LONG-TERM EFFECTS OF ZINC ON MRP2 FUNCTION IN KILLIFISH (*Fundulus heteroclitus*) BRAIN CAPILLARIES

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A substantial obstacle to understanding regulation of integrated tissue function over the mid- to long-term is the limited viability of mammalian tissue in vitro. One solution to the problem is the use of tissues from poikilotherms, which exhibit extended viability and function in vitro. We have taken this approach in our studies of brain capillary transport function, the goal being to identify signals that alter the blood-brain barrier. To this end, we recently validated a new and long-lived comparative model for studying drug transport across the blood-brain barrier (Miller, D.S. et al, Am. J. Physiol., 282:R191-R198, 2002). Using brain capillaries isolated from two poikilotherms, a teleost (killifish) and an elasmobranch (dogfish shark, *Squalus acanthias*), confocal microscopy and fluorescent substrates, we found, as in mammals (Miller, D.S. et al, Mol. Pharm. 58:1357-1367, 2000), evidence for involvement of two ATP-driven, xenobiotic export pumps, p-glycoprotein and Mrp2, in transport from CNS to blood. Last summer, using killifish brain capillaries, we established that at least four parallel signaling pathways rapidly down-regulate Mrp2 and p-glycoprotein function (Bauer, B. et al, Bull MDIBL. 41:36, 2002).

Recent evidence indicates that ATP-driven drug export pumps can be regulated over the long-term by drugs acting through specific nuclear receptors, e.g., PXR and CAR, and by metals possibly acting through heat shock or metal-sensitive elements (Gerk, P.M. and Vore, M., J. Pharmacol. Exp. Therap. 302:407-415, 2002). Here we used the killifish brain capillary system to examine the possibility of long-term regulation of transport by the heavy metal, zinc. Isolated capillaries were incubated for 1-24 h in medium (modified marine teleost saline) without (control) or with 1 μM $ZnCl_2$. After incubation, accumulation (60 min) of 2 μM sulforhodamine 101 free acid (Mrp2 substrate) in individual killifish capillary lumens was measured using confocal microscopy and quantitative image analysis. In control capillaries, luminal fluorescence was about 25 times higher than in the medium and it remained constant for the entire 24 h period, again demonstrating the extended viability of the preparation. Zn significantly ($n=10$ capillaries, $P<0.01$) increased mean luminal sulforhodamine 101 accumulation by 23 ± 8%, 44 ± 6%, 47 ± 8% after 6, 12 and 24 h of exposure, respectively.

These are the first experiments demonstrating long-term regulation of barrier function in intact brain capillaries in vitro and the first to show metal up-regulation of drug efflux transporter function in brain. At present it is not clear whether increased Mrp2 expression underlies the change in transport observed or whether other transporters, e.g., p-glycoprotein, are similarly affected. These preliminary results have important implications with regard to drug therapy. The data suggest that, as in excretory tissues, drug transporter function at the blood-brain barrier can be up-regulated by xenobiotics. In this case, increased transport function would imply a tighter barrier and improved protection against neurotoxic chemicals, but also increased difficulty in delivering therapeutics to the brain. Supported in part by DFG FR1211/8-1 to GF, a New Investigator Award to GF and by the MDIBL Center for Membrane Toxicity Studies (ES 03828).