematic of the procedure used for projecting human brain penetration. Both *in vitro* and *in vivo* data can be used for increased confidence.

Table 1
Compound Properties

Variable	Compound A	Compound B
Mw (Da)	337.81	446.5
cLogP	3.54	4.37
cLogD (pH = 7.4)	N/A	2.98
TPSA	38.1	61.6
Ionization state	Neutral	Base
RRCK × 10^6 (cm/s)	21.4	17.7
Transcellular permeability × 10^6 (cm/s)	285	28.2
MDR efflux ratio	1.16	2.77
fu_p	0.029	0.21
fu_b	0.032	0.17
Ratio of unbound brain to plasma AUC (0-tlast)	0.8	0.4

Molecular Operating Environment 2D (MOE2D, 2008) descriptors and Moriguchi descriptors[19] as substitutes for the *in vitro* experimental input. Obviating the need for a specific assay extends the utility of the approach. The model construction used 81 compounds.[18] Similar to the work of Tsinman et al.,[18] the present effort also divided the drugs into ionization classes using the reported p*K*a values.[18] On account of the small number of data points, care was taken to identify only 2 statistical significant descriptors per class. JMP® 7.0 was used to evaluate the descriptors and to generate the linear regression models.

In Silico *Model of* In Vitro *Active Efflux Data*

Various methods have been put forward to extract the efflux rate from *in vitro* assays. Tran et al.[20,21] developed a thorough analysis which defined the kinetic parameters; however, the process was resource intensive and provided extraneous information (e.g., saturation at free concentrations unachievable *in vivo*). In contrast, Kalvass[22] reduced the complexity of the system to a level of detail commensurate to the PBPK model. Neither analysis considered the unstirred water layer of the assay or the potential for paracellular diffusion between the cells. The model from Kalvass was, therefore, extended to include these aspects. Figure 2 is a schematic of the model structure, and Equations 1-5 define the system. C_a, $C_{s,a}$, C_c, $C_{s,b}$, and C_b refer to the concentrations in the apical well, at the surface of the cell on the apical side, in the cell, at the basolateral cell surface, and in the basolateral well, respectively. $P_{UWL,a}$, $P_{UWL,b}$, $P_{bi,a}$, $P_{bi,b}$, P_{act}, and P_{para} refer to the permeability of the unstirred water layer (apical or basolateral), cell bilayer (apical or basolateral), active efflux, and paracellular diffusion respectively. V_a, V_s, V_c, and V_b refer to the volumes of each compartment separated by cross-sectional area SA. Note that the volume associated with the cell surface should be set at a vanishingly small value. Equations 6 and 7 describe the relation between the measured *in vitro* value for permeability (P_{AB} and P_{BA} refer to apical-to-basolateral and basolateral-to-apical permeability, respectively) and the processes captured in Figure 2. Note that traditionally the transwell assay is run at sink conditions implying that the concentration in the receiver compartment can be considered to be negligible.

$$\frac{dC_a}{dt} \times \frac{V_a}{SA} = -P_{UWL,a} \times (C_a - C_{s,a}) \quad \text{Eq.1}$$

$$\frac{dC_{s,a}}{dt} \times \frac{V_s}{SA} = P_{UWL,a} \times (C_a - C_{s,a}) - P_{bi,a} \times (C_{s,a} - C_c) - P_{para} \times (C_{s,a} - C_{s,b}) + P_{act} \times C_c \quad \text{Eq.2}$$