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Mitochondrial dysfunction is responsible for the intestinal calcium absorption inhibition induced by menadione

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Abstract

Menadione (MEN) inhibits intestinal calcium absorption by a mechanism not completely understood. The aim of this work was to find out the role of mitochondria in this inhibitory mechanism. Hence, normal chicks treated with one i.p. dose of MEN were studied in comparison with controls. Intestinal calcium absorption was measured by the in situ ligated intestinal segment technique. GSH, oxidoreductase activities from the Krebs cycle and enzymes of the antioxidant system were measured in isolated mitochondria. Mitochondrial membrane potential was measured by a flow cytometer technique. DNA fragmentation and cytochrome *c* localization were determined by immunocytochemistry. Data indicate that in 30 min, MEN decreases intestinal Ca^{2+} absorption, which returns to the control values after 10 h. GSH was only decreased for half an hour, while the activity of malate dehydrogenase and α-ketoglutarate dehydrogenase was diminished for 48 h. Mn^{2+}-superoxide dismutase activity was increased in 30 min, whereas the activity of catalase and glutathione peroxidase remained unaltered. DNA fragmentation and cytochrome *c* release were maximal in 30 min, but were recovered after 15 h. In conclusion, MEN inhibits intestinal Ca^{2+} absorption by mitochondrial dysfunction as revealed by GSH depletion and alteration of the permeability triggering the release of cytochrome *c* and DNA fragmentation.

Keywords: Intestine; Mitochondria; Menadione; Calcium absorption; Apoptosis

1. Introduction

Intestinal Ca^{2+} absorption occurs by two different transport processes named paracellular and transcellular pathways [1]. The latter one is finely regulated by diverse hormones and nutrients, being $1,25(OH)_2D_3$ or calcitriol the major regulator [2]. Calcitriol regulation is altered by intestinal glutathione (GSH) depletion caused either by DL-buthionine-*S*,*R*-sulfoximine (BSO) or menadione (MEN) administration, as previously reported [3,4]. The effect of MEN on intestinal Ca^{2+} absorption is acute, transient, and is accompanied by an increment of antioxidant defenses such as superoxide dismutase (SOD) and catalase (CAT) activities in the enterocyte cytoplasm. In contrast, chronic MEN administration neither affects the intestinal Ca^{2+} absorption nor the activity of those antioxidant enzymes [5]. This is in agreement with studies performed by Chiou et al. [6], who have found that the acute cardiac and renal toxicity of MEN in rats is more severe than the cumulative toxicity.

MEN or vitamin K_3 is a synthetic compound that is applied in anticancer therapy and in the treatment of osteoporosis [6,7]. MEN metabolism involves redox cycling, which results in the release of various oxygen species, such as superoxide anions and hydroxyl free radicals [8,9]. The mechanisms involved in the inhibition of intestinal Ca^{2+} absorption by MEN are not completely known, but we have demonstrated that MEN triggers an important signal of hydroxyl free radicals in the enterocytes treated in vitro with the quinone [4].

Toxic MEN effects through apoptotic mechanisms have been shown in several tissues. Caricchio et al. [10] have demonstrated that MEN provokes apoptosis of Jurka human T cell lines through activation of the Fas/Fas ligand system. In acinar

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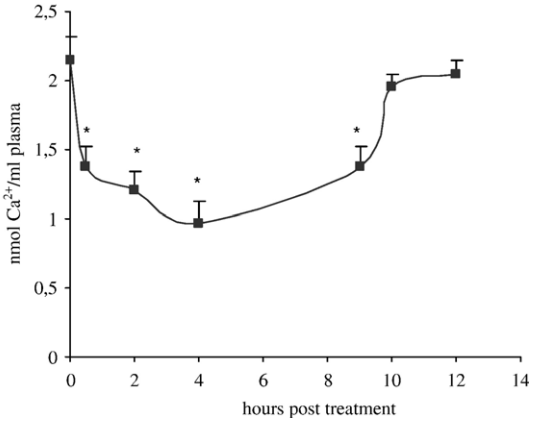

Fig. 1. Time effects of MEN administration on intestinal Ca^{2+} absorption. One milliliter of 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, containing 1.85×10^5 Bq $^{45}Ca^{2+}$, pH 7.2, was introduced into the lumen of the ligated intestinal segment from each individual chick. After half an hour, plasma ^{45}Ca was measured in a liquid scintillation counter. Absorption was defined as the appearance of $^{45}Ca^{2+}$ in the blood. Dose: 2.5 μmol MEN/kg of b.w. Values are the means± . standard errors of samples from 6 animals each. *$p<0.05$ for comparison with control.

cells from pancreas, MEN requires intracellular Ca^{2+} release and induction of the permeability transition pore in the mitochondria to initiate the process of apoptosis [11]. The opening of the permeability transition pore is thought to be favored by oxidative stress via oxidation of intracellular GSH and other sulfhydryl groups [12]. Toxic exposures to MEN have also produced a marked reduction in both pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (α-KGDH) activities, followed by diminished mitochondrial transmembrane potential prior to murine cortical neuronal death [13].

The role of intestinal mitochondria in the inhibitory effect of MEN on intestinal Ca^{2+} absorption is unknown. However, it is quite possible that intestinal mitochondria could be involved because they are considered the major source of ROS [14], which originate as subproducts from the electron transport chain. There is also a GSH pool in the mitochondria, independent of the cytosolic GSH pool [15], which could be altered by MEN changing the thiol redox state and, consequently, modifying the mitochondrial metabolism. Besides, an independent system of mitochondrial antioxidant enzymes could counteract the quinone effects on the redox couples. Based on these considerations, the aims of this study were, first, to establish the time dependence of the inhibitory effects of MEN on intestinal Ca^{2+} absorption; second, to investigate whether intestinal mitochondria metabolism is altered by MEN; third, to determine the possible participation of apoptotic mechanisms activated by mitochondria under quinone treatment; and finally to find out the responses of the mitochondrial antioxidant mechanisms triggered by MEN.

Table 1
Effect of MEN administration on malate dehydrogenase and α-ketoglutarate dehydrogenase activities from chick intestinal mitochondria

	mMDH U/mg protein	α-KGDH mU/mg protein
Control	1.19±0.03 (9)	27.76±0.90 (9)
+MEN 0.5 h	1.01±0.03 (7)*	19.16±1.89 (7)*
+MEN 1 h	0.97±0.04 (9)*	20.32±1.30 (9)*
+MEN 15 h	1.03±0.45 (7)*	14.48±2.33 (5)*
+MEN 48 h	0.91±0.08 (5)*	19.54±1.36 (5)*

Chicks were injected i.p. with 2.5 μmol MEN/kg b.w. Values are means±S.E. ( )= number of determinations in different pools from 3 chick duodenal mucosa each. *$p<0.05$ vs. control.

Table 2
Effect of MEN administration on total glutathione content and superoxide dismutase, catalase and glutathione peroxidase activities from chick intestinal mitochondria

	mGSH nmol/mg protein	Mn^{2+}-SOD U/mg protein	mCAT U/mg protein	mGPx U/mg protein
Control	2.87±0.16 (9)	0.54±0.08 (6)	8.67±0.46 (6)	0.17±0.01 (4)
+MEN 0.5 h	1.84±0.27 (9)*	2.38±0.19 (5)*	6.90±0.27 (4)	0.21±0.02 (4)
+MEN 1 h	2.29±0.18 (6)	0.81±0.12 (5)	8.50±0.15 (6)	0.22±0.02 (4)
+MEN 15 h	2.88±0.66 (5)	0.80±0.28 (5)	9.02±0.31 (4)	0.24±0.03 (4)

Chicks were injected i.p. with 2.5 μmol MEN/kg of b.w. Values are means±S.E. ( )= number of determinations in different pools from 3 chick duodenal mucosa each. *$p<0.05$ vs. control.

2. Materials and methods

2.1. Animals

One-day-old Cobb Harding chicks (*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 divided into two groups: a) normal chicks (controls), and b) normal chicks treated i.p. with 2.5 μmol of MEN/kg of b.w. at different times as indicated in the Results section. The protocol was conducted according to the Guide for Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. They were killed by cervical dislocation and the excised duodenae were rinsed with cold 0.15 mol/L NaCl and enterocytes or intestinal mitochondria were isolated as described below.

2.2. Intestinal Ca^{2+} absorption

Chicks were laparotomized under ether anesthesia and a 10 cm segment of duodenum was ligated following the technique previously described [3]. One milliliter of 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, containing 1.85×10^5 Bq $^{45}Ca^{2+}$, pH 7.2, was introduced into the lumen of the ligated intestinal segment. 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.

2.3. Mitochondrial isolation

Mitochondria were isolated from intestinal mucosa of both groups of animals by differential centrifugation as described previously [16]. Protein was determined by the method of Gornall et al. [17].

2.4. Spectrophotometric procedures

All the enzyme activities were assayed in supernatants of mitochondrial extracts prepared by freezing and thawing the mitochondria three times. The activity of α-KGDH was assayed in supernates of mitochondrial extracts containing 1 mM dithiothreitol and 50 μL of chicken serum/mL buffer. The assay medium contained 50 mM MOPS, pH 7.0, 1 mM NAD, 1 mM $MgCl_2$,