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 (pION 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) Microtiter plates are automatically coated with an phospholipid-based solution, mimicking the composition of brain membrane components (BBB-1 Lipid, pION INC).

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. 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 with the in situ rat perfusion permeability data reported in Summerfield et al. was affected by an error in the permeability units reported originally in ref. 3.

Validation of BBB PAMPA

A set of compounds with rat in situ brain permeability data2 (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) values. BBB data without any additional in silico enhancement correlated linearly with published data with a slope close to 1 and r² ~ 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™ (corrected for the unit error) was necessary for the PAMPA data. Pre-coated PAMPA plates from BD Biosciences did not produce results adequately correlating with rat brain perfusion data.

pION’s Approach for Optimizing BBB PAMPA

Learning from the research that led to the successful Double-Sink™ PAMPA model for predicting human GIT permeability4, 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.

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.5 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 result in sufficient correlation with rat in situ brain perfusion data5.

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 pION 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 account5.

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REFERENCES