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Coordinate Regulation of Hepatic Bile Acid Oxidation and Conjugation by Nuclear Receptors

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Abstract: Bile acids play important functions in the maintenance of bile acid homeostasis. However, due to their detergent properties, these acids are inherently cytotoxic and their accumulation in liver is associated with hepatic disorders such as cholestasis. During their enterohepatic circulation, bile acids undergo several metabolic alterations, including amidation, hydroxylation, sulfonation, and glucuronidation. Most of these transformations facilitate the excretion of bile acids into the bile (amidation and sulfonation) or into the blood for subsequent urinary elimination (hydroxylation, sulfonation, and glucuronidation). In this review, the role of various nuclear receptors and transcription factors in the expression of bile acid detoxification enzymes is summarized. In particular, the coordinate manner in which the xenobiotic sensors pregnane X receptor and constitutive androstane receptor, the lipid sensors liver X receptor, farnesoid X receptor, peroxisome proliferator-activated receptor alpha, and vitamin D receptor, and the orphan receptors hepatocyte nuclear factor 4α and small heterodimer partner regulate bile acid detoxification is detailed. Finally, we conclude by discussing the importance of these transcription factors as promising drug targets for the correction of cholestasis.

Keywords: Nuclear receptor; bile acid metabolism; cholestasis; amidation; oxidation; glucuronidation; sulfonation

Introduction

Bile acids are synthesized from cholesterol in the liver, secreted into the bile, and delivered in the lumen of the small intestine where they serve as detergents for the absorption of dietary lipids, cholesterol, and fat-soluble vitamins. In humans, conversion of cholesterol produces the primary bile acids, chenodeoxycholic (CDCA) and cholic (CA) acids.[1] In the intestine, bile acids are absorbed with nutrients and are transported back to the liver via the portal circulation.[1] However, primary bile acids escaping reabsorption are converted by the intestinal microflora to secondary deoxycholic (DCA) and lithocholic (LCA) acids, a portion of which is also absorbed.[2,3] The enterohepatic recirculation allows the recovery of 95% of bile acids secreted in the intestine, and the 5% lost is replaced by neosynthesis in the liver. In fact, conversion into bile acids represents 90% of the amount of cholesterol that is metabolized daily.

However, bile acids are cytotoxic when their concentrations reach high levels, and this toxicity increases with the hydrophobicity of bile acids.[4] Cholestasis, or impaired bile flow, one of the most common and devastating manifestations of liver diseases,[5] is associated with intracellular

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accumulation of toxic bile acids and consecutive cell damage.[5,6] The causes of intrahepatic cholestasis are as varied as genetic alterations, inflammatory disorders (primary biliary cirrhosis (PBC) or primary sclerosing cholangitis), pregnancy, infiltrative or granulomatous diseases, alcohol, and drugs.[5,6] Cholestasis is clinically characterized by elevated plasma concentrations of biliary constituents, such as bile acids and their sulfate or glucuronide conjugates.[7,8] Pharmacological modalities available in the management of patients with chronic cholestasis are limited, and clinicians frequently have nothing other than ursodeoxycholic acid (URSO, UDCA) to offer their patients.

Under normal conditions, accumulation of bile acids in hepatocytes is avoided through a tight control of bile acid synthesis, transport, and metabolism, and this control is allowed by a series of feedback and feedforward autoregulatory processes.[9] Such mechanisms involve the participation of a series of nuclear receptors which function as ligand-inducible transcription factors. Among the nuclear receptor family, studies performed during the past 10 years evidenced the key roles that the farnesoid X receptor (FXR), liver X receptors (LXRs), peroxisome proliferator-activated receptor alpha (PPARα), vitamin D receptor (VDR), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) play for the maintenance of bile acid homeostasis. These lipid and xenobiotic sensors belong to the nuclear receptor 1 (NR1) family and are activated by small hydrophobic molecules (reviewed in refs 2 and 10). In the presence of their ligands, they form active heterodimers with the retinoid X receptor (RXR, NR2B1) to bind the promoter region of target genes on response elements (RE), which usually consist of the repetition of AGGTCA-like sequences arranged in direct, inverted, or everted repeats separated by 1 to 6 nucleotides.[2,10] Ligands of these metabolic receptors potentially offer novel approaches in the treatment of various liver disorders. Consequently, the multiple and coordinate actions that these receptors exert on genes encoding bile acids synthesizing enzymes, as well as transporters, have been extensively studied and very recently reviewed.[1,2,9−12] By contrast, bile acid metabolism received less attention; therefore the present review will summarize the most recent data on the control of bile acid metabolizing enzyme expression.

Overview of the Enzymes That Catalyze Bile Acid Hydroxylation and Conjugation Reactions. During their enterohepatic recirculation, bile acids undergo several metabolic alterations, such as amidation, hydroxylation, glucuronidation, or sulfonation. Indeed, both neosynthesized primary or reabsorbed primary and secondary bile acids undergo conjugation and deconjugation reactions in the liver. With this mechanism, taurine- and glycine-conjugated bile acids can be deconjugated and, subsequently, hydroxylated and reconjugated with glucuronide or sulfate groups.[1]

Amidation involves the addition of an amino acid, usually glycine or taurine, in amine linkage to carbon 24 of bile acids.[1] Ninety-eight percent of the bile acids excreted from the liver are amidated, thus illustrating the importance of bile salt secretion into bile.[1] In addition to being efficiently excreted, glycine and taurine conjugates of bile acids also exhibit higher hydrophobicity and stronger detergent properties to facilitate lipid and vitamin absorption in the intestine. Bile acid amidation involves at least two enzymes: the bile acid CoA synthetase (BACS) forms the bile acid CoA-thioesters which serve as substrates for conjugation with taurine and glycine. This reaction is catalyzed by the bile acid-CoA:amino acid *N*-acetyltransferase (BACAT) enzyme (Figure 1).[13] The important role played by BACAT in the maintenance of bile acid homeostasis is further demonstrated by the causative relation between inactivating mutations in its gene and familial hypercholanemia, a syndrome characterized by elevated serum bile acid levels and fat malabsorption.[14] Recycling of tauro- or glyco-bile acids is performed either in the intestine through the action of bacterial enzymes or in the liver by the bile acid-CoA thioesterase (BACTE) enzyme.[15,16] Interestingly, tauro- and glyco-

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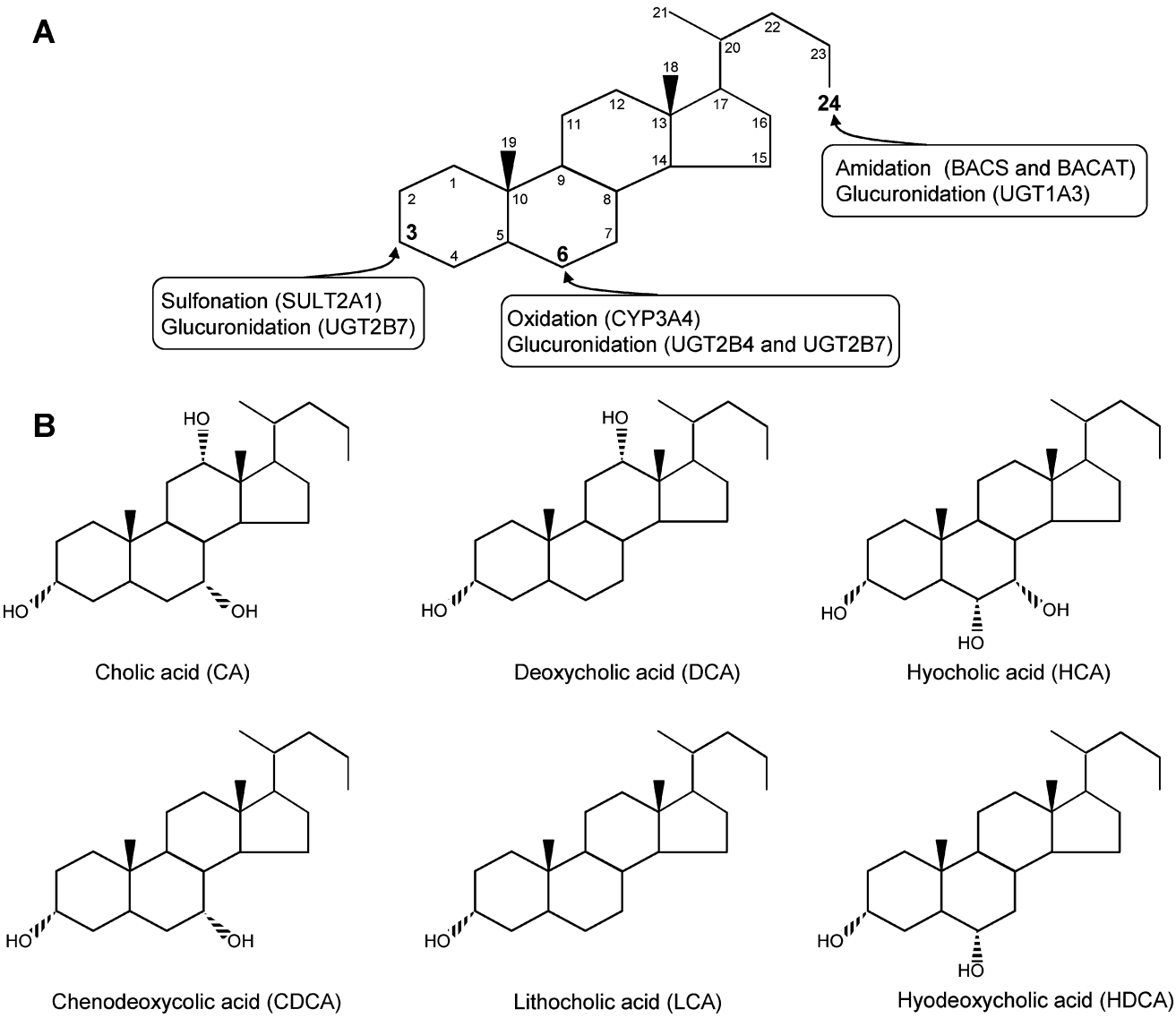

Figure 1. Oxidation, sulfonation, glucuronidation, and amidation reactions principally involve the 3α- and 6α-hydroxy positions and the 24-carboxy position of bile acids. (A) Sulfonation produces 3α-sulfated bile acids, whereas 6α-hydroxylated metabolites are the major conversion products of primary and secondary bile acid oxidation catalyzed by CYP3A4. UGT2B4 conjugates these oxidized metabolites, while UGT1A3 transfers the glucuronosyl groups on the 24-carboxyl position of primary, secondary, and hydroxylated bile acids. UGT2B7 forms both 3α- and 6α-glucuronide metabolites. The amidation reaction also involved their 24-carboxyl group. (B) Structure of primary, secondary, and hydroxylated bile acids. BACAT: bile acid-CoA:amino acid *N*-acetyltransferase. BACS: bile acid CoA synthetase. CYP3A4: cytochrome P450 3A4. SULT2A1: sulfotransferase 2A1. UGT2B4, -2B7, -1A3: UDP-glucuronosyltransferase 2B4, 2B7, 1A3.

conjugated bile acid are biologically active molecules that serve as potent activators of the bile acid sensors.[17]

An important metabolic alteration of primary and secondary bile acids occurring in the liver corresponds to ring hydroxylation, a phase I metabolic reaction that increases their solubility and therefore reduces their toxicity. In addition, hydroxylated bile acids are better substrates for glucuronidation, although nonhydroxylated acids can also be glucuronide conjugated.[18] Among the human cytochrome P450 enzymes, CYP3A4 (Cyp3a11 in mice) was reported as the predominant enzyme for bile acid oxidation, whereas CYP3A5 also catalyzes bile acid oxidation, but at a much

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lower rate.[19,20] CYP3A4 catalyzes the hydroxylation of different acids, including CDCA, LCA, DCA, and UDCA and their amidated metabolites, at different positions leading to the formation of 3-oxo-, 1β-, 6α-, and 22-hydroxy bile acids[4,19] (Figure 1). However, CYP3A4 activity increases with the hydrophobicity of bile acids. Indeed, hyocholic (HCA) and hyodeoxycholic (HDCA) acids, the 6α-hydroxylated products of CDCA and LCA, respectively, are the predominant excreted bile acids found in human urine.[8,21,22] On the other hand, the recombinant CYP3A4 displays higher activity but lower affinity for reacting with tauro-conjugated bile acids when compared to the unconjugated compounds.[19]

Glucuronidation and sulfonation are two major phase II metabolic reactions involved in the inactivation of a huge variety of endo- and xenobiotics.[23,24] The sulfonation reaction, which consists of the transfer of a sulfonyl group from 3-phosphoadenosine-5-phosphosulfate to the acceptor molecule, is catalyzed by members of the sulfotransferase (SULT) enzyme family.[24] Similarly, addition of the glucuronosyl group from the UDP-glucuronic acid to glucuronidated compounds is performed by the 18 UDP-glucuronosyltransferase (UGT) enzymes.[23] Both glucuronidation and sulfonation produce more hydrophilic and less toxic molecules that are efficiently excreted into the bile or urine.[23,24] Whereas 3α-sulfated bile acids are relatively abundant in a normal situation,[25] both sulfonation and glucuronidation become major eliminating pathways in cholestatic patients.[8] Among the human SULT enzymes, SULT2A1 (sult2a9 in mice) plays a predominant role in the formation of 3α-sulfated bile acids, such as LCA[26] (Figure 1). Glucuronide conjugation involves mainly three UGT enzymes: UGT2B4 catalyzes the glucuronide conjugation at the 6α-hydroxy position of acids, such as HDCA,[27] whereas UGT2B7 transfers the glucuronosyl moiety to the 3α- and 6α-hydroxyl groups of primary, secondary, and hydroxylated bile acids.[28,29] UGT1A3 is almost unique in modifying the C24-carboxyl groups of bile acids, such as CDCA, LCA, and HDCA, to form acyl glucuronide derivatives.[28,29]

PXR and CAR: Two Major Controllers of Bile Acid Metabolizing Enzymes. PXR (NR1I2) and CAR (NR1I3) were initially identified as xenobiotic sensors involved in the regulation of phase I and phase II enzymes and drug transporters.[30] PXR is highly expressed in the liver and moderately in the intestine, both of which are active sites for the detoxification of endo- and exogenous chemicals.[31] PXR ligands include xenobiotics such as rifampicin and paclitaxel as well as natural and synthetic steroids such as pregnenolone and dexamethasone. Interestingly, PXR is also a receptor for cholestatic bile acids, such as LCA.[32] CAR is another nuclear receptor abundantly expressed in liver and intestine, and activated by xenobiotics (reviewed in ref 10). CAR−RXR and PXR−RXR heterodimers can bind to an overlapping range of hexameric repeat DNA configurations within target promoters.[33] There are extensive cross-talks between PXR and CAR: PXR can regulate *CYP2B* genes through recognition of the phenobarbital response element. Reciprocally, CAR can bind to and activate the PXR

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response element within the *CYP3A4* promoter.[34] This functional symmetry between the two metabolic sensors may provide a two-layered resistance mechanism against the toxic compounds that the CYP enzymes metabolize.[33]

Numerous studies establish the crucial role that CAR and PXR play in bile acid detoxification, and among the different receptors, PXR is considered to be the predominant regulator of CYP3A4 expression. In human hepatic cells, activation of PXR and CAR increases the expression of CYP3A4 (reviewed in ref 33). In addition to hydroxylation, PXR ligands also activate the expression of the bile acid sulfating and glucuronidating SULT2A1 and UGT1A3 enzymes in hepatic cells.[35−37] Despite the lack of direct evidence, CAR may also be an inducer of the UGT1A3-dependent glucuronidation of bile acids. Indeed, CAR is a strong inducer of the bilirubin conjugating UGT1A1 enzyme,[38,39] an effect mediated by CAR binding to a phenobarbital response element within the UGT1A1 gene promoter.[40] Since this response element is conserved among human *UGT1A* gene promoters, including UGT1A3, it was suggested that CAR activation may also induce UGT1A3 expression and activity.[41]

The regulation of bile acid metabolic enzymes was further confirmed in transgenic animal models expressing the human PXR receptor. Indeed, transgenic mice expressing human PXR or CAR were completely resistant to LCA-induced liver damage in contrast to wild type animals.[33,42,43] These anticholestatic effects are associated with an increased expression of *Cyp3a11*, *sult2a9*, and *Ugt1a* genes in PXR transgenic animals and of sult2a9, but not Cyp3a11, in animals expressing the human CAR.[33,35,38,42,43] Interestingly, *PXR* gene disruption was accompanied by an increased *Cyp3a11* gene expression at the basal level,[32,42,44] but prevented the pregnenolone-16α-carbonitrile (PCN) induction of Cyp3a11,[32,42] and the hepatic sensitivity of PXR-null animals to LCA was not significantly different from that of wild type mice.[33] As for Cyp3a11, the hepatic concentration of SULT2A mRNA was strikingly elevated in PXR-null animals,[45] whereas Sonoda et al. reported that PCN treatment failed to activate sult2a9 expression in these animals.[35] This suggests that, in the absence of activators, rodent PXR represses Cyp3A and Sult enzyme expression, while becoming an inducer when ligand-activated.

All these observations indicate that PXR and CAR activation induces multiple LCA detoxifying enzymes (Figure 2) and provides strong protection against LCA toxicity during cholestasis. On the other hand, the cholestatic response of CAR-null animals is less clear, with one study reporting no effect of the gene depletion[46] and another indicating that CAR depletion resulted in increased sensitivity to bile acid-induced cholestasis.[47] Nevertheless, the double PXR- and CAR-null mice clearly showed massive liver damage and lower activation of metabolizing enzymes such as Cyp3a11.[46,47] Overall these in vivo models establish the coordinate manner in which PXR and CAR prevent bile acid toxicity, and designate these receptors as drug targets for the treatment of cholestasis. However, all these animal models exhibit some limits to further understand the role of these xenobiotic sensors in the regulation of bile acid metabolism. Indeed,

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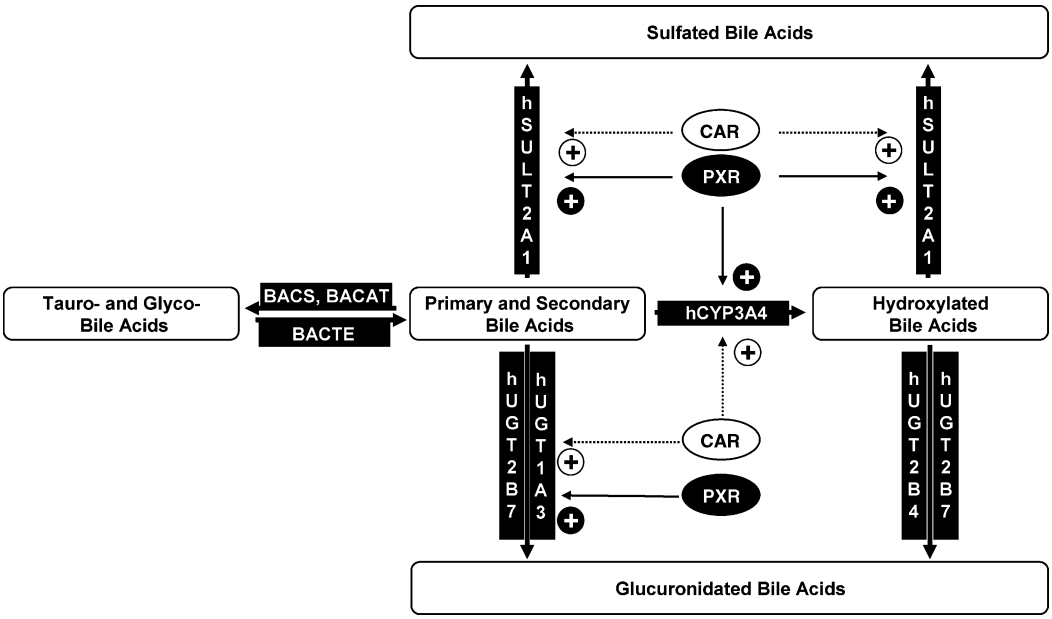

Figure 2. PXR and CAR coordinately regulate hepatic bile acid oxidation and phase II conjugation in humans. In human liver PXR activation induces the expression of CYP3A4, SULT2A1, and UGT1A3 enzymes, whereas CAR stimulates *UGT1A3, CYP3A4*, and *SULT2A1* gene expression. Plain and dashed arrows discriminate between effects of PXR and CAR agonists, respectively. BACAT: bile acid-CoA:amino acid *N*-acetyltransferase. BACS: bile acid CoA synthetase. BACTE: bile acid-CoA thioesterase. CAR: constitutive androstane receptor. hCYP3A4: human cytochrome P450 3A4. PXR: pregnane X receptor. hSULT2A1: human sulfotransferase 2A1. hUGT2B4, -2B7, -1A3: human UDP-glucuronosyltransferase 2B4, 2B7, 1A3.

bile acid glucuronidation is almost absent in rodents,[48] where additional hydroxylation is a preferred metabolic pathway.[49] Furthermore, the true orthologues of human UGT2B4 and UGT2B7 genes in rodents are undetermined, while the murine *Ugt1a3* gene contains a premature stop codon which prevents the formation of a functional protein.[50] For instance, the in vivo relevance of UGT regulation by PXR or CAR activators, in terms of bile acid glucuronidation, cannot be established using classical animal models. Nevertheless, the recent generation of humanized mice expressing the entire human UGT1A locus provides a relevant model for such studies.[48] It will therefore be of interest to investigate the resistance of such animals to toxic bile acids, and to generate transgenic animals expressing both human UGT and nuclear receptors to investigate the in vivo consequences of the regulation of bile acid conjugating UGT enzymes by these ligand-activated transcription factors.

FXR and LXR Regulate Bile Acid Metabolism in an Opposite Manner. LXRs (NR1H2,3) and FXR (NR1H4) are intracellular sensors for sterols and bile acids, respectively. LXRs are activated by physiological levels of oxysterols, such as 24(*S*)-hydroxycholesterol and 24,25-epoxycholesterol, whereas FXR ligands are the primary bile acids CDCA and CA, and their tauro-conjugates (reviewed in ref 12). Consistent with their role as lipid sensors, FXR and LXRs are highly expressed in enterohepatic tissues, where they exert opposite actions on the same target gene.[12] By functioning in a coordinate manner, these two nuclear receptors allow the maintenance cholesterol, carbohydrate, and bile acid homeostasis.[12] Numerous studies established FXR as a bile acid sensor which regulates a network of genes encoding enzymes involved in the synthesis, metabolism, or transport of bile acids (reviewed in ref 12). While inducing bile acid exporting transporters in hepatocytes, FXR reduces the expression of the rate-limiting bile acid synthesizing enzyme, CYP7A1 (reviewed in ref 2). This regulatory process involves, at least in part, the FXR-dependent induction of the small heterodimer partner (SHP), a receptor which forms inactive complexes with the liver receptor homologue 1 (LRH-1), an orphan nuclear receptor that is known to positively regulate *CYP7A1* expression (reviewed in ref 1). FXR also controls the expression of almost all genes involved in bile acid oxidation and/or conjugation. In rats, the high-affinity synthetic FXR ligand GW4064 resulted in an increased expression of BACS and BACAT enzymes,[51] whereas functional FXR response elements were identified

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in the corresponding human genes.[51] The role of FXR in the bile acid dependent induction of hydroxylation is less well understood. In human hepatoma HepG2 cells, treatment with the synthetic FXR agonist GW4064 induced CYP3A4 mRNA levels.[52] In contrast, disruption of the endogenous *FXR* gene in mice resulted in increased Cyp3a11 expression,[53,54] in higher hepatic LCA 6α-hydroxylation (but reduced 6β-hydroxylation),[45] and in elevated urinary excretion of hydroxylated bile acids when animals were under cholestatic conditions[54] or fed with a CA-enriched diet.[44] These observations suggest that FXR acts as a negative regulator of basal Cyp3a11 expression in mice. However, GW4064 is able to further enhance hepatic Cyp3a11 levels in wild type and PXR-null, but not in FXR-null animals, indicating that FXR may even participate in the bile acid induced *Cyp3a11* gene activation.[52] It was postulated that the increased expression of Cyp3a11 observed in FXR-null animals reflects the disrupted bile acid transport system and the accumulation of cholic acid that occurs in these animals.[52,54,55] CA may in turn activate the *Cyp3a11* gene in a PXR-dependent manner,[52] an effect which is further increased in mice fed with CA. Such a compensatory mechanism is supported by the observation that the double knock-out PXR−FXR-null mice are unresponsive to a LCA-enriched diet in terms of Cyp3a11 induction.[44] Overall, these observations indicate that FXR, as well as PXR, contributes to a complex mechanism that allows the feedforward detoxification of bile acid through hydroxylation (Figures 2 and 3).

The role of FXR in the regulation of bile acid sulfonation is also unclear. Identification of a functional FXR response element within the human *SULT2A1* gene promoter initially suggested that FXR also stimulates bile acid sulfonation in human liver.[56] However, a recent study reports that CDCA failed to induce SULT2A1 expression in human hepatocytes.[57] Furthermore, disruption of the *FXR* gene in mice resulted in increased hepatic levels of SULT mRNAs and proteins, as well as in elevated bile concentrations of 3α-sulfated bile acids in animals fed with a LCA-enriched diet.[45] These observations suggest that FXR functions as a repressor rather than an inducer of sulfonation, at least in rodents.

On the other hand, FXR is also a regulator of bile acid glucuronidation.[58,59] Interestingly, FXR regulates bile acid conjugating UGT enzymes, in a tissue- and isoform-specific manner. Indeed, treatment of human hepatic cells with FXR activators increases the expression and activity of the 6α-hydroxylated bile acid glucuronidating UGT2B4 enzyme,[59] without affecting the expression of UGT1A3, an enzyme which glucuronidates LCA and CDCA at their 24-carboxyl position.[28,29] By contrast, in colon carcinoma Caco2 cells, LCA-activated FXR was identified as a negative regulator of the *UGT2B7* gene, which encodes an isoform involved in the formation of 3-hydroxy-glucuronidated bile acids.[58]

These findings indicate that FXR prevents bile acid induced toxicity in liver through inducing the formation of 6α-hydroxylated bile acids and their subsequent glucuronidation without affecting the glucuronidation of hydrophobic bile acids (Figure 3). Interestingly, LXR activation results in the opposite effect. Indeed, Handschin et al.[60] previously suggested that LXR negatively regulates human *CYP3A4* gene expression by competing with PXR for binding to the same response element. In accordance with such a negative regulation, Cyp3a11 expression was reported as increased in LXRα/β-null animals.[61] Furthermore, we recently observed that LXRα activators fail to modulate the expression of the UGT2B4 enzyme, while stimulating the UGT1A3-dependent glucuronidation of CDCA and LCA.[62] These observations imply that, in the presence of FXR activators, 6α-hydroxylation of bile acids and subsequent hepatic

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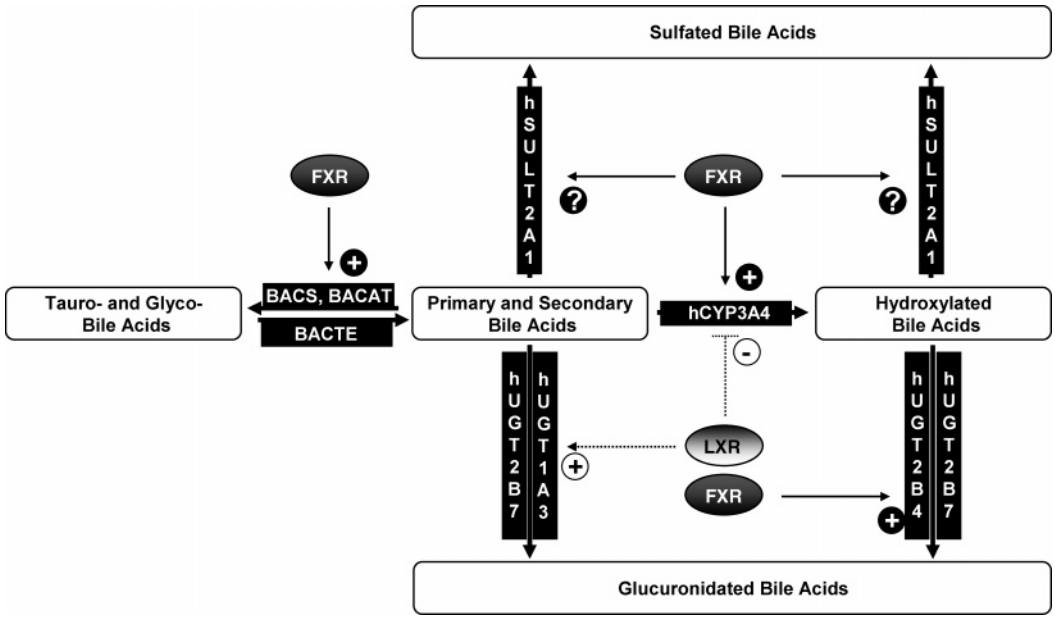

Figure 3. FXR and LXR control hepatic bile acid oxidation, amidation, and glucuronidation. Upon ligand activation, FXR positively regulates the hepatic expression of BACS, BACAT, CYP3A4, and UGT2B4 enzymes, whereas its role in the control of the bile acid sulfotransferase, SULT2A1, is unclear. By contrast, LXR negatively interferes with the PXR-dependent activation of the *CYP3A4* gene promoter, while inducing the expression of the UGT1A3 enzyme. A potential role of LXR on the SULT2A1-dependent sulfonation of bile acids has never been reported. In the intestine FXR negatively regulates UGT2B7 expression.[58] Plain and dashed arrows discriminate between effects of FXR and LXR agonists, respectively. BACAT: bile acid-CoA:amino acid *N*-acetyltransferase. BACS: bile acid CoA synthetase. BACTE: bile acid-CoA thioesterase. hCYP3A4: human cytochrome P450 3A4. FXR: farnesoid X receptor. LXR: liver X receptor. hSULT2A1: human sulfotransferase 2A1. hUGT2B4, -2B7, -1A3: human UDP-glucuronosyltransferase 2B4, 2B7, 1A3.

glucuronidation may be enhanced, whereas LXRα activators may favor glucuronidation of the primary and secondary bile acids instead of hydroxylation (Figure 3).

VDR, the Third Bile Acid Sensor, Also Controls Bile Acid Hydroxylation and Sulfonation. VDR (NR1I1) is a receptor for the active form of vitamin D, the 1α,25-dihydroxyvitamin D3, which regulates calcium homeostasis and bone metabolism. Interestingly, recent findings indicate that VDR is strongly activated by LCA, its 3- and 6-keto and glyco-metabolites.[63] In colon cancer cells, VDR was identified as a strong inducer of CYP3A4,[61] and since LCA possesses carcinogenic activities in this tissue, it is believe that the VDR-mediated induction of CYP3A4 expression by LCA is part of a self-protection mechanism against colon cancer.[63,64] However, VDR was recently reported as an inducer of the hepatic expression of bile acid hydroxylation and sulfonation in hepatic HepG2 cells,[26,56,65] suggesting that VDR also serves as an additional feedforward mechanism to protect the liver against cholestatic disorders (Figure 4).

PPARα Regulates Bile Acid Amidation, Sulfonation, and Glucuronidation. The PPAR subfamily consists of three distinct subtypes termed PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3) which display tissue-selective expression patterns reflecting their biological functions.[66] PPARα is expressed preferentially in tissues where fatty acids are catabolized, such as the liver (reviewed in ref 67). Natural eicosanoids derived from arachidonic acid and the hypolipidemic fibrates activate PPARα.[68] Solaas et

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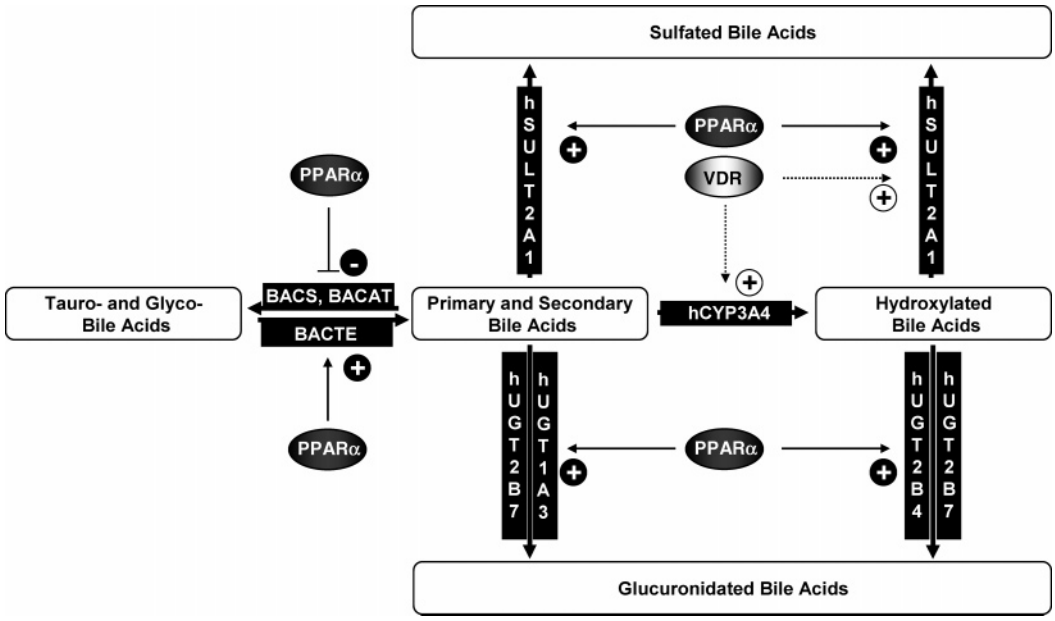

Figure 4. PPARα and VDR are also key controllers of bile acid metabolism in the liver. In mice, PPARα activation reduces the hepatic expression of the BACS and BACAT enzymes, while activating the *BACTE* gene. In human liver, PPARα ligands induce the expression of SULT2A1 and UGT2B4. VDR is also a strong activator of bile acid sulfonation and hydroxylation. Plain and dashed arrows discriminate between effects of PPARα and VDR agonists, respectively. BACAT: bile acid-CoA:amino acid *N*-acetyltransferase. BACS: bile acid CoA synthetase. BACTE: bile acid-CoA thioesterase. hCYP3A4: human cytochrome P450 3A4. PPARα: peroxisome proliferator activated receptor alpha. hSULT2A1: human sulfotransferase 2A1. hUGT2B4, -2B7, -1A3: human UDP-glucuronosyltransferase 2B4, 2B7, 1A3. VDR: vitamin D receptor.

al. recently documented that PPARα activation results in a reduction of taurine-conjugated bile salt synthesis.[15] Using wild type and PPARα-null mice, they demonstrated that PPARα activation induced the hepatic synthesis and activity of BACTE and reduced the activity of the peroxisomal BACAT enzyme in a PPARα-dependent manner.[15] These results suggest that, in mice, PPARα activation decreases the synthesis of taurine-conjugated bile acids. PPARα positively regulates the hepatic expression and activity of the SULT2A1 and UGT2B4 enzymes.[57,69] Overall, these observations suggest that, in cholestatic patients, PPARα activators may detoxify bile acids by stimulating their phase II conjugation (Figure 4). This is of particular interest since fibrates are currently investigated for beneficial effects in the treatment of PBC, and recent studies demonstrated that these drugs are significantly effective in improving liver function in patients with asymptomatic and symptomatic PBC.[70−73]

Other Receptors Involved in the Maintenance of Bile Acid Homeostasis. In addition to the receptors discussed above, various transcription factors also exert important roles in bile acid homeostasis. Among them, the hepatocyte nuclear factor (HNF)4α (NR2A1) is a liver-enriched nuclear receptor that plays a major role in hepatocyte differentiation and maintenance (reviewed in ref 74). In contrast to the above discussed receptors, HNF4α functions as a homodimer.[74] In addition to being strongly associated to the control of glucose homeostasis, HNF4α recently emerged as a key regulator of bile acid synthesis (reviewed in ref 74). HNF4α directly regulates the murine orthologues of the BACS and BACAT enzymes, since HNF4α-null mice exhibit markedly decreased expression of these enzymes.[75] Furthermore, HNF4α is a critical regulator of both basal and drug-induced CYP3A4 expression, as illustrated by the dose-dependent reduction of CYP3A4 in human hepatocytes silenced for HNF4α

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expression.[76] Not only does HNF4α activate FXR, PXR, and RXRα gene expression,[77] but also it is a major component of the transcriptional machinery recruited by ligand-activated PXR, CAR, and LXRα.[10,78,79] Indeed, a recent study revealed that HNF4α is recruited in the PXR-activated regulatory complex to the *CYP3A4* gene promoter.[80] Interestingly, the formation of such a complex is inhibited by SHP.[80] SHP is an unusual receptor which does not exhibit DNA binding activity, but forms inactive heterodimers with other nuclear receptors, including LRH-1, CAR, PXR, LXRα, and HNF4α (reviewed in ref 10). SHP is thus susceptible to repress the ligand-dependent activation of these receptors' target genes, including those encoding bile acid synthesizing and metabolizing enzymes.

Clinical Significance and Perspectives. All the in vitro and in vivo studies discussed in the above sections reported that bile acid oxidation and conjugation are tightly regulated in order to reduce the toxicity of bile acids. In addition, most of these receptors also modulate bile acid synthesis and transport, two major aspects of bile acid maintenance discussed elsewhere.[1,2,9,10,12,74] These studies provide a strong understanding of the molecular mechanisms involved in the control of bile acid homeostasis, and identify ligand-activated nuclear receptors as promising drug targets for the correction of bile acid level abnormalities associated with liver disorders. Indeed, PXR and CAR activators are already used clinically for challenging cholestatic patients. The best example regards the PXR ligand rifampicin, which is an efficient treatment of pruritus in cholestatic patients.[81] Rifampicin treatment increases the urinary excretion of 6α-hydroxylated bile acids and their glucuronide conjugates in healthy subjects,[82] and stimulates CYP3A4 and MRP2 expression in patients with symptomatic cholesterol gallstones.[83] By contrast, rifampicin treatment fails to modulate UGT2B4, UGT2B7, SULT2A1, and BACAT expression in these patients.[83] UDCA, which is one of the most effective drugs for the treatment of cholestatic patients,[81] was recently reported as an activator of PXR and was efficient to activate the *CYP3A4* gene promoter.[53] However, a recent study reported that UDCA fails to induce CYP3A enzyme activity in normal subjects and early-stage PBC patients,[84] thus suggesting that CYP3A4 is not induced by UDCA in humans. On the other hand, patients treated with phenobarbital, a CAR activator, exhibited elevated plasma levels of 4β-hydroxycholesterol, a cholesterol metabolite formed by CYP3A4.[4] These observations support the potential of PXR and CAR ligands as therapeutic stimulators of bile acid metabolism in cholestatic patients.

By contrast, the clinical efficiency of other receptor ligands for reducing bile acid toxicity remains to be established. In fact, direct pharmaceutical targeting of multiple but complementary nuclear receptors, so-called "nuclear receptor therapy", might be a novel option in treatment of cholestatic diseases.[85] Novel approaches may be aimed at designing dual PXR/VDR agonists to induce several pathways of bile acid metabolism (oxidation, conjugation, transport).[86] Similarly, the use of combination of rifampicin and FXR activators (6-ethyl chenodeoxycholic acid, 6ECDCA or GW4064) might reduce or even normalize bile acid concentration, leading to curable cholestasis.[87] It might be of concern that nuclear receptors also regulate other metabolic processes and nonspecific stimulation could have unanticipated deleterious effects in human therapy. Indeed, dual targeting of PXR and CAR in mice increased serum alanine aminotransferase levels additionally compared to the effects of bile duct ligation.[88] The interspecies differences in activation patterns of nuclear receptors make direct extrapolation of these observations

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extremely difficult, and the relevance of this effect in humans remains to be established.

On the other hand, the mechanisms of action of FXR, which were described in detail in the previous sections as well as in other recent reviews,[1,10,12,89] seem to make this particular nuclear receptor an attractive target for the treatment of chronic cholestatic conditions or cholesterol gallstones. Liu et al.[90] have used GW4064 in two animal models of liver injury: the α-naphthylisothiocyanate (ANIT) induced intrahepatic cholestasis and bile duct ligation induced extrahepatic cholestasis. In both models, pretreatment with GW4064 caused a significant improvement of various markers of hepatic inflammation and cholestasis. The significance of these findings, particularly in ANIT-induced liver damage, has been questioned and should be interpreted with caution since this agent causes a widespread liver necrosis; thus this model does not adequately reflect chronic cholestasis in humans.[89] Nevertheless these findings may imply a potential hepatoprotective role of FXR agonists in liver damage caused by other agents which induce liver necrosis such as paracetamol. More recently, Fiorucci et al.[91] have analyzed the potential anticholestatic properties of another FXR agonist, 6-ECDCA, in 17α-ethynylestradiol (E(2)17alpha) induced cholestasis in rats. 6-ECDCA was found to cause a restoration of bile flow in this model of cholestasis by stimulating the expression of bile acid transporters, and by further enhancing the E(2)17alpha-dependent induction of SHP expression[92] and the subsequent reduction of CYP7A1 mRNA levels.[91] CDCA was used for the treatment of cholesterol gallstones many years before the discovery of FXR. Despite its reasonable efficacy it was eventually withdrawn from the market because of toxicity.[93] Certainly, its effectiveness lends further support to the idea of treating cholesterol gallstones (CGS) with FXR agonists. No effective pharmacological treatment of CGS exists; thus pharmacological agents facilitating the dissolution of cholesterol gallstones would be very welcome. Increased canalicular secretion of cholesterol is considered to be a primary event triggering the formation of cholesterol gallstones when its concentration exceeds the bile's capacity to solubilize it within micellae. FXR agonists which facilitate bile flow, decrease the synthesis of bile acids, and stimulate their elimination may play an important role in restoring a biliary balance between bile salts, phospholipids, and cholesterol. Moschetta et al. have recently shown that FXR−/− mice fed a lithogenic diet are more prone to form cholesterol crystals,[94] an effect that was counteracted by concomitant administration of GW4064 in wild type animals.

Conclusion

Recent advances in the understanding of the regulatory processes of the enzymes at the basis of bile acid elimination evidenced the potential of xenobiotic- and lipid-activated nuclear receptors as promising drug targets for bile acid detoxification. Therefore these NR agonists have the potential to increase the very limited armamentarium of pharmacological agents which are beneficial in the management of chronic cholestatic conditions. Hopefully, further work will help to better characterize these agents, their potential side effects, and their usefulness in contemporary hepatology.

Abbreviations Used

ANIT, α-naphthylisothiocyanate; BACAT, bile acid-CoA: amino acid *N*-acetyltransferase; BACS, bile acid CoA synthetase; BACTE, bile acid-CoA thioesterase; CA, cholic acid; CAR, constitutive androstane receptor; CDCA, chenodeoxycholic acid; CYP, cytochrome P450; DCA, deoxycholic acid; 6-ECDCA, 6-ethyl chenodeoxycholic acid; FXR, farnesoid X receptor; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; HNF, hepatocyte nuclear factor; LCA, lithocholic acid; LRH-1, liver receptor homologue-1; LXR, liver X receptor; NR, nuclear receptor; PCN, pregnenolone-16α-carbonitrile; PPAR, peroxisome proliferator activated receptor; PXR, pregnane X receptor; RE, response element; RXR, retinoid X receptor; SHP, small heterodimer partner; SULT, sulfotransferase; UDCA, ursodeoxycholic acid; UGT, UDP-glucuronosyltransferase; VDR, vitamin D receptor.

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