Glutathione export from skate (*Raja erinacea*) liver is unaffected by taurocholic acid

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Reduced glutathione (GSH) is a tripeptide that plays a central role in a multitude of biochemical processes, and disturbances in its homeostasis are implicated in the etiology and progression of a number of diseases[3]. GSH is synthesized in every cell of the body, but the liver is quantitatively the major site of synthesis. After its synthesis, much of the hepatic GSH is secreted into blood plasma and bile for delivery to other tissues; however, the mechanism by which GSH is transported across cell membranes is not clearly defined. Recent studies have proposed a role for some members of the organic anion transporting polypeptide family of proteins (OATP or SLC21A family), and of the multidrug resistance-associated protein family (MRP or ABCC), in cellular GSH export[1]. The SLC21A transporters work as secondary-active carriers, and studies have shown that rat Oatp1 and Oatp2 can export GSH[1,5,6]. The human ABCC family includes a total of nine ATP-dependent organic anion pumps (MRP1 to MRP9), as well as the gene defective in cystic fibrosis (*CFTR*) and the sulfonylurea receptor genes (*SUR1, SUR2*). To date, MRP1, MRP2, MRP4 and MRP5 have been shown to accept GSH as a substrate or co-substrate[1,4,7-10]. Rius and coworkers[10] recently demonstrated that GSH is cotransported with monoanionic bile salts by MRP4 when expressed in membrane vesicles from V-79 cells. These findings thus suggested that this basolateral transport protein may contribute to GSH export from liver cells into blood. In this model, the co-transported bile salt would be rapidly reabsorbed from blood back into the cell by the various basolateral bile salt transporters (e.g., the Na^+-dependent bile salt transporter, NTCP, and various OATPs), while GSH would be released into circulating blood. However, this mechanism is highly inefficient from an energetic standpoint: the cell would expend both ATP and the Na^+ electrochemical gradient to affect GSH transport, when the GSH could simply be released down its own electrochemical gradient. Nevertheless, if this hypothesis were correct, GSH export into blood should be stimulated by either bile salt availability and/or bile salt transport rate across the basolateral membrane. To test this prediction, the present study measured sinusoidal GSH export in the isolated perfused skate liver in the presence and absence of taurocholic acid in the perfusate. Because skate livers retain viability for many hours of perfusion, detailed measurements of sinusoidal GSH release can be made in this model system[11].

Male little skates were collected by net from Huntsman's Bay in Maine and maintained for up to 4 days in tanks equipped with flowing seawater. Livers were removed from the skates and perfused in an erythrocyte-free, recirculating perfusion system at 15°C as previously described[11]. The perfusion medium consisted of 100 ml of well-oxygenated, heparinized elasmobranch Ringer solution containing 5 mM glucose, 5 mM Hepes/Tris, pH 7.5, and 0.4 mM acivicin. The latter compound is an inhibitor of γ-glutamyl transpeptidase, and was added to prevent GSH degradation. The medium was continuously filtered and aerated with humidified air. The filter upstream from the perfusate reservoir consisted of a 200-μm silk-screen mesh stretched over a small funnel, while the downstream filter was a Millipore filter holder containing a prefilter (AP25-042-00). Bile was collected at 60-min intervals in tubes containing 0.1 ml of 4% sulfosalicylic acid to prevent GSH oxidation. A 0.5 ml aliquot of perfusate was also taken every hour for analysis. The perfusion was carried out for 6 h; after 1 h, 1 μmole of taurocholic acid was added to the recirculating medium every 30 min (2 μmole/h). Bile volume was measured gravimetrically, assuming a density of one. GSH in bile and perfusate was measured by an enzymatic recycling assay containing 5,5'-dithio-bis(2-nitrobenzoic acid) and glutathione reductase[2].

Hepatic tissue viability was monitored by measuring K^+ content in perfusate using flame photometry, and by recording portal perfusion pressure.

As expected, the addition of taurocholic acid at 2 μmole/h produced a significant choleresis in skate livers, and this bile salt was not toxic to the liver. Bile flow rates in control livers were between 2-3 $\mu l \cdot g^{-1} \cdot h^{-1}$, but this was increased to 5-6 $\mu l \cdot g^{-1} \cdot h^{-1}$ in livers that received taurocholate. However, taurocholate had no affect on GSH release into the sinusoidal circulation or into bile. GSH was released into the recirculating perfusate at a rate of approximately 3 $nmol \cdot g^{-1} \cdot h^{-1}$ in control livers, and this was unaffected by taurocholate. Likewise, GSH was secreted into bile at 0.5-1.0 $nmol \cdot g^{-1} \cdot h^{-1}$ in control livers, and this was unaffected by taurocholate. Because of the higher bile flow rate with taurocholate, the GSH concentration in bile was proportionately lower. Hepatic tissue GSH levels were also unaffected by taurocholate (0.96±0.12 and 0.97±0.14 μmol/g liver, for control and taurocholate-treated, respectively: values are means ± SE, n = 5 and 4, respectively).

These results do not support the hypothesis that GSH export from the liver is mediated by GSH-bile salt co-transport. Thus, additional studies are required to define the physiological significance of the MRP4-mediated GSH-bile salt cotransport observed in isolated membrane systems. (Supported by ES03828, ES01247, DK34989, DK25636, and DK48823).

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