A Dynamic Humanized drug resistant In Vitro Blood-Brain Barrier Model to assess the permeability of relevant CNS drugs

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ABSTRACT

CNS disorders (e.g., Alzheimer's, epilepsy, Parkinson's, etc.) have a big impact on society, in terms of both incidence and quality of life. On average, the development of a CNS drug from the preliminary basic research to the FDA validation and introduction into the market is a process that takes 4 years longer than for non-CNS drugs due to the increased difficulty of drug design and the development of reliable testing protocols. For this reason, we developed a humanized Dynamic *In Vitro* BBB model (hDIV-BBB) which closely mimics the BBB *in vivo*. In this system, vascular endothelial cells (EC) are cultured in the lumen of hollow microporous fibers in the presence of abluminal astrocytes. The capillaries are exposed to pulsatile flow in the lumen which induces and maintains EC polarity.

By using this novel DIV-BBB, we compared the transendothelial permeation properties of sucrose, phenytoin and diazepam in humanized blood-brain barrier (BBB) models based on co-cultures of primary human astrocytes (HA) and human control and drug resistant (MDR1 over-expressing) brain microvascular endothelial cells (HBMEC control and HBMEC-epi isolated from surgical specimen of patients undergoing temporal lobectomy for intractable epilepsy).

HBMEC and HA were co-cultured for 28 days using polypropylene capillaries. HBMEC were exposed to physiological levels of shear stress generated by intraluminal media flow. Permeability to 3H sucrose, 14C phenytoin and 14C diazepam were measured in control and drug resistant BBB models with and without pre-treatment with the MDR1 inhibitor XR9576. BBB integrity was monitored by trans-endothelial electrical resistance measurements. Sucrose permeability was ≈ 4 x10-7 cm/sec in all systems. Phenytoin permeability ranged from 1.74 x10-5 cm/sec in control to 1.54 x10-6 in drug resistant BBB models. Pretreatment of drug resistant BBB models with XR9576 restored the phenytoin permeability to control values (8.47 x10-6 cm/sec). Permeability to diazepam was ≈ 4.75 x10-3 cm/sec in control BBB models, 1.21 x10-3 cm/sec in drug resistant BBBs and 2.28 x10-3 cm/sec when the drug resistant BBBs where pre-treated with the MDR1 inhibitor. These results demonstrate that the humanized DIV-BBB recapitulates the in vivo rank order for drug permeability and also reproduces a "drug resistant" BBB phenotype, thus making it an ideal vector to study brain penetration of new CNS drugs.

Conclusion

These results show that the humanized DIV-BBB recapitulates the physiological permeability properties of the BBB *in vivo* and is also capable of reproducing a drug resistant BBB phenotype. These unique characteristics make the DIV-BBB an ideal model to study brain penetration of a wide range of xenobiotics as well as the effect of pathological vascular changes on BBB integrity and function.

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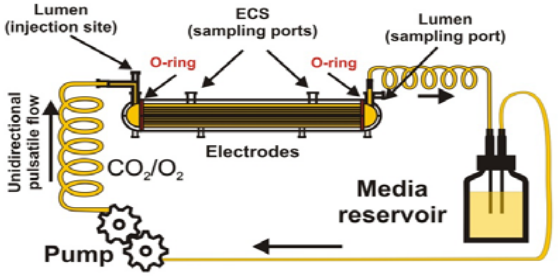
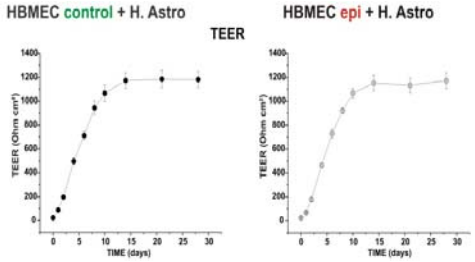
The Dynamic *in vitro* BBB model

A



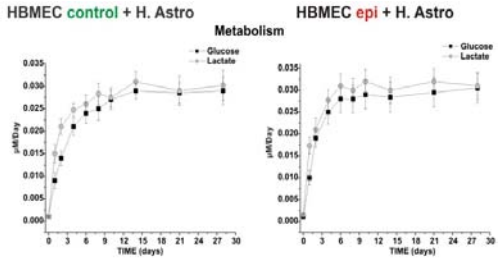
(A) Diagrammatic representation of the DIV-BBB. A bundle of porous polypropylene hollow fibers is suspended in the DIV-BBB chamber. The hollow fibers are in continuity with a medium source through a flow path consisting of gas-permeable silicon tubing. Two three-way stopcocks positioned on either side of the module regulate the access to the luminal compartment.

B



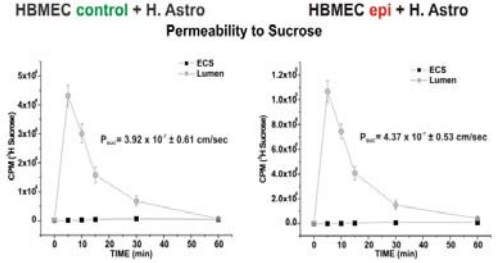
(B): Microvascular endothelial cells from control and epileptic brain develop a very tight barrier characterized by a stable high TEER (> 1100 Ohm cm2).

C



(C) Glucose consumption and lactate production in both control and epileptic co-cultures demonstrate an almost identical metabolic pathway (aerobic).

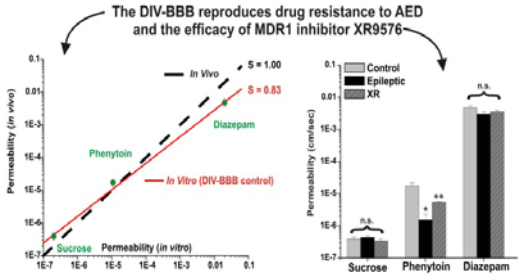
D



(D) Permeability measurements to [3H]-sucrose in control and epileptic *in vitro* BBB models. Note that both models develop a very stringent barrier to this well established paracellular marker.

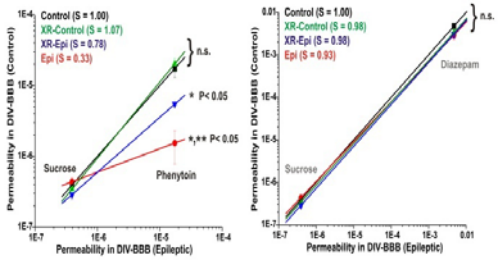
Summary of permeability experiments

E



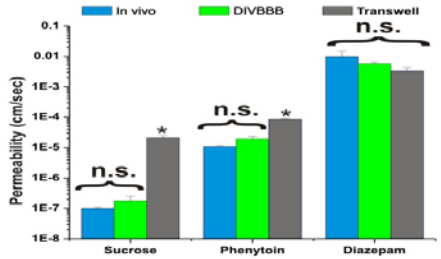
(E) Permeability of sucrose, phenytoin and diazepam in control DIV-BBB. The dash line indicates the idealized permeability linear fit of these compounds if the experiment would have been performed *in vivo*.

F



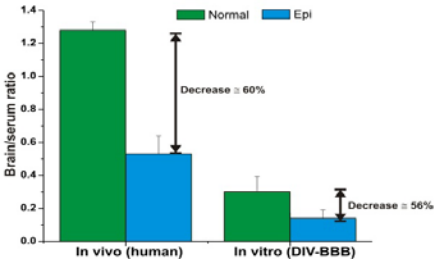
(F) The epileptic *in vitro* BBB is 10 fold less permeable to phenytoin than control. Pre-treatment with XR9576 partially abolish this difference. Permeability of diazepam across the epileptic and control *in vitro* BBB is not statistically different and is not affected by XR9576.

G



(G) Summary of permeability of the compounds tested across the *in vitro* BBB comprised of either normal or epileptic brain microvascular endothelial cells under different experimental conditions.

H



(H) Phenytoin distribution in patients versus DIV-BBB.