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 were pre-treated with the MDR1 inhibitor. These results demonstrate that the humanized DIV-BBB recapitulates the in vivo 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.

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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