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Humanized *in vitro* blood-brain barrier models to screen for brain penetration of antiepileptic drugs

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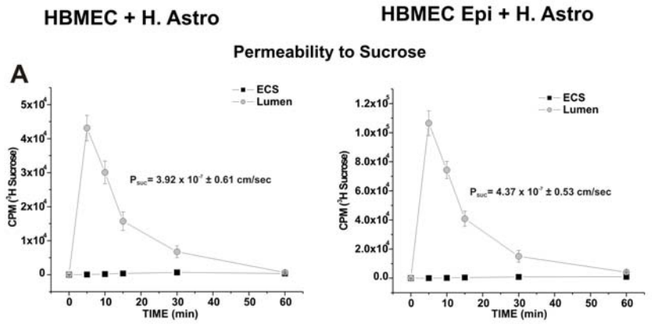
ABSTRACT

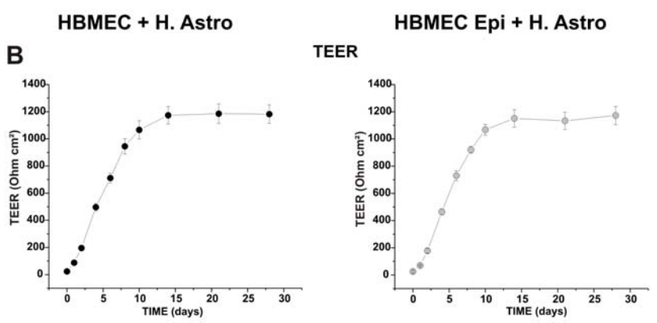
The blood-brain barrier (BBB) is crucial for drug delivery, bio-defense and pathogenesis of chronic neurological diseases which are often associated with cerebrovascular dysfunction and changes in BBB integrity. This is important given the role of the BBB in maintaining the brain homeostasis since the involvement of neuron-inflammatory processes or vascular pathological events such as hypertension, stroke and heart failure can lead to a leaky BBB which may ultimately worsen the neurological conditions [1-5]. A novel biotechnological breakthrough for the study of neurotoxicity and the BBB is the use of a reproducible *in vitro* model that recapitulates the functional and structural properties of the BBB *in situ* [6]. For this purpose, we compared the trans-endothelial permeation properties of sucrose, phenytoin and diazepam in co-cultures of human astrocytes (HA) and human control and drug resistant (MDR1 over-expressing) brain microvascular endothelial cells isolated from brain tissue resection of patients undergoing temporal lobectomies for intractable epilepsy (HBMEC control and HBMEC epi) [7]. 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 ^{3}H sucrose, ^{14}C phenytoin and ^{14}C 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 (TEER). Cell viability was assessed by measurement of glucose consumption and lactate production. Sucrose permeability was in the range of ≈ $4x10^{-7}$ cm/sec regardless the origin of the endothelium used (normal or epileptic brain) to establish the BBB *in vitro*. Phenytoin resulted to be 10 fold less permeable ($1.54x10^{-6}$ cm/sec) in drug resistant BBB models than controls ($1.74x10^{-5}$ cm/sec). In addition, while pre-treatment with XR9576 did not affect phenytoin permeability in control, epileptic co-cultures demonstrated ≈ 3.5 fold increase in phenytoin permeability after exposure to the MDR1 blocker. Permeability to diazepam in DIV-BBB established from control and epileptic brain microvascular endothelium where not significantly different (P > 0.05) and pre-treatment with the MDR1 inhibitor XR9576 did not affect the permeability to diazepam regardless the expression level of MDR1 (P > 0.05). This is in agreement with a study *in vivo* [8] demonstrating that diazepam is not an MDR1 substrate. 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 our *in vitro* BBB model an ideal vector to study brain penetration of a wide range of xenobiotics as well as the effect of pathological vascular changes on BBB integrity and function.

Support contributed by: ARDF and Philip Morris USA and Philip Morris International external research awards to Luca Cucullo and by *NIH-2RO1 HL51614, NIH-RO1 NS43284 NIH-RO1 NS38195 and Philip Morris USA and Philip Morris International external research awards to Damir Janigro*

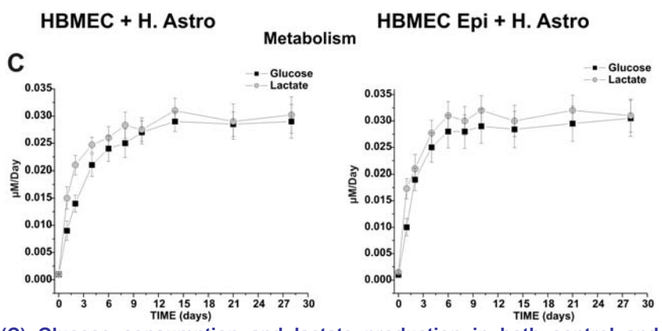
HBMEC from control and epileptic brain co-cultured with astrocytes and exposed to flow develop BBB properties



(A) 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.

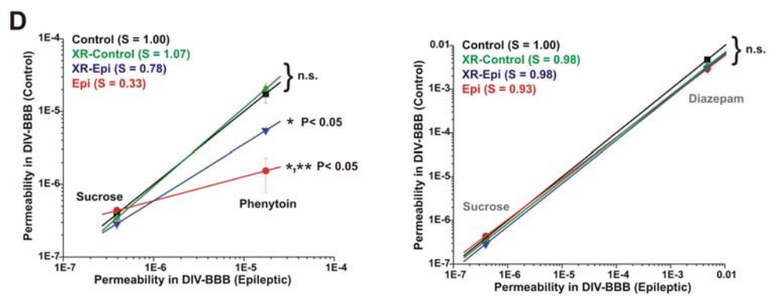


(B): microvascular endothelial cells from control and epileptic brain develop a very tight barrier characterized by a stable high TEER (> 1100 Ohm cm2).

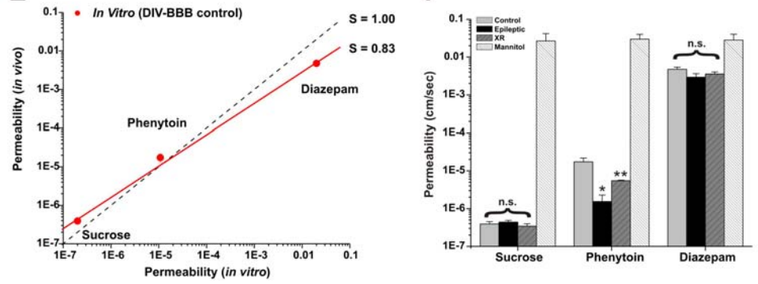


(C) Glucose consumption and lactate production in both control and epileptic co-cultures demonstrate an almost identical metabolic pathway (aerobic).

Summary of the permeability experiments



(D) 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.

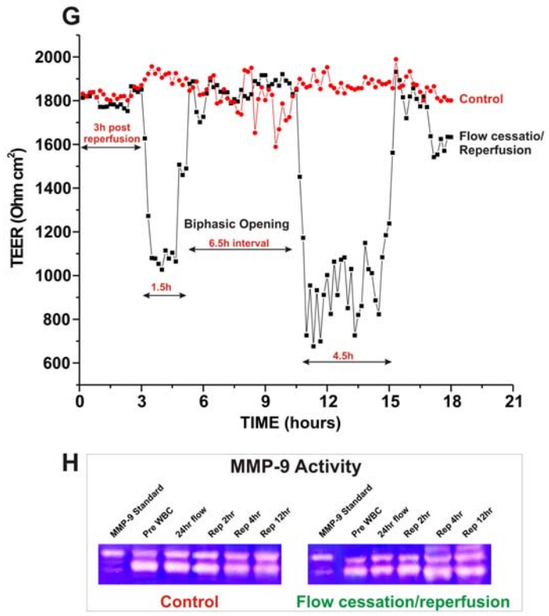


(E) Permeability of sucrose, phenytoin and diazepam in control DIV-BBB. The dash line indicates the idealized permeability linear fit of these compounds if the experiment would have been performed *in vivo*. (F) Summary of permeability of the compounds tested across the *in vitro* BBB comprised of either normal or epileptic brain microvascular endothelial cells under different experimental conditions.

CONCLUSIONS

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.

DIV-BBB and WBC in an experiment of flow cessation/reperfusion



(G) Effect of loss of shear stress during normoxia–normoglycemia on blood–brain barrier integrity in humanized DIV-BBB with flow adapted WBC. (H) MMP-9 levels in control and experimental models

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