

Blood-Brain Barrier Penetration: Evaluation of *In Vitro* Parameters for Classification



Hinnerk Boriss¹, Robert R. Annand², Katya Tsaion²

¹Sovicell GmbH, Deutscher Platz 5b, 04103 Leipzig, Germany, hbo@sovicell.com

²Apredica, Inc., 313 Pleasant Street, Watertown, MA 02472, USA



Introduction

Early assessment of compound availability in the central nervous system (CNS) is essential for CNS drugs and useful for optimizing the toxicity profile of non-CNS drugs. Extent and rate are the key factors which determine how compounds cross the blood-brain barrier. This study compares the utility of the *in vitro* techniques PAMPA BBB and TRANSIL Brain Absorption for the prediction of rate and extent of brain penetration and for classification of CNS- and CNS- compounds.

Materials and methods

TRANSIL assays were conducted for plasma protein binding using TRANSIL HSA and AGP Binding kits. Brain membrane affinity was determined with the TRANSIL Brain Absorption kit. Assay compound concentrations were 2 μ M. All samples were quantified using LC/MS/MS.

For PAMPA BBB filter membranes in 96-well plates were coated with porcine brain lipid (4 μ L/well of a 20 mg/mL solution in dodecane). Donor wells had an initial compound concentration of 10 μ M in PBS (pH 7.4), the acceptor wells contained PBS, and the assay was run undisturbed over a period of 18 hours. The concentration in the donor and acceptor wells was determined by LC/MS/MS.

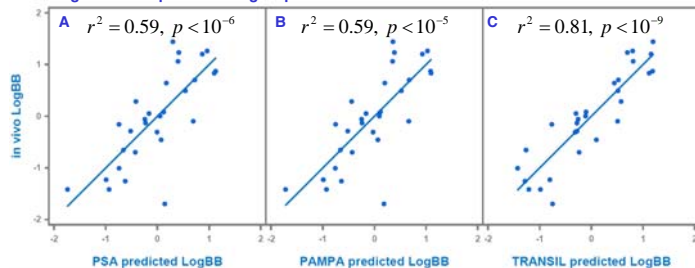
General linear models were used to predict the brain free fraction and the logBB.

1. Extent: logBB

The brain-to-plasma distribution coefficient describes the extent of brain penetration. To predict the *in vivo* logBB we used (A) as a base model polar surface area (PSA) and plasma protein binding (logit transformed free fraction: logKB/F), and in an extended model (B) additionally PAMPA BBB permeability (Papp), as well as in a third model (C) the brain membrane affinity (MA_{brain}) instead of Papp.

- A) $\log BB = A \cdot PSA + B \cdot \log K_{B/F} + C$
- B) $\log BB = A \cdot P_{app} + B \cdot PSA + C \cdot \log K_{B/F} + D$
- C) $\log BB = A \cdot \log MA_{brain} + B \cdot PSA + C \cdot \log K_{B/F} + D$

Figure 1: Comparison of logBB predictions



2. Extent: Brain Free Fraction

With increasing lipophilicity compounds logBB increases and unspecific binding in brain increases, which in turn decreases the brain free fraction (fig. 2). Hence, to determine the extent of brain penetration in the sense of available compound, we predicted the brain free fraction the brain membrane affinity with the TRANSIL assay kit (fig. 3).

Figure 2: Relationship between the brain free fraction and logBB

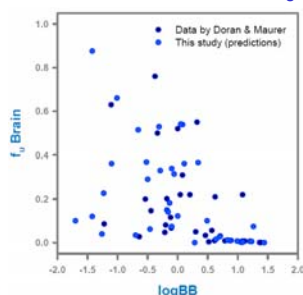
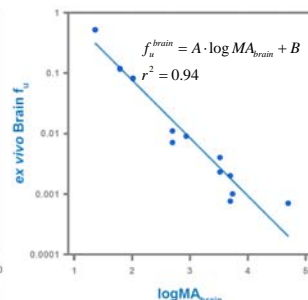


Figure 3: TRANSIL prediction of the brain free fraction



Conclusions

Brain membrane affinity in conjunction with PSA and plasma free fraction yields better predictions of the extent of brain penetration than PAMPA BBB.

Brain membrane affinity alone is a good predictor of the brain free fraction.

Rate and extent based CNS classification of compounds using brain availability and brain membrane affinity yields 86% correctly classified compounds, while classification based on PAMPA BBB rates alone yields only 62% correctly classified compounds and 10% non-categorized.

3. Classification by Rate

The PAMPA BBB assay were used to assign compounds to low, intermediate, and highly permeable compounds indicating their ability to cross the blood brain barrier. See table 1 for the class assignments.

$$c = \begin{cases} \text{low} & \text{if } P_{app} \leq 2 \cdot 10^{-6} \text{ cm/sec} \\ \text{med} & \text{if } 2 \cdot 10^{-6} \text{ cm/sec} > P_{app} > 4 \cdot 10^{-6} \text{ cm/sec} \\ \text{high} & \text{if } P_{app} \geq 4 \cdot 10^{-6} \text{ cm/sec} \end{cases}$$

4. Classification by Rate and Extent

Rate and extent are considered critical for brain penetration. The rate can be estimated by PAMPA BBB (P_{app}) and by the log transform of the affinity to brain membranes (logMA). The best estimate for the extent is the free concentration in brain (c_u). The free concentration in brain is proportional to the product of the B/P ratio and the brain free fraction, which we denote brain availability.

The rate and extent estimates are used to classify the test compounds as CNS+ or CNS- compounds (fig. 4). We used the rate estimated by PAMPA BBB in figure 4A, and the TRANSIL derived rate in figure 4B. Only the TRANSIL derived rate estimate results in clear separation of CNS compounds versus non CNS compounds. Data and compounds used are shown in Table 1.

Both the PAMPA BBB assay as well as the TRANSIL Brain Absorption assay only measure passive transport, so compounds for which active transport plays a significant role in BBB penetration might be misclassified by either method.

Figure 4A: Classification based on PAMPA rate and TRANSIL extent

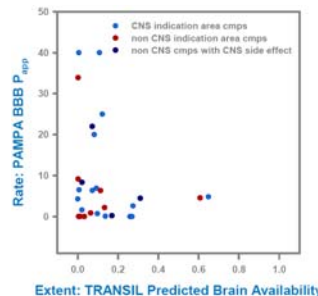


Figure 4B: Classification based on TRANSIL rate and extent

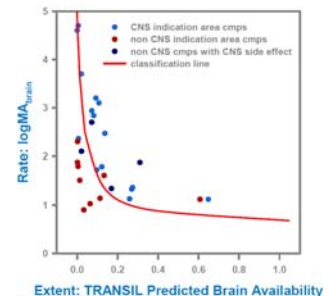


Table 1: Measured and predicted data for 29 commercially available compounds as well as their classification according to PAMPA BBB rates

Compound	PAMPA class	Drug type	Class
Amiripytline	high	anti-depressant	CNS
Carbamazepine	high	antiepileptic, anticonvulsant	CNS
Desipramine	high	anti-depressant	CNS
Diphenhydramine	high	sedative, hypnotic	CNS
Imipramine	high	anti-depressant, Alzheimer	CNS
Promazine	high	antipsychotic	CNS
Trifluoperazine	high	antipsychotic	CNS
Verapamil	high	calcium channel blocker	CNS
Acetaminophen	low	analgesic and antipyretic	CNS
Antipyrin	low	analgesic	CNS
Propofol	low	sedative	CNS
Thiopamide	low	histamine receptor antagonist	CNS
Caffein	med		CNS
Chlorpromazine	med	antipsychotic	CNS
Eserine	high	parasympathomimetic	CNS
Thioridazine	high	antipsychotic	CNS
Chlorambucil	high	chemotherapy	non CNS
Indomethacin	high	anti-inflammatory	non CNS
Nalidixic acid	high	bacteriostatic	non CNS
Atenolol	low	beta blocker	non CNS
Cimetidine	low	antihistamine	non CNS
Ranitidine	low	antihistamine	non CNS
Tolbutamide	low	type II diabetes	non CNS
Pinidolol	med	beta blocker	non CNS
Procaine	high	local anesthetic	non CNS
Propranolol	high	beta blocker	CNS side effect
Theophylline	low	COPD	CNS side effect
Pyrimidine	high	antihistamine	CNS side effect
Quinidine	high	anti-rhythmic agent	CNS side effect