REVIEW

Molecular mechanisms triggered by bile acids on intestinal Ca^{2+} absorption

Ana Marchionatti, María Rivoira, Valeria Rodríguez, Adriana Pérez and Nori Tolosa de Talamoni

Laboratorio "Dr. Fernando Cañas", Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, INICSA (CONICET-Universidad Nacional de Córdoba), Pabellón Argentina, 2do. Piso, Ciudad Universitaria, 5000 Córdoba, Argentina

*Send correspondence to:

Prof. Dr. Nori Tolosa de Talamoni

Cátedra de Bioquímica y Biología Molecular

Facultad de Ciencias Médicas

INICSA (CONICET-Universidad Nacional de Córdoba)

Pabellón Argentina, 2do. Piso

Ciudad Universitaria

5000 Córdoba

Argentina

Phone number: +54-351-4333024

Fax Number: +54-351-4333072

E-mail: ntolosatalamoni@yahoo.com.ar

ABSTRACT

Bile acids (BAs) are synthesized in the liver and are among the main components of bile. For many years, it was thought that they have only a role as emulsifiers of fat in the small intestine facilitating the absorption of lipids and lipid soluble vitamins. Lately, they are also considered important signaling molecules, not only by regulating their own synthesis, but also having a role in several metabolic diseases. In this review we focus on the effect of deoxycholic, ursodeoxycholic and litocholic acids and their combination upon the intestinal Ca^{2+} absorption. To make the actions of those BAs clear on this physiological process, an overview of current information about the mechanisms by which the intestinal Ca^{2+} occurs is described. Finally, detailed statements related to the modulation of redox state, apoptosis and autophagy as molecular mechanisms involved in the action of BAs on intestinal Ca^{2+} absorption are discussed. Although the mechanisms are still not completely understood, we provide the latest knowledge regarding the effect of BAs on intestinal Ca^{2+} absorption and discuss the possible therapeutic applications that might evolve as a result.

Key Words: bile acids, intestinal Ca^{2+} absorption, NaDOC, UDCA, LCA, oxidative stress, autophagy, nitrosative stress, apoptosis

INTRODUCTION

Bile acids (BAs) are synthesized in the liver and are among the main components of bile [1]. It is well known that during digestion bile and BAs neutralize the chyme and serve as emulsifiers of fat in the small intestine, which facilitate the absorption of lipids and lipid soluble vitamins [2]. For many years it was thought that they have only that function in the human body. Lately, they are also considered important signaling molecules, not only by regulating their own synthesis, but also having a role in diseases such as metabolic syndrome, obesity, and diabetes [3]. BAs derive from cholesterol and share certain common features: 1) the steroid nucleus formed by six-membered rings (A, B and C) and a five-membered ring (D), 2) a short side chain with a terminal carboxylic group branched to ring D. The molecular diversity in the BA pool is due to the presence of one or more hydroxyl or keto groups on the steroid core, mainly on the carbons forming rings A and B [4]. Sometimes, subtle changes in the configuration provoke marked dissimilarity with regard to their physicochemical and biological properties. Ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) only differ in the configuration of the hydroxyl group at C-7 (β in UDCA and α in CDCA), but UDCA is a hepatocyte protector while CDCA is highly toxic [5,6]. The list of functions of BAs will most likely continue to grow because of a variety of different BA receptors, and a wide distribution of those receptors in the body [7].

There are two main synthetic pathways for BA formation, which are called the "classical" and the "alternative" pathways. The first pathway is also called "neutral" because the intermediate metabolites are neutral sterols; the alternative pathway forms acidic intermediate metabolites. Both ways lead to production of cholic acid (CA) and

CDCA, which are named primary BAs. Part of these acids are biotransformed by the intestinal microflora to secondary BAs, deoxycholic (DCA) and litocholic acids (LCA), which originate from CA and CDCA, respectively [8]. The primary BAs are the most potent endogenous ligands for the nuclear receptor farnesoid X receptor (FXR), while the secondary BAs mainly activate the plasma membrane receptor TGR5 [9]. UDCA is a minor BA, formed in the human colon by bacterial epimerization of CDCA through 7-oxo-LCA [10]. Besides, the 7-oxo-LCA can be reabsorbed in the distal intestine and transported back to the liver, where it is transformed into CDCA and to UDCA, in less extension [11].

High concentrations of hydrophobic BAs such as DCA have been associated with various liver diseases and colon cancer [12]. In contrast, UDCA is a hydrophilic BA, which increases bile flow, changes the hydrophobicity of the BA pool and modulates the immune response [13]. It is widely used as a therapeutic drug for patients with cholestatic liver diseases or with gallstones. UDCA is the only drug approved to treat patients with primary biliary cirrhosis (PBC), but there is controversy with regard to its efficacy for treating patients with primary sclerosing cholangitis [14].

At intestinal level, the BAs have different responses, which depend on the type and dosage of each BA, the number and class of receptors and other unknown factors. The sodium salt of DCA (NaDOC) was shown to perturb membrane structures by alteration of membrane microdomains [15]. Miyaki et al. [16] have demonstrated that both CDCA and NaDOC increase the release of prostaglandin E2 from colon cancer cells by stimulating synthesis and downregulating catabolism. The transepithelial electrical resistance in the Caco-2 cell line has been found to be decreased by NaDOC through ROS generation and other signaling mechanisms [17]. Apoptosis in Caco-2 cells as in other human colon cancer

cell lines were also induced by NaDOC [18]. Alrefai et al [19] have observed inhibition of human intestinal apical Cl^-/OH^- exchange activity in Caco-2 cells by taurodeoxycholate and glycochenodeoxycholate *via* Ca^{2+}, PI3 kinase-, and PKC beta I-dependent pathways. Ward et al [20] have recently found that UDCA exerts anti-inflammatory actions in the colon. The authors have demonstrated that LCA, derived from UDCA in the colon, was more effective than UDCA in preventing dextran sulfate sodium-induced colitis, which suggests that LCA may be an important mediator of UDCA effects.

In the following topics we will discussed the molecular mechanisms of intestinal Ca^{2+} absorption and how these mechanisms are affected by BAs as well as their underlying molecular events.

MOLECULAR MECHANISMS OF INTESTINAL CALCIUM ABSORPTION

The intestinal Ca^{2+} absorption is an ATP dependent process that occurs mainly in the small intestine [21]. Since the Ca^{2+} solubility is an important factor, the duodenum seems to be the site with maximum solubility because the average pH is 6.0, which is the lowest of the entire intestine [22].The sojourn time of Ca^{2+} in each intestinal segment is another factor affecting the intestinal Ca^{2+} absorption. The chime remains in the jejunum, and especially in the ileum, for periods of about 4 h, which causes important amounts of Ca^{2+} to be absorbed [23]. The order of Ca^{2+} absorption rate occurs in the following way: duodenum > jejunum> ileum [24]. Colonic Ca^{2+} absorption might be important in pathological conditions such as in short bowel syndrome [25]. Although Ca^{2+} solubility is low in the ileum, the total amount of Ca^{2+} absorption is greater in the ileum because the residence time in this segment is 10 times longer than in the more proximal intestinal segments [26, 27]. The integrity and functionality of the intestinal mitochondria are important to produce an

optimal intestinal Ca^{2+} absorption since they are important sources of ATP [28]. Vitamin D is a major responsible for intestinal Ca^{2+} absorption by activating the vitamin D receptor (VDR), which is highly expressed in the small intestine and colon [29]. VDR levels are remarkably higher in intestinal epithelial cells than in other cells [30]. Calcitriol or $1,25(OH)_2D_3$, the active metabolite derived from vitamin D with hormonal action, is one of the main regulators of the human genome controlling the transcription rate of hundreds of genes in a tissue-specific fashion. It acts as a transcription factor within the cell nucleus and as an inducer of non-genomic signaling in the cytosol [29]. Other hormones and dietary factors also influence the intestinal Ca^{2+} absorption [31-34].

The efficiency of intestinal Ca^{2+} absorption depends on the physiological needs of Ca. It increases under conditions such as growth, pregnancy, lactation, whereas aging decreases cation absorption [35-37]. Also, when the Ca^{2+} intake is low, the efficiency of intestinal Ca^{2+} absorption increases [38].

The accepted main mechanisms of intestinal Ca^{2+} absorption are the transcellular and the paracellular pathways. Both mechanisms are regulated by hormones, nutrients and other factors. The transcellular pathway comprises the Ca^{2+} entry across the brush border membrane (BBM) of the enterocytes or intestinal epithelial cells, the intracellular diffusion from one pole to the other of the enterocytes and the Ca^{2+} exit through the basolateral membrane (BLM). In the BBM there are Ca^{2+} epithelial channels, called transient receptor potential vanilloid-family member 6 (TRPV6) and transient receptor potential vanilloid-family member 5 (TRPV5), which would be involved in the Ca^{2+} uptake from the lumen to inside the cell across the BBM [39]. TRPV6 predominates in the intestine, whereas TRPV5 in the kidney. $Ca_v1.3$ is another channel from the BBM, which would play a dominant role

under depolarizing conditions during digestion, mainly when diet is plentiful in Ca^{2+} [40]. However, some authors consider that $Ca_v1.3$ is not critical for active intestinal Ca^{2+} absorption *in vivo* in mice [41]. Calbindin D_{9k} in mammals and calbindin D_{28k} in birds are cytoplasmic proteins carrying Ca^{2+} as ferries from the BBM to the BLM [42]. Calbindins also buffer Ca^{2+}, which allows the maintenance of intracellular Ca^{2+} concentrations below 10^{-7} M preventing cell death by apoptosis [43, 44]. Whether calbindin acts as Ca^{2+} ferry in the intestine is a controversial issue, but not its role as a Ca^{2+} buffer. In calbindin D_{9k} null mice, neither basal nor calcitriol-induced intestinal calcium absorption is reduced [45, 46]. However, VDR-independent upregulation of intestinal calbindin D_{9k} in TRPV6 transgenic VDR KO mice suggests that this protein may buffer intracellular Ca^{2+} during intestinal Ca^{2+} absorption [47]. For the final step, Ca^{2+} extrusion from the cell through the BLM is an energy dependent process, which is mainly mediated by the plasma membrane calcium ATPase 1b ($PMCA_{1b}$) and, in less extension, by the Na^+/Ca^{2+}-exchanger 1 (NCX1). Vitamin D deficiency reduces the $PMCA_{1b}$ expression whereas vitamin D repletion or low Ca diets increase its protein expression [48, 49]. Another novel protein 4.1R, which co-localizes with $PMCA_{1b}$, could have an important role in the transcellular Ca^{2+}pathway, but its physiological function needs to be clarified [50]. Deletion of $PMCA_{1b}$ or 4.1R diminishes both basal and calcitriol-induced intestinal Ca^{2+} absorption [50, 51]. The $PMCA_{1b}$ expression and activity are higher in villus tip enterocytes than in villus crypt cells, which allows mature enterocytes to have the greatest ability for transcellular Ca^{2+} movement [52]. NCX1 is the predominant isoform in the intestine, which can function in either a forward mode (Ca^{2+} extrusion) or in a reversed mode (Ca^{2+} entry), depending on the Na^+ and Ca^{2+} gradients and the membrane potential [53]. In our laboratory, we have

demonstrated that calcitriol increases the intestinal NCX1 activity in vitamin D-deficient chicks through genomic and non-genomic mechanisms. The genomic mechanism would occur via nuclear VDR and increased transcription of *NCX1* gene and protein synthesis. Non genomic effects are mediated by PKA and PKC activation. The physiological role of the rapid effects and the interrelationships between both molecular mechanisms remain unknown [54].

The paracellular Ca^{2+} pathway occurs through the tight junctions (TJ), which are intercellular structures formed by the apical and lateral plasma membranes of adjacent enterocytes building charge and size selective pores [55]. It is a non-saturable process, which depends on the concentration and the electric gradient across the epithelium. Claudin-2 and Cldn-12 seem to be responsible, at least in part, for transporting Ca^{2+} through this pathway [56]. This route is switched on in conditions of high Ca^{2+} intake due to a short residence time in the gut and a down-regulation of proteins involved in the transcellular pathway [57]. There is evidence that the expression of paracellular TJ genes is regulated by the transcellular calbindin protein, which indicates that active and passive Ca^{2+} transport pathways may work cooperatively [58].

PHYSIOLOGICAL ROLE OF BILE ACID ON INTESTINAL CA^{2+} ABSORPTION

More than a century ago, scientists believed that the bile was involved in the intestinal Ca^{2+} absorption. Some of them pointed out that the bile could enhance the Ca^{2+} transport only as a result of an effect of vitamin D on the cation absorption [59]. Webling and Holdsworth [60] reported that BAs and detergents enhance Ca^{2+} absorption in ileum of normal and vitamin D-deficient chicks. However, Taylor et al. [61] did not find anti-

rachitic effects of ox bile by feeding it for several weeks to fowls on a rachitogenic diet. Limlomwongse et al. [62] have demonstrated that bile and Na taurocholate enhanced the Ca^{2+} transport in rats. Nevertheless, they could not find changes in net absorption due to concurrent increase in the Ca^{2+} efflux. Buts et al. [63] have observed in cholestatic rats that oral administration of tauroconjugated BAs significantly decreased Ca^{2+} transport in the proximal portion of the intestine. These authors consider that the inhibitory effect could be due to the formation of complexes of BAs with free Ca^{2+}. Walshe et al. [64] have demonstrated that deoxycholate in rat intestine reduced the Ca^{2+} uptake and produced extensive microvillous and intracellular damage to the enterocytes. Sanyal et al. [65] have demonstrated that premicellar taurocholate enhanced Ca^{2+} uptake from all regions of rat small intestine, but they did not find out about the mechanisms involved. As noticed, the data are controversial, but probably differences in the bile composition from different species, variability in the components of the diet, variations in the exposure times and ways of administration, solubility of BAs and other factors could explain the results. It has been shown that the effects of BAs are not equivalent in terms of bioactivity and the conjugation of BAs changes their properties, absorption and responses [66]. Therefore, it is quite possible that each BA has its own effect on the intestinal Ca^{2+} absorption and the total bulk of BAs from the bile produces a response depending on the proportions of different BAs. Based on these considerations we have analyzed either in mammals or in birds the influence of NADOC, UDCA, LCA and their combinations on the absorption of the cation at the duodenal level, the site with maximum Ca^{2+} transport rate.

We have found that NaDOC inhibited intestinal Ca^{2+} absorption, effect that was rapid (15 min) and dependent on the concentration. The response occurred through

downregulation of Ca^{2+}-ATPase mRNA and the protein expression of Ca^{2+}-ATPase, calbindin D_{28k} and Na^{+}/Ca^{2+} exchanger, all molecules involved in the transcellular Ca^{2+} pathway [67]. These findings not only confirmed previous data obtained by Walshe et al. [64], but also detected which Ca^{2+} pathway was altered by the salt bile. The experimental model did not allow us to study whether this effect continued with the time or not, which would be important to know in terms of deciphering if unfavorable changes in bone health could be occurring.

With regard to UDCA, Verma et al. [68] have reported that PBC patients display low spinal and femoral neck BMD and reduced fractional Ca^{2+} absorption, being the latter effect partially corrected by using this BA as a therapy. However, they did not find out about the molecular mechanisms involved in this response. In addition, the possibility that UDCA could improve the intestinal Ca^{2+} absorption under physiological conditions was not investigated either. In our laboratory, we have found that UDCA enhanced the intestinal Ca^{2+} absorption in healthy rats or chicks. UDCA produced enhancement of VDR protein and gene expression, which suggests that VDR is involved in the intestinal Ca^{2+} absorption stimulated by UDCA. In biliary epithelial cells from human liver, UDCA increased cathelicidin expression *via* VDR activation [69], which also shows the relationship between UDCA and VDR. It is known that the BAs have several different receptors to produce a diversity of responses [70]. The farnesoid X receptor and the G protein-coupled bile acid receptor 1 seem to be essential to modulate the expression and activity of genes involved in the maintenance of intestinal integrity [71]. If they are involved in the responses to NaDOC and/or UDCA for modulating the intestinal Ca^{2+} absorption needs to be determined.

The transcellular Ca^{2+} pathway is affected by UDCA, but in the opposite way to that triggered by NaDOC, which indicates that UDCA is good and NaDOC is bad for the intestinal Ca^{2+} absorption [72]. UDCA causes a small increase in the protein expression of calbindin D_{28k}, Ca^{2+}-ATPase, and Na^{+}/Ca^{2+} exchanger, and a striking enhancement in the mRNA from *cb*, *pmca*$_{1b}$ and *ncx1* genes. These data suggest that UDCA stimulates the gene transcription and the translation of proteins involved in the transcellular Ca^{2+} pathway. The interesting point is that the combined treatment of NaDOC and UDCA does not cause any effect on the intestinal Ca^{2+} absorption, which indicates that UDCA prevents the inhibitory effect of NaDOC on the intestinal Ca^{2+} absorption either in rats or in chicks [72]. We have recently reported that the inhibitory and stimulatory actions of NaDOC and UDCA on intestinal cation absorption occur by decreasing or increasing the Ca^{2+} uptake only by mature enterocytes, respectively [73].Therefore, it is quite possible that certain degree of differentiation in the enterocytes is necessary to respond to these BAs, which could be related to the number of appropriate receptors and/or metabolic machinery involved in the response.

Makishima et al. [74] have demonstrated that LCA is another ligand of VDR, but the affinity of LCA is weaker than that of calcitriol [75], and the orientation of LCA in the ligand-binding pocket is opposed to that of $1,25(OH)_2D_3$ [76]. Furthermore, LCA does not alter the intestinal Ca^{2+} absorption, but it protects the intestinal Ca^{2+} absorption in the presence of NaDOC. By itself, LCA does not change the gene and protein expression of molecules presumably involved in the transcellular pathway, which is in concordance with the lack of effect of LCA on the intestinal Ca^{2+} absorption. Nevertheless, the inhibitory effects of NaDOC on the protein expression of $PMCA_{1b}$, NCX1 and CB D_{28K} are avoided

by simultaneous treatment with LCA [77]. In vitamin D deficient mice, Nehring et al. [78] have found that LCA enhanced the gene expression of TRPV6, calbindin D_{9k} and Ca^{2+}-ATPase. These authors claim that LCA can act as a vitamin D substitute only in vitamin D deficient states because LCA does not compete with any VDR ligand. In addition, LCA may induce a VDR conformation distinct from $1,25(OH)_2D_3$ exhibiting selective physiological functions [79].

As known, in intestine LCA also binds modestly to farnesoid X receptor (FXR), which is known to induce genes involved in the enteroprotection [80]. Besides, it is well known that BAs also produce non receptor mediated effects [81]. In other words, the participation of putative receptors of LCA involved in the protection of the intestinal Ca^{2+} absorption in the presence of hydrophobic BAs needs to be clarified.

MOLECULAR MECHANISMS BY WHICH THE BILE ACID ALTER THE INTESTINAL CA^{2+} ABSORPTION

It seems that multipathways are involved in the variety of responses of intestine to absorb Ca^{2+}under the effect of single or combined BAs. NaDOC inhibits the intestinal Ca^{2+} absorption affecting molecules of the transcellular Ca^{2+} pathways as a consequence of oxidative/nitrosative stress, which leads to apoptosis and autophagy. The enhancement of oxidative stress has been confirmed by an increase in ROS production and in protein carbonyl groups, a decrease in the GSH content and alterations in the permeability (swelling) [67]. In addition, the restoration of intestinal Ca^{2+} absorption to control values by an antioxidant such as quercetin confirms that the inhibition of that physiological process caused by NaDOC is mediated by oxidative stress indeed [67]. The role of NaDOC as an

inducer of oxidative stress in colon has been largely reported [82, 83]. The increment in the NO content and in the iNOS protein expression in intestine of NaDOC-treated rats reveals that the nitrergic system is also involved in the response of intestinal Ca^{2+} absorption. The release of NO by BAs has been previously observed in enteric neurons [84], and in human colon exposed to intraluminal irritation with DCA [85]. As a result of oxidative/nitrosative stress, NaDOC leads to exacerbate the process of apoptosis, which was revealed by an increase in DNA fragmentation and in cytochrome c loss from the mitochondria [67]. Cytochrome c forms part of the electron transport chain and is indirectly involved in ATP production [86]. Therefore, the cytochrome c loss from the mitochondria would not only cause apoptosis activation but also ATP depletion, which would contribute to explaining the deterioration of the intestinal Ca^{2+} absorption since this is an active process. Due to certain evidence that BAs alter the electron transport chain [87, 88], it is quite possible that NaDOC partially inhibits the electron transport chain and the remnant functioning of the pathway could be employed to cause apoptosis, which is also an ATP-driven process. NaDOC not only triggers the mitochondrial apoptotic *via*, as revealed by the cytochrome c release from the mitochondria, but also the extrinsic apoptotic pathway as judged by an increase in the protein expression of FAS, FASL, caspase-8 and caspase-3 activity and a decrease in the procaspase-8 protein expression. Although the intestine tries to compensate the damage caused by NaDOC through enhancement in the activity of antioxidant enzymes such as SOD, CAT and GPx, their catalytic actions are not enough and there is an impaired oxidant/antioxidant balance, which induces the cascade of apoptotic events continuing until DNA fragmentation.

Autophagy is an important mediator of pathological responses and interacts by cross-talk with ROS and RNS in both cell signaling and protein damage [89]. It consists of degradation and recycling of organelles and other cellular components in the cytoplasm [90], which are sequestered into double-membrane structures called autophagosomes [91]. Autophagy is another mechanism involved in the NaDOC action on the intestine, as suggested by an increase in the LC3 II protein and in the number of acidic vesicular organelles [73]. LC3 is a mammalian homologue of yeast Apg8p that localizes in the autophagosome membranes [92], and is considered a marker of autophagosomes [93]. It has been found that LC3 II is a molecule involved in the autophagy caused by traditional Chinese medicine [94], ethanol [95], curcumin [96], serum inhibited gene [97] and other drugs and conditions. When autophagy is inducted, LC3 is conjugated to phosphatidylethanolamine and targeted to autophagic membranes. Hence, changes in LC3 localization have been used to measure autophagy [98].Although autophagy might be a double-edged sword, because it can promote survival or cell death of stressed cells, in those NaDOC-treated animals, the triggering of autophagy did not ameliorate the effects of the NaDOC on nitrosative and oxidative stress. Furthermore, it is quite possible that the exacerbation of NaDOC-induced autophagy leads to cell death.

As mentioned above, UDCA is a beneficial BA for intestinal Ca^{2+} absorption. The combined therapy of NaDOC and UDCA does not modify the intestinal Ca^{2+} absorption, which proves that UDCA prevents the inhibitory effect of NaDOC [67]. This combination abrogates the enhancement of intestinal Ca^{2+} absorption caused by UDCA [72]. The stimulatory effect of UDCA is in part due to a decrease in the protein expression of molecules involved in the extrinsic apoptotic pathway such as FAS and FASL, which

suggests that the number of cells that normally die in the intestine is reduced by UDCA leading to a higher number of enterocytes with absorptive capacity.

The anti-apoptotic effects of UDCA were previously observed in hepatocytes, mainly due to modulating the mitochondrial transmembrane potential, the ROS production [99], the stabilization of p53, the degradation of NF-kB and the expression of BCL-2 family members [100]. In the duodenum, UDCA alone does not alter the GSH content, the protein carbonyl or the SOD and CAT activities. Similarly, the nitrergic system remains unaltered after UDCA treatment. In contrast, UDCA abrogates changes in the oxidative/nitrosative system caused by NaDOC as well as the apoptotic events triggered by this BA. Therefore, UDCA treatment in patients with PBC or other liver disorders with cholestasis might also be a benefit on the intestinal Ca^{2+} absorption, because it avoids the inhibitory effect caused by hydrophobic BAs such as NaDOC, neutralizes the oxidative/nitrosative stress and suppresses apoptosis. With regard to autophagy, UDCA does not modify it but UDCA prevents the enhancement of autophagy caused by NaDOC. This is another mechanism by which UDCA protects the intestinal Ca^{2+} absorption. In agreement with that, it has been recently reported that the deregulated autophagy induced by glycochenodeoxycholic acid in cholangiocytes was suppressed by pre-treatment with UDCA and tauro-UDCA [101]. Figs. 1 shows a schematic representation of mechanisms involved in the effects of NaDOC, UDCA or their combination on the intestinal Ca^{2+} absorption.

As previously pointed out, LCA does not affect the intestinal Ca^{2+} absorption, but it blocks the inhibition of the intestinal Ca^{2+} absorption caused by NaDOC. The mechanism of this protection was by avoiding the reduction of the transcellular Ca^{2+} movement,

apparently through blocking the oxidative stress and apoptosis [77]. In fact, LCA prevents an increase in the superoxide anion content and in the protein carbonyl content produced by NaDOC, and avoids an enhancement produced by NaDOC on the protein expression of FAS and FASL and on the caspase-3 activity, an executive protease highly involved in apoptosis. In addition, LCA ameliorates the changes in mitochondrial membrane permeability (swelling) provoked by NaDOC. It is really intriguing that LCA, which is considered a toxic hydrophobic BA, could behave as a protective BA on the intestinal Ca^{2+} absorption. In humans, the toxicity of LCA is in question because of the efficient detoxification [102], so it is quite possible that LCA or derivates could be used to protect the intestinal Ca^{2+} absorption under oxidant conditions. A schematic representation of molecular mechanisms triggered by NaDOC, LCA or both that affect the intestinal Ca^{2+} absorption is shown in Fig. 2.

In conclusion, the response of the intestine to absorb Ca^{2+} is affected by BAs, but it is different according to the type and dose of BA. When there is a single administration, NaDOC has an inhibitory effect, UDCA is an stimulator whereas LCA does not have any influence. However, the combination of BAs modifies the response. Either UDCA or LCA protects the intestine against the oxidative injury caused by NaDOC by blocking of the oxidative/nitrosative stress, apoptosis and autophagy. The effects of other BAs on the intestinal Ca^{2+} absorption are unknown and it deserves to be investigated.

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Legends to Figures

Fig. 1. Schematic representation of the molecular mechanisms involved in the effects of NaDOC, UDCA or their combination on the intestinal Ca^{2+} absorption. The image represents enterocytes and their intercellular spaces. Ca^{2+} ions enter the enterocyte (transcellular pathway) or cross between cells (paracellular pathway). NaDOC= sodium deoxycholate, UDCA = ursodeoxycholic acid, AP = alkaline phosphatase, SOD =

superoxide dismutase, NCX1 = sodium calcium exchanger 1, PMCA = plasma membrane Ca^{2+}-ATPase, C-VDR = calcitriol-vitamin D receptor complex, RXR = retinoid X receptor, NO = nitric oxide, iNOS= inducible nitric oxide synthase, FAS= FAS receptor, FASL= FAS ligand, LC3 = light chain 3, GSH = glutathione, white arrows = effects of NaDOC, ⊣ = inhibitory effect

Fig. 2. Schematic representation of the molecular mechanisms involved in the effects of NaDOC, LCA or their combination on the intestinal Ca^{2+} absorption. Images represent enterocytes and their intercellular spaces. Ca^{2+} ions enter the enterocyte (transcellular pathway) or cross between cells (paracellular pathway). NaDOC = sodium deoxycholate, LCA = litocholic acid, AP = alkaline phosphatase, SOD = superoxide dismutase, NCX1 = sodium calcium exchanger 1, PMCA = plasma membrane Ca^{2+}-ATPase, C-VDR = calcitriol-vitamin D receptor complex, RXR= retinoid X receptor, NO = nitric oxide, iNOS = inducible nitric oxide synthase, FAS = FAS receptor, FASL = FAS ligand, LC3 = light chain 3, GSH = glutathione, white arrows = effects of NaDOC, ⊣ = inhibitory effect