LOW BILIARY EXCRETION OF INDOCYANINE GREEN IN PERFUSED SKATE (*Raja erinacea*) LIVER: POSSIBLE ROLE OF THE MULTIDRUG RESISTANCE P-GLYCOPROTEIN-2, Mdr2, IN HEPATIC ELIMINATION OF THIS HYDROPHOBIC ANIONIC DYE

Ned Ballatori[1], David J. Seward[1], Catherine Bennet[2], and James L. Boyer[3]
[1]Dept. of Environmental Medicine, Univ. of Rochester School Medicine, Rochester, NY 14642
[2]Georgetown University, Washington, DC 20057
[3]Liver Center, Department of Medicine, Yale Univ. School of Medicine, New Haven, CT 06520

The liver is a major site for the clearance, metabolism, and excretion of potentially toxic compounds of both endogenous and exogenous origin. Plasma membrane transport proteins on the basolateral (sinusoidal) domain of hepatocytes serve to efficiently clear chemicals from circulating blood plasma, whereas apical (canalicular) proteins mediate their export into bile. All of the canalicular transport proteins identified to date belong to the ATP-binding cassette (ABC) superfamily, and include the multidrug resistance-associated protein-2 (MRP2/Mrp2), the bile salt export pump (BSEP/Bsep), and the multidrug resistance gene products (MDR1 and MDR3 in humans, and Mdr1a, Mdr1b, and Mdr2 in rodents). MRP2/Mrp2 mediates the biliary excretion of a wide variety of amphipathic anionic substrates, principally glucuronide, sulfate, and glutathione conjugates, whereas BSEP/Bsep mediates biliary excretion of bile salts. MDR1/Mdr1 is also a multispecific transport pump, mediating export of many hydrophobic chemicals from liver cells into bile. In contrast, MDR3/Mdr2 is a phospholipid flippase, delivering phosholipids from the inner to the outer leaflet of the canalicular membrane. Bile salts facilitate the release of these lipids from the canalicular membrane and the formation of phospholipid/cholesterol vesicles and mixed lipid/bile salt micelles within the canalicular lumen. MDR3/Mdr2 is thus a key regulator of whole body cholesterol elimination and bile acid homeostasis. In addition, this transporter may contribute to biliary elimination of hydrophobic organic anions or cations that bind to or partition into biliary vesicles or micelles (Scharschmidt and Schmid, J. Clin. Invest. 62:1122-1131, 1978). Sequestration of hydrophobic compounds into these structures decreases the concentration of the monomeric forms in bile, and thus stimulates further export from the cell.

In support of this hypothesis, a recent study by Huang and Vore (Drug Metab. Dispos. 29(5):634-637, 2001) reported that Mdr2 is required for biliary excretion of a tricarbocyanine dye containing two polar sulfonate groups and a quaternary ammonium group (indocyanine green, ICG). This conclusion was surprising because most investigators had assumed that ICG's physicochemical properties would make it an excellent substrate for the anion transporter Mrp2. However, previous studies of ICG distribution in bile indicated that it is extensively (90-100%) associated with phospholipid vesicles and mixed lipid/bile salt micelles (Scharschmidt and Schmid, J. Clin. Invest. 62:1122-1131, 1978). Huang and Vore (Drug Metab. Dispos. 29(5):634-637, 2001) tested whether Mrp2 is involved in ICG excretion from the liver by comparing the biliary excretion of ICG and another organic anion, estradiol-17beta-D-glucuronide (E(2)17G), in isolated perfused livers from wild-type and from Mdr2-deficient female mice. They observed that biliary excretion of ICG (0.4 micromol) was reduced by 90% in Mdr2-/- mice relative to wild-type mice, whereas the biliary excretion of E(2)17G was increased by 30% in Mdr2-/- mice, indicating that the absence of Mdr2 differentially influences the biliary excretion of these organic anions, and that phospholipid vesicles and mixed micelles in bile are essential for biliary excretion of ICG.

To further test the hypothesis that Mdr2 contributes to biliary excretion of ICG, the present study measured the biliary elimination of ICG in the isolated perfused liver of the little skate, *Raja erinacea*. The skate does not secrete significant amounts of phospholipids and cholesterol into bile, and appears to lack a functional Mdr2 protein (Oude Elferink et al., unpublished observations). Thus, biliary excretion of ICG in the skate should be lower than that of organic anions that are substrates for the organic anion pump, Mrp2. Biliary ICG excretion in the skate was compared with biliary excretion of another anionic dye, dibromosulfophthalein (DBSP), a substrate for Mrp2.

ICG and DBSP both have high extraction ratios in mammalian livers, and are excreted unchanged into bile in high concentrations.

Skate livers were isolated and perfused as previously described (Simmons et al. Biochem. Phamacol. 42:2221-2228, 1991). The recirculating perfusate consisted of 100 ml of elasmobranch Ringer supplemented with 5 mM D-glucose, 5 mM Hepes/Tris, pH 7.5, and 0.25% bovine serum albumin. ICG or DBSP were added after one hour of perfusion at an initial concentration of 10 μM (1 micromol/liver). Bile and perfusate samples were collected every hour for 8 h. Bile volume was measured gravimetrically, assuming a density of one. ICG concentrations were measured spectrophotometrically at 805 nm, after dilution of the samples in elasmobranch Ringer containing 0.25% bovine serum albumin. DBSP was measured spectrophotometrically at 585 nm, after dilution with 0.1 M sodium pyrophosphate buffer, pH 8.2.

ICG and DBSP were rapidly removed from the recirculating perfusate, as expected (Boyer et al. Am. J. Physiol. 230:974-981, 1976). These compounds were added to perfusate at the 1 h time interval, and one hour later less than 4% of the DBSP remained in the perfusate (<0.4 μM), whereas about 10-15% of the ICG (1.0-1.5 μM) remained, indicating a slightly faster clearance of DBSP. Given the slow bile flow rate in the skate, these dyes did not appear in collected bile until the third collection interval (from 1-2 h after administering the dyes). DBSP was excreted into bile more efficiently than ICG such that over this collection interval the amount of DBSP in bile was 4-times that of ICG. However, both of these compounds were concentrated in bile, indicating active hepatobiliary transport. DBSP reached its maximum biliary concentration at the 7th collection interval, and started declining by the 8th hour, whereas ICG concentration continued to rise until the end of the experiment.

These data indicate that ICG is excreted into skate bile relatively slowly, and its concentration in bile does not reach the high levels observed for the Mrp2 substrate DBSP, or for other Mrp2 and Bsep substrates (Boyer et al. Am. J. Physiol. 230:974-981, 1976). Because skate liver secretes only very low amounts of phospholipid and cholesterol into bile, these data support the hypothesis that Mdr2-mediated lipid transport contributes to biliary excretion of hydrophobic compounds such as ICG (Supported by ES03828, ES01247, DK34989, DK25636, DK48823, and by NSF-REU DBI0139190).