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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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Chapter3

The Role of Lithocholic Acid in the Regulation of Bile acid Detoxification, Synthesis and Transport proteins in Rat and human Intestine and Liver Slices

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Submitted

Abstract

The effects of lithocholic acid (LCA), a common ligand of VDR, FXR and PXR, on the regulation of proteins involved in bile acid metabolism (CYP3A isozymes), synthesis (CYP7A1) and transport (MRP3, MRP2, BSEP, NTCP), as well as the nuclear receptors (FXR, PXR, LXR α , HNF1 α , HNF4 α and SHP) that are involved in their regulation, were studied in the rat and human precision-cut intestine and liver slices at the level of mRNA as quantified by qRT-PCR. The LCA effects were compared to those from specific PXR, FXR and VDR ligands. LCA induced CYP3A1 and CYP3A9 in the rat jejunum, ileum and colon, CYP3A2 only in the ileum, and CYP3A9 expression in the liver only. LCA induced CYP3A4 in the human ileum and not in liver. Based on the data obtained with specific VDR, PXR and FXR ligands, we conclude that LCA induced the expression of detoxification enzymes via by the VDR (CYP3A1, CYP3A2 and CYP3A4) and PXR (CYP3A9) both in the rat and the human intestine. The expression of rMRP2 was induced in the colon but not in the jejunum and ileum that for rMRP3 was not affected for segments of the intestine in the rat, whereas in human ileum slices, LCA induced hMRP3 and hMRP2 expression. In the rat liver, LCA decreased rCYP7A1, rLXR α and rHNF4 α expression, and induced rSHP expression but did not affect rBSEP and rNTCP expression, whereas in the human liver, a small but significant decrease was found for hHNF1 α expression. These data suggests profound species differences in the effects of LCA on bile acid transport, synthesis and detoxification. The LCA-induced altered enzymes and transporter expressions in the intestine may have consequences for drug disposition in conditions with elevated LCA levels such as cholestasis.

Keywords: Cytochrome P450, induction, intestinal slices, liver slices, lithocholic acid.

Introduction

Lithocholic acid (LCA) is a toxic secondary monohydroxy bile acid (BA) formed by the bacterial biotransformation (7 α -dehydroxylation) of the primary BA, chenodeoxycholic acid (CDCA) in the terminal part of the small intestine (12, 24). LCA is passively absorbed by the intestinal mucosal cells (35, 51, 55), metabolized and excreted either back into the intestinal lumen or into the portal blood. LCA is reported to be carcinogenic in the intestine and cholestatic in the liver of animals and man (Javitt, 1966; Fisher et al., 1971; Narisawa et al., 1974). It is metabolized by cytochrome P450 (CYP) enzymes in the intestine and the liver of humans (CYP3A4) and rats (CYP3A1, CYP3A2, CYP3A9, CYP2C6, CYP2C11 and CYP2D1) to 6 α - and 6 β -hydroxy metabolites, respectively (3, 17). Recently, 3-keto-5 β -cholanic acid (3KCA) was identified as the major LCA metabolite with human recombinant CYP3A4 isozyme (7). Moreover LCA is conjugated by sulfotransferase into sulfolithocholic acid (27).

Because LCA is predominantly formed in the terminal part of the small intestine and the ileal mucosal cells are exposed to very high concentrations of LCA. Nevertheless, most of the studies on LCA biotransformation were performed in liver microsomes of different species, and data are not available for intestine. Furthermore, the LCA-mediated regulation of the CYP isozymes involved in its metabolism is incompletely understood in the intestine and the liver. Makishima et al. (41), Wolf et al. (59) and Staudinger et al. (53) independently showed that LCA and its metabolite, 3KCA can bind and transactivate the vitamin D receptor (VDR), the pregnane X receptor (PXR) and the farnesoid X receptor (FXR) upon binding of these nuclear receptors (NR's) to their respective response elements in the promoters of the target genes as a heterodimer with retinoic acid X receptor α (RXR α). Hence, it is hypothesized that LCA and its metabolites may coordinately regulate bile acid detoxification, synthesis and transport proteins in the intestine and the liver.

In addition to the well known involvement of the PXR and glucocorticoid receptor (GR) in the regulation of CYP3A isoforms, 1 α ,25-dihydroxy vitamin D₃ (1,25(OH)₂D₃), a VDR ligand was recently reported to regulate the CYP3A isozymes involved in the detoxification of LCA in human and rat intestinal cell lines and human foetal intestine explants (9, 20, 50, 56). Recently, we observed 1,25(OH)₂D₃-dependent regulation of the rat CYP3A isozymes in the small intestine, colon and liver, and CYP3A4 in the human ileum and liver using precision-cut slices (33). Our results were consistent with the induction of CYP3A1 in duodenum, jejunum and ileum of Sprague-Dawley rats treated with intraperitoneal (ip) injection of 1,25(OH)₂D₃ for four days (11, 60).

Bile acids (BAs) are synthesized from cholesterol in the liver by the sequential activity of 12 enzymes in which CYP7A1 catalyzes the first rate limiting step (49). Upon amidation with glycine and taurine, BA's are secreted into the intestine via the bile and reabsorbed in the terminal part of the small intestine and transported back to the liver, a

process known as the enterohepatic cycle (28). During the enterohepatic cycle, BAs and their conjugates are transported across the membranes of hepatocytes and enterocytes by members of the ATP binding cassette (ABC) transporter family, multidrug resistance-associated protein (MRP) 2, 3 and 4, bile salt export pump (BSEP; ABCB11), apical sodium dependent bile acid transporter (ASBT; SLC10A2), sodium dependent taurocholate co-transporting polypeptide (NTCP; SLC10A1) and OST α -OST β (2, 16, 25, 26, 46, 52, 54, 61). Together, these transporters contribute to the maintenance of the bile acid pool *in vivo*. BA synthesis and transport are regulated at the transcriptional level by various nuclear receptors such as PXR, FXR, CAR and VDR (2, 19, 29, 32, 37, 45).

The effects of LCA on the regulation of enzymes and transporters had been studied in the liver of rodents after LCA administration (6). In man, however, no direct data is available but the role of LCA was indirectly studied in human cholestatic livers (62, 63). In these *in vivo* studies, it is difficult to control the exposure of LCA to the different organs and it is not possible to discriminate between direct effects of LCA and indirect effects of cholestasis or other potential confounding factors. Also, no data is available on the role of LCA in the regulation of enzymes and transporters involved in BA homeostasis for the human and rat intestine. Therefore we conducted a systematic study to investigate the direct effects of LCA on the regulation of BA detoxification enzymes and transporters in the rat and human intestine and liver, the BA synthesis enzyme, CYP7A1, in rat and human livers, and the nuclear receptors/transcription factors involved in the regulation of these proteins at the level of mRNA by exposing precision-cut organ slices to different concentrations of LCA. Previously, this *in vitro* model was shown to be a valuable model to study regulation of genes of interest by ligands for several NR in liver (23, 30, 47) and intestine (33, 34, 43, 57) under identical conditions. Further, we compared the LCA mediated effects with those induced from other, specific nuclear receptor ligands, CDCA for FXR, PCN for PXR, DEX for GR and PXR, and budesonide (BUD) for GR.

Materials and methods

Chemicals and Reagents. Lithocholic acid and chenodeoxycholic acid were purchased from Calbiochem, San Diego, CA, dexamethasone was from Genfarma bv, Maarssen. Pregnenolone-16 α carbonitrile, budesonide and the solvents; ethanol, methanol and dimethylsulfoxide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO); Gentamicin sulfate and Williams medium E with glutamax-I and amphotericin B (Fungizone)-solution were obtained from Gibco (Paisley, UK). D-glucose, HEPES were procured from ICN Biomedicals, Inc. (Eschwege, Germany). University of Wisconsin organ preservation solution (UW) was obtained from Du Pont Critical Care, Waukegab, Illinois, USA. Low gelling temperature agarose was 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 (10x), PCR nucleotide mix (10 mM), AMV RT (22 U/ μ l) and RNasin (40 U/ μ l); were purchased from Promega

Corporation, Madison WI, USA. SYBR Green and Taqman Master Mixes (2x) were purchased from Applied Biosystems, Warrington, UK, Abgene Westbrug and Eurogentech, respectively. All primers were purchased from Sigma-Genosys by order on demand. All reagents and materials used were of the highest purity that was commercially available.

Animals. Male Wistar (HsdCpb:WU) rats weighing about 230-250 g were purchased from Harlan (Horst, The Netherlands) and were allowed to acclimatize for 7 days before experimentation. 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 experimental protocols were approved by the Animal Ethical Committee of the University of Groningen.

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 (47). After surgical resection, human liver tissue was immediately placed in ice-cold University of Wisconsin (UW) organ storage solution. Human liver donor (n=5) characteristics are as reported earlier (33). Further, two additional human livers were used for LCA experiments and their donor characteristics (human livers HL6 and HL7) are illustrated in table 3. The human ileum was obtained as part of the surgical waste after resection of the ileocolonic part of the intestine in colon carcinoma patients; the donor characteristics were identical to those reported earlier (33). After surgical resection, the ileum tissue was immediately placed in ice-cold Krebs-Henseleit buffer supplemented with 10 mM HEPES, 25 mM sodium bicarbonate and 25 mM D-glucose, pH 7.4 (KHB). The research protocols were approved by the Medical Ethical Committee of the University Medical Center, Groningen, with informed consent of the patients.

Preparation of Rat and Human Intestinal Slices. Rat intestinal slices were prepared as published before (33, 57). In brief, 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 lumenally flushed with carbogenated ice-cold KHB, filled and embedded with 3% low gelling agarose using a 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- μ m and wet weight of 2-3 mg (without agarose) with standard settings (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 previously for human intestinal slices (33, 58). In brief, ileum tissue was stripped of the muscular layer and the mucosal tissue was embedded in low gelling 3% agarose in saline. Precision-cut slices of approximately 200- μ m thick were prepared as described above for rat intestine.

Preparation of Rat and Human Liver Slices. Human and rat liver slices were prepared as described earlier (33), from cylindrical cores of liver tissue (8 mm) using the Krumdieck tissue slicer (cycle speed 40: interrupted mode). The thickness of the liver slice was kept at ~ 200-300- μ m (wet weight of 10-12 mg). The slices were stored in ice-cold UW solution on ice prior to the start of the experiment, which usually varies from 1-3 h from sacrificing the rat and for human livers 2-3 h post surgery.

Induction Studies

Rat and Human Intestinal Slices. Precision-cut slices from rat intestine (jejunum, ileum and colon) and human ileum were incubated individually in 12-well sterile tissue culture plates (Grenier bio-one GmbH, Frickenhausen, Austria) containing 1.3 ml Williams medium E supplemented with D-glucose to a final concentration of 25 mM, gentamicin sulfate, 50 μ g/ml amphotericin/fungizone, 250 μ g/ml and saturated with carbogen at 37° C, continuously gassed with carbogen and shaken at 80 rpm. Rat intestinal slices were incubated with LCA (final concentrations 5 and 10 μ M), CDCA (final concentration, 50 μ M), DEX (final concentrations, 1 and 50 μ M) and PCN (final concentration, 10 μ M) added as a 100-times concentrated stock solution in methanol (LCA and CDCA) and DMSO (DEX and PCN), and incubated for 12 h. Human ileum slices were incubated with LCA (final concentration 10 μ M) and CDCA (final concentration 50 μ M) added as a 100-times concentrated stock solution in methanol and incubated for 8 and 24 h. Control slices from rat intestine and human ileum were incubated in supplemented Williams medium E with 1% methanol or 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 quantitative real-time PCR (qRT-PCR) analysis. Samples were stored in -80° C freezer until RNA isolation. These experiments were replicated in 3-5 rats and with human ileum samples from 3-5 donors.

Rat and Human Liver Slices. Rat and human liver slices were incubated individually in 6-well sterile tissue culture plates (Grenier bio-one GmbH, Frickenhausen, Austria) containing 3.2 ml Williams medium E supplemented with D-glucose to a final concentration of 25 mM, gentamicin sulfate (50 μ g/ml) and saturated with carbogen at 37° C, continuously gassed with carbogen and shaken at 80 rpm. Rat liver slices were incubated with LCA (final concentration, 50 μ M), DEX (final concentration, 50 μ M), BUD (final concentrations, 10 and 100 nM) and PCN (final concentration, 10 μ M) added as 100-times concentrated stock solution in methanol (for LCA and CDCA) and DMSO (for DEX, BUD and PCN). Control slices were incubated in supplemented Williams medium E with 1% methanol and DMSO without inducers for 8 and 24 h. Human liver slices were incubated with LCA and DEX (final concentration, 50 μ M) added as 100-times concentrated stock solution in methanol and DMSO, respectively. Control slices were

incubated in supplemented Williams medium E with 1% methanol or DMSO without inducers for 24 h. 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 with liver samples from 4-5 human liver donors.

RNA Isolation and qRT-PCR. Total RNA from the rat and human intestine and liver samples was isolated with RNAeasy mini columns from Qiagen by following manufacturer's instruction. RNA concentration and quality 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 260/280 and 260/230 was always above 1.8 for all the samples. The total RNA (2 µg/50 µl) was reverse-transcribed into template cDNA with Promega reverse-transcription kit from Promega Corporation, Madison WI, USA according to the earlier published method (33).

qRT-PCR was performed for the rat and human genes using primer sequences listed in table 1 and 2, respectively, using SYBR Green detection system as reported earlier by Khan et al. (33). Primer sequences used for CYP3A1, CYP3A2 and CYP3A9 analysis were as reported earlier by Mahnke et al.,(40). All primer sets were analyzed using BLASTn to ensure primer specificity for the gene of interest (<http://www.ncbi.nlm.nih.gov/BLAST/>). Furthermore appropriate controls were analyzed for all the primer sets to determine dimer formation of the primer and homogeneity of the PCR products. The comparative threshold cycle (C_T) method was used for relative quantification of the mRNA. C_T is 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 reference gene (villin for intestine and GAPDH for liver) (ΔC_T), which was compared to the ΔC_T of the corresponding solvent control ($\Delta\Delta C_T$). Data are expressed as fold induction or repression of the gene of interest according to the formula $2^{-(\Delta\Delta C_T)}$. No significant differences were observed in the expression of genes of interest between control incubations with and without the solvents (methanol and DMSO), therefore all control incubation data was analyzed as one experimental group.

Statistical analysis. All experiments were performed in 3-5 rats and in 5-7 human tissue samples. All values were expressed as mean \pm S.E.M. All data were analyzed by the paired student's *t*-test or Mann-Whitney *U*-test to detect the effect of the exposure to the ligands. The student's *t*-test was used to analyze the rat data where the error distribution was found to be normal with equal variance. The non parametric Mann-Whitney *U*-test was used for experiments where non-equal error distribution and high variance (e.g. expression of CYP3A1 and CYP3A2 genes in Wistar rats and all genes in human tissues)

were observed. Statistical analysis was performed on fold induction as well as on $\Delta\Delta C_T$ with similar results. A P value < 0.05 was considered as significant.

Table 1 Oligonucleotides for quantitative Real-Time PCR, rat genes (SYBR Green analysis)

Gene	Forward primer (5' – 3')	Reverse primer (5' – 3')
r Villin	GCTCTTTGAGTGCTCCAACC	GGGGTGGGTCTTGAGGTATT
r GAPDH	CTGTGGTCATGAGCCCCTCC	CGCTGGTGCTGAGTATGTCG
r - CYP3A1	GGAAATTCGATGTGGAGTGC	AGGTTTGCCTTTCTCTTGCC
r CYP3A2	AGTAGTGACGATTCCAACATAT	TCAGAGGTATCTGTGTTTCT
r CYP3A9	GGACGATTCTTGCTTACAGG	ATGCTGGTGGGCTTGCCCTC
r CYP7A1	CTGTCATACCACAAAGTCTTATGTCA	ATGCTTCTGTGTCCAAATGCC
r BSEP	TGGAAAGGAATGGTGTATGGG	CAGAAAGGCCAGTGCATAACAGA
r Ntcp	CTCCTCTACATGATTTTCCAGCTTG	CGTCGACGTTCTGTTCTTTCTTG
r MRP2	CTGGTGTGGATTCCCTTGG	CAAAACCAGGAGCCATGTGC
r MRP3	ACACCGAGCCAGCCATATAC	TCAGTTTACATTTGCCTGTC
r SHP	CTATTCTGTATGCACCTTCTGAGCCC	GGCAGTGGCTGTGAGATGC
r HNF1 α	CTCCTCGTACTGCAAGAAACC	TTGTCACCCAGCTTAAGACTCT
r HNF4 α	CCAGCCTACACCACCTGGAGTT	TTCTCAGCTCCTCCTGAA
r LXR α	TGCAGGACCAGCTCCAAGTA	GAATGGACGCTGCTCAAGTC
r LRH-1	GCTGCCCTGTGGACTACAC	TGTAGGGCACATCCCCATTC
r PXR	GATGATCATGTCTGATGCCGCTG	GAGGTTGGTAGTTCAGATGCTG
r FXR	CCAACCTGGGTTTCTACCC	CACACAGCTCATCCCCTTT

r, rat

Table 2 Oligonucleotides for quantitative Real-Time PCR, human genes (SYBR Green and Taqman[®] analysis)

Gene	Forward primer (5' – 3')	Reverse primer (5' – 3')
h Villin	CAGCTAGTGAACAAGCCTGTAGAGGAGC	CCACAGAAGTTTGTGCTCATAGGC
h GAPDH	ACCCAGAAGACTGTGGATGG	TCTAGACGGCAGGTCAGGTC
h CYP3A4	GCCTGGTGCTCTCTATCTA	GGCTGTTGACCATCATAAAAG
h CYP7A1	GCTGTTGTCTATGGCTTATTCTT	GCCCAGGTATGGAATTAATCCA
h BSEP	CAGTTCCTCAACCAGAACAT	TTTGATCATTTCGCTCTCGATG
h Ntcp	CTCAAATCCAAACGGCCACAAT	CACATGCACAAGAGAATGATGATC
h MRP2	CGGACAGCATCATGGCTTCT	ACTCCTTCTTGGCCAAGTTG
h MRP3	GTCCGCAGAATGGACTTGAT	TCACCACTTGGGGATCATT
h SHP	TGAAAGGGACCATCTCTTCA	CAATGTGGGAGGCGGCT
h HNF1 α	CAGAAAGCCGTGGTGGAGAC	GACTTGACCATCTTCGCCACA
h HNF4 α	CCTGGAATTTGAGAATGTGCAG	AGGTTGGTGCCTTCTGATGG
h LXR α	CCCTTCAGAACCCACAGAGATC	GCTCGTTCGCCAGCATTTT
h PXR	CCCAGCCTGCTCATAGGTTT	GGGTGTGCTGAGCATTGATG
h FXR	AGAGATGGGAATGTTGGCTGA	GCATGCTGCTTCACATTTTTC
h GAPDH	Assay-on-Demand [™] ID - Hs99999905 ml Probe sequence (5' FAM -3' NFQ) - GCGCCTGGTACCAGGGGCTGCTTTT	

h, human

Results

Regulation of CYP3A isozymes by LCA in rat intestine and liver slices. Incubation of rat intestinal slices (jejunum, ileum and colon) with LCA induced CYP3A1 expression along the length of the intestine, showing a very high induction in the jejunum (400-fold at 10 μ M of LCA; $P < 0.05$) and ileum (550-fold at 10 μ M of LCA; $P < 0.05$), and a moderate induction in the colon (3.5-fold at 10 μ M of LCA; $P < 0.05$) (Fig. 1A). LCA induced CYP3A2 in the rat ileum slices but not in the jejunum and colon slices (Fig. 1B). Induction of CYP3A2 in the rat ileum slices by LCA was found to be significant at 5 μ M (5-40-fold;

$P < 0.05$). At 10 μM of LCA, CYP3A2 induction was even higher but showed a high variation among the rats ranging from 6- to 124-fold and therefore failed to reach statistical significance. Furthermore, LCA induced CYP3A9 in jejunum, ileum and colon slices (2-3-fold; $P < 0.001$) with a slightly higher effect in the colon slices (Fig. 1C).

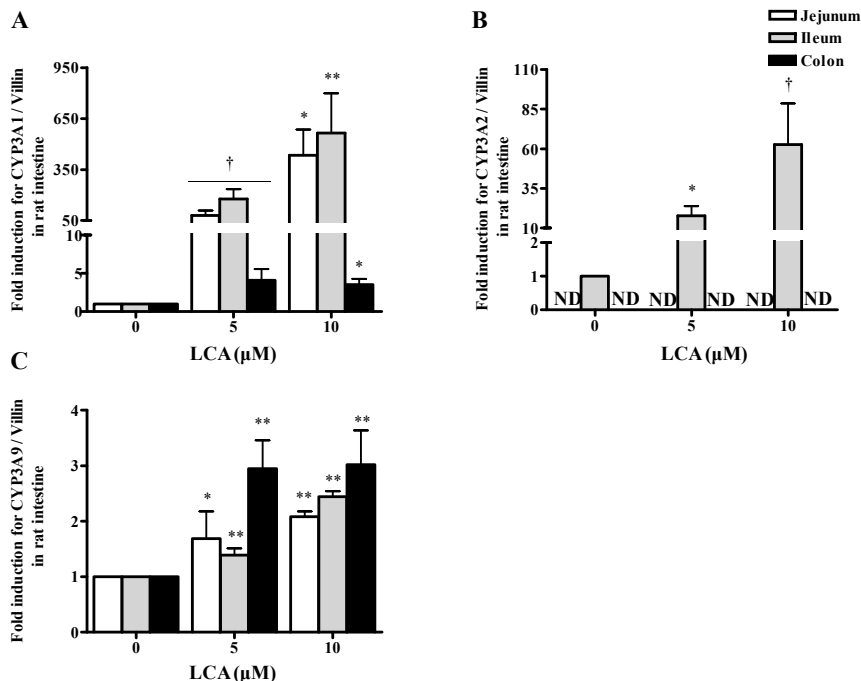


Figure 1. Rat jejunum, ileum and colon tissue-slices were exposed to LCA (5 and 10 μM) for 12 h after which total RNA was isolated and mRNA expression of CYP3A1 (A), CYP3A2 (B) and CYP3A9 (C) were evaluated by qRT-PCR. After normalizing for villin expression the results were compared to the 12 h incubated control slices of the same segment. Results showed mean \pm 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 ** $P < 0.001$. “†” denotes induction of CYP3A1 and CYP3A2 in all experiments, but failed to reach statistical significance due to high variation between the experiments, ND-not detectable.

In contrast to the intestinal slices, the expression of CYP3A1, CYP3A2 and CYP3A9 mRNA in the rat liver slices was not affected by LCA during 8 h of incubation. However, when liver slices were exposed to LCA for 24 h, CYP3A9 expression was induced by 2-fold, whereas CYP3A1 and CYP3A2 expression remained unaltered (Fig. 2). As expected, the PXR ligands dexamethasone (DEX) and pregnenolone-16 α carbonitrile (PCN) significantly induced the expression of CYP3A1 (100-fold) and CYP3A9 (5-8-fold; $P < 0.001$), but not CYP3A2 (Fig. 2). Budesonide (BUD), a synthetic GR ligand significantly induced CYP3A9 expression (2-3-fold; $P < 0.001$) and decreased CYP3A2 expression, but did not influence CYP3A1 expression (Fig. 2). These results with PCN, DEX and BUD show that the PXR and GR mediated pathways are intact in rat liver slices.

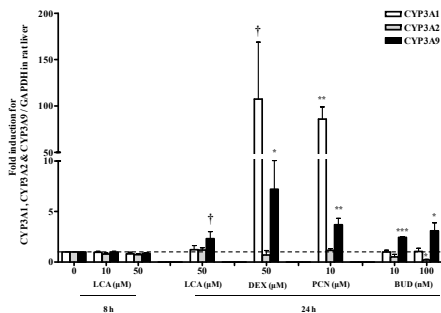


Figure 2. Rat liver slices were exposed to LCA (10 to 50 μM) for 8 h and 24 h; and with DEX (50 μM), PCN (10 μM) and BUD (10 and 100 nM) for 24 h, after which total RNA was isolated and mRNA expression of CYP3A1, CYP3A2 and CYP3A9 was evaluated by qRT-PCR. After normalizing for GAPDH expression, the results were expressed as fold-induction and compared with the 8 h and 24 h incubated control slices. Results showed mean \pm S.E.M. of 3-5 rats; 3 slices were incubated per condition in each experiment. Significant differences towards the control incubations are indicated with * $P < 0.05$, ** $P < 0.001$ and *** $P < 0.0001$. “†” denotes induction of CYP3A9 in all experiments, but failed to reach statistical significance due to high variation between the experiments.

Expression and regulation of CYP3A4 in human ileum and liver slices. CYP3A4 expression was stable during control incubation of the human ileum slices for 8 h, but was decreased in human ileum and liver slices after 24 h of incubation (Fig. 3A). Incubation of human ileum slices with LCA (10 μM) significantly induced CYP3A4 expression (9- and 5-fold induction during 8 h and 24 h, respectively; $P < 0.05$) (Fig. 3B). Incubation of human liver slices with LCA (50 μM) induced CYP3A4 mRNA expression in four out of seven livers, and slightly reduced it in the other three livers (Fig. 3B and table 3).

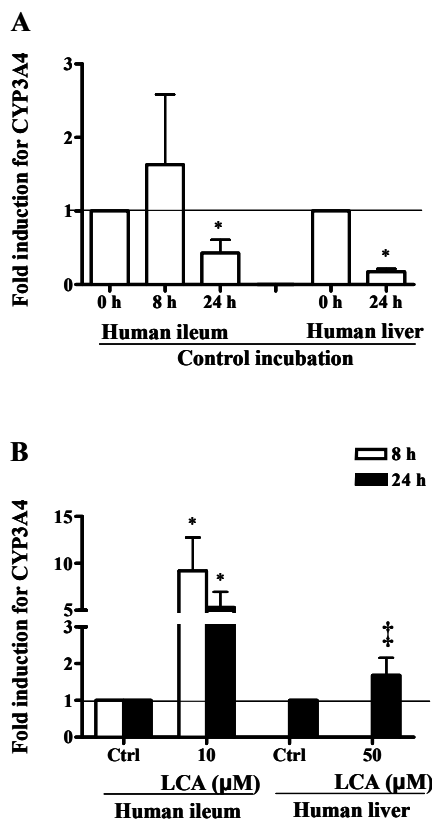


Figure 3. Slices from human ileum and liver were exposed to control incubation (A), LCA, 10 μM and 50 μM (B), respectively for 8 h and 24 h, after which total RNA was isolated and mRNA expression of CYP3A4 was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with villin for ileum and GAPDH for liver, and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean \pm S.E.M. of 4 human ileum and 7 liver donors; in each experiment 3 ileum and liver slices were incubated per condition. Significant differences towards the control incubations are indicated with * $P < 0.05$. “†” denotes induction of CYP3A4 in 4 out of 7 experiments.

Expression and regulation of rMRP2 and rMRP3 in rat intestine slices. In rat intestine, rMRP2 and rMRP3 transporters are expressed in reciprocal gradients along the length of the intestine with rMRP2 mRNA expression showing a decreasing gradient from the jejunum towards the colon, and rMRP3 expression showed an increasing gradient from the jejunum to the colon (Fig. 4). During control incubations of rat jejunum, ileum and colon slices in Williams Medium E without ligands, rMRP2 expression was significantly increased in jejunum and ileum, but decreased in colon slices (Fig. 5A). rMRP3 expression was significantly increased in jejunum, ileum as well as in colon slices during control incubation (Fig. 5C). LCA induced the expression of rMRP2 compared to the control incubations in colon slices but not in jejunum and ileum slices (Fig. 5B). LCA did not affect the rMRP3 expression along the length of the intestine (Fig. 5D). In contrast, CDCA significantly decreased the expression of rMRP2 in jejunum and ileum slices but induced rMRP2 in colon slices (Figs. 5B and D), whereas CDCA increased the rMRP3 expression in ileum slices only but did not affect the rMRP3 expression in jejunum or colon (Figs. 5B and D). DEX significantly induced the rMRP2 expression in the jejunum and colon slices and decreased the expression of rMRP3 in jejunum and ileum but not in colon slices (Fig. 5B and D). Similar to DEX, PCN significantly induced the rMRP2 expression in the jejunum and colon slices (Fig. 5B) but PCN did not affect the rMRP3 expression along the length of the rat intestine (Fig. 5D).

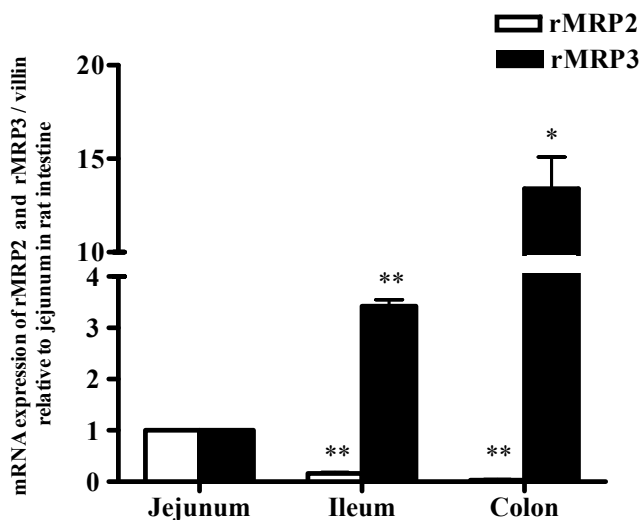


Figure 4. mRNA expression of rMRP2 and rMRP3 transporters relative to villin expression in intestinal tissue (jejunum, ileum and colon) of the Wistar rat. The mRNA expression of rMRP2 and rMRP3 transporters relative to villin in ileum and colon was expressed relative to that in the jejunum, which was set to unity. Each bar represents the results of three animals \pm S.E.M. Significant differences between ileum and colon compared to jejunum are indicated with * $P < 0.05$ and ** $P < 0.001$.

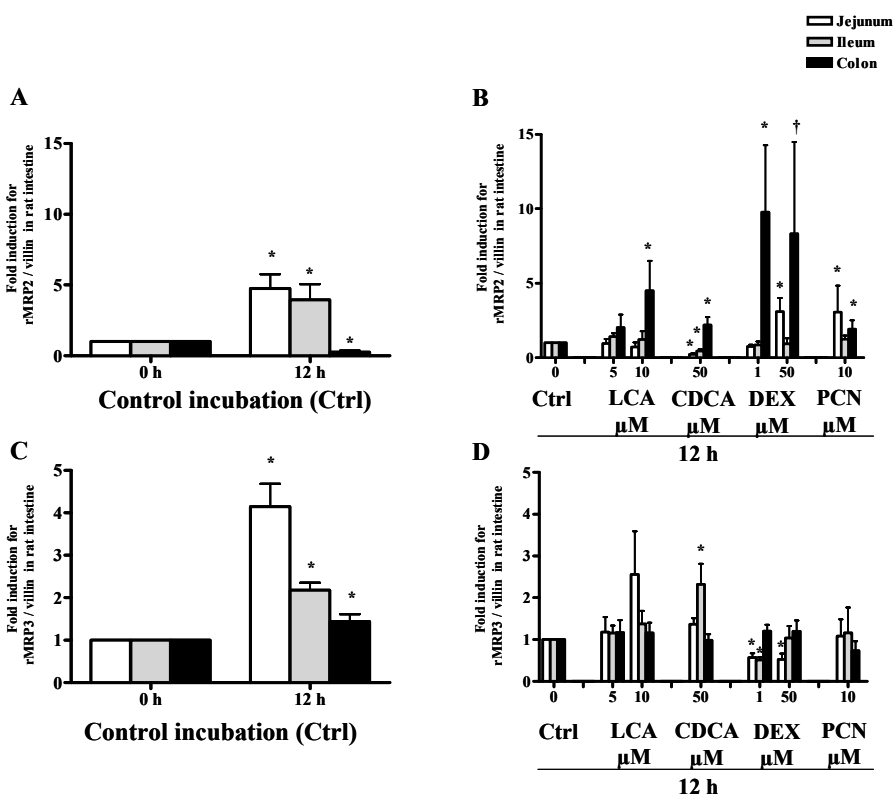


Figure 5. Rat jejunum, ileum and colon tissue-slices were exposed to LCA (5 μ M and 10 μ M), CDCA (50 μ M), DEX (1 μ M and 50 μ M) and PCN (10 μ M) for 12 h after which total RNA was isolated and mRNA expression of rMRP2 (A and B) and rMRP3 (C and D) were evaluated by qRT-PCR. Results were expressed as fold induction after normalizing with villin expression and compared to the control incubated slices of the same segment for 12 h, which was set to 1. Results showed mean \pm S.E.M. of 3-5 rats; in each experiment, 6 slices were incubated per condition. Significant differences compared to the control incubations are indicated with * $P < 0.05$. “†” denotes induction of rMRP2 in all experiments, but failed to reach statistical significance due to large variation between the experiments.

Expression and regulation of hMRP2 and hMRP3 in human ileum slices. In the human ileum slices, the hMRP2 expression was significantly increased during control incubation for 8 h and returned to control values at 24 h, whereas hMRP3 was increased after 24 h (Fig. 6A). LCA induced hMRP3 mRNA expression by 4-fold after 8 h of incubation and hMRP2 expression by 4-fold after 24 h of incubation as compared to the solvent treated controls (Figs. 6B and C). CDCA did not affect the expression of hMRP2 and hMRP3 in human ileum slices during 8 h of incubation but upon prolonged (24 h) exposure of human ileum slices to CDCA, hMRP3 but not hMRP2 expression was induced (Figs. 6B and C). DEX induced hMRP2 expression in all the tested human ileum samples without affecting hMRP3 expression after 24 h incubation (Figs. 6B and C).

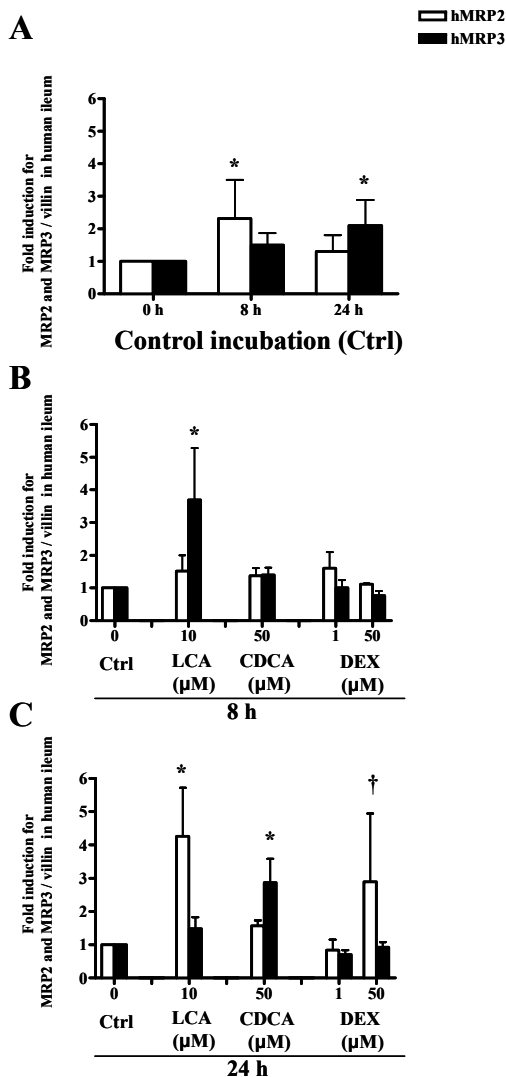


Figure 6. Human ileum slices were exposed to LCA (10 μ M), CDCA (50 μ M) and DEX (1 μ M and 50 μ M) for 8 h and 24 h after which total RNA was isolated and mRNA expression of hMRP2 and hMRP3 (A, B and C) were evaluated by qRT-PCR. Results were expressed as fold induction after normalizing with villin expression and compared to the control incubated slices for 8 h and 24 h, which was set to 1. Results showed mean \pm S.E.M. of 4-5 human ileum donors. In each experiment, 3 ileum slices were incubated per condition. Significant differences compared to the control incubations are indicated with * $P < 0.05$. “†” denotes induction of hMRP2 in all experiments, but failed to reach statistical significance due to large variation between the experiments.

Expression and regulation of the bile acid synthesis enzyme, transporters and nuclear receptors in rat liver slices. The expression of rCYP7A1 mRNA in rat liver slices was highly sensitive to incubation. rCYP7A1 mRNA expression was decreased by 90% during 8 h of incubation, and upon 24 h incubation, rCYP7A1 mRNA was barely detectable (Fig. 7A). Incubation of rat liver slices with LCA for 8 h significantly decreased the rCYP7A1 expression when compared to control incubated slices (Fig. 7B). Furthermore, LCA induced rSHP and decreased rHNF1 α , rLXR α and rLRH-1 expression without affecting the rHNF4 α expression after 8 h of incubation (Figs. 8B and C). Prolonged exposure of rat liver slices to LCA for 24 h significantly decreased rHNF4 α expression (Fig. 8C). LCA decreased the rPXR and rFXR mRNA expression (Figs. 8B and C). DEX but not PCN

significantly decreased the rCYP7A1 expression with concomitant induction of rSHP (Figs. 7B and 8B). Furthermore, DEX but not PCN induced the rPXR expression in liver slices upon 8 h of incubation (Fig. 8B), whereas, both DEX and PCN significantly decreased the expression of rLXR α , rPXR, rFXR, rHNF1 α , rHNF4 α and rLRH-1 upon 24 h of incubation (Fig. 8C).

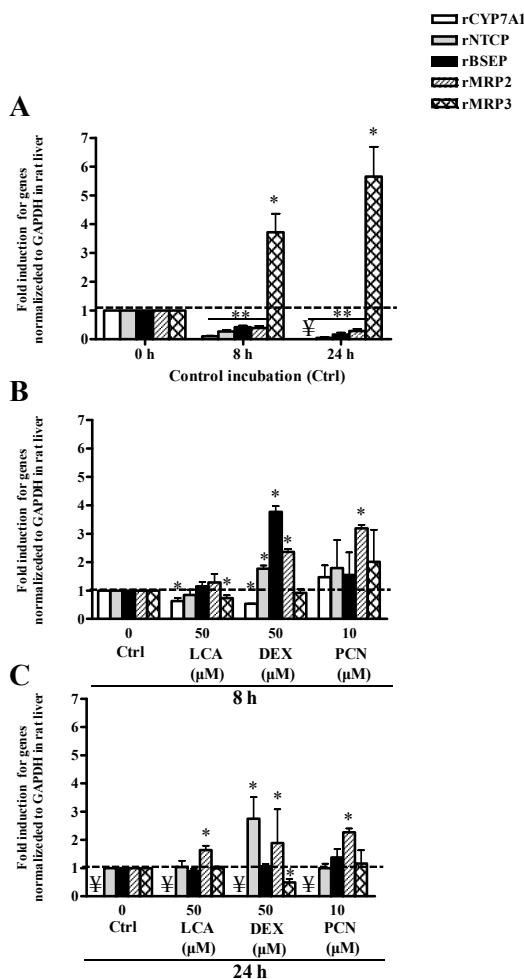


Figure 7. Slices from rat liver were exposed to LCA (50 μ M), DEX (50 μ M) and PCN (10 μ M) for 8 and 24 h, after which total RNA was isolated and mRNA expression of rCYP7A1, rBSEP, rNTCP, rMRP2 and rMRP3 were evaluated for control (0 h, 8 h and 24 h) (A), (B) 8 h and (C) 24 h incubations by qRT-PCR. Results were expressed as fold-induction after normalizing with rGAPDH and compared with the control slices that were incubated for 8 h and 24 h, which was set to unity. Results showed mean \pm S.E.M. of 3-5 rats; 3 slices were incubated per condition in each experiment. Significant differences compared to the control incubations are indicated with * $P < 0.05$ and ** $P < 0.001$. “ Ψ ” indicates rCYP7A1 is not detectable in samples incubated for 24 h. Note: The data for rPXR induction in livers slices for 8 h is used from our recent publication (Khan A.A. et al., 2009).

In the rat liver slices, the expression of rBSEP and rNTCP mRNA was decreased during control incubation (Fig. 7A). LCA did not affect rBSEP and rNTCP expression (Figs. 7B and C), whereas DEX but not PCN induced rNTCP and rBSEP expression (Fig. 7B and C). Furthermore, the induction of rBSEP by DEX was completely abolished upon 24 h incubation (Fig. 7C). During incubation of rat liver slices, rMRP2 expression was

decreased and rMRP3 expression was increased, (Fig. 7A). LCA decreased the expression of rMRP3 without affecting the expression of rMRP2 in liver slices upon 8 h incubation, but induced rMRP2 expression upon 24 h incubation (Figs. 7B and C). DEX and PCN induced the rMRP2 but not the rMRP3 expression (Figs. 7B and C).

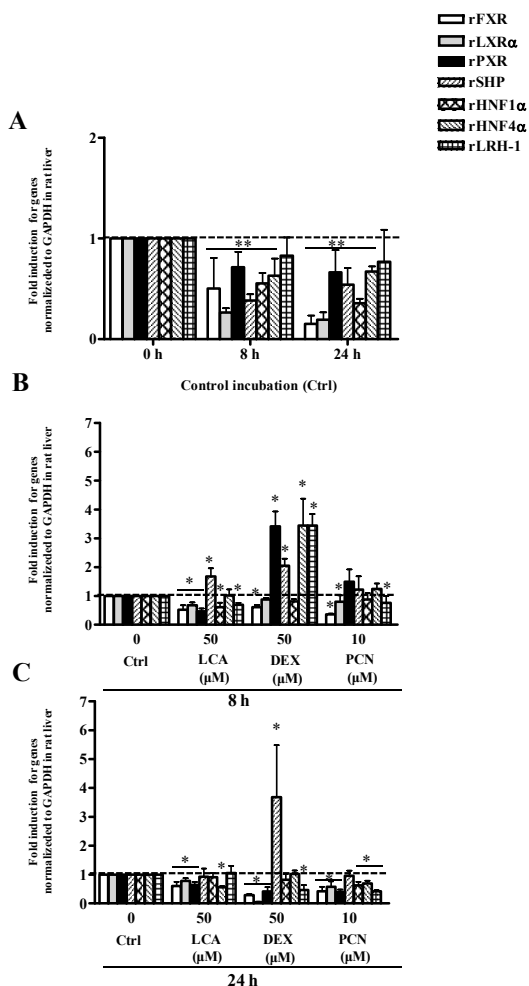


Figure 8. Slices from rat liver were exposed to LCA (50 μ M), DEX (50 μ M) and PCN (10 μ M) for 8 and 24 h, after which total RNA was isolated and mRNA expression of rFXR, rLXR α , rPXR, rSHP, rHNF1 α , rHNF4 α , and rLRH-1 was evaluated for control (0 h, 8 h and 24 h) (A), 8 h (B) and 24 h (C) incubations by qRT-PCR. Results are expressed as fold-induction after normalizing with rGAPDH and compared with the control incubated slices for 8 and 24 h, which was set to unity. Results showed mean \pm S.E.M. of 3-5 rats; 3 slices were incubated per condition in each experiment. Significant differences compared to the control incubations are indicated with * $P < 0.05$ and ** $P < 0.001$.

In the rat liver slices, the expression of rBSEP and rNTCP mRNA was decreased during control incubation (Fig. 7A). LCA did not affect rBSEP and rNTCP expression (Figs. 7B and C), whereas DEX but not PCN induced rNTCP and rBSEP expression (Fig. 7B and C). Furthermore, the induction of rBSEP by DEX was completely abolished upon 24 h incubation (Fig. 7C). During incubation of rat liver slices, rMRP2 expression was decreased and rMRP3 expression was increased, (Fig. 7A). LCA decreased the expression of rMRP3 without affecting the expression of rMRP2 in liver slices upon 8 h incubation,

but induced rMRP2 expression upon 24 h incubation (Figs. 7B and C). DEX and PCN induced the rMRP2 but not the rMRP3 expression (Figs. 7B and C).

Expression and regulation of the bile acid synthesis enzyme, transporters and nuclear receptors in human liver slices. In the human liver slices, the expression of most genes including hMRP2 and hMRP3 and CYP7A1 was constant during control incubations for 24 h, only NTCP and FXR expression was decreased and SHP expression was increased (Figs. 9A and B).

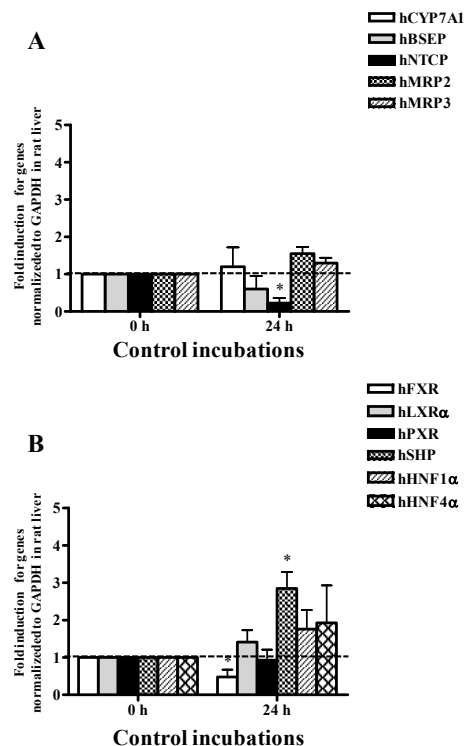


Figure 9. Slices from human liver were incubated for 24 h, after which total RNA was isolated and mRNA expression of hCYP7A1, hBSEP, hNTCP, hMRP2 and hMRP3 (A) and hFXR, hLXRα, hPXR, hSHP, hHNF1α and hHNF4α, (B) was evaluated by qRT-PCR. Results are expressed as fold-induction after normalizing with hGAPDH and compared with the un incubated samples, which was set to unity. Results show mean \pm S.E.M. of 5-7 human liver donors. In each experiment 3 slices were incubated per condition. Significant differences compared to the control incubations are indicated with * $P < 0.05$.

The effects of LCA on the expression of bile acid synthesis enzyme and transporters, and the transcription factors regulating their expression in human liver slices were quite variable among the individual livers and therefore the data is given for each individual liver in table 3 as fold induction with respect to the solvent incubated controls. This high variation does not seem to be caused by differences in viability of the livers (as judged by ATP concentration and morphology), nor does it seem to be related to the type of donor (transplantation or partial hepatectomy), or to the expression level of hVDR, hFXR or hPXR. The expression levels of these NR's varied up to 30 fold between the livers for hFXR and up to 16 fold for hPXR (results not shown), whereas hVDR was low but detectable (Ct 33-39) in 4 livers and undetectable (Ct < 40) in 2 livers. In each individual liver, a fold induction of > 1.5 is considered as up regulation and a fold induction of < 0.7 is

considered as down regulation. Furthermore, the expression of a gene is considered as induced or down regulated, if it is induced or repressed in 50% of the human livers, and the others being non responsive.

In contrast to the findings in rat liver slices, incubation of human liver slices with LCA did not consistently up regulate CYP3A4 expression (up regulation in four, down regulation in three livers) (table 3), whereas DEX showed a strong up regulation of CYP3A4 expression in all the human livers (table 4). In addition LCA did not have a consistent effect on hCYP7A1 (decrease in three out of seven livers), hSHP expression, (induction in only two out of seven livers), hHNF1 α expression (reduced in three out of five livers), hLXR α (decreased in three out of five livers) and hHNF4 α (decreased in four out of six livers) (table 3). DEX induced hHNF4 α expression in three out of four livers, but hLXR α expression was not affected by DEX (table 4).

The effect of LCA on the expression of hBSEP, hNTCP, hPXR, hFXR, hMRP2 and hMRP3 is neither significant nor consistent (table 3). DEX significantly induced hBSEP and moderately induced hPXR expression in all the livers, and induced hNTCP in four out of five livers, but had no effect on hMRP2 and hMRP3 expression (table 4).

Discussion

In this report, rat and human precision-cut intestinal and liver slices were used to characterize the role of LCA in the regulation of genes involved in bile acid detoxification, transport and synthesis. Our data on the effects of 1,25(OH) $_2$ D $_3$ (33, 34), BUD, GW4064, CDCA, PCN and DEX show that VDR, FXR, PXR and GR pathways are intact in the tissue slices. The observed changes in the expression of the CYP P450 isozymes and transporters during control incubations indicate that apparently the basal expression is normally maintained by ligands that are absent in the culture medium. The pattern of LCA-mediated induction of CYP3A1 and CYP3A2, and CYP3A4 in the rat and the human intestine, respectively (Figs. 1A, 1B, 1C and 3B) resembles that of the VDR ligand, 1,25(OH) $_2$ D $_3$, and was clearly different from that of FXR, PXR or GR ligands as reported earlier by us (33) and others (20), confirming the role of VDR in the regulation of CYP3A isozymes by LCA in the rat and human intestine. However, the higher induction of CYP3A1 mRNA by LCA in the rat ileum compared to the colon cannot be explained by differences in VDR expression, as VDR was shown to be higher in colon (33), thus other factors such as the presence of activators/repressors may also play a role. The induction of CYP3A9 by LCA in the rat intestine is likely mediated via PXR, as the enzyme was induced by other PXR ligands, PCN and DEX, but not by 1,25(OH) $_2$ D $_3$, the VDR ligand, or the CDCA, FXR ligand (33). The high induction of CYP3A9 in the colon is consistent with the higher expression of PXR (33). In human ileum slices, LCA induced CYP3A4 expression (Fig. 3B). However, the nuclear receptor (NR) involved in the induction of CYP3A4 by LCA is not conclusive from our studies since CYP3A4 was induced by PXR,

GR and VDR ligands (20, 33). The involvement of VDR rather than PXR is suggested recently by Matsubara et al. (44), who showed that the regulation of human CYP3A4 by LCA in HepG2 cells is specifically mediated by VDR and not by PXR.

In the rat liver slices, LCA did not affect CYP3A1 and CYP3A2 mRNA expression (Fig. 2), which is congruent with the results obtained previously with the VDR ligand, 1,25(OH)₂D₃ (33). The absence of a VDR mediated induction of CYP3A isozymes can be explained by the localization of VDR in the bile duct epithelial cells but not in the hepatocytes as reported earlier by Gascon-Barre et.al. (42), and confirmed in our studies (chapter 4), whereas most of the CYP3A expression is present in the hepatocytes. However, the LCA-induced CYP3A9 expression upon 24 h incubation was similar to that from the PXR ligands, PCN and DEX (Fig. 2), suggesting a PXR response. This delayed response in the induction of CYP3A9 by LCA suggest that this effect might not be directly mediated by LCA, but by its metabolite, 3KCA, as it was shown that LCA itself is a poor PXR ligand, and needs to be metabolized to 3KCA prior to PXR binding (41, 53).

In contrast to the observations in rat liver, but consistent with those in human ileum, LCA induced the CYP3A4 expression in human liver slices of four out of seven liver donors (Fig. 3). This induction could, in principle, be explained by the observed expression of VDR and its involvement with LCA in human hepatocytes (21). But the induction of CYP3A4 did not correlate with the VDR expression in these livers (table 3). Therefore it is unlikely that the LCA mediated induction of CYP3A4 in these four livers is mediated by VDR; other nuclear receptors such as PXR with its ligands DEX (table 4) and rifampicin (47), could explain this induction data. This variability in effect may be caused by the inter individual variation in activity of the enzymes that are involved in the metabolism of LCA, sulfotransferases (27) and CYP3A4 (36), which may result in variations in the effective exposure to LCA. Subsequent to characterizing the effect of LCA on CYP3A isozymes in rat and human intestine and liver, we studied the effect of LCA on the regulation of rMRP2 and rMRP3 in intestinal slices, since these transporters play an important role in the excretion of monovalent and conjugated bile acids across the apical and basolateral membranes, respectively, of enterocytes (8, 10, 25, 26, 61) and the basolateral transporter, OST α -OST β (5, 13). Recently, we showed that LCA decreased the expression of OST α and OST β in the rat ileum and induced OST α and OST β in the rat colon and liver, and in the human ileum and liver (Khan et. al., 2009b). The reciprocal expression of rMRP3 and rMRP2 along the length of the rat intestine with rMRP3 expression decreasing from jejunum and rMRP2 expression increasing towards the colon (Fig. 4), together with the basolateral localization of OST α -OST β in the ileum (4), suggest that BA transport in the colon is directed more towards the lumen, whereas in the small intestine basolateral transport is predominant. The finding that LCA did not affect the rMRP3 expression in rat jejunum, ileum and colon slices but induced rMRP2 expression in colon slices (Figs. 5B and D) and decreased rOST α -rOST β in rat ileum (34) suggests that the luminal excretion of BA's is further stimulated by LCA. Although the VDR ligand,

1,25(OH)₂D₃ also induced rMRP2 without affecting rMRP3 expression (A.A. Khan et. al., unpublished observation), we cannot conclude whether the effect of LCA on rMRP2 is mediated by VDR, PXR or FXR, as DEX, PCN and CDCA also induced rMRP2 expression in the rat colon (Fig. 5B). In the mouse colon, however, Mrp3 was reported to be induced by 1,25(OH)₂D₃ (45), suggesting species differences in the regulation of MRP3. Thus, in the rat, LCA favours its own detoxification and transport into the lumen of the colon by inducing rMRP2 but not rMRP3 expression (Figs. 5B and D), hence excretion via the faeces. In contrast, the primary BA, CDCA, stimulates absorption of bile salts by induction of rMRP3 and OST α -OST β expression and repression of rMRP2 expression in rat jejunum and ileum (Figs. 5B and D), 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 hMRP2 expression, whereas CDCA favours the basolateral transport of BAs by inducing hMRP3 expression in ileum slices (Figs. 6B and C), which is consistent with the earlier reports (29). As CDCA did not affect hMRP2 expression in human ileum slices, the LCA effects are not likely to be mediated by FXR.

In addition to the evaluation of the effects of LCA on the detoxification enzymes and MRP transporters in rat and human intestine, we studied the effect of LCA in rat and human liver on CYP7A1, the rate limiting enzyme in bile acid synthesis, and on the bile acid transporters, NTCP, BSEP, MRP2 and MRP3 and the NR/transcription factors involved in the regulation of these proteins. In rat liver slices, LCA decreased the rCYP7A1 expression, with simultaneous induction of rSHP, as expected for an FXR ligand, and in line with the effects of CDCA (chapter 4). Furthermore, in rat liver slices, LCA also affected the SHP-independent pathways of rCYP7A1 regulation by decreasing the expression of rHNF1 α , rHNF4 α , rLXR α and rLRH-1 (Figs. 8B and C), that are essential for the expression of rCYP7A1 (1, 14, 15, 22, 38). However, the effects of LCA are not likely to be mediated by PXR since by PCN, the PXR ligand, failed to alter the expression of rCYP7A1 and rSHP. The effects of LCA on rCYP7A1 seem to decrease with increasing incubation time, which might be due to the metabolism of LCA into metabolites which are less efficient agonists of FXR but have a higher affinity towards PXR. Therefore, the FXR effects of LCA, such as repression of bile acid synthesis may precede the PXR effects, like CYP3A9 induction. Furthermore, we found that LCA, like CDCA decreased the expression of rFXR and rPXR in rat liver slices (Fig.8B) (chapter 4), an observation not reported previously for the rat liver. Thus, in addition to decreasing the expression of rCYP7A1, LCA also decreased the rFXR expression, probably by a feedback loop mediated by the decreased expression of rHNF1 α , (Figs. 8B and C), which is required for its basal expression (39). The LCA dependent rSHP induction and concomitant repression of rPXR is consistent with results found in human hepatocytes and in mice with SHP-mediated repression of PXR (48), but in contrast with the increased expression of SHP and PXR found in mice fed with CDCA and GW4064 (31).

Table 3 Summary of the effects of 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	HL3	HL4	HL5	HL6	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 / μ g of protein \pm SD		*10.4 \pm 1.5	*5.7 \pm 1.9	*12.1 \pm 1.0	11.1 \pm 0.9	*3.3 \pm 1.2	*9.7 \pm 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
	□	HNF1 α	↔	↔	0.3	↔	↔	ND	↔	0.78	0.10	[†] 0.042
	↔	HNF4 α	↔	0.4	0.2	0.4	2.4	ND	0.4	0.80	0.34	0.523
	↔	LXR α	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
	↔	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	

HL-human liver; ND-not done; NDE-not detectable; N/A – not available; ↔ - No significant effect; ↓- significant repression; “*” data is taken from Khan et al.(33); 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.

Table 4 Summary of the effects of 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	HL3	HL4	HL5	Mean	S.E.M.	P	
Gender		Female	N/A	Male	Female	Female				
Age		54	N/A	65	72	64				
ATP pmol / μ g of protein \pm SD		*10.4 \pm 1.5	*5.7 \pm 1.9	*12.1 \pm 1.0	*3.3 \pm 1.2	*9.7 \pm 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
	↑	HNF1 α	↔	↔	1.6	1.7	ND	1.35	0.20	0.079
	↑	HNF4 α	1.9	↔	6.5	3.9	ND	3.31	1.24	0.071
	↑	PXR	↔	↔	2.3	↔	1.6	1.5	0.21	0.043
	↑	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

HL-human liver; ND-not done; NDE-not detectable; N/A – not available; ↔ - No effect; ↑- significant induction or induction in \geq 50% of the livers; “*” data is taken from Khan et al.(33); All values are expressed as fold induction with respect to their solvent incubated controls.

In human liver slices, the effects of LCA on the NRs were highly variable, as observed for CYP3A4. The decreased HNF1 α , HNF4 α and LXR α , as found in rat liver slices, was observed in only 3-5 of the tested human livers. (table 3 and Figs. 8B and C). Unlike in rat liver slices, LCA did not affect the hMRP2 expression in human livers (Figs. 7B and C, table 3). In addition, the LCA mediated effects on the expression of hSHP, hBSEP and hNTCP in human livers were not consistent, but CDCA effects were consistent with an intact FXR pathway (chapter 4). Hence, it is concluded that LCA does not act as an FXR ligand in the human liver, as also reported by others (2, 30). These results indicate that the cholestatic effects of LCA are at least partly mediated by direct effect of LCA itself on the regulation of hBSEP but not on hMRP2. Furthermore, DEX induced hNTCP (table 4), similar to that of rNTCP (Figs. 8B and C), which is probably mediated by GR and consistent with an earlier report (18). Hence, the GR pathway is intact in human liver slices. DEX also induced hBSEP, similar to rBSEP (table 4 and Fig. 8B), which is not reported before.

In conclusion, LCA plays an important role in the feed forward regulation of its detoxification pathways in the rat and human intestine by inducing CYP3A isozymes, thereby increasing its metabolism. In addition, it increases the luminal efflux of conjugated (toxic) bile acids via rMRP2 into the colon, while simultaneously preserving the primary bile acid pool by inducing the expression of rOST β in the colon and rMRP3 in the ileum. Distinct species differences were observed for the effects of LCA in the rat and human liver. In the rat liver, LCA decreases bile acid synthesis and excretion but its effects in the human liver were inconsistent and need further investigation. Thus, LCA as a promiscuous ligand for FXR, VDR and PXR, regulates bile acid synthesis, metabolism and transport in the rat intestine and liver and the human intestine. This altered expression of transporters and CYP3A enzymes may also have consequences for the disposition of drugs, especially in situations when LCA is increased such as during cholestasis.

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