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Ursodeoxycholic and deoxycholic acids: A good and a bad bile acid for intestinal calcium absorption

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ABSTRACT

The aim of this study was to investigate the effect of ursodeoxycholic acid (UDCA) on intestinal Ca^{2+} absorption and to find out whether the inhibition of this process caused by NaDOC could be prevented by UDCA. Chicks were employed and divided into four groups: (a) controls, (b) treated with 10 mM NaDOC, (c) treated with 60 μg UDCA/100 g of b.w., and (d) treated with 10 mM NaDOC and 60 μg UDCA/100 g of b.w. UDCA enhanced intestinal Ca^{2+} absorption, which was time and dose-dependent. UDCA avoided the inhibition of intestinal Ca^{2+} absorption caused by NaDOC. Both bile acids altered protein and gene expression of molecules involved in the transcellular pathway of intestinal Ca^{2+} absorption, but in the opposite way. UDCA aborted the oxidative stress produced by NaDOC in the intestine. UDCA and UDCA *plus* NaDOC increased vitamin D receptor protein expression. In conclusion, UDCA is a beneficial bile acid for intestinal Ca^{2+} absorption. Contrarily, NaDOC inhibits the intestinal cation absorption through triggering oxidative stress. The use of UDCA in patients with cholestasis would be benefited because of the protective effect on the intestinal Ca^{2+} absorption, avoiding the inhibition caused by hydrophobic bile acids and neutralizing the oxidative stress.

Introduction

Bile acids (BAs) are among the main components of bile. Their amphipathic nature ensures fat solubilization and emulsification, thus promoting lipid digestion. They are also considered as cell signaling molecules binding two specific receptors, which have selective affinity to different types of BAs leading to the diverse roles of BAs [1]. There are two synthetic pathways in the human liver to convert cholesterol into BAs. Both ways lead to production of cholic acid (CA)[2] and chenodeoxycholic acid (CDCA), which are called primary BAs. Part of these acids are transformed by the intestinal flora to secondary BAs, deoxycholic (DCA) and litocholic acids (LCA), which originate from CA and CDCA, respectively [2]. In the human colon, CDCA is partially converted into ursodeoxycholic acid (UDCA) (Fig. 1) [3]. It is interesting to note that BAs exhibit distinct biological effects. UDCA and CDCA only differ in the configuration of the hydroxyl group at C-7 (β in UDCA and α in CDCA); however, UDCA is a hepatocyte protector while CDCA is highly toxic [4,5]. Several liver diseases and colon cancer have been associated with high concentration of hydrophobic BAs [4]. The toxicity of BAs has been related to membrane damage and to nondetergent effects, such as oxidative stress and apoptosis. Contrarily, UDCA and its taurine conjugated form are hydrophilic compounds, which have shown to modulate hepatocyte injury induced by hydrophobic BAs. The beneficial effects of UDCA are protection against cytotoxicity due to more toxic BAs, stimulation of bile flow, antioxidant activity and inhibition of cell apoptosis [6]. These properties have made that UDCA was initially used for gallstone dissolution and is currently used as the first choice treatment for a number of cholestatic liver diseases, mainly primary biliary cirrhosis (PBC) [7]. Its beneficial effects have been extended to other tissues such as placenta [8], retina [9] colon [10], and the biochemical and molecular underlying mechanisms are continued to be characterized.

The major secondary BA in humans is DCA or its salt, sodium deoxycholate (NaDOC), which are in the millimolar range in the fecal water. In our laboratory, we have demonstrated that high concentrations of NaDOC inhibit intestinal Ca^{2+} absorption through down regulation of proteins involved in the transcellular pathway of the cation. NaDOC triggers this response due to the production of oxidative stress and mitochondria mediated apoptosis [11]. Based upon the beneficial properties of UDCA described above, it

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[2] *Abbreviations used:* BAs, bile acids; BBM, brush border membrane; BLM, basolateral membrane; CA, cholic acid; CAT, catalase; CB, calbindin D_{28k}; CDCA, chenodeoxycholic acid; DAB, 3,3′-diaminobenzidine; DCA, deoxycholic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione; LCA, litocholic acid; NaDOC, sodium deoxycholate; NBT, nitro blue tetrazolium; NCX1, Na^{+}/Ca^{2+} exchanger; PBC, primary biliary cirrhosis; $PMCA_{1b}$, plasma membrane Ca^{2+}-ATPase; RIPA, radio immuno precipitation assay buffer; SOD, superoxide dismutase; UDCA, ursodeoxycholic acid; VDR, vitamin D receptor.

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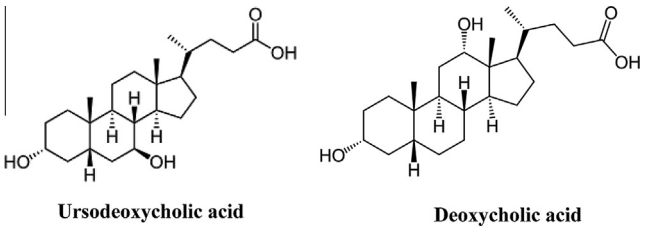

Fig. 1. Molecular structures of UDCA and NaDOC on the left and right, respectively.

is quite possible that it could prevent the inhibitory effect of NaDOC on intestinal Ca^{2+} absorption. UDCA therapy has been demonstrated to enhance fractional Ca^{2+} absorption in PBC [12], but the underlying mechanisms were not studied. The possibility that UDCA could improve the intestinal Ca^{2+} absorption under physiological conditions also needs to be investigated.

The intestinal Ca^{2+} absorption occurs mainly in the small intestine, which is responsible for about 90% of overall Ca^{2+} absorption [13]. Two mechanisms are involved in the intestinal Ca^{2+} absorption: paracellular (passive) and transcellular (active) pathways. The last one comprises the following steps: (1) Ca^{2+} entrance to the brush border membrane (BBM) through the epithelial Ca^{2+} channels TRPV6 and TRPV5, (2) cation movement from the BBM to the basolateral membrane (BLM), which is mediated by binding to the calcium binding protein calbindin D_{28k} (CB) in birds or calbindin D_{9k} in mammals, and (3) Ca^{2+} exit through the BLM involving the plasma membrane Ca^{2+}-ATPase ($PMCA_{1b}$) and/or the Na^+/Ca^{2+} exchanger (NCX1) [14].

The aim of this study was to determine the effect of UDCA on the chick intestinal Ca^{2+} absorption as well as the underlying mechanisms and to ascertain the potentiality of UDCA to prevent the inhibition of the cation absorption caused by NaDOC. In addition, the effect of UDCA alone or in the presence of NaDOC on intestinal Ca^{2+} absorption was also studied in rats.

Material and methods

Animals

One-day-old Cobb Harding chicks (*Gallus gallus domesticus*) were obtained from Indacor S.A. (Rio Ceballos, Córdoba, Argentina) and were fed a commercial normal avian diet (Cargill, S.A.C.I., Pilar, Córdoba, Argentina). At 4 weeks of age, they were divided into four groups: (a) controls, (b) chicks treated with 10 mmol/L NaDOC, (c) chicks treated with 60 μg UDCA/100 g of b.w., and (d) chicks treated with 10 mmol/L NaDOC and 60 μg UDCA/100 g of b.w. at the same time. Chicks were laparotomized under anesthesia and a 10 cm segment of duodenum was ligated as previously described [15]. One milliliter of phosphate buffer saline (PBS, vehicle), NaDOC, UDCA or NaDOC + UDCA was introduced with a syringe in the distal portion of the sac, and the loop was reintroduced in the abdomen for 30 min. Some experiments were performed in 2 month old male Wistar rats, which were treated using identical protocol. The studies were conducted according to the Guide for Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. They were killed by cervical dislocation and the excised duodenae were rinsed with cold 0.15 mol/L NaCl and mucosa or intestinal mitochondria were isolated, as described below.

Intestinal Ca^{2+} absorption

After injecting NaDOC, UDCA, NaDOC + UDCA or vehicle in the duodenal lumen for 30 min. and rinsing the intestinal segment with saline solution, one milliliter of 150 NaCl, and 1 mmol/L $CaCl_2$, containing 1.85×10^5 Bq $^{45}Ca^{2+}$, pH 7.2, was introduced into the lumen of the ligated duodenal segment. After half an hour, blood was withdrawn by cardiac puncture, centrifuged and the plasma $^{45}Ca^{2+}$ was measured in a liquid scintillation counter. Absorption was defined as appearance of $^{45}Ca^{2+}$ in blood [15]. Similar experiments were done by injecting only UDCA or vehicle at variable doses for different periods of time.

Spectrophotometric procedures

All enzymes activities were assayed in supernatants of duodenal homogenates. Superoxide dismutase (Mg^{2+}-SOD), EC 1.15.1.1, and catalase (CAT), EC 1.11.1.6, activities were performed in diluted aliquots of the supernatants of intestinal homogenates (1:5). Mg^{2+}-SOD activity was determined in 1 μmol/L EDTA, 50 mmol/L potassium phosphate buffer, pH 7.8, 13 mmol/L methionine, 75 μmol/L nitro blue tetrazolium (NBT) and 40 μmol/L riboflavin [16]. CAT activity was assayed in 50 mmol/L potassium phosphate buffer pH 7.4 and 0.3 mol/L H_2O_2 [17]. Alkaline phosphatase, E.C. 3.1.3.1., was measured in water homogenates (1:10) of intestinal mucosa using *p*-nitrophenyl phosphate as substrate in 0.5 mol/L diethanolamine buffer pH 9.8. This was performed by following an adaptation of the method of Walter and Schütt [18]. Results of enzyme activities are expressed in U/mg of protein. The protein carbonyl content was determined by using 2,4-dinitrophenylhydrazine in a aliquot from homogenates of scraped duodenal mucosa diluted in a isolation buffer (50.3 HEPES, 127 KCl, 1.36 EDTA, 0.5 MgSO4, and 0.183 mmol/L PMSF, pH 7.4) following the procedure of Levine et al. [19]. Total GSH content was also determined in supernatants from intestinal homogenates [20]. The data related to the GSH content and protein carbonyl content are expressed in nmol/mg of protein.

Western blot analysis

Immunoblotting analysis of NCX1, $PMCA_{1b}$, calbindin D_{28k} and VDR was performed using mucosal homogenates from chick duodenum. Suspensions were done in RIPA (radio immuno precipitation assay buffer) lysis buffer (1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate in PBS, containing 1 PMSF and 1 mmol/L NaF), and then centrifuged. Afterwards, proteins (100 μg) were denatured for 5 min. at 95 °C and separated in 12% (w/v) SDS–polyacrylamide minigels for calbindin D_{28k} and VDR and in 8% (w/v) SDS–polyacrylamide minigels for the other proteins [21]. Gels containing the separated proteins were immersed in the transfer buffer (25 Tris–HCl, and 192 mmol/L glycine, 0.05% w/v SDS and 20% v/v methanol) [22]. Nitrocellulose membranes (0.45 μm) were blocked for 1.5 h with 2% w/v nonfat dry milk in 0.5 mol/L Tris-buffered saline solution and incubated overnight at 4 °C with the specific primary antibody at 1:1000 dilution in each case. The antibodies were: anti-NCX 1 monoclonal antibody PPS019 (R&D Systems, Minneapolis, MN, USA), anti $PMCA_{1b}$ (human erythrocyte clone 5F10 A7952 SIGMA Saint Louis, MO, USA), anti CB (anti calbindin D_{28k}, polyclonal antibody produced in rabbit, SIGMA Saint Louis, MO, USA) and anti-VDR monoclonal antibody 9 A7 (Affinity Bioreagents, Golden, CO, USA). After three washings, appropriate secondary biotinylated antibodies were incubated at room temperature for 1 h. Then, the blots were washed three times and streptavidin–biotin conjugate (Histostain-SP Broad Spectrum, Invitrogen CA, USA) was added. Detection was performed using 3,3′-diaminobenzidine (DAB) as a chromogen. Monoclonal antibody anti-GAPDH (clone GAPDH-71.1) from Sigma–Aldrich, St. Louis, MO, USA, was used to detect GAPDH as a marker to normalize the relative expression of the other proteins. The band intensities were quantified using an Image Capturer EC3 Imaging System, Launch

Visionworksls software in order to obtain the relative expression of proteins.

RNA isolation and analysis of $pmca_{1b}$, ncx1, cb and vdr gene expression by qRT-PCR

Total RNA isolation was performed with TRIZOL reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). RNA concentration and purity were determined by spectrophotometry. Quantitative RT-PCR amplification was performed in a thermocycler (Quantitative PCR thermocycler Stratagene Mx 3000P, Agilent Technologies, Inc., Santa Clara, CA, USA). Amplification mixture (total volume: 25 μL) contained 0.5 μg RNA, 0.3 μmol/L each primer, 0.4 μL of the diluted reference dye, 1.0 μL of reverse transcriptase (RT)/RNase block enzyme mixture and 12.5 μL of 2× Brilliant IISYBR Green QRT-PCR master mix (Stratagene, Agilent Technologies, Inc., Santa Clara, CA, USA). The following protocol was used: 1 cycle at 50 °C for 30 min., 1 cycle at 95 °C for 10 min., 40 cycles as follows: denaturation at 95 °C for 30 s, annealing at 60 °C for 60 s and extension at 72 °C for 30 s. The amount of PCR products formed in each cycle was evaluated on the basis of SYBR Green fluorescence. Primer sequences for amplification of chick *ncx1* gene were as follows: forward 5′-GACTGTTTCCAACCTCACAC-3′ and reverse 5′-TAGCAACCTTTC CGTCCATC-3′, which amplified a 490 bp fragment. Primers sequences for chick $pmca_{1b}$ gene were: forward 5′-GTTGTGCAGATAATTATGCTGC-3′ and reverse 5′-GGAA TTTCAA GCGGCTAGTTGG-3′, which amplify a 120 bp long sequence. Primers sequences for chick *cb* gene were: forward 5′-ATGGATGGGAAGGAGCTACAA-3′ and reverse 5′-TGGCACCTAAAGA ACAACAGGAAAT-3′, which amplify a 194 bp long sequence. Primers sequences for chick *vdr* gene were: forward 5′-TGTT TTCCAACCTGGACCTC -3′ and reverse 5′-CTCCATGGTGAAGGAC TGGT-3′, which amplify a 245 bp long sequence. Cycle-to-cycle fluorescence emission readings were monitored and quantified using the $\Delta\Delta Ct$ method [23]. The amount of copy numbers of mRNA from each gene was normalized relative to that of 18S.

Changes in mitochondrial membrane permeability (swelling)

Mitochondria were isolated from intestinal mucosa of control chicks by differential centrifugation, as previously reported [24]. Isolated intestinal mitochondria (3 mg protein) were incubated in 3 mL of respiratory buffer (0.1 NaCl, 10 MOPS, 1 glutamate, and 1 mmol/L malate pH 7.4) for 10 min at 25 °C and monitored at 540 nm in a Beckman Coulter DU 640 spectrophotometer (USA). Basal values of mitochondrial absorbance were measured for 5 min. and the optical density was followed for 5 more min, after addition of 1 NaDOC and/or 0.06 mmol/L UDCA or NaDOC + UDCA [25].

Statistics

Data are expressed as means ± SE. Results were evaluated by one-way analysis of variance (ANOVA) and the Bonferroni's test as a *post hoc* test. Differences were considered statistically significant at $p < 0.05$. All the analyses were carried out by using SPSS software (version 17.0) for Windows XP (SPSS, Inc., Chicago, IL, USA).

Results

UDCA enhanced chick intestinal Ca^{2+} absorption, which was dependent on time and dose

Fig. 2 shows that the chick intestinal Ca^{2+} absorption was affected by UDCA administered in the luminal compartment of chick duodenal sacs. This effect was dose dependent. At doses of 15 or 30 μg UDCA/100 g of b.w., the intestinal Ca^{2+} absorption was not altered, at 45 μg there was a tendency to be increased, but at 60 μg or higher dose the process was significantly enhanced by the BA. The alteration in the cation absorption has been determined after 30 min. treatment. When the time of treatment was shorter, less effect of the BA was detected (data not shown).

UDCA avoided the inhibition of chick intestinal Ca^{2+} absorption caused by NaDOC

The intestinal Ca^{2+} absorption was significantly inhibited by 10 mmol/L NaDOC injected in the chick intestinal lumen, which agreed with previous data [11]. However, this effect was avoided when 60 μg UDCA/100 g of b.w. was concomitantly administered with NaDOC, being the values similar to those from the control chicks. In contrast, UDCA alone enhanced the intestinal Ca^{2+} absorption over the control values (Fig. 3).

NaDOC and UDCA altered protein and gene expression of molecules involved in the transcellular pathway of intestinal Ca^{2+} absorption, but in the opposite way

Protein expression of $PMCA_{1b}$, NCX1 and CB, all proteins involved in the transcellular pathway of intestinal Ca^{2+} absorption, was modified in the opposite way by 10 mmol/L NaDOC and 60 μg UDCA/100 g of b.w. when they were injected in a single

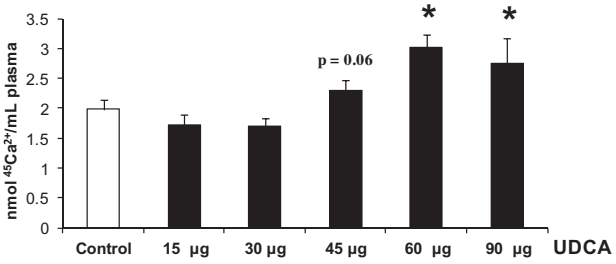

Fig. 2. Effect of different UDCA doses (expressed as μg/100 g of b.w.) on intestinal Ca^{2+} absorption in chicks. After injecting UDCA or vehicle in the duodenal lumen for 30 min. and rinsing the intestinal segment with saline solution, one milliliter of 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, containing 1.85×10^5 Bq $^{45}Ca^{2+}$, pH 7.2, was introduced into the lumen of the ligated intestinal segment for 30 min. Then, blood was withdrawn by cardiac puncture, centrifuged and the plasma $^{45}Ca^{2+}$ was measured. Values represent means ± SE from five chicks for each experimental condition. $^{*}p < 0.05$ vs. control and treated with 15, 30 or 45 μg UDCA; $p = 0.06$ vs. control.

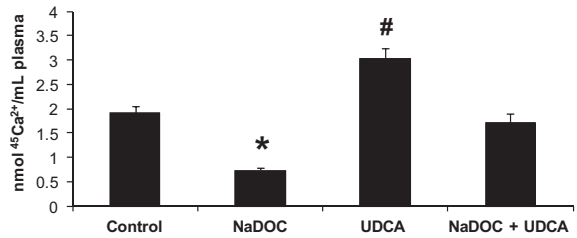

Fig. 3. Effect of different treatments on chick intestinal Ca^{2+} absorption. After injecting 10 mmol/L NaDOC, 60 μg UDCA/100 g of b.w, 10 mmol/L NaDOC + 60 μg UDCA/100 g of b.w or vehicle in the duodenal lumen for 30 min and rinsing the intestinal segment, one milliliter of 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, containing 1.85×10^5 Bq $^{45}Ca^{2+}$, pH 7.2, was introduced into the lumen of the ligated intestinal segment for 30 min. Then, blood was withdrawn by cardiac puncture, centrifuged and the plasma $^{45}Ca^{2+}$ was measured. Values represent means ± SE from five chicks for each experimental condition. $^{*}p < 0.05$ vs. control, UDCA and NaDOC + UDCA. $^{\#}p < 0.05$ vs. control and NaDOC + UDCA.

way. NaDOC diminished the protein expression of $PMCA_{1b}$, NCX1 and CB, whereas UDCA increased the protein expression of all of them. However, the protein expressions of these three molecules were identical to those from the control group when the combined treatment was employed (Fig. 4). With regards to the gene expression of these proteins after the same treatments, the data indicate that the gene expressions of *$pmca_{1b,}$ ncx1* and *cb* were increased about 3-fold in mucosa from UDCA or UDCA + NaDOC treated chicks as compared to those from the control group. By contrast, NaDOC decreased significantly the gene expressions of *$pmca_{1b}$* and *cb* without altering *ncx1* gene expression (Fig. 5).

UDCA and UDCA plus NaDOC increased VDR protein and gene expression

As can be seen in Fig. 4, VDR protein expression was not modified by NaDOC. However, UDCA increased twofold the VDR protein expression, which was slightly lower when UDCA was co-administered with NaDOC. With regards to VDR gene expression, UDCA and UDCA *plus* NaDOC produced more than twofold increase, while NaDOC alone did not cause any effect (Fig. 5).

UDCA avoided the inhibition of the intestinal alkaline phosphatase activity provoked by NaDOC in chick intestine

The activity of intestinal alkaline phosphatase from homogenates of mucosa was decreased by NaDOC injection into the chick duodenal lumen. By itself, UDCA did not modify the enzymatic activity. However, the co-administration of NaDOC and UDCA avoided the inhibitory effect of NaDOC on the enzyme activity (Fig. 6).

Changes in mitochondrial membrane permeability (swelling) caused by NaDOC were ameliorated by the simultaneous treatment with UDCA

Changes in the absorbance of mitochondrial suspensions at 540 nm were used to monitor mitochondrial swelling due to changes in the mitochondrial permeability. Fresh isolated mitochondria of duodenal mucosa from control chicks were exposed to NaDOC (1 mmol/L), UDCA (0.06 mmol/L) or to both drugs (1 mmol/L NaDOC *plus* 0.06 mmol/L UDCA) at the same time (Fig. 7). A decrease in the absorbance was observed after NaDOC addition to the mitochondrial incubation, showing this effect dose dependency (data not shown). UDCA by itself did not modify the absorbance of the mitochondrial suspensions. However, the co-treatment avoided partially the intense alteration in the absorbance caused by NaDOC (Fig. 7).

UDCA did not alter the intestinal redox state but aborted the oxidative stress produced by NaDOC in chick intestine

As depicted in Table 1, NaDOC decreased the intestinal GSH content and increased protein carbonyl content and the SOD

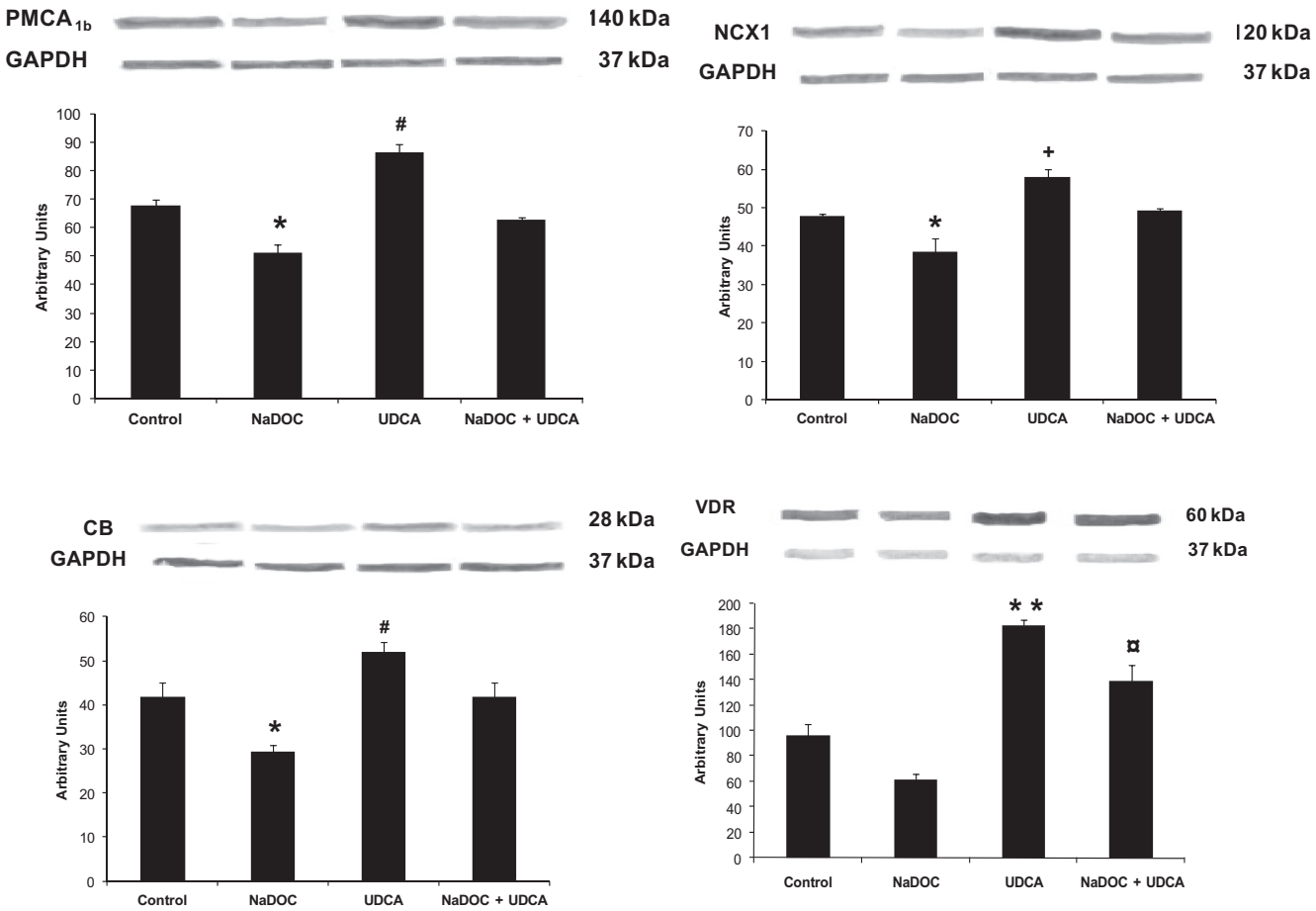

Fig. 4. Effect of 10 mmol/L NaDOC, 60 μg UDCA/100 g of b.w, 10 mM NaDOC + 60 μg UDCA/100 g of b.w on $PMCA_{1b}$, NCX1, CB and VDR expressions was analyzed after 30 min by Western blot in pool of mucosa from three chick duodenae for each experimental condition. One hundred micrograms of duodenal mucosa homogenate suspension was separated by SDS–PAGE and immunoblotted with the corresponding antibodies followed by detection with DAB as a chromogen. Three independent experiments were accomplished. Values are expressed as means ± S.E. $^{*}p < 0.05$ vs. control, UDCA and NaDOC + UDCA; $^{\#}p < 0.05$ vs. control and NaDOC + UDCA; $^{+}p < 0.05$ vs. control; $^{**}p < 0.01$ vs. control, NaDOC and NaDOC + UDCA; $^{¤}p < 0.01$ vs. control and NaDOC.

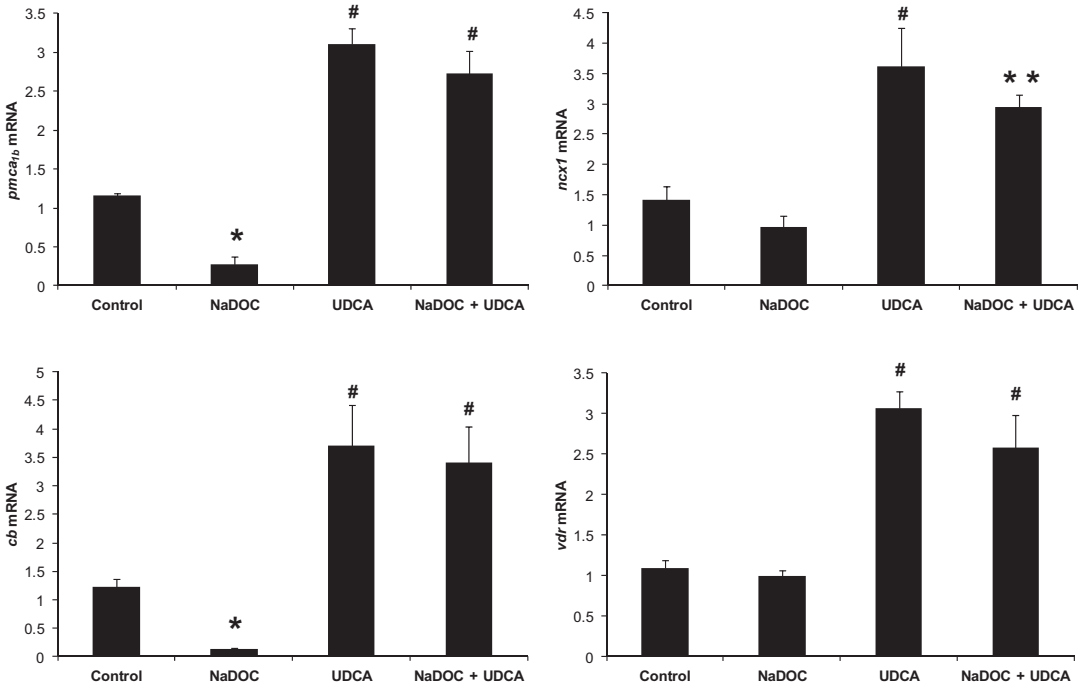

Fig. 5. Real time quantitative PCR analysis of chick duodenae mRNA expression levels of *pmca*$_{1b}$, *ncx1*, *cb* and *vdr* after 30 min of treatment. Gene expression levels represent the relative mRNA expression compared with the 18S mRNA. Values represent means ± SE from 10 chicks for each experimental condition.$^{*}p < 0.05$ vs. control; $^{\#}p < 0.01$ vs. control and NaDOC; $^{**}p < 0.05$ vs. control and NaDOC.

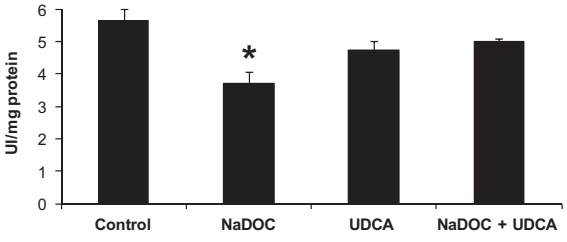

Fig. 6. Effect of 10 mmol/L NaDOC, 60 μg UDCA/100 g of b.w, 10 mmol/L NaDOC + 60 μg UDCA/100 g of b.w or vehicle in the duodenal sac on alkaline phosphatase activity after 30 min. treatment. Alkaline phosphatase was measured in water homogenates (1:10) of intestinal mucosa using *p*-nitrophenyl phosphate as substrate in 0.5 M diethanolamine buffer pH 9.8. Values represent means ± SE from four chicks for each experimental condition. $^{*}p < 0.05$ vs. control, UDCA and NaDOC + UDCA.

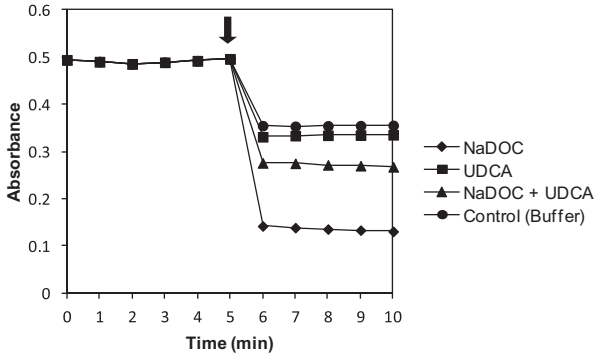

Fig. 7. Optical density changes (swelling) in isolated mitochondria from chick enterocytes. Basal values of mitochondrial absorbance were measured for 5 min and optical density was followed for five more min after addition of 1 mmol/L NaDOC, 0.06 mmol/L UDCA and 1 mmol/L NaDOC + 0.06 mmol/L UDCA. Arrow: treatment addition. The graph represents one of three separated experiments.

activity without altering the CAT activity. The values of these variables under UDCA administration were not different to those from the control group. Nevertheless, UDCA blocked the alterations caused by NaDOC returning the values almost to those from the control ones.

UDCA also enhanced the intestinal Ca^{2+} absorption in rats

Similarly to the data obtained in chicks, 10 mmol/L NaDOC diminished the intestinal Ca^{2+} absorption in rats, which was also avoided by simultaneous treatment with 60 μg UDCA/100 g of b.w. UDCA alone increased the intestinal Ca^{2+} absorption in rats (Fig. 8).

Discussion

The present study clearly demonstrates that UDCA administration into the luminal compartment of chick duodenal sacs causes enhancement of intestinal Ca^{2+} absorption. The underlying mechanism seems to be the stimulation of the transcellular pathway of intestinal Ca^{2+} absorption, as judged by the increased expression of genes and proteins involved in this way. The effect occurs at doses below the pharmacological optimum dosage to treat the PBC (10–15 mg/kg/day) [26]. The final concentration of UDCA added into the chick or rat duodenal lumen was around 610 μmol/L, which is a concentration much lower than that of the bile gallbladder (5 mmol/L), but much higher than that of the fecal water (13 μmol/L) [3,27]. The enhancement of intestinal Ca^{2+} absorption by UDCA is an agreement with a membrane "fluidification" due to a decrease in the cholesterol/phospholipid ratio and an increase in the unsaturation index of total lipid fatty acids, as previously described in rat intestine [28].

This study shows for the first time that UDCA enhances the intestinal Ca^{2+} absorption in healthy animals. The response is not only triggered in avian, but also in mammals. In humans with PBC, pharmacological doses of UDCA have been shown to increase

Table 1
Effect of 10 mmol/L NaDOC, 60 μg UDCA/100 g of b.w, 10 mmol/L NaDOC + 60 μg UDCA/100 g of b.w or vehicle in the duodenal sac at 30 min. on GSH and protein carbonyl content and CAT and SOD activities.

Groups	GSH (nmol/mg protein)	PROTEIN CARBONYL (nmol/mg protein)	CAT (CAT U/mg protein)	SOD (SOD U/mg protein)
Control	4.38 ± 0.44	0.89 ± 0.18	20.33 ± 1.81	5.80 ± 0.55
NaDOC	2.92 ± 0.25*	2.22 ± 0.53*	17.72 ± 1.35	8.98 ± 2.16*
UDCA	3.88 ± 0.25	0.78 ± 0.18	18.76 ± 1.57	4.54 ± 1.68
NaDOC + UDCA	3.77 ± 0.11	0.63 ± 0.13	17.09 ± 1.60	5.08 ± 1.48

Values represent means ± SE from seven chicks for each experimental condition
* $p < 0.05$ vs. control, UDCA and NaDOC + UDCA.

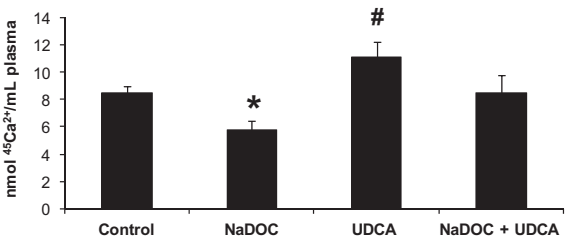

Fig. 8. Effect of different treatments on intestinal Ca^{2+} absorption in rats. After injecting 10 mmol/L NaDOC, 60 μg UDCA/100 g of b.w, 10 mmol/L NaDOC + 60 μg UDCA/100 g of b.w or vehicle in the duodenal lumen for 30 min. and rinsing the intestinal segment, one milliliter of 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, containing 1.85×10^5 Bq $^{45}Ca^{2+}$, pH 7.2, was introduced into the lumen of the ligated intestinal segment for 10 min. Then, blood was withdrawn by cardiac puncture, centrifuged and the plasma $^{45}Ca^{2+}$ was measured. Values represent means ± SE from 5 rats for each experimental condition. *$p < 0.05$ vs. control, UDCA and NaDOC + UDCA. #$p < 0.05$ vs. control and NaDOC + UDCA.

the intestinal cation absorption [12]. As known, vitamin D is the main stimulator of the intestinal Ca^{2+} absorption through the involvement of its nuclear receptor VDR. For many years, it was thought that endogenous ligands for VDR were only vitamin D compounds such as calcitriol. Makishima et al. [29] demonstrated that the bile acid LCA and its derivatives could activate human and mouse VDRs. LCA also activates chicken VDR, but weakly [30]. The enhancement of VDR protein and gene expression by UDCA suggests that VDR is also involved in the intestinal Ca^{2+} absorption stimulated by UDCA. The relationship between UDCA and VDR has been previously observed. UDCA has been shown to increase cathelicidin expression in biliary epithelial cells from human liver through VDR activation, effect that was blunted by siRNA strategy [31].

The intestinal Ca^{2+} absorption occurs by two mechanisms: paracellular (passive) and transcellular (active) pathways. Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger are the two proteins involved in the Ca^{2+} exit from enterocytes when the cation movement occurs by the transcellular pathway. UDCA administration causes a small increase in the expression of both proteins, and a high increment of steady state mRNA levels from *pmca*$_{1b}$ and *ncx1* genes. In addition, the gene and protein expressions of CB, a protein presumably involved in the Ca^{2+} transport from the BBM to the BLM from the enterocytes, are also increased. Probably, UDCA stimulates the gene transcription of proteins involved in the transcellular Ca^{2+} pathway, although a downregulation of their mRNAs degradation cannot be excluded. The higher expression of $PMCA_{1b}$, NCX1 and CB proteins after the UDCA treatment would indicate that the translation of these proteins is also enhanced. Another possibility would be that UDCA causes a decrease in protein degradation. The ability of UDCA to affect less the protein expression than the gene expression of $PMCA_{1b}$, NCX1 and CB could be only a matter of timing. Contrarily to our expectations, UDCA alone does not alter the activity of intestinal alkaline phosphatase. This enzyme, located in the intestinal BBM, has been proposed to have a role in the intestinal Ca^{2+} absorption modulated by vitamin D_3, but its participation has not been always demonstrated [32–34].

When UDCA is administered in combination with NaDOC, the intestinal Ca^{2+} absorption is similar to that from the control chicks, which proves that UDCA prevents the inhibitory effect of NaDOC on the intestinal Ca^{2+} absorption [11]. The combination of both BAs abrogates the enhancement of intestinal Ca^{2+} absorption caused by UDCA, either in chicks or in rats.

GSH is a tripeptide essential to have an adequate intestinal Ca^{2+} absorption [15]. We have demonstrated the inhibition of intestinal Ca^{2+} absorption in chick duodenum by using GSH depleting drugs such as DL-buthionine- S,R-sulfoximine, menadione and NaDOC [15,34–36,11]. UDCA appears not to modify the chick intestinal GSH levels, but blocks the GSH depletion produced by NaDOC. Similarly, UDCA alone does not change the protein carbonyl content, but avoids the increase caused by NaDOC. Both GSH depletion and enhancement of protein carbonyls produced by NaDOC are expressions of oxidative stress, which confirm previous findings of this laboratory [11]. Probably, as a compensatory mechanism, NaDOC increases the SOD activity, which is arrested by simultaneous treatment with UDCA. SOD and CAT activities are not modified by UDCA itself. The present data clearly indicate that UDCA blocks the oxidative stress caused by NaDOC in chick intestine, which could explain at least partially the absence of inhibitory effect of NaDOC on the intestinal Ca^{2+} absorption when both BAs are administered. It is interesting to note that the protective mechanism triggered by UDCA seems to be switched on under oxidative stress conditions, leading the cells to the normalization of redox status, and consequently, to the normal intestinal Ca^{2+} absorption.

To conclude, UDCA is a beneficial BA for intestinal Ca^{2+} absorption either in chicks or rats. By contrast, NaDOC causes an inhibitory effect on intestinal cation absorption through triggering oxidative stress. The combined treatment neutralizes both responses, probably because UDCA protects the intestine against the GSH depletion and protein carbonyl increment produced by NaDOC. The stimulatory effect of UDCA alone on intestinal Ca^{2+} absorption seems to be mediated by VDR leading to enhancement of protein and gene expressions of molecules involved in the transcellular pathway of intestinal Ca^{2+} absorption. The use of UDCA in patients with PBC or other liver disorders with cholestasis would be benefited because of the protective effect on the intestinal Ca^{2+} absorption, avoiding the inhibitory effect caused by hydrophobic BAs such as NaDOC and neutralizing the oxidative stress.

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