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Regulation of ileal bile acid-binding protein expression in Caco-2 cells by ursodeoxycholic acid: Role of the farnesoid X receptor

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Abstract

Ursodeoxycholic acid (UDCA) is beneficial in cholestatic diseases but its molecular mechanisms of action remain to be clearly elucidated. Other bile acids, such as chenodeoxycholic (CDCA), are agonists for the nuclear farnesoid X receptor (FXR) and regulate the expression of genes relevant for bile acid and cholesterol homeostasis. In ileal cells CDCA, through the FXR, up-regulates the expression of the ileal bile acid-binding protein (IBABP), implicated in the enterohepatic circulation of bile acids. We report that UDCA (100 and 200 μM) induced a moderate increase of IBABP mRNA (≈10% of the effect elicited by 50 μM CDCA) in enterocyte-like Caco-2 cells and approximately halved the potent effect of CDCA (50 μM). On the contrary, UDCA reduced by 80–90% CDCA-induced IBABP transcription in hepatocarcinoma derived HepG2 cells. We confirmed that these effects on IBABP transcription required the FXR by employing a cell-based transactivation assay. Finally, in a receptor binding assay, we found that UDCA binds to FXR expressed in CHO-K1 cells (K_d = 37.7 μM). Thus, UDCA may regulate IBABP in Caco-2 cells, which express it constitutively, by acting as a partial agonist through a FXR mediated mechanism. The observation that in HepG2 cells, which do not express constitutively IBABP, UDCA was able to almost completely prevent CDCA-induced activation of IBABP promoter, suggests that tissue-specific factors, other than FXR, may be required for bile acid regulation of FXR target genes.

Keywords: Bile acids; Ursodeoxycholic acid; Chenodeoxycholic acid; Caco-2 cells; Ileal bile acid-binding protein; mRNA; Farnesoid X receptor

1. Introduction

UDCA is increasingly used to treat a variety of cholestatic liver diseases [1–4]. Its therapeutic benefits have been attributed to several concurrent mechanisms of action, including protection against cytotoxic effects of hydrophobic bile acids [5], stimulation of hepatobiliary secretion [3], decrease of intestinal bile acid absorption [6] and inhibition of liver cell apoptosis [7,8]. Nevertheless, the molecular mechanisms by which UDCA acts still remain to be clearly elucidated.

Hydrophobic bile acids such as CDCA and DCA acid are agonists for the nuclear FXR and regulate the expression of genes whose products are important for bile acids and cholesterol homeostasis [9–11]. Agonist-bound FXR, in the liver, activates gene expression of the bile salt export pump [12–15] and of the orphan receptor small heterodimer partner, which in turns represses the transcription of *Cyp7a* [16–18]. In the intestine CDCA, through the FXR, up-regulates the expression of the cytosolic IBABP [19]. This protein contains a deep bile acid-binding pocket [20] and contributes to transcellular bile acid movement through enterocytes [21]. FXR expression is restricted to liver, kidney, colon, small intestine and the adrenal cortex [22]. FXR binds as a heterodimer with retinoid X receptor to response elements consisting of an inverted repeat of the canonical AGGTCA hexanucleotide core motif [23] or separated by a single base [21,24,25]. FXR/retinoid X receptor heterodimers can also recognize other DNA motifs with varying affinity, such as direct repeats of this core sequence with different spacing [26]. In addition, an everted

Abbreviations: CDCA, chenodeoxycholic acid; *Cyp7a*, cholesterol 7α-hydroxylase; DCA, deoxycholic acid; FCS, fetal calf serum; FXR, farnesoid X receptor; IBABP, ileal bile-acid binding protein; LDH, lactate dehydrogenase; RLU, relative light units; UDCA, ursodeoxycholic acid

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repeat of the core motif separated by eight nucleotides was recently shown to mediate the induction of the multidrug resistant-associated protein 2 by bile acids [27]. Finally, it was recently shown that apolipoprotein AI is regulated by FXR via a monomeric form [28]. Therefore, reported functional FXR response elements display a large heterogeneity.

Cell-based reporter assays have been widely used to establish the agonistic activity of CDCA and DCA on FXR, but no activation with UDCA and other bile acids such as muricholic and cholic acid has been detected [9,10,29]. According to Ellis et al. [30] UDCA, unlike CDCA, does not bind to FXR since it does not suppress *Cyp7a* mRNA in cultures of primary hepatocytes. However, this was not confirmed by Lew et al. [31]. These authors, using an FXR scintillation proximity binding assay, showed that UDCA binds to this orphan receptor and may act as a partial agonist in comparison to CDCA. They also reported that in HepG2 cells UDCA behaves as a very weak FXR agonist in inducing bile salt export pump expression or lowering *Cyp7a* mRNA levels.

The activity of UDCA alone or in the presence of endogenous bile acids, such as CDCA, on IBABP expression has not yet been investigated. This information appears important considering that both compounds are present simultaneously in the pool of body bile acids after therapeutic administration of UDCA.

In this study, we found that UDCA causes a moderate increase of IBABP mRNA in enterocyte-like Caco-2 cells and partially blocks the potent effect of CDCA. Employing a cell-based transactivation assay, we confirmed that the effect on IBABP transcription requires the presence of FXR. Finally, in a receptor binding assay, we showed that UDCA binds to the FXR expressed in CHO-K1 cells and unlabeled CDCA competed with its binding. Thus, UDCA may regulate IBABP expression by acting as a partial agonist via FXR.

2. Materials and methods

2.1. Materials

The bile acids CDCA and UDCA, as sodium salts, were purchased from Sigma–Aldrich. Stock solutions of bile acids (10^{-2} M) were prepared in absolute ethanol and diluted in cell culture medium. The final ethanol concentration added to the cells was 0.05% (v/v; the same ethanol concentration, as vehicle, was added to control cells). Bile acids were added to the flasks to final concentrations ranging from 10 to 600 μM. Cell culture medium and supplements were obtained from Invitrogen. All the other reagents were of analytical grade or of the highest purity available.

2.2. Determination of mRNA by RT-PCR

Caco-2 cells (at the 40th passage) were obtained from the European Collection of Animal Cells and cultured in Dulbecco's modified Eagle's medium (containing 4.5 g glucose/L) with 10% (v/v) charcoal-stripped FCS, 2 mM L-glutamine, 1% (w/v) non-essential amino acids, 100 IU of penicillin, 0.1 mg of streptomycin and 0.25 μg of amphotericin B/mL, and kept at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. The medium was replaced at 2-day intervals. Cells were grown to confluence in 75 cm^2 flasks and maintained for at least 21 days post-confluence to allow differentiation (dome formation). One day before experiments, Caco-2 cells were switched to phenol red-free media containing 10% charcoal-stripped FCS and treated for an additional 24 h with the indicated compounds. Cells were then detached using a cell scraper by incubating monolayers for 5 min with phosphate-buffered saline. Cell viability was assessed using the trypan blue dye exclusion method; about 90% of the cells were viable.

Total cellular RNA was extracted using Trizol® reagent and digested with RNase-free DNase (Invitrogen) for 15 min at 25 °C according to the manufacturer's instructions. Five micrograms were reverse-transcribed using oligo(dT) primers (Stratagene), 1 μL RNase inhibitor (1 U/μL) and 1 μL Maloney murine leukemia virus-RT (50 U/μL; Invitrogen). The reaction mixture was incubated for 60 min at 37 °C, after which reverse transcriptase was inactivated by heating at 94 °C for 5 min. The cDNA products were subjected to PCR in 50 μL of a reaction mixture containing cDNA (corresponding to 50 ng of total RNA) with primers (Invitrogen) specific for the human IBABP gene (GenBank Accession No. X90908; sense, 5′-GGCAAGTTCGAGATGGAGAG-3′; antisense, 5′-TGAGGTCTGGTGATAGTTGGG-3′; size, 294 bp) or for the human ribosomal protein L19 (GenBank Accession No. X63527; sense, 5′-CTAGTGTCCTCCGCTGTGG-3′; antisense, 5′-AAGGTGTTTTTCCGGCATC-3′; size, 168 bp; included as internal control), the buffer (50 mM KCl; 20 mM Tris–HCl, pH 8.4), 1 μL Taq DNA polymerase (2.0 U/μL; Invitrogen), 1 mM $MgCl_2$ and 0.2 mM dNTPs. The PCR reaction was run for 25 cycles (30 s at 94 °C, 30 s at 60 °C and 1 min at 72 °C); the protocol was preceded by 5 min incubation at 94 °C and followed by 10 min elongation at 72 °C. Pilot studies were conducted to establish the optimal primer concentrations and number of cycles and to ensure that the PCR reaction was still within the exponential phase of amplification for all transcripts. A negative control of cDNA, without the RT enzyme, was included for each sample. Appropriate blanks, lacking cDNA templates were included to serve as controls for cDNA contamination.

Amplified products were electrophoresed on 1.7% agarose gel containing ethidium bromide with a DNA ladder (Fermentas). Bands were visualized under UV light and gel image was captured and analyzed with the Kodak EDAS 120® digital system. The band intensity of the specific cDNA was normalized to that of the L19 internal control. The identity of PCR products was

confirmed by partial sequencing of the amplified bands (data not shown).

2.3. FXR transactivation assay

The mouse IBABP (p-496/Luc) reporter plasmid containing nucleotides −496 to +40 of the gene encoding mouse IBABP and the plasmid encoding the human FXR were kindly provided by Prof. David Mangelsdorf (University of Texas, Dallas, TX). FXR cDNA was moved to the pcDNA 3.0 vector (pcDNA 3-FXR; Invitrogen). Caco-2 and HepG2 cells (obtained from Prof. N. Carulli, University of Modena, Italy and cultured in Minimum essential medium, Eagle, with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM sodium pyruvate, 90%; charcoal-stripped fetal bovine serum, 10%) were transfected in 6-well plates with the empty pCDNA 3.0 plasmid or with the pCDNA 3-FXR plasmid using the transfection reagent and stable clones were selected by culturing the cells in medium containing 400 μg/mL of geneticin (Invitrogen) for 3 weeks. Stable clones were isolated, grown in cell culture medium, then transiently transfected with the plasmid containing the IBABP promoter-Luc construct (2.5 μg/well). In each assay, 0.5 μ/well of pCMVβ-galactosidase (Clontech) were cotransfected as an internal standard for normalization of transfection efficiency. Briefly, 10^4 cells were seeded in 24-well plates using the cation polymer transfection reagent ExGen 500 following the manufacturer's specifications; then the cells were treated with various bile acids. For the competition study, UDCA was added 60 min before CDCA. Cells were harvested 24 h later, washed twice with phosphate-buffered saline and lysed with lysis buffer (Promega).

Luciferase activity was assayed using a commercial kit (Promega) by a luminometer and normalized by dividing the RLU by β-galactosidase activity. All experiments were run in triplicate and repeated at least four times.

2.4. FXR binding assay

Chinese hamster ovary (CHO-K1) cells were grown in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (1:1) with 10% charcoal-stripped fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin and 0.25 μg amphotericin B/mL (Invitrogen) at 37 °C in a 5% CO_2 atmosphere. Cells were seeded in 6-well plates at 10^4 cells/well the day before transfection. Cells were transfected with pCDNA 3-FXR or with the empty pCDNA 3.0 plasmid using ExGen 500 (Fermentas) and stable clones were selected by culturing the cells in medium containing 400 μg/mL of geneticin (Invitrogen) for 3 weeks. Confluent cells were then pelleted by centrifugation and lysed by NE-PER (Pierce). In saturation binding experiments, CHO-K1 cytosolic protein extracts (300 μg) were incubated overnight at 4 °C in triplicate with 0.5–90 μM [H^3]-UDCA (37 CI $mmol^{-1}$; NEN Life Sciences Products) with or without 1 mM cold UDCA in binding buffer (10 mM Tris–HCl at pH 7.4 containing 1.5 mM EDTA and 0.5 mM dithiothreitol [32]. Unbound ligand was removed with 10% charcoal-Norit A and 0.1% Dextran T-70. After incubation for 10 min at 4 °C, the samples were centrifuged (2500 × *g* for 10 min at 4 °C) and the bound radioactivity was quantified in a scintillation counter.

In competition binding experiments, CDCA was evaluated for its ability to inhibit [H^3]-UDCA (60 μM) binding to protein extracts. Non-specific binding was evaluated in the presence of 1 mM UDCA.

2.5. Cytotoxicity

Cells were grown in 6-well plates and treated with UDCA or CDCA as described above. Before harvesting cells for assay, 50 μL of medium were collected in order to test the cytotoxicity of bile acids by measuring LDH release using the CytoTox 96 assay kit (Promega). We also established the maximum LDH release, required in calculations to determine 100% release of LDH. LDH release is presented as a percentage of untreated, control cells.

2.6. Statistical analysis

All data are presented as mean ± S.E. for the indicated number of experiments. Statistical significance was determined by Duncan's multiple range test after ANOVA using GraphPad Prism, version 3.0 (GraphPad Software Inc.). *P*-values <0.05 were considered to be significant.

3. Results

3.1. CDCA and UDCA affect IBABP mRNA levels in Caco-2 cells

Consistently with other studies [9,10], in preliminary experiments we confirmed that CDCA raised IBABP mRNA levels in a time- and concentration-dependent manner. This up-regulation was detectable at 3 h and peaked at 24 h with a half-maximum (EC_{50}) of 30 μM (data not shown). UDCA started to raise IBABP mRNA at 100 μM and the highest non-toxic concentration (500 μM) resulted in partial induction of this gene, corresponding to ≈70% of that elicited by 50 μM CDCA (Fig. 1A). The EC_{50} was 360 μM (95% confidence limits 307–422 μM) Exposure to 100 μM UDCA caused a minimal increase of IBABP mRNA; however, when administered concomitantly, it virtually halved the effect of 50 μM CDCA (Fig. 1B). Higher concentration of UDCA (500 μM) caused an apparent reduction of ≈20% of CDCA-induced IBABP mRNA (Fig. 1B).

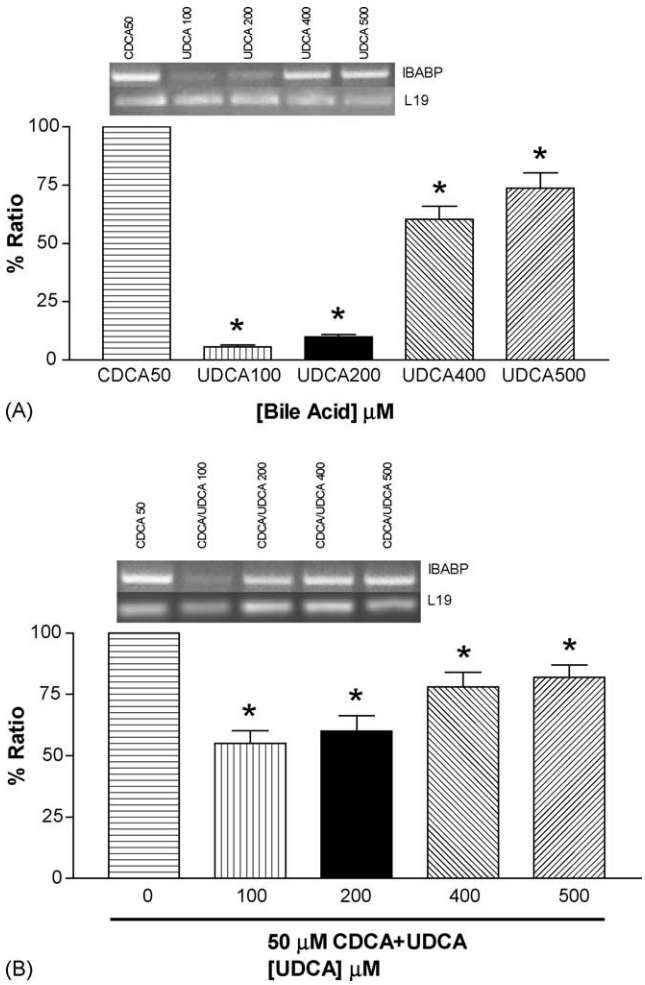

Fig. 1. Effects of bile acids on steady-state levels of IBABP mRNA in Caco-2 cells. (A) Concentration-dependent effect of UDCA. (B) UDCA partially antagonizes the effect of 50 μM CDCA. Cells were grown for 24 h in medium supplemented with the indicated molar concentration of bile acids. Steady-state levels of IBABP and L19 mRNA were established by RT-PCR (see Section 2). IBABP mRNA levels were normalized to those of L19 used as internal control. Values are the mean ± S.E.M. of four independent experiments done in duplicate. $^{*}P < 0.01$ vs. CDCA-treated cells (Duncan's multiple range test after ANOVA). Insets: a representative experiment is shown. Amplifications refer to a 294-bp fragment from mRNA encoding IBABP and a 168-bp fragment from mRNA encoding L19.

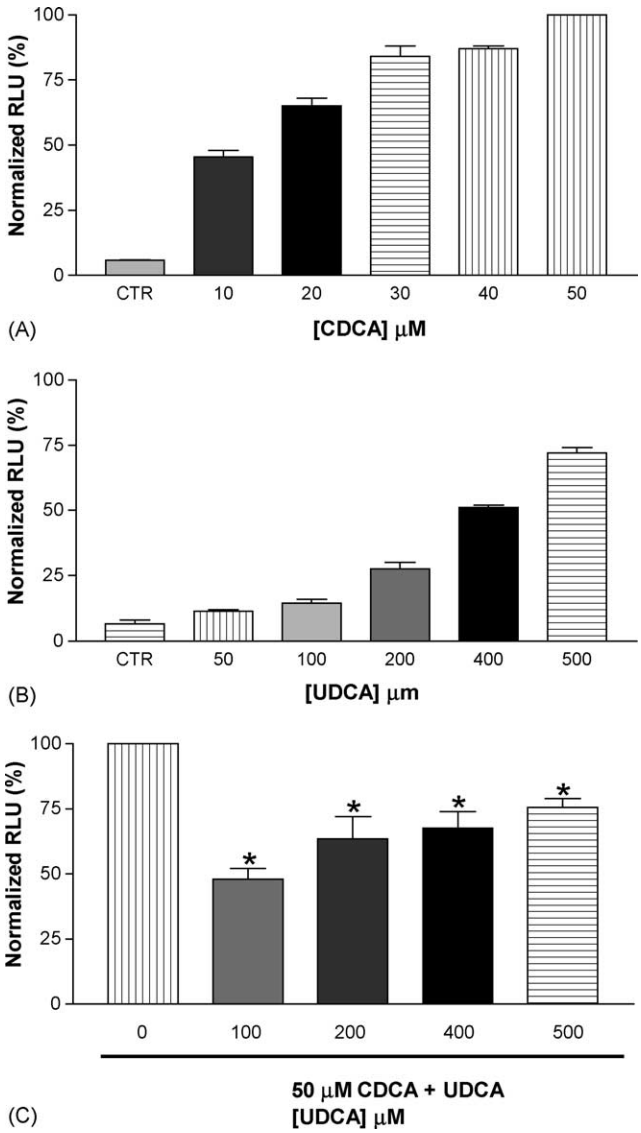

Fig. 2. Effect of CDCA and UDCA in FXR transactivation. Caco-2 cells stably transfected with FXR were seeded at a density of 10^4 cells/well in 24-well plates and transiently transfected with IBABP-Luc and pCMVβ-galactosidase plasmids; 24 h later the cells were exposed to the indicated concentrations of CDCA (A), UDCA (B) or 50 μM CDCA in the presence of different concentrations of UDCA (C). After 24-h treatment, the cells were harvested and the lysate was used to determine luciferase and β-galactosidase activity. Luciferase activity was normalized to β-galactosidase activity. Values are the mean ± S.E.M. of three experiments done in quadruplicate and expressed as % of normalized RLU vs. 50 μM CDCA. $^{*}P < 0.01$ vs. CDCA-treated cells (Duncan's multiple range test after ANOVA).

3.2. CDCA and UDCA in FXR transactivation with the IBABP promoter

IBABP gene expression is transcriptionally activated by FXR through an FXR-responsive element in the IBABP promoter [9,10]. To assess the action of UDCA on IBABP, Caco-2 and HepG2 cells were stably transfected with a plasmid expressing the human FXR and transiently transfected with another plasmid containing an IBABP promoter-driven luciferase construct. In agreement with previous studies [9,10], in cells transfected with both plasmids, CDCA (10–50 μM) increased luciferase activity in a concentration-related manner, with a maximum induction of 12 times in Caco-2 cells (Fig. 2A) and 15 times in HepG2 cells (data not shown). In the same experiments (see Fig. 2B), UDCA (10–500 μM) partially increased luciferase activity reaching, at 500 μM, 70% of the effect in Caco-2 cells elicited by 50 μM CDCA (Fig. 2A). UDCA, at concentrations that moderately activated luciferase (100–200 μM), reduced 50 μM CDCA-induced FXR-transactivation of 50% and 45%, respectively, in Caco-2 cells (Fig. 2C) and 80% and 90% in HepG2 cells (Fig. 3).

In a separate set of experiments, we investigated the cytotoxicity of CDCA and UDCA by estimating the

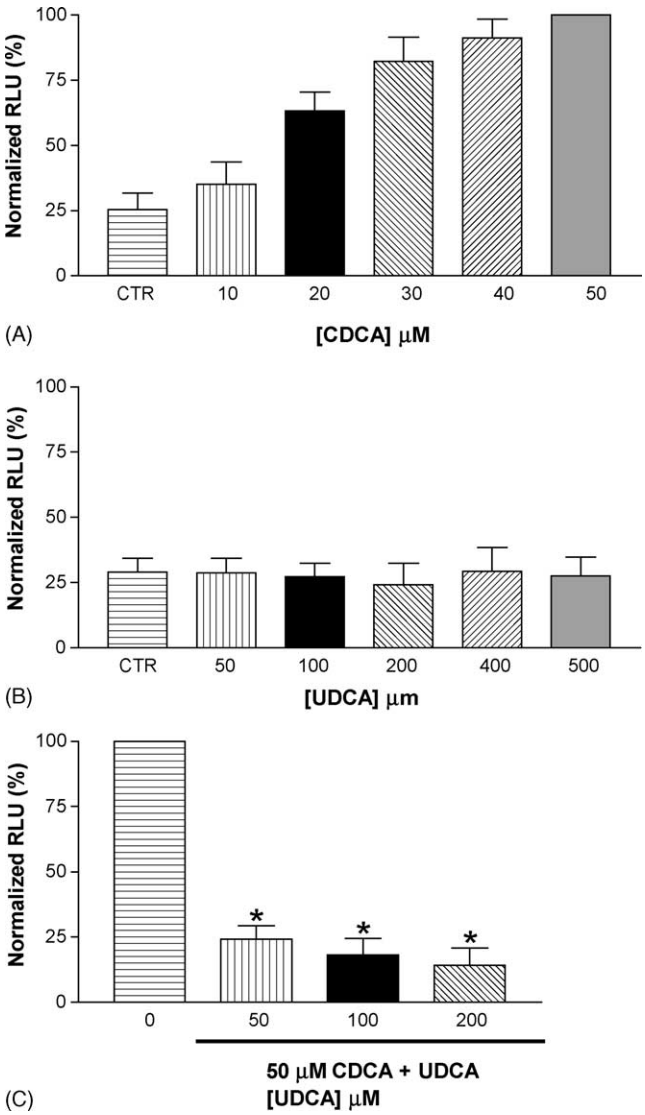

Fig. 3. Effect of CDCA and UDCA in FXR transactivation. HepG2 cells stably transfected with FXR were seeded at a density of 10^4 cells/well in 24-well plates and transiently transfected with IBABP-Luc and pCMVβ-galactosidase plasmids; 24 h later the cells were exposed to the indicated concentrations of CDCA (A), UDCA (B) or 50 μM CDCA in the presence of different concentrations of UDCA (C). After 24-h treatment, the cells were harvested and the lysate was used to determine luciferase and β-galactosidase activity. Luciferase activity was normalized to β-galactosidase activity. Values are the mean ± S.E.M. of three experiments done in quadruplicate and expressed as percentage of normalized RLU vs. 50 μM CDCA. $^*P < 0.01$ vs. CDCA-treated cells (Duncan's multiple range test after ANOVA).

release of LDH. In comparison to vehicle-treated cells, Caco-2 and HepG2 cell viability at 24 h was significantly reduced by 100 μM CDCA (respectively, 73 ± 4% and 76 ± 3%) whereas a higher concentration of UDCA (600 μM) was needed to cause a significant reduction of cell viability (respectively, 71 ± 5% and 72 ± 3%). Therefore, CDCA concentrations above 50 μM and UDCA concentrations above 500 μM were not included in this study.

3.3. Characterization of UDCA binding to FXR

To demonstrate the binding activity of UDCA to FXR, increasing concentrations of [^{3}H]UDCA were incubated with protein extracts from CHO-K1 cells transfected with a plasmid expressing FXR or with a control plasmid, as described under Section 2. As shown in Fig. 4A, specific saturable UDCA binding sites were detected in CHO-K1 cells expressing h-FXR but not in control cells. Linear regression analysis gave a K_d of 37.7 ± 6.2 μM and a B_{max} of 191.4 ± 6.56 fmol/mg protein. Unlabeled CDCA competed for [^{3}H]UDCA-specific binding, with a IC_{50} of 16.7 μM (95% confidence limits 3.36–33.9 μM) (Fig. 4B).

4. Discussion

IBABP is a small cytoplasmic protein that participates to bile acid absorption in the ileum [33–36]. Although the cellular function(s) of IBABP is not yet fully understood, it may facilitate bile acid trafficking and targeting towards the basolateral membrane [37]. However, Kok et al. [38] have reported that in FXR-deficient mice, despite ileal IBABP expression is down-regulated, the fractional turnover rate and cycling time of intestinal cholate were not affected. Future studies carried out on IBABP-deficient mice will help to better elucidate its contribution to bile acid kinetics and eneterohepatic circulation.

We found that UDCA may act as a partial FXR agonist in the regulation of IBABP gene expression in the human enterocyte-like Caco-2 cell line; it also partially reduced the effect of the potent agonist CDCA on IBABP mRNA. We showed that UDCA binds to the FXR expressed in CHO-K1 cells and CDCA can displace it. The effect elicited by UDCA on IBABP gene expression requires that this bile acid should cross the plasma membrane and should accumulate inside the cell to interact with the FXR. Several studies have reported that UDCA may be taken up by cultured cells of hepatic and non-hepatic origin involving, at least in part, a sodium-dependent transporter and/or by simple passive diffusion [39,40]. The transport of bile acids in Caco-2 cells may be dependent on time in culture and reaches a plateau after 28 days [41]; therefore, in agreement with this idea, we have employed Caco-2 cells maintained in culture for at least 21 days post-confluence (i.e., maintained in culture for 28–30 days) to favour UDCA uptake. Moreover, UDCA transport in intestinal cells has been studied in a variety of other systems, including isolated brush-border membranes [20] and in situ ileal perfusion preparations [42,43]. Taken together, these studies suggest that UDCA effectively may penetrate inside enterocyte-like Caco-2 cells.

The finding that UDCA binds to the FXR is in agreement with the study by Lew et al. [31] and confirms that UDCA may regulate IBABP transcription through this receptor, which is expressed in Caco-2 cells and in the ileum [44].

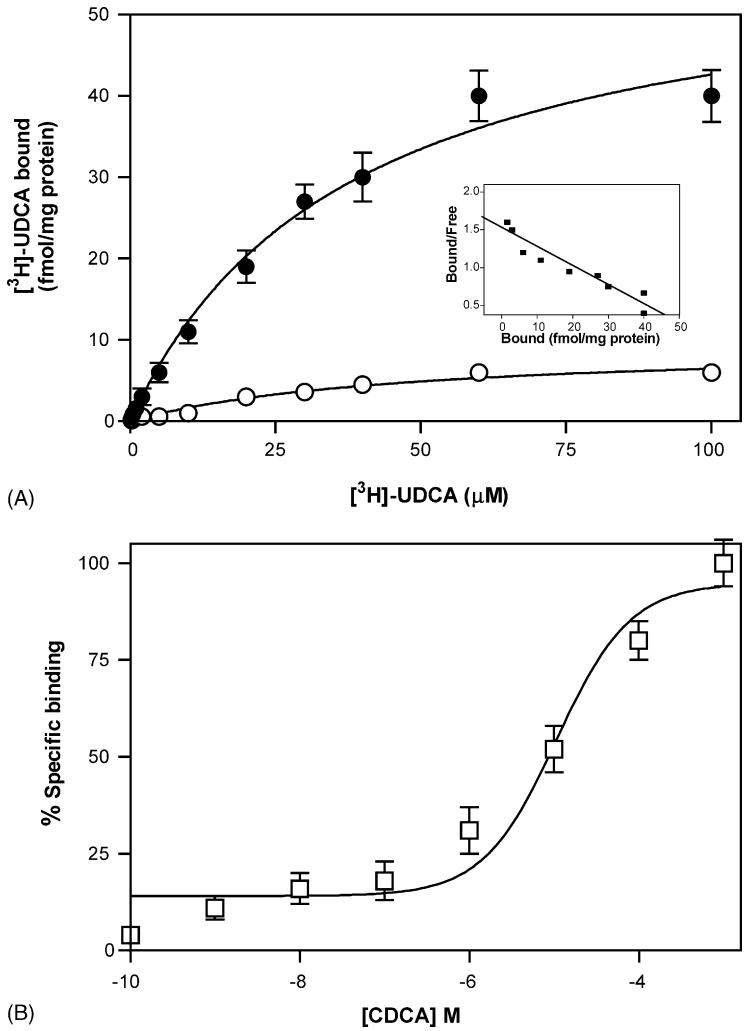

Fig. 4. (A) Saturation binding of [^{3}H]UDCA to protein extracts from CHO-K1 cells stably transfected with FXR (●) or with an empty vector (□). Non-specific binding was determined in the presence of 1 mM UDCA. Cells were incubated with increasing concentrations of the radioligand (5–90 μM) overnight at 4 °C as reported under Section 2. Inset, Scatchard transformation of specific [^{3}H]UDCA binding (■). Incubation was stopped as reported under Section 2. (B) CDCA displaces binding of [^{3}H]UDCA to cell membranes from CHO-K1 cells stably transfected with FXR. Cells were incubated with increasing concentrations of CDCA overnight at 4 °C as reported under Section 2 and the concentration of the radioligand was maintained constant (37 μM). In both panels, for the sake of clarity, only specific binding is shown. Values are the mean ± S.E.M. of three experiments done in triplicate.

However, several studies found UDCA had no effect on FXR-regulated IBABP transcription in cell-based assays, presumably because of the low concentrations used (<100 μM) [9,10].

In agreement with our results, Grober et al. [44] already reported that the IBABP promoter contains an FXR-responsive element, which interacts with the FXR/retinoid X receptor α heterodimer. They noted that, besides CDCA, glyco- and tauro-CDCA also transactivated the human I-BABP promoter when Caco-2 cells were co-transfected with a plasmid vector expressing the ileal bile acid transporter. Consistent with the idea that UDCA binding might result in a different FXR conformation which regulates the expression of FXR target genes differently, a structural study predicted that when UDCA (having a 7β-hydroxyl group) interacts with the FXR and would create a wider ligand binding pocket that would destabilize the protein structure, thereby preventing activation of the receptor by agonists such as CDCA which has a 7α-hydroxyl group [45].

UDCA-induced regulation of IBABP may also involve different cellular factors, other than FXR, which could influence its transcriptional activity. In fact, in HepG2 cells, which do not express constitutively IBABP, UDCA was able to almost completely prevent CDCA activation of IBABP/Luc reporter. Therefore, this latter cell model may represent an artificial system to investigate bile acid-induced IBABP regulation. In agreement with this hypothesis, Chen et al. [46] have reported that CDCA inhibits *Cyp7a* transcription through FXR in liver cells which have a constitutive expression of the *Cyp7a* gene; whereas this bile acid does not inhibit *Cyp7a* transcription in HEK293 cells which do not express this gene and were transfected with a *Cyp7a*/Luc reporter. These authors suggest that tissue specific factors other than FXR, are required for bile acid regulation of FXR-target genes expression, which

should be evaluated in suitable cell models. As observed by Chiang [29], tissue specific expression of nuclear factors (for instance small heterodimer partner, hepatocyte nuclear factor 4α- and α-fetoprotein transcription factor) may also provide specificity for bile acid regulated cascade mechanism of gene expression. Other potential cofactors of FXR function, which could have a tissue-specific expression, are: the steroid receptor co-activator-1, the peroxisome proliferators-activated receptor α and γ and the peroxisome proliferators-activated receptor-γ coactivator-1 [47]. Whether they are all physiologically important as mediators of FXR transactivation, and whether they perform promoter-specific functions or respond to bile acid-induced IBABP signalling in enterocyte cells remains to be elucidated in further studies. The presence of different tissue-specific regulatory mechanisms in Caco-2 and in HepG2 cells is further supported by the data of Singh et al. [48]; these authors, in fact, have reported that cytokines may differentially affect transcriptional regulation of apolipoprotein B in these two cell lines.

Our study may also help to explain one important aspect of the therapeutic use of UDCA. If a partial reduction of CDCA-regulated IBABP transcription by UDCA should occur in vivo it may reduce bile acid recovery in the intestine, resulting in larger amounts of CDCA and other endogenous bile acids in the feces. The finding that UDCA reduces ileal bile acid absorption – which is elevated in patients with primary biliary cirrhosis [6] – and increases fecal loss of CDCA in patients with cholesterol gallstones [49,50] adds further support to this theory.

The enterohepatic circulation of bile acids is a very efficient biological recycling system. In healthy human beings, the bile acid pool of 1.5–4.0 g is cycled 6–15 times: overall 17–40 g of bile acids are reabsorbed in the terminal ileum each day and they can reach an intestinal concentration up to 10 mM [3]. Following a chronic therapy with UDCA (500 mg bid were administered orally for 3 weeks), it amounts to ≈60% of the bile acid pool while the percentages of cholic acid, CDCA and DCA are all reduced significantly [51]. Therefore, in human beings UDCA can reach an intestinal concentration, which is adequate to modulate FXR transcription in enterocytes.

UDCA-regulated IBABP expression may well contribute, therefore, to the peculiar therapeutic profile of this drug, which is the result of different mechanisms of action at hepatic and intestinal level. Although UDCA is taken up in a concentration- and time-dependent manner in cultured cells [39,40], it does not affect the lipid membrane of Caco-2 cells [52]. UDCA protects the cells against the membrane-damaging effects of hydrophobic bile acids [5,53,54], inhibits apoptotic processes [7,55] and, unlike CDCA, does not raise *c-fos* mRNA in Caco-2 cells [56].

The idea that UDCA may partially antagonize bile acid-induced changes of the proteins involved in the transport of bile acids is further supported by Rost et al. [57] who reported that UDCA might prevent the impairment of hepatic function caused, in the rat, by the concomitant administration of cholic acid, by restoring hepatic transporter expression.

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