

ADVANCES IN HEPATOLOGY

Current Developments in the Treatment of Hepatitis and Hepatobiliary Disease

Section Editor: Eugene R. Schiff, MD

Canalicular Bile Secretion

James L. Boyer, MD
 Ensign Professor of Medicine
 Director, Liver Center
 Section of Digestive Diseases
 Department of Internal Medicine
 Yale School of Medicine

G&H Can you describe the role of bile canalicular secretion in the function of the healthy liver?

JB Canalicular bile secretion is a vital component of liver function and is divided, most simply, into a bile-salt-dependent fraction and a bile-salt-independent fraction. Bile salts are the primary driving source for canalicular bile secretion, and they are transported into bile by specific proteins through an energy-dependent process. When bile salts are transported into bile and are concentrated in very high amounts, they produce an osmotic effect that draws fluid into the lumen of the small (1 micron in diameter) bile canaliculi within the liver. This process stimulates the production and flow of bile. The bile-salt-independent fraction is largely dependent on the excretion of glutathione, a tripeptide that is excreted into the canalicular lumen in high concentrations by a different transport protein. Bile salts are transported by the bile salt export pump (BSEP), and glutathione is transported by the multidrug resistance protein type 2 (MRP2). These transport proteins are both adenosine triphosphate (ATP)-dependent transporters. There are other, less prominent determinants of bile-salt-independent secretion, but bile salts and glutathione comprise the major osmotically secreted substances that stimulate the formation of bile.

Once bile is secreted into the small canalicular spaces, it travels downstream into the intrahepatic bile ducts, and from there into the extrahepatic ducts and the intestine. In the intestine, bile takes up a second function, which is to aid in the absorption of dietary

lipids. The major solid components of bile are the bile salts, which are detergents and which emulsify dietary lipids and thus facilitate fat absorption.

Bile salts then undergo an enterohepatic circulation. They are re-absorbed, predominantly in the distal intestinal ileum, by a specific transport system, the ileal sodium dependent bile acid transporter, which takes up bile salts at the ileal luminal membrane. Bile salts then move across the ileum and into the mesenteric circulation via the heteromeric organic solute transporter, OST alpha-OST beta, located on the ileal cell's basolateral membrane. Once bile acids are returned to the liver, they are selectively removed from the circulation by a specific transporter protein, the sodium-dependent taurocholic cotransporter. This transporter has a high affinity for conjugated bile salts, whereas other transport proteins, known as organic anion transporting polypeptides, take up unconjugated bile acids into the liver, where they are again excreted into bile and continue their journey within the enterohepatic circulation. In man, the bile acid pool size is approximately 4 g in adults, and it circulates approximately 6 times in 24 hours. Thus, approximately 24 g of bile acids circulate through the enterohepatic system daily.

G&H Can you describe your historic research and collaboration with other specialists in defining the role of bile canalicular function?

JB When I embarked on this research more than 30 years ago, we had no knowledge of the basic mechanisms of bile formation. Gradually, with the help of talented colleagues and developments in molecular biology and genetics by others, enormous progress has been made.

Thirty years ago, I was interested in trying to obtain access to the canalicular lumen with microelectrodes in order to determine the electrical potential across the canalicular membrane. I had developed an isolated hepatocyte couplet system in short-term culture in our laboratory from rat liver, which I thought might be a suitable model to determine the primary driving forces for bile secretion. However, I was not trained as an electrophysiologist. Fortunately, I had recently met Dr. Jurg Graf, from Vienna, Austria, who was an electrophysiologist, and I was able to

persuade him to come to Yale for a short stay to see if we could succeed in micropuncturing the canalicular space in the couplets. He was remarkably successful in doing so and this marked the start of a 10-year collaboration where the electrical properties of the bile canalicular secretory system were defined. The hepatocyte couplet model has also proven to be useful in determining the choleric properties of compounds that stimulate canalicular secretion, as it is the primary bile secretory unit of the liver. In addition, this model allows for confounding effects of liver blood flow and the function of the bile ducts to be avoided.

Subsequently, we developed an isolated bile duct secretory unit, making it possible to clearly define whether a choleric compound stimulates secretion from the bile canalculus or the bile duct epithelium. These isolated preparations can also be used for localizing transport systems by immunofluorescent techniques, whereas the hepatocyte couplet model is particularly useful for visualizing the excretion of various fluorescent compounds into bile in living hepatocytes.

G&H How were animal models utilized in the subsequent continuation of this research?

JB Animal research on the mechanisms of bile formation began by collecting bile from cannulas placed in the bile ducts of rats, mice, dogs, and, eventually, man. Bile composition could be analyzed, and the rate of secretion and its stimulation by bile acids, hormones, and other choleric agents could be assessed. Subsequent research utilized isolated perfused livers from rats, and techniques were developed for isolating membranes from various liver domains, particularly the canalicular domain. Dr. Peter Meier from Zurich, Switzerland was a major contributor to this early work when he came to our laboratory and later pioneered the molecular characterization of bile acid and other organic anion transporters. These isolated membrane fractions could be formed into small vesicles, enabling the driving forces for solute transport to be defined. Indeed, before the molecular basis of bile secretion was discovered, it was determined that there was a sodium-dependent system for the uptake of bile acids in the liver and that an ATP-dependent transport system existed for the transport of bile acids into the canalicular space.

G&H How has this research allowed for a better understanding of specific hepatic disease states?

JB Major developments in molecular biology and sequencing of the human genome subsequently led to a fairly complete understanding of the molecular basis of bile formation and then to an understanding of cholestatic mechanisms. These developments came from the work of

many different laboratories. Animal models of cholestasis also led to the understanding of mechanisms of cholestasis at the molecular level. Dr. Michael Trauner, from Graz, Austria, who also spent a few years with us as a fellow, was particularly instrumental in these later developments.

The function of several BSEP and bilirubin export pumps that determine canalicular secretion was clearly defined when mutations in these specific transporters were discovered to be the cause of several cholestatic liver diseases. Mutations in bile transporters provided proof of principle regarding the function of these proteins. For example, mutations in BSEP result in progressive familial intrahepatic cholestasis type 2 (PFIC2) in infants and young children. Other mutations in BSEP can produce a benign recurrent form of cholestasis, known as benign recurrent intrahepatic cholestasis type 2. Polymorphisms in the BSEP have also been associated with drug-induced cholestasis and, in rare instances, in cholestasis of pregnancy. Some BSEP mutations result in folding defects that trap the protein within the endoplasmic reticulum, so that the protein cannot traffic to its functional location at the canalicular membrane. Other mutations do not affect the folding of the protein. Thus, it can be released from the endoplasmic reticulum, but it cannot traffic to the correct location in the canalicular membrane. Still others, such as those that produce intrahepatic cholestasis of pregnancy, allow the protein to reach the canalicular membrane but not to function normally, either because the protein is not fully expressed or it is inhibited by the high levels of estrogen that develop in the third trimester when cholestasis occurs. PFIC type 1 results from mutations in a P-type ATPase (ATP8B1), whereas PFIC-type 3 is caused by mutations in a phospholipid export pump in the canalicular membrane, known as the multidrug resistance-associated protein type 3 (MDR3). Mutations in MRP2 result in the Dubin-Johnson syndrome.

Current research is focused on the need to understand the role that polymorphisms and other genetic variants play in the level of expression of these transporters, and whether these genetic variants predispose the liver to cholestatic injury from drugs and other environmental insults.

G&H Has the discovery and mapping of these genetic polymorphisms allowed for new screening and therapeutic targets for the related disorders?

JB At present, it is necessary to sequence the individual genes to look for mutations and polymorphisms, but there are genetic screening tests under development that should enable more rapid determination of the most common mutations in some of these transport systems. They are usually autosomal recessive inherited disorders where both parents are heterozygotes and, thus, each

passes the mutation on to the child. However, female heterozygotes for MDR3 are also susceptible to cholestasis during pregnancy and to cholelithiasis. Research is also ongoing to develop drugs that function as molecular chaperones, which may enable the mutant proteins to get out of the endoplasmic reticulum of the hepatocyte, so they can traffic to the canalicular membrane to carry out their function.

G&H Are there other cholestatic diseases where research on canalicular secretion and the enterohepatic circulation could be of use?

JB The diseases discussed above are rare, inherited disorders. More common conditions are the acquired forms of cholestasis, which include primary sclerosing cholangitis, primary biliary cirrhosis, and drug-induced cholestatic liver injury. In these cholestatic liver diseases, the liver attempts to adapt to this injury by upregulating alternative pathways to eliminate bile salts. Cholestatic liver disease is injurious because bile salts cannot be excreted normally into bile. They therefore accumulate in the liver and, because they are detergents, they produce cell injury leading to oxidative stress, inflammation, fibrosis, cirrhosis, and eventually progressive liver failure and the need for a liver transplant.

The liver attempts to combat this damage by reversing its secretory polarity and upregulating the expression of bile acid transporters on the blood side of the liver cell. Three different transporters are affected, and two are ATP-dependent transporters (MRP3 and MRP4). These transporters are capable of moving bile salts, bilirubin conjugates, and other toxic substances back into the blood when they cannot be excreted into bile. A third basolateral membrane transporter, OST alpha-OST beta, was first isolated from the liver of a marine skate by my colleague Dr. Ned Ballatori and was later found to be the missing link in the enterohepatic circulation on the basolateral blood side of the terminal ileum, as well as the hepatocyte and the renal proximal tubule. OST alpha-OST beta facilitates the return of bile acids to the liver via the enterohepatic circulation and provides another salvage pathway in the liver for excretion of bile acids during cholestasis. The kidney, to the best of its ability, filters and eliminates bile acids into the urine. This is why serum bile acid levels rise in patients with cholestatic liver disease and why bile acids appear in the urine, where they are not normally present. However, unaided, the kidney cannot fully compensate for this excess of bile acids. Bile acid concentrations continue to rise in the liver and blood, and cholestasis eventually progresses.

Our current research goal is to develop a better understanding of how these bile acid transporters, which

serve as alternative pathways for elimination of bile acids, are regulated. If they can be upregulated pharmacologically, they could potentially provide better treatment for cholestatic liver diseases. This research focuses on the role of nuclear receptors in regulation of gene transcription. Our laboratory and others are studying the human orthologs of these transporters at the gene promoter level and are attempting to determine what nuclear receptors and their respective ligands can upregulate ortholog transcription. Our hope is to find small molecules that can be utilized as nuclear receptor ligands that will stimulate and enhance the expression of these salvage pathways. If we can up-regulate these transporters pharmacologically, before cholestatic damage becomes irreversible, the damaging effects of bile acids in the liver should be attenuated.

Another promising approach, about to move from animal studies to clinical trials, is the use of nor-ursodeoxycholic acid (nor-urso). Ursodeoxycholic acid, bile acid from the black bear, is currently the main medical treatment for cholestatic liver diseases because it reduces the toxicity of the bile. Nor-urso is a derivative of ursodeoxycholic acid and appears to have additional liver-preserving properties. Nor-urso ameliorates injury to the bile ducts in MDR2-deficient mice, which develop cholestatic disease similar to patients with primary sclerosing cholangitis. This bile acid is not normally present in bile but it has properties that reduce its detergency. It also seems to be reabsorbed by the bile duct epithelium, resulting in a cholehepatic circulation.

Another bile acid derivative has been developed by an Italian company that is a very potent ligand for the nuclear receptor FXR. FXR upregulates the expression of BSEP, MDR3, and other bile acid transporters, including OST alpha-OST beta. This compound (INT-747) reduces cholestasis in several animal models of cholestasis and is in phase II trials in patients with primary biliary cirrhosis.

Suggested Reading

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