Dynamic In-Vitro Blood-Brain Barrier Model in Epilepsy

It is becoming increasingly evident that multiple drug resistance to antiepileptic drugs is not due to an anomaly present in the epileptic brain but is rather a consequence of poor drug penetration across the blood-brain barrier [1-5]. Unfortunately, reliable animal models of multiple drug resistant epilepsy are a poor simulation of the complex pattern of multiple drug resistant pump expression in human epilepsy [6]. There are several in vitro models of the BBB currently employed to explore the permeability and potential efficacy of drugs targeting the brain [7;8]. The most commonly used system for EC culturing consists of a porous membrane support submerged in feeding media (e.g., Transwell apparatus). A major disadvantage of the system is the lack of physiologic shear stress due to the absence of intraluminal flow. The tightness of the barrier in this model is typically much less stringent than the BBB in vivo: thus, compounds that are excluded by the BBB in vivo (e.g., sucrose) readily diffuse across these endothelial monolayers. Today it is in fact well recognized the role played by intraluminal shear stress in brain microvessel endothelial cells differentiation as well as maintenance and induction of a BBB phenotype. We have developed an in vitro model based on the co-culture of human endothelial cells isolated from the blood-brain barrier of multiple drug resistant epileptic patients. These cells retain, in vitro, their abnormal capacity for drug extrusion (Fig 1).

In addition, these cells also express the newly discovered multiple drug resistance protein RLIP76 which is abundantly expressed in the human epileptic brain. Our studies have demonstrated the feasibility of BBB modeling under dynamic, quasi physiological conditions. Epileptic endothelial cells are grown in the presence of epileptic astrocytes under dynamic conditions to simulate the brain environment and reproduce the influences of blood flow (Fig 2) including blood cells on cell differentiation and profiles of traditional drug delivery. Experiments with well-characterized pharmacological agents (morphine, theophylline, mannitol, PNQX, paroxetine, dipyridamole etc.), have demonstrated that the DIV-BBB reproduces the salient physiological and pharmacokinetic properties of the in situ endothelium (Fig. 3).

With this system we can:
- Determine if a drug will penetrate the epileptic blood-brain barrier
- Determine the kinetic parameters of drug accumulation into the CNS
- Quantify the influence of individual transporters/extrusion molecules
- Verify the influence of protein binding
- Determine the efficacy of blockade of Pgp, MRPs, RLIP76 or other multiple drug resistance molecules
Future development includes the serial determination of intestinal absorption and liver metabolism on BBB passage. This is achieved by culture of gut epithelial cells and hepatocytes in reservoirs connected to the blood compartment of the in vitro epileptic BBB model.

Figure 3: BBB permeability in vivo vs. in vitro. Panel A shows that clinically relevant compounds (e.g., antitumoral) are excluded by the BBB in spite of their favorable chemical structure, while other compounds (glucose) are actively transported. Panel B: Sucrose permeability values in the DIV-BBB are much closer to the in vivo BBB compared to other models. Note how permeability values obtained in a DIV-BBB (panel C) are comparable to the permeability in vivo while in a static BBB model (panel D) permeability values are often misleading (significantly higher). Panel E shows phenytoin permeability obtained in a DIV-BBB.

Reference List