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Master regulation of bile acid and xenobiotic metabolism via the FXR, PXR and CAR trio

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1. ABSTRACT

Recent discoveries highlighted intriguing molecular pathways that regulate synthesis, uptake, metabolism and excretion of bile acids and xenobiotics. The knowledge of factors that control these homeostatic processes is of clinical relevance to better understand the drug-drug interacting scenario as well as to control cholesterol detoxification, cholestasis and other conditions. Here we present evidences for the existence of a gut-liver safety network whereby activation of the nuclear receptor FXR, PXR, CAR *trio* provides protection against accumulation of exogenous and metabolic *noxae*.

2. INTRODUCTION: BILE ACIDS AT A GLANCE

Bile acids (BAs) are C_{24} hydroxysteroid metabolites of cholesterol, synthesized in the pericentral hepatocytes via the coordinated action of enzymes located in the endoplasmic reticulum, cytosol, mitochondria and peroxisomes (1). During this process, the side chain of cholesterol undergoes oxidative cleavages while one or two hydroxyl groups are added to one face of the steroid nucleus to generate both a hydrophilic and a hydrophobic face. Thus, cholesterol molecules, which are insoluble membrane constituents, are converted into BAs, which are amphipathic water-soluble detergents. Newly synthesized

BAs are termed primary BAs and in humans consist of the dihydroxylated (3-alpha-OH, 7-alpha-OH) chenodeoxycholic acid (CDCA) and trihydroxylated (3-alpha-OH, 7-alpha-OH, 12-alpha-OH) cholic acid (CA). Before secretion into the canalicular lumen, primary BAs are conjugated at the terminal (C_{24}) carboxyl group with the amino acid taurine or glycine (2). This amidation process increases the amphipathic properties of primary BAs, which are less cytotoxic, more readily secretable into bile and more efficient promoters of the intestinal absorption of dietary lipids than unconjugated BAs (3). Indeed, in humans, conjugated BAs are the major solutes in bile, while unconjugated BAs are almost undetectable.

BAs are secreted by hepatocytes into the bile duct via an active transport system against a concentration gradient. Once in the bile duct, the minority of BAs is reabsorbed by biliary ductal cells (cholangiocytes) and returned to the hepatocytes through the periductal capillary plexus to be re-secreted across hepatocytes into bile. The majority of BAs reaches the gallbladder, where they are concentrated up to 1000 folds. From the gallbladder, after postprandial stimulus, BAs are released into the duodenum to allow the intestinal absorption of dietary lipids and lipid-soluble vitamins. Since BA biosynthesis is an expensive energetic process that involves more than 15 enzymes (4), 95% of BAs are absorbed from the distal ileum and returned to the liver through the portal vein to be re-secreted into bile (5). This cycle of secretion, absorption and re-secretion is called "enterohepatic circulation of BAs" and ensures that only 0.5g of BAs are lost *per* day via fecal excretion from the total BA pool of adult humans (3-4g). This loss is compensated by *de novo* hepatic synthesis of BAs from cholesterol, which contributes less than 3% to hepatic BA secretion (6).

Hepatocytes are polarized epithelial cells with an apical (canalicular) membrane facing the bile *canaliculus* and a basolateral (sinusoidal) membrane facing the blood stream. Both membranes are richly endowed with carrier proteins. Indeed, the vectorial hepatocellular secretion of BAs from blood into bile is driven by the coordinated expression of these distinct transport systems (7). The architecture of liver sinusoids allows the transit of BAs through endothelial fenestrae into the space of Disse, where hepatic basolateral uptake transporters extract BAs (8). Hepatic uptake involves two processes: cotransport with sodium coupled to the electron sodium gradient, and sodium independent BA/organic anion exchange (9,10). Notably, first-pass extraction of BAs is highly efficient, ranging between 75% and 90% of total BAs (11). In normal conditions, the uptake of BAs occurs mainly in periportal hepatocytes, which results in a lobular concentration gradient between zone 1 (periportal) and zone 3 (perivenous) hepatocytes (12).

While BAs are recycling in the gut-liver axis, bacterial enzymes present in the distal intestine modify their structure. With regard to the side chain, BAs undergo a deconjugation process with formation of unconjugated BAs and glycine or taurine. Some of these BAs are passively absorbed by enterocytes and shuttled back to the liver, where they are re-conjugated. With regard to the steroid nucleus, anaerobic bacteria present in the colon remove a hydroxyl group by bacterial dehydratases and transform CA in deoxycholic acid (DCA). Similarly, CDCA is converted to lithocholic acid (LCA). DCA and LCA are termed secondary BAs. Both primary and secondary BAs are in part absorbed from the colon and returned to the liver. Here, DCA is conjugated with glycine or taurine and is ready to circulate with primary BAs. DCA represents almost 20% of biliary BAs, while the remaining BAs are CDCA and CA. With regard to LCA, after up-take from the liver it undergoes a double conjugation since it is not only conjugated with glycine or taurine, but it is also sulfated. Sulfolithocholylglycine and sulfolithocholyltaurine BAs are secreted into bile, but unlike conjugated primary BAs or DCA, they are not efficiently absorbed in the distal ileum. Thus, double conjugated LCA molecules are promptly eliminated from the body, with beneficial effect since they are highly cytotoxic. Notably, in addition to the ileal BA transport system, a similar system exists in the proximal renal tubule, which absorbs the majority of BAs present in the tubular fluid. Thus, in physiological conditions, no BAs are eliminated with urine. This means that virtually all cholesterol is eliminated with the feces. However, the cholesterol elimination *via* its conversion to BAs accounts only for one third of total disposal; the remaining two thirds are eliminated as fecal cholesterol via bile.

Despite the beneficial effect of BAs in solubilizing dietary lipids and promoting their intestinal absorption, in preventing intestinal bacterial overgrowth (13) and regulating electrolyte secretion and absorption across the mucosal barrier (14), accumulation of BAs to high levels (either intracellular or extracellular) is detrimental. BA cytotoxicity increases linearly with hydrophobicity (15). CDCA and DCA are toxic since they are hydrophobic. Ursodeoxycholic acid (UDCA) is much more hydrophilic than CDCA or DCA and then less toxic. CA is between CDCA/DCA and UDCA. Thus, at low concentrations it is not cytotoxic, while at high concentrations it becomes toxic (16). Normally, BA uptake is followed by binding to intracellular proteins to reduce their concentration and foster elimination. When elimination of BAs is impaired, they accumulate intracellularly up to toxic levels: if the concentration exceeds the binding capacity of the cytosolic proteins, BAs can enter organelles such as mitochondria and induce damage leading to apoptosis or necrosis. Extracellularly, above their critical micellar concentration, BAs act as detergent molecules (15-19). At the physiological concentrations, BAs are theoretically able to damage the apical membrane of the hepatocytes and of the cells lining the biliary tract. However, the absence of such effects *in vivo* confirms the existence of cytoprotective mechanisms either at the level of the cell membrane or within biliary micelles. Indeed, increased concentration of phospholipids within bile salt micelles protects against BA cytotoxic effects in the canalicular lumen and in the gut (16,19). In line with these findings, mice with homozygous disruption of *multi drug resistant protein* 2 (*mdr2,* the phosphatidylcholine flippase) present no phospholipid in

Table 1. Selected HUMAN/rodent proteins involved in bile acid and xenobiotic metabolism-clearance

Gene family	Proteins	Substrates	Tissues
SLC10 (phase 0)	NTCP/Ntcp (*SLC10A1/Slc10a1*)	BAs	Liver
SLC0 (phase 0)	Oatp1a1 (*Slco1a1*)	BAs, xenobiotics	Kidney, liver
	OATP1A2 (*SLCO1A2*)	BAs, xenobiotics	Brain, kidney, liver
	Oatp1a3 (*Slco1a3*)	BAs, xenobiotics	Kidney
	Oatp1a4 (*Slco1a4*)	BAs, xenobiotics	Liver, blood-brain barrier
	Oatp1a5 (*Slco1a5*)	BAs, xenobiotics	Intestine
	OATP1B1 (*SLCO1B1*)	BAs, xenobiotics	Liver
	Oatp1b2 (*Slco1b2*)	BAs, xenobiotics	Liver
	OATP1B3 (*SLCO1B3*)	BAs, xenobiotics	Liver
CYP (phase I)	CYP3A4/Cyp3a11	BAs, xenobiotics	Liver, intestine, kidney, brain, lung
	CYP7A1/Cyp7a1	BAs	Liver
	CYP8B1/Cyp8b1	BAs	Liver
	CYP3A5/Cyp3a13	Xenobiotics	Liver, intestine, kidney, brain, lung
	CYP2B6/Cyp2b9	Xenobiotics	Liver, heart
SULT (phase II)	SULT2A1/Sult2a1	BAs, xenobiotics	Liver, adrenals
UGT (phase II)	UGT2B4	BAs, xenobiotics	Liver, kidney, lung
	UGT2B7	BAs, xenobiotics	Liver, kidney, intestine
	UGT1A3	BAs, xenobiotics	Liver, intestine
	UGT1a6/Ugt1a6	Xenobiotics	Brain, intestine, kidney,liver, lung
ABC (phase III)	MDR1/Mdr1 (*ABCB1/Abcb1*)	Xenobiotics	Liver, intestine, bile duct
	MDR3/Mdr2 *(ABCB4/Abcb4)*	PC	Liver
	BSEP/Bsep *(ABCB11/Abcb11)*	Monoanionic conjugated BAs	Liver
	MRP2/Mrp2 *(ABCC2/Abcc2)*	Dianionic conjugated BAs	Liver, bile duct, kidney
	MRP3/Mrp3 *(ABCC3/Abcc3)*	BAs	Liver, bile duct, intestine
	MRP4/Mrp4 *(ABCC4/Abcc4)*	BAs	Liver, kidney
OST (phase III)	OST-alpha/beta/Ost-alpha/beta	BAs	Liver, intestine, bile duct, kidney

bile and exhibit severe BA-induced hepatocyte damage *in vivo* (20).

3. BILE ACID AND XENOBIOTIC METABOLISM PHASES

The liver is the main organ of our body that ensures BA and xenobiotic metabolism-clearance, a process that is systematically described into four phases (Table 1). Phase 0 covers hepatic uptake of BAs and xenobiotics from the systemic circulation. Hepatic clearance of BAs and xenobiotics from the portal blood takes place at the basolateral membrane of hepatocytes and it is in part mediated by the organic anion transporting polypeptides (OATPs). These sodium-independent membrane transporting proteins use a transport mechanism based on anion exchange, which couples the efflux of bicarbonate, glutathione or glutathione-S-conjugates to the influx of a wide range of amphipathic organic molecules, including both xenobiotics and conjugated and unconjugated BAs (9,21-23). Moreover, some OATPs may function as bi-directional transporters when the intracellular concentration of the substrates is high, serving, when request, as an extrusion system of potentially toxic compounds (24).

To date, 36 OATPs have been identified in humans, rat and mouse that according to the new OATP classification system, belong to the solute carrier O (*SLCO*, where O stay for OATP) gene superfamily (25). This OATP (*SLCO*) superfamily of membrane transporters is further divided into families and subfamilies. OATPs within the same family share ≥ 40% amino acid sequence identities and are designed by Arabic numbering [OATP1 (*SLCO1*), OATP2 (*SLCO2*), OATP3 (*SLCO3*), OATP4 (*SLCO4*), OATP5 (*SLCO5*), and OATP6 (*SLCO6*)]. OATP subfamilies include members that share ≥ 60% amino acid sequence identities and are designed by letters, e.g. subfamilies OATP1A (*SLCO1A*), OATP1B (*SLCO1B*), OATP1C (*SLCO1C*), OATP2A (*SLCO2A*), OATP2B (*SLCO2B*), OATP3A (*SLCO3A)* etc. Indeed, based on the chronology of identification, additional continuous Arabic numbering is used to classify individual non-orthologous proteins of the same subfamily, e.g. OATP1A2 (*SLCO1A2*), Oatp1a3 (*Slco1a3*), etc. Notably, for many rat/mouse *Oatp* genes no orthologous human *OATP* genes have been yet identified (26).

Although some members of the OATP superfamily are exclusively expressed at the basolateral membrane of hepatocytes to elicit hepatic uptake of BAs and xenobiotics from the portal blood (27), other OATPs are also expressed in the intestine, kidney, lung, heart and blood-brain barrier (26). Moreover, since most OATPs transport a wide spectrum of substrates with partially overlapping specificity, an important role in overall drug absorption and disposition has been suggested for these membrane transporters. Thus, selective inhibition of specific hepatic OATP members could be exploited to increase oral bioavailability of drugs that have a high OATP-mediated hepatic first-pass clearance. In this regard, it has been shown that the drug rifampicin mainly inhibits human OATP1B3 (*SLCO1B3*) (28,29).

On the other hand, Na^+-dependent uptake is mediated by the solute carrier 10A1 (*SLC10A1*), a member of the solute carrier family 10 (*SLC10*), also termed Na^+-taurocholate cotransport protein (NTCP) (30-32). This membrane transporter mediates sodium-coupled uptake of BAs, accounting for more than 80% of conjugated but less than 50% of unconjugated BA uptake (33).

Following hepatic uptake, BAs and xenobiotics undergo phase I oxidation reactions, which result in

biotransformed, more polar products, hence suitable for elimination. BA and xenobiotic oxidation is catalyzed by members of the cytochrome P450 (CYPs) superfamily, which are heme proteins expressed in the endoplasmatic reticulum that use electrons from NAD(P)H to catalyze activation of molecular oxygen, leading to regiospecific and stereospecific oxidative attack of a plethora of substrates.

Presently there are more than 270 different *CYP* gene families, with 18 recorded in mammals. These families, and derived subfamilies, are arranged on the base of percentage identity in aminoacid sequence (34). Enzymes that share ≥ 40% identity are classified into a particular family designed by an Arabic numeral, whereas those sharing ≥ 55% identity are assigned to the same subfamily designed by a letter following the Arabic numeral, e.g. CYP3A4, CYP2B2. Individual members of a family or subfamily are labeled again by Arabic numeral. To represent an individual member, a new P450 sequence has to differ by more than 3%. Only four mammalian P450 gene families (*CYP1- 4*) code for enzymes that metabolize both xenobiotics and endogenous lipophilic substrates. P450s belonging to the others mammalian CYP families typically do not metabolize exogenous chemicals but just endogenous substrates into physiological important pathways. Indeed, if CYP7A1 and CYP8B1 are required for BA biosynthesis from cholesterol, members of the CYP3A and CYP2B subfamilies are responsible for the metabolism of more that 60% and 20% of prescription drugs, respectively.

The *P450* genes are regulated in several ways and at multiple levels by both endo- and xenobiotics. Structurally diverse exogenous chemicals increase P450 protein levels by stimulating *P450* gene expression with an important impact on P450-dependent drug metabolism, pharmacokinetics and drug-drug interaction. Thus, xenobiotics can induce their own phase I biotransformation through CYPs. Furthermore, exogenous chemicals can also activate their own phase II metabolism and phase III clearance through other families of cytosolic enzymes and membrane transporters. Phase II enzymes are responsible for conjugation of phase I products with endogenous molecules to further increase their water solubility. Members of the cytosolic sulfotransferase (SULT) and UDP-glycosyltransferase (UGT) superfamilies catalyze these reactions.

SULT enzymes catalyze the transfer of a sulfonyl group from the donor molecule 3'-phosphoadenosina 5'-phosphosulfate (PAPS) to amino or hydroxyl groups of lipophilic molecules with formation of sulfamate or sulfate conjugates, respectively. To date, at least 10 human isoforms and 11 mouse isoforms have been identified (35,36), some of which are involved in the sulfation of hydroxysteroids, such as LCA (37-39).

UGT enzymes catalyze the addition of glycosil groups (i.e. glucose, galactose, glucuronic acid, etc.) to lipophilic substrates such as steroids, fat-soluble vitamins, environmental toxicants, drugs and BAs. Members of the *UGT* superfamily have been named on the basis of a divergent evolution, with each gene given the root symbol *UGT,* followed by an Arabic number to indicate the family (e.g. *UGT1*), a letter to identify the subfamily (e.g. *UGT1A*), and again an Arabic number to denote the individual gene within that subfamily (e.g. *UGT1A1*) (40). To date, 117 mammalian *UTG* genes have been identified and are divided into four families (*UGT1, UGT2, UGT3, UGT8*) (41). Members of the *UGT1*and *UGT2* families code for enzymes that are most efficient in using UDP glucuronic acid as glycosyl donor (42,43). While other UGTs are involved in biosynthetic pathways, such as UGT81A1 in the synthesis of cell-membrane components (11), UGT1 and UGT2 enzymes are invaluable in detoxifying endogenous and exogenous chemicals, because the glycosylated products are more water soluble, hence more readily excreted.

After hepatic phase I and phase II metabolism, the resulting conjugated BAs and xenobiotics are enough polar to be easily eliminated from the body, but not yet ready to leave the liver by passive membrane diffusion. Indeed, phase III, which is the last step of the metabolism-clearance process, deals with BA and xenobiotic active secretion into the bile for eventual fecal elimination. This process is mediated by members of the ATP-binding cassette (ABC) transporter family. These proteins are membrane transporters that use the energy of ATP hydrolysis to translocate a wide variety of hydrophilic substrates across the plasma membrane in a single direction, typically out of the cytoplasm. *ABC* genes are widely dispersed in the genome and show a high level of amino acid sequence homology among eukaryotes. Phylogenetic analysis has allowed the division of this family into seven subfamilies, six of which are found in both mammalian and the *S. cerevisiae* genomes. The human genome contains 48 ABC genes arranged among subfamilies that are indicated by capital letter to distinguish them from the rodent subfamilies that are indicated by small letter. Moreover, also common names are available for most of the mammalian *ABC* genes.

Among the hepatic ABC proteins responsible for the secretion of BAs and xenobiotics into the bile there is the bile salt export pump [BSEP (*ABCB11*)] for the monoanionic conjugate BAs, the multidrug-related protein 2 [MRP2, (*ABCC2*)] for the dianionic conjugated BAs and multidrug protein 1 [MDR1, (*ABCB1*)] for xenobiotics. All these ABC transporters are expressed at the canalicular membrane of the hepatocyte, like the human multidrug protein 3 [MDR3, (*ABCB4*)] or the rodent orthologous multidrug protein 2 [Mdr2, (*Abcb4*)], which is involved in biliary phosphatidylcholine secretion. On the contrary, ABC transporters such as the multidrug-related protein 3 [MRP3, (*ABCC3*)] and multidrug-related protein 4 [MRP4, (*ABCC4*)] are expressed at the basolateral membrane. These proteins, together with another basolateral transporter, the organic anion transporter alpha and beta (OST-alpha/beta), represent an alternative route for the liver to spillover BAs when the canalicular secretion is impaired. Notably, in addition to the liver, some ABC transporters are also expressed in the intestine and kidney

to coordinately regulate BA and xenobiotic distribution and disposal (Figure 1).

4. NUCLEAR RECEPTORS AT THE CROSSROAD OF BILE ACID AND XENOBIOTIC METABOLISM

Hepatic, intestinal and renal metabolism-clearance of BAs, prescription drugs and other xenobiotics is of critical importance to avoid accumulation of potentially toxic lipophilic compounds in our body. This task is mainly orchestrated by the farnesoid X receptor (FXR), the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR). This trio of ligand-activated transcription factors belongs to the nuclear receptor superfamily and it is highly expressed in the enterohepatic system and kidney where it regulates the expression of enzymes and membrane transporters responsible for endobiotic and xenobiotic detoxification processes.

Unlike the classical steroid hormone receptors, which are selectively activated by their ligands at nanomolar concentrations and regulate the expression of their target genes as homodimers, FXR, PXR and CAR modulate gene expression by forming heterodimer with the retinoid X receptor (RXR) and are activated by micromolar concentration of ligands. All three nuclear receptors were initially cloned without knowing their endogenous ligands and were termed “orphan nuclear receptors”. Later, BAs were identified as endogenous ligands for FXR. On the contrary, endogenous ligands that can activate PXR and CAR at physiological concentrations are yet unknown, hence these NR are defined as (orphan) xenobiotic receptors.

As members of the nuclear receptor superfamily (44), FXR, PXR and CAR proteins share a common structure that consists of different domains of homology based on regions of conserved sequence and function. Among these, the DNA binding domain (DBD) is the most highly conserved region followed by the C-terminal ligand-binding domain (LBD), while the N-terminal trans-activation domain (AF1) and the ligand induced trans-activation domain (AF2) present in the LBD are the most heterogenous regions. Indeed, after binding to the LBD, a hydrophobic pocket that can accommodate different ligands, BAs and xenobiotics can induce FXR, PXR and CAR activation, which result in the stimulation of the AF1 and AF2 domains to interact with co-activator proteins to induce gene expression. Notably, unlike steroid receptors that upon activation by their own ligands make homodimers that shuttle from the cytoplasm to nucleus to regulate the expression of their target genes, FXR and PXR are already located on the DNA where they make heterodimers with RXR, and only after ligand binding can run into conformation changes that allow them to interact with co-activator proteins. The DNA sequences on the promoter region of the target genes recognized by NR homo- or heterodimers are termed hormone responsive elements (HRE). These responsive elements contain symmetric (palindromic) repeats of hexameric half-site sequences. Non-steroid and orphan receptors recognize the consensus 5’-AGGTCA-3’ half-site and the corresponding direct (DR), inverted (IR) or everted repeats (ER) are differentiated by the number of base-pairs spacing the two half-sites, which is indicated by an Arabic number. Thus, FXR/RXR heterodimer preferentially binds to IR1 motif, although IR0, DR1, DR3 and ER8 motifs are also bound (45), while PXR/RXR and CAR/RXR heterodimers bind to DR3, DR4, IR6, ER6 and ER8 motifs (46,47).

4.1. FXR

FXR is an adopted member of the nuclear receptor superfamily highly expressed in the liver, intestine, kidney and adrenals. It was isolated from a rat-liver cDNA library and named after the initial finding that super physiological concentrations of farnesol, an intermediate in the mevalonate biosynthesis, were able to weakly activate FXR (48). Later, it was shown that physiological concentrations of BAs bind and activate FXR (49-51). CDCA strongly activates FXR, DCA and LCA exert intermediate effects, while CA and UDCA are week activators. FXR plays a role of master regulator of BA homeostasis by modulating BA synthesis, conjugation, secretion and absorption. Thus, activation of FXR in the enterohepatic system is important to entrain the enterohepatic circulation of BAs and to regulate the bile flow, but also to restrain BA toxicity. In fact, BAs are strong detergents and when they accumulate at high concentrations damage cell membrane, impair liver function, potentially leading to cirrhosis and cholestasis, a condition characterized by reduced bile flow and accumulation of bile constituents in the liver and blood. The importance of FXR in mediating BA detoxification has been highlighted in FXR-null mice that exhibit hepatic toxicity when fed a diet enriched in BAs (52,53). Indeed, BAs regulate their own metabolism and enterohepatic circulation in a feed-forward mode and their own synthesis in a feed-back mode through the transcriptional mediator FXR (see below).

In addition to be involved in BA metabolism, to some extent FXR plays also a role in xenobiotic detoxification. Xenobiotics that accumulate in the liver are, in fact, secreted into bile through membrane transporters regulated by FXR for the secretion of BAs. In this way, FXR modulates the secretion of xenobiotics into bile, the route for their fecal elimination. Thus, FXR is also involved in phase III xenobiotic detoxification.

4.2. PXR

The orphan nuclear receptor PXR, also termed SXR (steroid and xenobiotic receptor) when referred to humans, was isolated in 1998 as candidate xenobiotic receptor postulated to regulate the expression of *CYP3A* (54-57). Three related observation suggested PXR as xenobiotic receptor. First, PXR is highly expressed in the liver and small intestine, two tissues involved in xenobiotic metabolism through the expression of CYP enzymes. Second, genes encoding CYP enzymes, as is the case of *CYP3A*, present PXR responsive elements in their promoters. Finally, although PXR received its name by the initial finding that pregnenolone and its derivate 16-alpha-carbonitrile (PCN) can activate it, a wide range of structural diverse exogenous chemicals such as rifampicin, taxol, dexamethasone, tamoxifen, phytoestrogens and the herb

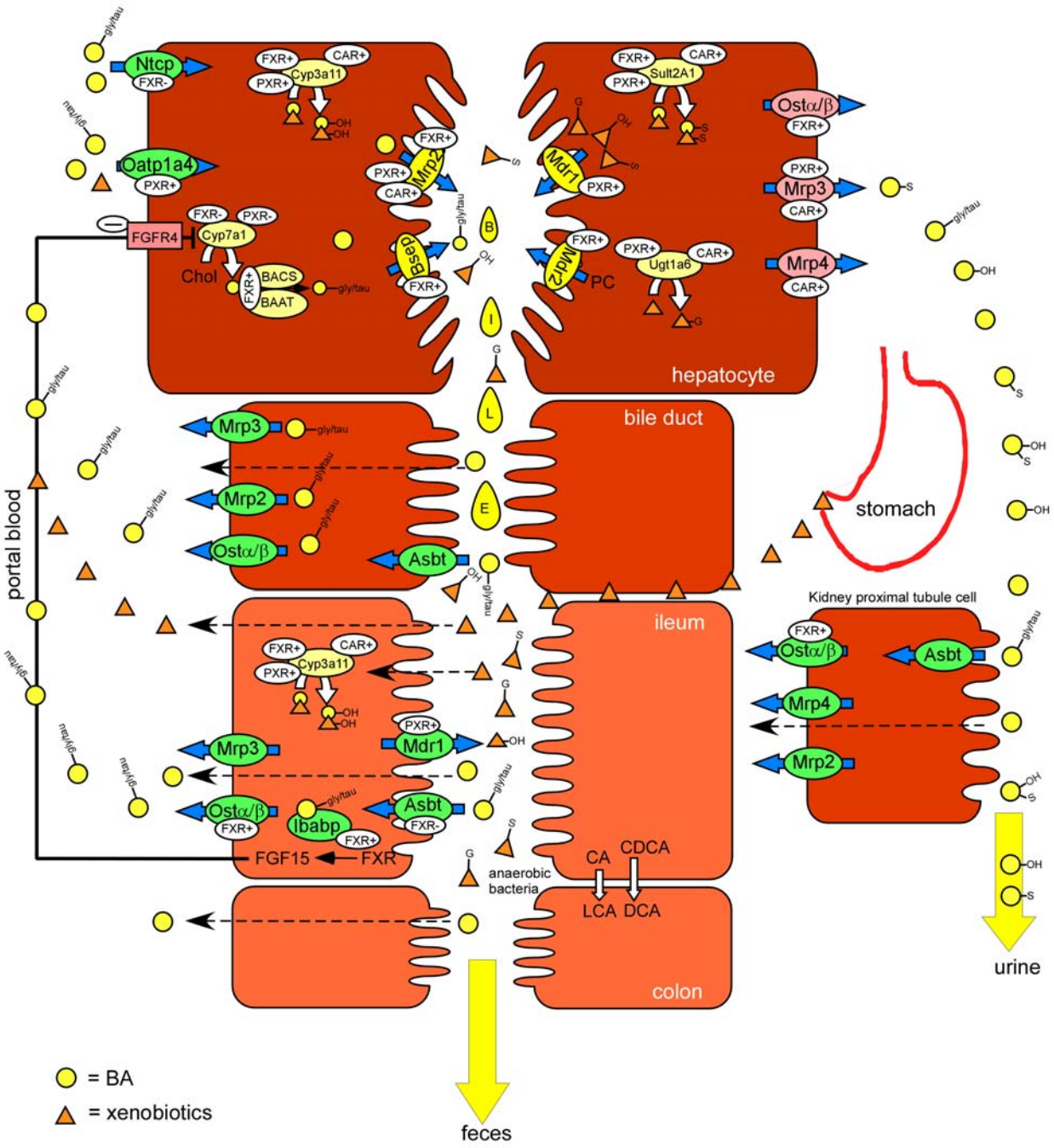

Figure 1. Regulation of bile acid and xenobiotic metabolism-clearance in rodents via FXR, PXR and CAR. BAs are synthesized in the liver from cholesterol and secreted into the gallbladder after conjugation with glycine or taurine. All these steps are regulated by FXR, which can repress *Cyp7a1* expression while inducing the expression of *BACS* and *BAAT* for BA conjugation, and *Bsep*, *Mrp2* and *Mrd2* for bile formation. Before BAs are concentrated in the gallbladder, a small amount of BAs is passively (unconjugated) or actively (conjugated) reabsorbed from cholangiocytes. In the latter case, the canalicular membrane transporter Asbt is responsible for the uptake of conjugated BAs that are secreted into the periductal capillary plexus via the basolateral transporters Mrp3, Mrp2, Ost-alpha/beta, to be return to the liver. The majority of BAs concentrate in the gallbladder and after postprandial stimulus are delivered into the small intestine to allow the absorption of lipophilic nutrients. After reaching the distal ileum, most conjugated BAs are actively reabsorbed by the canalicular transporter Asbt, while some unconjugated BAs are passively reabsorbed from the distal ileum and the proximal colon where secondary BAs (LCA, DCA) are produced by anaerobic bacteria. Only a very small amount of BAs is lost with feces. After being taken-up by Asbt, BAs induce FGF15 expression in the

ileum via FXR, to communicate to the liver the need to stop BA synthesis by down-regulating *Cyp7a1* expression. At the same time, BAs are shuttled to the basolateral membrane of the ileal enterocyte by the cytosolic transporter Ibabp. Afterward, BAs are secreted in the portal blood via Mrp3 and Ost-alpha /beta transporters to travel back to the liver and complete the enterohepatic circulation. After being taken-up from the liver by Ntcp, BAs activate FXR and repress their own synthesis by down-regulating *Cyp7a1* expression via SHP. Also, PXR can repress *Cyp7a1* expression when BAs accumulate in the liver to toxic levels. During cholestasis, several adaptive mechanisms are coming into play. PXR and CAR help FXR to induce phase I (Cyp3a11) and phase II (Sult2a1) BA metabolism, besides to stimulate BA secretion via Mrp2. Moreover, when canalicular secretion is impaired, FXR, PXR, and CAR can also induce an alternative basolateral secretion (through Ost-alpha /beta, Mrp3 and Mrp2) that diverts BAs in the blood stream for final elimination of hydroxylated and sulfonated BAs with urines. FXR protects also the intestine from the toxic effects of accumulating intracellular BAs by repressing *Asbt* expression while inducing that of *Ibabp* and *Ost-alpha/beta* to spill over BAs from the enterocyte. During cholestasis, by inducing *Oatp1a4* expression, PXR allows the liver to conserve a minimal ability to metabolize BAs and xenobiotics when *Ntcp* expression is repressed by FXR via SHP. Orally dosed drugs are absorbed by the intestine where they can activate PXR or CAR to induce their own metabolism via Cyp3a11 and further secretion into the gut lumen via Mdr1 as hydroxylated products ready for fecal elimination. Those drugs that can reach the liver are withdrawn by Oatp1a4 and activate hepatic PXR and CAR, which in turn induce both phase I (Cyp3a11) and phase II (Sult2a1, Ugt1a6) drug metabolism, as well as phase III hepatic clearance via Mdr1 for the secretion of hydroxylated, sulfonated and glucuronidated drugs into the bile for fecal elimination.

Saint John's wort is eventually able to induce PXR transcriptional activity. The ability of PXR to bind different compounds is facilitated by the unusual ligand-binding pocket of this nuclear receptor that is both smooth and large. The idea that PXR functions as xenosensor has been confirmed in PXR-null mice. These mice, when treated with dexamethasone or PCN do not exhibit induction of *CYP3A* (58) and fail to show protection against drug-sedatives that are metabolized and inactivated by CYP3A such as zoxazolamine and tribromoethanol (59). On the contrary, transgenic mice for a human activated PXR showed constitutive induction of *CYP3A* resulting in protection against these drugs (58).

Although PXR has been identified as a xenobiotic sensor, further studies have suggested PXR also as an endobiotic receptor. In fact BAs, such as LCA, can bind and activate PXR. Upon activation, PXR induces LCA phase I metabolism through the up-regulation of *CYP3A* expression. Thus, PXR transgenic mice are protected against liver toxicity from LCA (59). These findings suggest a feed-forward regulatory pathway, where potentially toxic endogenous products such as BAs can induce their own phase I metabolism through PXR. In addition to *CYP3A* genes, PXR regulates also the expression of genes encoding for phase II enzymes such as glutathione-S-transferase (*GST*), *UGT* and *SULT* genes besides to genes encoding for ABC and OATP membrane transporters. Thus, PXR function as master regulator of endo- and xenobiotics by coordinating their oxidation, conjugation and secretion/excretion.

4.3. CAR

In 1994, an orphan nuclear receptor was initially termed MB67 (60). Interestingly, the initial characterization of MB67 showed that it was able to activate gene transcription in the absence of any ligand. Later, it was demonstrated that high concentrations of androstane metabolites repressed the constitutive activity of this receptor. Indeed, to keep with the tradition of naming nuclear receptor on the base of their ligand, MB67 is now termed constitutive androstane receptor (CAR).

CAR is highly expressed in the liver and intestine, just like PXR and FXR, and its role as xenobiotic receptor as been suggested in 1998 when it was found to be deactivated by androstane metabolites and to regulate the expression of *CYP2B* genes. The activity of CAR as xenobiotic receptor has been confirmed in CAR-null mice, which display no upregulation of *CYP2B10* expression by xenobiotics (61).

As it is the case of PXR, inducers of CAR activity are not only xenobiotics but also endogenous molecules. In fact bilirubin, LCA and steroids can induce the transcriptional activity of this nuclear receptor, although indirectly. Upon activation, CAR regulates the expression of genes encoding for enzymes involved in the oxidative metabolism (*CYP*s), conjugation (*GSTs, UGTs, SULTs)* and transport (*ABCs*, *OATPs*) of xeno- and endobiotics such as BAs. Thus, CAR regulates an overlapping set of genes with PXR. For example, PXR induces *CYP2B* genes while CAR increases the expression of *CYP3A* genes. The mechanism of this cross-regulation has been attributed to the presence of shared responsive elements for these two xenobiotic sensors on the promoter region of these genes (62-64).

Although PXR and CAR share some common ligand and have an overlapping partner of target genes, the way of activation for these two nuclear receptors is different (65). If PXR, as FXR, is in fact located in the nucleus and is activated upon ligand binding, CAR is present in the cytoplasm and only after activation shuttles to the nucleus to regulate the expression of its target genes. The cytoplasmatic CAR retention protein (CCRP) is a co-chaperon for CAR that interacts with the ligand-binding domain of this nuclear receptor and is responsible for its retention in the cytoplasm. The heat shock protein 90 (HSP90) is also part of this cytoplasmatic complex and it is a well-known chaperon for the retention of steroid receptor in the cytoplasm. Notably, CAR makes a complex with HSP90 only in the presence of CCRP suggesting that CCRP recruits CAR to the CCRP-HSP90 complex (66).

CAR can be activated either directly, as with the hepatomitogen 1,4-bis [2-(3,5 dichloropyridyloxy) benzene (TCPOBOP), or indirectly, as with phenobarbital, bilirubin,

Table 2. Nuclear receptor regulation of bile acid and xenobiotic metabolism-clearance

Receptor	Ligands	Target genes (phase)	Physiological effects
FXR (NRH14)	CDCA, CA DCA, LCA, GW4064 (synthetic)	*SHP* *BSEP/Bsep (III)* *MRP2/Mrp2 (III)* *OATP1B3 (0)* *OST-alpha/beta/Ost-alpha/beta(III)* *UGT2B4 (II)* *UGT2B7 (II)* *SULT2A1 (II)* *MDR3(III)* *CYP3A4(I)*	Induction of BA and xenobiotic metabolism and secretion; induction of BA and xenobiotic hepatic uptake; induction of alternative basolateral BA excretion during cholestasis
SHP (NR0B2)	None	*CYP7A1/Cyp7a1* *CYP8B1/Cyp8b1* *ASBT/Asbt* *Ntcp (0)*	Repression of BA synthesis; repression of BA basolateral uptake in the liver, intestine and bile duct.
PXR (NR12)	Rifampicin in human (synthetic) PCN in rodents (synthetic) LCA UDCA St. John's wort Dexamethasone Clotrimazole	*MRP2/Mrp2 (III)* *MDR1 (III)* *Oatp1a4 (0)* *CYP3A4/Cyp3a11 (I)* *CYP2B (I)* *SULT2A1/Sult2a1 (II)* *Sult2a9 (II)* *CYP7A1* *UGT1A1/Ugt1a1 (II)* *Ugt1a6 (II)* *UGT1A3 (II)*	Induction of BA, xenobiotic and bilirubin metabolism;induction of BA and xenobiotic canalicular secretion; induction of BA hepatic uptake; induction of alternative basolateral BA excretion during cholestasis; repression of BA synthesis.
CAR (NR1I3)	Bilirubin TCPOBOP Yin Cin Phenobabital	*MRP2/Mrp2 (III)* *MRP3 (III)* *MRP4/Mrp4 (III)* *CYP2B (I)* *CYP3A4/Cyp3a11 (I)* *Sult2a1, Sult2a9 (II)* *UGT1A1/Ugt1a1 (II)* *Ugt1a6 (II)* *UGT1A1/Ugt1a1 (II)* *CYP7A1*	Induction of BA, xenobiotic and bilirubin metabolism; induction of BA canalicular secretion; induction of alternative basolateral BA excretion during cholestasis.

CA, and steroids. These compounds, unlike TCPOBOP, activate nuclear translocation of CAR by a phosphorilation-dephosphorilation pathway that involves the okadaic acid-sensitive protein phosphatase 2A (66,67). Thus, the fact that CAR can respond to cytoplasmatic signals that affect its nuclear translocation and the fact that it has a ligand-independent constitutive activity when localized in the nucleus makes CAR unique among the orphan nuclear receptor family.

In summary, FXR, PXR and CAR act in concert to establish a metabolic safety network against BA and xenobiotic toxicity by repressing BA synthesis and by inducing genes responsible for phase I (hydroxylation), phase II (conjugation) and phase III (excretion) metabolism of endo- and xenobiotic molecules. Indeed, by binding and activating FXR, PXR and CAR, BAs and xenobiotics stimulate in a feed-forward mode the expression of enzymes and membrane transporters, which are responsible for their detoxification and clearance (Table 2). In order to present the regulatory pathways in a meaningful way, we decided to organize the next paragraph subdividing the issues in feed-back repression of bile acid synthesis, bile acid and xenobiotic uptake, metabolism and secretion with the final angle of intestinal bile acid absorption.

5. REGULATION OF BILE ACID AND XENOBIOTIC METABOLISM VIA FXR, PXR AND CAR

5.1. Feed-back repression of bile acid synthesis.

Starting from cholesterol, BAs are synthesized in the liver through a biosynthetic pathway that consists of at least 15 enzymatic steps (1). The "classical" pathway leads to the formation of the primary BAs CA and CDCA, which, in humans, are synthesized roughly in similar amounts. An "alternative" pathway, which is ubiquitous, is responsible for the production of oxidized cholesterol molecules that are converted predominately to CDCA in the liver (4).

Prior to secretion from the hepatocytes into bile, free BAs are amidated with either glycine or taurine at the C_{24}. This reaction involves the initial activation of CDCA and CA to the corresponding CoA-thioesters by the BA CoA synthase (BACS) enzyme. Indeed, activated BAs are substrates for the BA-CoA:amino acid *N*-aceyltransferase (BAAT) enzyme, which catalyzes BA conjugation with taurine or glycine (68). Recycling of tauro- or glycol-BAs is performed in the intestine by the action of bacterial enzymes as well as in the liver by the BA-CoA thioesterase (BACTE) enzyme (69,70). Notably, conjugated BAs are potent endogenous activators of FXR (50), which in turn is responsible for the up regulation of BACS and BAAT (71).

CYP7A1/Cyp7a1 is the rate-limiting enzyme in the "classical" pathway and it is tightly regulated at the transcriptional level by different molecules such as drugs, hormones, oxysterols and also BAs (72). Notably, the rodent *Cyp7a1* promoter is positively regulated by the oxysterol receptor liver X receptor (LXR) after activation by oxysterols (73,74), while no effect on the regulation of human *CYP7A1* expression by LXR has been found (75). Since CYP7A1 promotes the catabolism of cholesterol into BAs, the ability of LXR to increase *Cyp7a1* expression in rodents makes these animals more resistant than humans to

hypercholesterolemia induced by a high-cholesterol diet. In line with these findings, transgenic mice for human *CYP7A1* in a mouse *Cyp7a1* knock-out background become hypercholesterolemic when fed with a high-cholesterol diet (76,77).

In addition to LXR, also PXR and CAR regulate the expression of *CYP7A1*. Both receptors are activated by precursors of cholesterol such as isoprenoids and squalene (78,79). Thus, when cholesterol synthesis is blocked, the activation of PXR and CAR by accumulated cholesterol precursors results in the repression of *CYP7A1* with consequent inhibition of cholesterol catabolism to BAs. The goal of this molecular mechanism is to prevent that cholesterol levels drop too low when its biosynthesis is impaired.

BAs are able to repress their own synthesis in a negative feed-back way that involves FXR. Indeed, by activating FXR in the liver, BAs induce the expression of SHP (small heterodimer partner), an orphan nuclear receptor lacking a DNA-binding domain, that interacts and represses the activity of another orphan nuclear receptor, the liver receptor homolog-1 (LRH-1), which is a potent inducer of *CYP7A1* expression (80-82). Thus, SHP-LRH-1 interaction determines the reduced expression of *CYP7A1*. Nevertheless, it was shown that BAs are still able to repress *Cyp7a1* in SHP-null mice indicating the presence of redundant mechanisms for the repression of *CYP7A1* by BAs. Also, given the importance of BA pool size more than intracellular BA concentration *per se*, a recent gut-liver pathway has been demonstrated. In the murine intestine, FXR induces the expression of the fibroblast growth factor 15 (FGF15), which after reaching the liver binds to the FGFR4 receptor and induces a c-jun N-terminal kinase-dependent pathway establishing a crosstalk between intestine and liver to regulate BA homeostasis by means of reduced *Cyp7a1* expression (83). Indeed, in the murine model Asbt$^{-/-}$ mice, which present the major features of human intestinal BA malabsorption, treatment with FXR agonists or FGF15 decreases the BA neo-synthesis, normally increased in conditions of malabsorption. This finding gives us the possibility of a new putative strategy to treat patients affected by BA malabsorption (84). This intriguing intestinal FXR-FGF15 pathway has been proved to be important in the ileal BA feed-back control (85) at the basis of postprandial gallbladder relaxation (86) that follows the end steps of lipid digestion and absorption in the gut (87). Other SHP-independent pathways for the repression of *CYP7A1* could be mediated by PXR. Indeed, it has been observed that xenobiotics and BAs are able to induce PXR activity and cause the down-regulation of *CYP7A1* expression both *in vitro* and *in vivo* (88,89). However, the exact mechanism for PXR repression of *CYP7A1* is unclear.

In addition to CYP7A1/Cyp7a1, another important cytochrome P450 in the classical pathway is CYP8B1. This enzyme controls the ratio of CA to CDCA by regulating the synthesis of CA. As for *CYP7A1*, also *CYP8B1* is repressed by FXR (90). By inducing SHP, FXR reduces the expression of *CYP8B1* decreasing the transcriptional activity of HNF4-alpha. Thus BAs can repress their own synthesis in a feed-back fashion by reducing *CYP7A1* and *CYP8B1* expression via FXR-SHP cascade, with the gut-liver axis playing a pivotal role only for *Cyp7a1*. Indeed, evidences from liver-specific and intestine-specific FXR-null mice have shown that FXR mediated BA synthesis repression needs both hepatic and intestinal expression of FXR since, conversely to *Cyp8b1* which is efficiently repressed by the activation of FXR in the liver with the subsequent increase of SHP, *Cyp7a1* is not decreased by this pathway but its repression requires the induction of FGF15 hormone mediated by the intestinal FXR (91). Moreover, studies in hepatic specific knockout mice have shown that the absence of LRH1 can reduce only the expression of *Cyp8b1* without affecting *Cyp7a1* levels. These findings demonstrate a key regulatory role of LRH1 for the composition of BA pool but not for its size since it is not involved in the feed-back repression of BA synthesis (92,93).

5.2. Bile acid and xenobiotic uptake (phase 0)

Hepatic uptake of exogenous chemicals is the rate-limiting step for their detoxification and bioavailability. Moreover, regulation of hepatic BA uptake is also important during cholestasis. At each meal, BAs recycle from the distal ileum to the liver where they are taken-up by the NTCP and OATP transporters (94). Extraction of BAs from the portal system is the last step in the enterohepatic circulation of BAs and is essential for bile formation since 95% of the biliary excreted BAs are those reabsorbed in the distal ileum and re-introduced in the portal circulation. Thus, the expression of the hepatic basolateral membrane transporters is reduced when the liver is overloaded with BAs, as in cholestasis (95-97) or in murine model of this liver disease (98,99). Limiting the hepatic uptake of BAs during cholestasis may be considered a hepatocellular adaptive response to reduce intracellular BA induced toxicity. In this respect, the study of the transcriptional regulation of NTCP via nuclear receptor is of great translational value. Nevertheless, a considerable difference might exist among humans, mice, and rats in the *NTCP* regulation (100). The rat *Ntcp* promoter is transcriptionally activated by the RXR-alpha:RAR-alpha (101-103) heterodimer, with a negative modulation via FXR and SHP (104). However, BAs are able to downregulate *Ntcp* also in absence of SHP (105), thus underlining potential SHP-independent mechanisms in the negative regulation of *Ntcp* (106). On the other hand, FXR appears to be necessary for the BA-induced repression of *Ntcp* (107). Finally, there are no RXR-alpha:RAR-alpha response elements in the human *NTCP* promoter (100), thus pointing to RXR-alpha:RAR-alpha independent mechanisms for BA downregulation of *NTCP* in human cholestatic liver diseases (96,98).

In case of the Na^+ -independent transporters, rat liver express three Oatps [Oatp1a1 (*Slco1a1*), Oatp1a4 (*Slco1a4*), Oatp1b2 (*Slco1b2*)], while four hepatic OATPs [OATP1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*), OATP1A2 (*SLCO1A2*), OATP2B1 (*SLC02B1*)] are present in the human liver. Oatp1a1 [also known as Oatp1 (Slc21a1)] was cloned from a rat liver cDNA library (108).

It is selectively expressed at the hepatic basolateral membrane and has the same BA and xenobiotic specificity of the mouse Oatp1a1 (109). As for *Ntcp*, the expression of *Oatp1a1* is down-regulated in both HNF1-alpha (110) and HNF4-alpha (111) knock-out mice as well as after CA feeding in cholestatic liver (112), suggesting a possible involvement also for FXR in the regulation of *Oatp1a1* expression. Although the role of FXR in *Oatp1a1* regulation remains to be determined, PXR is probably not involved since neither phenobarbital nor PCN have effect on *Oatp1a1* expression (113-115).

Oatp1a4 [also known as Oatp2 (*Slc21a5*)] was first cloned from rat brain. Later, it was also found in the hepatic basolateral membrane where it shows overlapping substrate specificities to Oatp1a1 regarding to BAs. Unlike Oatp1a1, which shows a more lobular distribution, Oatp1a4 is mainly expressed in perivenous hepatocytes (116,117). Thus, Oatp1a1 is responsible for the Na^+ -independent BA uptake at the level of periportal hepatocytes that occurs under normal conditions (12,118). However, in murine models of cholestasis, which are associated with down-regulation of *Oatp1a1* and *Ntcp* (119) pericentral hepatocytes in zone 3 of the sinusoids are recruited for BA transport (118). Thus, Oatp1a4 may have an important role when Oatp1a1 and Ntcp activity or expression is impaired (120). From a transcriptional point of view, also *Oatp1a4* is down-regulated in HNF1-alpha knock-out mice, but unlike *Ntcp* and *Oatp1a1*, its expression is induced by phenobarbital and PCN via PXR (113-115). This finding increases the complexity of the regulation of the adaptive response via nuclear receptor during cholestasis. Indeed, PCN treatment via PXR has been shown to protect rats from cholestasis induced by LCA, a highly toxic secondary BAs that has been associated also to human cholestasis. The complexity of this mechanism is in the fact that by inducing *Oatp1a4* expression, PXR would increase the hepatic intracellular LCA uptake with a putative increase of intracellular toxicity of this bile acid. However, one could speculate that the increase in LCA uptake is important for clearing the blood from cytotoxic BAs and for increasing its consequent biotransformation into a more polar product that can be easily eliminated from the body. In fact, urines of patients suffering from cholestasis have elevated levels of 6-hydroxylated LCA. Notably, PXR is a low affinity receptor for a subset of BAs, but high levels of LCA, as found in cholestasis, can activate PXR. Thus, if on one hand during cholestasis *Oatp1a1* and *Ntcp* are repressed to alleviate liver injury by limiting BA overload, on the other hand LCA that accumulates in the liver can activate PXR to induce *Oatp1a4* and maintains the ability of the liver to clear the blood from BAs and xenobiotics.

Oatp1b2 [also known as Oatp4 (*Slc21a10*)] is exclusively expressed at the hepatic basolateral membrane. It mediates similar Na^+ -independent BA and xenobiotic transport of Oatp1a1 and Oatp1a4, although with different affinities (121). Recent findings suggest that also *Oatp1b2* is positively regulated by HNF1-alpha (122). In humans, the most relevant OATPs for hepatic BA and xenobiotic uptake are OATP1B1 and OATP1B3. OATP1B1 [also known as OATP-C (*SLC21A6*)] is the main Na^+ -independent BA uptake system (21,25). As for *Ntcp*, the repression of *OATP1B1* during cholestasis is mediated by FXR through SHP. In fact, by interfering with HNF4-alpha activity, SHP can reduce the expression of HNF1-alpha, a target gene of HNF4- alpha, which is a strong activator of the *OATP1B1* promoter. In addition, also without the involvement of FXR and SHP, BAs can reduce nuclear binding activity and gene expression of *HNF4-alpha*, and consequently *OATP1B1* expression (123). OATP1B3 [also known as OATP8 (*SLC21A8*)] is a multispecific transport system involved in hepatic uptake of xenobiotics but not BAs (124). It is induced by FXR and it is believed to maintain hepatic uptake of xenobiotics even during cholestasis, while BA uptake systems are down-regulated. Thus, xenobiotics can enter the liver through OATP1B3 to undergo oxidation, conjugation and secretion into bile to be eliminated from the body.

Taken together, the studies on the regulation of hepatic BA uptake systems suggest that BAs can negatively regulate their own uptake in a feed-back fashion through the activation of a cascade involving the transcription factors HNF4-alpha, HNF1 -alpha and RAR-alpha, via an FXR-SHP dependent or independent pathway. Activation of FXR in the liver blocks BA uptake and induces their secretion into bile or portal vein, highlighting the protective role of FXR against cellular accumulation of these potentially toxic endogenous products. At the same time, hepatic activation of FXR promotes xenobiotic uptake allowing the liver to metabolize drugs and other exogenous chemicals even under cholestatic conditions. Finally, by inducing hepatic basolateral efflux of BAs during cholestasis, FXR shifts BA elimination from feces to urine.

5.3. Bile acid and xenobiotic metabolism (phase I and phase II)

Organisms have evolved a series of defense mechanisms to counteract the harmful effects of xenobiotics and endogenous products that accumulate to toxic levels. Thus, toxic levels of BAs, as in cholestasis, and xenobiotics can stimulate in a feed-forward mode their own metabolism by inducing the expression of *CYP, SULT* and *UGT* genes, responsible for phase I and II reactions.

In the liver, CYP3A4/Cyp3a11, is the predominant enzyme involved in this process, catalyzing the hydroxylation of a different subset of BAs, such as CDCA, LCA, DCA and their amidated derivates, at different positions leading to 3-oxo-, 1-beta-, 6-alpha- and 22-hydroxy BAs (125,126). Notably, CYP3A4/Cyp3a11 activity increases with the hydrophobicity of BAs, which is directly proportional to BA toxicity. In addition to BA metabolism, CYP3A4/Cyp3a11 is also important for the metabolism of xenobiotics and although the liver is the main organ predisposed to the biotransformation of exogenous chemicals, in the intestine CYP3A4/Cyp3a11 plays a key role in the first-pass metabolism of orally dosed drugs (127).

Phase II glucuronidation and sulfonation reactions are also important in the detoxification process (128,129). Associated to hydroxylation reactions, they

produce even more hydrophilic and less toxic molecules that are efficiently secreted into bile or urine. Thus, while 3-alpha-sulfated BAs are relatively abundant in healthy individuals (130), cholestatic patients show high levels of sulfonated and glucuronidated BAs (131), highlighting the important role of these metabolic pathways. Among SULT enzymes, SULT2A1 and Sult2a9 are key enzymes in phase II metabolism that catalyze sulfoconjugation of xenobiotics and BAs (57,132) and are predominantly responsible for the formation of 3-alpha-sulfated BAs. This process plays a protective role against BA toxicity as demonstrated in mice treated with LCA (133). Sulfated LCA is in fact less cytotoxic than LCA both *in vitro* and *in vivo* (134). Indeed, the role of SULT2A1 in subjects with cholestasis is of critical importance. BAs that accumulate in the liver of these subjects are turned more polar by SULT2A1 in a way that after being pumped out from the liver they are easily eliminated with urine.

Regarding glucuronide conjugation, three UGTs are meanly involved in BA conjugation. These are UGT2B4 for the glucuronidation at the 6-alpha-hydroxy position (135), UGT2B7 for the 3-alpha- and 6-alpha-hydroxy positions (136,137), and UGT1A3 for the modification of C_{24}-carboxyl groups to form acyl glucuronide derivates (136,137).

In the field of nuclear receptors, two major controllers of phase I and phase II metabolism are PXR and CAR. Both receptors are expressed in the liver and intestine (56), where they provide a two-layered resistance mechanism against toxic compounds. In human hepatic cells, activation of PXR and CAR induce *CYP3A4* expression (138).

The role of FXR in BA hydroxylation is less understood. Human hepatic HepG2 cells treated with the synthetic FXR ligand GW4064 increases *CYP3A4* expression (139), while in FXR knock out mice it was observed an increase in *Cyp3a11* expression (140,141), LCA 6-alpha-hydroxylation (133) and urinary secretion of hydroxylated BAs under cholestatic conditions (140) or when animals are fed with a CA-containing diet (44). Thus, FXR seems to act as negative regulator of *Cyp3a11* basal expression in rodents. However, since GW4064 induces *Cyp3a11* expression in both wild type and PXR-null mice, but not in FXR knock-out mice, activation of this BA sensor is important for the induction of *Cyp3a11* (139).

In humans and rodents, activation of PXR by ligands such as rifampicin regulates both hydroxylation and conjugation (142). Indeed, in addition to CYP3A4/Cyp3a11, PXR activation induces also the expression of sulfating and glucuronating SULT2A1 and UGT1A3/Ugt1a1 enzymes (143-145). Activation of CAR by ligands induces *Cyp1a11, Sult2a1* and Ugt1a1 and, although there is no direct evidence, it may be that the expression of *UGT1A3* is also induced by CAR. In fact, CAR can bind to *UGT1A1* promoter and induce the expression of this gene involved in conjugation of bilirubin (146,147). Since the CAR responsive element in the *UGT1A1* promoter gene is conserved among *UGT1A* genes, including *UGT1A3,* it is possible that CAR induces also this gene (148).

In addition to the hydroxylation process, the role of FXR is also unclear regarding the sulfonation process. Responsive elements for FXR have been found in the promoter of *SULT2A1* gene suggesting that FXR may induce BA sulfonation (39). However, it was also shown that CDCA fails to induce *SULT2A1* expression in human hepatocytes (149). Moreover, in FXR-null mice, both SULT2a mRNA and proteins levels, as well as 3-alpha-sulfonated BAs, are increased when animals are fed with a LCA-enriched diet (133). Thus, at least in rodents, FXR seems to function as repressor of BA sulfonation.

By regulating UGTs enzymes, FXR controls also BA glucuronidation in a tissue and isoform specific way (150,151). Activation of FXR in human hepatic cells induces the expression of *UGT2B4* for the glucuronidation of 6alpha-hydroxylated BAs (150), without affecting the expression of UGT1A3, an enzyme responsible for the glucuronidation of LCA and CDCA at their C_{24}-carboxyl position (136,137). Thus, activation of FXR induces 6-alpha-hydroxylation and subsequent glucuronidation without affecting the metabolism of hydrophobic BAs. Notably, *UGT2B4* is the only target of FXR described so far whose transcription can be induced by FXR without its heterodimeric partner RXR (150). Thus, by sequestering FXR, RXR may reduce the pool of monomeric FXR able to bind its single hexameric responsive element on *UGT2B4* promoter. In colon carcinoma CaCO2 cells, FXR acts as negative regulator of *UGT2B7*, a gene that encodes for an enzyme involved in the formation of 3-hydroxy-glucuronidated BAs (151). Although it seems that BAs such as LCA and CDCA can reduce *UGT2B7* expression *in vitro* (151), the ability of these BAs to repress its expression *in vivo* remains unclear.

The important role of PXR and CAR in regulating BA metabolizing enzymes has been highlighted in transgenic mice expressing human PXR and CAR. These animal models are resistant to LCA-induced liver damage due to the induced expression of *Sult2a9*, *Ugt1a6* and *Cyp3a11* genes (58,145,147,152). Interestingly, although PXR-null mice do not show induction of *Cyp3a11* and *Sult2a9* after treatment with PXR ligands, they have an increased basal expression of *Cyp3a11* and *Sult2a9* (58,89,133,153), and their sensitivity to LCA is not different from wild type mice (138). Thus, in the absence of PXR activators, *Cyp3a11* and *Sult2a9* expressions are repressed, while after ligand activation, PXR becomes an inducer of these metabolizing enzymes. Overall, these observations suggest that genes that encode for phase I and phase II enzymes under the control of PXR are low expressed in normal condition. These enzymes are induced after activation of PXR by exogenous chemicals or BAs reaching toxic concentrations to promote the detoxification of these endo- and xenobiotics. This would explain why sulfonated and glucuronidated BAs are present during cholestasis while they are almost absent in healthy subjects.

The *in vivo* models discussed above establish a critical role for PXR and CAR in preventing xenobiotic and BA toxicity. Indeed, they represent intriguing models for studying drug metabolism and developing new pharmacological approaches for the management of BA-related diseases such as cholestasis. However, these animal models exhibit some limits to fully understand the regulation of xenobiotic and BA metabolism in humans. For instance, BA glucuronidation is almost absent in rodents (154) where additional hydroxylation is the major metabolic pathway (155). Furthermore, the true orthologous of human *UGT2B4* and *UGT2B7* genes in rodent are unknown and the *Ugt1a3* gene contains a premature stop codon that determines the formation of a no-functional protein (156). Thus, to study the relevance of *UGT* gene regulation by PXR and CAR for xenobiotic and BA glucuronidation, a humanized mouse expressing the entire human *UGT1A* locus has been generated (154). However, other humanized mice for the different *UGT* genes still have to be generated. Moreover, a fully humanized model to study this aspect of BA and xenobiotic metabolism would require the generation of transgenic mice expressing both human UGT enzymes and human PXR and CAR receptors.

5.4. Bile acid and xenobiotic secretion (phase III)

After BAs and xenobiotics are turned more hydrophilic in the liver, they are pumped into bile as a route for fecal elimination. BSEP/Bsep and MRP2/Mrp2 mediate the export of BAs at the canalicular membrane of hepatocytes. The regulation of these transporters is of critical importance to prevent BA accumulation in the liver and consequent hepatic injury. Thus, BAs induce in a feed-forward fashion their own efflux in bile by up-regulating the expression of *BSEP* and *MRP2*, as shown in mice fed with a diet enriched in CA (112,157). The transcriptional mediator of BA up-regulation of *BSEP* is FXR (158-160). FXR-null mice have a reduced basal expression of *Bsep* and BAs are not able to induce *Bsep* expression under this genotype (52,141,157). The importance of the FXR-driven upregulation of *Bsep* has been recently highlighted also in a mouse model of cholesterol gallstone disease (161,162). Unlike *BSEP*, the expression of *MRP2* is not only induced by BAs, but also by PXR and CAR ligands (153,157,163). Indeed, FXR binds with high affinity to the rat *Mrp2* promoter in responsive elements that are also binding sites for PXR and CAR (163) and the human *MRP2* gene is also induced by FXR and PXR ligands (163). Thus, not only BAs, but also drugs able to activate PXR and CAR can regulate the expression of *MRP2*, with potential implications for drugs in BA homeostasis. Thus, while canalicular BA secretion via BSEP is regulated only by FXR, modulation of *MRP2* expression is more complex and involves the entire trio, if not multiple nuclear receptors.

After synthesis, hydroxylation and conjugation, BAs are normally secreted into bile by canalicular transporters. However, BAs can also be pumped out of the liver into the portal blood through an alternative basolateral way, which becomes the major route for hepatic elimination of BAs when canalicular secretion is compromised. The alternative basolateral export of BAs is mediated by members of the MRP family such as MRP3 and MRP4, besides organic solute transporter OST-alpha/beta (164). Notably, in normal conditions these basolateral transporters are expressed at very low levels, since canalicular secretion is the preferential route used by the liver to secrete BAs. On the contrary, when the liver is loaded with BAs, like in experiments with BA feeding, and in murine models of cholestasis or in human cholestasis, basolateral transporters are dramatically induced (107,165,166). MRP3/Mrp3, MRP4 and Ost-alpha/beta transport sulfate and glucuronidated BAs that are eliminated from the liver through the basolateral membrane into the portal system for urinary elimination. Thus, hyper-activation of these transporters during cholestasis may account for the shift toward renal excretion of BAs as a major mechanism for their elimination. The overexpression of murine Mrp3 and Mrp4 after bile duct ligation or BA feeding is independent of FXR (141,157,167) while it has been speculated that PXR could mediate their expression (141). Indeed, PXR ligands can induce *MRP3* but not *MRP4* expression (168,169). With regard to CAR, it seems to regulate both *Mrp3* (167,170) and *Mrp4* (167,169,171).

Although FXR does not regulate the expression of *MRP3* and *MRP4,* it can contribute to the hepatic basolateral export of BAs during cholestasis through the up-regulation of OST-alpha/beta. Indeed, recent studies have shown two functional responsive elements for FXR in the human *OST-alpha* and *OST-beta* gene (172). Moreover, studies in FXR knock-out mice have shown a reduced basal expression of *Ost-alpha/beta* when compared to their wild-type littermate and the induction of *Ost-alpha/beta* expression was abolished after bile duct ligation, administration of FXR ligand or feeding CA (173).

FXR, PXR and CAR are required for the coordination of adaptive basolateral BA efflux under BA over-load in cholestatic conditions. In fact, basolateral excretion is an alternative way for BA elimination in order to protect the liver from accumulation of toxic BAs. Induction of this adaptive pathway along with induction of phases I and II BA detoxification pathways explains the shift toward renal BA elimination in patients with cholestasis. Although CAR, unlike PXR and FXR, is not activated by BAs, other compounds that accumulate in the liver during cholestasis such as bilirubin can activate to some extent CAR (174,175), explaining the over expression of CAR target genes as *Mrp4* during cholestasis.

5.5. Intestinal bile acid absorption.

After reaching the distal ileum, conjugated BAs are actively and efficiently absorbed into the portal system and returned back to the liver to complete their enterohepatic circulation. On the contrary, unconjugated BAs are only partially absorbed by passive diffusion. Active absorption of BAs takes place at the ileal enterocyte brush border membrane through the apical sodium-dependent BA transporter [ASBT, (*SLC10A2*)] (176,177).

Once BAs are taken-up by the enterocyte, ileal BA binding protein (IBABP) shuttles BAs from the apical to basolateral membrane (178-180). This intracellular transport activity of IBABP is challenged by the evidence that its absence in mice does not negatively interfere with intestinal absorption and entero-hepatic cycling of CA (53). BAs are transported through the basolateral membrane of the enterocytes into the portal system by the heteromeric organic solute transporter OST-alpha/beta (164). This entire process is orchestrated by FXR, which is highly expressed in the distal ileum. Upon activation by BAs, FXR appears to down-regulate the expression of *ASBT* while inducing the expression of *IBABP* (53,181) and *OST-alpha/beta* (182,183). With regard to *IBABP* and *OST-alpha/beta*, FXR responsive elements have been found on their promoters and their expression is directly regulated by FXR. Differently, the repression of *ASBT/Asbt* is indirectly regulated by FXR through SHP. The regulation of the expression of *ASBT* seems to be different among species. In mice, but not in rats that lack of a LRH-1 responsive element on the *Asbt* promoter, BAs exert a negative feedback on *Asbt* expression by FXR via SHP-mediated repression of LRH-1 activity (184). In humans, SHP represses *ASBT* expression by interfering with the heterodimer RXR-alpha/RXR-alpha, which is involved in the up-regulation of *ASBT* (185).

In addition to the intestine, transport systems for BA re-absorption exist also in bile ducts and in kidneys. Cholangiocytes contain several transport systems for the absorption and secretion of BAs. While unconjugated BAs can passively enter cholangiocytes, conjugated BAs are re-absorbed via Asbt. Basolateral export of BAs is mediated by Mrp3 and Ost-alpha/ beta, which is supposed to be the counterpart of Asbt (186). During cholestasis, the expression of these transporters is up-regulated. Thus, increased *Asbt* expression is important for the removal of BAs from the stagnant bile in the biliary duct during cholestasis due to bile duct obstruction.

In the kidney, BAs are reabsorbed by *ASBT* localized at the apical membrane of proximal renal tubular cells and then secreted by the basolateral OST-alpha/beta into the systemic circulation (186-188). Thus, in normal conditions no BAs are excreted into urine. On the contrary, during cholestasis, when BAs are secreted from the liver into the portal blood, passive glomerular filtration, due to elevated serum BA levels, and repression of Asbt (189) can account for the lost of BAs with urine. Moreover, since BA feeding and bile duct ligation (BDL) not only decrease *Asbt* (189), but also increase *Mrp2* and *Mrp4* expression in the apical tubular membrane (190-192), it may be that passive glomerular filtration is aided by active tubular secretion of sulfated and glucuronidate BAs through these two ABC transporters. Although these events represent an important alternative route for BA elimination during cholestasis (193), the knowledge regarding the transcriptional regulation of these membrane transporters by nuclear receptors is limited to OST-alpha /beta, which are directly induced by FXR (172).

6. CLINICAL RELEVANCE OF FXR, PXR AND CAR IN ENDOBIOTIC AND XENOBIOTIC METABOLISM

Today, more than 10% of prescription drugs available on the pharmaceutical market exert their effects by modulating nuclear receptor activity. Examples with proven clinical utility are drugs targeting the glucocorticoid receptor for the management of inflammatory disorders, the estrogen and androgen receptors for the treatment of breast and prostate cancer, retinoid receptors for leukemia and peroxisome proliferators-activated receptor for diabetes and dyslipidemia. In this scenario, activators of FXR are also being evaluated for their potential in the treatment of liver diseases associated with impaired BA homeostasis. Moreover, although the primary role of PXR is to mediate the response of our body to xenobiotic stress, it could be a pharmacological target because of its ability to induce also endobiotic detoxification. Finally, CAR, which shares several overlapping properties with its close relative PXR, seems to respond also to metabolic and nutritional stress suggesting intriguing possibilities also for this nuclear receptor as a drug target.

6.1. Cholestasis

Bile secretion is an important excretory route of conjugated drugs and endogenous waste products. When cessation or impairment of bile flow occurs, a medical condition termed cholestasis is determined with retention of toxic biliary compounds such as BAs, bilirubin and xenobiotics in the liver, where they can induce fibrosis, eventually leading to cirrhosis and ultimately liver failure. The causes of cholestasis are broad; however, modifications in expression or function of transport systems required for bile formation represent a hallmark of this disease. These modifications can be acquired because of inflammation, drug administration (tamoxifen, chlorpromazine, macrolides), and anatomical obstruction of the bile duct or may be due to hereditary defects. In this respect, progressive familial intrahepatic cholestasis type 1 (PFIC1), also called Byler disease, affects subjects with mutations in FIC1, a putative aminophospholipid transferase (194). PFIC2 is characterized by loss of function mutations in *BSEP*, the canalicular BA export pump (195). Finally, PFIC3 is observed in patients with mutations in *MDR3*, the canalicular phospholipid flippase responsible for transporting phospholipids into bile (196).

Physiologic hepatic reaction against cholestasis consists of adaptive responses aimed at reducing BA synthesis and increasing detoxification and clearance of BAs, bilirubin and toxins. Thus, in animal models of cholestasis and in patients with cholestatic liver disease, *CYP7A1* and *CYP8B1* are down-regulated in parallel to increased phase I and phase II reactions. Simultaneously, *NTCP* and *OATP* expression is minimized while that of *MRP3*, *MRP4* and *OST-alpha/beta* is induced to favour BA secretion versus BA uptake at the basolateral membrane. In normal conditions, *MRP3, MRP4* and *OST-alpha/beta* are only weakly expressed to allow BAs to exit the liver from the canalicular membrane trough MRP2 and BSEP, which are also up-regulated during cholestasis to increase BA

secretion into bile. However, when BSEP or MRP2 do not work properly, as in the case of cholestasis, the only alternative way for hepatic BA secretion becomes the basolateral membrane. By this route, BAs are secreted into the systemic circulation and this accounts for the shift of BA elimination from feces to urine.

Although the intrinsic hepatic adaptive response in enzyme and transporter expression during cholestasis is beneficial in limiting hepatic accumulation of BAs, bilirubin and xenobiotics, it cannot prevent liver damage. Nevertheless, one could think to enhance or even initiate earlier the adaptive response in the history of cholestatic disease to get further beneficial effects. In this respect, the discovery of FXR, PXR and CAR as master regulators of BA and xenobiotic metabolism renders this trio of nuclear receptors a suitable pharmacological target for the management of cholestasis. Regarding to FXR, its activation can mediate many of the adaptive changes observed during cholestasis, including up-regulation of *BSEP*, *MDR3*, *MRP2*, *IBABP*, *OST-alpha/beta* and down-regulation of *NTCP*, *CYP7A1* and *CYP8B1*, suggesting a protective role for this BA sensor against cholestasis. In line with this, FXR- null mice are unable to modulate FXR-regulated events in response to CA feeding resulting in increased liver toxicity and mortality, further emphasizing the essential role of FXR in the adaptive response against cholestasis (52). Thus, a growing interest for developing FXR agonists has emerged during recent years and some compounds have been shown to be protective in rat and mouse models of cholestasis. Indeed, treatment with GW4064, a potent and specific synthetic ligand for FXR, reduces inflammation, bile duct proliferation and liver damage induced by alpha-napthylisothiocynate and BDL (197), two experimental setups commonly used to induce cholestasis. Moreover, both GW4064 and 6-ethyl chenodeoxycholic acid, another potent synthetic ligand for FXR, protect rats from ethinyl estradiol-induced cholestasis by increasing the expression of *Mrp2*, *Bsep*, and reducing the expression of *Cyp7a1*, *Cyp8b1* and *Ntcp* (198). Notably, both GW4064 and 6-ethyl chenodeoxycholic acid are not able to protect the liver of FXR-null mice from experimental cholestasis, underlining the specificity and necessity of FXR for the adaptive response in cholestasis.

In addition to FXR, animal studies suggest that also PXR and CAR are important in the adaptive response in cholestasis. In fact, both PXR- and CAR-null mice are predisposed to more significant cholestatic liver injury than littermate wild-type (199). Indeed, agonists of this nuclear receptor may be of therapeutic interest. With regard to PXR, activation of this xenobiotic sensor by the potent ligand rifampicin has been shown to improve liver function in patients with primary biliary cirrhosis (PBC) (200,201). Also, after BDL, CAR and PXR are able to reduce serum BA and bilirubin concentration, through an increased urinary disposal. On the other hand, the clinical value of PXR and CAR agonists is somewhat limited by the evidence that they increase the serum levels of markers of hepatocyte injury (ALT), and display no effect on liver morphology (167). Of note, the agonists for CAR and PXR were already used in the clinical setting, time before their pharmacological activity was known. Phenobarbital (a CAR agonist) was used in patients with Crigler Najjar type 2 to induce the residual activity of the enzyme UGT1A1. Additionally, rifampicin (a PXR agonist) was employed as a palliative for pruritus in cholestasis (202). Since PXR inhibits *CYP7A1* expression (203), the benefit observed after rifampicin treatment could be related to the reduced BA synthesis. Moreover, activation of PXR by rifampicin can also induce *CYP3A*, *UGT1A1* and *MRP2* mRNA levels, as observed after two weeks treatment in patients with gallstone disease prior to surgery (204,205). Notably, the induction of *CYP3A* and *UGT1A1* by PXR agonists should also account for increased BA glucuronides found in urine of healthy volunteers (142).

Finally, also CAR is involved in the adaptive response during cholestasis by mediating both BA and bilirubin clearance. CAR plays an important role in the detoxification of BAs by stimulating their sulfation through the induction of Sult2a9 (152) and Sult2a1 (171), and hepatic BA secretion at the basolateral membrane through Mrp4 (171). These effects are not observed in CAR-null mice. Activation of CAR is necessary and sufficient to mediate resistance to the hepatotoxic effects of LCA (152). With regard to bilirubin detoxification, activation of CAR by Yin Shi Huang herb in humanized CAR mice, but not in CAR-null mice, induces all the steps required for hepatic bilirubin clearance, including bilirubin uptake transporter Oatp1b3, cytosolic binding proteins Gsta1 and GSTA2, bilirubin conjugating enzyme Ugt1a1 and bilirubin conjugate export pump Mrp2. Moreover, activation of CAR by Yin Shi Huang herb and phenobarbital reduces jaundice in neonates and lowers both BA and bilirubin serum levels in patients with primary biliary cirrhosis (175,206). However, CAR appears to be a key regulator of acetaminophen (APAP) hepatotoxicity in mice and probably also in humans. Indeed, the activation of murine and human CAR by its agonists or acetaminophen itself leads to increased expression of three APAP-metabolizing enzymes and to the subsequent increase of its toxicity (207). By contrast, the role of PXR in this process is not clear since in two different studies on single and double knock-out (CAR and PXR knock-out mice) the results are opposed, although both CAR and PXR are involved in protection against hydrophobic bile acid such as LCA (208, 209).

In summary, FXR, CAR and PXR display coordinated protective effects in cholestasis. Indeed, combination of specific ligands for these nuclear receptors may be useful to maximize the expression of their target genes involved in BA, bilirubin and xenobiotic detoxification. Interestingly, whether during cholestasis hepatic uptake of BAs is reduced, xenobiotics continue to be taken up by the liver. Thus, theoretically pharmacological activation of FXR, PXR and CAR would not only protect the liver from BA accumulation, but also from the harmful effects of xenobiotics by inducing their biotransformation and excretion, if not at the canalicular membrane via MDR1, at least at the basolateral membrane via MRP3, MRP4 and OST-alpha/beta for final elimination with urine.

6.2. Cholesterol detoxification

Cholesterol is an essential precursor of steroid hormones and it is required for cell membrane formation. On the other hand, cholesterol may elicit harmful effects through its metabolites such as oxysterols, bile alcohols and BAs. These products of cholesterol catabolism are in fact responsible for premature cardiovascular diseases, neurological disorder and liver diseases. Thus, efficient clearance of excess of cholesterol and its metabolites is of critical importance. Although PXR is defined as a xenobiotic receptor that serves to defend against potentially toxic exogenous chemicals, it also induces detoxification of metabolic intermediates produced by the body itself. *In vitro* assays have showed that chemicals with a steroid backbone, such as oxysterols, are able to activate PXR. Moreover, mice lacking sterol 27-hydroxylase, an enzyme involved in the conversion of cholesterol to BAs, have elevated concentrations of BA precursors such as 5-beta-cholestane-3-alpha, 7-alpha, 12-alpha-triolo, and consequently higher PXR activity. Indeed, PXR can be activated *in vivo* by cholesterol metabolites in a feed-forward fashion that induces their own detoxification, suggesting a protective role of PXR against cholesterol toxicity.

The protective role of PXR in cholesterol metabolism has been shown *in* PXR-null mice fed a diet enriched in cholesterol and CA (210). Feeding rodents with this kind of diet increases hepatic cholesterol and catabolic products because of the ability of CA to both stimulate intestinal cholesterol absorption and decrease hepatic cholesterol breakdown. Surprisingly, when challenged with a high-cholesterol diet, PXR-null mice, which do not show a phenotype under normal conditions, died after two months of treatment. On the contrary, no lethality was observed in wild-type mice (210). Thus, PXR seems to protect against the acute toxicity of a high-cholesterol diet. This effect could be explained in two ways. First, activators of PXR such as PCN increase the concentration of cholesterol into bile, suggesting that PXR may regulate cholesterol homeostasis. Second, PXR can protect against acute cholesterol toxicity by increasing the hepatic expression of *Cyp3a11* and *Oatp1a4* under a cholesterol/CA diet in wild-type but not in PXR-null mice. Thus, this kind of diet eventually produces an agonist for PXR. *Cyp3a11* is involved in the hydroxylation of xenobiotics and endogenous steroids, including cholesterol (211). Oatp1a4 is a transporter located on the sinusoidal membrane of hepatocytes to transport into the liver conjugated and unconjugated BAs and steroids that have to be metabolized and excreted from the body. Interestingly, activation of CAR, which induces *Cyp3a11* expression but not *Oatp1a4* expression, does not rescue the observed phenotype in PXR-null mice when fed the cholesterol/CA diet. Thus, it has been supposed that PXR protects from acute cholesterol toxicity thought Oatp1a4.

Cholesterol toxicity is always thought to occur over long periods of time as in cardiovascular disease, but these studies on PXR-null mice have also revealed the existence of acute cholesterol toxicity under a high-cholesterol diet and in the absence of PXR. Unfortunately, so far it is unknown which chemical kills PXR-null mice under a high-cholesterol diet and which is the PXR ligand generated by this experimental diet. Moreover, it is also unclear if these findings can be translated to humans, and if polymorphisms and mutations of PXR in humans may account for alterations in cholesterol metabolism and consequent liver disease. If so, pharmacological or genetic approaches could be developed to restore liver function.

6.3. Drug-Drug interaction

Every day we confront with exogenous chemicals such as toxins, environmental pollutants, and prescription and non-prescription drugs. Detoxification and clearance of these xenobiotics from our body are of critical importance to avoid their accumulation up to toxic levels. This process is largely mediated by the supergene family of cytochrome 450 (CYP) enzymes, which catalyzes the conversion of such compounds to polar derivates that are more readily eliminated (212,213). Among CYP enzymes, CYP3A and CYP2B are of particular clinical relevance since there are involved in the metabolism of almost 60% and 20% of prescription drugs, respectively. The metabolic versatility, combined with their inducibility by many different pharmaceutical compounds, represents the molecular basis for the vexing clinical problem of drug-drug interaction. This problem is well known when P450 inducers such as glucocorticoids, phenobarbital or rifampicin are administrated with drugs that are normally metabolized by CYP enzymes. Thus, since CYP enzymes recognize a large spectrum of drugs, a compound that induces CYPs is potentially able to influence the metabolism of any co-administered drug. In this regard, St. Jone's wort, a popular herbal remedy for depression, by activating CYP3A increases the metabolism and decreases the bioavailability of co-consumed drugs such as oral contraceptives, the immunosuppressant cyclosporine and the HIV protease inhibitor indinavir (214-216).

The molecular mechanism for drug induction of *CYP3A* and *CYP2B* gene has been elusive for many decades. This was due in part to the fact that a diverse range of chemical structures trigs the xenobiotic response, and in part to the fact that, unlike for conserved hormonal responses, the induction of *CYP* genes is species specific. For instance, the anti-glucocorticoid PCN induces *Cyp3A* only in rodents while the antibiotic rifampicin is a specific *CYP3A* inducer in humans but not in rodents. This divergence among species is mainly due to differences in the LBD of the xenosensor nuclear receptors PXR and CAR that mediate the transcriptional activation of *CYP* genes by functioning as pleiotropic receptors for promiscuous endo- and xenobiotics. Probably, the evolutionary need of responding to a different set of ingested nutrients and xenobiotics may account for these structural and pharmacological differences. Animal models with humanized xenobiotic response would be of significant practical use in drug development. In fact, rodents are used in the preclinical phase of new candidate drugs to assess toxicity, therapeutic index and pharmacokinetic. However, rodents are highly unreliable predictors of human drug response and drug-drug interaction problems because of the species-specificity of the xenobiotic response.

The generation of humanized transgenic mice for PXR on a mouse PXR-null background (58) has represented a milestone in view of a murine toxicological model that could be used in preclinical phase studies for the screening of new human candidate drugs. These mice allow the study of toxicity, therapeutic index, pharmacokinetics and drug-drug interaction of new developed drugs in the exclusive presence of the human PXR receptor. Thus, they exhibit response to human inducers such as rifampicin and show similar pharmacokinetics of CYP3A regulation (58).

Given the problem of drug-drug interaction and its unpredictability, a drug that also activates PXR would be of lower benefit than a PXR neutral drug. Indeed, a therapeutically active but PXR-neutral drug would represent the best choice during drug screening. In this context, a humanized mouse model for PXR would represent an important step for the development of safer drugs. In the past years, a body of evidence has highlighted an important role not only for PXR, but also for CAR in the regulation of drug-metabolizing *CYP* enzymes by functioning as pleiotropic receptor for promiscuous endo- and xenobiotics. CAR, like PXR, regulates the expression of genes involved in drug metabolism and exhibits species-dependent ligand specificity. For example, TCPOBOP and androstenol affect CAR activity in mice but not in humans, while the antimycotic clotrimazol is an efficacious deactivator of human but not mouse CAR (217). Notably, both xenobiotic receptors are highly expressed not only in the liver but also in the gut. The intestine is another important district of our body involved in the absorption, metabolism and clearance of xenobiotics. It represents the first-pass drug metabolism for orally dosed drugs by expressing drug metabolizing enzymes and drug transporters. Thus, a more completely humanized mouse model for the xenobiotic response should express the human xenobiotic receptors in both liver and intestine. Even better, to create a valuable complement to humanized PXR mice, a double knockout mouse for PXR and CAR crossed with a double transgenic mouse for human PXR and CAR will give the possibility to simultaneously monitor the xenobiotic response to a drug that activates both these nuclear receptors using just one mouse model. This would make the mouse model even more close to the human condition.

7. CONCLUSIONS AND PERSPECTIVES

In this review we highlighted the transcriptional regulatory role of the nuclear receptor trio FXR, PXR and CAR in the maintenance of BA and xenobiotic metabolism and clearance. Recently discovered molecular pathways have better elucidated the different adaptive responses to dietary and metabolic noxae. Studies predominantly performed in mice have shown that activation of FXR, PXR and CAR network is efficient in protecting the liver from cholesterol-derivatives or -precursor toxic molecules as well as xenobiotics and BA accumulation during cholestasis. We believe that it is now time to move forward. If one considers cholestasis as a systemic disorder, the reduction of bile components reaching the intestine will definitively play a causative or aggravating role in this condition. Intriguing scenarios have recently revitalized the connection between intestine and liver with a novel view on the entero-endocrinology. Thus, reactivation of the signaling in a bile depleted gut-liver axis might result in a clinical benefit for cholestatic patients. Strategies aiming at targeting cholestasis through intestinal specific routes are timely solicited at this stage.

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Abbreviations: ABC, ATP-binding cassette; AF, activation function domain; ASBT, apical sodium-dependent bile acid transporter; BAs, bile acids; BACAT, bile acid-CoA:amino acid *N*-aceyltransferase; BACS, bile acid CoA synthase; BDL, bile duct ligation; BSEP, bile salt export pump; CA, cholic acid; CAR, constitutive androsane receptor; CCRP, cytoplasmatic CAR retention protein; CDCA, chenodeoxycholic acid; CYP, cytochrome P450; DBD, DNA binding domain; DCA, deoxycholic acid; DR, direct repeat; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FXR, farnesoid X receptor; GST, glutathione-S- transferase; HNF, hepatocytes nuclear factor; HRE, hormone response element; HSP, heat shock protein; IBABP, ileal bile acid binding protein; LBD, ligand binding domain; LCA, lithocolic acid; LRH-1, liver receptor homolog 1; MDR, multidrug protein; MRP, multidrug-related protein; N-CoR, nuclear receptor corepressor; NR, nuclear receptor; NTCP, Na^+ - taurocholate cotransport protein; OATP, organic anion transporting polypeptide; OST, organic solute transporter; PBC, primary biliary cirrhosis; PCN, pregnenolone-16a-carbonitrile; PFIC, progressive familial intrahepatic cholestasis; PXR, pregnane X receptor; RAR, retinoid acid receptor; RXR, retinoid x receptor; SHP, small heterodimer partner; SLC, solute carrier; SULT, sulfotransferase; SXR, steroid xenobiotic receptor; TCPOBOP, 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene; UDCA, ursodeoxycholic acid; UGT, UDP-glycosyltransferase

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