Prediction of *in situ* Blood-Brain Barrier Permeability using BBB PAMPA Model

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INTRODUCTION

Central nervous system (CNS)-targeting drugs need to cross the blood-brain barrier (BBB) in order to reach their therapeutic receptors inside the brain. If a reliable, reasonably low-cost, compound-sparing, and high-throughput method could be developed to predict the potential of pharmaceutical discovery compounds penetrating the BBB, then such a product would be an important contribution to the assortment of the discovery tools available for pharmaceutical industry.

This study presents new developments achieved in BBB lipid formulation for the PAMPA assay and comparison between BBB PAMPA and recently published rat *in situ* perfusion permeability data. It also discusses applicability of commercially available pre-coated PAMPA plates from BD Biosciences for the prediction of *in situ* rodent brain perfusion uptake kinetics.

BBB PAMPA METHOD

The robotic sample preparation system of the PAMPA Evolution Instrument (*p*ION INC) uses either the Beckman FX or TECAN Evo workstation, and a 96-well UV scanning plate spectrophotometer. Magnetically-stirred **STIRWELL**™ 96-well microtitre plate "sandwiches" are used. (Figure 1 a, b) Microfilters are automatically coated with a phospholipid-based solution, mimicking the composition of brain membrane components (BBB-1 Lipid, *p*ION INC).

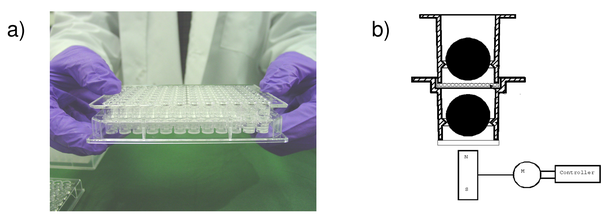

Figure 1. PAMPA "sandwich" (a) and one permeation cell of a double-sided stirred 96-well microtitre plate "sandwich" (b).

Following an hour permeation period, the sandwich is disassembled and the permeability coefficients are calculated from the relative sample concentrations in the donor and acceptor wells, with correction for mass balance, compound ionization, and the aqueous boundary layer. These are referred to as *intrinsic* permeability coefficients (P_o^{PAMPA}), and are expressed in cm/s units.

RESULTS AND DISCUSSIONS

Original (Wyeth) Approach to BBB PAMPA

The first application of PAMPA used to differentiate between CNS+ and CNS- compounds was described by Li Di *et al.*[1,2] The authors used a formula of 2% wt/vol of porcine brain lipid extract in dodecane to demonstrate that the PAMPA assay can successfully "bin" CNS+ and CNS- compounds. This strategy is schematically represented in Figure 2. Unfortunately, the comparison of these data[2] with the *in situ* rat perfusion permeability data reported in Summerfield *et al.*[3] was affected by an error in the permeability units reported originally in ref. 3.

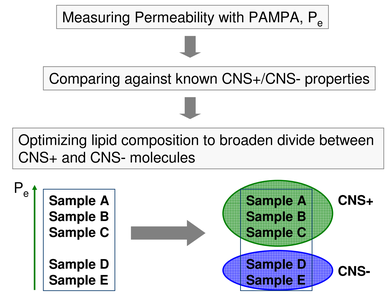

Figure 2. Diagram of the Wyeth approach to apply PAMPA for BBB application.

*p*ION*'s Approach for Optimizing BBB PAMPA*

Learning from the research that led to the successful Double-Sink™ PAMPA model for predicting human GIT permeability[4], a similar approach was applied to optimize the lipid model and conditions for predicting rodent *in situ* brain perfusion uptake kinetics. Figure 3 illustrates the research strategies in optimizing the BBB PAMPA model. Achieving correlation between PAMPA results and *in situ* Pgp deficient (mdr1a(-/-)) mice brain perfusion data, see Figure 4, was the main target of the project[5].

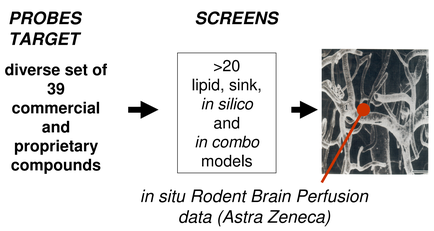

Figure 3. Diagram illustrating the "training" of PAMPA for BBB application.

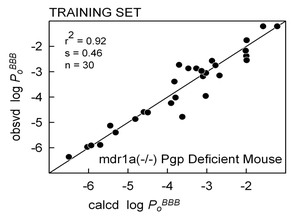

Figure 4. Shows the best *in combo* model for predicting rodent brain perfusion data, based on the 30-compound knockout mouse training set[5].

Validation of BBB PAMPA

A set of compounds with rat *in situ* brain permeability data[3] (corrected for the unit error) was used as an external data set to test the model. The lipid composition was further refined to minimize the need for *in silico* addition to the model. BBB PAMPA assays were performed on most of these compounds applying stirring in all individual wells at different pH conditions to correct for ionization and aqueous boundary layer effects and obtain intrinsic permeability (P_0) values. BBB PAMPA data without any additional *in silico* enhancement correlated linearly with published data with a slope close to 1 and r^2 ~ 0.7; see Figure 5. It was also demonstrated that in a high throughput setting, a single measurement at pH 7.4 could be enough assuming that stirring with the Gut-Box™ (*p*ION INC) is provided to mimic aqueous boundary layer (ABL) thickness of the brain perfusion experiments.

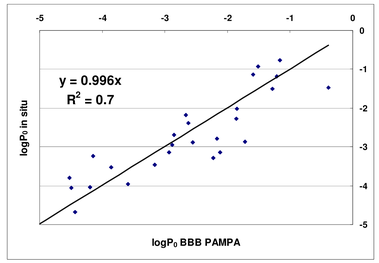

Figure 5. Correlation between intrinsic BBB PAMPA and published[3] *in situ* data corrected for ionization effect. No additional calculation treatment has been applied to the data.

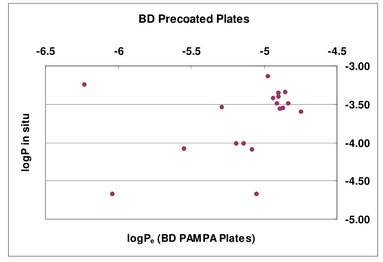

Figure 6. Values obtained with pre-coated PAMPA plates from BD Bioscience compared to published[3] *in situ* rat perfusion data.

Pre-coated PAMPA Plates from BD Biosciences

Pre-coated PAMPA plates were used to evaluate their potential for predicting brain perfusion kinetic. Unfortunately, due to the geometry of the Millipore/BD Biosciences PAMPA "sandwich" it was not possible to implement stirring. This significantly limited the capability of correcting results for the ABL and ionization effects. Figure 6 demonstrates that correlation was not satisfactory when comparing effective BD PAMPA values to the reported published data.

CONCLUSIONS

The results obtained from BBB PAMPA provided better predictability of *in situ* brain penetration than cell-based MDCK permeability assay according to the publication by Summerfield *et al.*[3] and were considered quite satisfactory because no additional *in silico* treatment was necessary for the PAMPA data.

Pre-coated PAMPA plates from BD Biosciences did not produce results adequately correlating with rat *in situ* brain perfusion data[3].

In high-throughput settings BBB PAMPA can be utilized at a single pH 7.4 if the efficient stirring is applied to minimize ABL influence.

BBB PAMPA reagent kit is available from *p*ION along with a set of test compounds to ensure proper assay running.

Additional improvement can be achieved if partitioning of compounds to the brain tissue is taken into account[5].

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