Brain Tissue Binding of Drugs: Evaluation and validation of solid supported porcine brain membrane vesicles (TRANSIL)

Kathleen Böhme, Daniel Nimptsch, Hinnerk Boriss

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

Introduction

Early assessment of drug candidate availability in the Central Nervous System (CNS) is essential for CNS drug development and useful for optimizing the toxicity profile of non-CNS drugs. The extent of brain tissue binding is an important optimization parameter because the stronger the binding of a drug candidate to brain tissue, the lower will be the unbound fraction of the drug that can freely interact with target receptors.

We present a novel matrix-free high-throughput method for estimating the unbound fraction of drug in brain. The method was evaluated by comparing free fractions (fu_{brain}) obtained using the TRANSIL Brain Absorption assay and current standard equilibrium dialysis technologies for a test set of 65 drugs. Moreover, we established a "four-in-one" cocktail incubation method that further enhances the throughput capacity.

Methods

- The affinity of the test compounds to brain lipid membranes was determined using a TRANSIL Brain Absorption kit (Sovicell). Each test compound was incubated for 2 min at a concentration of 0.5 µM and quantification was performed by UPLC-MS/MS.
- Cocktail incubations were processed analogously but set up with a total concentration of 2 µM made up of 0.5 µM for each out of the four test compounds per well.
- Interspecies comparability of brain lipid composition of porcine and rat tissue homogenates was analyzed by ^{31}P NMR spectroscopy.

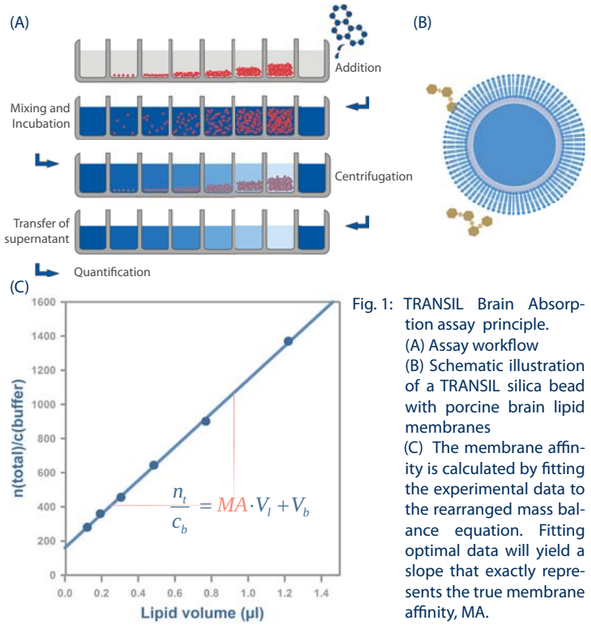

Fig. 1: TRANSIL Brain Absorption assay principle.
(A) Assay workflow
(B) Schematic illustration of a TRANSIL silica bead with porcine brain lipid membranes
(C) The membrane affinity is calculated by fitting the experimental data to the rearranged mass balance equation. Fitting optimal data will yield a slope that exactly represents the true membrane affinity, MA.

Brain Lipid Composition

Our TRANSIL assay utilizes porcine brain membranes for the determination of brain tissue binding, while rat homogenates are routinely used for dialyses approaches. To ensure the comparability of the results obtained from these species we investigated the lipid composition of both brain extracts. ^{31}P NMR revealed only minor differences between rat and pig brain, which indicates that there are virtually no species differences in brain tissue binding.

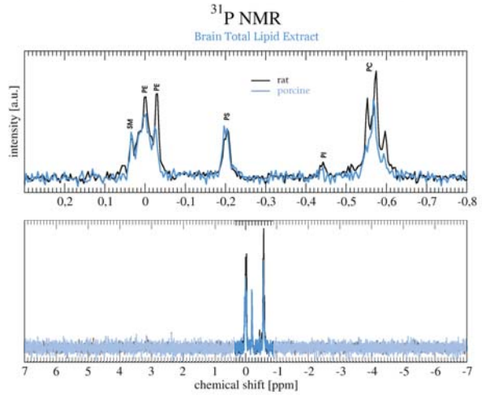

Fig. 2: ^{31}P NMR spectra of rat and porcine brain homogenates. The global NMR spectra is shown at the bottom, while the upper panel dissolves the area of the different brain lipids found in both extracts.

Measurement of Brain Tissue Binding

The comparison of the measurements of brain free fractions (fu_{brain}) using the TRANSIL Brain Absorption assay and equilibrium dialysis methods for a test set of 65 drugs (27 marketed and 38 GlaxoSmithKline proprietary drugs) revealed a good correlation (r^2 >0.93) of fu_{brain} between both methods.

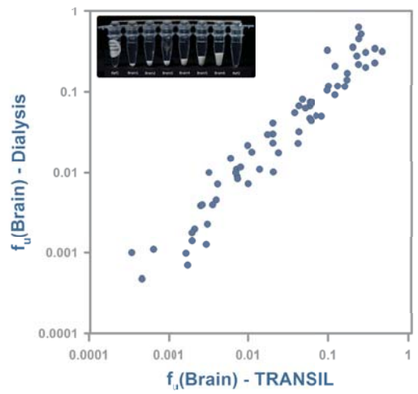

Fig. 3: Correlation of brain tissue binding estimates obtained with the TRANSIL Brain Absorption assay kit and dialysis with brain homogenate (r^2 >0.93). The set of 65 drugs is structurally heterogeneous and spans a clogP from 0.6 to 6.2 and a polar surface area from 3 to 26.

Multiplexing approach

To further increase sample throughput of the TRANSIL brain absorption system, we investigated the possibility of assaying multiple compounds in parallel. The results obtained with a "four-in-one" cocktail incubation approach displayed comparable percentages of free fractions in brain for 25 CNS-active drugs when evaluated individually or as a mixture. While the discrete incubation method enables to run 12 compounds per plate, the pooling facilitates assaying up to 48 compounds per 96 well plate. A good correlation (r^2 =0.98) was observed for the discrete and cocktail experiments of the chemically diverse test set, despite considering a wide range of brain free fractions.

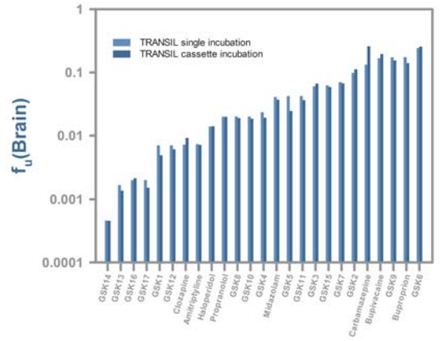

Fig. 4: Comparison of brain free fractions between cocktail and discrete TRANSIL Brain Absorption measurements. The test set of 25 compounds comprises new chemical entities with CNS target activity as well as commercial available drugs.

Conclusion

- High similarity in brain lipid composition of rat and pig extracts indicates the comparability of pig fu_{brain} and rat fu_{brain}.
- Brain membrane affinity is a good predictor of the brain free fraction.
- The TRANSIL Brain Absorption assay yields highly comparable brain tissue binding estimates to equilibrium dialysis with brain homogenate (r^2=0.93).
- At the same time, the TRANSIL assay is
  - Extraordinarily fast, requiring only 15 minutes incubation time and 5 minutes preparation (dialysis: 5 h incubation and 100 min handling)
  - Matrix-free method, hence, less issues with compound stability
  - Multiplexing increases throughput and cost effectiveness while retaining accuracy

References

Longhi R, Corbioli S, Fontana S, Vinco F, Braggio S, Helmdach L, Schiller J, and Boriss H (2011). Brain Tissue Binding of Drugs: Evaluation and Validation of Solid Supported Porcine Brain Membrane Vesicles (TRANSIL) as a Novel High-Throughput Method. Drug Metabolism and Disposition (39): 312-321.

Sovicell
Deutscher Platz 5b 04103 Leipzig t. +49 341 520 44 0 f. +49 341 520 44 12 e. info@sovicell.com www.sovicell.com