NEW CONCEPTS IN GASTROENTEROLOGY

Nuclear Receptor Ligands: Rational and Effective Therapy for Chronic Cholestatic Liver Disease?

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The regulation of genes that are essential to many hepatic metabolic and transport functions are mediated in large part by the action of small molecules that function as nuclear receptor ligands.[1] This process has been labeled *chemical genomics*.[2] These ligands bind to their specific nuclear receptors, activating the receptor that then binds to specific elements in a gene's promoter resulting in stimulation or inhibition of gene expression.[3] In the liver many drugs, metabolites, and herbal compounds exert their biologic properties as ligands for nuclear receptors.[4,5] Some familiar examples of drugs that activate nuclear receptors include phenobarbital and St John's wort. Both drugs induce hepatic drug metabolizing enzymes by acting as ligands for the pregnane X receptor (PXR), which binds to specific response elements in the promoter of cytochrome P-450 3A (CYP3A), a major hepatic microsomal drug–metabolizing enzyme. This induction then indirectly affects the metabolism and systemic effects of a wide variety of other compounds metabolized by CYP3A. An extract of 4 different plants, Yin Zhi Huang, has been used traditionally for treating neonatal jaundice in China and is a ligand for the constitutive androstane receptor (CAR) that up-regulates the expression of several different liver transporters and enzymes involved in the hepatic clearance of serum bilirubin.[5] Indeed, more than 10% of medically useful drugs are known to exert their biologic behavior by binding to ligand-binding domains on specific nuclear receptors.[4] Table 1 shows some of the most important nuclear receptors and their physiologic ligands that determine the hepatic transport and metabolism of a variety of xenobiotics and endogenous substrates.

In this issue of GASTROENTEROLOGY, Marschall et al[6] report that rifampicin and ursodeoxycholic acid (UDCA), two well-known drugs used in the treatment of cholestatic liver disease, each stimulate the transcription of a distinct set of genes that together decrease bile acid uptake, enhance bile acid detoxification and excretion, and stimulate the clearance of bilirubin. Rifampicin increased the expression of CYP3A4, uridine 5′-glucuronosyl transferase (UGT1A1), and multidrug-resistance–associated protein (MRP2) whereas UDCA stimulated the bile salt export pump (BSEP), and multidrug resistance protein (MDR3), and MRP4. Although these effects were observed in patients undergoing cholecystectomy who otherwise were healthy, these separate but complementary effects on gene and protein expression are predicted to have beneficial effects if they occurred in patients with cholestatic liver disease. These findings thus suggest that there is therapeutic benefit from combining drugs such as rifampicin and UDCA that target nuclear receptors that have coordinated effects on the transcriptional regulation of hepatobiliary excretory mechanisms.[7] How does this occur?

The hepatic clearance of bile acids and bilirubin can be divided into 4 phases that include the following: phase 0, hepatic uptake; phase I, metabolism (eg, hydroxylation); phase II, detoxification (eg, conjugation); and phase III, excretion. Nuclear receptors and their ligands that are the major determinants of the functional expression of genes that determine these pathways are shown in Figure 1, together with enzymes that control bile acid synthesis.

In the enterohepatic circulation, the hepatic uptake of conjugated bile acids (phase 0) is mediated predominantly by the sodium taurocholate cotransporting polypeptide NTCP (SLC10A1), whereas unconjugated bile acid uptake is facilitated by several organic anion transporting polypeptides (OATPs) on the sinusoidal membrane, but predomi-

Abbreviations used in this paper: ABCG5/8, cholesterol export pump; BSEP, bile salt export pump; CAR, constitutive androstane receptor; CYP, cytochrome P-450; FTF, fetal transcription factor; FXR, farnesoid X receptor; GST, glutathione-S-transferase; LXR, liver X receptor; MDR, multidrug resistance protein; MRP, multidrug-resistance–associated protein; NTCP, sodium taurocholate co-transporting polypeptide; OATP, organic anion transporting polypeptides; PXR, pregnane X receptor; RAR, retinoic acid receptor; SULT, sulfotransferase; UGT, uridine 5′-glucuronosyl transferase; VDR, vitamin D receptor.

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Table 1. Nuclear Receptors, Important Ligands, and Target Genes

Nuclear receptor	Ligand(s)	Some major target genes
RXRα (retinoid X receptor)	9-cis-retinoic acid	Heterodimeric partner of other class II receptors
RXRα partners		
FXR (farnesoid X receptor)	Bile acids	BSEP, SHP, UGTs, SULTs, MRP2, MDR3
PXR (pregnane X receptor)	Xenobiotics, Ursodeoxycholic acid, Rifampacin	CYP3A, OATP-C, MRP2, MRP4, GST
CAR (constitutive androstane receptor)	Xenobiotics, Phenobarbital	CYP3A, OATP-C, MRP2, MRP4, UGT, SULTs, GSTs
LXR (liver X receptor)	Oxysterols (metabolites of cholesterol)	CYP7A, CYP8B, ABCG5/8
RAR (retinoic acid receptor)	All-trans retinoic acid	NTCP, MRP2
Others		
SHP-1 (short heterodimeric partner)	None	Inhibits CYP7A, CYP8B, NTCP
FTF (fetal transcription factor)	? bile acids	CYP7A, CYP7A and 8B1, MRP3
VDR (vitamin D receptor)	Vitamin D, Lithocholic acid	CYP3A, SULTs
HNF-1 (hepatocyte nuclear factor 1)	None	NTCP, CYP7A

nantly by OATP1B1 (SLC01B1), formally known as OATP-C. Normally, bile acids then are excreted into bile via BSEP (ABCB11), an adenosine triphosphate–binding cassette family member located on the apical canalicular membrane (phase III). Bile acids also are synthesized in the hepatocyte from the conversion of cholesterol via CYP7A1, leading to the synthesis of the 2 primary bile acids: cholic acid and chenodeoxycholic acid.[8] Bilirubin is thought to be taken up by OATP1B1, bind to glutathione-S-transferase, and undergo conjugation by hepatic microsomal UGTs to monoglucuronides and then diglucuronides (phase II). These conjugation reactions increased the hydrophobicity of this hydrophobic end product of heme metabolism, enabling it to be excreted into bile by MRP2 (ABCC2), a member of the multidrug resistance–associated protein gene family located at the canalicular membrane of the hepatocyte (phase III).

In animal models of cholestasis and in patients with cholestatic liver disease, sodium dependent taurocholate co-transporting polypeptide (NTCP) and most OATPs are down-regulated to minimize bile acid uptake.[9] Bile acid synthesis is diminished by the down-regulation of CYP7A1 and CYP8B, which reduces the conversion of cholesterol to bile acids. The more hydrophobic toxic secondary bile acids, lithocholate and deoxycholate, accumulate in the enterohepatic circulation and undergo phase I 6-α hydroxylation reactions in the liver via CYP3A1. Subsequently, phase II detoxifying conjugation reactions form more water-soluble bile acid glucuronides and sulfates, respectively, via the enzymatic activity of UGTs and sulfotransferases (phase II). Under cholestatic conditions, BSEP generally continues to be expressed functionally whereas MRP3 and MRP4, two alternative phase III export pumps for bile acid and bilirubin conjugates, are up-regulated on the basolateral membrane.[10,11] Normally, MRP3 and MRP4 are expressed only weakly but they appear to function during cholestasis to allow these more water-soluble bilirubin and bile acid conjugates to be transported back into the systemic circulation to be excreted by the kidney. Although these adaptive responses in transporter expression are beneficial, they clearly are unable to compensate fully for the primary defects in cholestasis, and progressive liver injury ultimately occurs in many cholestatic diseases. Nevertheless, if these adaptive responses could be enhanced or possibly initiated earlier in the natural history of cholestatic liver disease, they might have additional benefits. This is where nuclear receptor therapy is proposed to play a role, particularly ligands for the farnesoid X receptor (FXR), CAR, and PXR.[12,13]

FXR (NR1H4) is activated by bile acids. The hydrophobic bile acid CDCA is its most potent physiologic agonist. FXR is expressed highly in the liver, intestine, adrenal glands, and kidney, and up-regulates many of the steps in the enterohepatic circulation of bile acids including BSEP, the ileal bile acid–binding protein (FABP6), the hepatic canalicular membrane phospholipid flippase (MDR3, ABCB4), and MRP2. MDR3 is the export pump for phosphotidylcholine and mutations in MDR3 result in progressive cholestasis.[14] FXR also activates transcription of the nuclear receptor short heterodimeric protein, which in turn inhibits transcription of CYP7A and CYP8B, thereby providing feedback inhibition of bile acid synthesis (Figure 1).[15] These adaptive changes mediated by FXR protect the liver from the injurious effects of the hepatic accumulation of bile acids during cholestasis. Thus, there has been considerable interest in the development of nontoxic synthetic FXR agonists for the treatment of cholestatic liver disease and several compounds have been shown to be hepatoprotective in rat and mouse models of cholestasis. The administration of a synthetic FXR agonist, GW4064, reduces markers of liver damage, inflammation, and bile duct proliferation induced by α-napthylisothiocyanate and