ISOLATED FISH BRAIN CAPILLARIES: A COMPARATIVE MODEL TO STUDY BLOOD-BRAIN BARRRIER FUNCTION

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The cells of the central nervous system are particularly sensitive to chemical injury and thus require a highly regulated extracellular environment. In this regard, the brain capillary endothelium is a formidable barrier to the entry of xenobiotics into the central nervous system. This barrier protects the CNS from toxic chemicals, but also denies entry to therapeutics. A critical impediment to understanding transport function in brain capillaries has been the lack of suitable in vitro techniques that both retain viability and that allow the investigator to measure luminal accumulation of diffusible solutes, which reflects specific function of excretory transporters. We recently developed a simple but powerful system in which to study drug transport across the capillary wall (Miller et al., Mol. Pharm. 58:1357-1367, 2000). It consists of freshly isolated, intact brain capillaries, fluorescent substrates and confocal imaging. This system was used to follow bath to lumen transport of selected fluorescent xenobiotics in capillaries from rat and pig. Such transport was found to be mediated by two ATP-driven drug export pumps, p-glycoprotein and Mrp2. A major shortcoming in the use of isolated brain capillaries from mammals is limited viability. To circumvent this problem we have turned to comparative models from poikilotherms. All vertebrates are known to possess a functional blood-brain barrier, although anatomical differences have been noted. In all vertebrates, excluding elasmobranch fishes, the anatomical barrier to macromolecules is at the level of the tight junctions between endothelial cells; in elasmobranchs, it appears to be at the level of the glia, which surround the endothelium (REF). The present report describes initial transport experiments with brain capillaries isolated from a teleost (killifish, *Fundulus heteroclitus*) and an elasmobranch (dogfish shark, *Squalus acanthias*).

Initial attempts to isolate fish capillaries using modifications of the mammalian methods of Miller et al (op cit) proved fruitless, since fish microvessels fragmented when subjected to the purification procedure (tissue homogenization and density gradient centrifugation followed by separation on a glass bead column). However, viable, intact capillaries could be imaged in crude tissue homogenates without further purification. Capillaries were exposed to fluorescent drugs and drug derivatives in a buffered, physiological saline. Compounds tested were substrates for transport by p-glycoprotein (fluorescent cyclosporin A, verapamil and ivermectin derivatives) and Mrp2 (fluorescent methotrexate derivative and sulforhodamine 101). Luminal accumulation of the dyes within individual capillaries was measured using confocal microscopy and quantitative image analysis (Miller at al., op. cit.). In some experiments, capillaries were fixed and processed for immunostaining with antibodies to mammalian p-glycoprotein and Mrp2; these antibodies specifically react with the killifish and shark transporters (Miller et al., Am J. Physiol. 275:R697-705, 1998; Masereeuw et al., Mol. Pharm. 57:59-67, 2000 and Miller, unpublished data).

Isolated brain capillary segments from killifish and shark brain were 5-7 μm in diameter and up to several hundred μm in length. Most capillaries possessed open lumens and some trapped red cells. Capillaries from shark were surrounded by a single layer of cells not seen in killifish capillaries. This may mean that an intact glial coat survives isolation, but additional functional and ultrastructural studies are needed before this can be established with certainty.

Isolated brain capillaries from killifish and shark brain excluded fluorescent dextrans for over 5 hours, demonstrating an intact passive barrier. In killifish brain capillaries, luminal accumulation of fluorescent derivatives of cyclosporine A (CSA) and verapamil was concentrative, specific and energy-dependent (inhibition by KCN). Luminal accumulation of these fluorescent drug derivatives was reduced by PSC 833, but not by leukotriene C4 (LTC_4), indicating the involvement of p-glycoprotein. Importantly, the ability of control capillaries to transport the CSA derivative was unchanged over 20 h, demonstrating the extended viability of the preparation. Luminal accumulation of the fluorescent organic anions, sulforhodamine 101 and fluorescein-methotrexate, was also concentrative, specific and energy-dependent. Transport of these compounds was reduced by LTC_4, but not by PSC 833, indicating the involvement of a multidrug resistance-associated protein (most likely Mrp2). Similar results were obtained using capillaries isolated from dogfish shark brain. Immunostaining localized p-glycoprotein and Mrp2 to the luminal surface of the killifish brain capillary endothelium, which is the correct location to prevent xenobiotic entry into CNS and pump xenobiotics out of brain tissue.

The present initial results are interesting in two respects: First, they validate a new and long-lived comparative model for studying drug transport across the blood-brain barrier in vitro; as in mammals (Miller et al, op.cit.), they implicate two ATP-driven xenobiotic export pumps, p-glycoprotein and Mrp2, in transport from CNS to blood. Second, they show close functional similarities between capillaries isolated from teleosts and elasmobranchs. For sharks, this implies that the junctional barrier at the level of glia survives isolation, a suggestion that awaits confirmation at the ultrastructural level. Supported by DFG FR1211/8-1 to GF and the MDIBL CMTS (ES 03828).