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The protective effect of zinc sulphate pretreatment against duodenal ulcers in the rat

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Exogenously administered zinc compounds have been shown to possess antiulcer activity in the development of gastric lesions. The aim of this study was to investigate the effects of zinc sulphate pretreatment of rats on cysteamine-induced duodenal ulcers and to correlate them with changes in zinc serum and tissue levels. Atomic absorption spectrophotometry was used to determine zinc serum and tissue concentrations in all animal groups. Cysteamine produced marked duodenal ulceration in control animals 24 h after application, with an increase in endogenous zinc tissue concentrations and a marked decrease in serum concentrations. Zinc sulphate (20, 40 or 80 mg kg^{-1}) applied per os one hour prior to cysteamine application inhibited the development of duodenal lesions in a dose-related manner. The application of zinc sulphate in a single intraperitoneal (i.p.) application (80 mg kg^{-1}) did not, however, prevent the formation of duodenal lesions. In order to assess zinc absorption from the gastrointestinal tract, one group of rats received a single oral dose of zinc sulphate (80 mg kg^{-1}) without cysteamine application. The observations of this study seem to indicate that zinc plays an important cytoprotective role in duodenal ulcer disease.

Keywords: cysteamine, cytoprotection, duodenal ulcer, rat, zinc

Introduction

Peptic ulcer disease (PUD) presents one of the most common, still unresolved, social and medical problems worldwide. Present knowledge about PUD includes numerous factors in etiology and pathogenesis such as genetic, neural, humoral, iatrogenic and infective. The key step in the pathogenesis of PUD is a disruption in the active equilibrium of aggressive factors and self protective mucosal processes (Isenberg *et al.* 1995). Antiulcer drugs used in today's therapy possess multipharmacological properties, although the antisecretory or cytoprotective feature is more emphasized (Brunton 1996).

Zinc compounds have proved their beneficial cytoprotective effect through numerous experimental works which have been carried out over several years (Escolar *et al.* 1987, Cho 1989) and zinc compounds have been included in antiulcer treatment in humans (Frommer 1975, Lorenzo 1986, Bosch & Jimenez 1990). The effects of zinc salts have been extensively investigated in various animal models of gastric ulceration (Cho 1989); however, their effects on the duodenum have still to be resolved.

In this communication we have used a cysteamine-induced duodenal ulcer model in the rat since this agent causes rapid epithelial cellular damage resulting in the formation of duodenal ulcers within 6 to 12 h (Pfeiffer *et al.* 1987a). Furthermore, cysteamine ulcers have a similar pathomorphologic history to

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human ulcers (Szabo 1978). Following the study of the dynamics of endogenous zinc ions (Troskot *et al.* 1996), the present work was designed to examine the possible direct cytoprotective effect of zinc sulphate against duodenal lesions produced by cysteamine.

Materials and methods

Female Wistar rats (home bred) weighing 180–250 g were randomly assigned to different groups ($N = 10$ rats per group) and housed in a temperature ($22 \pm 1°C$) 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, USA) was dissolved in distilled water and administered subcutaneously at a dose of 400 mg kg^{-1}. One hour prior to cysteamine application, rats were pretreated with a single per os application of zinc sulphate 20, 40 or 80 mg kg^{-1} or a single intraperitoneal (i.p.) injection of zinc sulphate 80 mg kg^{-1}. A similar volume (5 ml kg^{-1}) of 0.9% NaCl was given per os to a group of animals which served as a cysteamine control group. To determine zinc absorption, one group of animals received a single oral application of zinc sulphate in a dose of 80 mg kg^{-1}, without cysteamine (zinc sulphate control). Ten unstressed animals served as a healthy control group.

All animals were sacrificed 24 h after cysteamine application. Duodenal lesions were assessed, and blood and duodenal tissue samples taken for 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 the 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 animals was expressed as the ulcer index (Cho & Ogle 1977).

Whole blood samples for the assessment of zinc concentrations were drawn from the carotid arteries and centrifuged using a standard centrifuge at 3000 rpm 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 with 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 with 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 1^{-1} was compared with the reference value of $2.30 \pm 0.08 \times 10^{-6}$ mol l^{-1}.

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 show that the pretreatment with zinc sulphate was able to prevent the development of duodenal ulceration in a dose-dependent manner. However, the route of application also seems to have an important role (Table 1, Figure 1).

In the cysteamine control group, animals not pretreated with zinc sulphate, cysteamine-induced duodenal ulcers developed in 80% of rats, with an ulcer index of 6.3 mm^2 (Figure 1). No lesions developed in the group of animals that received zinc sulphate in the highest per os dose of 80 mg kg^{-1}. In the group of animals treated with zinc sulphate at a dose of 40 mg kg^{-1}, 50% of rats developed duodenal ulcers, with an ulcer index of 3.5 mm^2 (Figure 1). In the lowest per os dose group (20 mg kg^{-1} body weight), duodenal ulcers developed in the same percentage of animals as in the cysteamine control group (80%); however, the ulcer index was 4.6 mm^2 (Figure 1). The dose of 80 mg kg^{-1} administered by the i.p. route did not exert such a protective effect as the same dose given orally, but the

Table 1. The mean ± SEM values of serum and zinc tissue concentrations and the 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	2.355 ± 0.632^{b}	2.427 ± 0.327^{b}	0
Cysteamine control	$1.224 \pm 0.374^{a,c}$	$3.600 \pm 0.483^{a,c}$	6.3
Zinc sulphate control	2.404 ± 0.241^{b}	2.118 ± 0.276^{b}	0
80 mg kg^{-1} per os	1.789 ± 0.204^{b}	2.303 ± 0.164^{b}	0
40 mg kg^{-1} per os	$1.574 \pm 0.305^{a,c}$	2.269 ± 0.188^{b}	3.5
20 mg kg^{-1} per os	$1.573 \pm 0.327^{a,c}$	2.210 ± 0.105^{b}	4.6
80 mg kg^{-1} i.p.	$1.149 \pm 0.267^{a,c}$	2.396 ± 0.153^{b}	4.0

[a] $P < 0.05$ versus healthy control.
[b] $P < 0.05$ versus cysteamine control.
[c] $P < 0.05$ versus zinc sulphate control.

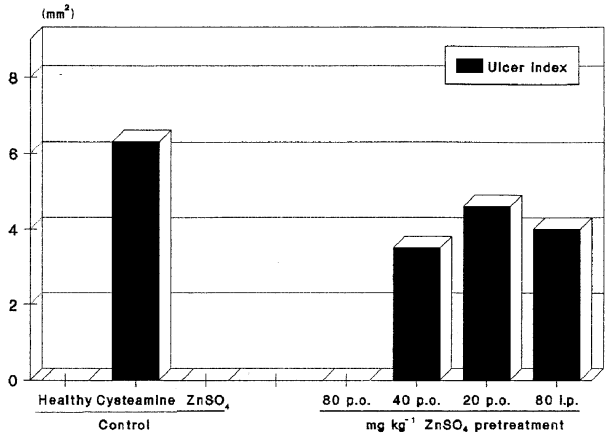

Figure 1. The development of duodenal ulcers induced by the subcutaneous application of cysteamine (400 mg kg^{-1}). Values represent the ulcer index (mm^2) of 10 rats in each experimental group.

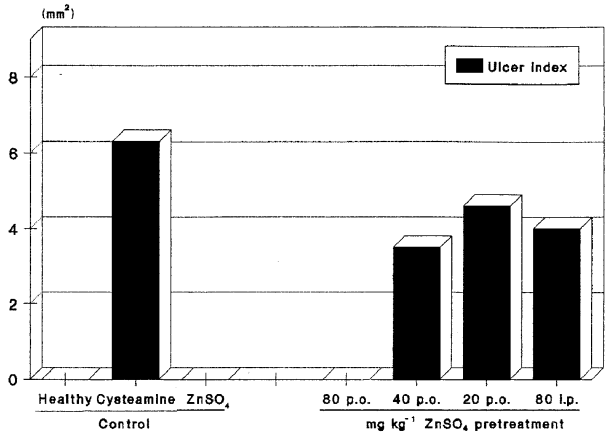

Figure 2. Zinc serum (mol l^{-1}) and tissue concentration values (mol kg^{-1}) in the investigated experimental groups. The values represent the means of 10 rats in each group; horizontal lines indicate the SEM. $^{a}P < 0.05$ versus the healthy control group; $^{b}P < 0.05$ versus the cysteamine control group; $^{c}P < 0.05$ versus the zinc sulphate control group.

ulcer index (4.0 mm^2) was significantly lower than in the cysteamine control group (Figure 1).

The values of the serum and tissue zinc concentrations of the investigated groups are reported in Table 1 and Figure 2. Only in the group pretreated with zinc sulphate at a dose of 80 mg kg^{-1} per os did the zinc serum value show a significant difference from the cysteamine control group. No statistical significance in serum values was noted among the remaining groups which were pretreated with zinc sulphate when analyzed between themselves, but a significant difference did exist when these groups were compared with healthy control animals.

Tissue zinc concentrations all showed significantly lower values in the investigated groups when compared with the cysteamine control group. No difference, however, was noted when groups were compared amongst themselves, nor when they were compared with the healthy control group.

Zinc sulphate, when applied solely, did not cause the development of duodenal ulcers (Figure 1, Table 1). The serum concentration in this group was significantly higher than in the cysteamine control animals as well as in the experimental groups which received zinc sulphate pretreatment at doses of 40 and 20 mg kg^{-1} per os and 80 mg kg^{-1} i.p. No difference was noted when compared with the group that received zinc sulphate at a dose of 80 mg kg^{-1} per os nor when compared with healthy control animals. The zinc tissue concentration was significantly lower than in the cysteamine control group, but no difference could be noted when compared with the groups with zinc pretreatment nor with the healthy control group.

Discussion

The results of the present study indicate that zinc sulphate exhibits a potent dose- and route-dependent cytoprotective effect in cysteamine-induced duodenal ulceration.

Cysteamine-induced duodenal ulcers in rats have been widely used to evaluate the effects of antiulcer agents, because histopathological changes of this ulcer model closely resemble those in human chronic duodenal ulcers (Szabo 1978). The mechanism by which cysteamine induces duodenal ulcers is complex. Centrally, it antagonizes dopamine (Glavin & Szabo 1990) while peripherally it increases gastric secretion, induces duodenal dismotility (rapid duodenal transit), decreases epithelial bicarbonate production and decreases mucosal blood flow. All these factors reduce the acid neutralization capacity and mucosal resistance, and contribute to duodenal ulceration (Szabo & Cho 1988).

Zinc compounds have been proven to preserve defense mechanisms and protect against ulcer development in the gastric mucosa. 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* 1987b, Cho 1989), inhibition of histamine release from mast cells within the

gastric mucosa (Ogle & Lau 1979), improvement of microcirculation (Lloris *et al.* 1988), an increase in the production of gastric mucus (Esplugues *et al.* 1985) and stimulation of prostaglandin biosynthesis (Navarro *et al.* 1988).

Recently, we demonstrated that the onset, development and spontaneous healing of cysteamine-induced duodenal ulcer lesions were associated with certain shifts in endogenous zinc serum and tissue levels. Briefly, prior to ulcer formation (up to 6 h), a significant increase was noted in serum zinc values. With the onset of duodenal lesions (between 6 and 12 h), zinc serum concentrations significantly decreased, while there was an increase in duodenal tissue concentrations. Compared with healthy control animals, 24 h following cysteamine application, serum zinc levels reached the lowest values noted while at the same time the largest tissue concentrations were recorded. Despite the correlations between endogenous zinc tissue concentrations and ulcer forming processes in the duodenal mucosa (Troskot *et al.* 1996), the mechanism by which a cytoprotective effect is achieved is not fully understood (Navarro *et al.* 1990, Cordova 1994, Kashiwagi *et al.* 1995).

In the present study, therefore, we once again used the cysteamine duodenal ulcer model to evaluate the direct cytoprotective effects of zinc sulphate pretreatment in preventing the formation of duodenal ulcers.

Comparing the results obtained in the healthy control group and the zinc sulphate control group, it is obvious that there is no significant difference between zinc serum and tissue concentrations in both groups. This confirms the existence of an absorption/excretion balance which is characteristic of healthy, unstressed animals (Onosaka *et al.* 1986). The zero ulcer index in the zinc absorptive control group confirms that zinc, by itself, is not an ulcerogenic compound.

Cysteamine, as an ulcerogenic stressor, caused the depletion of serum zinc concentrations and an increase in zinc tissue concentrations, with an ulcer index of 6.3 mm^2. It is well known that various types of stress lower the serum concentration of zinc (Hambridge *et al.* 1986), but the synthesis of metallothioneins is increased in both enterocytes and hepatocytes as a reaction to stress (Kotsonis & Klaassen 1979) as well as following oral and parenteral application of zinc (Onosaka *et al.* 1986). The function of metallothionein, however, remains elusive, although many possibilities have been proposed. It has been thought to detoxify heavy metals, stabilize membranes, or regulate zinc and copper metabolism; it has been suggested to be a radical ion scavenger, shown to be the source of zinc for newly synthesized apoenzymes, and postulated to serve as a regulator molecule in gene expression (Eaton *et al.* 1980, Heilmaier & Summer 1985, Heilmaier *et al.* 1987, Vallee & Falchuk 1993).

Animals in the pretreatment groups were given zinc sulphate one hour prior to cysteamine (stressor). Upon assessment of ulcer indexes in the experimental groups, 24 h after cysteamine application, the largest per os dose (80 mg kg^{-1}) group showed no macroscopic visible duodenal lesions (ulcer index = 0). Lower per os doses of zinc sulphate and i.p. application showed weaker protective effects (Figure 1, Table 1). The low zinc serum concentration in all these groups, except to some extent the 80 mg kg^{-1} per os group, resemble the cysteamine control value. Suprisingly, zinc tissue concentrations in all these groups did not follow the same characteristic increase as was noted in the cysteamine control group and in other experimental models (Cordova 1994, Kashiwagi *et al.* 1995).

Based upon the observations of the ulcer indexes, we can conclude that the zinc pretreatment is effective in preventing the development of duodenal lesions; however, it is obvious that the protective effect is dependent upon the route of application. It is well known that zinc is absorbed in the duodenum, jejunum and ileum by both passive diffusion and carrier-mediated processes in enterocytes, and is influenced by various physiological (age, lactation, pregnancy, etc.) and pathological (starvation, inflammation, stress, infection) conditions. On the contrary, i.p. application represents an unnatural route of application for this metal, evading all the previously mentioned, well-balanced absorptive mechanisms (Vallee & Falchuk 1993, Whitehead *et al.* 1996). Thus, in our opinion, it is only natural to expect that the same dose of $ZnSO_4$ (80 mg kg^{-1}) applied i.p. exerts a less potent protective effect (ulcer index 4.0 mm^2) compared with per os application (ulcer index 0 mm^2).

The exact mechanism by which zinc ions exert their protective effects in this model can be speculated. It has been shown that zinc sulphate pretreatment reduces gastric acid secretion and also reduces H^+ back diffusion into and Na^+ leakage from the gastric mucosa (Cho 1989). Zinc ions have been shown to play a critical physiological role in the structure and function of biomembranes. Zinc stabilizes membranes by reacting with SH groups of the plasma membrane proteins to form stable mercaptopeptides which are important for the integrity of plasma membranes (Cho 1989). Cysteamine

duodenal ulcerogenic potency seems to be associated with two carbon (–C–C–) groups containing reactive radicals, (–SH, $–NH_2$) (Szabo 1978). The question remains: can zinc, among its other possible protective mechanisms, prevent cysteamine ulcers by reacting with the cysteamine –SH group?

To conclude, the results of this study indicate a protective effect of zinc sulphate pretreatment in cysteamine-induced duodenal ulcers; however, this protective effect was not achieved by measurable accumulation of zinc in the duodenal tissue.

References

Blanusa M, Breski D. 1981 Comparison of dry and wet ashing procedure for cadmium and iron determination in biological material by atomic absorption spectrophotometry. *Talanta* **28**, 681–684.

Bosch F, Jimenez E. 1990 Post-marketing surveillance of zinc acexamate in peptic ulcer treatment. *Clin Trials J* **27**, 301–312.

Brunton LL. 1996 Agents for control and treatment of gastric acidity and treatment of peptic ulcers. In: Hardnen JG, Goodman-Gilman A, Limbird LE, eds. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill; 901–916.

Cho CH. 1989 Current views of zinc as a gastrohepatic protective agent. *Drug Develop Res* **17**, 185–197.

Cho CH, Ogle CW. 1977 The effects of zinc sulphate on vagal-induced mast cell changes and ulcers in the rat stomach. *Eur J Pharmacol* **43**, 315–322.

Cordova A. 1994 Zinc content in selected tissues in streptozocin-diabetic rats after maximal exercise. *Biol Trace Elem Res* **42**, 209–216.

Eaton DL, Stacey NH, Wong KL, Klaassen CD. 1980 Dose-response effect of various metal ions on rat liver metallothionein, glutathione, heme oxygenase and cytochrome p-450. *Toxicol Appl Pharmacol* **55**, 393–402.

Escolar G, Camarasa J, Navarro C, Vernetta C, Bulbena O. 1987 Antiulcerogenic activity of zinc acexamate in different experimental models. *Meth Find Exptl Clin Pharmacol* **9**, 423–427.

Esplugues JV, Bulbena O, Escolar G, Marti-Bonmati E, Esplugues J. 1985 Effects of zinc acexamate on gastric mucosal resistance factors. *Eur J Pharmacol* **109**, 145–151.

Falchuk KH, Hilt KL, Vallee BL. 1988 Zinc in biological samples by atomic absorption spectrophotometry. *Methods Enzymol* **158**, 422–434.

Frommer DJ. 1975 The healing of gastric ulcers by zinc sulphate. *Med J Aust* **2**, 793–796.

Glavin GB, Szabo S. 1990 Dopamine in gastrointestinal disease. *Dig Dis Sci* **35**, 1153–1161.

Hambidge KM, Casey CE, Krebs NF. 1986 Zinc. In: Mertz W, ed. *Trace Elements In Human and Animal Nutrition*. London: Academic Press; vol 2, 1–137.

Heilmaier HE, Summer KH. 1985 Metallothionein content and zinc status in various tissues of rats treated with iodoacetic acid and zinc. *Arch Toxicol* **56**, 247–251.

Heilmaier HE, Drasch GA, Kretschmer E, Summer KH. 1987 Metallothionein, cadmium, copper and zinc levels of human and rat tissues. *Toxicol Letters* **38**, 205–211.

Isenberg JL, McQuaid KR, Laine L, Walsh JH. 1995 Acid peptic disorders. In: Yamada T, ed. *Textbook of Gastroenterology*. Philadelphia: JB Lippincott Co., 1347–1430.

Kashiwagi M, Akimoto H, Goto J, Aoki T. 1995 Analysis of zinc and other elements in rat pancreas, with studies in acute pancreatitis. *J Gastroenterol* **30**, 84–89.

Kotsonis FN, Klaassen CD. 1979 Increase in hepatic metallothionein in rats treated with alkylating agents. *Toxicol Appl Pharmacol* **51**, 19–27.

Lloris JM, Esplugues JV, Sarria B, *et al*. 1988 Effect of zinc sulphate on gastric mucosal blood flow in gastric emptying in the rat. *J Pharm Pharmacol* **40**, 60–61.

Lorenzo MJV. 1986 Zinc acexamate and ranitidine in the short and mid-term management of gastroduodenal ulcers. *Curr Ther Res* **39**, 19–29.

Momcilovic B, Belonja B, Sham BG. 1975 Effect of the matrix of the standard on results of atomic absorption spectrophotometry of zinc in serum. *Clin Chem* **21**, 588–590.

Navarro C, Escolar G, Banos JE, Casanovas LI, Bulbena O. 1988 Effect of zinc acexamate on gastric mucosal production of prostaglandin E2 in normal and stressed rats. *Prost Leucotr Essential Fatty Acids* **33**, 75–80.

Navarro C, Ramis A, Sendros S, *et al*. 1990 Relationship between gastric levels and antiulcerogenic activity of zinc. *Arch Int Pharmacodyn* **307**, 119–129.

Ogle CW, Lau HK. 1979 Disodium cromoglycate: its influence on gastric ulcers produced by stress in rats. *IRCS Med Sci* **7**, 393–394.

Onosaka S, Min KS, Fukuhara C, *et al*. 1986 Concentrations of metallothionein and metals in malignant and nonmalignant tissues in human liver. *Toxicology* **38**, 261–268.

Pfeiffer CJ, Pfeiffer DC, Szabo S. 1987a Early ultrastructural changes in rat duodenal mucosa associated with cysteamine-induced ulcer. *Exp Mol Pathol* **46**, 102–113.

Pfeiffer CJ, Bulbena O, Esplugues JV, *et al*. 1987b Antiulcer and membrane stabilizing actions of zinc acexamate. *Arch Int Pharmacodyn* **285**, 148–157.

Szabo S. 1978 Animal model of human disease: duodenal ulcer disease. *Am I Pathol* **93**, 273–276.

Szabo S, Cho CH. 1988 From cysteamine to MPTP: structure activity studies with duodenal ulcerogenes. *Toxicol Pathol* **16**, 205–212.

Troskot B, Simicevic VN, Dodig M, *et al*. 1996 Endogenous zinc concentrations in cysteamine-induced duodenal ulcers in the rat. *BioMetals* **9**, 371–375.

Vallee BL, Falchuk KH. 1993 The biochemical basis of zinc physiology. *Physiol Rev* **73**, 79–118.

Whitehead MW, Thompson RPH, Powell JJ. 1996 Regulation of metal absorption in the gastrointestinal tract. *Gut* **39**, 625–628.