Unconjugated bilirubin is a curse at high concentrations, producing apoptosis and cell death, but a boon at more physiological levels, protecting cells against oxidant damage

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Both hydrophobic bile salts1,2 and unconjugated bilirubin (UCB)3 induce apoptosis in cultured cells at moderately elevated concentrations and cell necrosis at higher concentrations. Retention of bile salts in cholestasis is believed to cause secondary damage to hepatocytes,4 and retention of UCB in severe neonatal jaundice is known to cause bilirubin encephalopathy.3 For both agents, the cytotoxicity results from damage to mitochondrial membranes, with collapse of the transmembrane potential and generation of a mitochondrial membrane permeability transition,5,6 and unscheduled cell death.

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IS BILIRUBIN A CURSE OR A BOON?

Bilirubins were once regarded as toxic wastes, of importance only when excessively high levels of UCB led to the development of encephalopathy.3 It is now appreciated that bilirubins and biliverdin are extremely potent antioxidants,21,22 and that these antioxidant properties may be cytoprotective,19-20 even at physiological concentrations. A new finding of the Granato paper8 was that the synthetic ditaurate conjugate of bilirubin, a satisfactory surrogate for the natural glucuronosyl conjugates, was nearly as potent as UCB in protecting hepatocytes against toxicity from GCDC.

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EXPERIMENTAL CONSIDERATIONS

Like many previous in vitro studies of the cytotoxicity of UCB8 and bile salts,19 the studies of Granato and colleagues8 who performed experiments at concentrations of the unbound fraction far above those that are clinically relevant. Although the incubation media contained 18 μmol/l bovine serum albumin, concentrations of UCB and GCDC greatly exceeded the high affinity binding capacity of 1 mol/mol albumin. The final unbound concentrations of UCB (Bf) were also far above aqueous saturation,10 so the pigment must have been heavily self-aggregated.11,12 Unbound concentrations of GCDC were also well above those seen in plasma in cholestasis, questioning the relevance of the results to clinical conditions.10 Furthermore, although UCB was recrystallised, polar derivatives had not been extracted with alkali,14 and surface active impurities, which may have contributed to the toxicity, were not removed from GCDC by available methods.15,16

Differential Susceptibilities to Bilirubin Toxicity

Granato and colleagues8 reported that rat hepatocytes were unaffected by Bf levels approximately three orders of magnitude higher than those that cause cell damage in rodent astrocytes and neurons,24 confirming in vivo observations in jaundiced Gunn rats that oxidative phosphorylation and respiration in the liver is unimpaired even in animals with bilirubin encephalopathy and kernicterus.25 Apparently, the resistance of the liver to UCB toxicity allows that organ to benefit from the antioxidant properties of the pigment, even at very high UCB levels. In the CNS, regional differences in sensitivity to UCB toxicity are manifested by selective accumulation of UCB only in certain areas of the brain in kernicteric neonates and Gunn rats.5 What might account for these differences?

Cells might be protected against accumulation of UCB by transporters that export UCB from the cell back into the blood and by conjugation and/or oxidation of UCB to non-toxic polar derivatives.23 When the cellular export and metabolism of UCB is insufficient to keep its total intracellular concentration low, cells might be protected by intracellular proteins that bind UCB,3 or by changes in mitochondrial membrane composition.24

Transporters

Both hepatocytes and a variety of CNS cells express two classes of ATP binding cassette (ABC) transporters, multidrug resistance P-glycoproteins (MDR/PGPs)
and the multidrug resistance associated proteins (MRPs) that can protect cells against accumulation of toxic substances by exporting them back into the plasma. For unconjugated organic anions, the most important of such exporters are MDR1/mdr1, MRP1/Mrp1, and MRP3/Mrp3. In the hepatocyte, MDR1 is localized apically, and MRP1 and 3 basolaterally. In the cholestatic liver, or after infusion of bile acids, the two Mrps are upregulated to limit accumulation of organic anions into the hepatocyte. Persistent hyperbilirubinaemia likewise enhances the conjugation and excretion of bilirubin because binding of UCB to the constitutive androstane receptor upregulates expression of ligandin, UGT1A1, and Mrp2. It is not known if Mdr1a/b or Mrp 1 and 3 are induced by UCB.

ABC transporters in the capillary endothelium (blood-brain barrier) and/or the choroid plexus (CP) epithelium (blood-cerebrospinal fluid (CSF) barrier) have been shown to play an important role in limiting the accumulation of organic anions and other small molecules in the CNS and CSF, respectively. As yet however there is only weak evidence that UCB is carried by any ABC transporters. Although some data have suggested that Mdr1a and MDR1 limit the accumulation of UCB in CNS cells, these results are inconclusive due to the use of vastly excessive UCB concentrations. UCB is reportedly a weak substrate for MDR1, and a role for MR P1 in UCB transport has been proposed, but not directly proven. Even if they do transport UCB, the presence of Mrp1 and Mdr1 in the blood-brain barrier and CP cannot explain the preferential accumulation of UCB in only selected areas of the kerinetic brain.

Conjugation

Hepatocytes are the major site of bilirubin conjugation, mediated by the specific microsomal UDPGA-bilirubin glucuronosyl transferase, UGT1A1. In the brain, CP epithelial cells express glucuronosyl transferases for a variety of other organic anions but UGT1A1 has yet to be demonstrated either here or in astrocytes or neurones. Even if present, UGT1A1 in the CP could play no role in protecting cultured astrocytes or neurones from UCB toxicity in vitro. In the paper by Granato and colleagues, bilirubin conjugation by their cultured hepatocytes was extremely low and thus unlikely to account for the resistance of these cells to UCB toxicity.

Oxidation

In the liver, UCB can be oxidised by the microsomal mixed function monooxygenases, CYP1A1 and CYP1A2, but basal expression is low, although readily inducible by indole-3-carbinol and aryl hydrocarbons. In many regions of the brain, low constitutive levels of CYP1A2 are found in neurones and of CYP1A1 in the capillary endothelium of the CP and arachnoid. CYP1A1 in liver is transcriptionally induced by elevated UCB levels in j Gunn rat pups, mediated by binding of UCB or an oxidation product to the aryl hydrocarbon receptor. In the latter paper, significant upregulation of CYP1A1 occurred after exposure of hepatoma cells to UCB under conditions similar to those used by Granato and colleagues, suggesting that increased oxidation of UCB contributed to the resistance of hepatocytes to UCB toxicity.

Cytosolic binding proteins

In the hepatocyte, tight binding to two different cytosolic glutathione S-transferases, Y protein (ligandin) and Z protein, limits the free cytosolic concentrations of UCB and bile salts, respectively. Ligandin is little expressed in the CNS, suggesting that the abundance of ligandin in hepatocytes may be a key factor in their greater resistance to UCB toxicity compared with neurones and astrocytes. The reputed antioxidant potency of UCB bound to cytosolic binding proteins could account for the protective effect of UCB against GCDC mediated apoptosis in hepatocytes, which is mediated by generation of reactive oxygen species.

Mitochondria from rat livers are themselves more resistant than mitochondria from rat brain to impairment of respiration and oxidative phosphorylation by UCB concentrations. Even though the net uptake of UCB is similar in both preparations, these findings suggest that intrinsic changes in the mitochondrial membrane contribute to the greater resistance of hepatocytes to apoptosis caused by hydrophobic bile salts. Additionally, hepatocytes and liver mitochondria from bile duct ligated rats are more resistant to the mitochondrial permeability transition than are the specimens from sham operated controls, and this may be mediated by an increase in mitochondrial cardiolipin content.

CONCLUSIONS

UCB is a curare at high concentrations, producing apoptosis and cell death, but is boon at more physiological levels, protecting cells against oxidant damage. Differences in organ or cell susceptibility to bilirubin toxicity may be related to differences in expression of ABC transporters that can export UCB, conjugation and oxidation of the pigment, binding of the pigment to cytosolic binding proteins, and intrinsic differences in the composition of mitochondrial membranes. Conjugation and oxidation convert the pigment to polar non-toxic derivatives which themselves may be antioxidants and/or regulate the metabolism and transport of bilirubin and bile salts. Binding of UCB to ligandin decreases the concentration of the toxic bound fraction and may enhance the antioxidant potency of the bound fraction. Much additional work, preferably performed with UCB at relevant concentrations, is needed to verify or disprove these possibilities.

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