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Regulation of metabolizing enzymes and transporters for drugs and bile salts in human and rat intestine and liver

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Khan, A. A. (2009). *Regulation of metabolizing enzymes and transporters for drugs and bile salts in human and rat intestine and liver: a study with precision-cut slices*. s.n.

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Chapter 8
General Discussion and Conclusions

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1 Introduction

The research described in this thesis is focused on the regulation of drug metabolizing enzymes (DMEs) and drug transporters (DTs) that are involved in drug metabolism and transport as well as in bile acid synthesis, detoxification and transport in human and rat liver and intestine. To predict drug metabolism and transport of drugs in man, animal studies are widely applied and among the rodents, the rat is the most preferred preclinical animal species used in the pharmacological and toxicological evaluation of new chemical entities (NCE) in drug discovery. However interspecies differences make this extrapolation hazardous and unreliable. Moreover the expression of DMEs and DTs is subject to regulation by ligand-activated nuclear receptors (NRs) and nuclear factors (NFs). Data on regulation of DMEs and DTs in animals can be obtained from *in vivo* studies and data on regulation of DMEs and DTs in man is hard to obtain. For human studies occasionally tissue from patients is studied but it remains undetermined to what extent confounding factors of the disease interfere with the results, and moreover secondary effects of ligands cannot be discriminated from primary effects. Therefore *in vitro* methods are required to study the direct effects of drugs on the regulation of DMEs and DTs. *In vitro* methods to study regulation of DMEs and DTs in liver are available, such as primary human hepatocytes, immortalized cell lines and precision cut liver slices (PCLS) but for the intestine very few successful methods were available, such as enterocytes and intestinal derived immortalized cell lines (Caco-2) and data on regulation in the intestine are scarce. Recently, we validated precision cut intestinal slices (PCIS) as an *in vitro* model to study activity and regulation of DMEs. In this model all the cell types are represented and the cell-matrix contacts remain intact. They can be prepared from human liver and intestine in a similar way and allow studying the regional differences in the activity and regulation of DMEs and DTs in the intestine. Therefore we set out to study the regulation of DMEs and DTs in human and rat tissue slices, prepared from liver and from different regions of the intestine.

In this chapter, the results on induction and repression of DMEs, DTs and the NRs / NFs involved in the regulation of DMEs and DTs by ligands of the pregnane X receptor (PXR; NR1I2), glucocorticoid receptor (GR; NR3C1), farnesoid X receptor (FXR; NR1H4) and vitamin D receptor (VDR; NR1I1) in human intestine and liver are summarized and discussed. Furthermore, the data obtained in human tissue are compared to those in rat tissue and the observed species differences are discussed.

The most preferred route of administration of drugs is the oral route. Although, the liver is the most important organ for the first pass metabolism of orally ingested drugs, nowadays the intestine is also recognized as a major drug metabolism organ with the identification of high levels of DMEs and DTs, and clinical drug interactions are reported at the level of intestinal metabolism for drugs such as cyclosporine, midazolam and verapamil based on pharmacokinetic studies in man (27, 42, 72). During drug discovery, it is

important to screen the NCEs for its intestinal drug metabolic profile along with the liver metabolic profile. After oral administration, the enterocytes are exposed to very high concentrations of the ingested drugs in the intestinal lumen and they can have a significant impact on the bioavailability of these drugs. Induction and repression of DMEs and DTs in response to environmental stimuli and co-administered drugs may influence the oral bioavailability of drugs. This needs to be investigated during drug discovery and development and not only in the preclinical animal species but also in humans. This is particularly of importance for drugs which have a narrow therapeutic window because of the imminent danger of decreased efficacy or adverse drug reactions, such as described for immunosuppressive agents like cyclosporin or tacrolimus. These drugs are substrates for CYP3A4 and the efflux pump, multi drug resistance protein 1 (MDR1 or Pgp). Induction and repression of CYP3A4 and MDR1 by co-administered drugs can have a profound impact on the bioavailability, efficacy and toxicity of these compounds (27, 42, 72). Hence, understanding the underlying mechanisms for the induction and repression of DMEs and DTs in the intestine and the liver is of paramount importance. Many mechanisms are known to be involved in the regulation of the expression of DMEs and DTs, and constitutive, induced and repressed expression of genes is largely controlled at the level of transcription by the involvement of nuclear receptors, such as the PXR, constitutive androstane/active receptor (CAR), GR, VDR, aryl hydrocarbon receptor (Ahr) and Nrf2 (40, 41, 48, 51, 57, 70). **In this thesis, we aimed to investigate the VDR mediated induction and repression of DMEs and DTs involved in drug and bile acid detoxification, transport and synthesis proteins in human and rat liver and intestine using precision-cut tissue slices as *in vitro* model.** To obtain more insight in the role of VDR and to elucidate the role of other nuclear receptors in bile acid synthesis and disposition, the effects of the VDR ligands, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and lithocholic acid (LCA) were compared with those of specific ligands for various other nuclear receptors, chenodeoxycholic acid (CDCA) and GW4064 for FXR, pregnenolone-16α carbonitrile (PCN) for PXR, budesonide (BUD) for glucorticoid receptor (GR) and dexamethasone (DEX) for GR and PXR.

In vitro studies like reporter gene assays are powerful tools to characterize the nuclear receptor response elements (NRRE's) in the promoters of the DMEs and DTs. Primary cultures of rat and human hepatocytes and enterocytes, and immortalized human cell lines such as HepG2, LS180 and Caco-2 are widely applied to study the effect of various ligands on DMEs and DTs (15, 34, 35, 43, 54, 56). However, the induction and repression of DMEs and DTs in the intestine and liver in response to ligands of the NRs is not only determined by the presence of NRRE's in the target genes but also by the expression levels of the NRs, co-activators and repressors in the tissues, which in their turn are also subjected to regulation and are not always expressed at their original levels in these cell lines. Moreover, the exposure of the particular cell to the ligand plays an important role. This exposure is determined by the extracellular concentration and by the rate of uptake, metabolism and excretion of the ligand. This may vary between the different

organs, and hence the response may vary concomitantly. Therefore in **chapter 2 and 4**, we characterized the expression of the nuclear receptors, VDR, PXR and FXR at the level of mRNA along the length of the rat intestine and found significant differences in different regions of the intestine, which we further confirmed at the level of protein by immunohistochemistry for VDR. VDR mRNA was found to be highly expressed in the enterocytes of the rat intestine with an increasing gradient from jejunum to colon, and also in human ileum and colon. In the rat and the human liver, the VDR mRNA expression was found to be very low but detectable. The cellular localization of the VDR protein in the liver showed a species difference: in the rat liver VDR is localized exclusively in the bile duct epithelial cells and endothelial cells, whereas in the human liver, VDR protein could also be detected in the hepatocytes along with the bile duct epithelial cells and the endothelial cells. This gradient in expression is not found for every NR. The expression of PXR and FXR also showed a gradient along the length of the intestine with the highest expression in colon, but their expression was 2-10 fold higher in the liver compared to the intestine. However taking into account that the enterocytes represent ~ 17-25% of the intestinal tissue and the hepatocytes represent ~ 80% of the liver tissue, the expression of PXR and FXR in enterocytes is rather similar to that in hepatocytes. Previous data from our laboratory showed that the CAR expression did not vary much along the length of the rat intestine (**van de Kerkhof et al., thesis 2007, unpublished observation**). Future research should elucidate the relative expression of these NRs in human jejunum, ileum, colon and liver. Currently the expression of the NRs is further investigated at the protein level by immunohistochemistry.

2 Regulation of CYP3A isozymes by VDR, PXR, GR and FXR in rat and human intestine and liver

The cytochrome P450 (CYP P450) 3A isozymes are the most abundant CYP isozymes expressed in the liver and the intestine, and are responsible for the metabolism of 60% of the marketed drugs and a number of endogenous compounds such as bile acids (18, 23, 50). In **chapter 2**, we studied the role of the four nuclear receptors in the regulation of CYP3A isozymes at the level of mRNA in different regions of the rat intestine and liver, and the human ileum and liver with VDR-, PXR-, GR- and FXR-specific ligands: $1,25(OH)_2D_3$, LCA, PCN, DEX, BUD and CDCA using precision-cut tissue slices. In the rat intestine, CYP3A1 expression is very sensitive to the VDR ligand, and to a lesser extent to PXR and GR ligands and the induction showed a gradient in the different segments of the intestine which was different for the different ligands but did not correlate with the gradient of the respective NRs. For the VDR ligand, the gradient was in the rank order of ileum (Il) > jejunum (J) = colon (Co). The CYP3A2 expression was found to be exclusively regulated by the VDR ligand and only in the ileum. The CYP3A9 expression in the liver and in all regions of the intestine appears to be mainly regulated by PXR and GR ligands but not by the VDR ligand. In rat liver slices, $1,25(OH)_2D_3$ did not alter the expression of CYP3A isozymes, which can be explained by the specific localization of

VDR in the bile duct epithelial cells and the very high expression of CYP3A isozymes in the hepatocytes. A potential up regulation of CYP3A isozymes in bile duct epithelial cells is therefore difficult to detect. The $1,25(OH)_2D_3$ mediated induction of CYP3A1 mRNA in rat intestinal slices, presumably mediated through VDR is confirmed in *in vivo* studies in Wistar rats treated with 1200 pmol/kg/day $1,25(OH)_2D_3$ intraperitoneally **(chapter 5)**, which is in line with the earlier reports of Xu et al.(73) and Chow et al. in Sprague-Dawley rats treated with $1,25(OH)_2D_3$ intraperitoneally (640 – 2560 pmol/kg/day) (14). However, induction of CYP3A1 mRNA *in vivo* was found to be high in the jejunum compared to the ileum, where it showed a large variation. Furthermore, CYP3A1 mRNA was not induced in the colon of these rats and CYP3A2 mRNA was not induced in the ileum. These results are in line with the results of Chow et al. (14). The apparent contrasting results of the *in vivo* studies to those of the slice experiments, where, $1,25(OH)_2D_3$ showed a significant induction of CYP3A1 mRNA along the length of the intestine and CYP3A2 in ileum **(chapter 2 and 3),** might be due to the concentration gradients in the intestinal blood, resulting in a different exposure of the enterocytes along the length of the intestine.

Further, in **chapter 2** we studied the $1,25(OH)_2D_3$-mediated regulation of CYP3A4 in human ileum and liver. $1,25(OH)_2D_3$ significantly induced the CYP3A4 expression in human ileum and liver slices, similar to earlier studies in Caco-2, LS180 HepG2 cells and human hepatocyte cultures (20, 24, 49, 62, 63). However, $1,25(OH)_2D_3$ mediated induction of CYP3A4 in human liver shows a high variation among the human liver donors which can be attributed to the variation in the VDR expression. Furthermore, both in the ileum and the liver, CYP3A4 was up regulated by VDR, PXR and GR ligands.

Our results suggest that prediction of the inducing potential of drugs based on reporter gene assays should not rely strictly on whether or not the drug under study is a ligand for a certain NR, or whether the promoter of the target gene harbors the NRRE. Also the expression level of the NR in the target organ is not predictive for the inducing potential. The uptake, metabolism and excretion of the ligand as well as the availability of co-activators or repressors in the specific tissue may play a decisive role in the regulation of DMEs and DTs. For example, the VDR ligand, $1,25(OH)_2D_3$, is likely to be highly metabolized in the colon compared to the jejunum due to the higher expression of CYP24A1 in rat colon compared to jejunum **(Chow et al., un published observation)**, which may explain its relatively low effect of $,25(OH)_2D_3$ in the colon despite the high VDR expression. All these factors may show interspecies and inter organ differences.

In **chapter 3**, we investigated the effects of another VDR ligand, LCA (47), a toxic bile acid formed by the bacterial biotransformation of the primary bile acid CDCA in the terminal part of the ileum (16, 30) on the regulation of CYP3A isozymes in the rat intestine and liver, and the human ileum and liver at the level of mRNA. LCA, similar to $1,25(OH)_2D_3$ (**chapter 2**) induced the expression of CYP3A1 along the length of the intestine with the highest induction in ileum slices, and CY3A2 in ileum slices only.

Furthermore, in contrast to $1,25(OH)_2D_3$, LCA also induced the expression of CYP3A9 in liver slices and along the length of the intestine with a moderately higher induction in the colon slices. This effect is presumably mediated through PXR, as $1,25(OH)_2D_3$ did not regulate this isoform and can be explained by the observation that LCA and its metabolite, 3-keto-5β-cholanic acid (3KCA) also bind to PXR (66). The PXR mediated induction of the CYP3A9 isozyme in the rat intestine and liver was further confirmed by the effects of the synthetic PXR ligands, PCN and DEX in rat intestine and liver slices **(chapter 2 and 3)**. Unexpectedly, LCA did not induce CYP3A1 expression in rat liver slices, although CYP3A1 is regulated by PXR ligands such as PCN and DEX in rat intestine and liver slices **(chapter 2 and 3)** but an induction of CYP3A9 was found, presumably via PXR. Further experiments are needed to explain this discrepancy. In addition, we found indications for a role for GR in the regulation of the rat CYP3A9, as it was also up regulated by the GR ligand, BUD in the rat intestine and liver **(chapter 2 and 3)**, which to the best of our knowledge has not been reported before and deserves further investigations. Furthermore, in human ileum slices, LCA similar to $1,25(OH)_2D_3$ induced CYP3A4 **(chapter 2 and 3)**. However, surprisingly, the induction of CYP3A4 by LCA in human liver slices did not correlate with the expression of VDR and LCA even decreased the expression of CYP3A4 in three out of four VDR positive livers. These results suggest that the induction of CYP3A4 by LCA in human livers is mediated through other nuclear receptors, such as the PXR, which in man is referred as steroid X receptor / pregnane activated receptor (SXR/PAR) and FXR, since DEX and CDCA significantly induced CYP3A4 in human liver slices, which is consistent with the earlier reports in human hepatocytes (28). The effect of VDR, FXR, SXR/PAR and GR ligands on CYP3A4 regulation in individual human livers is summarized in Tables 1, 2, 3 and 4. The results obtained with the various nuclear receptor ligands in human liver and ileum are highly variable with respect to extent of the regulation, but are qualitatively consistent with previously published data, showing the validity of the model. In spite of that, the LCA effects did not seem to correspond to the known effects of VDR, PXR and FXR ligands **(chapter 3)** (Tables 1, 2, 3 and 4). Toxicity of LCA in the human liver slices at these doses cannot be excluded, although the significant up regulation of the mRNA of OSTα and OSTβ seems in contradiction with toxicity **(chapter 6)**. Further experiments are needed to elucidate the mechanism of the effects of LCA in the human liver.

In **chapter 7**, we investigated the interaction of CDCA, with, $1,25(OH)_2D_3$ and LCA, on the regulation of CYP3A isozymes involved in LCA detoxification. CDCA is known to induce the expression of VDR but in spite of inducing VDR **(chapter 7)** it decreased the induction of CYP3A1 and CYP3A2 in the presence of known VDR ligands, $1,25(OH)_2D_3$ and LCA. These enzymes are involved in BA detoxification. Hence, our results suggest a possible novel mechanism of enhancement of LCA-mediated toxicity in ileum by inhibiting the VDR-mediated induction of CYP3A isozymes by the simultaneous presence of CDCA and LCA in the rat ileum. Further studies are in progress to investigate whether a similar mechanism plays a role in the regulation of CYP3A4 in the human ileum.

3 Regulation of bile acid transporters by VDR, PXR, GR and FXR in rat and human intestine and liver.

In **chapter 4, 5 and 6**, we studied the regulation of the BA transporters as well as the NRs involved in the regulation of these proteins in rat and human ileum and liver by VDR, PXR, GR and FXR ligands. BAs are synthesized from cholesterol (60) in the liver by a cascade of 12 reactions, initiated by the first and rate limiting step, cholesterol 7α-hydroxylation, catalyzed by CYP7A1 (13) to form CDCA and cholic acid (CA), which upon amidation are transported into the bile canaliculi by the bile salt export pump (BSEP) and secreted into the intestine via the bile. About 95 % of the bile acids are actively re-absorbed in the terminal ileum by the coordinate action of apical sodium dependent bile acid transporter (ASBT; SLC10A2) and the basolateral heterodimeric organic solute transporter (OSTα-OSTβ) (5, 65). In the liver, bile acids are taken up into hepatocytes by the sodium dependent co transporting polypeptide (NTCP; SLC10A1) and members of the organic anion transporting polypeptide (OATP) family. Bile acid synthesis and secretion in the liver, reabsorption in the ileum and uptake in the liver constitute the enterohepatic cycle (33), which maintains the bile acid pool *in vivo* and is coordinately modulated by regulation of the proteins involved in the synthesis, transport and reabsorption of BAs. BAs play an important role in the absorption of lipids and lipid soluble vitamins in the upper part of the intestine.

The reabsorption of BAs in the terminal part of the ileum is mediated by the ASBT, which is highly regulated at the level of transcription by various nuclear receptors. The peroxisome proliferator activated receptor α (PPARα; NR1C1) and the GR were reported to mediate the expression of ASBT in rat and human ileum (37, 38, 53). Recently, Chen et al. (11) reported the involvement of the VDR in the regulation of ASBT in the rat ileum, but data on the involvement of VDR in the expression of ASBT in the human ileum is lacking up to now. Furthermore, in mice, guinea pigs, and rabbits ASBT was reported to be negatively regulated in the ileum by the reabsorbed BAs mediated through the FXR (45, 46, 52, 71). The ligand activated GR, PPARα and VDR bind to their respective response elements in the ASBT promoter as a homodimer or heterodimer with retinoic acid X receptor α (RXRα; NR1B1) (38, 69) and induce the ASBT expression. The BA-activated FXR forms a heterodimer with RXRα and induces the expression of short heterodimer protein (SHP; NR0B2), which down regulates the ASBT expression by inhibiting the activity of liver receptor homologue–1 (LRH-1; NR5A2), which is essential for the basal expression of ASBT. In the rat, the regulation of the ASBT gene by BAs is controversial with reports suggesting positive regulation (29, 67), negative regulation (21, 61) or no regulatory effects (3). Recently, Chen et al. (10) found that, in contrast to the mouse and the human ASBT promoter, the LRH-1 binding site is absent in the rat ASBT promoter. This explained the absence of a negative feedback regulation of ASBT by the BA-liganded FXR in the rat, as the FXR-SHP-LRH1 cascade cannot play a role in the rat (10).

In chapter 5, we characterized the VDR, PXR, GR and FXR mediated regulation of ASBT and the NRs regulating its expression in rat and human ileum and liver precision-cut tissue slices. Consistent with earlier reports (37, 53), the GR ligand DEX significantly induced the expression of ASBT in rat and human ileum and liver slices, suggesting an intact and responsive GR pathway. The VDR ligand, $1,25(OH)_2D_3$ significantly decreased the expression of ASBT mRNA in rat ileum and liver slices, and in the human ileum but not in the human liver. The $1,25(OH)_2D_3$ mediated ASBT repression in rat ileum slices is in contrast to the data of Chen et al.(11), who showed a positive VDRE in rat ASBT promoter and induction of ASBT mRNA expression and activity in the ileum of Sprague-Dawley rats with high doses of $1,25(OH)_2D_3$. We speculate that this difference might be due to a difference in sensitivity between strains of rats as treatment of Wistar rats *in vivo* with $1,25(OH)_2D_3$ did not affect the ASBT expression in ileum **(chapter 5)**. Moreover, more recent experiments in Sprague-Dawley rats could not confirm this mRNA induction, but showed an increased protein expression at the highest dose only (14). The differences observed in the regulation of ASBT between the *in vitro* (ileum slices) and *in vivo* experiments in Wistar rats might be ascribed to a lower exposure of ileocytes to the effective concentration of $1,25(OH)_2D_3$ in ileum *in vivo* after the intraperitoneal administration, as we also found a much lower and highly variable CYP3A1 induction *in vivo* as compared to the high and consistent induction *in vitro* in ileum slices cultured in the presence of $1,25(OH)_2D_3$ **(Chapters 2 and 5).** We did not measure the plasma concentration of $1,25(OH)_2D_3$, but we can estimate, based on the data of Brown et. al. (9) who measured serum levels after ip injection of radiolabelled $1,25(OH)_2D_3$ that the serum levels in our rats are 3-5 nM and are lower than those in the *in vitro* incubations.

Interestingly, we found a repression of HNF1α in rat ileum slices treated with $1,25(OH)_2D_3$ **(Chapter 5)**, since HNF1α is essential for the basal expression of ASBT (64). The repression of ASBT may be explained by this repression of HNF1α. These results were further confirmed with the natural VDR ligand LCA, which also showed a significant repression of ASBT in rat ileum with concomitant repression of HNF1α. Like in rat ileum slices, $1,25(OH)_2D_3$ decreased the ASBT expression in rat liver slices, in line with the co-localization of VDR and ASBT in the biliary epithelial cells. $1,25(OH)_2D_3$ also decreased the ASBT expression in the human ileum slices, whereas in the human liver slices ASBT expression remained unaltered, which is as yet unexplained but indicates species and organ specific differences in the regulation of ASBT in rat and human ileum and liver.

The FXR ligands, CDCA and GW4064 did not affect the ASBT expression in rat ileum slices, indicating that FXR does not play a significant role in the ASBT regulation in the rat ileum in spite of an intact FXR pathway as shown by the SHP induction in these samples. This can be attributed to the reported lack of LRH1 binding site in the rat ASBT promoter (10), which is essential for the feedback regulation by FXR through the FXR-SHP-LRH-1 cascade (52). However, CDCA induced the ASBT expression in rat liver slices, which indicates an LRH-1-independent pathway in the biliary epithelial cells. In line

with the results published by Bergheim et al. (7), CDCA significantly decreased the ASBT expression in human ileum slices with concomitant induction of SHP (7), but did not affect the ASBT expression in the human liver, which may indicate a lower expression of FXR in the biliary epithelial cells than in the enterocytes.

Subsequent to characterizing the regulation of ASBT in rat and human ileum and liver by VDR, FXR, GR and PXR nuclear receptor ligands, we studied the regulation of basolateral and canalicular bile acid transporters involved in the excretion of bile acids in the rat and human ileum and liver in **chapter 6**. Several basolateral bile acid transporters such as truncated ASBT (tASBT), MRP3 and MRP4, which shows an affinity towards BAs have been proposed to be involved in bile acid excretion (35, 44, 59, 68). However, the organic solute transporter (OST) consisting of two half transporters, α and β (OSTα and OSTβ) is recently reported as the main bile acid transporter and its expression on the basolateral membranes parallels the ASBT expression along the length of the intestine, bile duct epithelial cells and renal proximal tubular cells in mouse, rat and human (4, 17). Furthermore Ostα knockout mouse showed perturbed bile acid homeostasis (6, 58). The regulation of OSTα and OSTβ is largely unknown and therefore we studied the regulation of OSTα and OSTβ in the rat and human intestine and the liver. Incubation of rat and human ileum and liver slices with the FXR ligand, CDCA showed the induction of the OSTα and OSTβ genes, confirming earlier observations (43). Furthermore, our experiments showed for the first time that CDCA also induced the OSTα and OSTβ gene expression in rat jejunum and colon. The rat but not the human OSTα and OSTβ genes in the ileum were negatively regulated by the VDR ligands, $1,25(OH)_2D_3$ and LCA. This repression of rat OSTα and OSTβ genes by $1,25(OH)_2D_3$ led to the postulation of a negative VDRE in the rat promoters, which remains to be confirmed. OSTα and OSTβ are positively regulated by the GR ligand, DEX but not by the PXR ligand. In conclusion, rat and human OSTα and OSTβ genes are positively regulated by FXR and GR ligands., and, in the rat the VDR ligand decreases the expression of both apical and basolateral bile acid transporters, ASBT, OSTα and OSTβ . Further studies are needed to evaluate the role of VDR ligands in the reabsorption of bile acids in the ileum of Wistar rats.

In addition to OSTα and OSTβ, multi drug resistance associated protein 2 (MRP2) and MRP3 play a role in the excretion of monovalent and conjugated bile acids across the apical and basolateral membranes of the enterocytes, respectively (5, 8, 12, 17, 31, 32, 74). Therefore, we studied the regulation of MRP2 and MRP3 transporters by VDR, FXR, GR and PXR ligands. In **chapter 5 and 6**, we showed that LCA, a VDR ligand with affinity toward FXR and PXR did not affect the MRP3 expression in ileum slices but induced MRP2 expression **(chapter 2).** These results suggest that LCA decreases the BA uptake in rat ileum by decreasing the ASBT and increases the luminal excretion of BAs in colon by induction of MRP2. Thus, in the rat LCA favours its own detoxification and transport into the lumen of the colon by inducing MRP2 but not MRP3 expression hence excretion via the faeces. In contrast, the primary BA, CDCA, which is an FXR ligand stimulates absorption

of bile salts by induction of MRP3 and OSTα-OSTβ expression and repression of MRP2 expression in rat jejunum and ileum, favouring the reclamation of bile acids in the small intestine. Also in the human intestine, LCA may induce the luminal transport of BAs by inducing MRP2 expression, whereas CDCA favours the basolateral transport of BAs by inducing MRP3 expression in ileum slices. As CDCA did not affect MRP2 expression in human ileum slices, the LCA effects are not likely to be mediated by FXR but seem to be mediated by VDR, since $1,25(OH)_2D_3$ induced MRP2 expression by 2.5-fold (un published data).

In **chapters 3 and 4**, we studied the regulation of BA transporters in rat and human liver by VDR, FXR and GR ligands. These results obtained in the human liver are summarized in the Tables 1, 2, 3 and 4. $1,25(OH)_2D_3$ significantly decreased the NTCP expression in human liver slices, without affecting the expression of BSEP, MRP2 and MRP3. The repression of NTCP by $1,25(OH)_2D_3$ can be attributed to the decrease in HNF4α, which is essential for its basal expression (26). Our results are the first to show that human NTCP is significantly decreased by the $1,25(OH)_2D_3$ and further studies are needed to elucidate whether this is indeed mediated via HNF4α down regulation. In line with earlier reports, CDCA, significantly decreased NTCP and induced BSEP expression in rat and human liver slices (2, 19), although the decrease in NTCP in human liver slices was not significant which is in line with previous data from our lab (36). Furthermore, CDCA induced MRP2 expression in rat liver slices where as in human liver slices CDCA induced MRP3 but not MRP2 which is in line with the earlier reports by Inokuchi et al. (35), who characterized a bile acid response element in the human MRP3 promoter. The GR ligands, DEX and BUD induced the NTCP expression, which is in line with the earlier reports (22). Furthermore our results show that GR ligands induce BSEP expression in rat and human liver slices, which is not reported earlier.

4 Regulation of the bile acid synthesis enzyme, CYP7A1 by VDR and FXR ligands in rat and human liver.

BAs, as mentioned in the previous section are synthesized from cholesterol (60) in the liver by a cascade of 12 reactions, initiated by the first and rate limiting step, cholesterol 7α-hydroxylation, catalyzed by CYP7A1. Recently, an altered expression of bile synthesis proteins and transporters in the liver were observed in Sprague-Dawley rats treated with $1,25(OH)_2D_3$ (14) despite the lack of localization of VDR in parenchymal cells (25). Thus, we hypothesized that changes in the expression of proteins involved in bile acid synthesis and disposition after ip administration of $1,25(OH)_2D_3$ in the rat liver (14) might be secondary to the increased bile acid flux due to increased bile acid absorption. To test this hypothesis, in **chapter 4,** we treated rat and human liver slices with the VDR ligand, $1,25(OH)_2D_3$, and also with the primary bile acid, CDCA as the FXR ligand. CDCA but not $1,25(OH)_2D_3$ significantly decreased the CYP7A1 expression with simultaneous induction of SHP in rat liver slices. Furthermore, CDCA but not $1,25(OH)_2D_3$ decreased

the expression of HNF1α, HNF4α and LRH1. Thus, in rat liver CDCA regulates CYP7A1 expression by affecting both SHP dependent pathways and SHP independent pathways, but 1,25$(OH)_2D_3$ is not involved in CYP7A1 regulation. However, in human liver slices, 1,25$(OH)_2D_3$ significantly decreased the expression of CYP7A1. The repression of CYP7A1 was also found after treatment with CDCA with simultaneous induction of SHP. Furthermore, 1,25$(OH)_2D_3$ significantly decreased the expression of HNF4α without affecting SHP and HNF1α expression. Thus, in human liver 1,25$(OH)_2D_3$ affects the SHP independent pathways. Our results showed for the first time, that 1,25$(OH)_2D_3$ decreased the expression of HNF4α in human liver slices, which may contribute to a decrease in the expression of CYP7A1 (1). In conclusion, the expression of CYP7A1 was decreased by the VDR ligand, 1,25$(OH)_2D_3$ in the human but not in the rat liver, whereas the FXR ligand, CDCA decreased CYP7A1 expression both in human and rat liver. This species difference in CYP7A1 regulation is consistent with the differential localization of VDR in the rat liver (exclusively in non-parenchymal cells) and human liver (non-parenchymal cells and hepatocytes). These contrasting effects of VDR and FXR ligands on the regulation of bile acid homeostasis proteins in the rat liver support the hypothesis that the decrease in CYP7A1 protein and activity by 1,25$(OH)_2D_3$ in Sprague-Dawley rats, that occurred with the simultaneous induction of SHP reported by Chow et.al. (14) was secondary to the increased bile acid absorption, subsequent to the induction of ileal ASBT. However, the induction of ileal ASBT and increase in BAs in portal blood was not confirmed in our studies with Wistar rats, which can be ascribed to strain related differences and the lower dose of 1,25$(OH)_2D_3$ and needs further investigation.

5 Regulation of VDR, PXR and FXR expression in rat and human ileum and liver.

In **chapter 2, 3, 4 and 7**, we also characterized the regulation of the nuclear receptors VDR, PXR and FXR by their respective ligands in rat and human intestine and liver. The GR ligands, DEX and BUD induced the expression of PXR in rat and human intestine and liver. However, the induction of PXR in rat liver seemed to be transient, observed at 8 h of incubation but not at 24 h incubation. This can be attributed to the simultaneous induction of SHP by GR ligands, as SHP is known to repress the PXR expression (55). The induction of SHP by GR ligands is not reported before. The FXR ligand, CDCA decreased the expression of PXR in rat in line with the increased SHP expression, but not in human liver slices, which is in contrast to that of earlier reports (39). Furthermore, our results in **chapter 4** suggest that CDCA decreases the expression of FXR in rat liver slices but not in human liver slices, which might probably act as feedback loop by the increased bile acid synthesis in rat liver. These results are not reported before and needs further investigation.

In chapter 7, we investigated the regulation of VDR in rat and human ileum and liver and showed that 1,25$(OH)_2D_3$ and CDCA significantly induced the expression of

VDR in rat ileum and liver. Because LCA did not show these effects, these results suggest that the regulation of VDR by $1,25(OH)_2D_3$ and CDCA is not likely to be mediated by VDR or FXR activation. The involvement of Protein kinase C α (PKCα) could not be confirmed for the rat ileum but incubation of rat liver slices with the PKCα agonist, phorbol-12-myristate-13-acetate (PMA) significantly induced the VDR expression. Also in the human ileum VDR is induced by $1,25(OH)_2D_3$ and PMA but not by CDCA and LCA. Further, glucocorticoids decreased the VDR expression in rat and human liver and also in human ileum but not in rat ileum, which indicates a novel cross talk between VDR and GR.

6 Conclusions and future perspectives

In this thesis, we have demonstrated that the DMEs and DTs involved in the regulation of bile acid detoxification and synthesis, and bile acid transporters is regulated by **VDR, PXR, GR and FXR** nuclear receptors in a tissue specific manner by culturing rat and human intestine (jejunum, ileum and colon) and liver precision-cut slices in the presence of specific nuclear receptor ligands. During culturing of the slices in the medium, the expression of DMEs and DTs are altered, showing both up and down regulation **(chapter 2, 3, 4, 5, 6 and 7)**. As the mRNA expression of DMEs and DTs are regulated *in vivo* by natural inducers or repressors these changes can be explained by lack of these natural regulatory factors. For example the lack of bile acids in our culture medium can explain the changes in MRP2 and MRP3 expression in rat intestinal slices during control incubations **(chapter 3)**. However the slices remain responsive to inducers. Therefore, for the characterization and understanding of the role of NRs in the regulation DMEs and DTs in principle it might be useful to modify the culture medium to minimize the alteration of DMEs and DTs in the slices. During incubation the intestinal slices are equally exposed to the inducers from both the apical (luminal) and basolateral (blood) side, which is different from the *in vivo* situation, where orally taken drugs and endogenous bile acids are present at high concentrations in the intestinal lumen and the consequences need to be evaluated. Furthermore, significant interspecies differences were observed in the regulation of DMEs, DTs and NRs between rat and human intestine and liver and thus extrapolation of data from preclinical species to man is hazardous and underline the necessity of relevant *in vitro* models for human studies. The results presented in this thesis are based on the observed changes in mRNA levels of DMEs, DTs and NRs involved in bile acid synthesis, transport and detoxification in response to various nuclear receptor ligands measured by quantitative real time PCR (qRT-PCR). Although qRT-PCR is a powerful technique in measuring the changes in the gene expression, the changes in the mRNA expression levels might not always lead to the proportional changes in the protein expression and hence the activity. In future experiments, the changes in gene expression induced by the various nuclear receptor ligands need to be characterized at the level of protein either by western blot or by immunohistochemistry. To further gain insight in the role of various nuclear factors regulating the expression of genes at the transcription level, siRNA transfections in the

slices can be performed to momentarily knock out the particular transcription factor and to study the effects on a particular pathway.

Table 1 Summary of the effects of $1,25(OH)_2D_3$ (200 nM) on the expression genes in human livers; n= 4-5 human liver donors; criteria for induction and repression are 1.5-fold and 0.7-fold, respectively.

Human livers			HL1	HL2	HL5	HL6	HL3	HL4	HL7	Mean	S.E.M.	S *t*-Test
Gender			Female	N/A	Male	N/A	Female	Female	Female			
Age			54	N/A	65	N/A	72	64	42			
ATP pmol / µg of protein ± SD			*10.4 ± 1.5	*5.7 ± 1.9	*12.1 ± 1.0	11.1 ± 0.9	*3.3 ± 1.2	*9.7 ± 1.8	ND			
VDR (ΔC_T)			+ ve	+ ve	+ ve	+ ve	- ve	- ve	ND			
Gene												
$1,25(OH)_2D_3$	↑	CYP3A4	2.3	↔	1.5	1.9	0.3	ND	↔	1.30	0.28	0.294
	↓	CYP7A1	0.7	↔	0.7	↔	0.3	ND	↔	0.67	0.08	0.002
	↔	SHP	↔	1.5	0.27	↔	↔	ND	↔	0.91	0.15	0.556
	↔	HNF1α	↔	↔	↔	↔	1.5	ND	0.6	0.99	0.13	0.925
	↓	HNF4α	↔	↔	0.5	↔	0.5	ND	0.7	0.72	0.08	0.004
	↔	LXRα	0.7	↔	0.7	ND	2.16	ND	ND	1.12	0.31	0.640
	↔	PXR	↔	↔	↔	ND	0.66	ND	ND	0.82	0.06	0.004
	↔	FXR	0.7	↔	0.7	ND	↔	ND	ND	0.84	0.09	0.066
	↔	VDR	↔	0.5	↔	↔	NDE	NDE	ND	0.90	0.17	0.596
	↔	BSEP	0.5	0.7	↔	↔	1.96	ND	1.5	1.13	0.21	0.551
	↔	NTCP	0.4	↔	↔	↔	0.4	ND	↔	0.78	0.13	0.100
	↔	MRP2	0.5	↔	↔	↔	1.5	ND	↔	1.01	0.14	0.950
	↔	MRP3	↔	↔	0.7	0.7	NDE	ND	1.38	0.98	0.13	0.840
	↔	ASBT	↔	0.6	↔	ND	0.6	ND	ND	0.92	0.19	0.580
	↔	OSTα	0.5	2.9	0.5	ND	0.4	ND	ND	1.07	0.60	0.875
	↓	OSTβ	0.6	↔	0.4	ND	0.4	ND	ND	0.69	0.21	0.074

HL-human liver; ND-not done; NDE-not detectable; N/A – not available; TX – transplantation liver; PH partial hepatectomy liver; ↔ - No effect; ↓-repression; ↑-induction; "" data is taken from Khan et al.(2009a); All values are expressed as fold induction with respect to their solvent incubated controls.*

Table 2 Summary of the effects of chenodeoxycholic acid (CDCA) on the expression genes in human livers; n= 4-5 human liver donors; criteria for induction and repression are 1.5-fold and 0.7-fold, respectively.

Human livers			HL1	HL2	HL5	HL3	HL4	Mean	S.E.M.	S *t*-Test
Gender			Female	N/A	Male	Female	Female			
Age			54	N/A	65	72	64			
ATP pmol / µg of protein ± SD			*10.4 ± 1.5	*5.7 ± 1.9	*12.1 ± 1.0	*3.3 ± 1.2	*9.7 ± 1.8			
VDR (ΔC_T)			11.7	16.19	14.8	NDE	NDE			
Gene										
CDCA	↑	CYP3A4	1.7	1.5	1.8	2.7	9.0	3.34	1.43	0.140
		CYP7A1	0.7	0.07	0.3	↔	0.1	0.45	0.21	0.033
	↑	SHP	3.4	2.1	↔	4.76	6.57	3.55	0.99	0.033
	↔	HNF1α	↔	0.7	0.1	↔	ND	0.77	0.23	0.281
	↔	HNF4α	0.6	↔	0.1	2.4	ND	0.98	0.50	0.717
	↓	LXRα	↔	↔	0.6	0.5	↔	0.97	0.17	0.846
	↔	PXR	0.7	0.7	2.8	2.2	0.3	1.35	0.48	0.485
	↔	FXR	0.7	↔	↔	↔	↔	0.92	0.11	0.500
	↔	VDR	↔	↔	0.3	NDE	NDE	0.748	0.22	0.293
	↑	BSEP	2.3	↔	6.6	1.9	2.1	2.77	0.98	0.110
	↓	NTCP	0.4	↔	0.5	1.9	0.3	0.78	0.29	0.473
	↔	MRP2	↔	0.7	↔	2.14	↔	1.09	0.30	0.456
	↑	MRP3	↔	2.3	1.5	NDE	2.1	1.80	0.24	0.007
	↔	ASBT	↔	1.5	0.2	↔	↔	1.03	0.22	0.909
	↑	OSTα	10.7	24.3	11.9	4.8	25.2	15.38	4.01	0.007
	↑	OSTβ	117	91	132	14	204	112	30.69	0.007

HL-human liver; ND-not done; NDE-not detectable; N/A – not available; TX – transplantation liver; PH partial hepatectomy liver; ↔ - No effect; ↓-repression; ↑-induction; "" data is taken from Khan et al.(2009a); All values are expressed as fold induction with respect to their solvent incubated controls*

Table 3 Summary of the effects of dexamethasone (DEX) on the expression genes in human livers; n= 4-5 human liver donors; criteria for induction and repression are 1.5-fold and 0.7-fold, respectively.

Human livers			HL1	HL2	HL5	HL3	HL4	Mean	S.E.M.	S *t*-Test
Gender			Female	N/A	Male	Female	Female			
Age			54	N/A	65	72	64			
ATP pmol / µg of protein ± SD			*10.4 ± 1.5	*5.7 ± 1.9	*12.1 ± 1.0	*3.3 ± 1.2	*9.7 ± 1.8			
VDR (ΔC_T)			11.7	16.19	14.8	NDE	NDE			
Gene										
DEX	↑	CYP3A4	17.6	4.0	15.0	1.7	9.1	9.52	3.05	0.023
	↔	CYP7A1	1.9	0.3	0.2	1.7	0.1	0.83	0.39	0.677
	↔	SHP	↔	↔	2.8	↔	6.8	2.52	1.13	0.215
	↑	HNF1α	↔	↔	1.6	1.7	ND	1.35	0.20	0.079
	↑	HNF4α	1.9	↔	6.5	3.9	ND	3.31	1.24	0.071
	↔	LXRα	↔	↔	↔	↔	1.5	1.02	0.15	0.908
	↑	PXR	↔	↔	2.3	↔	1.6	1.5	0.21	0.043
	↔	FXR	0.3	↔	0.4	2.3	↔	1.05	0.37	0.899
	↓	VDR	0.4	0.4	0.2	ND	NDE	0.31	0.05	0.000
	↑	BSEP	8.7	2.3	15.2	3.3	3.6	6.61	2.40	0.048
	↑	NTCP	6.5	1.5	4.2	2.3	↔	3.04	1.03	0.083
	↔	MRP2	↔	1.9	0.6	↔	↔	1.2	0.22	0.387
	↔	MRP3	0.5	↔	2.0	ND	0.5	0.95	0.35	0.861
	↑	ASBT	3.6	2.0	1.5	2.2	1.9	2.23	0.36	0.010
	↓	OSTα	0.7	0.2	0.5	0.6	0.6	0.51	0.08	0.000
	↑	OSTβ	1.8	5.4	↔	↔	4.6	2.71	0.95	0.109

HL-human liver; ND-not done; NDE-not detectable; N/A – not available; TX – transplantation liver; PH partial hepatectomy liver; ↔ - No effect; ↓-repression; ↑-induction; "" data is taken from Khan et al.(2009a); All values are expressed as fold induction with respect to their solvent incubated controls.*

Table 4 Summary of the effects of lithocholic acid (LCA) on the expression genes in human livers; n= 4-7 human liver donors; criteria for induction and repression are 1.5-fold and 0.7-fold, respectively.

Human livers			HL1	HL2	HL5	HL6	HL3	HL4	HL7	Mean	S.E.M.	*p*
Gender			Female	N/A	Male	N/A	Female	Female	Female			
Age			54	N/A	65	N/A	72	64	42			
ATP pmol / µg of protein ± SD			*10.4 ± 1.5	*5.7 ± 1.9	*12.1 ± 1.0	11.1 ± 0.9	*3.3 ± 1.2	*9.7 ± 1.8	ND			
VDR (ΔC_T)			11.7	16.2	14.8	16.2	NDE	NDE	ND			
Gene												
LCA	↔	CYP3A4	0.6	0.6	1.8	0.5	2.2	3.2	2.0	1.54	0.38	0.175
	↔	CYP7A1	↔	↔	0.3	0.5	↔	0.1	1.9	0.90	0.25	0.683
	↔	SHP	↔	0.7	0.7	↔	2.6	3.1	↔	1.40	0.38	0.312
	↔	HNF1α	↔	↔	0.3	↔	↔	ND	↔	0.78	0.10	†0.042
	↔	HNF4α	↔	0.4	0.2	0.4	2.4	ND	0.4	0.80	0.34	0.523
	↔	LXRα	0.6	0.7	0.4	ND	↔	↔	ND	0.77	0.19	0.166
	↔	PXR	0.7	0.3	1.7	ND	1.5	0.4	ND	0.93	0.30	0.794
	↔	FXR	0.6	↔	0.5	ND	↔	↔	ND	0.86	0.16	0.336
		VDR	↔	0.5	0.5	0.3	NDE	NDE	ND	0.51	0.12	0.017
	↔	BSEP	0.4	↔	1.7	↔	0.7	0.2	↔	0.84	0.19	0.413
	↔	NTCP	0.3	0.30	↔	0.1	2.3	0.1	2.2	0.86	0.36	0.712
	↔	MRP2	0.5	↔	0.5	↔	↔	↔	1.5	1.03	0.16	0.867
	↔	MRP3	↔	↔	↔	↔	NDE	↔	↔	1.03	0.09	0.741
	↔	ASBT	↔	0.6	0.16	ND	↔	2.3	ND	1.08	0.40	0.796
	↑	OSTα	1.5	2.6	↔	ND	3.9	5.2	ND	2.87	0.85	0.014
	↑	OSTβ	2.6	1.7	2.5	ND	6.2	4.2	ND	3.42	0.89	0.004

HL-human liver; ND-not done; NDE-not detectable; N/A – not available; TX – transplantation liver; PH partial hepatectomy liver; ↔ - No effect; ↓-repression; ↑-induction; "" data is taken from Khan et al.(2009a); All values are expressed as fold induction with respect to their solvent incubated controls.* † *indicates all samples showed down regulation but to a different extent, which is outside the criteria for induction and repression.*

References

1.**Abrahamsson A, Gustafsson U, Ellis E, Nilsson LM, Sahlin S, Bjorkhem I, and Einarsson C.** Feedback regulation of bile acid synthesis in human liver: importance of HNF-4alpha for regulation of CYP7A1. *Biochem Biophys Res Commun* 330: 395-399, 2005.
2.**Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, and Suchy FJ.** Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem* 276: 28857-28865, 2001.
3.**Arrese M, Trauner M, Sacchiero RJ, Crossman MW, and Shneider BL.** Neither intestinal sequestration of bile acids nor common bile duct ligation modulate the expression and function of the rat ileal bile acid transporter. *Hepatology* 28: 1081-1087, 1998.
4.**Ballatori N, Christian WV, Lee JY, Dawson PA, Soroka CJ, Boyer JL, Madejczyk MS, and Li N.** OSTalpha-OSTbeta: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. *Hepatology* 42: 1270-1279, 2005.
5.**Ballatori N, Fang F, Christian WV, Li N, and Hammond CL.** Ost{alpha}-Ost{beta} is required for bile acid and conjugated steroid disposition in the intestine, kidney, and liver. *Am J Physiol Gastrointest Liver Physiol* 295: G179-186, 2008.
6.**Ballatori N, Fang F, Christian WV, Li N, and Hammond CL.** Ostalpha-Ostbeta is required for bile acid and conjugated steroid disposition in the intestine, kidney, and liver. *Am J Physiol Gastrointest Liver Physiol* 295: G179-G186, 2008.
7.**Bergheim I, Harsch S, Mueller O, Schimmel S, Fritz P, and Stange EF.** Apical sodium bile acid transporter and ileal lipid binding protein in gallstone carriers. *J Lipid Res* 47: 42-50, 2006.
8.**Brady JM, Cherrington NJ, Hartley DP, Buist SC, Li N, and Klaassen CD.** Tissue distribution and chemical induction of multiple drug resistance genes in rats. *Drug Metab Dispos* 30: 838-844, 2002.
9.**Brown AJ, Ritter CS, Holliday LS, Knutson JC, and Strugnell SA.** Tissue distribution and activity studies of 1,24-dihydroxyvitamin D2, a metabolite of vitamin D2 with low calcemic activity in vivo. *Biochem Pharmacol* 68: 1289-1296, 2004.
10.**Chen F, Ma L, Dawson PA, Sinal CJ, Sehayek E, Gonzalez FJ, Breslow J, Ananthanarayanan M, and Shneider BL.** Liver receptor homologue-1 mediates species- and cell line-specific bile acid-dependent negative feedback regulation of the apical sodium-dependent bile acid transporter. *J Biol Chem* 278: 19909-19916, 2003.
11.**Chen X, Chen F, Liu S, Glaeser H, Dawson PA, Hofmann AF, Kim RB, Shneider BL, and Pang KS.** Transactivation of rat apical sodium-dependent bile acid transporter and increased bile acid transport by 1alpha,25-dihydroxyvitamin D3 via the vitamin D receptor. *Mol Pharmacol* 69: 1913-1923, 2006.
12.**Cherrington NJ, Hartley DP, Li N, Johnson DR, and Klaassen CD.** Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2, and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. *J Pharmacol Exp Ther* 300: 97-104, 2002.
13.**Chiang JY.** Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. *J Hepatol* 40: 539-551, 2004.
14.**Chow ECY, Maeng HJ, Liu S, Khan AA, Groothuis GMM, and Pang KS.** 1a,25-Dihydroxyvitam D_3 Triggered Vitamin D Receptor and Farnesoid X Receptor-like Effects in Rat Intestine and Liver *In Vivo. Biopharmaceutics and Drug Disposition*, 2009.*(In Press)*
15.**Crestani M, Karam WG, and Chiang JY.** Effects of bile acids and steroid/thyroid hormones on the expression of cholesterol 7 alpha-hydroxylase mRNA and the CYP7 gene in HepG2 cells. *Biochem Biophys Res Commun* 198: 546-553, 1994.
16.**Danielsson H and Gustafsson J.** Biochemistry of bile acids in health and disease. *Pathobiol Annu* 11: 259-298, 1981.
17.**Dawson PA, Hubbert M, Haywood J, Craddock AL, Zerangue N, Christian WV, and Ballatori N.** The heteromeric organic solute transporter alpha-beta, Ostalpha-Ostbeta, is an ileal basolateral bile acid transporter. *J Biol Chem* 280: 6960-6968, 2005.
18.**de Waziers I, Cugnenc PH, Yang CS, Leroux JP, and Beaune PH.** Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *J Pharmacol Exp Ther* 253: 387-394, 1990.
19.**Denson LA, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, and Karpen SJ.** The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* 121: 140-147, 2001.
20.**Drocourt L, Ourlin JC, Pascussi JM, Maurel P, and Vilarem MJ.** Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J Biol Chem* 277: 25125-25132, 2002.
21.**Dumaswala R, Berkowitz D, and Heubi JE.** Adaptive response of the enterohepatic circulation of bile acids to extrahepatic cholestasis. *Hepatology* 23: 623-629, 1996.

22.**Eloranta JJ, Jung D, and Kullak-Ublick GA.** The human Na+-taurocholate cotransporting polypeptide gene is activated by glucocorticoid receptor and peroxisome proliferator-activated receptor-gamma coactivator-1alpha, and suppressed by bile acids via a small heterodimer partner-dependent mechanism. *Mol Endocrinol* 20: 65-79, 2006.
23.**Faber KN, Muller M, and Jansen PL.** Drug transport proteins in the liver. *Adv Drug Deliv Rev* 55: 107-124, 2003.
24.**Fukumori S, Murata T, Taguchi M, and Hashimoto Y.** Rapid and drastic induction of CYP3A4 mRNA expression via vitamin D receptor in human intestinal LS180 cells. *Drug Metab Pharmacokinet* 22: 377-381, 2007.
25.**Gascon-Barre M, Demers C, Mirshahi A, Neron S, Zalzal S, and Nanci A.** The normal liver harbors the vitamin D nuclear receptor in nonparenchymal and biliary epithelial cells. *Hepatology* 37: 1034-1042, 2003.
26.**Geier A, Martin IV, Dietrich CG, Balasubramaniyan N, Strauch S, Suchy FJ, Gartung C, Trautwein C, and Ananthanarayanan M.** Hepatocyte nuclear factor-4alpha is a central transactivator of the mouse Ntcp gene. *Am J Physiol Gastrointest Liver Physiol* 295: G226-233, 2008.
27.**Glaeser H, Drescher S, Hofmann U, Heinkele G, Somogyi AA, Eichelbaum M, and Fromm MF.** Impact of concentration and rate of intraluminal drug delivery on absorption and gut wall metabolism of verapamil in humans. *Clin Pharmacol Ther* 76: 230-238, 2004.
28.**Gnerre C, Blattler S, Kaufmann MR, Looser R, and Meyer UA.** Regulation of CYP3A4 by the bile acid receptor FXR: evidence for functional binding sites in the CYP3A4 gene. *Pharmacogenetics* 14: 635-645, 2004.
29.**Higgins JV, Paul JM, Dumaswala R, and Heubi JE.** Downregulation of taurocholate transport by ileal BBM and liver BLM in biliary-diverted rats. *Am J Physiol* 267: G501-507, 1994.
30.**Hirano S, Masuda N, and Oda H.** In vitro transformation of chenodeoxycholic acid and ursodeoxycholic acid by human intestinal flora, with particular reference to the mutual conversion between the two bile acids. *J Lipid Res* 22: 735-743, 1981.
31.**Hirohashi T, Suzuki H, Ito K, Ogawa K, Kume K, Shimizu T, and Sugiyama Y.** Hepatic expression of multidrug resistance-associated protein-like proteins maintained in eisai hyperbilirubinemic rats. *Mol Pharmacol* 53: 1068-1075, 1998.
32.**Hirohashi T, Suzuki H, Takikawa H, and Sugiyama Y.** ATP-dependent transport of bile salts by rat multidrug resistance-associated protein 3 (Mrp3). *J Biol Chem* 275: 2905-2910, 2000.
33.**Hofmann AF.** The enterohepatic circulation of conjugated bile acids in healthy man: quantitative description and functions. *Expos Annu Biochim Med* 33: 69-86, 1977.
34.**Hylemon PB, Gurley EC, Stravitz RT, Litz JS, Pandak WM, Chiang JY, and Vlahcevic ZR.** Hormonal regulation of cholesterol 7 alpha-hydroxylase mRNA levels and transcriptional activity in primary rat hepatocyte cultures. *J Biol Chem* 267: 16866-16871, 1992.
35.**Inokuchi A, Hinoshita E, Iwamoto Y, Kohno K, Kuwano M, and Uchiumi T.** Enhanced expression of the human multidrug resistance protein 3 by bile salt in human enterocytes. A transcriptional control of a plausible bile acid transporter. *J Biol Chem* 276: 46822-46829, 2001.
36.**Jung D, Elferink MG, Stellaard F, and Groothuis GM.** Analysis of bile acid-induced regulation of FXR target genes in human liver slices. *Liver Int* 27: 137-144, 2007.
37.**Jung D, Fantin AC, Scheurer U, Fried M, and Kullak-Ublick GA.** Human ileal bile acid transporter gene ASBT (SLC10A2) is transactivated by the glucocorticoid receptor. *Gut* 53: 78-84, 2004.
38.**Jung D, Fried M, and Kullak-Ublick GA.** Human apical sodium-dependent bile salt transporter gene (SLC10A2) is regulated by the peroxisome proliferator-activated receptor alpha. *J Biol Chem* 277: 30559-30566, 2002.
39.**Jung D, Mangelsdorf DJ, and Meyer UA.** Pregnane X receptor is a target of farnesoid X receptor. *J Biol Chem* 281: 19081-19091, 2006.
40.**Kliewer SA, Goodwin B, and Willson TM.** The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev* 23: 687-702, 2002.
41.**Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, Perlmann T, and Lehmann JM.** An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* 92: 73-82, 1998.
42.**Kolars JC, Awni WM, Merion RM, and Watkins PB.** First-pass metabolism of cyclosporin by the gut. *Lancet* 338: 1488-1490, 1991.
43.**Landrier JF, Eloranta JJ, Vavricka SR, and Kullak-Ublick GA.** The nuclear receptor for bile acids, FXR, transactivates human organic solute transporter-alpha and -beta genes. *Am J Physiol Gastrointest Liver Physiol* 290: G476-485, 2006.
44.**Lazaridis KN, Tietz P, Wu T, Kip S, Dawson PA, and LaRusso NF.** Alternative splicing of the rat sodium/bile acid transporter changes its cellular localization and transport properties. *Proc Natl Acad Sci U S A* 97: 11092-11097, 2000.

45.**Li H, Chen F, Shang Q, Pan L, Shneider BL, Chiang JY, Forman BM, Ananthanarayanan M, Tint GS, Salen G, and Xu G.** FXR-activating ligands inhibit rabbit ASBT expression via FXR-SHP-FTF cascade. *Am J Physiol Gastrointest Liver Physiol* 288: G60-66, 2005.
46.**Lillienau J, Crombie DL, Munoz J, Longmire-Cook SJ, Hagey LR, and Hofmann AF.** Negative feedback regulation of the ileal bile acid transport system in rodents. *Gastroenterology* 104: 38-46, 1993.
47.**Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, and Mangelsdorf DJ.** Vitamin D receptor as an intestinal bile acid sensor. *Science* 296: 1313-1316, 2002.
48.**Matic M, Mahns A, Tsoli M, Corradin A, Polly P, and Robertson GR.** Pregnane X receptor: promiscuous regulator of detoxification pathways. *Int J Biochem Cell Biol* 39: 478-483, 2007.
49.**Matsubara T, Yoshinari K, Aoyama K, Sugawara M, Sekiya Y, Nagata K, and Yamazoe Y.** Role of vitamin D receptor in the lithocholic acid-mediated CYP3A induction in vitro and in vivo. *Drug Metab Dispos* 36: 2058-2063, 2008.
50.**Morel F, Beaune PH, Ratanasavanh D, Flinois JP, Yang CS, Guengerich FP, and Guillouzo A.** Expression of cytochrome P-450 enzymes in cultured human hepatocytes. *Eur J Biochem* 191: 437-444, 1990.
51.**Nakata K, Tanaka Y, Nakano T, Adachi T, Tanaka H, Kaminuma T, and Ishikawa T.** Nuclear receptor-mediated transcriptional regulation in Phase I, II, and III xenobiotic metabolizing systems. *Drug Metab Pharmacokinet* 21: 437-457, 2006.
52.**Neimark E, Chen F, Li X, and Shneider BL.** Bile acid-induced negative feedback regulation of the human ileal bile acid transporter. *Hepatology* 40: 149-156, 2004.
53.**Nowicki MJ, Shneider BL, Paul JM, and Heubi JE.** Glucocorticoids upregulate taurocholate transport by ileal brush-border membrane. *Am J Physiol* 273: G197-203, 1997.
54.**Okuwaki M, Takada T, Iwayanagi Y, Koh S, Kariya Y, Fujii H, and Suzuki H.** LXR alpha transactivates mouse organic solute transporter alpha and beta via IR-1 elements shared with FXR. *Pharm Res* 24: 390-398, 2007.
55.**Ourlin JC, Lasserre F, Pineau T, Fabre JM, Sa-Cunha A, Maurel P, Vilarem MJ, and Pascussi JM.** The small heterodimer partner interacts with the pregnane X receptor and represses its transcriptional activity. *Mol Endocrinol* 17: 1693-1703, 2003.
56.**Pandak WM, Stravitz RT, Lucas V, Heuman DM, and Chiang JY.** Hep G2 cells: a model for studies on regulation of human cholesterol 7alpha-hydroxylase at the molecular level. *Am J Physiol* 270: G401-410, 1996.
57.**Pascussi JM, Gerbal-Chaloin S, Drocourt L, Maurel P, and Vilarem MJ.** The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim Biophys Acta* 1619: 243-253, 2003.
58.**Rao A, Haywood J, Craddock AL, Belinsky MG, Kruh GD, and Dawson PA.** The organic solute transporter alpha-beta, Ostalpha-Ostbeta, is essential for intestinal bile acid transport and homeostasis. *Proc Natl Acad Sci U S A* 105: 3891-3896, 2008.
59.**Rius M, Hummel-Eisenbeiss J, Hofmann AF, and Keppler D.** Substrate specificity of human ABCC4 (MRP4)-mediated cotransport of bile acids and reduced glutathione. *Am J Physiol Gastrointest Liver Physiol* 290: G640-649, 2006.
60.**Russell DW and Setchell KD.** Bile acid biosynthesis. *Biochemistry* 31: 4737-4749, 1992.
61.**Sauer P, Stiehl A, Fitscher BA, Riedel HD, Benz C, Kloters-Plachky P, Stengelin S, Stremmel W, and Kramer W.** Downregulation of ileal bile acid absorption in bile-duct-ligated rats. *J Hepatol* 33: 2-8, 2000.
62.**Schmiedlin-Ren P, Thummel KE, Fisher JM, Paine MF, Lown KS, and Watkins PB.** Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1alpha,25-dihydroxyvitamin D3. *Mol Pharmacol* 51: 741-754, 1997.
63.**Schmiedlin-Ren P, Thummel KE, Fisher JM, Paine MF, and Watkins PB.** Induction of CYP3A4 by 1 alpha,25-dihydroxyvitamin D3 is human cell line-specific and is unlikely to involve pregnane X receptor. *Drug Metab Dispos* 29: 1446-1453, 2001.
64.**Shih DQ, Bussen M, Sehayek E, Ananthanarayanan M, Shneider BL, Suchy FJ, Shefer S, Bollileni JS, Gonzalez FJ, Breslow JL, and Stoffel M.** Hepatocyte nuclear factor-1alpha is an essential regulator of bile acid and plasma cholesterol metabolism. *Nat Genet* 27: 375-382, 2001.
65.**Shneider BL, Dawson PA, Christie DM, Hardikar W, Wong MH, and Suchy FJ.** Cloning and molecular characterization of the ontogeny of a rat ileal sodium-dependent bile acid transporter. *J Clin Invest* 95: 745-754, 1995.
66.**Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, and Kliewer SA.** The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A* 98: 3369-3374, 2001.
67.**Stravitz RT, Sanyal AJ, Pandak WM, Vlahcevic ZR, Beets JW, and Dawson PA.** Induction of sodium-dependent bile acid transporter messenger RNA, protein, and activity in rat ileum by cholic acid. *Gastroenterology* 113: 1599-1608, 1997.

68.**Sun AQ, Ananthanarayanan M, Soroka CJ, Thevananther S, Shneider BL, and Suchy FJ.** Sorting of rat liver and ileal sodium-dependent bile acid transporters in polarized epithelial cells. *Am J Physiol* 275: G1045-1055, 1998.
69.**Thompson PD, Jurutka PW, Haussler CA, Whitfield GK, and Haussler MR.** Heterodimeric DNA binding by the vitamin D receptor and retinoid X receptors is enhanced by 1,25-dihydroxyvitamin D3 and inhibited by 9-cis-retinoic acid. Evidence for allosteric receptor interactions. *J Biol Chem* 273: 8483-8491, 1998.
70.**Timsit YE and Negishi M.** CAR and PXR: the xenobiotic-sensing receptors. *Steroids* 72: 231-246, 2007.
71.**Torchia EC, Cheema SK, and Agellon LB.** Coordinate regulation of bile acid biosynthetic and recovery pathways. *Biochem Biophys Res Commun* 225: 128-133, 1996.
72.**von Richter O, Greiner B, Fromm MF, Fraser R, Omari T, Barclay ML, Dent J, Somogyi AA, and Eichelbaum M.** Determination of in vivo absorption, metabolism, and transport of drugs by the human intestinal wall and liver with a novel perfusion technique. *Clin Pharmacol Ther* 70: 217-227, 2001.
73.**Xu Y, Iwanaga K, Zhou C, Cheesman MJ, Farin F, and Thummel KE.** Selective induction of intestinal CYP3A23 by 1alpha,25-dihydroxyvitamin D3 in rats. *Biochem Pharmacol* 72: 385-392, 2006.
74.**Zelcer N, van de Wetering K, de Waart R, Scheffer GL, Marschall HU, Wielinga PR, Kuil A, Kunne C, Smith A, van der Valk M, Wijnholds J, Elferink RO, and Borst P.** Mice lacking Mrp3 (Abcc3) have normal bile salt transport, but altered hepatic transport of endogenous glucuronides. *J Hepatol* 44: 768-775, 2006.