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Assessment of a commercially available *in vitro* model of the blood-brain barrier

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ABSTRACT

Purpose: A commercially available, ready to use, *in vitro* model of the blood-brain barrier (BBB) was evaluated as a potential tool in early drug discovery to predict brain permeation. This *in vitro* model uses primary cultures of three main cell types including rat brain capillary endothelial cells, pericytes and astrocytes. Upon thawing and minimal activation time, the kit retains monolayer integrity and expression of both efflux and uptake transporters located at the BBB.

Methods: To assess the ease of use and the predictive power of these *in vitro* BBB kits, a variety of commercially available compounds and in-house Abbott compounds were tested in both uni-directional and bi-directional permeability experiments. Compounds were also tested in other screening assays commonly used in early CNS drug discovery such as the bi-directional MDCK-MDR1 efflux assay and early screening PK in which unbound brain to plasma ratios are determined and used to predict brain impairment (BI = $C_{u,plasma}/C_{u,brain}$).

Results: Permeability data from the BBB kits were then compared to in house *in vivo* brain impairment studies (data not shown) as well as literature values for brain permeability as assessed by *in situ* brain perfusion studies. While there was limited correlation to overall brain impairment, improved correlation is seen with *in vivo* brain permeability.

Conclusions: This kit offers another tool for researchers pursing central nervous system drugs as they attempt to achieve adequate CNS exposure and understand the complexity of predicting BBB permeation. The predictive power of these kits is better suited for assessing brain permeation rates which are rarely determined in early drug discovery because the pre-existing assays are labor and time intensive.

INTRODUCTION

Understanding and predicting the penetration of new chemical entities across the blood-brain barrier remains one of the greatest challenges in CNS drug discovery. Current models used early in the drug discovery process such as the MDCK-MDR1 bi-directional assay are amenable to high throughput and can be quite predictive once *in vivo* data are corrected for plasma and brain binding (Figure 1). The MDCK-MDR1 cells however are epithelial cells and are structurally quite different than the endothelial cells that make up the BBB[1,3]. While transfection of the MDCK cells with the MDR1 gene that encodes the efflux transporter P-glycoprotein (P-gp) yields a cell line very capable of predicting P-gp involvement and generally gives a good *in vitro* to *in vivo* correlation, there are other transporters and permeability characteristics that may be more accurately assessed by a brain endothelial cell culture model. Most brain endothelial models however have not been embraced by the pharmaceutical industry because they are labor intensive, costly and generally low throughput. Furthermore, many isolated brain endothelial systems do not maintain the barrier properties required for permeability studies, nor do they preserve their transporter function. Novel attempts at creating improved brain endothelial cell based *in vitro* systems are emerging and may have a place in the pharmaceutical industry.

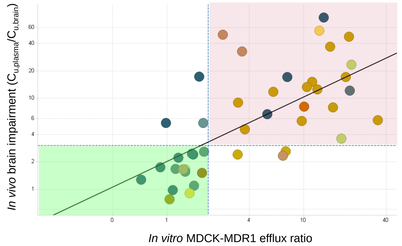


Figure 1. Representative example of the brain impairment IVIVC generally used in early CNS discovery at Abbott. Compounds falling in the bottom left quadrant have good brain penetration predicted by low efflux ratios; whereas compounds in the top right quadrant show varying degrees of brain impairment as indicated by *in vitro* efflux ratios > 2.

MATERIALS AND METHODS

Materials:
Ready-to-use BBB kits (RBT-24H) were graciously provided by PharmaCo-Cell Co, Ltd. (Nagasaki, Japan). DMEM F-12 media supplemented with FBS, heparin, bFGF, ITS, hydrocortisone and gentamicin were provided as part of the BBB kit. Commercially available test compounds were purchased from Sigma Aldrich (St. Louis, MO). Permeability experiments were performed in 24-well Corning base plates (part number 3524) (Corning, NY).

TEER measurement:
Prior to the commencement of the permeability experiments, TEER values of each well were assessed to ensure that adequate monolayer integrity had been achieved. A World Precision Instruments REMS autosampler was used (Sarasota, FL). Unit area resistance was calculated using the effective surface area of the 24-well type inserts (0.33cm^2). All wells reached the required TEER > 150 Ω x cm^2.

LC/MS/MS:
Analysis of the test compounds was performed using a Shimatzu LC with a gradient solvent program and Fortis Pace C18 5 µm (2.1 x 30 mm) column followed by tandem mass spectrometry using a Sciex 5500 triple quad. Prior to analysis, samples were quenched with 50:50 methanol:acetonitrile containing internal standard.

Thawing and Activation of BBB kit:
Thawing and activation of the kits begins with removal from the -80 degree freezer and addition of warm media. A media change is required the following day and the kits are allowed to incubate for 3 days, which can ideally be scheduled over the weekend. Kits are then ready to be used in permeability experiments on days 4-7.

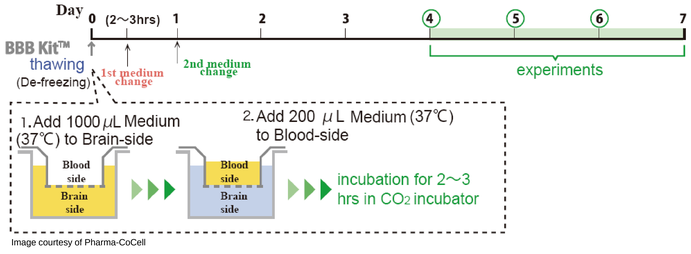
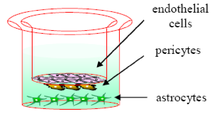


Image courtesy of Pharma-CoCell

Triple co-culture advantage:
The PharmaCo-Cell product uses a synergistic model in which Wistar rat brain endothelial cells are cultured on permeable inserts, pericytes are grown on the underside of the inserts and astrocytes are cultured on the base plates. This triple culture of multiple cell types more accurately represents the *in vivo* anatomical situation than standard endothelial culture and facilitates cross-talk and results in enhanced expression of tight junction proteins[1]. While expression of efflux transporters are preserved[1], the functional activity is reduced, making the model less sensitive to detect P-gp substrates than models such as the MDCK-MDR1 model[3] (Figure 2).



Permeability studies:
Test compound were added to the luminal side (top chamber) at concentrations of 1-10uM (0.1% DMSO) and allowed to incubate for 30 minutes. At the end of the incubation samples were taken from both the top and bottom chambers and an *in vitro* apparent permeability was calculated as well as a percent recovery.

RESULTS

Figure 2. Efflux ratios of two known P-gp substrates (quinidine and verapamil) and two non-substrates carbamazepine and indomethacin in both the MDCK-MDR1 and brain endothelial cell based BBB kit.

Compound	Efflux Ratio (MDCK-MDR1)	Efflux Ratio (BBB kit)
quinidine	19.4	1
verapamil	7.9	0.8
carbamazepine	1.1	1
indomethacin	0.9	1.2

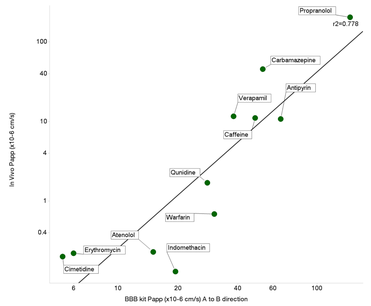


Figure 3. The luminal to abluminal permeability of eleven commercially available drugs were tested in the provided BBB kits over the course of a 30 minute incubation and compared to the *in vivo* apparent permeability reported in the literature[2]. *In vivo* permeability values across the BBB are rarely calculated in early discovery because the gold standard for calculating them, the *in situ* brain perfusion technique is labor intensive and low throughput. Good correlation (r^2=0.778) was observed and may suggest that this BBB kit could serve as a surrogate to *in situ* brain perfusion.

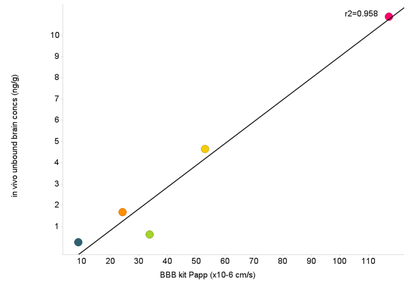


Figure 4. Based on the positive correlation seen between the *in vitro* BBB kit permeability and *in vivo* BBB permeability as assessed by *in situ* brain perfusion, a few internal Abbott compounds were selected to be tested in the BBB kit. These compounds are from an early CNS discovery project and are expected to have different permeability rates based on their unbound brain concentrations in rat after a 1 mg/kg IP dose. These *in vivo* data are used for the comparison, as the *in situ* brain perfusion technique is not readily available in-house.

SUMMARY / CONCLUSIONS

A novel, commercially available, ready-to-use kit for assessing permeability across the BBB was investigated. Detailed instructions are included with the kit and activation of the kit as well as execution of a permeability assay is quite straightforward. Initial studies indicated good correlation between literature reported *in vivo* BBB permeability rates and permeability rates generated using the kit. These data suggest that the BBB kit could be used as a surrogate for more labor intensive and low throughput techniques for assessing BBB permeability such as *in situ* brain perfusion. In addition to commercially available test compounds, five internal Abbott compounds from an early CNS discovery project were assessed. These compounds had no brain impairment and we were attempting to see if the BBB kit could differentiate them. Based on unbound brain concentrations following a 1mg/kg IP dose, the kits did quite well predicting which compound would have the best brain penetration. Of note however, is the somewhat disappointing finding that the functional expression of known transporters at the BBB such as P-gp is still quite low and the system is incapable of identifying substrates such as quinidine and verapamil which are easily detected with other assays used in early CNS discovery like the MDCK-MDR1 assay. The MDCK-MDR1 assay is amenable to high throughput, relatively straightforward to run, quite accurately predicts brain impairment, and may be the preferred screening method positioned early in the screening funnel. However, this novel BBB kit may hold value as compounds progress and teams are looking to better understand the BBB permeability characteristics of their compounds. Furthermore, a tool such as this could also be used to better understand false positives and false negatives when building an IVIVC as shown in Figure 1. Interestingly, the five internal compounds tested were all false positives in the MDCK-MDR1 assay, meaning they showed an efflux ratio > 2, but did not experience *in vivo* brain impairment, suggesting that their BBB permeability may have both efflux and influx components.

REFERENCES

[1] Nakagawa *et al*. (2009) *Neurochem Inter* 54: 253-263, [2] Dagenais *et al*. (2009) *Euro J Pharm Sci* 38: 121-137, [3] Hellinger *et al*. (in press 2012) *Euro J Pharm Biopharm*