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Effect of the H, K–ATPase inhibitor, esomeprazole magnesium, on gut total antioxidant capacity in mice

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Abstract

Antioxidant depletion is believed to be a mechanism involved in the pathophysiology of several upper gastrointestinal disorders, and H, K–ATPase inhibitors can alter free radical production by neutrophils. We hypothesized that the H, K–ATPase inhibitor esomeprazole magnesium would decrease gut free radical production with a concomitant increase in gut total antioxidant capacity. A/J mice (n = 10/group) received either vehicle (control) or one of three concentrations of esomeprazole magnesium in vehicle by once-daily gavage for 10 days. Using tissue extracts from stomach and colon, total antioxidant capacity, lipid peroxide levels, and constitutive Cu/Zn–superoxide dismutase were measured using validated assays. There was a dose-related increase in total antioxidant capacity (analysis of variance, $P <$ 0.001) in stomach, but there was no change in the colon. In the assessment of free radical production, there was a trend toward decreased lipid peroxide levels in stomach from mice receiving esomeprazole. In stomach, Cu/Zn–superoxide dismutase activity was increased (ANOVA: p=.03) in mice receiving esomeprazole. In conclusion, gastric total antioxidant capacity and Cu/Zn–superoxide dismutase activity are increased by esomeprazole, and these changes may result in part from decreased free radical production. The present results support the notion that the pharmacological effects of this agent on upper intestinal tissue are more complex than previously thought, and appear to involve both enzymatic and nonenzymatic tissue antioxidants. © 2004 Elsevier Inc. All rights reserved.

Keywords: Antioxidant; Superoxide dismutase; Mice; H; K–ATPase inhibitor; Esomeprazole; Stomach

1. Introduction

Free radical production and tissue micronutrient antioxidant depletion have emerged as common mechanisms in the pathophysiology of several upper intestinal diseases. Preliminary evidence has shown that *Helicobacter pylori (H. pylori)* in humans is associated with increased free radical production and decreased gastric nonenzymatic antioxidant levels [1–5]. A chemiluminescent study has shown increased reactive oxygen species in stomach from patients with *H. pylori* gastritis, which then decreased after successful eradication therapy [2]. In an in vitro study [5], Beil et al. showed that *H. pylori* reduced cellular concentrations of glutathione in the absence of cell necrosis, suggesting that *H. pylori* may be a source for production of reactive oxygen metabolites. A previous study had demonstrated depletion of zinc–superoxide dismutase in esophagus from patients with active reflux esophagitis [6].

Several groups have begun to investigate potential antioxidant properties of the H, K–ATPase inhibitor omeprazole. Omeprazole itself has also been shown to react with hypochlorous acid in vitro [7]. In mice, Kawamura et al. [8] have shown that omeprazole suppresses superoxide production by neutrophils. Using polymorphonuclear neutrophils from humans, Wandall has reported that omeprazole inhibited superoxide anion generation [9]; however, omeprazole did not scavenge superoxide anion generated in a cell free system. Suzuki et al. [10] examined the effects of omeprazole on human neutrophils both in vivo in volunteers and in vitro on isolated cells. In these two systems, omeprazole appeared to inhibit in a dose-dependent fashion the production of oxygen-derived free radicals by neutrophils. In a recent study, Biswas et al. showed that omeprazole blocks the generation of hydroxyl radical in a rat model [11].

In a previous study using an acute, 10-day mouse model, we observed a marked increase in gastric and colonic total

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antioxidant capacity induced by supplementation with omeprazole [12]. Omeprazole however had no apparent effect on tissue levels of glutathione [12]. In clinical studies, an isomer of omeprazole, esomeprazole magnesium, has been recently shown to be more effective in the treatment of upper intestinal disorders [13].

Based on these studies, we hypothesized that esomeprazole magnesium would decrease free radical production in the gut, as estimated by gut lipid peroxide levels, and would therefore increase gut total antioxidant capacity. In this 10-day acute study, we examined the effects of increasing doses of esomeprazole magnesium on total antioxidant capacity, lipid peroxide levels, and Cu/Zn–superoxide dismutase activity in murine stomach and colon.

2. Methods and materials

2.1. Preparation of the animal model

All in vivo animal studies were performed at the Durham Veterans Affairs Medical Center and Duke University; permission for these studies was granted by the Animal Studies Subcommittee of the Durham Veterans Affairs Medical Center. Mice of the A/J strain, 1 month of age, were obtained from Jackson Laboratories (Bar Harbor, ME). A/J inbred mice were used because this strain appears to be resistant to development of diseases associated with oxidative stress and the A/J mouse strain has a stable tissue antioxidant content. These effects are expected to reduce the chance of misinterpretation of data.

Mice were raised on an experimental diet comprising 45.5% fat, 16.5% protein, and 38.1% complex carbohydrates (Research Diets, Inc., New Brunswick, NJ). This diet meets the American Institute Nutrition requirements for mice with regard to mineral and vitamin content, and mimics the high-fat diet of the American public.

In this 10-day supplementation experiment, mice (n = 10/group) received the following: 1) as a control group, only the vehicle for dissolution of esomeprazole (PEG-400) given orally once daily by gastric gavage; 2) esomeprazole 0.5 mg/kg in an equivalent volume of PEG-400 given orally once daily by gastric lavage; 3) esomeprazole 5 mg/kg in an equivalent volume of PEG-400 given orally once daily by gastric lavage; and 4) esomeprazole 50 mg/kg in an equivalent volume of PEG-400 given orally once daily by gastric lavage. In previous rodent studies, 44 mg/kg/day was the minimal dose of omeprazole shown to produce maximal acid suppression [14].

At 10 days, all mice were killed by injection of Nembutal (Abbott Laboratories, Inc., North Chicago, IL 60064) (5 mg/100 g body weight) before procurement of the stomach and colon. After trisection all tissues were frozen on dry ice and stored at −76°C until extraction for assays.

2.2. Total antioxidant capacity

Extraction of tissue samples for total antioxidant capacity was performed using the method of Pellegrini et al. [15] as we have previously described [16]. Each tissue layer was homogenized in ice-cold 4.31% sulfosalicyclic acid solution containing 0.25% EDTA. After centrifugation for 10 minutes at 4°C at 20,000 × g, the supernatant was frozen at −76°C. Total antioxidant capacity was determined in the supernatant [15,16]. This assay is based on the absorbance of 2,2′azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS radical cation) using Trolox, a water-soluble analogue of α-tocopherol, as an external standard. The absorbance recorded at 734 nm after 6 minutes is proportional to the antioxidant capacity. The total antioxidant capacity value in a sample is then estimated as Trolox-Equivalent Antioxidant Capacity.

2.3. Lipid peroxide measurement

Reactive oxygen-derived metabolites in each specimen were estimated by determining lipid peroxide levels using a modified version of the Yagi method [17], as described by our group [18]. Tissues were homogenized in 20 mmol/L Tris HCl buffer (pH 7.4) at 4°C. Supernatant from centrifuged homogenate was frozen at –76°C before determination.

2.4. Cu/Zn–superoxide dismutase activity

Cu/Zn–superoxide dismutase activity was determined using a spectrophotometric assay [19] obtained from Cal-Biochem-Novabiochem AG (Darmstadt, Germany). Briefly, in this assay, each tissue was homogenized in absolute ethanol:chloroform (V/V: 62.5/37.5) at 4°C, and then centrifuged at 3000 × g for 2 minutes. Supernatant was frozen and stored at −76°C before assay. In this assay, sample is incubated for 1 minute at 37°C with a mercaptan scavenger in buffer (2-amino-2-methyl-1, 3-propanediol, 50 mmol/L, with 3.3 nmol/L boric acid and 0.11 mmol/L DTPA, pH = 8.8); a chromogenic reagent is added and change in absorbance at 525 nm at 10-second intervals for 30 seconds is determined. As a blank control, buffer was used in duplicate samples with the chromogenic reagent to determine the change in absorbance at 525 nm at 30 seconds. Cu-Zn–superoxide dismutase activity was determined from the ratio of rate of sample to average rate of blank.

2.5. Statistical analysis

Total antioxidant capacity, Cu/Zn–superoxide dismutase activity, and lipid hydroperoxide levels were expressed per gram of wet tissue. Mean values and standard errors were calculated for each group of results using a standardized computer program (StatView, SAS Institute Inc., Cary, NC). Results from the four groups of mice were examined

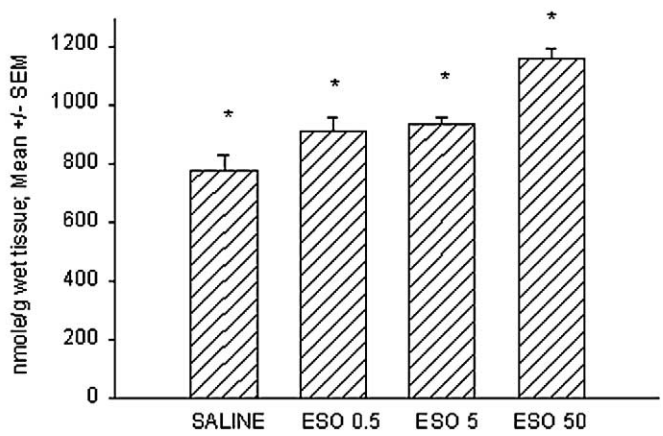

Fig. 1. Supplementation with esomeprazole magnesium (ESO) at 0.5-, 5 -, and 50-mg/kg/day and gastric total antioxidant capacity in mice. Gastric total antioxidant capacity was significantly increased (*$P < 0.001$ by analysis of variance) by treatment with increasing doses of esomeprazole.

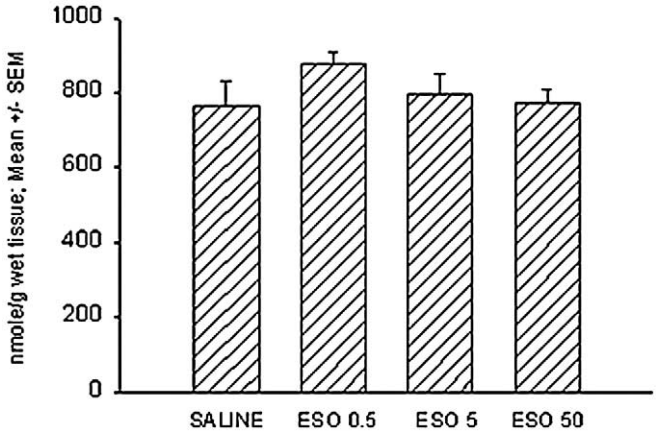

Fig. 2. Supplementation with esomeprazole magnesium (ESO) at 0.5, 5, and 50 mg/kg/day and colonic total antioxidant capacity in mice. Colonic total antioxidant capacity was not significantly changed by treatment with esomeprazole.

by one-way analysis of variance (ANOVA). The Fisher PLSD (Protected Least Significant Difference) and Scheffé F test were then used for multiple comparisons within the four groups of mice. A probability value of $P \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Total antioxidant capacity

As shown in Fig. 1, there was a distinct dose–response effect on total antioxidant capacity of the stomach induced by increasing concentrations of esomeprazole magnesium (ANOVA, $P < 0.001$). At 10 days, the 50-mg/kg/day dose of esomeprazole increased gastric total antioxidant capacity by 50%. According to the Fisher PLSD test, each of the three doses of esomeprazole produced increased gastric total antioxidant capacity (for each test, $P < 0.05$) compared to the control animals. By contrast, total antioxidant capacity in colon was similar in all four groups (Fig. 2).

3.2. Lipid peroxide levels

As shown in Fig. 3, there was a trend toward decreased lipid peroxide levels in the stomach of mice treated with esomeprazole magnesium (ANOVA, $P = 0.14$). By comparing the control group to the group receiving esomeprazole at 0.5 mg/kg/day, the Fisher PLSD statistic revealed a value of $P = 0.07$. By comparing the control group to the group receiving esomeprazole at 50 mg/kg/day, Fisher PLSD testing also revealed a value of $P = 0.07$; these results suggest that esomeprazole could decrease free radical production in the stomach. However, lipid peroxide levels in the colon were similar in all four groups (Fig. 4).

3.3. Cu/Zn–superoxide dismutase activity

As shown in Fig. 5, Cu/Zn–superoxide dismutase activity was increased in the stomach of mice treated with esomeprazole magnesium (ANOVA, $P = 0.03$). By Fisher PLSD testing, comparison of the 0.5 mg/kg/day dose to controls produced a test statistic of $P = 0.007$; comparison of the 5-mg/kg/day dose to controls produced a test statistic of $P = 0.028$; and comparison of the 50-mg/kg/day dose to controls produced a test statistic of $P = 0.08$.

4. Discussion

This study has shown that esomeprazole magnesium supplementation in mice induced increased total antioxidant capacity of the stomach and also increased gastric Cu/Zn–

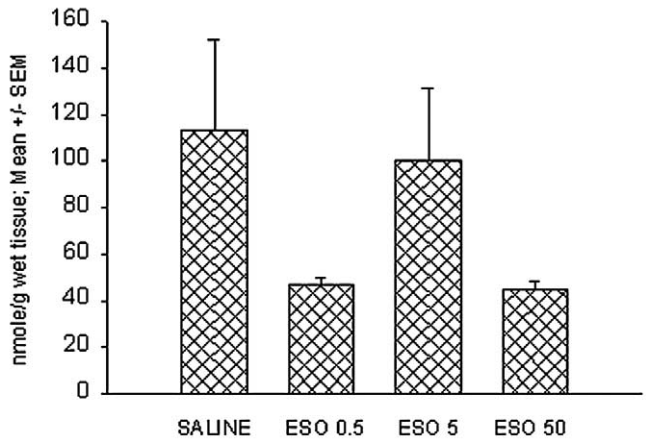

Fig. 3. Supplementation with esomeprazole magnesium (ESO) at 0.5, 5, and 50 mg/kg/day and gastric lipid peroxide levels in mice. There was a trend toward decreased lipid peroxide levels (*$P = 0.14$ by analysis of variance) by treatment with increasing doses of esomeprazole.

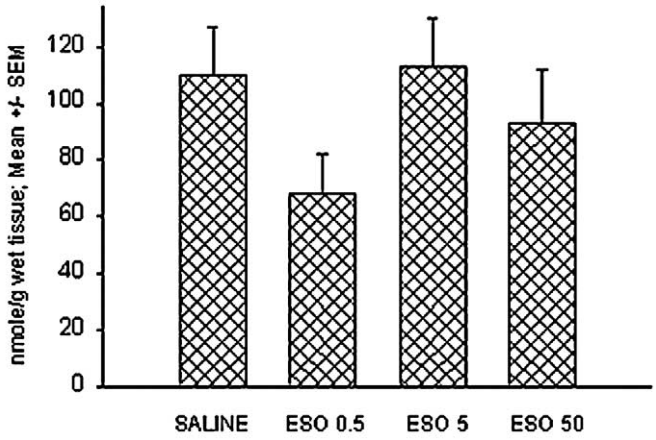

Fig. 4. Supplementation with esomeprazole magnesium (ESO) at 0.5, 5, and 50 mg/kg/day and colonic lipid peroxide levels in mice. Colonic lipid peroxide levels were not significantly changed by treatment with esomeprazole.

superoxide dismutase activity. A trend toward decreased gastric lipid peroxide levels with esomeprazole supplementation supported the view that reducing free radical production is only a partial explanation for increased gastric total antioxidant capacity induced by esomeprazole magnesium.

The pharmacological relationship between H, K–ATPase inhibitors and tissue micronutrient antioxidants is of paramount importance for at least two reasons. The first is that H, K–ATPase inhibitors are considered internationally to be a major class of therapeutic agents. The second is that understanding the effects of H, K–ATPase inhibitors on the pathophysiology of upper gastrointestinal disorders requires a better understanding of the interaction between H, K–ATPase inhibitors and the gut antioxidant system. In addition, the role of trace metals in the biological activity of H, K–ATPase inhibitors has not been fully investigated. The finding from this present study that esomeprazole magnesium increases gastric Cu/Zn–superoxide dismutase activity suggests the importance of examining the role of zinc in the biological activity of this class of agents.

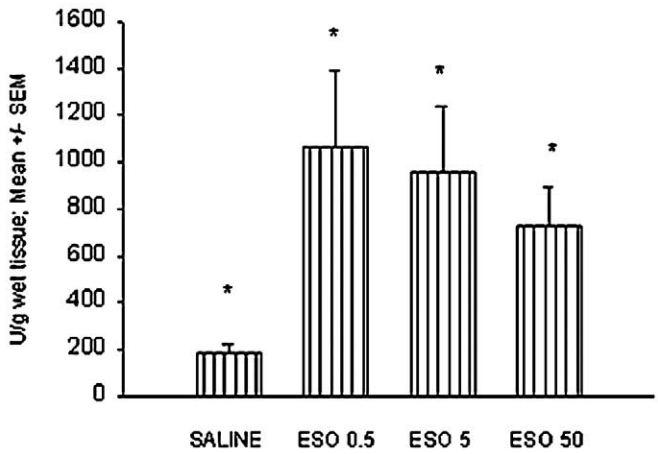

Fig. 5. Supplementation with esomeprazole magnesium (ESO) at 0.5, 5, and 50 mg/kg/day and gastric Cu/Zn superoxide dismutase levels in mice. Superoxide dismutase levels were significantly increased (ANOVA: $*P = 0.03$) by treatment with esomeprazole.

Mammalian tissues contain biochemically linked, free radical scavenging systems including enzymes (i.e., catalase), copper–zinc containing proteins (i.e., constitutive superoxide dismutase), and tissue micronutrients (i.e., ascorbate and glutathione) [20]. The relative contributions of these systems to the total antioxidant capacity in the gut is presently under investigation. A recent review has suggested that ingestion of dietary antioxidants may also play an important protective role in the gut [21].

Limited human studies examine the effects of H, K–ATPase inhibitors on either gut antioxidant levels or the bioavailability of dietary antioxidants. One report identified reduced gastric juice and serum levels of ascorbate in patients receiving omeprazole and suggested that this finding might represent a potential risk factor for development of gastric cancer [22]. This prior study is, however, difficult to interpret. It was not clear whether reduced levels of ascorbate in gastric juice in patients receiving omeprazole was related to decreased secretion of ascorbate or increased intraluminal destruction of ascorbate. In addition, the authors did not measure gastric glutathione levels even though tissue levels of ascorbate and glutathione are known to form a balance in antioxidant homeostasis [23].

A recent study has shown that under standardized conditions, the estimated total antioxidant capacity value is reflective of the individual antioxidants present in a given sample [24]. We have previously shown that omeprazole does not alter gut levels of glutathione [12]. It has also been reported that this assay for total antioxidant capacity does not include an assessment of the enzymatic component of the antioxidant activity [25]. Thus, the total antioxidant capacity measured in our study may actually underestimate the total effect of esomeprazole supplementation on the micronutrient antioxidant status of the experimental animals. Clearly, esomeprazole increases both gastric nonenzymatic and enzymatic antioxidant capacity in this murine model. Further work is required to determine the origin of increased gastric non-enzymatic total antioxidant capacity induced by treatment with esomeprazole magnesium.

Superoxide dismutases are metalloenzymes that catalyze conversion of superoxide ion into oxygen and hydrogen peroxide. The extraction method that was used in this work was not sensitive enough for the detection of mitochondrial Mn–superoxide dismutase. The potential biological importance of the increase in Cu/Zn–superoxide dismutase activity induced by esomeprazole magnesium is not presently clear. In animal models of colitis, a superoxide dismutase mimetic has been shown to reduce the appearance of diarrhea and loss of body weight [26]. By contrast, studies of the overexpression of Cu/Zn–superoxide dismutases in cell lines [27,28] support the development of pathophysiological changes including increased lipid peroxidation and predisposition to undergo apoptosis. We have recently initiated

studies of esomeprazole magnesium in human subjects to try to understand the potential importance of increases in Cu/Zn–superoxide dismutase activity and tissue micronutrients in the upper gut.

In summary, in mice, esomeprazole magnesium induced an increase in total antioxidant capacity and Cu/Zn–superoxide dismutase activity in stomach. There was a trend toward decreased lipid peroxide levels in stomach. The present results support the notion that the pharmacological effects of this agent on upper intestinal tissue are more complex than previously thought, and appear to involve both enzymatic and nonenzymatic tissue antioxidants.

Acknowledgments

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