FUNCTIONAL EVIDENCE FOR THE MULTIDRUG RESISTANCE ASSOCIATED PROTEIN (MRP2; cMOAT) IN CULTURED SKATE HEPATOCYTES

Gert Fricker[1], Heike Gutman[2], David Seward[3], Agathe Droullé[4], David S. Miller[5] and James L. Boyer[6]

[1]Institut f. Pharmazeut.Technologie und Biopharmazie, INF 366, 69120 Heidelberg, Germany
[2]University Hospital, 4031 Basel, Switzerland
[3]Williams College, Williamstown, MA 01267; [4]Lycee Th.. Roosevelt, 51100 Reims, France
[5]Laboratory of Cellular and Molecular Pharmacology, NIEHS, Research Triangle Park, NC 27709
[6]Dept. of Medicine and Liver Center, Yale Univ. School of Medicine, New Haven, CT 06520 -8019

Certain cells develop resistance to multiple therapeutic agents when they express genes encoding specific plasma membrane transport proteins known as mdrs (p-glycoproteins or multidrug resistance proteins) and mrps (multidrug resistance related proteins). These transporters mediate the efflux of a variety of drugs from cells (Kusuhara et al., *J.Pharm.Sci.*87: 1025-1040, 1998), a phenomenon that results in resistance to chemotherapeutic agents in tumors that overexpress these proteins. Mdrs and mrps are also found in high levels in normal epithelial tissues such as liver, kidney, small intestine or brain capillary endothelial cells. In mammalian liver, mdrs and particularly the mrp homologue, mrp2 (also known as cMOAT, the canalicular multispecific organic anion transporter) are highly expressed in the bile canalicular membrane, where they mediate the biliary excretion of a variety of xenobiotics.

Recent work from this laboratory (Ballatori et al., *Bull. MDIBL* 37: 85-86, 1998) indicates that cultured skate hepatocytes maintain a polarized distribution of canalicular and basolateral membrane domains as determined by morphological and functional studies. In the present study we have assessed the functional activity of the canalicular membrane transport protein, mrp2, in long-term 7 day cultures of skate hepatocytes as part of the characterization of this model system.

After isolation, skate hepatocytes were maintained in culture dishes in a complete skate hepatocyte culture medium (CM-1), modified from a rectal gland cell culture medium (Valentich, *J. Tiss. Cult. Meth.* 13: 49-162, 1991; Ballatori et al., *Bull.MDIBL* 37: 85-86, 1998), supplemented with antibiotics. After 7 days the cells were incubated with medium containing 1 μM fluorescein-methotrexate, (FL-MTX) a specific fluorescent substrate for mrp2 (cMOAT; Miller and Pritchard, *J. Exp. Zool.* 279: 462-70, 1997; van Aubel et al., *Mol. Pharmacol.* 53: 1062-1067, 1998) and examined by confocal fluorescence microscopy. Within 5 minutes of incubation, the dye could be observed within the canalicular spaces between the hepatocytes. With time, the canalicular lumens expanded and the fluorescence intensity increased within the lumens to levels that greatly exceeded those in the cells, indicating active secretion of FL-MTX (Fig. 1). When the cell monolayers were incubated with 0.5 μM leukotriene C_4, a potent inhibitor of mrp2, the canalicular accumulation of FL-MTX was nearly abolished (Fig. 2).

These findings indicate that the canalicular transport protein, mrp2, is functionally maintained at 7 days of cell culture and that skate hepatocytes remain differentiated with respect to this major secretory function while in long term culture. These studies suggest that this skate hepatocyte culture system may be a useful model for investigating the regulation of hepatic secretion of drugs and xenobiotics.

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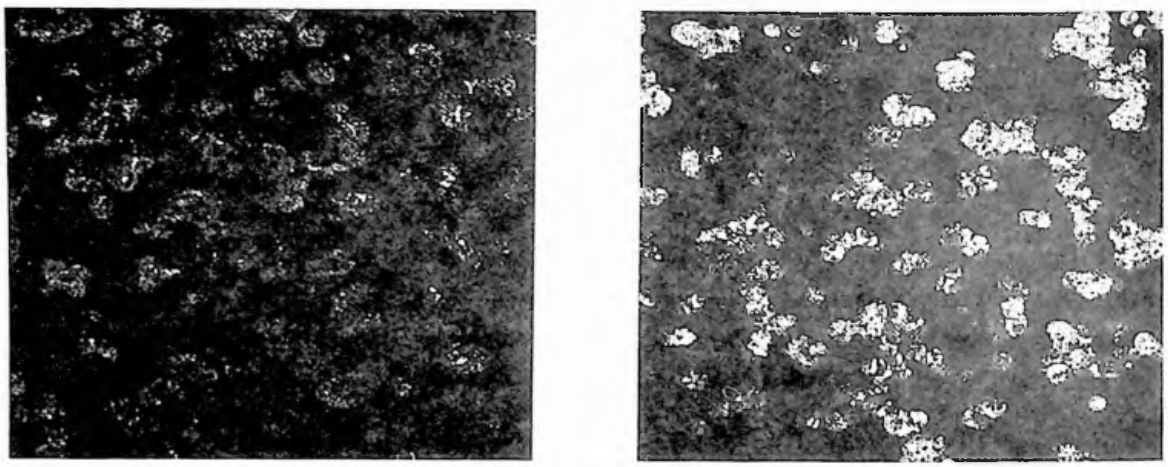
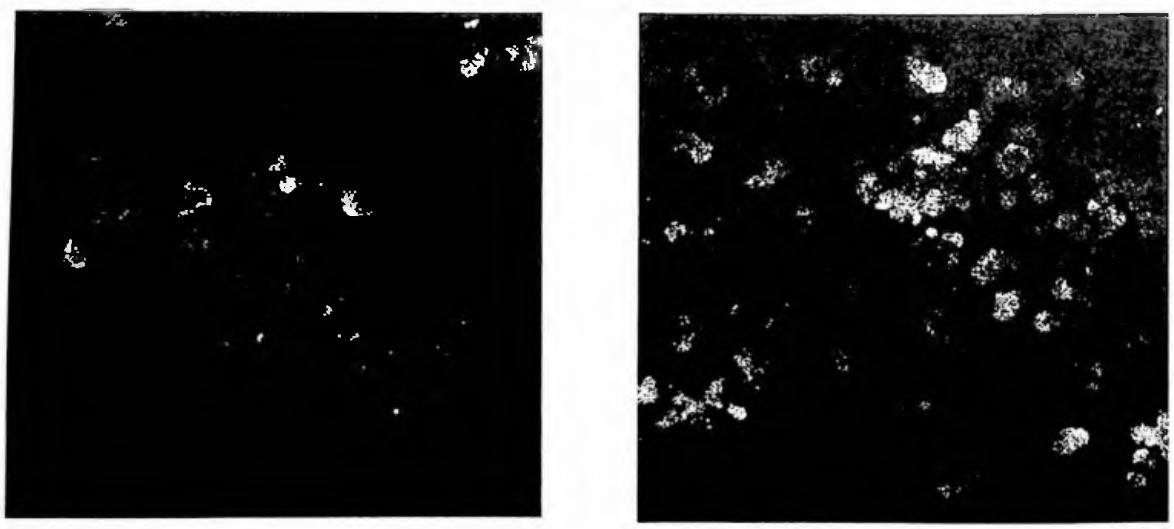


5 min 20 min

Figure 1: Time dependent secretion of Fl-methotrexate across canalicular plasma membranes after incubation of hepatocytes with 1 μM fluorescent compound



5 min 20 min

Figure 2: Time dependent secretion of Fl-methotrexate across canalicular plasma membranes after incubation of hepatocytes with 1 μM fluorescent compound in the presence of 0,5 μM cMOAT (mrp2) substrate leukotriene C_4. Secretion into canalicular spaces is nearly abolished.