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REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

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Inherited Disorders of Bilirubin Transport and Conjugation: New Insights Into Molecular Mechanisms and Consequences

Serge Erlinger,[1] Irwin M. Arias,[2] and Daniel Dhumeaux[3]

[1]University of Paris 7, Paris, France; [2]National Institutes of Health, Bethesda, Maryland; and [3]Henri Mondor Hospital, Créteil, University of Paris-Est, Créteil, France

Inherited disorders of bilirubin metabolism might reduce bilirubin uptake by hepatocytes, bilirubin conjugation, or secretion of bilirubin into bile. Reductions in uptake could increase levels of unconjugated or conjugated bilirubin (Rotor syndrome). Defects in bilirubin conjugation could increase levels of unconjugated bilirubin; the effects can be benign and frequent (Gilbert syndrome) or rare but severe, increasing the risk of bilirubin encephalopathy (Crigler–Najjar syndrome). Impairment of bilirubin secretion leads to accumulation of conjugated bilirubin (Dubin–Johnson syndrome). We review the genetic causes and pathophysiology of disorders of bilirubin transport and conjugation as well as clinical and therapeutic aspects. We also discuss the possible mechanisms by which hyperbilirubinemia protects against cardiovascular disease and the metabolic syndrome and the effects of specific genetic variants on drug metabolism and cancer development.

Keywords: Crigler–Najjar Syndrome; Hepatic Storage Disease; Glucuronosyl Transferase; Bile Secretion; Kernicterus.

Hereditary hyperbilirubinemias range from benign to lethal, and all are caused by defective bilirubin transport or conjugation by the liver. Bilirubin is the end product of heme catabolism. It belongs to the superfamily of tetrapyrrolic compounds, one of the most highly conserved groups of molecules in living organisms. Bilirubin is poorly water soluble. In blood, it circulates bound to serum albumin, presumably to prevent the toxicity of free (unbound) bilirubin. Unbound bilirubin is rapidly and selectively taken up by hepatocytes and then conjugated to glucuronic acid into bilirubin glucuronides by uridine diphosphate (UDP)-glucuronosyl transferase before being secreted into bile through the hepatocyte canalicular membrane via an adenosine triphosphate (ATP)-dependent transporter.

In clinical practice, hereditary hyperbilirubinemias can be separated into predominantly unconjugated and predominantly conjugated forms. These conditions result from mutations of transporters or enzymes involved in the hepatic bilirubin elimination pathway. The aim of this review is to describe these inherited disorders, with a particular focus on their molecular mechanisms. Studies of bilirubin metabolism have broader implications, showing the beneficial effects of moderate hyperbilirubinemia (due to the antioxidant properties of bilirubin) and other consequences of mutations on drug metabolism and cancer susceptibility. We will not discuss hereditary hemolytic unconjugated hyperbilirubinemia, which is caused by bilirubin overproduction, or several genetically mediated cholestatic diseases such as progressive familial intrahepatic cholestasis or Alagille syndrome, which can also lead to hyperbilirubinemia.

Bilirubin Transport and Conjugation by the Liver

Uptake by Hepatocytes: Passive Diffusion or Active Transport?

Unconjugated bilirubin is lipid soluble and should thus readily cross biological membranes. However, passive diffusion alone would not account for the remarkable specificity of hepatic uptake. One possible explanation for this specificity is the presence in hepatocytes of cytoplasmic proteins with a higher affinity than albumin for bilirubin. One such protein was identified and characterized in the late 1960s and early 1970s by Arias et al and was named Y protein or ligandin.[1] Kinetic studies suggested that bilirubin binding to this protein was not involved in initial cellular uptake but rather reduced bilirubin efflux from the cytosol back into the space of Disse, thus resulting in intrahepatocytic bilirubin accumulation. More recent studies have attempted to identify bilirubin transport proteins in the hepatocyte basolateral membrane, particularly among the organic anion transport proteins (OATPs). They belong to the OATP superfamily, which is also called the solute carrier organic anion transporter (SLCO) superfamily.[2] The human SLCO superfamily comprises 11 members grouped

Abbreviations used in this paper: **ATP, adenosine triphosphate; BSP, bromosulphophthalein; CN, Crigler–Najjar; DJS, Dubin–Johnson syndrome; GS, Gilbert syndrome; ICG, indocyanine green; MRP2, multidrug related protein 2; OATP, organic anion transport protein; OMIM, Online Mendelian Inheritance in Man; RS, Rotor syndrome; SLCO, solute carrier organic anion transporter; UDP, uridine diphosphate; UGT1A1, uridine diphosphate glucuronosyl transferase 1A1.**

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into 6 families encoded by *SLCO* genes. Bilirubin is a substrate for OATP1B1 (Online Mendelian Inheritance in Man [OMIM]*604843) and OATP1B3 (OMIM*605495).[2] Human OATP1B1 and OATP1B3 can transport conjugated and, possibly, unconjugated bilirubin in vitro.[3,4] Studies in humans, and particularly genome-wide association studies, suggest that polymorphisms that reduce OATP1B1 or OATP1B3 activity are associated with higher serum levels of both conjugated and unconjugated bilirubin.[5,6] *Oatp1a* and *Oatp1b* knockout mice lacking the Oatp1a and 1b transporters have serum bilirubin levels more than 40 times higher than those of their wild-type counterparts.[7] Serum bilirubin in these mice is mostly conjugated, probably (see the following text) because of defective reuptake of bilirubin glucuronide.[8] However, serum levels of unconjugated bilirubin are 2-fold higher in *Oatp1a/1b* knockout mice than in their wild-type counterparts.[7] This suggests that *Oatp1a/1b* proteins may contribute to unconjugated bilirubin uptake by hepatocytes. Human embryonic kidney cells (HEK293) permanently expressing recombinant OATP1B1 (formerly OATP2) showed uptake of [^{3}H]monoglucuronosyl bilirubin, [^{3}H]bisglucuronosyl bilirubin, and [^{3}H]sulfobromophthalein, with K_m values of 0.10, 0.28, and 0.14 μmol/L, respectively.[3] However, this observation could not be reproduced with unconjugated bilirubin in HeLa or HEK293 cells transfected with OATP1B1.[9] Further studies are thus needed to clarify the respective roles of passive diffusion and carrier-mediated transport in unconjugated bilirubin uptake by hepatocytes.

Conjugation

After its uptake and binding to ligandin, bilirubin is transferred to the smooth endoplasmic reticulum, where it is conjugated into bilirubin glucuronides by UDP–glucuronosyl transferase 1A1 (UGT1A1). The process of bilirubin conjugation and the function of UDP–glucuronosyl transferases have been extensively reviewed recently.[10–12]

UGT1A1 (OMIM*191740) (Figure 1) appears to be the only enzyme that glucuronidates bilirubin. Indeed, mutations that completely suppress UGT1A1 activity result in a total absence of bilirubin glucuronides.[11] UGT1A1 is a transmembrane protein located mainly on the smooth endoplasmic reticulum. It has a binding site for bilirubin and another one for glucuronic acid, with both sites located on the luminal face of the endoplasmic reticulum membrane.[11] Glucuronic acid is derived from uridine diphospho-glucuronic acid, which itself is derived from UDP glucose. The location of the binding sites implies that both bilirubin and glucuronic acid are transported from the cytosol into the lumen of the endoplasmic reticulum. UGT1A1 catalyzes the conversion of bilirubin to bilirubin monoglucuronide and then to bilirubin diglucuronide. Bilirubin glucuronides then move to the cytoplasm, probably through a specific endoplasmic reticulum membrane transporter (Figure 2, *inset*), where they bind to ligandin, albeit with far lower affinity than unconjugated bilirubin.

Secretion Into Bile and Plasma

Once back in the cytosol, bilirubin diglucuronide can diffuse toward either the canalicular pole or the sinusoidal pole of the hepatocyte. At the canalicular pole, it is efficiently secreted into bile, mostly by the ATP-dependent MRP2/ABCC2 transporter.[13,14] This protein mediates the canalicular secretion of several organic anions, including bilirubin glucuronides, dyes such as sulfobromophthalein (BSP) and indocyanine green (ICG), divalent bile salts, and reduced glutathione.[13,14] Other transporters, particularly Abcg2,[15] may be involved in bilirubin secretion across the canalicular membrane.

Interestingly, a substantial fraction of bilirubin glucuronide is rerouted to the sinusoidal pole and secreted back into plasma by another transporter, Abcc3.[8] From there, it can be taken up again by hepatocytes via Oatp1b1/3.[8] It has been proposed that this reuptake process may take place in downstream hepatocytes (hepatocytes located near the central vein) to prevent saturation of the biliary secretory capacity of upstream hepatocytes (hepatocytes located near the portal tracts).[8]

A schematic representation of bilirubin transport and conjugation by hepatocytes is provided in Figure 2.

Hereditary Hyperbilirubinemias

Hereditary hyperbilirubinemias may be caused by increased bilirubin production, mostly as a result of hyperhemolysis, or decreased bilirubin clearance. This review will be limited to conditions associated with decreased bilirubin clearance.

Decreased bilirubin clearance may be caused by (1) defective bilirubin uptake by hepatocytes, leading to unconjugated hyperbilirubinemia; (2) defective conjugated bilirubin reuptake, such as in Rotor syndrome (RS) (also known as hepatic uptake and storage disease; see the following text); (3) defective bilirubin conjugation, such as in Gilbert syndrome (GS), Crigler–Najjar (CN) syndrome, neonatal transient familial hyperbilirubinemia, and breast

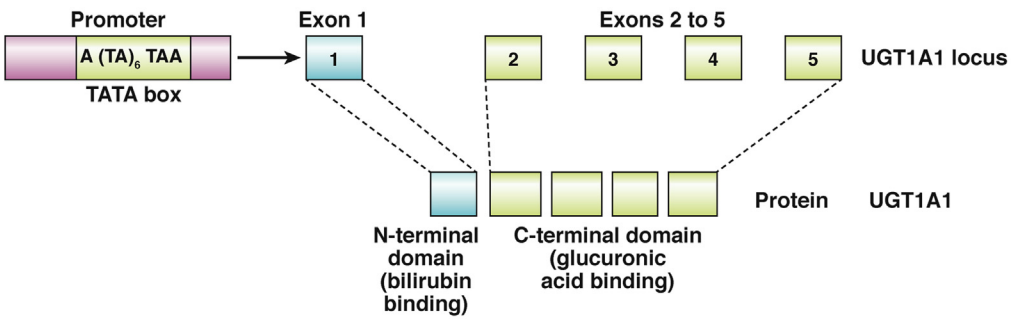

Figure 1. Schematic representation of the UGT1A1 locus and UGT1A1 protein.