Blood Brain Barrier Permeability Assay Background

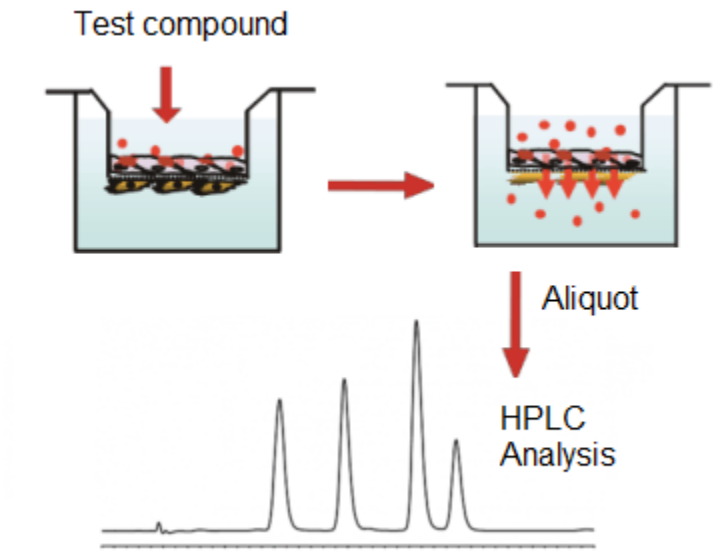
- The blood brain barrier (BBB) behaves as a highly selective semipermeable membrane separating circulating blood from the central nervous system while allowing the passage of glucose, water, and amino acids into the cerebrospinal fluid [1].
- The BBB protects brain nervous tissue from the fluctuation of plasma composition, from pathogenic agents, and maintains homeostasis of the brain parenchyma by restricting non-specific flux of ions, peptides, proteins and cells into and out the brain.
- The BBB is permeable to hydrophobic molecules and utilizes active transport to move ions, glucose, and other critical molecules across the membrane. When functioning properly, the blood brain barrier is a complex hurdle that must be overcome when delivering drugs to the brain.
- Assessment of BBB permeability of drug compounds is critical for drugs targeting regions of the brain; many drugs developed to treat Central Nervous System (CNS) disorders are unable to reach the brain parenchyma in therapeutically relevant concentrations.
- Poor penetration of the BBB is the cause for attrition for 95% of drugs developed for neurological disorders. As such, it is of great interest to explore potential molecules that can modulate BBB permeability [2].
- Disruption of the BBB is observed in many neurological disorders, including multiple sclerosis, stroke, Alzheimer's disease, epilepsy, and traumatic brain injury, and is frequently induced by neuroinflammation [1].
- The BBB is a complex system, which is difficult to mimic *in vitro*, which typically had to be addressed with costly, low-throughput animal studies.
- Utilizing a novel BBB *in vitro* model from Neuromics, we offer an *in vitro* assay capable of assessing the penetration kinetics of molecules passing across the BBB.
- Additionally, we offer a complementary assay to assess modulation of BBB permeability due to drug treatment.
- This assay service leverages the novel Neuromics 3D Blood Brain Barrier Model and allows for both compound transport across the barrier to be studied as well as the effect of compounds on the structure and function of the BBB.

Protocol

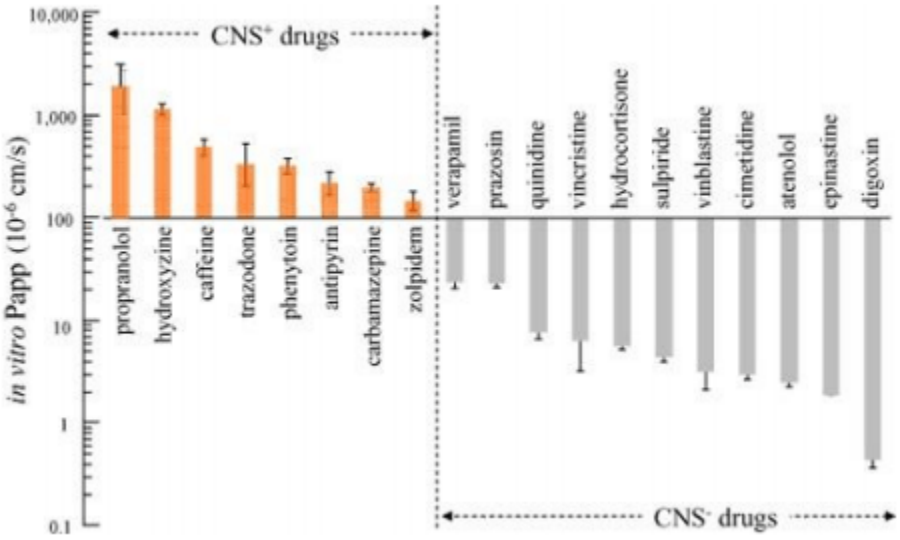
Instrument	ThermoFisher Varioskan Lux Agilent UPLC/DAD
Analysis Method	Plate Reader UPLC Analysis
Markers	70 kDa fluorescent dextran conjugate (for assessment of alterations to BBB permeability)
Cell model	Neuromics Human Blood Brain Barrier Model
Time points	30 min, 1 hr, 4 hr, 24 hr (custom time points available)
Test Article Concentration	Single point assay (1 µM) (custom concentrations available)
Number of Replicates	3 replicates per time point
Quality Controls	0.5% DMSO (vehicle control) Propanolol (positive control)
Test Article Requirements	50 uL of 20 mM solution or equivalent amount of solid
Data Delivery	Apparent Permeability of test compounds in either the apical or basal direction Change in Apparent Permeability of BBB due to effect of drug compound (optional)

General Procedure

1. 3D Blood Brain Barrier models are thawed and cultured for 4 days to establish viable barrier as measured by transendothelial electrical resistance (> 150 Ω x cm^2).
2. The test compound and fluorescent probe are added and the cultures are returned to incubate for designated times.
3. If assessment of alterations to BBB permeability due to drug treatment is required, fluorescent dextran conjugate is added to wells
4. Media is sampled for quantitation of test articles by UPLC, and for quantification of fluorecent probe by plate reader
5. Apparent permeability is calculated


Test compound
Aliquot
HPLC
Analysis

Data


10,000
1,000
100
10
1
0.1
in vitro Papp (10⁻⁶ cm/s)
CNS⁺ drugs
propranolol
hydroxyzine
caffeine
trazodone
phenytoin
antipyrin
carbamazepine
zolpidem
verapamil
prazosin
quinidine
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cimetidine
atenolol
epinastine
digoxin
CNS⁻ drugs

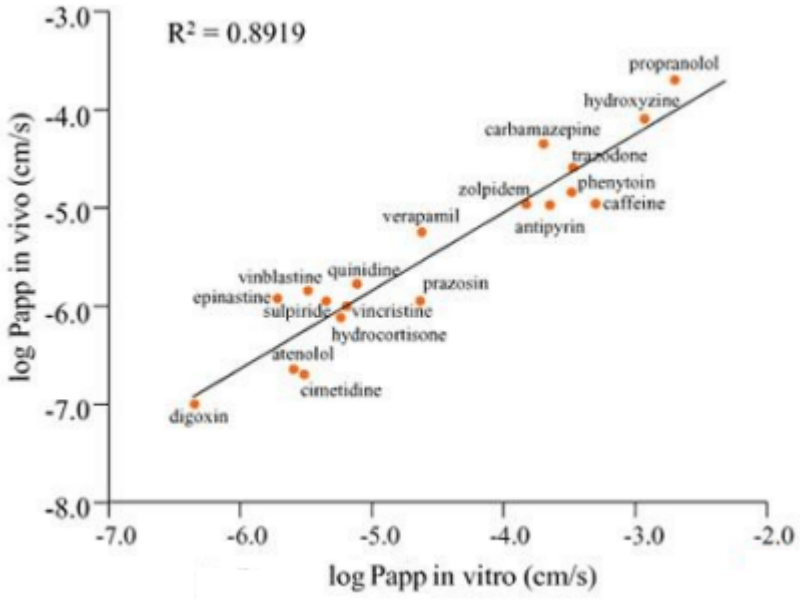

Figure 1. Apparent permeability (Papp) of various known drugs measured using *in vitro* BBB assay. CNS+ drugs show excellent Papp, CNS- drugs show very low Papp

Figure 2. *In vitro* results measuring apparent permeability of BBB to known drug compounds showed excellent correlation to *in vivo* results ($R^2 = 0.8919$)

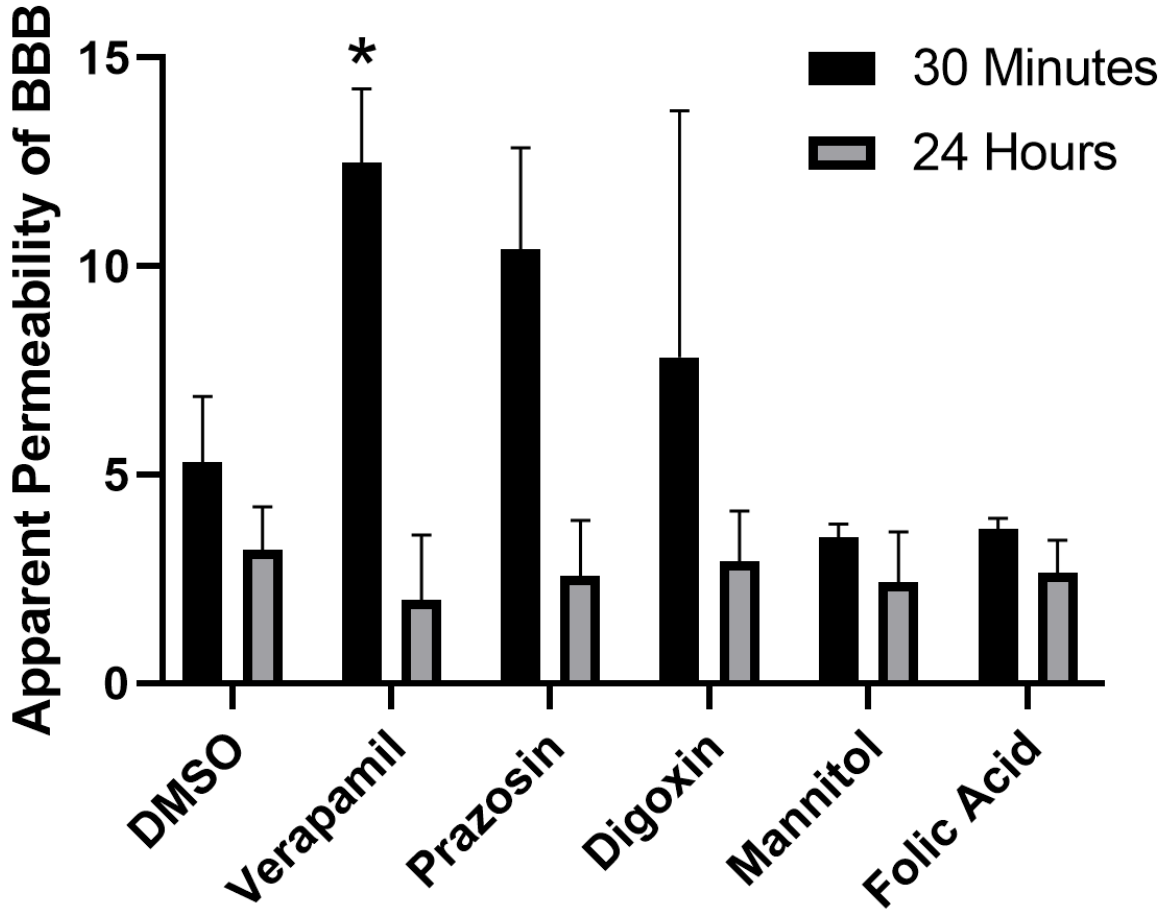

Figure 3. Alterations in apparent permeability of blood brain barrier due to drug treatment. Data presented as mean ± SEM x 10^{-6} cm/s. * Indicates $p<0.05$ for Fisher's LSD t-test comparisons to DMSO controls in a *post hoc* analysis following two-way ANOVA.

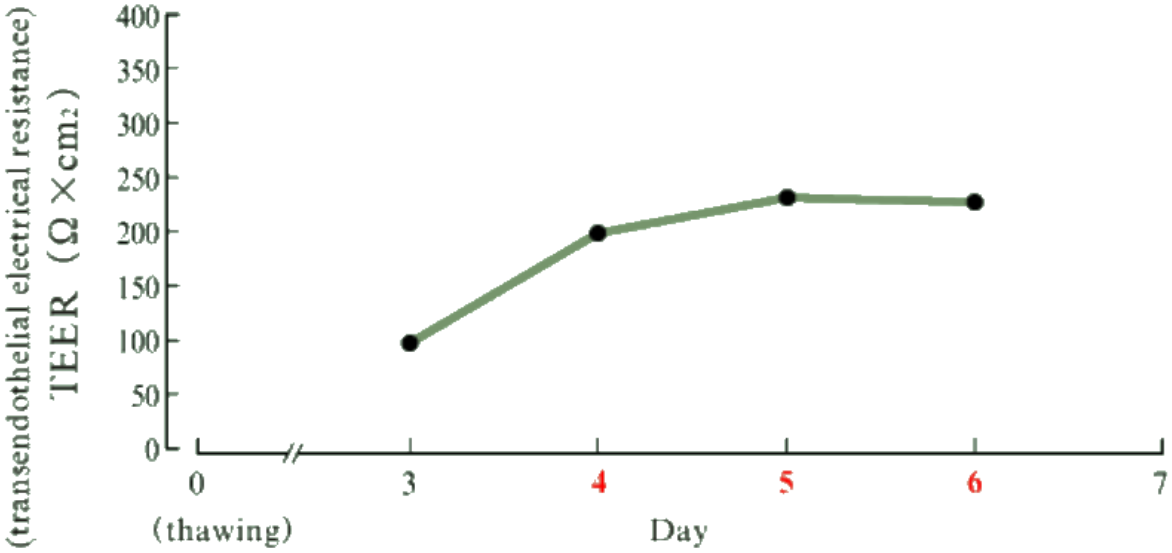

Figure 4. Transendothelial electrical resistance measurement of BBB model by day illustrating intact and functional barrier

References

1. Daneman, R. (2012). The blood–brain barrier in health and disease. *Annals of neurology*, *72*(5), 648-672.
2. Bourassa, P., Alata, W., Tremblay, C., Paris-Robidas, S., & Calon, F. (2019). Transferrin Receptor-Mediated Uptake at the Blood–Brain Barrier Is Not Impaired by Alzheimer's Disease Neuropathology. *Molecular pharmaceutics*, *16*(2), 583-594.