Glutathione depleting drugs, antioxidants and intestinal calcium absorption

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Author contributions: Moine L, Rivoira M, Díaz de Barboza G, Pérez A and Tolosa de Talamoni NT participated in information collection, analysis, information organization, writing, figure design, and final editing.

Conflict-of-interest statement: No conflicts of interest, financial or otherwise, are declared by the authors.

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Manuscript source: Invited manuscript

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Received: August 28, 2018
Peer-review started: August 28, 2018
First decision: October 9, 2018
Revised: October 24, 2018
Accepted: November 2, 2018

Abstract

Glutathione (GSH) is a tripeptide that constitutes one of the main intracellular reducing compounds. The normal content of GSH in the intestine is essential to optimize the intestinal Ca\textsuperscript{2+} absorption. The use of GSH depleting drugs such as DL-buthionine-S,R-sulfoximine, menadione or vitamin K\textsubscript{3}, sodium deoxycholate or diets enriched in fructose, which induce several features of the metabolic syndrome, produce inhibition of the intestinal Ca\textsuperscript{2+} absorption. The GSH depleting drugs switch the redox state towards an oxidant condition provoking oxidative/nitrosative stress and inflammation, which lead to apoptosis and/or autophagy of the enterocytes. Either the transcellular Ca\textsuperscript{2+} transport or the paracellular Ca\textsuperscript{2+} route are altered by GSH depleting drugs. The gene and/or protein expression of transporters involved in the transcellular Ca\textsuperscript{2+} pathway are decreased. The flavonoids quercetin and naringin highly abrogate the inhibition of intestinal Ca\textsuperscript{2+} absorption, not only by restoration of the GSH levels in the intestine but also by their anti-apoptotic properties. Ursodeoxycholic acid, melatonin and glutamine also block the inhibition of Ca\textsuperscript{2+} transport caused by GSH depleting drugs. The use of any of these antioxidants to ameliorate the intestinal Ca\textsuperscript{2+} absorption under oxidant conditions associated with different pathologies in humans requires more investigation with regards to the safety, pharmacokinetics and pharmacodynamics of them.

Key words: Glutathione; Transcellular and paracellular Ca\textsuperscript{2+} pathways; DL-buthionine-S,R-sulfoximine; Fructose rich diet; Menadione; Sodium deoxycholate; Glutamine; Ursodeoxycholic acid; Melatonin; Quercetin; Naringin

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The normal content of glutathione (GSH) in the intestine is essential to optimize the intestinal Ca\textsuperscript{2+} absorption. The use of GSH depleting drugs such as DL-buthionine-S,R-sulfoximine, menadione or vitamin K\textsubscript{3}, sodium deoxycholate or diets enriched in fructose, which induce several features of the metabolic syndrome, produce inhibition of the intestinal Ca\textsuperscript{2+} absorption. The flavonoids quercetin and naringin highly abrogate the inhibition of intestinal Ca\textsuperscript{2+} absorption, not only by restoration of the GSH levels in the intestine but also by their anti-apoptotic properties. Ursodeoxycholic acid, melatonin and glutamine also block the inhibition of Ca\textsuperscript{2+} transport caused by GSH depleting drugs.

INTRODUCTION

Ca\textsuperscript{2+} absorption is one of the most important intestinal functions since the intestine is the only entrance gate of the cation into the body. This physiological process together with the renal Ca\textsuperscript{2+} reabsorption and the bone Ca\textsuperscript{2+} resorption maintain the Ca\textsuperscript{2+} homeostasis. An appropriate Ca\textsuperscript{2+} homeostasis preserves bone integrity, metabolic balance and avoids epithelial cancers such as breast, colon and prostate cancer\textsuperscript{[4-7]}. A poor intestine absorption caused by infection, inflammation or a pathology in the intestine morphology may cause an adverse Ca\textsuperscript{2+} balance\textsuperscript{[5]}, which under chronic conditions leads to a deleterious bone mineralization. The intestinal Ca\textsuperscript{2+} absorption occurs along the entire intestine, but the small intestine is responsible for about 90% of overall Ca\textsuperscript{2+} absorption, and the order of Ca\textsuperscript{2+} absorption rate is duodenum > jejunum > ileum. The colon is only responsible for less than 10% of the total Ca\textsuperscript{2+} absorbed, but it appears to become important under pathological conditions\textsuperscript{[8]}. Ca\textsuperscript{2+} is absorbed in the intestine by active and passive transport systems. The transcellular Ca\textsuperscript{2+} absorption is an active process and occurs via cation influx into the enterocyte, intracellular shuttling, and basolateral extrusion\textsuperscript{[9]}. Ca\textsuperscript{2+} absorption can also occur via a passive, paracellular route, where the movement of the cation between epithelial cells is made through tight junction (TJ) proteins, which facilitate or block the Ca\textsuperscript{2+} movement\textsuperscript{[10]}. The active transport of Ca\textsuperscript{2+} is mainly regulated by the biologically active form of vitamin D\textsubscript{3}, 1,25(OH)\textsubscript{2}D\textsubscript{3} (calcitriol)\textsuperscript{[9]}, by previous activation of a vitamin D receptor (VDR)\textsuperscript{[9]}. When VDR was deleted specifically in the intestine (VDR\textsuperscript{−/−}) of mice, the intestinal Ca\textsuperscript{2+} absorption was decreased, the bone mineralization is inhibited and bone fractures were increased\textsuperscript{[10]}. Thus, intestinal VDR is not only essential for intestinal Ca\textsuperscript{2+} absorption, but also for bone formation.

As previously reported, the transcellular Ca\textsuperscript{2+} movement involves the participation of transient receptor potential vanilloid type 6 (TRPV6) and transient receptor potential vanilloid type 5 in the step across the brush border membrane from enterocytes, calbindin D\textsubscript{9k} (CB D\textsubscript{9k}) as a ferry from one pole to the other pole of the cells and the plasma membrane Ca\textsuperscript{2+}-ATPase (PMCA\textsubscript{9k}) and the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX1) for cation extrusion\textsuperscript{[11]}. The molecules involved in the paracellular Ca\textsuperscript{2+} movement are not completely known, but there is certain evidence that the proteins of the TJ such as claudin-2 and claudin-12 facilitate the Ca\textsuperscript{2+} transport\textsuperscript{[12,13]}. In contrast, either gene or protein expression of cadherin-17 are decreased in mice’s and rat’s intestines during low Ca intake\textsuperscript{[14]}, as well as in Caco-2 cells after treatment with calcitriol\textsuperscript{[15]}

When Ca\textsuperscript{2+} intake is low, the cation entry occurs through the transcellular pathway; whereas high luminal Ca\textsuperscript{2+} content (> 2-6 mmol/L) switches on the paracellular route due to a short sojourn time in the intestine and a down-regulation of molecules involved in the transcellular pathway\textsuperscript{[16,17]}. The expression of paracellular TJ genes seems to be regulated by the calbindin protein, which suggests that the active and passive Ca\textsuperscript{2+} transport pathways may work cooperatively\textsuperscript{[18]}. A reduction in more than 70% in the active intestinal Ca\textsuperscript{2+} absorption, 55% in CB D\textsubscript{9k} expression and 90% in TRPV6 expression was observed in VDR null mice\textsuperscript{[19]}

Although calcitriol is the main regulator of intestinal Ca\textsuperscript{2+} absorption, other hormones also contribute to altering this process as parathyroid hormone, glucocorticoids, estrogen, growth hormone, etc. In addition, many dietary and pharmacological compounds also modify the intestinal Ca\textsuperscript{2+} transport\textsuperscript{[20]}. We have demonstrated that the normal content of the tripeptide glutathione (GSH) in enterocytes is essential for an optimal intestinal Ca\textsuperscript{2+} absorption, which was proved either in birds or in mammals\textsuperscript{[21,22]}. GSH depletion produced by different ways generates a low GSH/glutathione disulfide (GSSG) ratio leading to oxidative stress and apoptosis of enterocytes by exacerbation of reactive oxygen species (ROS) production\textsuperscript{[23]}. Clausen et al\textsuperscript{[24]} have reported that GSH plays an important role in the opening of the TJ of intestinal epithelium enhancing the paracellular transport. In this review we will analyze the role of GSH in the intestine, the molecular mechanisms by which GSH depleting drugs inhibit the intestinal Ca\textsuperscript{2+} absorption and the prevention or restoration of these effects by drugs that act through normalization of intestinal GSH content.

GSH SYNTHESIS AND ITS

PHYSIOLOGICAL ROLE IN THE INTESTINE

The intestinal mucosa comprises the surface monolayer...
of self-renewing epithelial cells and the lamina propria with the vascular, immune and structural components. In the small intestine there are invaginations called crypts of Lieberkühn and prominences into the lumen called villi with differentiated cells. The crypts contain proliferative stem cells and Paneth cells responsible for the innate immunity and antibacterial defense and for providing essential signals to intestinal stem cells. The intestinal epithelium is a single heterogeneous layer of different cells: enterocytes (80% of total cells), enteroendocrine cells (1% of all epithelial cells), Goblet cells (4% in duodenum, secretory cells) and tuft cells (secretory cell type). They originate in the crypts, migrate toward the villi during differentiation and then suffer spontaneous apoptosis and shedding when reaching the villus tip after terminal differentiation. Only enterocytes are involved in the Ca\(^{2+}\) transport from the intestinal lumen to the lamina propria. Although Ca\(^{2+}\) uptake occurs in all enterocytes, the mature cells from the tip and the middle part of the villi are mainly involved in the transcellular Ca\(^{2+}\) movement. Ca\(^{2+}\) uptake is stimulated by calcitriol or low Ca diets either in the mature enterocytes or in the undifferentiated cells from the crypt, but the most differentiated cells exhibit a higher response. The GSH content in the intestine is in the millimolar range as occurs in other cells; however, the tripeptide concentration varies according to the degree of maturation of cells. Surprisingly, mature enterocytes have lower GSH content than the immature cells. GSH exists as the biologically active reduced-thiol form, and its oxidation to GSSG is associated with oxidative stress (OS). The GSH/GSSG ratio is around 100/1; when GSSG increases, this ratio decreases causing an oxidative shift in the cellular redox state. Intestinal GSH redox homeostasis is maintained by de novo synthesis, regeneration from GSSG and GSH uptake that derives from the dietary disulfides, detoxification of xenobiotics, metabolism of peroxidized lipids and maintenance of the mucus fluidity. Tsunada et al have shown that chronic administration of lipid peroxides interferes with the regulation of enterocyte death and proliferation in vivo; these disruptive effects were reversed by GSH supplementation after normalization of GSH/GSSG redox balance (Figure 1).

Intracellular GSH is distributed in different compartments. Cytosolic GSH is the source of GSH pool of the mitochondria, endoplasmic reticulum and nucleus. The GSH/GSSG ratio in the cytoplasm varies between 30/1 to 100/1, whereas in the endoplasmic reticulum (ER) is between 3/1 and 1/1, which indicates that in ER the system GSH/GSSG is more oxidized than that from the cytoplasm. The steady-state redox potential of the GSH/GSSG system is about -330 mmol/L to -300 mmol/L in the mitochondrial matrix and -260 mmol/L to -200 mmol/L in the cytoplasm. The endoplasmic reticular GSH/GSSG redox potential is about -150 mmol/L, which is in midway between cytoplasmic and plasma values. The nuclear GSH/GSSG redox potential remains unknown but there is certain evidence that it can be somewhat more reducing than that from the cytoplasm. Proliferative cells have more negative steady-state redox potential and differentiated cells have more positive cells and cells undergoing apoptosis and necrosis have a more oxidized steady-state potential (around -170 mmol/L to -150 mmol/L). In other words, the cellular compartments have different GSH/GSSG ratios, and the life cell cycle is also associated with different thiol redox potentials, which can alter the cellular functions. Since enterocytes show different degrees of differentiation and then suffer apoptosis and shedding in a range of 4–7 d, the redox potential of GSH/GSSG couple must change quite rapidly in order to facilitate the variety of functions of those cells during the lifespan.

GSH DEPLETION AND THE INTESTINAL CALCIUM ABSORPTION

Many years ago, Mårtensson et al demonstrated that...
GSH was required for intestinal function. They observed that chronic depletion of mucosal GSH by buthionine sulfoximine (BSO), a specific inhibitor of GCL\(^\text{[47]}\), caused severe degeneration of epithelial cells from jejunum and colon, which was prevented by oral GSH or GSH monoester. We have shown that BSO alters the Ca\(^{2+}\) transfer from intestinal lumen-to-blood in vitamin D supplemented chicks but does not affect that of vitamin D-deficient chicks, which indicate that the effects of BSO on intestinal Ca\(^{2+}\) absorption were dependent on the vitamin D status of the animal. The reversibility of this inhibition was proved by adding GSH monoester, an indication that intestinal GSH is essential to have an optimal intestinal Ca\(^{2+}\) absorption\(^\text{[51]}\). At that time, the molecular mechanisms involved in the effect of BSO on the intestinal Ca\(^{2+}\) absorption remained unknown. Later on, we have demonstrated that GSH depleting drugs inhibit the intestinal Ca\(^{2+}\) transport not only in birds but also in mammals\(^\text{[48]}\). The tripeptide depletion might increase the oxidation of sulfhydryl groups that are important to maintain the functionality of proteins involved in the Ca\(^{2+}\) transport, such as occurs with the PMCA\(_{10}\)\(^\text{[21]}\). In addition, other GSH depleting drug such as vitamin K\(_3\) or menadione (MEN) causes inhibition of intestinal Ca\(^{2+}\) absorption, which is related to OS, as judged by a decrease in GSH content and an increment in the total carbonyl group content. This inhibitory effect of MEN on intestinal Ca\(^{2+}\) absorption begins in half an hour, lasts for several hours and finishes after 10 h of treatment\(^\text{[49]}\). The quinone inhibits two enzymes presumably involved in Ca\(^{2+}\) transcellular pathway such as the intestinal alkaline phosphatase (IAP), located in the brush border membrane, and PMCA\(_{10}\), protein that carries Ca\(^{2+}\) outside the cell, as mentioned above. The enzyme inhibition might be due to alterations produced by ROS, which are triggered by GSH depletion caused by its consumption in the redox cycle of the quinone\(^\text{[50]}\). When MEN is metabolized, it may undergo one or two-electron reduction. If it suffers one-electron reduction, there is formation of a very unstable semiquinone radical, which reacts rapidly with molecular oxygen resulting in regeneration of the parent compound and production of a superoxide anion that yields H\(_2\)O\(_2\) via enzymatic or spontaneous dismutation. Two-electron reduction of MEN produces a hydroquinone, a pathway that constitutes a detoxification mechanism\(^\text{[51]}\). In both cases, GSH is the electron donor, which explains the tripeptide depletion after MEN treatment. Since the intestinal Ca\(^{2+}\) absorption is an active process, which requires ATP that is mainly provided by the mitochondria, we have analyzed the functionality of these organelles in the intestinal mucosa when animals were treated with MEN. In fact, we have detected that MEN produces mitochondrial dysfunction caused by GSH depletion, which alters the mitochondrial permeability resulting in the release of cytochrome c and DNA fragmentation, biomarkers of apoptosis through an intrinsic pathway. In other words, mitochondrial dysfunction is also involved in the mechanisms by which MEN inhibits transiently the intestinal Ca\(^{2+}\) absorption\(^\text{[49]}\). Later on, the system FASL/FAS/caspase-3, indicators of apoptosis via the extrinsic pathway, was also demonstrated to be activated by MEN\(^\text{[52]}\).

Sodium deoxycholate (NaDOC) is another GSH depleting drug that produces OS, as indicated by ROS generation and mitochondrial swelling leading to inhibition of intestinal Ca\(^{2+}\) absorption\(^\text{[53]}\). The effect of

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**Figure 1 Intestinal glutathione metabolism.** The \(\gamma\)-glutamyl cycle comprises the enzymatic reactions involved in the extracellular glutathione (GSH) degradation and the intracellular GSH synthesis: The enzymes \(\gamma\)-glutamyl transpeptidase and dipeptidase, localized in plasma membrane of enterocytes, cleave the extracellular GSH to their constituent amino acids. Within the enterocytes the GSH is synthesized \textit{de novo} by two reactions that consume adenosine triphosphate that are catalyzed by glutamate cysteine ligase and glutathione synthetase, sequentially. In the redox couple that ensures the maintenance of the cellular redox state participates the enzymes glutathione peroxidase and glutathione reductase. GSH: Glutathione; GSSG: Glutathione disulfide; GT: \(\gamma\)-glutamyl transpeptidase; GCL: Glutamate cysteine ligase; GS: Glutathione synthetase; GR: Glutathione reductase; GPX: Glutathione peroxidase; DP: Dipeptidase.
NaDOC is time and dose-dependent and is higher in mature enterocytes\textsuperscript{[53,22]}. NaDOC exerts its effect altering the transcellular Ca\textsuperscript{2+} pathway; the gene and protein expression of PMCA1b and the protein expressions of calbindin D\textsubscript{28k} (CB D\textsubscript{28k}) and NCX1 are decreased by this hydrophobic bile salt. NaDOC is a major component of the bile; in high concentrations it provokes liver damage during cholestasis and promotes colon carcinogenesis in experimental animals\textsuperscript{[58,59]}. The molecular mechanisms by which NaDOC alters the protein expression of molecules involved in the intestinal Ca\textsuperscript{2+} absorption seem to be also related to nitrosative stress, as indicated by increment in NO\textsuperscript{•} content and in induced nitric oxide synthase (iNOS) protein expression, and apoptosis, as shown by enhancement of the system FASL/FAS/caspase-8/caspase-3. In addition, an increase in acidic vesicular organelles (AVOs) and in LC3 II protein expression produced by NaDOC means that autophagy might be another mechanism triggered by this bile salt associated with the inhibition of the intestinal Ca\textsuperscript{2+} absorption\textsuperscript{[22]} (Table 1).

The administration of fructose rich diets (FRD) to normal rats, which induce several features of the metabolic syndrome, inhibit the intestinal Ca\textsuperscript{2+} absorption and induce vitamin D insufficiency\textsuperscript{[56,57]}. In our laboratory, we have found that the VDR protein expression is also diminished by the FRD\textsuperscript{[58]}. Since 1,25(OH)\textsubscript{2}D\textsubscript{3} and its receptor are depleted, the inhibition in the cation transport could be explained due to 1,25(OH)\textsubscript{2}D\textsubscript{3} is the main stimulator of the intestinal Ca\textsuperscript{2+} absorption. FRD alter both the transcellular and the paracellular pathways. The protein expression of TRPV6, CB D\textsubscript{9k} and PMCA\textsubscript{1b} as well as the enzyme activity of IAP are lower in animals fed FRD than in rats fed a normal diet. Similarly, the protein expressions of Claudin-2 and Claudin-12, molecules located in intestinal TJ, are also decreased in rats fed FRD\textsuperscript{[58]}. The intestinal GSH levels are decreased by the FRD, which would explain the increment in the superoxide anion and in the protein carbonyl content. This scenario is worsened by a decrease in the activities of superoxide dismutase and catalase, enzymes of the antioxidant defense, which result in impairment of the redox equilibrium contributing to altering the intestinal Ca\textsuperscript{2+} absorption. Other authors have also demonstrated that FRD decrease the GSH content and the antioxidant enzyme activities as well as vitamin C and vitamin E levels in rat liver and skeletal muscle\textsuperscript{[59]}. In addition, we have demonstrated that FRD increase the NO\textsuperscript{•} content and the nitrosylation of proteins of 22 and 38 kDa from rat intestine. He et al\textsuperscript{[60]} have demonstrated that FRD increase the expression of inducible NO\textsuperscript{•} synthase in the liver. Kannappan et al\textsuperscript{[61]} have shown that FRD augment nitrosothiols in the plasma, liver and skeletal muscle. All these findings indicate that an intake rich in fructose triggers nitrosative stress in a variety of tissues.

The inflammation is another mechanism triggered by FRD in rat intestine, as suggested by an increment in the intestinal protein expression of nuclear factor (NF)-\kappa B and interleukin (IL)-6. NF-\kappa B is a transcription factor that controls over 100 genes activated direct or indirectly by inflammation\textsuperscript{[61]}; IL-6 is a cytokine, whose gene has a promotor region with a site of binding for NF-\kappa B, and the nitrosylation of proteins of 22 and 38 kDa from rat intestine. He et al\textsuperscript{[60]} have shown that FRD increase the NO\textsuperscript{•} synthase in the liver.

In conclusion, any drug or disease associated with intestinal GSH depletion causes inhibition of intestinal Ca\textsuperscript{2+} absorption. This response is mediated by OS/nitrosative stress and inflammation, which could lead to cell death of enterocytes with capability to transport Ca\textsuperscript{2+} across the cells and in the paracellular route.

**Table 1 Glutathione depletion and the intestinal calcium absorption**

| Oxidative stress markers | Effect on intestinal Ca\textsuperscript{2+} absorption |
|--------------------------|--------------------------------------------------|
| BSO | ↓GSH ↓ROS and protein carbonyl ↓Mn\textsuperscript{2+}SOD and GPX Mitochondrial dysfunction ↓GSH ↑ROS ↓GSH ↑ | ↓Ca\textsuperscript{2+} transfer from lumen-to-blood ↓Intestinal Ca\textsuperscript{2+} absorption ↓mRNA PMCA1b ↓PMCA1b, CB D\textsubscript{28k} ↓TRPV6, PMCA1b, CBD\textsubscript{9k}, CLDN 2, CLDN12 and VDR protein ↓Intestinal Ca\textsuperscript{2+} absorption ↓Ca\textsuperscript{2+} transfer from lumen-to-blood ↓IAP and PMCA1b activities ↓IAP activity ↓TRPV6, PMCA1b, CBD\textsubscript{9k}, CLDN 2, CLDN12 and VDR protein expression |
| MEN | ↓GSH | ↓Ca\textsuperscript{2+} transfer from lumen-to-blood |
| NaDOC | ↓GSH ↓ROS Mitochondrial swelling, ↓SOD, CAT and GPX ↓NO\textsuperscript{•} ↓iNOS protein ↓GSH ↓HO\textsubscript{2} | ↓GSH ↑HO\textsubscript{2} ↓protein carbonyl and nitrotyrosine content ↓SOD and CAT ↓NO\textsuperscript{•} |
| FRD | ↓GSH ↑HO\textsubscript{2} | ↓GSH ↑HO\textsubscript{2} |

BSO: DL-buthionine-S,R-sulfoximine; CAT: Catalase; CB D\textsubscript{28k}: Calbindin D28k; CB D\textsubscript{9k}: Calbindin D9k; CLDN 2: Claudin 2; CLDN12: Claudin 12; FRD: Fructose rich diet; GPX: Glutathione peroxidase. GSH: Glutathione; IAP: Intestinal alkaline phosphatase. MEN: menadione. Mn\textsuperscript{2+}SOD: Mn\textsuperscript{2+}-superoxide dismutase. NaDOC: sodium deoxycholate.NCX1: Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. NO\textsuperscript{•}: Nitric oxide; PMCA1b: Plasma membrane Ca\textsuperscript{2+} ATPase; SOD: Superoxide dismutase; TRPV6: Transient receptor potential vanilloid type 6; VDR: Vitamin D receptor.

REVERSION/PREVENTION OF THE INHIBITION OF INTESTINAL CALCIUM ABSORPTION CAUSED BY GSH DEPLETION

The first approaches to revert or prevent the inhibition
of intestinal Ca\(^{2+}\) absorption caused by GSH depletion consisted in the use of GSH monoester in order to replenish the intestine with the tripeptide\[^{[22]}\]. In fact, this treatment leads to the normalization of the intestinal Ca\(^{2+}\) absorption. In addition, other strategies were also assayed because the intestinal GSH depletion could be generated not only by drugs but also by pathological conditions such as cholestasis and metabolic syndrome\[^{[58,63]}\].

Since GSH depletion causes exacerbation of ROS production, inflammation, apoptosis and autophagy, the reversion or prevention was thought to be blocked by flavonoids, molecules derived from natural sources with antioxidant, anti-inflammatory and antiapoptotic properties. Flavonoids are a class of phenolic metabolites produced by plants and fungi\[^{[64]}\]. Among them, quercetin (QT) is largely present in fruit, red wine, tea, vegetables and aromatic plants\[^{[65]}\], and exhibits all the biological properties mentioned above. Hence, QT is considered as a potential therapeutic agent for different diseases such as cancer, hypertension, inflammation, diabetes, thrombosis\[^{[66,68]}\]. Inoue et al\[^{[69]}\] have suggested that QT might improve the intestinal Ca\(^{2+}\) absorption because they have demonstrated in Caco-2 cells that QT increases the gene expression of TRPV6, which is a VDR target gene. We did not find that QT alone ameliorates the Ca\(^{2+}\) transport in the intestine, but we demonstrated that QT blocks the inhibition of the intestinal Ca\(^{2+}\) absorption caused by MEN via GSH depletion. Similarly, QT by itself does not change the intestinal GSH content, but it prevents the GSH depletion produced by the quinone\[^{[70]}\]. Boots et al\[^{[70]}\] have also observed that the effects of QT supplementation in patients with sarcoidosis appear to be more pronounced when the baseline levels of oxidative (malondialdehyde) and inflammatory (tumor necrosis factor \(\alpha\), IL-8, IL-10) markers are increased. So, it appears that the extent of the QT effects depends on the baseline of OS and inflammation. The protective mechanisms of QT on the intestinal Ca\(^{2+}\) absorption under oxidant conditions could be summarized in: (1) Normalization of intestinal redox state, (2) blockade of alterations in the mitochondrial membrane permeability (swelling), and (3) interference with the FASL/FAS/caspase-3 cascade activation. Taken together, it could be concluded that QT might be useful to prevent the inhibition of intestinal Ca\(^{2+}\) absorption caused by pro-oxidants or conditions that deplete GSH leading to OS and apoptosis\[^{[22]}\].

Naringin (NAR) is another flavonoid that abrogates the inhibition of intestinal Ca\(^{2+}\) absorption caused by oxidant conditions such as an experimental metabolic syndrome produced in rats by FRD\[^{[58]}\]. NAR is chemically known as naringenin 7-O-neohesperidoside and is present in different citrus being responsible for the bitterness in grapefruit, which is one of the richest sources of this flavonoid\[^{[71]}\]. NAR has been demonstrated to increase the GSH content either in the liver or in the intestine from mice exposed to whole-body irradiation\[^{[72]}\]. A meta-analysis also showed that NAR restores the GSH content in different parts of brain in various neurological ailments in rodents\[^{[73]}\]. We have demonstrated that NAR (40 mg/kg bw) can protect the intestinal Ca\(^{2+}\) absorption by blocking all the alterations in the redox state of the intestinal mucosa caused under oxidant conditions such as the intake of FRD by rats\[^{[80]}\]. With regard to GSH, NAR

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**Table 2** Antioxidants that preserve/restore the inhibition of intestinal Ca\(^{2+}\) absorption caused by glutathione depletion

| Drugs       | Normalized OS markers | Effect on inhibition of ICaA | Normalized component of ICaA | Apoptosis markers | References |
|-------------|-----------------------|-----------------------------|-----------------------------|-------------------|------------|
| GSH monoester | GSH total             | Restoration                  | PMCA1b, CB\(\alpha\), NCX1, CLDN 2 and CLDN 12 protein expression | ↓TUNEL index, ↓Caspase 3 activity | Tolosa de Talamoni et al\[^{[21]}\] |
| MEL         | GSH, ‘O’ Protein carbonyl, SOD, CAT and GPH activities | Prevention restoration | PMCA1b, CB\(\alpha\), NCX1, CLDN 2 and CLDN 12 protein expression | ↓Caspase 3 activity | Carpentieri et al\[^{[49]}\] |
| QT          | GSH total, GPH activity | Prevention restoration | CB\(\alpha\) and PMCA1b protein expression | ↓TUNEL index, ↓FAS, ↓FASL, Blocks mit swelling | Marchionatti et al\[^{[52]}\] |
| GLN         | GSH, ‘O’, Protein carbonyl, SOD and CAT activity | Prevention restoration | CB\(\alpha\) and PMCA1b protein expression | ↓TUNEL index, ↓FAS, ↓FASL, Blocks mit swelling | Moine et al\[^{[86]}\] |
| NAR         | GSH, ‘O’ NO+ Protein carbonyl and nitrotyrosine content, SOD and CAT activity | Prevention | IAP activity (partially) PMCA1b, CB\(\alpha\), NCX1, VDR, CLDN2 and CLDN12 protein expression | ↓Mit swelling, ↓FAS, ↓FASL, ↓Caspase-3 activity | Rodriguez et al\[^{[39]}\] |
| UDCA        | GSH, NO+ protein carbonyl, SOD activity, iNOS protein expression | Increase restoration | IAP activity, PMCA1b, CB\(\alpha\), NCX1, VDR, CLDN2 and CLDN12 protein expression | Caspase 3 activity | Rodriguez et al\[^{[22,46]}\] |

CAT: Catalase; CB\(\alpha\): Calbindin D28k; CB\(\alpha\): Calbindin D9k; CLDN 2: Claudin 2; CLDN12: Claudin 12; GLN: Glutamine; GPX: Glutathione peroxidase; GSH: Glutathione; IAP: Intestinal alkaline phosphatase; ICaA: Intestinal Ca\(^{2+}\) absorption; MEL: Melatonin; MEN: Menadione; Mit: Mitochondrial; NAR: Naringin; NCX1: Na\(^{+}/Ca\(^{2+}\) exchanger; NO: Nitric oxide; OS: Oxidative stress; PMCA1b: Plasma membrane Ca\(^{2+}\)-ATPase; QT: Quercetin; SOD: Superoxide dismutase; TRPV6: Transient receptor potential vanilloid type 6; UDCA: Ursodeoxycholic acid; VDR: Vitamin D receptor.
not only blocked its depletion produced by FRD but also increased almost twice the normal intestinal GSH content by a mechanism that needs to be clarified. NAR was able to abrogate all the described alterations provoked by FRD in rats through its anti-oxidant, anti-nitricergic and anti-inflammatory properties. The use of NAR to ameliorate the intestinal $Ca^{2+}$ transport under oxidant conditions associated with different pathologies holds a remarkable potential, but there are some obstacles in NAR clinical translation related to the extensive in vivo metabolism, low bioavailability and irregular absorption.\(^{[74]}\). On the other hand, it has been demonstrated that various phenolic antioxidants exhibit pro-oxidant properties at high doses.\(^{[75]}\). In fact, both flavonoids, quercetin and naringin, have antioxidant and pro-oxidant effects.\(^{[76]}\).

Melatonin (MEL) is one of the natural human antioxidant that has gained increasing attention. MEL is a hormone secreted by the pineal gland and other tissues such as bone marrow, skin and gastrointestinal tract. MEL is a lipophilic antioxidant of broad spectrum that has a high membrane permeability.\(^{[77]}\). The molecular mechanisms triggered by MEL seem to be different from those of the classical antioxidants such as vitamin $C$, vitamin $E$ and GSH; however, MEL synergizes with them in the scavenging of free radicals. The classical antioxidants undergo redox cycling so they have the potential to promote oxidation or prevent it. In contrast, MEL does not display redox cycling, thus, it does not stimulate oxidation; therefore it could be considered as a suicidal or terminal antioxidant. MEL may interact with free radicals forming several stable end products, which are excreted in the urine.\(^{[78]}\). MEL content is 400 times larger in the intestine than in the pineal gland,\(^{[79]}\), but the physiological significance of this is not very clear. Similarly to QT, MEL alone does not affect the intestinal $Ca^{2+}$ absorption, but it avoids or reverses the inhibitory effect of MEN or BSO.\(^{[80,81]}\). The GSH depletion caused by MEN was also prevented by MEL, counteracting the oxidative stress and apoptosis provoked by the quinone. MEL protects either the intestinal transcellular $Ca^{2+}$ pathway or the paracellular $Ca^{2+}$ route, but only under oxidant conditions. The modulation of transporters of $Ca^{2+}$ by MEL has also been reported in pancreatic acinar cells and in rat pituitary GH3 cells.\(^{[82]}\). In conclusion, MEL could be a drug for reversal of impaired intestinal $Ca^{2+}$ absorption produced by OS and apoptosis that occurs under pathophysiological conditions such as aging, celiac disease, intestinal bowel disease, cancer or other gut disorders, or by GSH depleting drugs (Table 2).

Since the amino acid glutamine (GLN) in the intestine is a fuel and a source of glutamate, substrate for GSH synthesis, and it has antioxidant and antiapoptotic properties,\(^{[83]}\), and has the advantage of being an oral nutritional supplement,\(^{[84,85]}\), we have thought that it could be used to prevent or reverse the intestinal $Ca^{2+}$ absorption inhibited by GSH depleting drugs. Similarly to other antioxidants, GLN alone does not modify the intestinal $Ca^{2+}$ absorption but it reverses the inhibition of the intestinal cation transport caused by MEN. The GLN protective action is dose and time dependent and also occurs when it is administered previous to MEN treatment. The normalization of the protein expression of CB $D_{20}$ and PMCA$_{1b}$ by GLN indicates that this amino acid protects the transcellular $Ca^{2+}$ pathway. The protection may be achieved because GLN restores the intestinal GSH content, normalizes the enzymatic activities of the antioxidant defense system and decreases the activation of the apoptotic pathway FASL/FAS/Caspase-3.\(^{[86]}\). In other words, the antioxidant and antiapoptotic properties of GLN facilitate the normalization of the intestinal $Ca^{2+}$ absorption under oxidant conditions. Whether GLN alters or not the intestinal $Ca^{2+}$ paracellular route and/or other mechanisms are involved in the protection of the intestinal $Ca^{2+}$ absorption is under investigation.

Ursodeoxycholic acid (UDCA) is a minor component of the bile and has hydrophilic properties.\(^{[87]}\). It is known that UDCA blocks the reactive oxygen species formation, the mitochondrial dysfunction and the death receptor induced apoptosis.\(^{[88]}\). It has been widely used for treatment of cholestatic liver diseases, mainly primary biliary cirrhosis (PBC).\(^{[89]}\). In our laboratory, we have explored the possibility that UDCA could prevent the inhibition of intestinal $Ca^{2+}$ absorption caused by NaDOC, a hydrophobic bile acid that causes GSH depletion in the duodenum, as mentioned in the previous section. Verma et al.\(^{[90]}\) have demonstrated that UDCA therapy enhances fractional $Ca^{2+}$ absorption in PBC. In agreement with these data, we have observed that UDCA alone improves the intestinal $Ca^{2+}$ absorption by increasing the amount of $Ca^{2+}$ transporters involved in the transcellular $Ca^{2+}$ pathway via activation of the VDR gene and protein expression. The effect of UDCA on $Ca^{2+}$ uptake by enterocytes has been shown to depend on the degree of differentiation of these cells, being higher in mature enterocytes. When UDCA is given simultaneously with NaDOC, the intestinal $Ca^{2+}$ absorption is similar to that from the control animals, which means that UDCA prevents the inhibition in the $Ca^{2+}$ transport caused by NaDOC. Although UDCA alone decreases FASL and FAS protein expression without changing the Caspase-8 protein expression and caspase-3 activity, it avoids the apoptotic effects of NaDOC through normalization of the protein expression of FASL, FAS, Caspase-8 and the enzyme activity of Caspase-3. Similarly, UDCA per se does not alter the intestinal NO$^+$ content, but it abrogates the increase in NO$^+$ and in iNOS protein expression provoked by NaDOC. In addition, UDCA avoids efficiently the enhancement in LC3$^+$ protein expression and in the number of AVOs in enterocytes caused by NaDOC, which means that UDCA attenuates the biomarkers of autophagy.\(^{[22]}\). The physiological significance of this response is not quite clear and needs to be clarified.

**CONCLUSION**

The $Ca^{2+}$ entrance to the organism is very important to maintain the $Ca^{2+}$-dependent functions and the correct mineralization of the skeleton. An optimal intestinal
Ca²⁺ absorption is reached when the GSH content in the intestine is in the normal range. Conditions associated with intestinal GSH depletion arising from administration of certain drugs or different diseases may inhibit the intestinal Ca²⁺ transport. This response could be prevented or restored by using flavonoids (QT, NAR), MEL, UDCA or GLN, which block the effects of the GSH depletion mainly through their antioxidant, antiapoptotic and anti-inflammatory properties. However, the use of these drugs to improve the intestinal Ca²⁺ absorption under oxidant conditions associated with different pathologies in humans requires more investigation with regards to the safety, pharmacokinetics and the pharmacodynamics of them.

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