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

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Chapter 9
Summary

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 the human and rat intestine and liver. To predict drug metabolism and transport 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), which also exhibit profound interspecies differences. The most important NRs regulating the expression of DME's and DT's are the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), the glucocorticoid receptor (GR), the vitamin D receptor (VDR), the aryl hydrocarbon receptor (Ahr) and nuclear factor E2-related factor 2 (Nrf2).

Data on the regulation of the expression of DMEs and DTs in animals can be obtained from *in vivo* studies but in man *in vivo* data is difficult 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 the 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. The available *in vitro* methods to study the regulation of DMEs and DTs in human liver and intestine are primary cells (hepatocytes and enterocytes), and immortalized cell lines (HepG2 and Caco-2). However, the primary cells are unstable with respect to the expression of DME's, DT's and NRs whereas immortalized cell lines exhibit inadequate expression of DME's, DT's and NRs. In this thesis, precision-cut liver slices (PCLS) and the recently validated precision-cut intestinal slices (PCIS) are used as an *in vitro* model to study the regulation of DME's and DT's in rat and human intestine and liver as well as the species-specificity and inter-organ differences herein. As these functions vary along the length of the intestine, slices were prepared from jejunum, ileum and colon.

The main focus of the thesis was on the VDR mediated effects by calcitriol ($1,25(OH)_2D_3$) and lithocholic acid (LCA) on the regulation of DME's and DT's. Further, to obtain more insight in the mechanism, the effects of the VDR ligands were compared with those of specific ligands for other nuclear receptors, chenodeoxycholic acid (CDCA) and GW4064 for FXR, and pregnenolone-16α carbonitrile (PCN) for PXR, budesonide (BUD) for GR and dexamethasone (DEX) for GR and PXR.

In **chapter 1**, the interspecies and inter-organ differences in the expression and regulation of DME's and DT's and the currently available models to study their impact on drug metabolism are summarized.

In **chapter 2**, the mRNA expression of the nuclear receptors VDR, PXR and FXR in the different regions of the rat and human intestine was characterized. In addition, the role of the VDR-, PXR-, GR- and FXR-specific ligands: $1,25(OH)_2D_3$, LCA, PCN, DEX, BUD and CDCA in the regulation of the mRNA expression of cytochrome P450 (CYP P450) 3A isozymes was studied in different regions of the rat intestine and liver, and in the human ileum and liver using precision-cut tissue slices. The CYP3A isozymes are responsible for the metabolism of 60% of the marketed drugs and of a number of endogenous compounds such as bile acids. In the rat intestine, CYP3A1 expression was found to be highly inducible by the VDR ligand, and to a lesser extent by PXR and GR ligands. The induction showed a gradient along the length of the intestine which was different for the different ligands but did not correlate with the gradient of the respective NRs. Furthermore, CYP3A2 expression was found to be exclusively regulated by the VDR ligand in the rat ileum only. CYP3A9 expression in the liver and in all regions of the intestine appeared to be mainly regulated by PXR and GR ligands but not by the VDR ligand. In contrast, the VDR ligand did not affect the expression of CYP3A isozymes in the rat liver. This organ specificity can be explained by the specific localization of VDR in the bile duct epithelial cells. In the rat liver VDR is not expressed in the hepatocytes, where CYP3A isozymes are highly expressed. In contrast to the rat CYP3A isozymes, human CYP3A4 was induced by the VDR ligand in ileum and liver, which can be explained by the observation that in the human liver VDR is expressed in the hepatocytes along with CYP3A4, which was confirmed at the level of protein by immunohistochemistry **(chapter 4).** These VDR ligand mediated *in vitro* effects on the regulation of CYP3A isozymes in the rat intestine and liver were confirmed *in vivo* by treating the rats with $1,25(OH)_2D_3$ **(chapter 5)**.

In **chapter 3**, the effects of another endogenous VDR ligand, LCA, on the regulation of CYP3A isozymes in the rat intestine and liver, and the human ileum and liver at the level of mRNA were investigated. LCA is a toxic bile acid formed by bacterial biotransformation of the primary bile acid CDCA in the terminal part of the ileum. In contrast to $1,25(OH)_2D_3$, LCA is also a ligand for PXR and FXR. Similar to $1,25(OH)_2D_3$ LCA induced the expression of CYP3A1 along the length of the intestine with the highest induction in ileum slices, and CYP3A2 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, which is presumably mediated by PXR. LCA induced CYP3A4 in human ileum slices, in contrast to $1,25(OH)_2D_3$, LCA did not affect the CYP3A4 expression in human liver slices. Furthermore, as LCA is also an FXR ligand, and FXR is known to play an important role in the regulation of bile acid homeostasis, the direct effects of LCA on the regulation of a bile acid synthesis enzyme, bile acid transporters and nuclear receptors were studied in rat and human liver slices and compared with the effects of CDCA, a high affinity FXR ligand **(chapter 4)** and these results are summarized in **chapter 8.**

In **chapter 4,** the influence of the VDR ligand, $1,25(OH)_2D_3$ on the regulation of bile acid homeostasis was compared with that of the FXR ligand, CDCA by measuring the mRNA expression of the bile acid transporters, the rate limiting enzyme in bile acid synthesis, CYP7A1 as well as the NRs involved in this regulation in rat and human liver. The VDR ligand, $1,25(OH)_2D_3$ decreased the expression of CYP7A1 and the basolateral transporter, sodium dependent taurocholate co-transporting protein (NTCP) expression in human liver slices but not in rat liver slices, without affecting the expression of the canalicular bile acid transporters BSEP and MRP2 in human and rat liver slices. In line with these findings, $1,25(OH)_2D_3$ decreased the expression of HNF4α, which is reported to be essential for the basal expression of NTCP in human but not in rat liver. As expected CDCA decreased the NTCP and induced the BSEP expression in rat and human liver slices, although the decrease in NTCP expression in human liver slices was not significant, which is in line with previous data from our lab. These data confirms the intactness of the FXR pathway in the liver slices during incubation. The species specific differences in the VDR effects in the regulation of CYP7A1 and NTCP in human and rat liver slices is attributed to the species differences in the cellular localization of VDR, which is expressed in human hepatocytes but not in the rat hepatocytes, which is confirmed at the level of protein by immunohistochemistry.

In chapter 5, the VDR, FXR and GR mediated regulation of the apical sodium dependent bile acid export transporter (ASBT) and the NRs regulating its expression in rat and human ileum and liver precision-cut tissue slices was characterized at the level of mRNA. 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**. (*Mol Pharmacol 69: 1913-1923, 2006)***, 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 $1,25(OH)_2D_3$. Interestingly, we found a repression of HNF1α in rat ileum slices treated with $1,25(OH)_2D_3$, which may explain the down regulation of ASBT since HNF1α is essential for the basal expression of ASBT. 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. However, the *in vitro* results of repression of ASBT were not found in Wistar rats *in vivo* treated with $1,25(OH)_2D_3$, where ASBT remained unaltered. These differences might be attributed to a lower exposure of the 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 $1,25(OH)_2D_3$ **(Chapter 2**). Also LCA decreased the expression of ASBT in the ileum of man and rat, which may be a VDR and/or an FXR effect as the FXR ligand CDCA also reduced the ASBT expression in the human ileum but not in rat ileum. In accordance with in vivo data, the GR ligands, DEX and BUD induced the ASBT expression in rat and human ileum slices with simultaneous induction of HNF1α, showing the viability of the slice model to show an induction of ASBT.

In **chapter 6,** the regulation of the basolateral bile acid transporter, organic solute transporter (OST) consisting of two half transporters, α and β (OSTα and OSTβ) involved in the absorption of bile acids in the rat and human ileum and by the cholangiocytes in the liver by VDR, FXR, GR and PXR nuclear receptor ligands was studied using precision cut rat and human intestinal and liver slices. 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. Further studies are needed to evaluate the role of VDR ligands in the reabsorption of bile acids in the ileum of Wistar rats.

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, a known VDR ligand 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 proteinkinase Cα (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 the rat and human liver and also in the human ileum but not in the rat ileum, which indicates a novel cross talk between VDR and GR.

In **Chapter 8**, the results of **chapters 2, 3, 4, 5, 6 and 7** were discussed and the major conclusions were summarized.

In summary, we have clearly demonstrated in this thesis that the enzymes and transporters involved in drug metabolism, bile acid detoxification and synthesis, and bile acid transport are regulated by the nuclear receptors **VDR, PXR, GR and FXR** 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. This *in vitro* model was found to reflect the *in vivo* established regulations properly. In contrast to *in vivo* where confounding secondary reactions may influence the regulation, the slice model

allows the study of direct effects of a ligand in different organs under identical circumstances, taking into account not only binding to the NR but also uptake, metabolism and excretion of the ligand as well as possible influences of co-activators and repressors. Furthermore, significant interspecies differences were observed in the regulation of DMEs, DTs and NRs between rat and human intestine and liver. This implicates that extrapolation of data from preclinical species to man is hazardous and underlines the necessity of relevant *in vitro* models for human studies. *In vitro* research with human tissue slices contributes to obtain human specific data and to reduce the number of experimental animals.