REVIEW

Bile Acids, Nuclear Receptors and Cytochrome P450

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Summary

This review summarizes the importance of bile acids (BA) as important regulators of various homeostatic mechanisms with detailed focus on cytochrome P450 (CYP) enzymes. In the first part, synthesis, metabolism and circulation of BA is summarized and BA are reviewed as physiological ligands of nuclear receptors which regulate transcription of genes involved in their metabolism, transport and excretion. Notably, PXR, FXR and VDR are the most important nuclear receptors through which BA regulate transcription of CYP genes involved in the metabolism of both BA and xenobiotics. Therapeutic use of BA and their derivatives is also briefly reviewed. The physiological role of BA interaction with nuclear receptors is basically to decrease production of toxic non-polar BA and increase their metabolic turnover towards polar BA and thus decrease their toxicity. By this, the activity of some drug-metabolizing CYPs is also influenced what could have clinically relevant consequences in cholestatic diseases or during the treatment with BA or their derivatives.

Key words

Bile acids • FXR • PXR • Cytochrome P450

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Introduction

The essential physiological role of bile and bile acids (BA) in digestion is to neutralize chyme and serve as emulsifiers of fat in small intestine. Thanks to their

amphiphilic nature, BA are emulsifiers which enable absorption of lipids and lipid soluble vitamins. The production and secretion of bile is regulated by intestinal paracrine hormones cholecystokinin and secretin and moreover autoregulation *via* negative feedback exists, too. BA and phospholipids stabilize micellar dispersion of cholesterol in the bile and facilitate cholesterol excretion as well as excretion of hydrophobic metabolites of xenobiotics, toxins and metals. In past decade, it has been postulated that bile acids may also regulate lipid and glucose homeostasis, thermoregulation, and immune response (Caudel *et al.* 2011). Especially the role of BA in immune response is undoubtedly involved in the therapeutic effects of some BA in cholestatic liver diseases (Roma *et al.* 2011, Buryova *et al.* 2013).

The most common human BA (Fig. 1) are cholic acid (CA), chenodeoxycholic acid (CDCA), in much less proportion also glycocholic acid, taurocholic acid (TCA), lithocholic acid (LCA), deoxycholic acid (DCA), and ursodeoxycholic acid (UDCA). BA and oxysterols are natural ligands of several nuclear receptors (NRs), membrane receptors and regulators of metabolism of lipids and glucose (Chiang 2004, Chiang 2009). At least, some of BA regulate above mentioned pathways *via* their farnesoid X receptor (FXR) agonistic activity. In particular, FXR agonists probably *via* production of glucagon-like peptides 1 and 2 increase insulin sensitivity, glucose uptake, and adipogenesis in extrahepatic tissues. Meanwhile, they increase fatty acid oxidation, decrease triglyceride, fatty acid, and cholesterol synthesis in the liver and increase insulin production in beta cells (Camilleri and Gores 2015, Adorini *et al.* 2012). BA as FXR agonists also decrease

activity of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase and thus, together with all above mentioned effects increase glucose tolerance and insulin sensitivity (Chiang 2013). BA also promote gut motility through TGR5 activation (Camilleri and Gores 2015) which is in agreement with reported adverse effects of therapeutically used BA (American Society of Health System Pharmacists 2016a, American Society of Health System Pharmacists 2016b). Primary BA are deconjugated and dehydroxylated by microflora, and these BA metabolites exert antimicrobial properties (Begley *et al.* 2005). Another physiological consequence of BA binding on FXR is increased synthesis of fibroblast growth factor-19 (FGF-19), which may reduce glycolysis and lipogenesis, improved insulin sensitivity, and reduce bile acid synthesis (Camilleri and Gores 2015).

Fig. 1. Unconjugated primary and secondary bile acids in humans.

In agreement with above mentioned physiological roles of BA, some BA may be also exploited or tested as therapeutic agents, mostly in cholestatic liver diseases, inflammatory bowel disease (Gadaleta *et al.* 2010), diabetes mellitus and metabolic disorders (part Therapeutic use of BA).

This review summarizes the importance of BA as important regulators of various homeostatic mechanisms with detailed focus on cytochrome P450 (CYP) enzymes.

Synthesis, metabolism and circulation of bile acids

BA are synthesized in the liver from cholesterol (“classical pathway”) in multiple steps catalyzed *via* CYPs, hydroxy-delta-5-steroid dehydrogenase (HSD3B7), Δ^{4-3} -oxosteroid-5 β -reductase (AKR1D1) and 3 α -hydroxysteroid dehydrogenase (AKR1C4) to form 5 β -cholestan-3 α ,7 α ,12 α -triol. Biosynthetic pathway of CDCA leads directly to 5 β -cholestan-3 α ,7 α -diol *via* AKR1D1 and AKR1C4. Subsequently, those by products are hydroxylated in the position 27 and the hydroxylic group is then oxidized to aldehydic and carboxylic group. The products are further ligated to coenzyme A (CoA) and the side chain is shortened by β -oxidation to release – propionyl-CoA and cholyl-CoA or chenodeoxycholyl-CoA. Under physiological conditions, CA and CDCA occurs as Na⁺ salts („bile salts“) (Chiang 2004). Then, primary BA are conjugated with glycine and taurine. UDCA is 7-epimer of chenodeoxycholic acid and therefore is more hydrophilic than its structural analogue CDCA (Fig. 1) and forms about 4 % of BA pool (Roma *et al.* 2011). Hydroxylation in the positions 6 α / β or 7 β increases water solubility and decrease toxicity of BA (Chiang 2013).

Apart from this “classic pathway”, alternative (“acidic”) biosynthetic pathway exists in humans, which forms less than 10 % of total BA. In this pathway, which is believed to occur also in extrahepatic tissues, cholesterol is hydroxylated by series of steps (e.g. CYP27A1, CYP46A1, HSD3B7, 3 β HSD), but finally has to be transported into the liver to complete synthesis of CA and CDCA (Chiang 2004, 2009; Russell 2000).

Conjugated BA are stored in gall bladder and secreted into the duodenum in response to ingestion of meal. Then, about 95 % of BA is reabsorbed in ileum, mostly by apical Na⁺-dependant bile salt transporter (SLC10A2 or ASBT) (Camilleri and Gores 2015b). Resorbed BA are transported to the liver in blood through *vena portae* (Chiang 2013). BA which are not reabsorbed undergo deconjugation and dehydroxylation by intestinal flora to form secondary BA, LCA, and DCA, of which the most is excreted in faeces (Chiang 2009, Chiang 2013).

Bile acids – ligands of nuclear receptors

For many years, BA were thought to be fat emulsifiers and digestive surfactants as the only function of

the BA in the human body. However, recent observations in the last decade documented that BA are involved in the regulation of more complex processes including bile production, glucose and lipid metabolism, and in the modulation of immune response (Renga *et al.* 2013, Trauner *et al.* 2010, Claudel *et al.* 2005). The regulatory role of BA is a result of their interaction with various types of receptors including both intracellular nuclear receptors (NR) and cell surface membrane receptors (MR). BA acids vary in their ability to bind and activate different types of receptors. CDCA together with DCA are the most potent activators of FXR (Parks *et al.* 1999, Makishima *et al.* 1999), while LCA is the most potent activator of membrane G-protein coupled receptor TGR5 (Sato *et al.* 2008). Except of regulation of energy metabolism and immune system reactions, stimulation of different NRs and MRs by BA helps to maintain bile acid homeostasis *via* targeting the processes of their synthesis, release, reabsorption or metabolism (Copples and Li 2016).

BA are activators/ligands of various NRs, at least their ability to influence FXR, pregnane X receptor (PXR), vitamin D receptor (VDR), and liver X receptor (LXR) is well documented (Table 1, Chiang 2013, Song and Liao 2000). On the other hand some NRs which do not interact with BA, such as constitutive androstane receptor (CAR), can significantly influence the metabolism of BA. Most of these NRs are highly expressed in tissues exposed to high levels of BA like are liver or intestine (Kliewer *et al.* 1998, Forman *et al.* 1995, Gascon-Barré *et al.* 2003, Qatanani and Moore 2005).

NRs are ligand activated transcription factors consisting of N-terminal DNA binding domain (DBD) necessary to recognize the DNA response element in the target genes and C-terminal ligand-binding domain (LBD) interacting usually with small hydrophobic molecules. Activation of NRs by bile acids results in restriction of BA synthesis and increase of their degradation and excretion.

Table 1. Bile acids, their derivatives and metabolites as ligands of nuclear receptors.

Receptor	Ligands	Reference
FXR	CDCA>DCA>LCA>CA>UDCA	Makishima <i>et al.</i> 1999, Parks <i>et al.</i> 1999)
	Bile alcohols, 6 α -ethylCDCA	Pellicciari <i>et al.</i> 2002
	5 β -cholanoic acid, 5 β -norcholanoic acid, and 5 α -cholanoic acid	Sepe <i>et al.</i> 2016
PXR	3-keto-LCA>LCA>CDCA>DCA>CA	Taudinger <i>et al.</i> 2001
	7 α -OH-4-cholesten-3-one	Goodwin <i>et al.</i> 2003
VDR	LCA, 3-keto LCA	Makishima <i>et al.</i> 2002
LXR	Cholestenoic acid	Song and Liao 2000
	6 α -hydroxylated BA	Song <i>et al.</i> 2000
	Oxysterols	Janowski <i>et al.</i> 1996, Lehmann <i>et al.</i> 1997

Farnesoid X receptor (FXR; NR1H4)

There are two genes (*FXR α* and *FXR β*) coding the FXR. *FXR β* represents a functional NR in mammalian species except of primates and humans, where the gene encodes the non-functional protein (Otte *et al.* 2003). *FXR α* encodes four isoforms of FXR different either in the use of promoters or in alternative splicing (Zhang *et al.* 2003). The structure of FXR corresponds with the general structure of NR with the LBD allowing receptor heterodimerization with retinoid X receptor (RXR) and interactions with co-regulators. After interaction with agonist, FXR binds with RXR response elements as heterodimer FXR/RXR or as

a monomer and regulates the gene expression (Ding *et al.* 2015).

FXR is mainly found in hepatocytes, enterocytes (Forman *et al.* 1995) and acts as a sensor in the enterohepatic system regulating BA homeostasis. BA are potentially toxic and their levels have to be strictly controlled. In the hepatocytes, FXR controls BA synthesis (*via CYP7A1*, *CYP8B1*), sinusoidal uptake, and canalicular secretion of BA. In the intestine, FXR regulates the absorption, trafficking from the apical to the basolateral membrane and basolateral efflux (Modica and Moschetta 2006). FXR plays a protective role against BA toxicity by feedback inhibition of *CYP7A1*, *CYP8B1*, and

CYP27A1 expression (Copples and Li 2016). The regulation of expression of above mentioned genes is not direct, but mediated *via* small heterodimer partner (SHP) interacting with liver-related homolog-1 (LRH-1) or hepatocyte nuclear factor 4 α in the liver (Kwong *et al.* 2015) and fibroblast growth factor 19 (FGF19) binding to FGF receptor 4 in the intestine (Kir *et al.* 2011).

BA can activate the FXR in their free and conjugated forms. The most powerful ligand of FXR is CDCA with the EC₅₀ approximately 10 μ mol/l (Ding *et al.* 2015). Other endogenous BA bind to FXR with lower affinity as follows: CDCA>DCA $\approx$ LCA>CA>UDCA (Makishima *et al.* 1999, Parks *et al.* 1999).

Pregnane X receptor (PXR; NR1I2)

PXR acts mainly as a xenobiotic sensor. It is activated by steroidal substances including glucocorticoids and its main role is to form a barrier protecting inner environment from xenobiotics. Therefore, its highest expression was found within liver and intestine (Kliwer *et al.* 1998).

The PXR LBC is substantially larger than in other NRs and is poor in the number of polar groups. Such characteristic explains the high promiscuity and variability of its ligands (Handschin and Meyer 2005). PXR is activated for example by glucocorticoids, steroids, macrolide antibiotics, antifungals, and some herbal extracts (Jones *et al.* 2000, Lehmann *et al.* 1998, Ihunnah *et al.* 2011). From bile acids the most potent ligand of PXR is LCA with the EC₅₀~10 μ mol/l (Staudinger *et al.* 2001). It is questionable whether BA can activate the receptor under physiologic conditions when their plasma levels are below 100 nmol/l. It is hypothesized, that sufficient concentration can be reached after rupture of intrahepatic bile duct in cholestasis, when PXR can increase BA clearance by *CYP3A* induction and decrease its biosynthesis by suppression of *CYP7A1* expression (Copples and Li 2016). After binding the ligand it is translocated into nucleus and associated with RXR to form a heterodimer activating gene transcription.

The range of regulated genes is wide including many of phase I (Table 2) and II enzymes, or uptake and efflux transporters. The role of PXR in the expression of drug metabolizing enzymes is well documented by the study of Kandel *et al.* (2016), who treated human hepatocytes from 6 donors with rifampicin, a well proved human PXR agonist. Results confirmed the protective role of PXR against BA toxicity as expression of most of the CYP biotransformation enzymes genes were

increased (Table 2) while the *CYP7A1*, involved in BA synthesis, was decreased to 0.32-fold in comparison to control (Kandel *et al.* 2016).

Table 2. The role of NRs in the regulation of CYP genes expression.

CYP	Receptor					Reference
	FXR	PXR	VDR	CAR	LXR	
1A1				↑		1
1A2				↑		1
2A6			↑	↑		1, 2
2A7				↑		1
2A9			↑			2
2A13				↑		1
3A1		↑				3
3A2		↑				3
3A4*	↑	↑	↑	↑		1, 4, 5, 6, 7, 8, 9
3A7		↑		↑		1
7A1	↓	↓	↓		↑	1, 10, 11, 12, 13
27A1	↓					10
2B6		↑		↑		1, 4, 9, 14
2B7		↑		↑		1
8B1	↓			↑		10
2C8		↑		↑		1
2C9		↑		↑		1, 4
2E1				↓		1

↑ – expression increased, ↓ – expression decreased, * – human *CYP3A4* is orthologue of mice *CYP 3A11*, therefore results from animal models are represented as change in *CYP3A4* expression. Ref.: 1 – Kandel *et al.* 2016, 2 – Drocourt *et al.* 2002, 3 – Kliwer *et al.* 1998, 4 – Drocourt *et al.* 2001, 5 – Gnerre *et al.* 2004, 6 – Goodwin *et al.* 2002, 7 – Goodwin *et al.* 2000, 8 – Makishima *et al.* 2002, 9 – Sberna *et al.* 2011, 10 – Eloranta and Kullak-Ublick 2005, 11 – Han *et al.* 2010, 12 – Lehmann *et al.* 1997, 13 – Staudinger *et al.* 2001, 14 – Xie *et al.* 2000.

Vitamin D receptor (VDR; NR1I1)

In spite of the fact that hepatocytes do not express VDR, its significant levels are found in non-parenchymal liver cells such as Kupffer cells or sinusoidal endothelial cells (Gascon-Barré *et al.* 2003). The typical ligand of VDR is cholecalciferol, while most of the BA including CDCA, CA, DCA, or muricholic acid does not activate VDR. Similarly to ligands of PXR, VDR can be activated by LCA and its metabolite 3-keto-LCA with the EC₅₀~8 μ M and 3 μ M, respectively (Makishima *et al.* 2002). It could be of clinical importance that therapeutically used UDCA can be converted to LCA (VDR and PXR agonist) by intestinal microflora (Xie *et al.* 2001, Staudinger *et al.* 2001).

The role of VDR to promote calcium and phosphate absorption is well known. Its protective role against BA toxicity similar to PXR or against infection of bile duct was documented in the past decade. Stimulation of VDR by both vitamin D and LCA induces production of antimicrobial peptide cathelicidin in the bile duct epithelial cells (D'Aldebert *et al.* 2009). Both ligands can also increase the expression of *CYP3A4* leading to elevated BA clearance (Makishima *et al.* 2002) while the expression of *CYP7A1* can be reduced through VDR activation (Han *et al.* 2010). Moreover, there is documented interaction between VDR and LXR α with antagonizing effects on the *CYP7A* gene, too (Jiang *et al.* 2006).

Liver X receptor alpha (LXR α ; NR1H3)

This NR can be found in the tissues with high metabolic activity such as liver, small intestine, kidney or adipocytes (Sato and Kamada 2011), in comparison to LXR β , which is ubiquitously expressed in all tissues (Teboul *et al.* 1995). LXR binds to its responsive elements as heterodimer associated with RXR and its influence on gene expression is tissue specific (Moschetta 2015).

Its known endogenous ligands are oxysterols and its physiologic role is the regulation of cholesterol, fatty acid, and glucose homeostasis (Zelcer and Tontonoz 2006). Oppositely to other mentioned NR, LXR activation leads to increased activity of *CYP7A1*, thus the formation of BA is increased. Together with increased cholesterol transport to bile *via* specific transporters and restriction of its absorption from intestine, the level of total plasma cholesterol is decreased. However, reduction in cholesterol levels *via* activation of LXR is associated with fatty liver and hypertriglyceridemia development (Moschetta 2015).

LXR is activated by cholestenoic acid with ED₅₀ of 200 nmol/l (Song and Liao 2000) and also by different oxysterols (Lehmann *et al.* 1997, Janowski *et al.* 1996) and 6 α -hydroxylated BA (Song *et al.* 2000) within the range of their physiologic levels.

Constitutive androstane receptor (CAR; NR1I3)

CAR is closely related to PXR, this NR acts similarly as xenobiotic sensors which regulate expression of genes significant for biotransformation and excretion of exogenous compounds. Both receptors are activated by toxic derivatives of endobiotic metabolism, too. However, CAR seems to be more sensitive to

endogenous stimuli (Bing *et al.* 2014). Typical exogenous ligands of CAR represent phenobarbital, 3,3',5,5'-Tetrachloro-1,4-bis(pyridyloxy)benzene (TCOBOP) (Timsit and Negishi 2007). When activated, it is translocated into nucleus *via* protein phosphate PP2A and association with RXR precede to binding to DNA (Timsit and Negishi 2007). BA do not seem to be direct ligands of CAR, nevertheless activation of this receptor increases activity of enzymes producing more hydrophilic and thus less hepatotoxic metabolites of BA (Beilke *et al.* 2009) and activates their excretion from hepatocytes (Wagner *et al.* 2005).

The genome-wide screening in the liver cells of human donors treated by CAR prototype ligand CITCO (6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime) revealed 11 CYP genes among top 25 influenced genes. In all cases CITCO increased gene expression in the range between 1.28- and 2.19-fold in ascending order as follows: 2C9<1A2<3A7<3A4<2C8<2A6<2A13<1A1<2A7<2B6<2B7 (Kandel *et al.* 2016). On the other hand, *CYP2E1* was downregulated with 0.86-fold decrease of its expression (Kandel *et al.* 2016).

Besides binding to nuclear receptors, BA are also ligands of other types of receptors, including membrane G protein-coupled receptors. The first G-protein coupled receptor known to interact with BA was TGR5, GP-BAR1, or M-BAR. This receptor is widely distributed in different tissues and stimulation leads to different effects depending on the tissue and signalling cascade mediating the signal (Duboc *et al.* 2014). TGR5 is involved in energy metabolism; it protects liver and intestine from inflammation and steatosis, and improves insulin sensitivity (Chiang 2013, Li and Chiang 2015). The influence of some of BA on the immune response is also essential in their therapeutic effect in cholestatic liver diseases (Poupon 2012, Poupon 2014). This effect is in case of UDCA probably mediated by interaction with toll-like receptors TLR4 and TLR9 and glucocorticoid receptors (Poupon 2012).

Regulation of CYP enzymes involved in the metabolism of bile acids

The synthesis of BA is limited mainly through activity of cholesterol 7 α -hydroxylase (*CYP7A1*), which is believed to be the only rate-limiting step in BA synthesis (Chiang 2013). The protein content and metabolic activity of *CYP7A1* is regulated by variety of

Table 3. Bile acids as regulators of CYP expression.

Bile acid	CYP											
	1A1	1A2	3A1	3A2	3A4 ⁺	3A7	3A9	7A1	27A1	2B6 ⁺	2C8	2E1
CDCA	↓ ⁷	↓ ⁷	↑ ^{5,6} ↓ ^{6*}	↑ ⁵ ↓ ^{6*}	↑ ^{3,6} ↓ ⁷		↓ ^{6*}	↓ ^{1,7}			↓ ⁷	↓ ⁷
DCA					↑ ³			↓ ¹				
UDCA					↑ ^{2,3}			↓ ^{1,2}		↑ ²		
LCA			↑ ⁵	↑ ⁵				↓ ¹				
CA					↑ ^{2,3}			↓ ²		↑ ²		
TUDCA					↑ ³							
TDCA									↓ ⁴			

↑ – expression increased, ↓ – expression decreased, ¹ – Roma et al. 2011, ² – Zollner et al. 2006, ³ – Schuetz et al. 2001, ⁴ – Rao et al. 1999, ⁵ – Khan et al. 2010, ⁶ – Khan et al. 2009, ⁷ – Krattinger et al. 2016. * – only in rat liver, + – human *CYP3A4* and *CYP2B6* are orthologue of mice *CYP3A11* and *CYP2B10*, respectively. Therefore results from animal models are represented as change in *CYP3A4* and *CYP2B6* expression.

factors. CYP7A1 metabolic activity is limited mainly by availability of its substrate cholesterol. Feeding the experimental animals with cholesterol lead to increase expression of CYP7A1, suggesting the stimulatory effect of substrate (“Km effect”) on CYP7A1 (Chiang 2013). On the other hand, negative feedback exists and most of BA are negative regulators of CYP7A1 (Gupta et al. 2001). CDCA, DCA and with much lesser potency also other BA, such as UDCA and LTA, inhibit transcription of *CYP7A1* gene. Minor BA, UDCA, which is used therapeutically, increases expression of murine *CYP3A11*, *CYP2B10* and human *CYP3A4* – these enzymes catalyze hydroxylation of primary BA towards less toxic (hydrophilic) BA (Roma et al. 2011, Schuetz et al. 2001).

As mentioned above, BA are ligands of several NR, including FXR and it seems that BA downregulate *CYP7A1* probably through activation of RXRα/FXR (Chiang 2013). FXR decreases *CYP7A1* expression by several indirect mechanisms, by induction of SHP, which in turn inhibits transactivation of *CYP7A1* and *CYP8B1* by hepatocyte nuclear factor 4α/HNF4α and liver-related homolog-1 (LRH-1). This is in line with finding of Peng et al. (2016), who reported that CYP7A1 is upregulated in young FXR nullizygous mice.

Moreover, activation of FXR further decreases intracellular BA content by increase of expression of canalicular BSEP (bile salt efflux pump, ABCB11). These feedback mechanisms seem to protect inner environment of hepatocytes from BA toxicity and liver damage. Taurodeoxycholic acid also downregulates *CYP27A1* via activation of HNF1α (Rao et al. 1999).

The regulation of BA synthesis and metabolism

seems to be very complex and some pathways are anticipatory, since activation of VDR may trigger decrease of SHP and this leads to induction of CYP7A1 (Chow et al. 2014). The expression of VDR itself in rat ileum and liver is regulated (among others) by some of BA (at least by CDCA), but not LCA. The VDR activation by 1,25-dihydroxycholecalciferol or LCA then leads to increase in transcription of *CYP3A1* and *CYP3A2* genes as well as CDCA treatment. However, when combined together with LCA the expression of *CYP3A1* and *CYP3A2* is reduced (Khan et al. 2010). It was also reported by the same team of authors that CDCA decreases expression of *CYP3A1*, *CYP3A2*, and *CYP3A9* mRNA in rat liver, whereas the expression of *CYP3A1* in the rat ileum is increased and *CYP3A2* and *CYP3A9* are not influenced within the whole rat intestine (Khan et al. 2009). This means that unlike UDCA, CDCA could decrease the metabolism and increase the liver toxicity of hydrophobic BA (Khan et al. 2010). Moreover, the same team of authors reported that CDCA induced CYP3A4 in human liver (Khan et al. 2009). In another study with the primary culture of human hepatocytes, CDCA decreased expression of *CYP3A4*, *CYP7A1*, *CYP2C8*, *CYP1A1*, *CYP2E1* and *CYP1A2* genes after 48 hours of incubation, as well as it decreased expression of the *AHR* and *PPARγ* gene (Krattinger et al. 2016). The overview of BA influence on CYP enzymes is summarized in the Table 3. The results of experiments focused on the regulatory role of BA in the CYP expression seem to be highly variable and dependent on the model used. Moreover, the effects of hydrophobic and hydrophilic BA may differ, as there are differences in

their selectivity and affinity to nuclear receptors (Table 1). This is also resembled by different clinical effects of DCA and CA on one hand and UDCA, TUDCA and nor-UDCA on the other hand. While CA and CDA appear to be hepatotoxic, UDCA prevents from liver damage. Surprisingly, the genetic polymorphisms of *CYP7A1* do not seem to influence the production of BA (Xiang *et al.* 2012).

Other endogenous regulators seem to play a significant role in BA synthesis, such as blood glucose levels, insulin, thyroid hormone, glucocorticoids and glucagon (Siljevik Ellis 2006, Twisk *et al.* 1995, Chiang 2009, Xiao *et al.* 2016). Glucose increases both *CYP7A1* transcription (Li *et al.* 2010) and *CYP7A1* metabolic activity by histone acetylation of gene promoter. The latter mechanism is also mediated by insulin (Li *et al.* 2012). Glucagon inhibits BA synthesis *via* block of *CYP7A1* expression (Song and Chiang 2006). In overall, fasting state seem to downregulate *CYP7A1*, while elevated glucose and insulin seems to increase expression or metabolic activity of *CYP7A1* (Chiang 2009, Chiang 2013).

Bile acids as regulators of CYP enzymes involved in the metabolism of xenobiotics

Through activation of FXR and PXR, BA are regulating rate-limiting step in their biosynthetic pathway (*CYP7A1*), as well as several transporters involved in BA elimination from hepatocytes, as mentioned above.

BA may also influence CYP enzymes, which are primarily involved in the drug metabolism, such as *CYP3A4*, *CYP2C8*, *CYP2E1* or *CYP1A2*.

Although PXR and CAR are primary regulators of CYP enzymes, other nuclear receptors of different types are also known to be involved in their regulation.

With respect to the proposed binding ability to the nuclear receptors, namely FXR, it is not surprising that BA may induce expression of *CYP3A4* (Schuetz *et al.* 2001), *SULT2A1* and *UGT2B4* genes (Poupon 2012) and increase *CYP3A4* metabolic activity (Schuetz *et al.* 2001).

In particular, FXR nullizygous mice exhibit downregulation of bile salt export pump and thus increase hepatocellular BA content, which in turn, may activate PXR and upregulate *CYP3A* (involved in their metabolism), but also *CYP2B* and some ABC transporters (Schuetz *et al.* 2001). It is also note of worth, that BA are not equipotent ligands of FXR (Table 1). Hydrophilic BA are not considered to be agonists of FXR (Chiang, 2013)

and sometimes are reported to be partial agonists, and sometimes also partial antagonists of FXR, such as UDCA (Modica *et al.* 2010).

It was reported that UDCA may induce the enzymes of *CYP3A* subfamily (Schuetz *et al.* 2001) and thus decrease concentrations of substrates of *CYP3A4* (such as cyclosporine A (CsA)) after UDCA treatment (Yan *et al.* 2008, Becquemont *et al.* 2006, Kurosawa *et al.* 2009, Uchida *et al.* 2014).

Interestingly, the rate of CsA elimination was not changed (Caroli-Bose *et al.* 2000), what implies for decreased bioavailability through induction of intestinal *CYP3A4* and P-glycoprotein by activation of PXR (Schuetz *et al.* 2001). PXR is known as potent inducer of phase I metabolic enzymes, phase II conjugation enzymes and phase III drug transporters (Chiang 2013). Among BA, both taurine-conjugate of UDCA (TUDCA) and UDCA were reported to be the most effective inducers of *CYP3A4* in primary human hepatocytes (Schuetz *et al.* 2001) which corresponds with their ability to decrease toxicity of more hydrophobic BA.

Information on the influence of UDCA on bioavailability of CsA are inconsistent and controversial, since there were published studies documenting decreased dosing of CsA when combined with UDCA, when AUC of CsA were increased twice (Gutzler *et al.* 1992). Similarly, the bioavailability of CsA was increased with either combination with TUDCA or CsA-TUDCA micellar solution (Balandraud-Pieri *et al.* 1997). This report may be explained in part by effect of excipient-based increase ion CsA bioavailability, since other micellar dispersions of CsA may also increase the bioavailability and AUC of CsA (Balandraud-Pieri *et al.* 1997).

Therapeutic use of BA

Dried bile of Chinese black bear was used as a remedy already during the dynasty of Tang in China (approximately 600-900 A.D.) (Guarino *et al.* 2013). Evidence-based use of bile acids as drugs dates about 30 years back (Beuers *et al.* 2015). In particular, CDCA was utilized as a treatment for gallstone dissolution, which was later displaced by UDCA due to the better safety profile and efficacy. CDCA caused diarrhea, increased serum total cholesterol and LDL-cholesterol, among other adverse effects (American Society of Health System Pharmacists 2016a). UDCA is currently the only drug approved by FDA for the treatment of primary

biliary cirrhosis (PBC, stage I. and II.) (Roma *et al.* 2011). UDCA delays progression and need for liver transplantation, increases survival and is well tolerated in PBC at the doses of 10-20 mg/kg/day (American Society of Health System Pharmacists 2016b, Poupon 2014, Roma *et al.* 2011). In some countries, UDCA is also recommended in primary sclerosing cholangitis (PSC) but evidence on benefit from treatment with UDCA is not as convincing as in PBC. Nevertheless, UDCA is reported to improve biochemical characteristics of disease, ameliorate inflammatory component of the disease, but it probably does not influence overall survival, liver histology nor time to the transplantation at the doses of 10-15 mg/kg/day (EASL 2009). UDCA is also registered for the dissolution of radiolucent, noncalcified gallbladder stones smaller than 20 mm in diameter at the doses of 10-114 mg/kg/day (American Society of Health System Pharmacists 2016b). UDCA is also recommended in the treatment of intrahepatal cholestasis in pregnancy (10-20 mg/kg/day). It alleviates pruritus and improves liver biochemical parameters in up to 80 % of patients (European Assoc Study 2009, Gabzdyl and Schlaeger 2015). Another use of UDCA is cystic fibrosis liver disease (CFLD, syn. CFALD – Cystic Fibrosis Associated Liver Disease, syn. CFAHD – Cystic Fibrosis Associated Hepatobiliary Disorders). UDCA improves biochemical parameters in cystic fibrosis patients, but not overall survival (Stauffer *et al.* 2014). Concerning off-label use, UDCA is recommended by European Society for Blood and Marrow Transplantation (ESBMT) as protective agent in Hepatic Veno-Occlusive Disease after hematopoietic stem cell transplantation (RR=0.34) (Dalle and Giral 2016). Due to the hepatoprotective effect of UDCA, there are some reports that UDCA improves biochemical parameters in non-alcoholic steatohepatitis (NASH), notably in higher doses (Xiang *et al.* 2013), but in overall there is not enough evidence for routine use of UDCA in NASH (Ratziu 2012, Georgescu and Georgescu 2007). UDCA in low doses may also prevent from colorectal cancer in patients with concomitant inflammatory bowel disease and PSC (Singh *et al.* 2013).

Some other derivatives of natural BA – “bile mimetics” – in particular FXR and TGR5 agonists, such as nor-UDCA and obeticholic acid, have been suggested to treat cholestatic liver disease (Poupon 2012, Adorini *et al.* 2012). Obeticholic acid (6 α -ethyl-chenodeoxycholic acid) is a semisynthetic derivative of CDCA and potent FXR agonist (Neuschwander-Tetri *et al.* 2015). It was

used in the treatment of PBC and NASH in patients non responding to UDCA (Camilleri and Gores 2015). Obeticholic acid also improved liver histology in NASH patients, but 23 % of the patients developed pruritus compared to 6 % in placebo group (Neuschwander-Tetri *et al.* 2015). Moreover, obeticholic acid (25 mg/day) improved liver enzymes, increased low density lipoproteins and decreased markers of fibrosis in patients with type II diabetes mellitus and non-alcoholic fatty liver disease (NAFLD) (Mudaliar *et al.* 2013). In another study, increase of total cholesterol with simultaneous decrease of HDL cholesterol was reported upon obeticholic acid treatment (Neuschwander-Tetri *et al.* 2015).

BA and BA sequestrants are currently investigated as possible adjustment to the treatment of diabetes mellitus (notably Type II) due to their ability to increase insulin sensitivity, and decrease gluconeogenesis (in particular through increase of glucagon like peptide 1) (Camilleri and Gores 2015).

Conclusions

BAs are essential physiological factors preserving homeostasis through their influence on nutrition, metabolism and excretion of both endo- and xenobiotics and their metabolites. BAs have also an important role in pathogenesis of cholestatic diseases and drug induced liver injury. Some of BA, their analogues and so called bile mimetics seem to be promising drugs. Apart of the effects of lipid absorption and xenobiotic excretion based on physico-chemical properties of BA, most of their regulatory and signalling properties are mediated through the nuclear receptors FXR, PXR, CAR, LXR and VDR, as well as membrane bound receptors TGR5 and S1PR2 (Coppole and Li 2016). The physiological role of interaction of BA with NRs is probably to decrease production of toxic hydrophobic BA and to increase their metabolic turnover towards polar and hydrophilic BA, to increase their excretion and thus decrease their toxicity in the hepatocytes. By this, the activity of some drug-metabolizing CYPs is also influenced since these are regulated by the same NRs, what could have clinically relevant consequences in cholestatic diseases or during the treatment with BA or their derivatives.

Conflict of Interest

There is no conflict of interest.

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