Pharmacology Biochemistry & Behavior, Vol. 13, pp. 41–44. Printed in the U.S.A.

Studies of Zinc and Histamine on Lysosomal Fragility: Possible Role in Stress Ulceration

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Received 11 January 1980

CHO, C. H., C. J. PFEIFFER AND A. CHEEMA. *Studies of zinc and histamine on lysosomal fragility: Possible role in stress ulceration.* PHARMAC. BIOCHEM. BEHAV. **13**(1) 41–44, 1980.—A combined in vivo and in vitro study was undertaken with rats to test the hypothesis that zinc would protect against cold water immersion—restraint gastric ulcers, and that this phenomenon was mediated in part by stabilization of lysosomal membranes. This postulate was confirmed by observed activity changes in released beta-glucuronidase in mucosal tissue, as well as by dose-response in vitro data on isolated hepatic lysosomes exposed to zinc. Histamine, a known ulcer-enhancing agent, induced the opposite effect and increased the lysosomal release of this marker acid hydrolase.

Zinc Stress ulcers Stomach Lysosomes β-Glucuronidase Histamine

GASTRIC ulcer disease in humans and experimental ulcers in animals has a complex etiology. A variety of changes in metabolic events within the gastric mucosa occur in association with ulcer, and it remains difficult to differentiate causal from secondary changes. It can be expected that further study of such biochemical alterations, as well as of pharmacologic agents which may enhance or inhibit gross ulceration in association with observed mucosal metabolic changes, will clarify ulcer pathogenesis.

Zinc sulfate is a compound that has recently been demonstrated to possess significant inhibitory effects on experimental ulcer in rats induced by a number of methods, including restraint in the cold room [4], reserpine [12,14], vagal stimulation [3], and pylorus ligation [11]. It is possible that this protective action of zinc is attributable to effects on cellular or organelle membrane systems, since the release of acid hydrolases from lysosomes has been associated with serotonin-induced ulceration [8], and with acid plus bile exposure to the canine gastric mucosa [18], and since the anti-ulcer agent, zinc, can also stabilize hepatic lysosomal membranes [6]. Further, zinc has been shown to inhibit the release of histamine from mast cells within the gastric mucosa during ulcer-invoking experimental conditions [11]. Therefore, the present experiment was undertaken to test whether or not zinc sulfate would inhibit ulceration in the rat induced by cold water immersion, which has been shown to be an efficient experimental method for eliciting acute gastric ulcers. In addition the activity of mucosal β-glucuronidase, as an indicator of lysosomal fragility, was assessed during normal and ulcerogenic conditions. In vitro analysis of the role of zinc as a protective agent and of histamine as an ulcer-enhancing agent was undertaken to clarify the above relationships.

METHOD

Animals

Male Wistar rats weighing 280–320 g of 11-week-old were normally maintained on conventional laboratory chow, but were fasted 24 hours (water ad lib) prior to restraint. Animals were randomly divided into groups (14 rats in each group). They were intraperitoneally administered zinc sulfate ($ZnSO_4 \cdot 7H_2O$, 11, 22 or 44 mg/kg, expressed as salt including water of crystallization) at 48 and 24 hours before stress. A similar volume of 0.9% saline was injected by the same route for control treatments.

Method of Stress

The method of restraint stress coupled with cold-water immersion stress was adopted in the present experiment. This method was originally devised by Takagi *et al.* [17] and was adapted by Pfeiffer [13]. Rats were placed into aluminum alloy restraint compartments at a fixed time in the morning to minimize variance attributable to biological rhythms. Animals were initially restrained in this manner in the vertical position at room-temperature (23°C) for 0.5 hours. Immediately after that, restraint was continued in ice cold

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TABLE 1

EFFECT OF ZINC SULFATE PRETREATMENT ON COLD-WATER IMMERSION (18°C AND RESTRAINT) INDUCED GASTRIC ULCERATION AND FREE β-GLUCURONIDASE ACTIVITY IN GASTRIC AND DUODENAL MUCOSAE

Pretreatment (IP)	Ulcer index* (mm)	Free β-glucuronidase* activity (Fishman units/ 100 mg wet wt. of tissue)	
		Stomach	Duodenum
A. Non-stressed control group (saline 1 ml/kg)	0.0 ± 0.00	63.0 ± 8.46	81.7 ± 9.44
B. Stressed groups (cold water immersion and restraint)			
Saline 1 mg/kg	5.5 ± 0.51‡	85.7 ± 6.02†	94.6 ± 14.26
$ZnSO_4$ 11 mg/kg	4.8 ± 0.84‡	81.0 ± 7.23	115.1 ± 14.11
$ZnSO_4$ 22 mg/kg	4.1 ± 0.97‡	71.5 ± 6.05	114.1 ± 12.14
$ZnSO_4$ 44 mg/kg	3.5 ± 0.63‡§	57.5 ± 6.92¶	81.5 ± 8.52

*Values indicate Mean ± SE of 14 rats.
†$p<0.05$, ‡$p<0.001$ when compared with the non-stressed, saline-treated group.
§$p<0.05$, ¶$p<0.01$ when compared with the stressed, saline-treated group.

(18°C) water. The water level was adjusted to the xyphoid level of the rats. Rats were restrained in water for 7 hours prior to sacrifice by intracranial injection of alcohol [15].

Ulcer Score

Immediately after sacrifice rat stomachs were opened along the greater curvature. Ulceration was assessed [3] by averaging the ulcer size across the largest diameter.

Assay of Mucosal β-Glucuronidase Activity

After quantitating ulcer indices, both the stomach and duodenum (10 cm from pylorus) were placed upon ice-cold glass strips and the following procedures were undertaken in a cold room (4°C). The mucosal layer of both organs was removed by a glass slide, and samples were individually homogenized with a fixed number of strokes in 5 ml of 0.25 M sucrose solution. The homogenate was centrifuged at 15,000 g for 20 min in a Sorvall RC-2B refrigerated ultracentrifuge. The supernatant was assayed for β-glucuronidase activity [16].

In Vitro Drug Studies

Rat liver lysosomes were isolated [7]. The lysosomes suspension was incubated with zinc (10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, or 10^{-3}M) or histamine (10^{-12}, 10^{-10}, 10^{-8}, 10^{-6}, or 10^{-4}M) for 40 min at 37°C, pH 7.4. Suspensions were centrifuged at 15,000 g for 20 min and the supernatant was used for measuring β-glucuronidase released during the incubation period. In order to assess the drug effect in response to time and the spontaneous release of β-glucuronidase from the lysosomes in the present system, the enzyme was measured at different time intervals (30, 60, 90 and 120 min) in the presence or absence of the optimal concentrations of drugs (10^{-3}M for zinc and 10^{-4}M for histamine).

Measurement of Lysosomal Protein

The protein content of the diluted lysosomal suspension was measured by the Bio-Rad protein assay at 595 nm [2].

Statistical Analysis

The data were analysed by means of Student's *t*-test, correlation coefficient and analysis of variance.

RESULTS

Cold water immersion for 7 hours elicited severe hemorrhagic ulceration in the glandular portion of the rat stomach. Table 1 indicates the effect of various doses of zinc sulfate on this ulcer model. Zinc sulfate pretreatment produced graded protection against restraint, water-immersion ulceration, with significant protection ($p<0.05$) at the highest (44 mg/kg) dose level. However, the drug did not provide complete protection as the ulcer index was still significantly above the control level ($p<0.001$, for all doses and with analysis of variance $F=1.45$ which is not significant). In Table 1 it is also apparent that the increase in ulcer index was accompanied with elevation in free β-glucuronidase in the gastric mucosae and they were found to be highly correlated (with correlation coefficient $r=0.969$) among the stressed groups. A significant increase of the enzyme was noted in the saline-treated group ($p<0.05$ when compared with saline non-stressed group). However, the increase in the activity of this enzyme of lysosomal origin in this tissue was markedly reduced by zinc sulfate (44 mg/kg) ($p<0.02$ when compared with the saline+stress group and with analysis of variance $F=3.83$ which is statistically significant). There was a low correlation between the ulcer index and the free β-glucuronidase in the duodenal mucosae ($r=0.431$).

In the in vitro study of isolated hepatic lysosomes, histamine enhanced the release of β-glucuronidase in a dose-dependent manner (Fig. 1). Significance was noted at the highest two doses ($p<0.01$ for 10^{-6}M and $p<0.001$ for 10^{-4}M). However, zinc sulfate induced the opposite effect, i.e., stabilization of lysosomes ($p<0.001$ at 10^{-3}M).

The release of β-glucuronidase occurred stepwise with respect to time (Fig. 2). The rate of enzyme release was maximal at the time interval between 30 to 60 min, and decreased after 90 min. Histamine significantly ($p<0.01$) enhanced the rate of β-glucuronidase release from hepatic

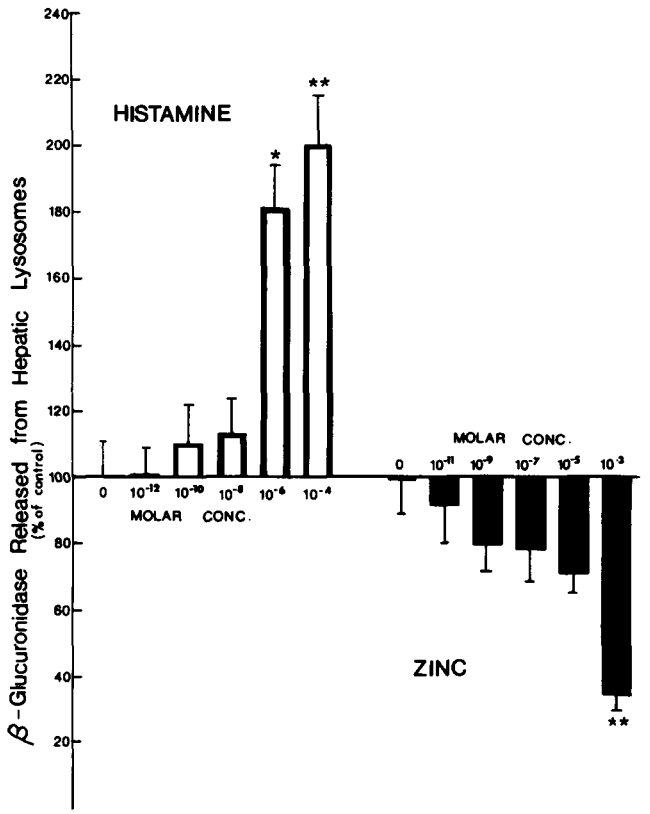

FIG. 1. Dose response effect of histamine and zinc on release of β-glucuronidase from isolated rat hepatic lysosomes. Each bar represents the mean ± SE of five tests, and significant differences from respective controls are indicated by $*p<0.01$ and $**p<0.001$.

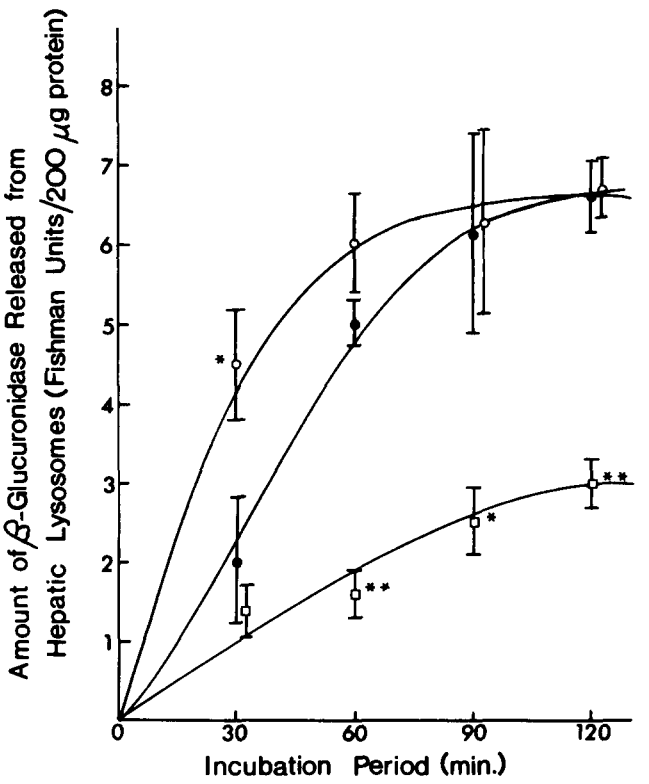

FIG. 2. Time-dependent release of β-glucuronidase from isolated hepatic lysosomes after exposure to histamine (10^{-4}M, open circles), to zinc (10^{-3}M, open squares), and during the control incubation (H_2O). Each point represents the mean ± SE of five tests, and significant differences from water control (center curve) are indicated by $*p<0.01$ and $**p<0.001$.

lysosomes during the first 60 min of incubation. Zinc sulfate at a concentration of 10^{-3}M stabilized lysosomal membranes and greatly reduced the release of this acid hydrolase, as illustrated in Fig. 2.

DISCUSSION

The hypothesis that zinc sulfate might depress gastric ulceration in the rat induced by cold water immersion during restraint was based upon experimental evidence that (a) zinc effectively prevented ulcers induced by other stress-related conditions [3, 4, 11, 12, 14], (b) zinc has been reported to reduce the labilization of lysosomal membranes [7,14], and (c) other workers have demonstrated that an increase in lysosomal fragility was associated with experimental gastric ulcer formation [8, 9, 18]. The present study has demonstrated partial protection by zinc sulfate of cold water immersion—restraint ulcer in the rat, and has demonstrated a possible mechanism for this protection, i.e., reduction of the fragility of lysosomes in the gastric mucosa. This phenomenon was further confirmed in vitro with isolated hepatic lysosomes.

It has earlier been postulated that zinc prevents stress ulcers in the rat by stabilizing mast cells and decreasing histamine release [3, 4, 11, 12]. This has also been shown recently for disodium chromoglycate which inhibits the anaphylactic release of histamine, and protected against gastric ulcers caused in Mastomys by the immunological release of histamine [1]. Though not assessed in the present study, prevention by zinc of histamine release may also have occurred during cold water immersion—restraint stress, and published data have suggested that histamine may be directly involved in gastric damage [5, 10, 11]. Thus, the present study further elucidates another possible mechanism by which histamine might be ulcerogenic or contributory to gastric mucosal damage, since this amine labilized lysosomal membranes (Figs. 1, 2), in additional to its known actions on gastric secretion and microvasculature.

It is possible that the protection of zinc against stress ulceration is by the phamacological action of stabilizing mast cells and lysosomes which are the sources of the ulcerogenic substances in tissues.

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