The role of the enterohepatic circulation of bile salts and nuclear hormone receptors in the regulation of cholesterol homeostasis: Bile salts as ligands for nuclear hormone receptors

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The coordinated effect of lipid activated nuclear hormone receptors; liver X receptor (LXR), bound by oxysterol ligands and farnesoid X receptor (FXR), bound by bile acid ligands, act as genetic transcription factors to cause feed-forward cholesterol catabolism to bile acids and feedback repression of bile acid synthesis, respectively. It is the coordinated action of LXR and FXR, each dimerized to retinoid X receptor, that signal nuclear DNA response elements to encode proteins that prevent excessive cholesterol accumulation and bile salt toxicity, respectively. LXR helps prevent hypercholesterolemia by enhancing transporters for cholesterol efflux that enhance reverse cholesterol transport, while FXR enhances intestinal reabsorption and preservation of bile salts by increasing the ileal bile acid binding protein. FXR also targets sodium taurocholate cotransport peptide and bile salt export pump (protein) genes to limit bile salt uptake and enhance export, respectively, which prevents bile salt toxicity. Other nuclear hormone receptors such as pregnan X receptor, which share the obligate partner, retinoid X receptor, and vitamin D receptor also function as bile acid sensors to signal detoxification by hydroxylation of toxic bile acids. Pharmacologically targeted receptor agonists (or antagonists) may be developed that alter cholesterol and bile salt concentrations by modulating nuclear hormone receptors and/or their coactivators or corepressors to positively affect cholesterol homeostasis and bile salt metabolism. It is the coordinated transcription factor action of LXR, which responds to ligand binding of circulating oxysterols in both liver and peripheral tissues, and FXR responding to bile salts within the enterohepatic circulation that make possible the regulation of cholesterol and bile acid homeostasis.

Key Words: *Bile acid; Cholesterol homeostasis; Nuclear hormone receptors*

Le rôle de la circulation entérohépatique des sels biliaires et des récepteurs de l'hormone nucléaire dans la régulation de l'homéostasie cholestérolique : Les sels biliaires comme ligands des récepteurs de l'hormone nucléaire

L'effet coordonné des récepteurs nucléaires de l'hormone activée par les lipides, du récepteur X hépatique (RXH), lié par les ligands d'oxystérol et le récepteur X famésoïde (RXF), liés par les ligands acidobiliaires, agit comme des facteurs de transcription génétique afin de provoquer, respectivement, un catabolisme cholestérolique non récurrent des sels biliaires et une répression à rétroaction de la synthèse de l'acide biliaire. C'est l'action coordonnée du RXH et du RXF, chacun étant dimérisé au récepteur X rétinoïde, qui signale aux éléments de réponse de l'ADN nucléaire d'encoder les protéines qui, respectivement, empêchent l'accumulation cholestérolique excessive et la toxicité des sels biliaires. Le RXH contribue à prévenir l'hypercholestérolémie en accentuant l'efflux cholestérolique des transporteurs, qui accroît le transport cholestérolique inversé, tandis que le RXF favorise la réabsorption intestinale et la préservation des sels biliaires en augmentant la liaison de la protéine à l'acide biliaire iléal. Le RXF cible également le peptide de cotransport du taurocholate sodique et les gènes de la pompe (à protéines) d'exportation des sels biliaires, respectivement, lesquels empêchent la toxicité des sels biliaires. D'autres récepteurs nucléaires de l'hormone, tels que le récepteur X pregnan, qui partage le partenaire strict, le récepteur X rétinoïde, et le récepteur de la vitamine D, fonctionnent également comme des senseurs de l'acide biliaire afin de signaler la détoxification par l'hydroxylation des acides biliaires toxiques. Des agonistes (ou antagonistes) récepteurs ciblés par la pharmacologie peuvent être mis au point pour altérer les concentrations de cholestérol et de sels biliaires en modulant les récepteurs de l'hormone nucléaire, leurs coactivateurs ou leurs corépresseurs à avoir un effet positif sur l'homéostasie cholestérolique et le métabolisme des sels biliaires. C'est l'action coordonnée des facteurs de transcription du RXH qui réagit à la liaison ligand des oxystérols circulants tant dans le foie que dans les tissus périphériques, et du RXF qui réagit aux sels biliaires dans la circulation entérohépatique qui rend possible la régulation du cholestérol et l'homéostasie de l'acide biliaire.

It is well known that bile salts (note: bile acids become bile salts at physiological cellular pH=7. In the present review, the term bile acid is used to denote their state at the time of synthesis while the designation bile salt is used to reflect their chemical state in body solutions as sodium and potassium salts of bile acids) have major biological effects related to their ability to solubilize cholesterol in bile and in the intestine, as well as other lipids such as monoglycerides, fatty acids and fat-soluble vitamins for luminal digestion and consequent intestinal absorption (1). The physiochemical mechanisms underlying these actions were worked out by seminal studies on biliary lipids and intraluminal intestinal digestion, thus providing a better understanding of the pathogenesis of gallstone disease (2) and luminal gut lipid malabsorption (3). However, the

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role of nuclear hormone receptors within the enterohepatic circulation of bile salts has not been fully appreciated, which relates to the molecular regulation of bile salt and cholesterol homeostasis that prevents intrahepatic bile salt toxicity and abnormal accumulation of cholesterol or their esters, respectively. Recently, through major advances in research relating to molecular signaling by ligand binding to nuclear hormone receptors, it is now apparent that bile salts themselves act as ligands to nuclear receptors and thereby play an increasingly important role in preserving cholesterol homeostasis in mammals, including man (4-8). The present review is intended to help the clinical gastroenterologist/hepatologist understand the role of the molecular regulation of bile salts and cholesterol homeostasis both within and complementary to the enterohepatic circulation of bile salts. Due to the limited scope of the present review and space limitations, additional reviews are listed in the bibliography for details of molecular structure and other nuclear hormone receptors that control other aspects of lipid metabolism (9-11).

EVOLUTIONARY IMPERATIVES FOR CHOLESTEROL METABOLISM

The need for bile salts in human evolution traces back to the need for cells to synthesize cholesterol as well as to control its excessive accumulation by metabolic degradation. As life progressed from non-nucleated prokaryotes to eurokaryotes, with the development of multiorganelle structures involving complex membrane functions, cholesterol was required as an essential membrane constituent and substrate for hormone synthesis. However, cellular control of sterol metabolism was necessary and required nuclear (genetic) regulation of diverse proteins functioning as enzymes, transporters and intracellular protein binders that regulate cholesterol metabolism. Nuclear receptors therefore evolved to orchestrate cholesterol homeostasis including its catabolism to bile acids (5,6,12,13). In addition, to the problem that cells had with degradation of the cholesterol sterol ring, they also had to cope with excessive cholesterol availability. For example, enhanced dietary cholesterol that became available for carnivorous mammals placed additional stresses on their cells so that sophisticated cellular adaptations were necessary to protect against excessive cholesterol accumulation.

CELLULAR ADAPTATIONS DURING EVOLUTIONARY DEVELOPMENT

Because eukaryotic cells could not degrade the cholesterol sterol ring, they developed at least 14 multistep enzymatic reactions to degrade cholesterol to bile acids to maintain normal intracellular cholesterol levels within narrowly defined physiological levels (14). To create cholesterol homeostasis (ie, to balance cholesterol input with output), cholesterol had to be catabolized to bile acids by both multiple hydroxylation steps and side chain shortening to accomplish the conversion of 27 carbon cholesterol molecules to 24 carbon bile acid molecules (14). This catabolism accounts for 50% of the conversion of cholesterol derived from dietary input, intestinal absorption, storage and synthesis. Because relatively small amounts of cholesterol are converted to hormones (13), the remainder of cholesterol output, apart from its catabolism to bile acids, occurs in large part by fecal elimination of unabsorbed dietary, biliary and effluxed cellular cholesterol to maintain cholesterol homeostasis. Bile acids, the catabolic end product of cholesterol, are much more hydrophilic than cholesterol and in fact act as detergents by forming mixed micelles with cholesterol and other lipids for their transport to and absorption from the intestine (2). Bile salts themselves can become toxic at high levels in cells, so that their intracellular levels must also be regulated in addition to that of cholesterol (15).

Insight into the physiological complexity contributing to the regulation of bile salt metabolism first became apparent with the description of an enterohepatic circulation of bile salts, which anatomically defined the movement of bile salts by following their synthesis as bile acids in the liver; secretion as bile salts into the intestine by the biliary tract; their avid reabsorption in the distal ileum; and consequent transport back to the liver in portal blood (16). More than 95% of bile salt secretion is thereby preserved with the body, so that less than 5% of the bile salt pool is lost by fecal elimination. Excessive bile salt loss also must be avoided, because bile salts have colonic effects on cyclic adenosine monophosphate-induced water secretion and will produce secretory diarrhea if lost excessively into the colon (ie, bile salt enteropathy) (17). This fine-tuned circulation of the bile salt pool of two to four grams, which cycles several times a meal and a total of 10 or more times daily, produces a total effective feed-forward secretion of 30 grams of bile salt per day. Thus, an economy of the enterohepatic circulation of bile salts is necessary for preservation of the bile salt pool (18). For this economy to be realized for the maintenance of a constant bile salt pool, feedback control of bile acid synthesis is also necessary to limit hepatic synthesis only to those bile salts that are lost via fecal elimination (Figure 1) (16). Early physiological experiments carried out by Redinger et al (16,18,19) revealed feedback regulation of synthesis including that of individual bile acids in primate models. Enzymatic regulation of cholesterol 7-alpha hydroxylase (CYP7A1) synthesis by bile acids was first described by Mosbach's laboratory in 1970 (20). However, the details of molecular nuclear regulation were only recently determined from more recent elegant studies that have revealed the molecular controls of enzyme regulation of feed-forward activation (12) and feedback repression of bile acid synthesis (21) that regulates cholesterol homeostasis.

MOLECULAR CONTROL OF CHOLESTEROL HOMEOSTASIS

The first descriptions of molecular regulation of intracellular cholesterol levels came from the laboratory of Brown and Goldstein (22), who found that membrane bound proteins called sterol regulating element binding proteins residing in the nuclear envelope and endoplasmic reticulum were activated by declining levels of cholesterol or their metabolites (ie, oxysterols), so that by cleavage of these sterol regulating element binding proteins, transcription factors were made available to target the nucleus and transactivate sterol responsive genes (22,23). These genes in turn encoded key enzymes including but not limited to 3-hydroxy-3-methylglutaryl coenzyme A reductase to enhance cholesterol synthesis. The second major description of transcription regulators of cholesterol homeostasis occurred with the discovery that nuclear hormone receptors acting as transcription factors regulate cholesterol metabolism in an opposing fashion. These receptors exist within a superfamily of ligand-activated nuclear hormone transcription factors that regulate enzymatic expression of cellular

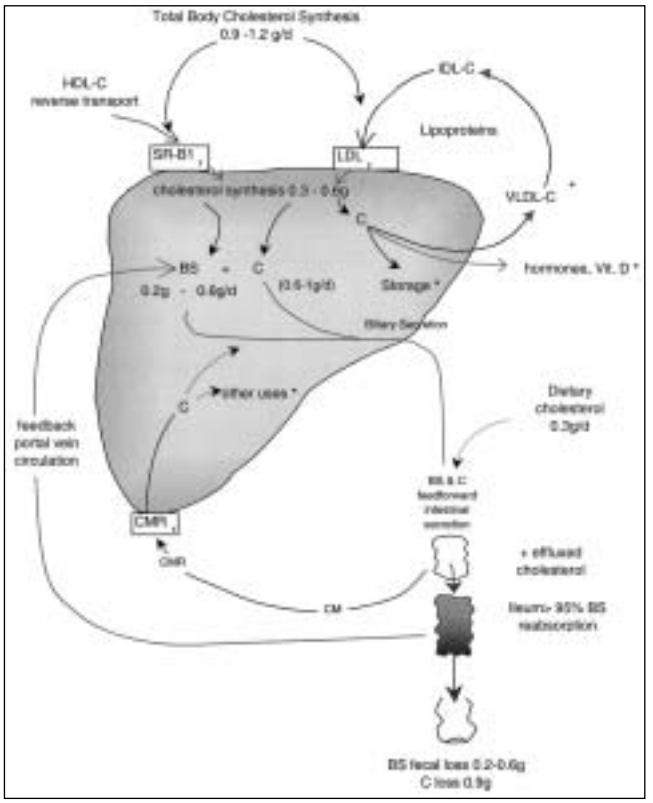

Figure 1) *Cholesterol (C) synthesis and its relationship to the circulation of bile salts (BS) is shown. Hepatic C is derived from newly synthesized cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL), diet via chylomicron remnants, and storage sources. The enterohepatic circulation of BS begins with feed-forward BS secretion after catabolism of hepatic cholesterol to bile acids. BS fecal losses equal bile acid synthesis because of avid bile acid ileal reabsorption and feedback repression of bile acid synthesis. Fecal C losses are the result of unabsorbed biliary and dietary intestinal C and effluxed C from enterocytes to maintain C balance/homeostasis. Boxed symbols, ie, [LDLr]* refer to the LDL receptor*, HDL scavenger receptor (SR)-B1, and chylomicron remnant (CMR) receptor in the liver for uptake of C by the liver. For C balance/homeostasis, C input (synthesis and diet) must equal C output (fecal BS and C losses). IDL Intermediate density lipoprotein; Vit D Vitamin D; VLDL Very low density lipoprotein*

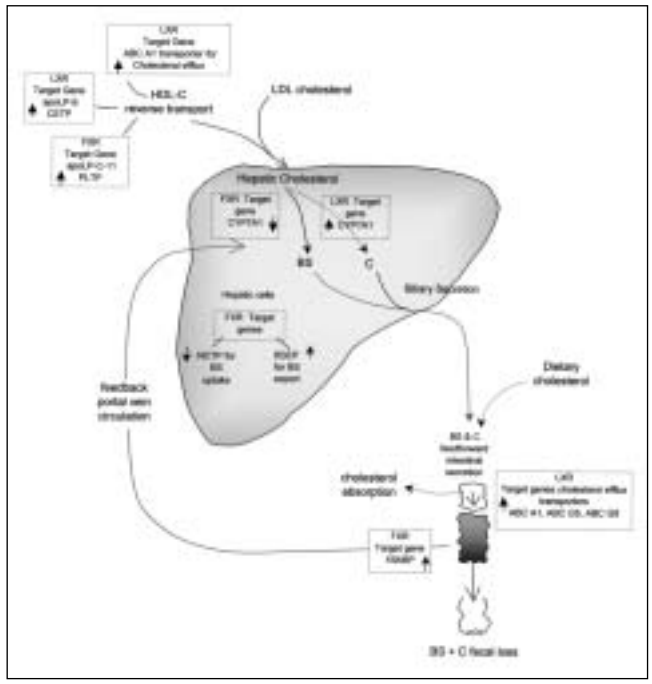

Figure 2) *The nuclear hormone receptor, liver X receptor (LXR), activates the target gene cytochrome P450 cholesterol 7-alpha hydroxylase (CYP7A1) to increase bile salt synthesis from cholesterol (C). It increases the target genes for the ATP-binding cassette protein (ABC)-A1 transporter to increase C efflux from peripheral cells for pickup of C by high density lipoprotein (HDL) and enhance reverse C transport to liver. LXR transactivation of apolipoprotein E and cholesterol ester transfer protein (CETP) genes and farnesoid X receptor (FXR) targeted genes for apolipoprotein C-11 and phospholipid transfer protein (PLTP) further enhance HDL-C transport. LXR also targets genes for enterocyte transporters ABC-G5/G8 to enhance C efflux for fecal C elimination from the intestine. FXR with small heterodimer receptor (SHP) activates target genes to repress CYP7A1 which regulates bile salt (BS) synthesis to levels needed to maintain bile salt pool size, and increases the target gene for ileal bile acid binding protein (IBABP) to enhance ileal bile salt absorption and preserve the bile salt pool. Concomitantly it decreases the hepatic target genes for sodium taurocholate contransport peptide (NTCP) controlling bile acid uptake while increasing that for bile salt export pump (protein) (BSEP) for bile acid export to protect against excessive bile salt toxicity. LDL Low density lipoprotein*

activity controlling many diverse functions such as embryonic development, cell differentiation and lipid homeostasis (24). Ligands or signaling molecules for these receptors are small lipophilic molecules within cells, which cross lipid cell membranes, bind to nuclear receptors, and activate transcriptional messages within nuclear DNA that encode targeted genes (Figure 2). As such, the superfamily consists of phylogenetically related nuclear receptor proteins that share DNA ancestral domain structures common to both early eurokaryotes and advanced organisms including mammals (24). These receptors contain a well-conserved central DNA binding domain that allows classification and a moderately conserved ligand binding domain that allows selective binding of specific ligands to nuclear hormone receptors. These in turn perform as transcription factors to activate DNA encoding of enzymes regulating homeostatic control of lipid metabolism including that of cholesterol homeostasis (Figure 3). The commonality of these receptor DNA domains allows the classification of nuclear receptors into six subfamilies (24). Subfamily I contains most of the nuclear hormone receptors found within and associated with the enterohepatic circulation of bile salts that function to control cholesterol and, even more broadly, many other aspects of lipid metabolism (25). The receptors in this subfamily include liver X receptor (LXR), farnesoid X receptor (FXR), pregnane X receptor (PXR), peroxisome proliferator activated receptor (PPARγ), and vitamin D receptor (VDR) which are further discussed in this review. Subfamily II contains retinoid X receptor (RXR), which dimerizes with and acts as an obligate partner to FXR, LXR and PXR (See Figure 3 for FXR/RXR dimer illustration), while Subfamily III contains steroid receptors for estrogen, androgen and progesterone receptors. Other subfamilies (IV, V and VI) contain orphan receptors, ie, receptors found without known ligands at the time of their discovery, which are also found in many tissues, including some

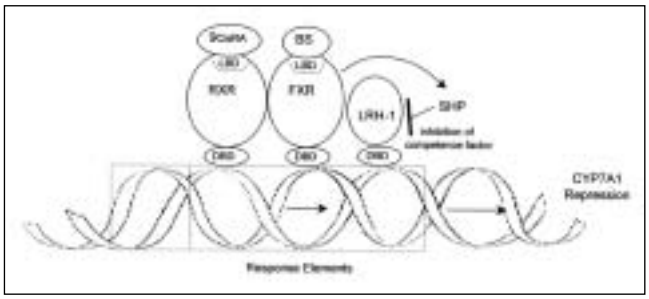

Figure 3) *An example of the heterodimer nuclear hormone receptor farnesoid X receptor (FXR) along with the retinoid X receptor (RXR) obligate partner is shown with their attached bile salt (BS) and 9 cis retinoic acid ligands (9CisRA). Ligand binding domains (LBD) and DNA binding domains (DBD) exist within these receptors, which interact with a nuclear response element with specific DNA motif to initiate transcription activity. Liver receptor homolog-1 (LRH-1), another nuclear receptor is a competence factor of LRH-1 {correct?}. The small heterodimer partner (SHP) is also activated by FXR. SHP without a DNA binding site is unable to bind to the nuclear DNA motif but by inhibiting LRH-1, it indirectly represses cytochrome P450 cholesterol 7-alpha hydroxylase (CYP7A1). Nuclear hormone receptors act as transcription factors and with coordinated receptor coactivators (LRH-1) and corepressors (SHP) encode feedback repression of bile acid synthesis*

within the enterohepatic circulation of bile salts, such as small heterodimer receptor (SHP) (discussed further in this review). Screening strategies had to be developed to identify ligands from known biological compounds for these orphan receptors so as to realize their physiological functions (12). Effective ligands for nuclear hormone receptor activation were consequently found to be small lipophilic hormone-like molecules obtained from tissue extraction or synthetically prepared compounds. As many as 45 nuclear hormone receptors have now been discovered in the human genome, but details of their molecular functions (eg, transcription regulation, coactivation, corepression) are not completely understood (26-28). Receptor agonists that can substitute for these physiological ligands have been found that can activate these transcription factors (5). Similarly, antagonists are being identified that interfere with normal receptor function (29). Table 1 contains a summary and characterization of such known receptor modulators (30-35). Nuclear hormone receptors are also capable of being activated or inhibited by molecules other than their ligands such as components of signal transduction pathways including immunomodulators (ie, inflammatory cytokines, G-proteins and kinases with their phosphorylation pathways) (36). Lipid activated nuclear hormone receptors, their ligands, and target genes involved in cholesterol and bile salt homeostasis have recently been discovered to be abundant in tissues within the territory of the enterohepatic circulation of bile salts (12) (Figures 1 and 2). These exist within the large subfamily that includes the non-steroidal nuclear hormone receptors, LXR and FXR, which are two major receptors involved in the regulation of cholesterol catabolism and elimination (5-7). Oxysterol intermediates of cholesterol catabolism (37) as well as bile salts (38), the final catabolic end products of cholesterol catabolism, have now been identified as ligands for the signal pathways of transcription factor expression and consequent activation of enzyme induction (37) or repression, respectively (38). The sequence of molecular events in these molecular pathways involves ligand binding to a heterodimer receptor complex, eg, FXR/RXR, (Figure 3), which signals a responsive element domain containing a specific DNA motif within nuclear DNA to transactivate transcription messages that encode target genes for the expression of protein enzymes. For further details of additional modifiers of nuclear hormone receptors, see the review by Aranda and Pascual (11).

LXR FUNCTIONS WITHIN THE ENTEROHEPATIC CIRCULATION

Oxysterol ligands, which are oxidized cholesterol metabolites, bind to the LXR, a specialized dimerized receptor that also needs to be complexed to an obligate partner RXR to function. The latter receptor is itself a common obligate partner to other members of Subfamily II noted earlier (24,39). The heterodimer receptor, LXR/RXR, while originally found in the liver, also exists in diverse tissue such as brain, macrophages and scavenger cells. In the liver, following ligand binding, it signals sequential genetic transcription messages that encode the target genes for the expression of CYP7A1. CYP7A1 upregulates bile acid synthesis from cholesterol by feed-forward genetic induction (encoding) of new bile acid synthesis. LXR also targets genes in enterocytes to cause cholesterol efflux by transporter proteins ATP-binding cassette proteins (ABC)-A1, ABC-G5 and G8 (34,35,40,41). In this fashion, additional cholesterol may be eliminated by fecal cholesterol excretion (Figures 1 and 2) (34-37). Mutations of genes encoding ABC-G5 and ABC-G8 transporters result in sitosterolemia as they are unable to efflux plant sterol sitosterol which then accumulates in serum (42).

NUCLEAR HORMONE RECEPTOR FUNCTIONS THAT ARE COMPLEMENTARY TO THE ENTEROHEPATIC CIRCULATION

Targeted gene regulation activated by LXR receptors also exists in peripheral tissues outside that of the enterohepatic circulation of bile salts to cause mobilization of cholesterol for return to the liver and further metabolism of cholesterol (35). Reverse cholesterol transport itself is a consequence of multiple nuclear hormone receptor actions which, as transcription factors, regulate lipid components within lipoproteins for reverse cholesterol transport (35,43). FXR signals enhanced apolipoprotein C-11 transcription (43) and phospholipid transfer protein (44-46), while LXR enhances cholesterol exchange transfer proteins (47), which facilitate cholesterol ester and phospholipid transfer or exchange to high density lipoprotein (HDL) from other lipoproteins for consequent transport of HDL cholesterol back to the liver. Further gene targets of LXR/RXR receptors include the ABC-A1 transporter that results in efflux of cholesterol out of macrophages and fibroblasts (35,40). In these peripheral tissues, effluxed cholesterol is taken up within their vascular beds by small naive HDL_4 particles, which aided by apolipoproteins E (48) and C11 (43), become larger mature HDL_2 particles by virtue of increased phospholipid and cholesterol content from phospholipid transfer protein and cholesterol exchange transfer proteins activity as well as by uptake and esterification of cholesterol by lecithin cholesterol acyl transferase. Furthermore, other nuclear hormones that are involved in lipid metabolism such as PPARγ are also able to enhance the expression of LXR target gene action on ABC-A1 transporters for cholesterol

TABLE 1
Nuclear hormone receptor modulators

Nuclear receptor	Agonist	Mechanism	Antagonist	Reference
LXR – NR1H3				
1. Endogenous ligands	Oxysterols	Ligand binding	Bile salts	4,5,12
		Target Genes:		
	27-OH-Chol	↑ CYP7A1	↓ CYP7A1	
	22-R-OH-Chol	↑ ABC-A1, G5/G8		34,35
	20-S-OH-Chol	↑ CETP		47
	25-S-25-Epoxy-Chol	↑ apo E		5,48
2. Pharmacological	Cholestyramine	↑ CYP7A1 (BS sequestrant)	None identified	5
3. Synthetic (Experimental)		All selective nonsteroidal		33
	GW3965	↑ ABC-A1		34
	T1317 (70901317)	↑ ABC-A1		36
	LG268	Retinoid (↑ ABC-A1)		30,34
	GW4064	↑ BSEP		32
	Acetyl Podocarpic Dimer	↑ ABC-A1		
FXR - NR1H4				
1. Endogenous ligands	Bile acids CDCA>LCA>DCA	Ligand binding		
		Target Genes:		
		↓ CYP7A1, ↑ apo C-11, ↑ PLTP		43,44
		↓ NTCP, ↑ BSEP, ↑ IBABP		54,56,57
2. Pharmacological	Ursodeoxycholic acid	Activates PXR, ↑ CYP3A		30,31
		Replaces hydrophobic BS	Guggelsterone	29
			Pregnanedione	
3. Synthetic (Experimental)		All nonsteroidal		
	GW4064	Isoxazole		
	GW9047	(Benzoic acid derivatives)		
	TTNPB	↓ CYP7A1		30,31,34
	LG268	Rexinoid (↓ CYP7A1)		36

In addition, please see reference 5 for a detailed review of agonist and antagonist actions. ABC A-1,G5/G8 ATP-binding cassette proteins A-1, G5 and G8; BS Bile salt; BSEP Bile salt export pump (protein); CDCA Chenodeoxycholic acid; CETP Cholesterol ester transfer protein; CYP3A Cholesterol 6α hydroxylase; CYP7A1 Cytochrome P450 cholesterol 7-alpha hydroxylase; DCA Deoxycholic acid; FXR Farnesoid X receptor – NR1H4; IBABP Ileal bile acid binding protein; LCA Lithocholic acid; LXR Liver X receptor – NR1H3; NTCP Sodium taurocholate cotransport peptide; PLTP Phospholipid transfer protein; PXR Pregnane X receptor – NR1I2

efflux (25). In this manner, cholesterol esters are transported for return to the liver for uptake through the scavenger receptor B1 and consequent hepatic catabolism to bile acids (Figure 1).

FXR FUNCTIONS WITHIN THE ENTEROHEPATIC CIRCULATION

Bile salt intracellular concentrations also need to be controlled within tissues of the enterohepatic circulation of bile salts to avoid intracellular bile salt toxicity. Another receptor, the FXR, is named after the lipid farnesol, which was its first discovered ligand (5). It is also dimerized to RXR (ie, FXR/RXR), and when activated by natural bile salt ligands initiates feedback control of bile acid synthesis (38,49). Chenodeoxycholic acid, lithocholic acid and deoxycholic acid are the most potent bile salt ligands. FXR, however, requires the assistance of another orphan receptor, the SHP, which is a heterodimer with no DNA binding domain (49). It is speculated that SHP inhibits the competence factor, liver receptor homolog-1, yet another deorphanized nuclear receptor (49-51), which in so doing allows FXR to indirectly repress bile acid synthesis by feedback control of CYP7A1 (Figures 2 and 3). An alternative explanation is that down-regulation of CYP7A1 involves jun N-terminal kinase, which after activation by bile salts, enhances SHP transcription (52). It is hypothesized that SHP, by forming an inhibitory heterodimer complex with liver receptor homolog-1, also autoregulates itself (38,49). Furthermore, dimerized FXR also up-regulates the ileal bile acid binding protein to enhance ileal reabsorption of bile salts (53,54). In this fashion, FXR mediates ileal bile acid binding protein gene expression to increase ileal bile acid absorption thereby preserving the existing bile salt pool as it concomitantly represses hepatic bile acid synthesis (54,55). FXR additionally decreases hepatic bile salt uptake by down-regulating the hepatic cell basolateral sodium taurocholate polypeptide transporter (56). Simultaneously, it up-regulates hepatic bile salt canalicular transport into bile by gene targeted protein induction of the bile salt export pump, thereby enhancing bile salt transport out of the liver (57). In

this fashion, an efficient bile salt pool is preserved while excessive bile salt levels are avoided in hepatocytes, all regulated by coordinated action of the nuclear hormone receptor FXR at liver and intestinal sites of action (51,54-57). Other nuclear hormone receptors such as steroid and xenobiotic receptor/PXR target the gene for induction of cholesterol 6-alpha hydroxylase (CYP3A), which enhances 6-alpha hydroxylation of toxic bile acids such as lithocholic acid (58,59). Hydroxylation increases hydrophilicity resulting in increased urinary and fecal lithocholic acid (LCA) losses (58-60).

SUMMARY AND FUTURE DIRECTIONS

The coordinated effects of nuclear hormone receptors bound by oxysterol ligands signal genetic transcription factors to cause feed-forward control of cholesterol catabolism to bile acids, while bile salts act as ligands for feedback repression of bile acid synthesis. Consequently, intrahepatic toxicity (due to increased bile salt levels) (5,15), abnormal lipoprotein transport (perturbed reverse cholesterol transport) (55), and decreased enterohepatic circulation of bile salts with an inadequate bile acid pool are avoided by coordinated effects of LXR/RXR and FXR/RXR receptors in liver, peripheral tissues and ileum, respectively. Cholestatic liver damage may be relieved by oral administration of ursodeoxycholic acid, which because of its greater hydrophilicity has lessened toxicity compared to that of more hydrophobic primary bile acids, cholic and chenodeoxycholic acid, which it replaces in the bile salt pool (61). In fact, other nuclear hormone receptors, such as PXR (59), play a role in detoxification of bile acids by enhancing CYP3A enzyme action, which by 6-alpha hydroxylation allows elimination of LCA in urine and feces (55,58,59). Ursodeoxycholic acid now has been found to enhance this PXR action as well (60). The intestinal VDR has also been discovered to be a nuclear hormone intestinal bile acid sensor of LCA, which also enhances the target gene expression of CYP3A to similarly detoxify LCA (62).

Potential new therapies such as pharmacologically targeted nuclear receptor antagonists such as guggelsterone inhibit FXR actions are bile acid suppression (29). Agonists such as ursodeoxycholic acids are now used to alter bile salt intracellular concentrations to positively affect bile acid and cholesterol homeostasis (60,61) (Table 1). An FXR antagonist of CYP7A1 repression might be better tolerated than a bile salt sequestrant such as cholestyramine, which acts as an LXR agonist by enhancing cholesterol catabolism to bile acids (5). Similarly, it may become possible to enhance reverse cholesterol transport by altering genetic up-regulation of ABC transporter activity by LXR agonists at either hepatic or extrahepatic levels so as to increase biliary cholesterol secretion for fecal elimination (63). Finally, ursodeoxycholic acid therapy has proven effective in reducing cholestasis in patients with inborn errors of bile aid metabolism that cause cholestatic liver disease (64). Gene transfer to replace defective or mutated enzymes might someday provide even better treatments when perfected (65). Thus, the promise of genetic engineering to alter nuclear membrane receptors, or the development of safe and effective agonists or antagonists at hepatic/intestinal or peripheral sites to enhance cholesterol catabolism or elimination, and decreased bile salt toxicity or pool size may well allow major therapeutic advances to correct dyslipidemia causing atherosclerosis, intrahepatic cholestasis, or other cholesterol/bile salt associated disorders such as gallstone disease.

It is the coordinated signaling and targeted gene action of the nuclear hormone receptor FXR within the enterohepatic tissues responding to ligand binding by circulating bile salts combined with LXR binding by oxysterols and its targeted gene actions in peripheral cells, liver and intestine, respectively, that achieve cholesterol homeostasis by enhancing reverse cholesterol transport, cholesterol catabolism to bile acids and enterocyte cholesterol efflux for fecal elimination. FXR targeted gene action assisted by other bile acid sensors, PXR and VDR, in the liver and intestine prevent bile salt toxicity and preservation of the bile salt pool to affect bile salt homeostasis within the enterohepatic circulation of bile salts.

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