Comparative Biochemistry and Physiology, Part A xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Effects of quercetin and menadione on intestinal calcium absorption and the underlying mechanisms

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ARTICLE INFO

Article history:
Received 5 June 2012
Received in revised form 12 September 2012
Accepted 12 September 2012
Available online xxxx

Keywords:
Antioxidant
Apoptosis
Calcium absorption
Chicken
Menadione
Mitochondria
Oxidative stress
Quercetin

ABSTRACT

Quercetin (QT) could be considered as a potential therapeutic agent for different diseases due to its antioxidant, anti-inflammatory, antiviral and anticancer properties. This study was designed to investigate the ability of QT to protect the chick intestine against menadione (MEN) induced injury *in vivo* and *in vitro*. Four-week old chicks (*Gallus gallus*) were treated i.p. with 2.5 μmol of MEN/kg b.w. or with i.l. 50 μM QT or both. QT protected the intestinal Ca^{2+} absorption against the inhibition caused by MEN, but QT alone did not modify. Glutathione (GSH) depletion provoked by MEN in chick enterocytes was abolished by QT treatment, whereas QT alone did not modify the intestinal GSH content. The enhancement of GSH peroxidase activity produced by MEN was blocked by QT treatment. In contrast, superoxide dismutase activity remained high after simultaneous treatment of enterocytes with MEN and QT. The flavonol also avoided changes in the mitochondrial membrane permeability (swelling) produced by MEN. The FasL/Fas/caspase-3 pathway was activated by MEN, effect that was abrogated by QT. In conclusion, QT may be useful in preventing inhibition of chick intestinal Ca^{2+} absorption caused by MEN or other substances that deplete GSH, by blocking the oxidative stress and the FasL/Fas/caspase-3 pathway activation.

1. Introduction

Quercetin (QT) is a flavonol ingested by man and animals with their regular diets and is considered beneficial for health (Mi et al., 2010) It is largely present in fruits, vegetables, aromatic plants, tea and red wine (Rice-Evans, 2001). QT exhibits various biological effects such as antioxidant, anti-inflammatory, antiviral and anticancer activities (Suzuki and Hara, 2009). These biological properties have made that QT could be considered as a potential therapeutic agent for different diseases, including cancer, viral infection, inflammation/allergy, hypertension and atherosclerosis (Park et al., 2003). QT promotes apoptosis of tumor cells, in part through depression of the heat shock protein 70, but may inhibit apoptosis in some nontumorigenic cells, such as in H_2O_2-induced apoptosis of mesangial cells, fibroblasts and epithelial cells (Ishikawa and Kitamura, 2000).

The gastrointestinal tract (GIT) is the first target after oral ingestion of QT (Trischitta and Faggio, 2006). There is not much information about the intraluminal concentration of QT in the GIT. It is assumed that its glycosides reach the intestine, where they are hydrolyzed by the enteric microflora, and then, the aglycone is conjugated intracellularly and released into the blood (Graf et al., 2006; Barnes et al., 2011). Natsume et al. (2009a) have observed that a low dose of QT produces changes in the gene expression profile of the mouse intestine such as an up-regulation of glutathione-S-transferases. The same group has also shown that QT suppresses the endoplasmic reticulum stress caused by calcium dynamics dysregulation by the inhibition of PI3K (Natsume et al., 2009b). The anti-inflammatory effects of QT on murine intestinal epithelial cells have been demonstrated to occur through mechanisms that inhibit cofactor recruitment at the chromatin of proinflammatory genes (Ruiz et al., 2007). The modulation of transport properties of intestine by QT is also possible as judged by the enhancement of the barrier function caused by the flavonol in the Caco-2 cell line via an increase in claudin-4 expression and in the assembly of zonnula occludens-2, occludin and claudin-1 (Amasheh et al., 2008; Suzuki and Hara, 2009). Barrenetxe et al. (2006) have shown that QT alters the intestinal enzymatic activity and nutrient uptake in healthy mice without impairing normal development, serum biochemical parameters or body weight. Sucrose and maltase activities were decreased by QT, whereas the activities of aminopeptidase N and alkaline phosphatase were increased. Recently, it has been observed that QT attenuates fasting and postprandial hyperglycemia in animal models of *Diabetes mellitus* by enhancing insulin sensitivity via α-glucosidase inhibition and enhanced insulin signaling (Kim et al.,

Abbreviations: QT, Quercetin; GIT, Gastrointestinal tract; MEN, Menadione; GSH, Glutathione; intraperitoneal, i.p.; intraluminal, i.l.; body weight, b.w.; Cu-Zn-SOD, Superoxide dismutase; GPX, Glutathione peroxidase; EDTA, Ethylendiaminetetracetic acid; NBT, Nitro blue tetrazolium; O_2^-, Superoxide anion.

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http://dx.doi.org/10.1016/j.cbpa.2012.09.007

2011). It has been also suggested that QT may improve Ca^{2+} absorption from epithelia considering that QT is a weak activator of the vitamin D receptor and stimulates TRPV6 gene expression in Caco-2 cells (Inoue et al., 2010).

Optimal calcium absorption is an important function of the intestine not only to maintain Ca^{2+} homeostasis, but also for proper mineralization of bone in the prevention of osteoporosis and osteoporotic fractures (Kumari et al., 2010). We have widely reported that normal intracellular glutathione (GSH) levels are essential to maintain a proper intestinal Ca^{2+} absorption (Tolosa de Talamoni et al., 1996; Marchionatti et al., 2001). We have shown that menadione (MEN) or vitamin K_3, which is used in the anticancer therapy and in the treatment of osteoporosis (Hattori et al., 2001; Graciani and Ximenes, 2012), inhibits transiently the intestinal Ca^{2+} absorption in normal chicks. This inhibitory effect is caused, at least in part, by GSH depletion leading to oxidative stress, mitochondrial dysfunction and apoptosis via the intrinsic pathway (Marchionatti et al., 2003; Marchionatti et al., 2008). Probably, MEN affects essential thiol groups or other groups from proteins involved in the intestinal transcellular Ca^{2+} pathway by enhanced ROS production, which was previously demonstrated (Marchionatti et al., 2003). Whether intestinal apoptosis via the extrinsic pathway is induced by MEN in intestine has not been investigated. Although enzymes of the antioxidant system enhance their activities to attenuate the oxidant effects of MEN, the transcellular Ca^{2+} movement is reduced affecting the global process of intestinal Ca^{2+} absorption (Marchionatti et al., 2008).

The aim of this study was to investigate the ability of QT to protect the chick intestine against MEN-induced injury *in vivo* and *in vitro* and to explore whether the modulation of the FasL/Fas/caspase-3 signalling pathway is involved. In addition, the total GSH content and the enzyme activities of the antioxidant system were also investigated in chick enterocytes treated with MEN, QT or both.

2. Experimental methods

2.1. Animals

One-day-old Cobb Harding chicks (*Gallus gallus domesticus*) were obtained from Indacor S.A. (Rio Ceballos, Cordoba, Argentina) and were fed a commercial normal avian diet (Cargill, S.A.C.I., Pilar, Provincia de Cordoba, Argentina). At 4 weeks of age, they were killed by cervical dislocation and the excised duodenae were rinsed with cold 0.15 M NaCl and enterocytes or intestinal mitochondria were isolated as described below. The protocols 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.

2.2. Chemicals

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. MEN and QT were always diluted in 0.15 M NaCl.

2.3. Intestinal Ca^{2+} absorption

Four week old chicks were divided into four groups: 1) normal chicks injected i.p. with 0.15 M NaCl for 30 min (controls), 2) normal chicks treated i.p. with 2.5 μmol of MEN/kg of b.w. for 30 min, 3) normal chicks treated i.l. with 1 mL of 50 μM QT for 30 min, and 4) normal chicks treated i.p. with 2.5 μmol of MEN/kg of b.w. simultaneously with 1 mL i.l. of 50 μM QT for 30 min. Chicks were laparotomized under anesthesia and a 10 cm segment of duodenum was ligated following the technique previously described (Tolosa de Talamoni et al., 1996). One mL of 150 mM NaCl, 1 mM $CaCl_2$, containing 1.85×10^5 Bq $^{45}Ca^{2+}$, pH 7.2, was introduced into the lumen of the ligated intestinal segment. After half an hour, blood was withdrawn by cardiac puncture, centrifuged and the plasma ^{45}Ca was measured in a liquid scintillation counter. Absorption was defined as appearance of $^{45}Ca^{2+}$ in the blood.

2.4. Duodenal villus tip cell isolation

Mature enterocytes were isolated as previously described (Centeno et al., 2004). Cellular viability was assayed by the Trypan blue exclusion technique. Enterocytes were divided into four groups: 1) controls, 2) treated with 500 μM MEN, 3) treated with 50 μM QT, and 4) treated simultaneously with 500 μM MEN and 50 μM QT. Incubation time was 30 min. Protein was determined by the method of Gornall et al. (1949).

2.5. Mitochondrial isolation

Mitochondria were isolated from intestinal mucosa of the four groups of animals by differential centrifugation as previously described (Tolosa de Talamoni et al., 1985).

2.6. Determination of GSH content

Total GSH content was assayed in supernates from homogenates of enterocytes. The determinations were carried out by the glutathione disulfide reductase-5,5′-dithiobis (2-nitrobenzoate) recycling procedure (Anderson, 1985).

2.7. Enzyme assays

Superoxide dismutase (Cu-Zn-SOD, EC 1.15.1.1), and glutathione peroxidase (GPX, EC 1.11.1.9) activities were performed in diluted aliquots of the supernates from homogenates of enterocytes. Cu-Zn-SOD activity was determined in 1 μM EDTA, 50 mM potassium phosphate buffer, pH 7.8, 13 mM methionine, 75 μM nitro blue tetrazolium (NBT) and 40 μM riboflavine. Cu-Zn-SOD activity was defined in terms of its ability of inhibiting the superoxide anion (O_2^-) dependent reaction due to the competition between SOD and NBT (Beauchamp and Fridovich, 1973). GPX activity was determined in 50 mM potassium phosphate buffer pH 7.8, 1 mM EDTA, 1 mM NaN_3, 1 mM GSH and 1 U GSH reductase. The activity was measured by following NADPH oxidation after addition of 1 mM NADPH (Cheng et al., 1999). Caspase-3 measurements were also accomplished in supernatants from homogenates of enterocytes following in a plate reader at 405 nm the absorbance of *p*-nitroaniline obtained from a caspase-3-substrate I (Calbiochem, San Diego, CA, USA) (Garcia-Calvo et al., 1998).

2.8. Western blots

Fas and FasL expressions were analyzed by Western blot procedure using supernates from homogenates of control and treated enterocytes. Homogenate suspensions were done in RIPA lysis buffer (1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate in PBS, containing 1 mM PMSF and 1 mM 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 (Laemmli, 1970). Gels containing the separated proteins were immersed in the transfer buffer (25 mM Tris–HCl, 192 mM glycine, 0.05% w/v SDS and 20% v/v methanol) (Towbin et al., 1979). Nitrocellulose membranes (0.45 μm) were blocked for 1 h with 2% w/v nonfat dry milk in 0.5 M Tris-buffered saline solution and incubated overnight at 4 °C with the following primary antibodies: mouse anti-CD95 monoclonal antibody (BD Pharmigen Biosciences, San José, CA, USA) at 1:1000 dilution or rabbit anti-FasL polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:1000 dilution. After three washings, appropriate biotinylated secondary antibodies were incubated at room temperature for 1 h. Then, the blots were washed three times and streptavidin–biotin conjugate (Zymed Laboratories Inc., Invitrogen, Carlsbad, CA, USA) was added. Detection was performed using 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 other proteins. The band intensities were quantified using an

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Image Capturer EC3 Imaging System, Launch Visionworksls software in order to obtain the relative expression of proteins.

2.9. Spectrophotometric assay of mitochondrial membrane permeability (swelling)

Isolated intestinal mitochondria (3 mg protein) were incubated in 3 mL of respiratory buffer (0.1 M NaCl, 10 mM MOPS, 1 mM glutamate, 1 mM malate pH 7.4) for 10 min at 25 °C and monitored at 540 nm in a Beckman Coulter DU 640 spectrophotometer (Fullerton, CA, USA). Basal values of mitochondrial absorbance were measured for 5 min and the optical density was followed for 5 more min, after addition of increasing concentrations of MEN (0.1–10 mM), QT (0.5–5 μM) or both MEN plus QT (Pastorino et al., 1993).

2.10. Statistics

Data are given as means ± SE. Comparisons between multiple groups were performed with one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. Differences were considered statistically significant at $P<0.05$. All the analyses were carried out by using SPSS for windows advanced Statistics 8.0 SPSS Inc. (Chicago, IL, USA).

3. Results

3.1. QT blocked the inhibitory effect of MEN on chick intestinal Ca^{2+} absorption

As previously shown, the i.p. injection of 2.5 μmol of MEN/kg of b.w. to one-month old chicks inhibited the intestinal Ca^{2+} absorption after 30 min of treatment. When i.l. 50 μM QT was administered simultaneously with i.p. MEN injection, the intestinal Ca^{2+} absorption was similar to the control values. QT alone did not either modify the chick intestinal Ca^{2+} absorption (Fig. 1A).

3.2. QT avoided the GSH depletion provoked by MEN in chick intestine

Normal levels of GSH are necessary to accomplish adequate intestinal Ca^{2+} absorption in chicks (Tolosa de Talamoni et al., 1996). We have observed that MEN consumes GSH in its redox cycle generating ROS production, which leads to oxidative stress and alteration of the intestinal Ca^{2+} absorption (Marchionatti et al., 2003). In agreement, the present study shows that GSH levels from chick enterocytes were depleted by 500 μM MEN added to the incubation medium. The concomitant treatment of 50 μM QT avoided the GSH depletion caused by MEN. However, intestinal total GSH levels were not altered by QT alone (Fig. 1B). When 50 μM QT was added to the incubation medium 30 min after MEN addition for either 30 or 60 min, the GSH levels were similar to those from control enterocytes (data not shown).

3.3. QT and MEN affected the activities of the enzymes involved in the antioxidant system in chick intestine

In order to find out whether the maintenance of the redox state was involved in the mechanism by which QT avoided the inhibitory effect of intestinal Ca^{2+} absorption caused by MEN, we determined the activities of SOD and GPX, enzymes of the antioxidant system. Either 500 μM MEN or 50 μM QT *in vitro* treatment increased the SOD activity from chick enterocytes. The combined *in vitro* treatment also increased the SOD activity from enterocytes, but this enhancement was not statistically different from that produced by each drug individually. In contrast, the increment of GPX activity produced by 500 μM MEN in enterocytes was blocked by simultaneous treatment with 50 μM QT. The flavonol alone did not modify the GPX activity, which was almost identical to

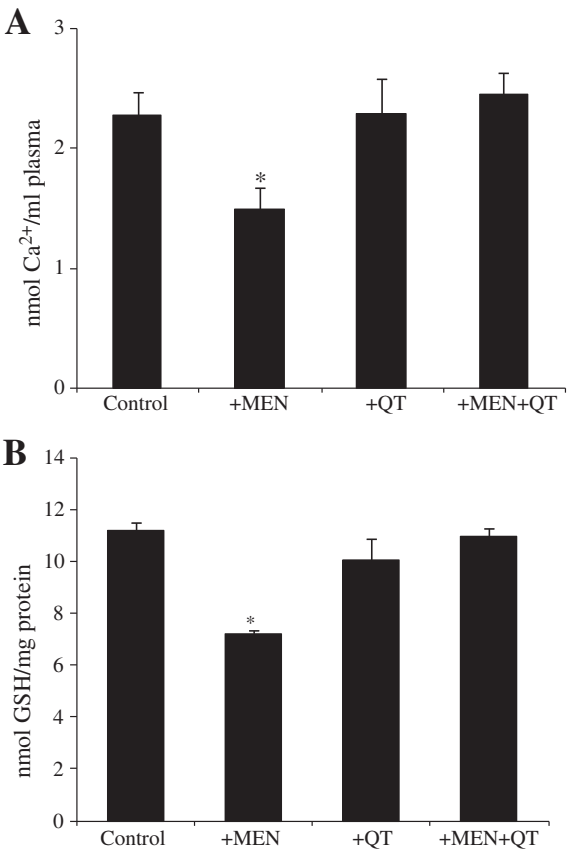

Fig. 1. Effects of MEN and QT on intestinal Ca^{2+} absorption and GSH content in chicks. A: Intestinal calcium absorption in chicks. MEN: 2.5 μmol/kg b.w. was i.p. injected 30 min before sacrifice. QT: 1 mL of 50 μM was i.l. injected in the duodenal sac for 30 min MEN + QT: 2.5 μmol MEN/kg b.w. was i.p. injected simultaneously with i.l. injection of 1 mL of 50 μM QT in the duodenal sac for 30 min. Absorption was defined as appearance of $^{45}Ca^{2+}$ in the blood. Values are expressed as means ± SE. Every mean corresponds to 7 different determinations. B: Glutathione (GSH) content from isolated enterocytes of chicks. MEN concentration: 500 μM. QT concentration: 50 μM. Incubation time: 30 min. Total GSH content was assayed in supernates from homogenates of enterocytes at 420 nm. Values are expressed as means ± SE. Each value corresponds to a pool of 3–4 chick intestines. Means correspond to 9 different determinations. * $P<0.05$ *vs* Control.

that found in control enterocytes (Table 1). Similar results were obtained when both drugs were incubated together for either 30 or 60 min (data not shown).

3.4. Apoptosis mediated by FasL/Fas/caspase-3 pathway and changes in mitochondrial permeability produced by MEN were abrogated by QT in chick intestine

Cell apoptosis is generally thought to play a central role in tissue injury in many pathological conditions. Among the biochemical features that characterize the apoptosis could be mentioned changes in the permeability of the mitochondria, caspase activation and alterations in the expression of pro- and anti-apoptotic molecules. To know whether apoptotic molecules involved in the extrinsic pathway could have a role in the inhibition of the intestinal Ca^{2+} absorption, Fas and FasL expressions were determined in enterocytes from chicks treated with MEN, QT or both. Western blot analysis reveals that either Fas or FasL protein expressions were augmented by 500 μM MEN, which was blocked by the concomitant 50 μM QT treatment. Fas and FasL expressions were not changed by the flavonoid alone (Fig. 2A and 2B).

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Table 1
Effect of menadione (MEN) and quercetin (QT) on antioxidant enzyme activities from isolated enterocytes of chick intestine.

	SOD U/mg of prot		GPX mU/mg of prot	
	n	Mean±SE	n	Mean±SE
Control	6	0.80±0.02	7	49.15±1.27
+MEN	5	1.37±0.06*	7	66.79±4.04*
MEN+QT 30 min	5	1.73±0.01*	5	43.33±1.38
+QT 30 min	5	1.42±0.02*	4	50.44±1.11

MEN concentration: 500 μM. QT concentration: 50 μM. Values are expressed as means±SE. Each value corresponds to a pool of 3–4 chick intestines. Every mean corresponds to "n" different determinations. * $P<0.05$ *vs* Control.

Caspase-3, an executive caspase involved in both extrinsic and intrinsic pathways of apoptosis, showed a similar pattern of activity; in other words, the caspase-3 activity was enhanced by 500 μM MEN, was not modified by 50 μM QT alone, but QT avoided the increase in the enzyme activity caused by the quinone (Fig. 3).

To find out if alterations in the mitochondrial membrane permeability could also be involved in the inhibition of the intestinal Ca^{2+} absorption provoked by MEN, 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 from enterocytes of control chicks were exposed to MEN (0.1–10 mM), QT (0.5–5 μM) or to both drugs at the same time (10 mM MEN and 0.5–5 μM QT). A decrease in the absorbance was observed after MEN addition to the mitochondrial incubation, showing this effect dose dependency. QT by itself slightly modified the absorbance of the mitochondrial suspensions. However, the co-treatment avoided the alterations in the absorbance caused by MEN (Fig. 4).

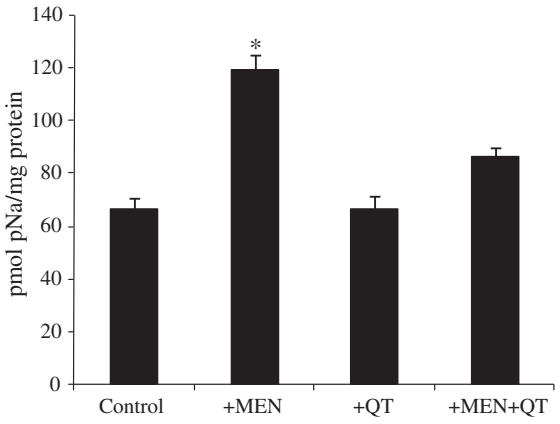

Fig. 3. Effects of MEN and QT on caspase-3 activity in chick enterocytes. MEN concentration: 500 μM. QT concentration: 50 μM. Incubation time: 30 min. Caspase-3 measurements were accomplished in supernates from homogenates of enterocytes at 405 nm. Values are expressed as means±SE. Each value corresponds to a pool of 3–4 intestines. Means correspond to 4 different determinations. * $P<0.05$ *vs* Control.

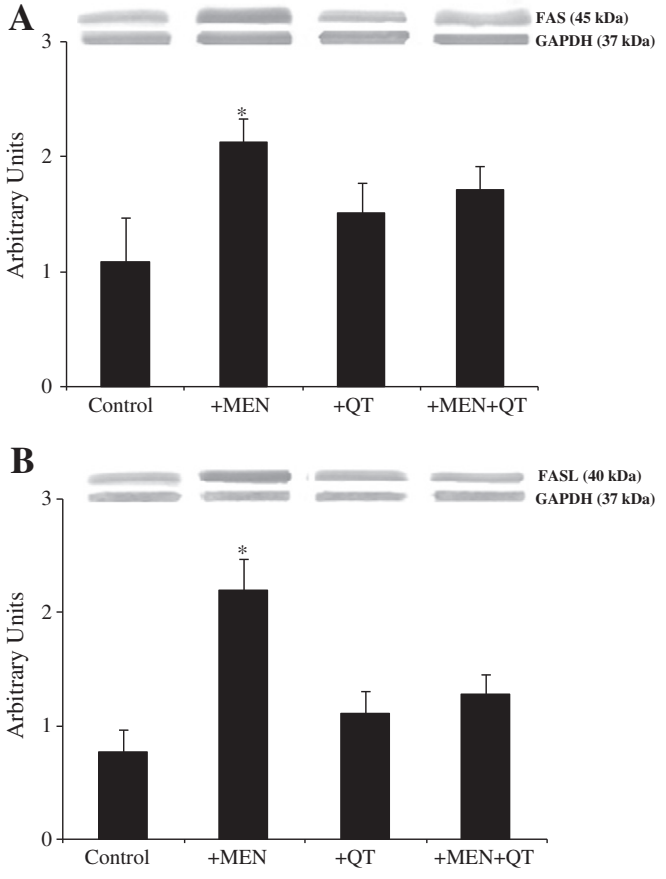

Fig. 2. Effects of MEN and QT on Fas and FasL expressions in chick enterocytes. (A) Fas and (B) FasL expressions from isolated enterocytes of chick duodenae were analyzed by Western blots. MEN concentration: 500 μM, QT concentration: 50 μM. Incubation time: 30 min. Values are expressed as means±SE. Each value corresponds to a pool of 3–4 intestines. Means correspond to 3 different determinations. * $P<0.05$ *vs* Control.

4. Discussion

Since considerable evidence indicates that oxidative damage contributes to the development of many diseases in humans and animals, the apparent disease-protective effects of QT or other flavonoids are extensively studied. The present data show that the flavonol QT abrogates the inhibitory effect of MEN on chick intestinal Ca^{2+} absorption. Some of the molecular mechanisms by which QT exhibits this protection appear to be: 1) restoration of intestinal redox state, 2) blockade of changes in the mitochondrial membrane permeability, 3) abolition of the FasL/Fas/caspase-3 cascade activation.

Accumulating proofs from our laboratory suggest that the intestinal Ca^{2+} absorption is a sensitive process to the oxidative stress (Marchionatti et al., 2001; Marchionatti et al., 2008). MEN is a quinone that undergoes a one-electron reduction to yield the corresponding

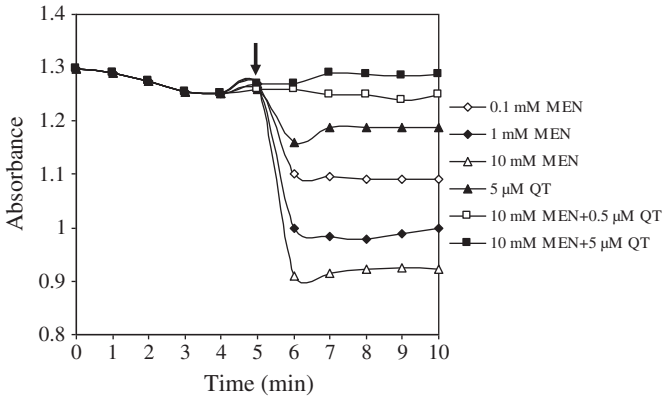

Fig. 4. Effect of MEN and QT in the mitochondrial membrane permeability. Optical density changes (swelling) (lowest OD = 0.92, highest O.D = 1.36) in isolated mitochondria from a pool of enterocytes from 7 chick intestines in each determination. Mitochondria were incubated with different concentrations of MEN, QT or both. Arrow: time of treatment addition. Values are expressed as means. Each mean corresponds to 6 different determinations.

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semiquinone. When the semiquinone radicals oxidize by molecular oxygen, a regeneration of the parent compound occurs and the production of superoxide anions yields H_2O_2 via enzymatic or spontaneous dismutation (Aherne and O'Brien, 2000). Besides, MEN in its redox cycle depletes GSH and activates the enzymes from the antioxidant system. In other words, the balance oxidation–reduction is altered and the oxidative stress is triggered leading to mitochondrial dysfunction (Marchionatti et al., 2008). Ca^{2+}-ATPase and other molecules presumably involved in the transcellular Ca^{2+} transport are inhibited not only by the oxidative stress, but also by inhibition of oxidoreductase activities from the Krebs cycle. This effect would lead to alter the electron transport chain functioning and the ATP production that is essential for the maintenance of the proper Ca^{2+} movement, process that occurs by an active transport. QT addition to the enterocytes avoids the GSH depletion caused by MEN, returning the tripeptide to normal levels. QT has been found to increase the expression of the rate limiting enzyme in the GSH synthesis by transactivation of γ-glutamylcysteine synthetase catalytical subunit promoter (Myhrstad et al., 2002), with a concomitant enhancement in the intracellular GSH concentration (Moskaug et al., 2005). Our data suggest that GSH depletion provoked by MEN might promote QT-induced GSH synthesis since QT alone does not increase the intestinal GSH content when tripeptide levels are adequate. Another possibility might be that QT detoxifies MEN by interacting with cellular defense systems such as those from phase I (CYP450 complex enzymes) or II (glutathione transferases and glucuronyl transferases) (Raucy, 2003). Superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidases (GPX) are important enzymes of the antioxidant defense system (Bouayed and Bohn, 2010). SOD decomposes superoxide radicals $O_2^{\bullet -}$ and produces H_2O_2, which is subsequently transformed into water by CAT or by GPX oxidizing GSH (Dröge, 2002; Lee and Choi, 2003). MEN increases the intestinal SOD and GPX activities. The latter enzyme also contributes to the GSH depletion provoked by the quinone. The restoration of GPX activity to the control values by QT could reinforce the returning of GSH levels to control ones. In contrast, SOD activity shows a tendency to be higher in enterocytes exposed to both MEN *plus* QT as compared to MEN alone. SOD participates in the first line of the antioxidant defense. It could occur that the reversion of the GSH depletion and the high SOD activity caused by QT contribute to the scavenger of the ROS production generated by MEN, which could block the MEN-induced apoptosis.

The relationship between oxidative stress and apoptosis is complex. There are several possibilities by which oxidative stress induces cell death such as activation of proapaptotic signaling pathways, perturbation of mitochondrial function and p53 gene expression (Laux and Nel, 2001). Regarding MEN action, we have previously shown that the quinone causes ROS production, DNA fragmentation and cytochrome c release from the mitochondria (Marchionatti et al., 2008). The present data suggest that the FasL/Fas/caspase-3 pathway is also involved in the MEN-induced apoptosis in chick intestine, which may also contribute to the inhibition of the intestinal Ca^{2+} absorption. The participation of the FasL/Fas system has been also observed in Jurka human T cell lines exposed to MEN (Caricchio et al., 1999; Laux and Nel, 2001). Caricchio et al. (1999), have demonstrated that the FasL/Fas involvement was linked to oxidative stress. Similarly, the high expression of either FasL or Fas in the chick intestine produced by MEN was associated with low levels of intracellular GSH. Furthermore, the repletion of GSH generated by QT treatment after MEN administration was accompanied by returning of the FasL and Fas to the control levels.

Caspase-3 constitutes a major pool of executor caspases. The enhancement in the catalytic activity of caspase-3 suggests that this enzyme is involved in the MEN induced injury in the intestine, effect that seems to be abolished by QT, but the flavonol alone does not modify the enzyme activity. Similarly, QT protected the H9c2 cardiomyoblast cells from H_2O_2-induced apoptosis by abolition of caspase-3 activation (Park et al., 2003). In granulose cells from chicken ovarian follicles, QT has been also found to attenuate cadmium-induced oxidative damage by inhibiting caspase-3 activity and other proapoptotic molecules (Jia et al., 2011).

Although the physiological intraluminal QT concentration is uncertain, it has been calculated that 500 mg QT *per os* in humans could result in mucosal concentrations of up to 200 μM QT (Amasheh et al., 2008). In our study, we used i.l. 50 μM QT, a concentration known to be much lower than its toxic concentration and to protect against oxidative stress induced by MEN in cardiomyoblasts (Park et al., 2003). Wätjen et al. (2005) have reported that QT has protective effects on H4IIE hepatoma cells against H_2O_2-induced citotoxicity, DNA strand breaks and apoptosis at concentrations as low as 10–25 μM. In contrast, doses higher than 50 μM QT produced DNA damage and apoptosis on those cells. De Marchi et al. (2009)) have demonstrated an ambivalent redox character of polyphenols, including QT, which can act either as an inhibitor or an inducer of alterations in the mitochondrial membrane permeability. The mitochondrial behavior of chick enterocytes indicates that in the range 0.5–5 μM QT, the flavonol blocks change in the mitochondrial membrane permeability induced by MEN. Since QT avoids GSH depletion caused by MEN, it is quite possible that the simultaneous treatment of QT and MEN does not affect the critical thiol groups, which seem to be involved in the mitochondrial pore opening (Chernyak and Bernardi, 1996).

Taken together, QT may be useful in preventing inhibition of intestinal Ca^{2+} absorption caused by MEN or other substances that deplete GSH leading to oxidative stress and apoptosis. This not only advances our understanding about the regulation of intestinal Ca^{2+} homeostasis under conditions of oxidative stress, but may also contribute to considering new therapeutic strategies during intestinal inflammation that accompanies the inflammatory bowel disease or postinfectious irritable bowel syndrome, all conditions that affect the intestinal Ca^{2+} absorption and other intestinal functions. Furthermore, these findings also offer new insight for improving and expanding QT applications.

Acknowledgments

This work was supported by Grants from FONCYT (PICT 2005–32464), CONICET (PIP 2010–12) and SECYT (UNC), Argentina. Prof. Dr. Nori Tolosa de Talamoni is a Member of Investigator Career from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). All authors participated in the conception, design and performance of the study as well as interpretation of data and drafting the manuscript. The authors thank to the students Martina Zuñiga Oliva and Milagros Nicollier for their help with some determinations. None of the authors had a personal conflict of interest.

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Please cite this article as: Marchionatti, A.M., et al., Effects of quercetin and menadione on intestinal calcium absorption and the underlying mechanisms, Comp. Biochem. Physiol., A (2012), http://dx.doi.org/10.1016/j.cbpa.2012.09.007