

Brain Availability Predicted by Membrane Affinity, PSA, and Plasma Protein Binding

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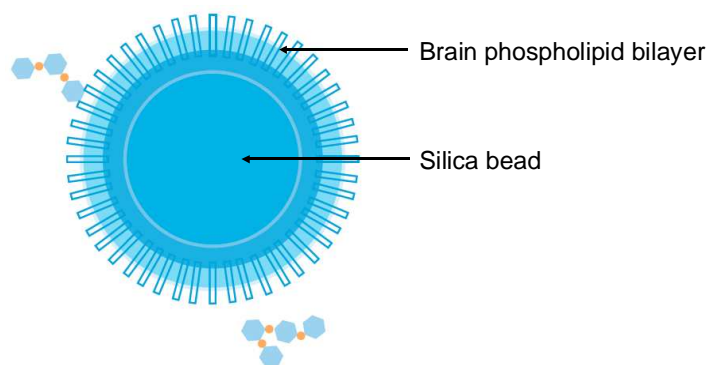
Introduction

Determining the unbound concentration of drug candidates in brain is essential in CNS projects to optimize the drug availability to potency ratio. Likewise, knowing the CNS penetration of drug candidates for all other indication areas at the stage of lead optimization assists in optimizing their toxicity profile.

Established measurements of the extent of free drug in brain are laborious, time consuming, and expensive. Hence there is a need for reliable, inexpensive, high-throughput *in vitro* assays to determine free drug in brain.

Materials and methods

All samples were quantified using HPLC-MS/MS with a Prontosil RP-C18 column and a Sciex API-2000 triple quadrupole mass spectrometer. Plasma protein binding was determined using TRANSIL HSA and AGP Binding kits. Brain membrane affinity was determined with the TRANSIL Brain Absorption kit. Predictions of the brain free fraction and the logBB were based on the brain membrane affinity, plasma protein binding and polar surface area. *In vivo* data for the brain-plasma distribution were obtained from Platts et al. (Eur. J. Med. Chem. 2001(9):719-730), and *ex vivo* data for the brain free fraction were obtained from Kalvass et al. (DMD 2005, 35(4):660-666).



Conclusions

Brain membrane affinity in conjunction with PSA and plasma free fraction is well suited to predict *in vivo* brain-plasma distribution coefficient logBB.

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

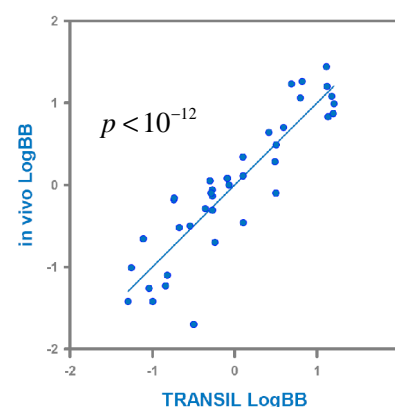
Polar surface area and membrane affinity explain different aspects of membrane permeability.

TRANSIL Brain Absorption can be used as an inexpensive high throughput tool to estimate the free concentration in brain.

1. LogBB Prediction

TRANSIL beads coated with brain lipid bilayers were used to determine compound's brain membrane affinity. Together with polar surface area and plasma protein binding, brain membrane affinity was used to build a model for predicting logBB.

$$\log BB = A \cdot \log MA_{\text{brain}} + B \cdot PSA + C \cdot \log K_{B/F} + D$$

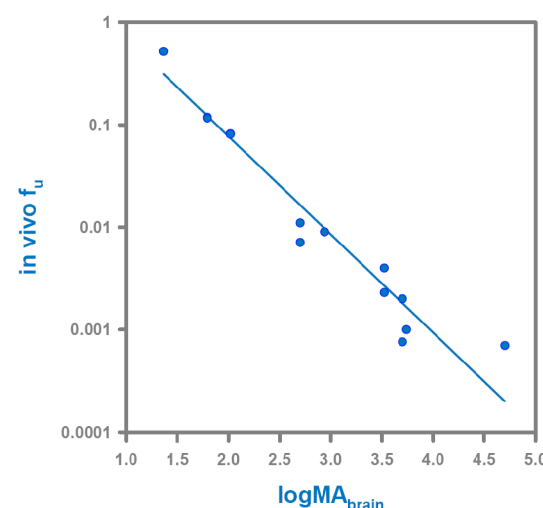


Variable	p-value
$\log MA_{\text{brain}}$	$6.9 \cdot 10^{-7}$
$\log K_{B/F}$	$2.1 \cdot 10^{-4}$
PSA	$1.2 \cdot 10^{-8}$

3. Brain Free Fraction Prediction

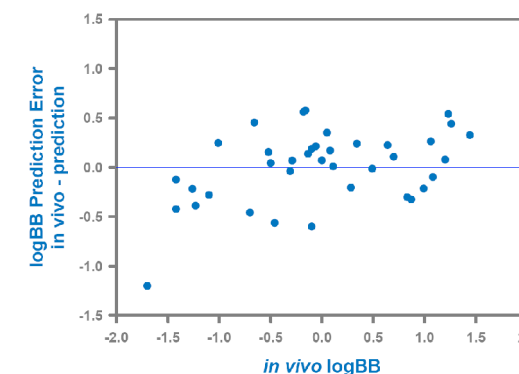
The brain lipid membrane affinity was also used to predict the free fraction in the brain interstitial fluid. To build the model *ex vivo* brain free fraction data were used in conjunction with data from the TRANSIL assay kit. We found an $r^2=0.94$ for the correlation with established methods.

$$f_u^{\text{brain}} = A \cdot \log MA_{\text{brain}} + B$$



2. Cross Validation

Leave one out cross validation was used to analyze the model performance. Prediction errors were evenly distributed over the *in vivo* logBB scale suggesting scale invariance of the model. The average prediction error was found to be 0.22 logBB units.



4. CNS+/CNS- Classification

The product of the brain-plasma distribution coefficient and the brain free fraction denotes the amount of compound freely available in brain. The brain membrane affinity also relates to the permeability rate across the blood-brain barrier. The figure below shows that these two measures can be used to separate compounds penetrating the brain from those that aren't.

