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Endogenous zinc concentrations in cysteamine-induced duodenal ulcers in the rat

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Exogenously administered zinc compounds have been shown to possess anti-ulcer activity against a wide variety of ulcerogenic agents, both in laboratory animal models and in human peptic ulcer disease. However, a strong possibility exists that endogenous zinc may also play an important role during noxious events by various mechanisms. Therefore, the aim of this study was to focus on the changes of endogenous zinc serum and tissue concentrations in cysteamine-induced duodenal lesions. We used atomic absorption spectrophotometry to determine the tissue and serum concentrations of zinc in normal (control) rats and those with cysteamine-induced duodenal ulcers. The results obtained in this study indicated that the onset, development and spontaneous healing of ulcer lesions were associated with certain shifts in zinc serum and tissue concentrations. Prior to ulcer formation, a significant increase was noted in serum zinc values. With the onset of duodenal lesions, zinc serum concentrations significantly decreased, while there was a significant increase in duodenal tissue concentrations when compared to healthy control animals. Zinc tissue concentrations decreased and returned to starting values by the end of the first week of spontaneous healing. This decrease in zinc tissue concentration corresponded to the healing rate of the duodenal ulcers. Serum zinc concentrations also returned to starting values within the first week period. These observations indicate and confirm that zinc could play an important role in duodenal ulcer disease and represent a natural defense system in the body.

Keywords: cysteamine, duodenal ulcer, rat, zinc concentrations

Introduction

Zinc is an essential element for most organisms (Elinder 1986). Compared with most other trace elements, it is relatively non-toxic *in vivo* (Borovansky & Riley 1989), and has been shown to be a functionally essential component of more than 300 enzymes including oxydoreductases, transferases, hydrolases, lyases, isomerases and lygases (Vallee & Falchuk 1993). Zinc ions are also important for the biosynthesis and catabolism of proteins. The synthesis of DNA and RNA is controlled by this element (Cho 1989).

The protective effect of zinc on the organism led to investigations of its influence on experimental stress lesions of the gastric mucosa. Oral administration of zinc salts has been shown to enhance the rate of healing in human gastric ulcers and to protect against experimental ulcers in different animal models including gastric ulceration induced by ethanol, cold-restraint stress, electric vagal

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stimulation, reserpine and acetic acid administration (Cho *et al.* 1976, Wong *et al.* 1986, Cho 1989). Previous reports have suggested that the antiulcerative effects of zinc could be due to several mechanisms such as: stabilization of biological membrane integrity including those of lysosomes (Pfeiffer *et al.* 1987a, Cho 1989), inhibition of histamine release from mast cells within the gastric mucosa (Ogle & Lau 1979), improvement of gastric microcirculation (Lloris *et al.* 1988, Cho *et al.* 1989), an increase in the production of gastric mucus (Esplugues *et al.* 1985) and stimulation of prostaglandin biosynthesis (Navarro *et al.* 1988).

However, little is known about the activity of endogenous zinc in the gastrointestinal tract during a noxious event. The work reported in this communication examines the activity of endogenous zinc in cysteamine-induced duodenal ulcer disease in the rat and the possibility that zinc, by its accumulation in the damaged tissue, could represent a natural defense system in the organism.

Table 1. The mean ± SEM of serum and tissue zinc concentrations and ulcer index in the investigated experimental groups

Group ($N = 10$)	Serum Zn concentration ($\times 10^{-5}$ mol l^{-1})	Tissue Zn concentration ($\times 10^{-3}$ mol kg^{-1})	Ulcer index (mm^2)
Healthy control (0 h)	2.36 ± 0.63[b]	2.43 ± 0.33[b]	0
Cysteamine application			
1 h	3.22 ± 0.59[a,b]	2.35 ± 0.37[b]	0
6 h	3.46 ± 1.10[a,b]	2.53 ± 0.68[b]	0
12 h	1.82 ± 0.62[b]	2.27 ± 0.33[b]	3.5
18 h	1.67 ± 0.60[b]	2.54 ± 0.54[b]	5.6
24 h	1.22 ± 0.37[a]	3.60 ± 0.48[a]	6.3
1 week	2.07 ± 0.32[b]	2.25 ± 0.10[b]	2.5
2 weeks	2.31 ± 0.40[b]	2.04 ± 0.23[a,b]	2.3
3 weeks	2.58 ± 0.30[b]	2.44 ± 0.23[b]	2.2
4 weeks	2.88 ± 0.41[b]	2.67 ± 0.49[b]	0

[a] $P < 0.05$ versus healthy control (0 h).
[b] $P < 0.05$ versus 24 h group.

Materials and methods

Female Wistar rats (home bred) weighing 180–250 g were randomly assigned to different groups and housed in a temperature- (22 ± 1°C) and humidity (65–70%) controlled room. They were provided with standard laboratory chow and free access to water. Before testing, animals were food fasted for 24 h with access to water *ad libitum*.

Duodenal ulcers were induced by an already established noxious regimen. Cysteamine (Sigma, St. Louis, MO) was dissolved in distilled water and administered subcutaneously at a dose of 400 mg kg^{-1} body weight (Seyle & Szabo 1973).

Animals receiving cysteamine were divided into several groups and sacrificed, by decapitation, at various time points (Table 1) in order to assess the dynamics of zinc serum and tissue concentrations during the phases of the onset, development and spontaneous healing of duodenal ulcers. Animals were sacrificed 1, 6, 12, 18 and 24 h and 1, 2, 3 and 4 weeks following cysteamine application. Duodenal lesions were assessed and blood and tissue samples taken for the determination of zinc concentrations.

Duodenal lesions were determined in the following manner. The degree of ulceration was assessed by averaging the size of hemorrhagic lesions measured across their largest diameters. In case of petechiae, five such lesions were taken as the equivalent of a 1 mm lesion. The total lesion length in each group of rats divided by the number of the animals was expressed as the ulcer index (Cho & Ogle 1978).

Whole blood samples for the assessment of zinc concentration were drawn from the carotid arteries and centrifuged using a standard centrifuge at 3000 r.p.m. for 10 min. Following the assessment of duodenal lesions, duodenal tissue samples were also taken. Serum zinc concentrations (mol l^{-1}) were determined using atomic absorption spectrophotometry (AAS) (Momcilovic *et al.* 1975, Falchuk *et al.* 1988). Tissue samples were mineralized by ashing procedure (Blanusa & Breski 1981, Falchuk *et al.* 1988) and zinc concentrations (mol kg^{-1}) also determined by AAS (Falchuk *et al.* 1988). Quality control was achieved by comparison to references for blood and tissue, respectively (Seronorm batch no. 116, Nycomed Pharma AS, Norway; SRM 1577, National Bureau of Standards, USA). For quality control of the analytical method, bovine liver was used as the reference sample for the determination of tissue samples. By our method $1.88 \pm 0.63 \times 10^{-3}$ mol kg^{-1} was compared to the reference value of $1.90 \pm 0.10 \times 10^{-3}$ mol kg^{-1}. When taking into consideration zinc serum values, by our method $2.22 \pm 0.17 \times 10^{-6}$ mol l^{-1} was compared to the reference value of $2.30 \pm 0.08 \times 10^{-6}$ mol l^{-1}.

Ten unstressed animals served as a healthy control group.

Results are expressed as mean ± SEM. The difference between means was compared using analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered significant at a level of $P \leq 0.05$.

Results

The results of our study confirm that, when applied subcutaneously, cysteamine induces duodenal lesions in rats. These lesions begin to develop 6–12h

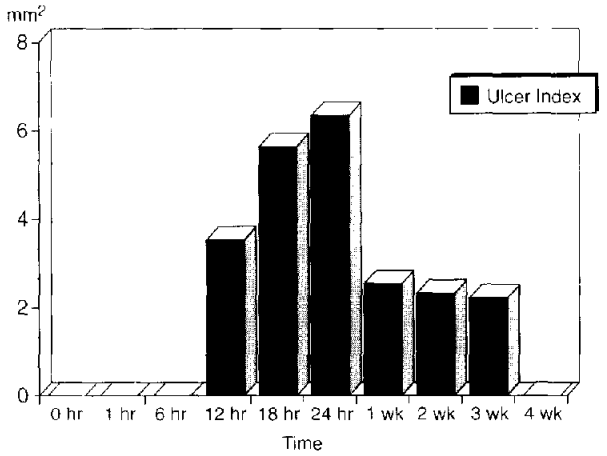

Figure 1. The development and spontaneous healing of duodenal ulcer lesions induced by the subcutaneous application of cysteamine (400 mg kg^{-1}). Values represent the ulcer index (mm^2) of 10 rats in each group.

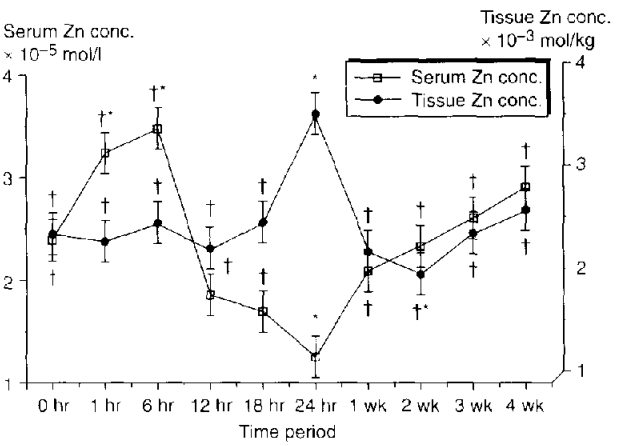

Figure 2. The dynamics of endogenous zinc serum (mol l^{-1}) and tissue concentrations (mol kg^{-1}). The values are the means of 10 rats in each group; vertical lines indicate SEM. $^*P < 0.05$ versus the healthy control group (0 h time period), $^+P < 0.05$ versus the 24 h group.

after cysteamine application. They vary in size and intensity ranging from petechiae to perforating ulcers and achieve their maximum diameter 24 h following cysteamine application. Lesions show a tendency to heal spontaneously within a period of 4 weeks with the most rapid healing rate being observed during the first week (Figure 1).

One hour following cysteamine application, a significant increase in the serum zinc concentration was noted. The serum concentration gradually increased till the 6 h time point, when a maximum zinc serum concentration was observed. No significant changes in the zinc tissue concentrations were noted in this time. During this time period no ulcer lesions could be identified in the duodenum (Table 1, Figures 1 and 2).

Between the 6 and 12 h period, a significant decline in the serum zinc concentrations was noted with practically no change in zinc tissue concentrations (Table 1, Figure 2). During this period ulcer lesions started to appear. Twelve hours after cysteamine application, a significant ulcer index of 3.5 mm^2 was obtained in the damaged duodenum (Figure 1).

The following period was characterized by the continuous decrease in serum zinc concentrations with the tendency of zinc tissue concentrations to increase. Eighteen hours after cysteamine application the ulcer index increased to a significant value of 5.6 mm^2 (Table 1, Figures 1 and 2).

Twenty four hours after cysteamine application, serum zinc levels reached the lowest values noted throughout the entire study. At the same time the largest tissue concentrations of zinc were recorded (Table 1, Figure 2). This time point was also characterized by the largest value of the ulcer index (6.3 mm^2) (Figure 1). Following this time, developed ulcers started to heal (Table 1, Figure 1).

With the start of the spontaneous healing of the developed ulcer lesions, there was a significant decrease in the zinc tissue concentrations which would continue till the end of the second week, parallel to a graduate and significant increase in the serum zinc concentrations which would continue till the end of the entire study (Table 1, Figure 2). During the first week ulcers healed most rapidly and the ulcer index decreased significantly (Figure 1). The spontaneous healing of these lesions continued till the end of the fourth week at which time all lesions healed (Table 1, Figure 1). Both the serum and zinc tissue concentrations returned to levels that were not significantly different from the healthy control values.

Discussion

Various kinds of stress are not specific and affect different organ systems (Seyle 1956, Szabo 1985). It is well known that the organism possesses many different endogenous substances that enable it to protect itself from various noxious events. One of the non-specific reactions of the organism to stress is the increase of endogenous zinc concentrations in various tissues. In addition to wounded skin, the increase of zinc tissue concentrations in stress has

already been confirmed in the heart, liver, kidney and muscles (Cordova 1994).

That zinc deficiency could affect wound healing was demonstrated accidentally (Poires 1957). In 1962, Savlov and co-workers (Savlov *et al.* 1962) proposed that zinc ions act at the wound site where they may be incorporated into enzyme systems. They found that radioactive zinc is preferentially concentrated in healing tissues. This concentration is greatest during the first 3 days after injury; it is then followed by a gradual decrease and by the 14th day the radioactivity of the wounded skin reaches the value of the normal skin. Further studies confirmed an acceleration of wound healing after zinc administration (Poires *et al.* 1967, Oberlas *et al.* 1971) and showed that the tensile strength of healing wounds was decreased in zinc-deficient rats (Oberlas *et al.* 1971, Sanstead & Shepard 1986). Chvapil & Zukowski (1975) suggested that the promotion of wound healing by zinc was probably related to its influence on protein, especially collagen precursor synthesis. Other investigators drew attention to the fact that zinc was more effective as a wound healing agent when combined with aminoacidic carriers (Lee *et al.* 1976).

Although the role of endogenous zinc in the gastrointestinal tract has not yet been fully investigated, it is obvious that zinc ions could also play an important role in gastrointestinal metabolism and disorders (Elinder 1986, Cho 1989, Vallee & Falchuk 1993). Studies that have been published over the years mainly focused on the beneficial activity of various zinc compounds on the prevention or healing of gastric lesions (Pfeiffer *et al.* 1987a, Cho 1989, Cho *et al.* 1989, 1992, Ito *et al.* 1995). Since little is known about the activity of endogenous zinc in the gastrointestinal tract, the aim of the present study was aimed to determine the activity of endogenous zinc in the duodenal tissue, using the well-established model of duodenal ulcers. Cysteamine causes rapid duodenal ulceration and is one of the best agents for producing consistent acute and chronic duodenal lesions (Szabo 1978).

Previous data obtained by Pfeiffer and co-workers, using scanning electron microscopy, demonstrated *in situ* cellular necrosis and surface cavitation of the duodenal mucosa 2–4 h after cysteamine treatment. Results indicated intracellular changes as early as 30 min after treatment and prior to damage of the columnar cell microvilli or epithelial tight junction (Pfeiffer *et al.* 1987b). At the light microscopic level, changes in surface epithelial cells, disturbance in Brunner's gland morphology and reductions in mucus glycoprotein content of superficial glands in the duodenal mucosa early after cysteamine administration have been noted (Poulsen *et al.* 1981, Tsuchihashi *et al.* 1985). The results of our study clearly indicate that, even though macroscopically visible duodenal ulcers appeared 6–12 h following cysteamine application (Figure 1), certain changes in the serum zinc concentrations could be recorded earlier (Table 1, Figure 2). Namely, the increase of serum zinc levels noted in the first 6 h of the experiment probably reflects the response of the organism to an unspecific noxious event. This response corresponds to the early microscopic changes seen in the duodenal mucosa (Pfeiffer *et al.* 1987b). With the formation of duodenal ulcers, the serum zinc concentration decreased, which corresponds to the findings of other authors (Lindeman *et al.* 1972).

A measurable increase of zinc tissue concentrations was observed in the damaged duodenal tissue with the maximum concentration being at the point of the largest ulcer index (Table 1). This corresponds to the findings of other authors who observed these phenomena in various other tissues (Savlov *et al.* 1962, Navarro *et al.* 1990, Cordova 1994). With the spontaneous healing of the developed ulcers, the values of zinc tissue concentrations decreased which points all together to the possibility that zinc represents a local mediator of tissue regeneration without regard to its mode of action. This finding is substantiated by the determination of exogenously administered zinc compounds accumulated in the gastric tissue. Namely, when the zinc content was studied in serial sections of stomachs perfused with zinc acexamate, a decreasing gradient of zinc in gastric tissue, from mucosa to serosa, was found in the luminal side (epithelium and submucosa) (Navarro *et al.* 1990).

Taking into account the possible mechanism of action of zinc ions (the stabilizing of biological membranes, the scavenging of free radicals as well as the requirement of zinc for the synthesis of DNA, RNA and proteins), its accumulation in the damaged target organs could be a multipurposeful one, leading to the preservation of undamaged tissue and the regeneration of already damaged tissues. All these data seem to point to the fact that endogenous zinc could represent a natural line of the body's defense system when exerted to stress.

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