



Comparison of effects of VDR versus PXR, FXR and GR ligands on the regulation of CYP3A isozymes in rat and human intestine and liver

Ansar A. Khan^{a,*}, Edwin C.Y. Chow^b, Anne-miek M.A. van Loenen-Weemaes^a, Robert J. Porte^c, K. Sandy Pang^b, Geny M.M. Groothuis^a

^a Pharmacokinetics, Toxicology and Targeting, Department of Pharmacy, University of Groningen, Ant. Deusinglaan 1, 9713 AV, Groningen, The Netherlands

^b Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, ON M5S 3M2, Canada

^c Department of Hepatobiliary Surgery and Liver Transplantation, University Medical Center Groningen (UMCG), University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands

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ABSTRACT

In this study, we compared the regulation of CYP3A isozymes by the vitamin D receptor (VDR) ligand 1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) against ligands of the pregnane X receptor (PXR), the glucocorticoid receptor (GR) and the farnesoid X receptor (FXR) in precision-cut tissue slices of the rat jejunum, ileum, colon and liver, and human ileum and liver. In the rat, 1,25(OH)₂D₃ strongly induced CYP3A1 mRNA, quantified by qRT-PCR, along the entire length of the intestine, induced CYP3A2 only in ileum but had no effect on CYP3A9. In contrast, the PXR/GR ligand, dexamethasone (DEX), the PXR ligand, pregnenolone-16 α carbonitrile (PCN), and the FXR ligand, chenodeoxycholic acid (CDCA), but not the GR ligand, budesonide (BUD), induced CYP3A1 only in the ileum, none of them influenced CYP3A2 expression, and PCN, DEX and BUD but not CDCA induced CYP3A9 in jejunum, ileum and colon. In rat liver, CYP3A1, CYP3A2 and CYP3A9 mRNA expression was unaffected by 1,25(OH)₂D₃, whereas CDCA decreased the mRNA of all CYP3A isozymes; PCN induced CYP3A1 and CYP3A9, BUD induced CYP3A9, and DEX induced all three CYP3A isozymes. In human ileum and liver, 1,25(OH)₂D₃ and DEX induced CYP3A4 expression, whereas CDCA induced CYP3A4 expression in liver only. In conclusion, the regulation of rat CYP3A isozymes by VDR, PXR, FXR and GR ligands differed for different segments of the rat and human intestine and liver, and the changes did not parallel expression levels of the nuclear receptors.

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1. Introduction

The cytochrome P450 enzymes constitute a family of heme protein oxygenases that display considerable similarities in their molecular weights, immunohistochemical properties, and substrate specificities (Gonzalez, 1988). The CYP3A isoforms play an important role in oxidation of endogenous steroids and toxic hydrophobic bile acids. In the rat, the CYP3A family consists of five isoforms: CYP3A1/CYP3A23 (Gonzalez et al., 1985), CYP3A2 (Gonzalez et al., 1986), CYP3A9 (Wang et al., 1996), CYP3A18 (Strotkamp et al., 1995) and CYP3A62 (Matsubara et al., 2004). These enzymes are expressed predominantly in the liver and in the enterocytes of the intestine (Kolars et al., 1994). The distribution of CYP3A isozymes in the rat appears to be sex-, tissue- and age-dependent. CYP3A2 and CYP3A18 are predominantly expressed in male rats (Gonzalez et al., 1986; Nagata et al., 1996; Strotkamp

et al., 1995), while CYP3A9 and CYP3A62 expression is higher in female rats. CYP3A1 and CYP3A2 are predominantly expressed in the rat liver, and CYP3A62, in female livers (Matsubara et al., 2004), whereas CYP3A9 is highly expressed in the intestine relative to the liver (Mahnke et al., 1997; Wang and Strobel, 1997). The human CYP3A family which is expressed in the liver is composed of at least four isozymes: CYP3A4, CYP3A5, CYP3A7 and CYP3A43 of which CYP3A4 is the predominant isozyme expressed in adult human liver (Guengerich et al., 1986). CYP3A4 and CYP3A5 isozymes are present along the human digestive tract, with CYP3A5 mainly present in the stomach and CYP3A4 along the intestine segments (Kolars et al., 1994).

The expression of CYP3A isoforms in rats and humans was reported to be modulated by exogenous and endogenous ligands through the pregnane X receptor (PXR) (Lu et al., 1972), the glucocorticoid receptor (GR) (Huss et al., 1999), and the vitamin D receptor (VDR) (Makishima et al., 2002; Thummel et al., 2001; Xu et al., 2006). Recently, a FXR response element (FXRE) was found in the human CYP3A4 promoter, and induction by CDCA, a FXR ligand, was noted (Gnerre et al., 2004). The 5' flanking promoter regions of

* Corresponding author. Tel.: +31 50 363 7565; fax: +31 50 363 3247.
E-mail address: a.a.khan@rug.nl (A.A. Khan).

the rat and human CYP3A are characterized by direct repeats spaced by three base pairs (DR3) and everted repeats spaced by six base pairs (ER6) (Gnerre et al., 2004; Hashimoto et al., 1993; Thummel et al., 2001). PXR, FXR and VDR directly bind to the respective response elements pursuant to the ligand binding and heterodimerization with retinoic acid X receptor α (RXR α) (Gnerre et al., 2004; Lehmann et al., 1998; Thompson et al., 1999). In contrast, the GR effects on CYP3A isozymes in rat and humans have been attributed indirectly to the induction of HNF4 α and PXR (Huss and Kasper, 1998).

The effects of various ligands on rat and human CYP3A enzymes in the intestine and liver have been studied *in vitro* in both primary cultured hepatocytes and enterocytes, and immortalized human cell lines such as HepG2 and Caco-2 cells. Immortalized intestinal cell lines derived from the different regions of the rat intestine were utilized to study the regulation of drug metabolizing enzymes (Zhang et al., 1997). However, these cell lines lack the normal expression of nuclear receptors (NRs), metabolic enzymes and transporters. For example, Caco-2 cells are PXR-deficient and exhibit reduced levels of drug metabolizing enzymes (Li et al., 2003). Furthermore, cell lines are unable to reflect the segmental expression of CYP3A isozymes and the gradients of activities along the length of the rat intestine (Liu et al., 2006; van de Kerkhof et al., 2005). The induction/repression of CYP3A isoforms in the intact liver and intestinal tissue in response to ligands of the NRs has not been extensively investigated. Such a response is dependent not only on the presence of NR response elements, but also on the expression levels of the NRs and exposure of the particular cell to the ligand. This exposure is the result of uptake, metabolism and excretion of the ligand and its metabolites and may differ between the various regions of the intestine and the liver as a result of differences in expression of uptake and excretion transporters and metabolizing enzymes. Different regions of the intestine and liver are exposed to different concentrations of the ligands *in vivo*. For an appreciation of the potential variation between the different organs and their sensitivity towards the NR ligands, these organs or tissues should be studied under identical conditions.

Therefore, in this study, we compared the effects of various NR ligands on the intestine and liver of the rat and human in precision-cut tissue slices. This model has been previously validated as a useful *ex vivo* model for induction studies (Olinga et al., 2008; van de Kerkhof et al., 2007b, 2008) that enables us to investigate the effects of inducing ligands under identical incubation conditions for the liver and intestine. We tested the hypothesis that the regulation of rat and human CYP3A isozymes by VDR ligands differed from those by PXR, GR and FXR ligands. We compared the induction potential of PXR, FXR and GR ligands to that of VDR ligand, 1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) on changes in mRNAs of the various CYP3A isoforms in the small intestine (jejunum and ileum), colon and liver of the rat and the CYP3A4 in human ileum and liver slices, and investigated whether these responses correlated to the expression levels of the NRs.

2. Materials and methods

2.1. Materials

1,25(OH)₂D₃ in ethanol was purchased from BIOMOL Research Laboratories, Inc., Plymouth Meeting, PA. Chenodeoxycholic acid was purchased from Calbiochem, San Diego, CA, dexamethasone was from Genfarma bv, Maarsse. The solvents: ethanol, methanol and DMSO were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO); Gentamicin and Williams medium E with glutamax-I and amphotericin B (Fungizone)-solution were obtained from Gibco (Paisley, UK). D-Glucose and HEPES were procured from ICN

Biomedicals, Inc. (Eschwege, Germany). Low gelling temperature agarose, pregnenolone-16 α carbonitrile and budesonide were purchased from Sigma–Aldrich (St. Louis, MO). RNAeasy mini columns were obtained from Qiagen, Hilden, Germany. Random primers (500 μ g/ml), MgCl₂ (25 mM), RT buffer (10 \times), PCR nucleotide mix (10 mM), AMV RT (22 U/ μ l) and RNasin (40 U/ μ l) were procured from Promega Corporation, Madison WI, USA. SYBR green and Taq Master Mixes were purchased from Applied Biosystems, Warrington, UK and Eurogentech, respectively. ATP Bioluminescence Assay kit CLS II is procured from Roche, Mannheim, Germany. All primers were purchased from Sigma Genosys. All reagents and materials used were of the highest purity that was commercially available.

2.2. Animals

Male Wistar (HsdCpb:WU) rats weighing about 230–250 g were purchased from Harlan (Horst, The Netherlands). Rats were housed in a temperature and humidity controlled room on a 12-h light/dark cycle with food (Harlan chow no 2018, Horst, The Netherlands) and tap water *ad libitum*. The animals were allowed to acclimatize for 7 days before experimentation. The experimental protocols were approved by the Animal Ethical Committee of the University of Groningen.

2.3. Rat liver and intestine

Under isoflurane/O₂/N₂O anaesthesia, the small intestine, colon and liver were excised from the rat. Small intestine and colon were immediately placed into ice-cold carbogenated Krebs–Henseleit buffer, supplemented with 10 mM HEPES, 25 mM sodium bicarbonate and 25 mM D-Glucose, pH 7.4 (KHB) and stored on ice until the preparation of slices. Livers were stored in ice-cold University of Wisconsin solution (UW) until slicing.

2.4. Human liver and ileum tissue

Pieces of human liver tissue were obtained from patients undergoing partial hepatectomy for the removal of carcinoma or from redundant parts of donor livers remaining after split liver transplantation as described previously by Olinga et al. (2008). Donor characteristics are given in Table 1. Human ileum was obtained as part of the surgical waste after resection of the ileo-colonic part of the intestine in colon carcinoma patients, donor characteristics are given in Table 2. After surgical resection, the ileum tissue was immediately placed in ice-cold KHB. The research protocols were approved by the Medical Ethical Committee of the University Medical Center, Groningen with informed consent of the patients.

Table 1

Characteristics of human liver donor used: ATP contents after 3 and 24 h of incubation (each value is a mean \pm S.D. of three slices per time point)^a.

Human liver (HL)	Gender	Age	ATP-content (pmol/ μ g of protein)	
			3 h	24 h
HL1 ^{b,c}	Female	54	9.2 \pm 0.5	10.4 \pm 1.5
HL2	Not available		4.2 \pm 0.9	5.7 \pm 1.9
HL3 ^c	Female	72	3.4 \pm 0.8	3.3 \pm 1.2
HL4	Female	64	7.2 \pm 1.2	9.7 \pm 1.8
HL5 ^c	Male	65	12.1 \pm 1.2	12.1 \pm 1.0
Mean \pm S.E.M.			7.2 \pm 1.6	8.2 \pm 1.6
P value				0.66

^a Data were expressed as mean \pm S.D.

^b Human liver tissue for immunohistochemistry of VDR.

^c Human livers responsive to CYP3A4 induction by 1,25(OH)₂D₃.

Table 2

Characteristics of human ileum donor used. The terminal ileum was obtained from colon carcinoma patients as part of tumor resection.

Human ileum (HIL)	Gender	Age	Medical history
HIL 1	F	85	Colon carcinoma; coronary disease
HIL 2	M	60	Colon carcinoma
HIL 3	F	61	Colon carcinoma
HIL 4		Not available	
HIL 5	F	69	Colon carcinoma

2.5. Preparation of slices

2.5.1. Rat and human intestinal slices

Rat intestinal slices were prepared as published before (van de Kerkhof et al., 2005). In brief, the rat jejunum (at 25–40 cm from the stomach), ileum (5 cm proximal to the ileocecal valve) and colon (large intestine, distal to the ileocecal valve) tissues were separated. The jejunum, ileum and colon were divided into approximately 3-cm segments. The lumen of the segments was flushed with ice-cold KHB that was aerated with carbogen. Thereafter, segments were tied at one end and filled with 3% low gelling agarose solution in saline that was kept at 37 °C, then cooled immediately in KHB allowing the agarose to solidify. Subsequently, the agarose filled segments were embedded in agarose solution filled pre-cooled embedding unit (Alabama R&D, Munford, AL, USA). The agarose filled solid embedded intestinal segments were then placed in the pre-cooled Krumdieck tissue slicer (Alabama R&D, Munford, AL, USA) containing carbogenated ice-cold KHB, and precision-cut slices were prepared with a thickness of approximately 200 μm and wet weight of 2–3 mg (without agarose) (cycle speed 40: interrupted mode). Slices were stored in carbogenated ice-cold KHB on ice until the start of the experiment which usually varies between 2 to 3 h after sacrificing the rat.

Human ileum slices were prepared according to the method described for the jejunum (van de Kerkhof et al., 2006). In brief, ileum tissue was stripped of the muscular layer and the mucosal tissue was transferred to carbogenated ice-cold KHB. Mucosal tissue was cut into rectangular pieces of ~6–8 mm wide and these were subsequently embedded in low gelling 3% agarose in saline using pre-cooled tissue embedding unit (Alabama R&D, Munford, AL, USA) allowing the agarose solution to solidify. Precision-cut slices of approximately 200-μm thick were prepared as described above for rat intestine.

2.5.2. Rat and human liver slices

Cylindrical cores of 8 mm were prepared from rat livers and human liver tissue by advancing a sharp rotating metal tube in the liver tissue and were subsequently placed in the pre-cooled Krumdieck tissue slicer. The slicing was performed in carbogenated ice-cold KHB. The thickness of the liver slice was kept at ~200–300 μm and a wet weight of 10–12 mg were prepared with the standard settings (cycle speed 40: interrupted mode) of the Krumdieck tissue slicer. Subsequently, slices were stored in ice-cold UW solution on ice prior to the start of the experiment, which usually varies from 1 to 3 h from sacrificing the rat and for human livers 2 to 3 h post-surgery.

2.6. Incubation of rat and human intestinal slices

Slices were incubated individually in the 12-well, sterile tissue culture plates (Grenier bio-one GmbH, Frickenhausen, Austria) containing 1.3 ml William's medium E supplemented with D-glucose to a final concentration of 25 mM, gentamicin sulfate (50 μg/ml), amphotericin/fungizone (250 μg/ml), and saturated with carbogen. The plates were placed in humidified plastic container kept at 37 °C and continuously gassed with carbogen and shaken at 80 rpm.

Rat intestinal slices were incubated for 12 h because the expression of villin and GAPDH remained unchanged up to 12 h, whereas in pilot experiments, the expression of villin was significantly decreased after 24 h of incubation, indicating loss of epithelial cells. Human ileum slices were incubated for 8 and 24 h, and showed that villin expression remained unchanged up to 24 h. Rat and human intestinal slices were incubated with 1,25(OH)₂D₃ (final concentrations 5–100 nM), CDCA (final concentration 50 μM), DEX (final concentrations 1 and 50 μM) and BUD (final concentration 10 nM). Furthermore, rat intestinal slices were also incubated with PCN (final concentration 10 μM). All ligands were added as a 100-times concentrated, stock solution in ethanol (for 1,25(OH)₂D₃), methanol (for CDCA) and DMSO (for DEX/BUD/PCN) and had no or only minor effects on villin expression. Higher concentrations of CDCA (100 μM) significantly reduced villin expression and considered toxic. Control slices were incubated in William's medium E with 1% ethanol, methanol and DMSO without inducers. From a single rat or human tissue sample, six (rat intestine) or three (human intestine) replicate slices were subjected to each experimental condition. After the incubation these replicate slices were harvested, pooled and snap-frozen in liquid nitrogen to obtain sufficient total RNA for qRT-PCR analysis. Samples were stored in –80 °C freezer until RNA isolation. These experiments were replicated in 3–5 rats and 3–5 human ileum donors.

2.7. Incubation of rat and human liver slices

Slices were incubated individually in 6-well, sterile tissue culture plates (Grenier bio-one GmbH, Frickenhausen, Austria) containing 3.2 ml William's medium E supplemented with D-glucose to a final concentration of 25 mM, gentamicin sulfate (50 μg/ml) and saturated with carbogen. The plates were placed in humidified plastic container kept at 37 °C and continuously gassed with carbogen and shaken at 80 rpm. Rat slices were incubated with 1,25(OH)₂D₃ (final concentrations 10–200 nM), CDCA (final concentrations 10–100 μM) and DEX (final concentrations 1–50 μM). Apart from the above inducers, rat liver slices were also incubated with PCN (final concentration 10 μM) and BUD (final concentrations 10–100 nM). All inducers were added as a 100-times concentrated stock solution in ethanol (for 1,25(OH)₂D₃), methanol (for CDCA) and DMSO (for DEX/PCN/BUD). Control rat and human liver slices were incubated in William's medium E with 1% ethanol, methanol, and DMSO, the vehicles. Rat and human liver slices were incubated for 8 and 24 h, respectively. From a single rat/single human liver donor three replicate slices were subjected to identical incubation conditions. At the end of the incubation these replicate slices were harvested, pooled and snap-frozen in liquid nitrogen to obtain sufficient total RNA for qRT-PCR analysis. Samples were stored in –80 °C freezer until RNA isolation. These experiments were replicated in 3–5 rats and 4–5 human liver donors.

2.8. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA from rat and human intestine and liver samples were isolated by using RNeasy mini columns from Qiagen according to the manufacturer's instruction. RNA quality and concentrations were determined by measuring the absorbance at 260, 230 and 280 nm using a Nanodrop ND100 spectrophotometer (Wilmington, DE, USA). The ratio of absorbance measured at 260 over 280 and 230 over 260 was always above 1.8. About 2 μg of total RNA in 50 μl was reverse transcribed into template cDNA using random primers (0.5 μg/ml), PCR nucleotide mix (10 mM), AMV RT (22 U/μl), RT buffer (10×), MgCl₂ (25 mM) and RNAasin (40 U/μl).

Real time quantitative PCR (qRT-PCR) was performed for genes of interest using primer sequences given in Table 3 by

Table 3
Oligonucleotides for quantitative real-time PCR, rat and human genes (SYBR Green and Taqman[®] analysis).

Gene	Forward primer(5'-3')	Reverse primer(5'-3')	Gene bank number
r Villin	GCTCTTTGAGTGCTCCAACC	GGGGTGGGCTCTGAGGTATT	XM.001057825
r GAPDH	CTGTGGTCATGAGCCCCTCC	CGCTGGTGCTGAGTATGTCG	XR.008524
r CYP3A1	GGAAATTCGATGGAGTGC	AGGTTTGCCTTCTCTTGCC	L24207
r CYP3A2	AGTAGTGACGATTCACATAT	TCAGAGGTATCTGTGTTTCT	XM.573414
r CYP3A9	GGACGATTCTTGCTTACAGG	ATGCTGGTGGGCTTGCCCTTC	U46118
r FXR	CCAACCTGGGTTTCTACCC	CACACAGCTCATCCCTTT	NM.021745
r PXR	GATGATCATGTCTGATGCCGCTG	GAGGTTGGTAGTTCAGATGCTG	NM.052980
h Villin	CAGCTAGTGAACAAGCCTGTAGAGGAGC	CCACAGAAGTTTGTGCTCATAGGC	NM.007127
h CYP3A4	GCCTGGTCTCCTCTATCTA	GGCTGTTGACCATCATAAAAG	DQ924960
h VDR	GGAAAGTGCAGAGGAAGCGGAGATG	AGAGCTGGGACAGCTCTAGGTCAC	NM.000376
r β actin ^a	Assay-by-Design [™] FAM labelled, Part number 4331348 (Applied Biosystems)		NM.031144
r VDR ^a	TGACCCCACTACGCTGACT	CCTTGAGAATAGCTCCCTGACT	24873
h GAPDH ^b	Assay-by-Design [™] ID, Hs99999905_m1		NM.002046
	Probe, 6FAM, GCGCTGGTCCACGAGGCTGCTTTT, NFQ		

r, rat genes and h, human genes.

^a Primer sets for rat Taqman[®] Gene analysis.

^b Primer sets for human Taqman[®] Gene analysis.

two detection systems based on the availability of primer sets; villin and GAPDH were used as house-keeping genes for intestinal epithelial cells and liver cells, respectively, and CYP3A1, CYP3A2, CYP3A9, PXR and FXR were analyzed by the SYBR Green detection system. Primer sequences used for CYP3A1, CYP3A2 and CYP3A9 analysis were identical to those reported earlier by Mahnke et al. (1997). All primer sets were analyzed using BLASTn to ensure primer specificity for the gene of interest (<http://www.ncbi.nlm.nih.gov/BLAST/>). For qRT-PCR using the SYBR Green detection system ~50 ng of cDNA was used in a total reaction mixture of 20 μ l of the SYBR Green mixture (Applied Biosystems, Warrington, UK). The PCR conditions are step 1: 95 °C for 10 min; and step 2: 40 cycles of 95 °C, 15 s; 56 °C, 60 s and 72 °C, 40 s, followed by a dissociation stage (at 95 °C for 15 s, at 60 °C for 15 s and at 95 °C for 15 s) to determine the homogeneity of the PCR product. Further, the control consisting of water (with water instead of total mRNA, which has been subjected to reverse transcription protocol) and the mRNA control (isolated mRNA which has not been subjected to reverse transcription protocol) were used to determine primer dimer formation and contamination of DNA in the isolated samples, respectively. Amplification plots and dissociation curves of the controls did not show any signal and dissociation product, suggesting the lack of primer dimer formation. In addition total RNA from the samples for the preparation of cDNA appeared to be free of DNA contamination. β -actin and VDR genes were analyzed by Taqman[®] analysis using primer sequences given in Table 3. For Taqman[®] analysis ~250 ng of cDNA was used in a total reaction mixture of 10 μ l Taq Master Mix (2 \times). The qRT-PCR conditions for Taqman[®] analysis were: step 1, 95 °C for 10 min; step 2, 40 cycles of 95 °C for 15 s and 60 °C for 60 s. All samples were analyzed in duplicates in 384 well plates using ABI7900HT from Applied Biosystems. The comparative threshold cycle (C_T) method was used for relative quantification since C_T was inversely related to the abundance of mRNA transcripts in the initial sample. The mean C_T of the duplicate measurements was used to calculate the difference in C_T for gene of interest and the house keeping gene, villin for intestine and GAPDH for liver (ΔC_T). This ΔC_T value of the treated sample was compared to the corresponding ΔC_T of the solvent control ($\Delta \Delta C_T$). Data are expressed as fold induction or repression of the gene of interest according to the formula $2^{-(\Delta \Delta C_T)}$.

2.9. ATP and protein content of the human liver slices

Viability of human liver slices during incubation was determined by measuring the ATP contents of the slices according to

the method described earlier by de Kanter et al. (2002). In brief, control human liver slices were incubated in 3.2 ml of William's medium E, supplemented with D-glucose to a final concentration of 25 mM, gentamicin sulfate (50 μ g/ml), and saturated with carbon, as described in Section 2.7 for 3 and 24 h. At the end of incubation time, three replicate slices were collected individually in 1 ml 70% ethanol (v/v) containing 2 mM EDTA (pH 10.9) and snap-frozen in liquid nitrogen and stored at -80 °C freezer until analysis. The samples were disrupted and homogenized by sonication, and ATP extracts were diluted 10 times with 0.1 M Tris-HCl containing 2 mM EDTA (pH 7.8) to reduce the ethanol concentration. The ATP content was measured using the ATP Bioluminescence Assay kit CLS II from Roche (Mannheim, Germany) in a 96-well plate Lucy1 luminometer (Anthos, Durham, NC, USA) using a standard ATP-calibration curve.

Protein content of the slices was estimated in three identical, replicate slices which were not used for incubation. The slices were digested with 5 M NaOH and homogenized, and subsequently diluted with water to result in a concentration of 0.1 M NaOH. The protein content of the diluted homogenate was determined by the Bio-Rad protein assay dye reagent method (Bio-Rad, Munich, Germany) using bovine serum albumin (BSA) for the calibration curve. The ATP content of the slice was expressed as pmol/ μ g of protein.

2.10. Statistics

All experiments were performed in 3–5 rats and in 4–5 human tissue samples. Values were expressed as mean \pm S.E.M. All data were analyzed by the unpaired student's *t*-test or Mann-Whitney *U*-test to detect differences between the means of different treatments. The Student's *t*-test was used to analyze the rat data where the error distribution was found to be normal with equal variance except for the CYP3A1 and CYP3A2 genes. Among experiments where non-equal error distribution and high variance (e.g. expression of CYP3A1 and CYP3A2 genes in Wistar rats and CYP3A4 in human tissues due to age and habits) were observed, the non-parametric Mann-Whitney *U*-test was used. Statistical analysis was performed on fold induction as well as on $\Delta \Delta C_T$ with similar results. The *P* value <0.05 was considered as significant.

3. Results

3.1. Expression of nuclear receptors in rat intestine and liver

VDR, PXR and FXR mRNA were detected in rat intestine as well as in liver. To analyze the expression of the NRs, enzymes and

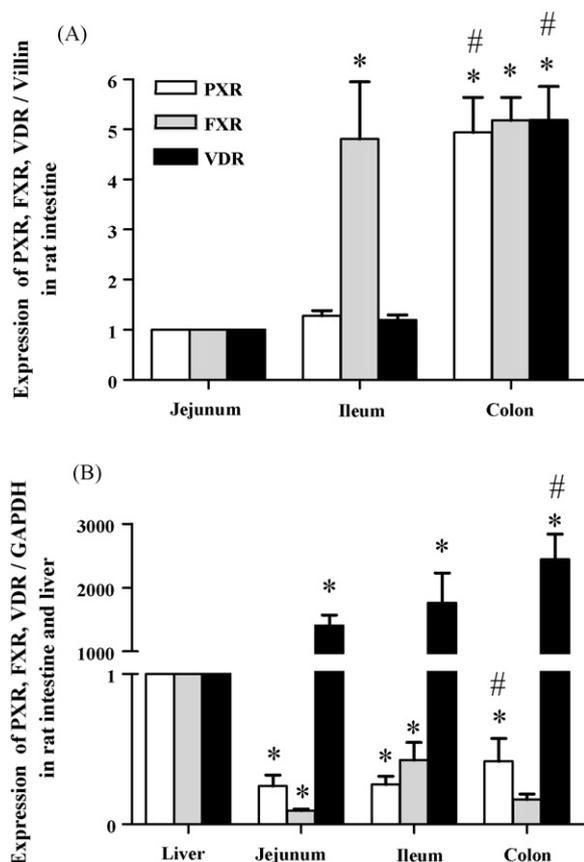


Fig. 1. Expression of PXR, FXR and VDR mRNA in rat intestine was normalized to that of villin (A); the value of jejunum/villin was set to 1. The expression of PXR, FXR and VDR mRNA in rat intestine and liver, after normalizing to GAPDH (B), with the liver value set to 1. Results were mean \pm S.E.M. of three rats. “*” denotes $P < 0.05$, compared to jejunum (A) or liver (B). “#” denotes $P < 0.05$, compared to ileum (A and B).

transporters per enterocyte, their expression is expressed relative to that of villin, which is present exclusively in the epithelial cells of the intestine. In rat intestine, PXR, FXR and VDR expression varied along the length of the small intestine and colon. The expression of PXR and VDR mRNA relative to villin was 5-fold higher in the colon compared to the jejunum and the ileum (Fig. 1A). FXR expression relative to villin was 5-fold higher in the ileum compared to the jejunum and was similar to that in the colon (Fig. 1A). For comparison of the expression in liver, GAPDH was used as a reference since villin is not expressed in hepatocytes. In the rat liver, the expression of FXR and PXR, relative to GAPDH, was significantly higher (2–10-fold) compared to those in the small intestine and colon (Fig. 1B). However, the mRNA expression of VDR relative to GAPDH in the rat liver was very low, about 0.1% compared to those in the small intestine and colon, and was detected at approximate C_T values of 32–34 cycles (Fig. 1B).

3.2. Expression and regulation of CYP3A isozymes in rat intestine and liver slices

3.2.1. Rat intestine slices

Among the CYP3A isozymes in the rat intestine, CYP3A9 was clearly expressed (C_T value \sim 19–21) in all segments: the expression of CYP3A9 in rat intestine per enterocyte was in the rank order of colon $>$ jejunum \geq ileum. CYP3A1 expression was low but

detectable (C_T values \geq 33 for CYP3A1) in all regions of the intestine. CYP3A2 was barely detectable in the ileum (\geq 35 for CYP3A2) but was undetectable in the jejunum and colon. Because CYP3A1 and CYP3A2 mRNA expression was decreased, whereas that of CYP3A9 expression was moderately elevated during incubation of the slices (data not shown), results on ligand-induced effects were expressed relative to “control” slices incubated with solvent for the same incubation period.

Increasing concentrations of the VDR ligand, 1,25(OH) $_2$ D $_3$ strongly induced CYP3A1 mRNA in all regions of the rat intestine (700-fold at 100 nM of 1,25(OH) $_2$ D $_3$ in jejunum, 15,000-fold for the ileum, and 1000-fold for the colon; $P < 0.05$) (Fig. 2A), but the mRNA expression of CYP3A9 remained unchanged (Fig. 2C). In contrast, PCN, DEX and BUD strongly induced CYP3A9 mRNA in the jejunum and ileum, and to a much lesser extent, in the colon (Fig. 2C). PCN and DEX but not BUD induced CYP3A1 in the ileum (Fig. 2A), but had no effect on CYP3A1 in the colon. Although PCN, BUD and DEX induced CYP3A1 mRNA in the jejunum samples, the results failed to reach statistical significance due to the high variation among the data (Fig. 2A). CDCA induced CYP3A1 mRNA only in the ileum and not in the jejunum and colon (Fig. 2A), and failed to affect the expression of CYP3A9 mRNA along the length of the intestine (Fig. 2C). CYP3A2 mRNA, though practically undetectable after incubation with PCN, BUD, DEX and CDCA, was highly induced by 1,25(OH) $_2$ D $_3$ in the ileum; however, CYP3A2 remained undetectable in the jejunum and colon for all situations (Fig. 2B).

3.2.2. Rat liver slices

In the rat liver, the expression of CYP3A1 and CYP3A2 was very high compared to that in the intestine, and was detected at a C_T value of 18–19, where as CYP3A9 was detected at a C_T value of 22. The expression of CYP3A1, CYP3A2, and CYP3A9 mRNAs was significantly decreased during incubation, but was not further affected by the presence of the solvent vehicle. Distinct from intestinal slices, incubation of rat liver slices with 1,25(OH) $_2$ D $_3$ did not change the expression of CYP3A1, CYP3A2 and CYP3A9 (Fig. 3A). DEX induced CYP3A1, CYP3A2 and CYP3A9 mRNA expression in rat liver slices in a concentration-dependent manner (Fig. 3C). PCN induced CYP3A1 and CYP3A9 but not CYP3A2 mRNA expression (Fig. 3C). However, BUD induced CYP3A9 expression without affecting those of CYP3A1 and CYP3A2 (Fig. 3C). CDCA significantly decreased the expression of CYP3A1, CYP3A2 and CYP3A9 with increasing concentration (Fig. 3B) to 0.7-fold, 0.5-fold and 0.7-fold, respectively.

3.3. Induction of PXR in rat intestine and liver slices

The expression of PXR, a known GR-responsive gene, was studied in the rat intestinal and liver samples treated with GR (DEX/BUD) and PXR (PCN) ligands. DEX and BUD but not PCN induced PXR expression in all the three regions of the intestine and in the liver (Fig. 4A and B). Furthermore, PXR induction by DEX (1 μ M) and BUD (10 nM) in the rat colon was lower compared to that in the jejunum and ileum, but the fold-induction at 50 μ M DEX, in the jejunum, ileum and colon was comparable (Fig. 4A).

3.4. Expression and regulation of CYP3A4 in human ileum and liver slices

CYP3A4 mRNA expression was constant up to 8 h of incubation in ileum slices, but decreased to 30–50% by 24 h, with only minor differences between the control and the solvent-treated slices (Fig. 5A). The FXR and PXR expression in human ileum and liver,

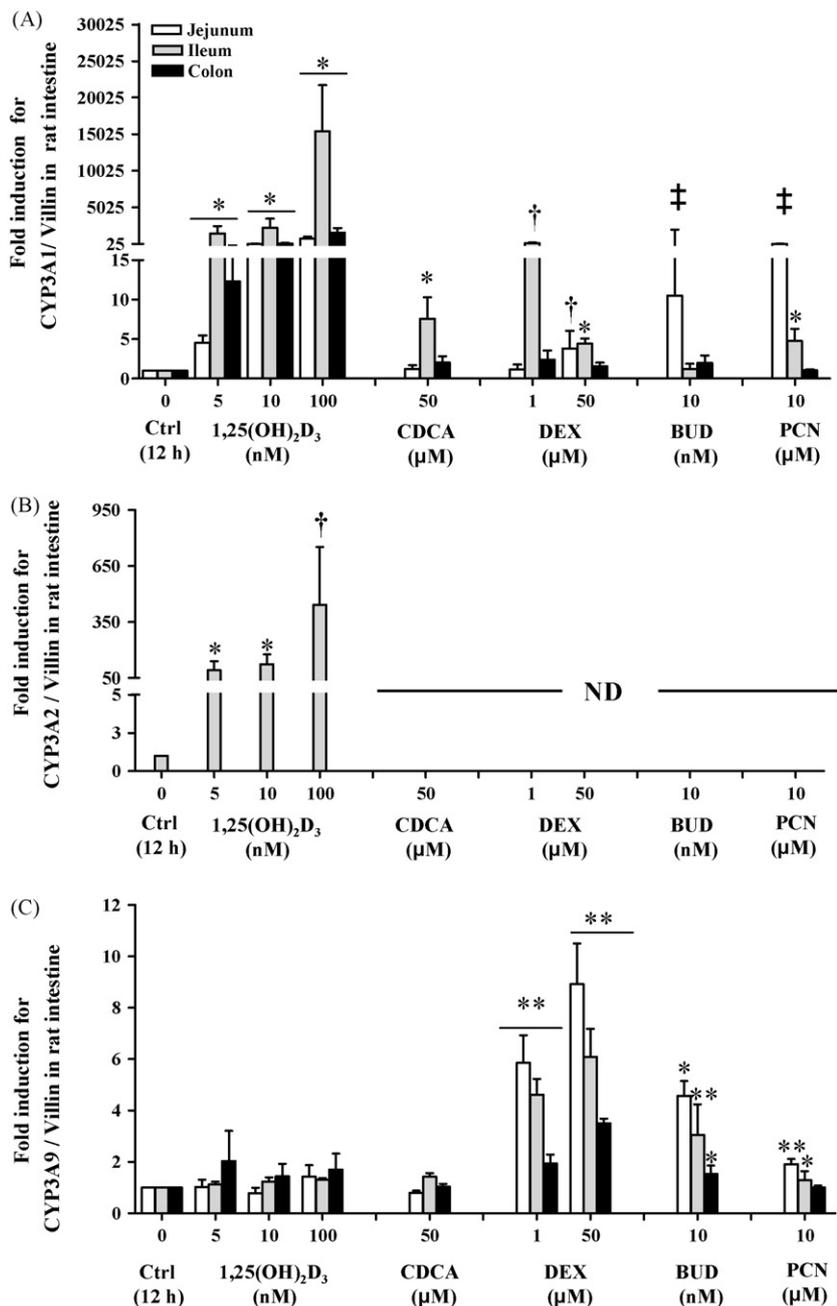


Fig. 2. Slices from rat jejunum, ileum and colon were exposed to 1,25(OH)₂D₃ (5, 10 and 100 nM), CDCA (50 μM), DEX (1 and 50 μM), BUD (10 nM) and PCN (10 μM) for 12 h, after which total RNA was isolated and mRNA expression of CYP3A1 (A), CYP3A2 (B) and CYP3A9 (C) were evaluated by qRT-PCR. Results were expressed as fold induction after normalizing with villin expression and compared to the control slices of the same segment that was also incubated for 12 h; the control value was set as 1. Results were mean ± S.E.M. of 3–5 rats; in each experiment, 6 slices were incubated per condition. Significant differences towards the control incubations are indicated with **P* < 0.05 and ***P* < 0.01. “†” denotes induction of CYP3A1 and CYP3A2 in all experiments, but the results failed to reach significance due to the high variation between the experiments, ND—not detectable; “††” denotes one or two out of three experiments showed induction.

when expressed relative to GAPDH, was higher in the liver compared to that in the ileum (1.5–4 fold); the opposite was observed for the VDR expression, which was significantly higher in the ileum than in the liver.

3.4.1. Human ileum

Incubation of ileum slices with increasing concentrations of 1,25(OH)₂D₃ induced CYP3A4 mRNA expression (Fig. 5B). DEX and BUD but not CDCA also induced CYP3A4 mRNA expression in the ileum slices (Fig. 5C and D).

3.4.2. Human liver

In human liver slices, CYP3A4 expression was significantly decreased to 10–20% upon incubation for 24 h in the solvent-treated controls (Fig. 6A). 1,25(OH)₂D₃ induced CYP3A4 in three out of four livers (Fig. 6B) (fold induction at 100 and 200 nM were: human liver (HL)1, 2.66/2.29; HL2, 2.89/0.83; HL3, 0.33/0.25; HL5, 1.43/1.41). CDCA and DEX induced CYP3A4 significantly in all the five human livers studied (Fig. 6C and D).

The effects of the ligands for VDR, PXR, GR and FXR on the regulation of CYP3A isozymes in rat intestine (jejunum, ileum and colon),

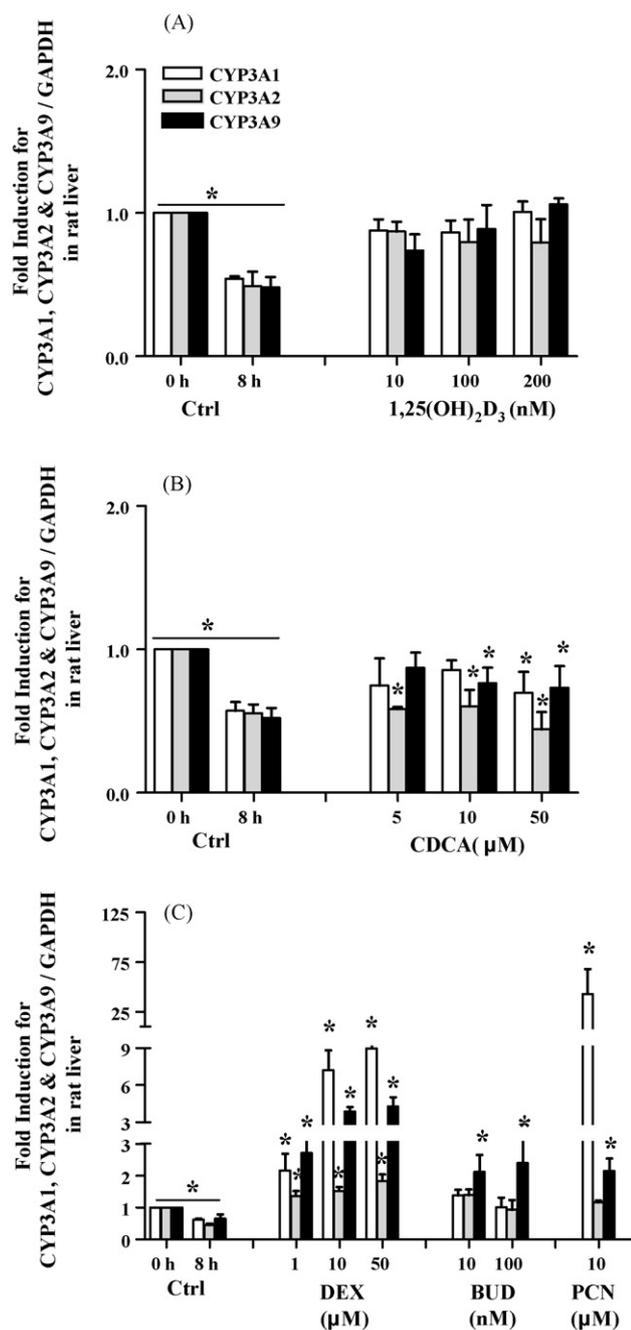


Fig. 3. Slices from rat liver were exposed to 1,25(OH)₂D₃ (10, 100 and 200 nM) (A), CDCA (50 μM) (B), and DEX (1, 10 and 50 μM), BUD (10 and 100 nM) and PCN (10 μM) (C) for 8 h, after which total RNA was isolated and mRNA expression of CYP3A1 (A), CYP3A2 (B) and CYP3A9 (C) were evaluated by qRT-PCR. Results were expressed as fold induction, after being normalized to the GAPDH expression, and compared with the control slices that were incubated for 8 h, whose value was set to 1. Results were mean ± S.E.M. of 3–5 rats; in each experiment, 3 slices were incubated per condition. Significant differences towards the control incubations are denoted by *, denoting $P < 0.05$.

rat liver, human ileum and liver are summarized as an overview in Table 4 to facilitate comparison of the effects of the various ligands in the different tissues.

4. Discussion

In this report, we compared the regulation of CYP3A isozymes by the VDR-specific ligand, 1,25(OH)₂D₃, in different regions of the

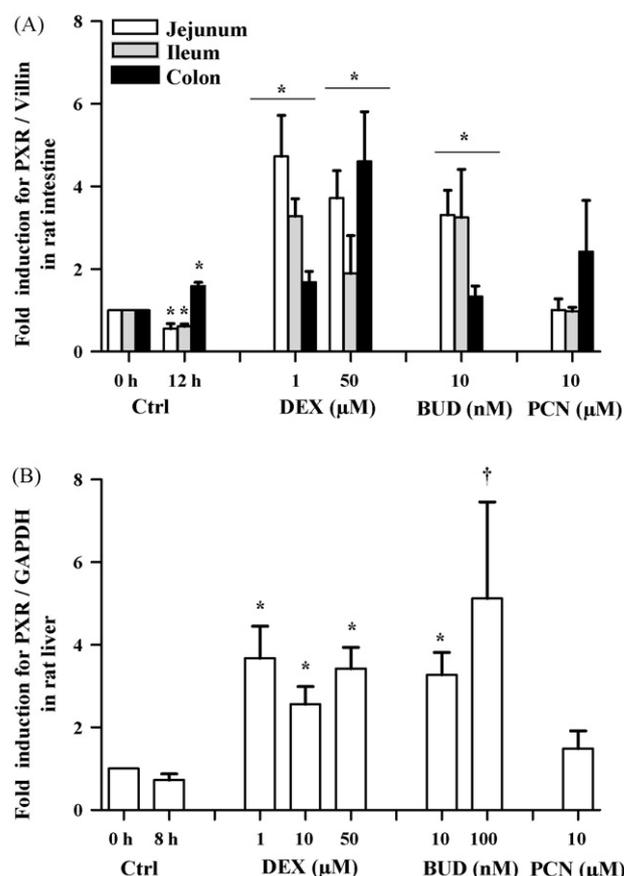


Fig. 4. Slices from rat intestine (jejunum, ileum and colon) were exposed to DEX (1 and 50 μM), BUD (10 and 50 μM), BUD (10 nM) and PCN (10 μM) (A) for 12 h. Liver slices were exposed to DEX (1, 10 and 50 μM), BUD (10 and 100 nM) and PCN (10 μM) (B) for 8 h after, which total RNA was isolated and mRNA expression of PXR was evaluated by qRT-PCR. Results were expressed as fold induction after being normalized to the villin for the intestine and GAPDH for liver expression, and compared with the control slices (values set to 1) that were incubated for 12 and 8 h, respectively. Results were mean ± S.E.M. of 3–5 rats; in each experiment 6 intestinal and 3 liver slices were incubated per condition. Significant differences towards the control incubations were denoted by * $P < 0.05$.

intestine and in the liver of the rat and humans with PXR-, GR- and FXR-specific ligands, and investigated whether the changes were related to expression levels of the NRs in the tissues. Most data concerning the regulation of CYP3A isoforms in the rat are restricted to the liver and the small intestine, mostly jejunum, and the data on the comparison of regulation of CYP3A isoforms across the intestinal tract, jejunum, ileum and colon is scarce. *In vivo*, the extent of exposure of the various organs to ligands of the NRs is rarely controlled, and it is difficult to discriminate between the direct and indirect effects of the ligands. In this study, we compared the regulation of gene expression in different segments of the intestine and liver under identical experimental conditions using precision-cut tissue slices (Olinga et al., 2008; van de Kerkhof et al., 2007a, 2008). Viability of the liver and intestinal slices during incubation was revealed by the stable expression of house-keeping genes, villin as specific gene for enterocytes, and GAPDH for the intestinal and liver tissue (data not shown). In addition, the ATP content of the human liver slices was assessed as an additional viability marker during incubation; these levels were found to be constant during incubation (Table 1). Furthermore, metabolism in tissue slices is comparable to *in vivo* (Graaf et al., 2007; van de Kerkhof et al., 2007b) with adequate expression of transporters and enzymes. Therefore, the uptake and metabolism of ligands is expected to be

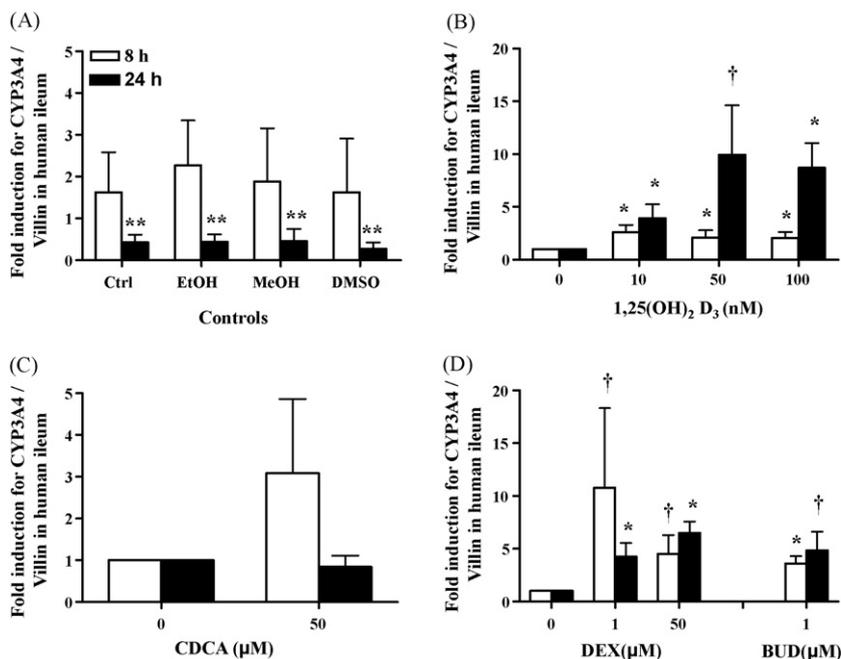


Fig. 5. Slices from human ileum were exposed to control solvents (ethanol, methanol, and DMSO) (A), 1,25(OH)₂D₃ (10, 50 and 100 nM) (B), CDCA (50 μM) (C), DEX (1 and 50 μM) and BUD (10 nM) (D) for 12 and 24 h, after which total RNA was isolated and mRNA expression of CYP3A4 was evaluated by qRT-PCR. Results were expressed as fold induction after being normalized to the villin and compared to the control slices (values set as 1) that were incubated for 12 and 24 h. Results were mean ± S.E.M. of 4–5 human ileum donors; in each experiment, 3 slices were incubated per condition. Significant difference towards the control incubations is denoted by **P* < 0.05 and ***P* < 0.01. “†” denotes induction of CYP3A4 in all experiments with high variation between the experiments, and failed to reach statistical significance.

similar to *in vivo* and to reflect species differences between human and rat. The concentrations of ligands for various nuclear receptors used in this study are similar to those used in earlier studies with metabolically active cells (Drocourt et al., 2002; Hoen et al., 2000), which seem to be adequate to elicit nuclear receptor-specific

induction responses, monitored by the induction of signature genes.

In rat intestine, the mRNA expression of PXR, FXR and VDR was found to be present in varying abundances along the length of the small intestine (jejunum and ileum), with the highest expression of

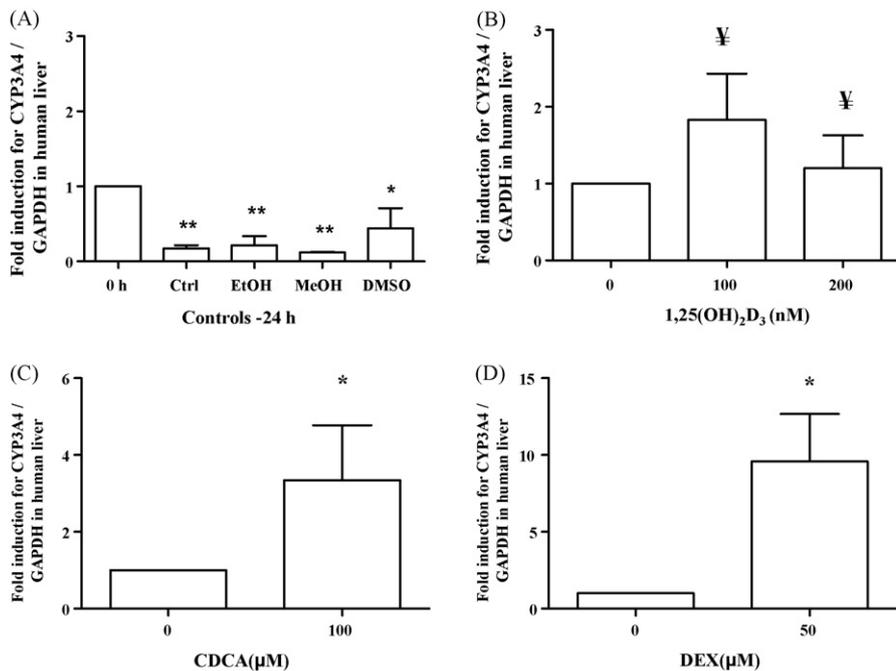


Fig. 6. Slices from human liver were exposed to control solvents (EtOH, MeOH and DMSO) (A), 1,25(OH)₂D₃ (100 and 200 nM) (B), CDCA (100 μM) (C) and DEX (50 μM) (D) for 24 h, after which total RNA was isolated and CYP3A4 mRNA expression was evaluated by qRT-PCR. Results were expressed as fold induction after being normalized to GAPDH and compared to the control slices (values set as 1) that were incubated for 24 h. Results were mean ± S.E.M. of 5 human liver donors for all ligands except for 1,25(OH)₂D₃ where *n* = 4; in each experiment 3 slices were incubated per condition. Significant differences towards the control incubations are indicated with **P* < 0.05 and ***P* < 0.01. “¥” denotes induction of CYP3A4 in three out of four human livers, which fails to reach statistical significance.

ulated by VDR, PXR and FXR ligands and CYP3A9 by PXR and GR ligands, dramatic differences in the extents of the induction were found in the different segments of the intestine (Table 4). These differences were apparently not related to the differential expression of the respective NRs. The colon, although endowed with an abundance of NRs, exhibited low induction potential of CYP3A isozymes compared to those of the jejunum and ileum.

The VDR-, PXR-, FXR- and GR-dependent regulation of CYP3A1, CYP3A2 and CYP3A9 mRNA in rat liver slices differed dramatically from intestinal slices. The expression of CYP3A1, CYP3A2 and CYP3A9 mRNA was unchanged in liver slices incubated with $1,25(\text{OH})_2\text{D}_3$, as found *in vivo* by Xu et al. (2006) and Chow et al. (2008). The lack of regulation of CYP3A1 in liver can be explained by the absence of VDR in rat hepatocytes, the major site of the target CYP genes, since VDR is found only in non-parenchymal cells and biliary epithelial cells (Gascon-Barre et al., 2003). This was confirmed by immunohistochemistry of rat liver slices in our studies where CYP3A1 was present exclusively in hepatocytes and CYP3A2, mainly in hepatocytes, and expressed at a much lower level in biliary epithelial cells (unpublished observations).

In contrast, PCN and DEX induced the expression of hepatic CYP3A1 and CYP3A9, and BUD induced CYP3A9, whereas CYP3A2 expression was modestly induced only by DEX. These data on the induction of CYP3A1 and CYP3A9 by the PXR ligands, PCN and DEX, agree with earlier reports on Sprague–Dawley rats (Huss and Kasper, 1998; Mahnke et al., 1997). However, the induction of CYP3A9 by BUD (GR), though suggested by Komori and Oda (1994), has not been reported earlier. Induction of CYP3A9 by PCN and DEX in the rat liver and intestine implies that CYP3A9 is likely regulated by PXR via a PXRE. However, it remains to be elucidated whether the inductive effect of BUD on CYP3A9 is directly mediated via GR and a GRE in the CYP3A9 promoter, or indirectly via upregulation of PXR and HNF4 α by BUD. The two stage induction by the GR on the upregulation of CYP via induction of PXR has been suggested for CYP3A1/23 (Huss and Kasper, 2000), and is a likely possibility since PXR induction was also observed with GR ligands (Fig. 4B).

Unlike the induction of CYP3A1 observed in the ileum, CDCA, an FXR ligand, showed repression of CYP3A1, CYP3A2 and CYP3A9 in rat liver slices. This might be due to the CDCA mediated repression of PXR (data not shown), which in turn, affects the basal expression of CYP3A isozymes in rat liver. These results are in stark contrast to the mouse studies of Jung et al. (2006), where *Cyp3a11* and *pxr* were positively regulated by FXR in mice that were treated with FXR agonists, cholic acid and GW4064, suggesting species difference in the regulation of these genes.

In the human ileum, CYP3A4 was significantly induced by VDR, PXR and GR ligands (Fig. 6B and D). Although the regulation of CYP3A4 *in vivo* by PXR and GR ligands in the human intestine is well known, we are the first to show the regulation of CYP3A4 in human intestinal tissue by the VDR ligand, $1,25(\text{OH})_2\text{D}_3$. These observations agree with findings in cultured monolayers such as Caco-2 and LS180 cells (Fukumori et al., 2007; Schmiedlin-Ren et al., 2001). Also in human liver slices, CYP3A4 is upregulated by $1,25(\text{OH})_2\text{D}_3$, but only in those three liver samples that showed expression of VDR, as reported for human hepatocytes (Drocourt et al., 2002). For the liver that did not express VDR, no upregulation of CYP3A4 by $1,25(\text{OH})_2\text{D}_3$ was found. This variation between samples was not due to viability differences because human liver slices treated with the PXR/GR ligand, DEX induced CYP3A4 in all the experiments, observations that were in agreement with earlier report (Lehmann et al., 1998). CDCA induced expression of CYP3A4 in human liver, confirming the FXR-dependent regulation in earlier reports by Gnerre et al. (2004). But this response was not observed in the ileum. It appears that all NRs: VDR, PXR, GR and FXR, are able to induce CYP3A4 in the human liver.

In summary, studies in tissue slices showed that the overall effects of ligands for NRs on regulation of CYP3A isozymes differed in different regions of the rat intestine and liver, and human ileum and liver slices, despite the incubation was conducted under identical circumstances. This difference appears not to be directly related to the different expression levels of the nuclear receptors involved. In the rat intestine, CYP3A1 expression is very sensitive to the VDR ligand, and to a lesser extent, to PXR and GR ligands, whereas CYP3A2 expression is exclusively regulated by the VDR. CYP3A9 expression both in the liver and in all regions of the intestine appears to be mainly regulated by PXR and GR but not by VDR. In human tissue, however, CYP3A4 in ileum and liver was upregulated by PXR, VDR and GR ligands. By contrast, CDCA elicited varying effects, ranging from decreased expression in rat liver, lack of effect in human ileum, and increased expression in rat ileum and human liver, effects that are not explained by the expression of FXR. Our results suggest that prediction of the inducing potential of drugs should not rely strictly on whether or not the drug under study is a ligand for a certain NR and the expression levels of this NR in the target organ. Uptake, metabolism and excretion of the ligand as well as the availability of co-activators or repressors in the specific tissue and species may play a decisive role.

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References

- Chan, S.D., Atkins, D., 1984. The temporal distribution of the $1\alpha,25$ -dihydroxycholecalciferol receptor in the rat jejunal villus. *Clin. Sci. (Lond.)* 67, 285–290.
- Chow, E.C.Y., Liu, S., Sun, H., Khan, A.A., Groothuis, G.M.M., Pang, K.S., 2008. Effects of $1\alpha,25$ -dihydroxyvitamin D₃ (calcitriol) and the vitamin D receptor (VDR) on rat enzymes, transporters and nuclear receptors. In: Proceedings of the AAPS Annual Meeting, Atlanta, GA.
- de Kanter, R., Monshouwer, M., Meijer, D.K., Groothuis, G.M., 2002. Precision-cut organ slices as a tool to study toxicity and metabolism of xenobiotics with special reference to non-hepatic tissues. *Curr. Drug Metab.* 3, 39–59.
- Drocourt, L., Ourlin, J.C., Pascussi, J.M., Maurel, P., Vilarem, M.J., 2002. Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J. Biol. Chem.* 277, 25125–25132.
- Feldman, D., McCain, T.A., Hirst, M.A., Chen, T.L., Colston, K.W., 1979. Characterization of a cytoplasmic receptor-like binder for $1\alpha,25$ -dihydroxycholecalciferol in rat intestinal mucosa. *J. Biol. Chem.* 254, 10378–10384.
- Fukumori, S., Murata, T., Taguchi, M., Hashimoto, Y., 2007. Rapid and drastic induction of CYP3A4 mRNA expression via vitamin D receptor in human intestinal LS180 cells. *Drug Metab. Pharmacokinet.* 22, 377–381.
- Gascon-Barre, M., Demers, C., Mirshahi, A., Neron, S., Zalzal, S., Nanci, A., 2003. The normal liver harbors the vitamin D nuclear receptor in nonparenchymal and biliary epithelial cells. *Hepatology* 37, 1034–1042.
- Gnerre, C., Blattler, S., Kaufmann, M.R., Looser, R., Meyer, U.A., 2004. Regulation of CYP3A4 by the bile acid receptor FXR: evidence for functional binding sites in the CYP3A4 gene. *Pharmacogenetics* 14, 635–645.
- Gonzalez, F.J., 1988. The molecular biology of cytochrome P450s. *Pharmacol. Rev.* 40, 243–288.
- Gonzalez, F.J., Kimura, S., Song, B.J., Pastewka, J., Gelboin, H.V., Hardwick, J.P., 1986. Sequence of two related P-450 mRNAs transcriptionally increased during rat development. An R. dre. 1 sequence occupies the complete 3' untranslated region of a liver mRNA. *J. Biol. Chem.* 261, 10667–10672.
- Gonzalez, F.J., Nebert, D.W., Hardwick, J.P., Kasper, C.B., 1985. Complete cDNA and protein sequence of a pregnenolone 16α -carbonitrile-induced cytochrome P-450. A representative of a new gene family. *J. Biol. Chem.* 260, 7435–7441.
- Graaf, I.A., Groothuis, G.M., Olinga, P., 2007. Precision-cut tissue slices as a tool to predict metabolism of novel drugs. *Expert Opin. Drug Metab. Toxicol.* 3, 879–898.
- Guengerich, F.P., Martin, M.V., Beaune, P.H., Kremers, P., Wolff, T., Waxman, D.J., 1986. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J. Biol. Chem.* 261, 5051–5060.
- Hashimoto, H., Toide, K., Kitamura, R., Fujita, M., Tagawa, S., Itoh, S., Kamataki, T., 1993. Gene structure of CYP3A4, an adult-specific form of cytochrome P450 in human livers, and its transcriptional control. *Eur. J. Biochem.* 218, 585–595.

- Hoen, P.A., Commandeur, J.N., Vermeulen, N.P., Van Berkel, T.J., Bijsterbosch, M.K., 2000. Selective induction of cytochrome P450 3A1 by dexamethasone in cultured rat hepatocytes: analysis with a novel reverse transcriptase-polymerase chain reaction assay section sign. *Biochem. Pharmacol.* 60, 1509–1518.
- Huss, J.M., Kasper, C.B., 1998. Nuclear receptor involvement in the regulation of rat cytochrome P450 3A23 expression. *J. Biol. Chem.* 273, 16155–16162.
- Huss, J.M., Kasper, C.B., 2000. Two-stage glucocorticoid induction of CYP3A23 through both the glucocorticoid and pregnane X receptors. *Mol. Pharmacol.* 58, 48–57.
- Huss, J.M., Wang, S.I., Kasper, C.B., 1999. Differential glucocorticoid responses of CYP3A23 and CYP3A2 are mediated by selective binding of orphan nuclear receptors. *Arch. Biochem. Biophys.* 372, 321–332.
- Jung, D., Mangelsdorf, D.J., Meyer, U.A., 2006. Pregnane X receptor is a target of farnesoid X receptor. *J. Biol. Chem.* 281, 19081–19091.
- Kolars, J.C., Lown, K.S., Schmiedlin-Ren, P., Ghosh, M., Fang, C., Wrighton, S.A., Merion, R.M., Watkins, P.B., 1994. CYP3A gene expression in human gut epithelium. *Pharmacogenetics* 4, 247–259.
- Komori, M., Oda, Y., 1994. A major glucocorticoid-inducible P450 in rat liver is not P450 3A1. *J. Biochem.* 116, 114–120.
- Lehmann, J.M., McKee, D.D., Watson, M.A., Willson, T.M., Moore, J.T., Kliewer, S.A., 1998. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* 102, 1016–1023.
- Li, Q., Sai, Y., Kato, Y., Tamai, I., Tsuji, A., 2003. Influence of drugs and nutrients on transporter gene expression levels in Caco-2 and LS180 intestinal epithelial cell lines. *Pharm. Res.* 20, 1119–1124.
- Liu, S., Tam, D., Chen, X., Pang, K.S., 2006. P-glycoprotein and an unstirred water layer barring digoxin absorption in the vascularly perfused rat small intestine preparation: induction studies with pregnenolone-16 α -carbonitrile. *Drug Metab. Dispos.* 34, 1468–1479.
- Lu, A.Y., Somogyi, A., West, S., Kuntzman, R., Conney, A.H., 1972. Pregnenolone-16-carbonitrile: a new type of inducer of drug-metabolizing enzymes. *Arch. Biochem. Biophys.* 152, 457–462.
- Mahnke, A., Strotkamp, D., Roos, P.H., Hanstein, W.G., Chabot, G.G., Nef, P., 1997. Expression and inducibility of cytochrome P450 3A9 (CYP3A9) and other members of the CYP3A subfamily in rat liver. *Arch. Biochem. Biophys.* 337, 62–68.
- Makishima, M., Lu, T.T., Xie, W., Whitfield, G.K., Domoto, H., Evans, R.M., Haussler, M.R., Mangelsdorf, D.J., 2002. Vitamin D receptor as an intestinal bile acid sensor. *Science* 296, 1313–1316.
- Matsubara, T., Kim, H.J., Miyata, M., Shimada, M., Nagata, K., Yamazoe, Y., 2004. Isolation and characterization of a new major intestinal CYP3A form, CYP3A62, in the rat. *J. Pharmacol. Exp. Ther.* 309, 1282–1290.
- Nagata, K., Murayama, N., Miyata, M., Shimada, M., Urahashi, A., Yamazoe, Y., Kato, R., 1996. Isolation and characterization of a new rat P450 (CYP3A18) cDNA encoding P450(6) β -2 catalyzing testosterone 6 β - and 16 α -hydroxylations. *Pharmacogenetics* 6, 103–111.
- Olinga, P., Elferink, M.G., Draaisma, A.L., Merema, M.T., Castell, J.V., Perez, G., Groothuis, G.M., 2008. Coordinated induction of drug transporters and phase I and II metabolism in human liver slices. *Eur. J. Pharm. Sci.* 33, 380–389.
- Sandgren, M.E., Bronnegard, M., DeLuca, H.F., 1991. Tissue distribution of the 1,25-dihydroxyvitamin D3 receptor in the male rat. *Biochem. Biophys. Res. Commun.* 181, 611–616.
- Schmiedlin-Ren, P., Benedict, P.E., Dobbins 3rd, W.O., Ghosh, M., Kolars, J.C., Watkins, P.B., 1993. Cultured adult rat jejunal explants as a model for studying regulation of CYP3A. *Biochem. Pharmacol.* 46, 905–918.
- Schmiedlin-Ren, P., Thummel, K.E., Fisher, J.M., Paine, M.F., Watkins, P.B., 2001. Induction of CYP3A4 by 1 α ,25-dihydroxyvitamin D3 is human cell line-specific and is unlikely to involve pregnane X receptor. *Drug Metab. Dispos.* 29, 1446–1453.
- Strotkamp, D., Roos, P.H., Hanstein, W.G., 1995. A novel CYP3 gene from female rats. *Biochim. Biophys. Acta* 1260, 341–344.
- Thompson, P.D., Hsieh, J.C., Whitfield, G.K., Haussler, C.A., Jurutka, P.W., Galligan, M.A., Tillman, J.B., Spindler, S.R., Haussler, M.R., 1999. Vitamin D receptor displays DNA binding and transactivation as a heterodimer with the retinoid X receptor, but not with the thyroid hormone receptor. *J. Cell. Biochem.* 75, 462–480.
- Thummel, K.E., Brimer, C., Yasuda, K., Thottassery, J., Senn, T., Lin, Y., Ishizuka, H., Kharasch, E., Schuetz, J., Schuetz, E., 2001. Transcriptional control of intestinal cytochrome P-4503A by 1 α ,25-dihydroxy vitamin D3. *Mol. Pharmacol.* 60, 1399–1406.
- van de Kerkhof, E.G., de Graaf, I.A., de Jager, M.H., Groothuis, G.M., 2007a. Induction of phase I and II drug metabolism in rat small intestine and colon in vitro. *Drug Metab. Dispos.* 35, 898–907.
- van de Kerkhof, E.G., de Graaf, I.A., de Jager, M.H., Meijer, D.K., Groothuis, G.M., 2005. Characterization of rat small intestinal and colon precision-cut slices as an in vitro system for drug metabolism and induction studies. *Drug Metab. Dispos.* 33, 1613–1620.
- van de Kerkhof, E.G., de Graaf, I.A., Groothuis, G.M., 2007b. In vitro methods to study intestinal drug metabolism. *Curr. Drug Metab.* 8, 658–675.
- van de Kerkhof, E.G., de Graaf, I.A., Ungell, A.L., Groothuis, G.M., 2008. Induction of metabolism and transport in human intestine: validation of precision-cut slices as a tool to study induction of drug metabolism in human intestine in vitro. *Drug Metab. Dispos.* 36, 604–613.
- van de Kerkhof, E.G., Ungell, A.L., Sjoberg, A.K., de Jager, M.H., Hilgendorf, C., de Graaf, I.A., Groothuis, G.M., 2006. Innovative methods to study human intestinal drug metabolism in vitro: precision-cut slices compared with using chamber preparations. *Drug Metab. Dispos.* 34, 1893–1902.
- Wang, H., Kawashima, H., Strobel, H.W., 1996. cDNA cloning of a novel CYP3A from rat brain. *Biochem. Biophys. Res. Commun.* 221, 157–162.
- Wang, H., Strobel, H.W., 1997. Regulation of CYP3A9 gene expression by estrogen and catalytic studies using cytochrome P450 3A9 expressed in *Escherichia coli*. *Arch. Biochem. Biophys.* 344, 365–372.
- Xu, Y., Iwanaga, K., Zhou, C., Cheesman, M.J., Farin, F., Thummel, K.E., 2006. Selective induction of intestinal CYP3A23 by 1 α ,25-dihydroxyvitamin D3 in rats. *Biochem. Pharmacol.* 72, 385–392.
- Zhang, Q.Y., He, W., Dunbar, D., Kaminsky, L., 1997. Induction of CYP1A1 by beta-naphthoflavone in IEC-18 rat intestinal epithelial cells and potentiation of induction by dibutyl cAMP. *Biochem. Biophys. Res. Commun.* 233, 623–626.
- Zierold, C., Mings, J.A., DeLuca, H.F., 2006. 19nor-1,25-dihydroxyvitamin D2 specifically induces CYP3A9 in rat intestine more strongly than 1,25-dihydroxyvitamin D3 in vivo and in vitro. *Mol. Pharmacol.* 69, 1740–1747.