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Effect of zinc sulphate on acetic acid-induced gastric ulceration in rats

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Abstract—The effects of zinc sulphate on gastric ulcer healing rate and mucosal mucus content of acetic acid-induced ulceration in rats have been assessed. Daily treatment with zinc sulphate progressively accelerated ulcer healing in a dose-dependent manner with a significant increase observed on day 15 after ulcer induction in rats treated with 44 and 88 mg kg^{-1} zinc sulphate. A significant increase in gastric mucosal adherent mucus was also observed in those animals treated with 88 mg kg^{-1} zinc sulphate. The results suggest that a minimum treatment period of 15 days is needed for the zinc sulphate to be effective, and that zinc ions may promote gastric ulcer healing by enhancing mucus formation to prevent acid back-diffusion into the gastric mucosa.

It has been shown that oral zinc sulphate accelerates the healing of wounds caused by excision of pilonidal sinuses (Pories et al 1967), and several workers have suggested that zinc may accelerate the healing of chronic leg ulcers (Husain 1969; Greaves & Skillen 1970; Sergeant et al 1970). Recent observations have shown the protective effect of zinc in gastric ulceration could be due to its ability to prevent gastric mucosal mast cell degranulation, with a concomitant decrease in histamine levels in the gastric secretion (Ogle & Cho 1977b). Zinc also depresses histamine release from the gastric mucosal mast cells following vagal over-reactivity which occurs under stressful conditions (Cho & Ogle 1977; Ogle & Cho 1977a). Furthermore, zinc pretreatment in normal rats causes an increase in gastric mucus content (Cho & Ogle 1978). Thus, it was decided to investigate whether zinc treatment would be able to hasten the healing of chronic ulcers induced by a topical application of acetic acid to rat stomachs.

Materials and methods

Female Sprague-Dawley rats (220–250 g) were fed a standard laboratory diet (Ralston Purina Co., USA), and kept in a room with controlled humidity (65–70%) and temperature (22 ± 1 °C).

Chronic ulcer induction. Rats were starved for 24 h before use, but were allowed free access to an 8% sucrose in 0·2% NaCl w/v solution, which was removed 1 h before experimentation. Experiments were conducted between 13.00 and 17.00 h because at this time the stomachs were almost empty and a full stomach was found to result in inconsistent ulcer formation. Under a light ether anaesthesia, a midline epigastric incision exposed the stomach and a cylindrical plastic mould (6·5 mm in diameter) was firmly placed upon the anterior serosal surface of the gastric wall. Sixty μL of 100% acetic acid was pipetted into the mould and allowed to remain for 60 s as described by Okabe et al (1970). The acid solution was then removed using a syringe; the mould was rinsed 3 times with 0·9% NaCl w/v (saline) to prevent possible damage to surrounding tissues close to the point of acid

application. The abdomen was closed and, once recovered, the animals were fed a normal diet on the day of the operation.

Experimental design. From the 2nd day after the operation zinc sulphate (22, 44 or 88 mg kg^{-1}, i.p.) was injected daily. Controls were injected with sodium sulphate (containing an equivalent weight of the sulphate ion as zinc sulphate 88 mg kg^{-1}) or a similar volume of saline (1 mL kg^{-1}). Rats were killed 5, 10 or 15 days after the operation by a sharp blow to the head; their stomachs were immediately removed and opened along the greater curvature. Mucosal ulcers were measured before mucus determination.

Measurement of gastric ulcers. The ulcers were examined under an illuminated magnifier (3×) and their size measured by a transparent grid (each grid was 1 mm^2) placed on the top of the glandular mucosal surface. The sum of the ulcer sizes in each group of rats was divided by the number of animals and expressed as the mean ulcer index.

Determination of gastric mucus. Following measurement of the gastric ulcers, the mucus adhering to the gastric glandular mucosa was assayed by the Alcian blue method (Corne et al 1974). The glandular segments were removed, weighed and placed in buffered Alcian blue solution (pH 5·8) for 2 h. Excess dye was removed by two rinses in 0·25 M sucrose solution. Dye complexed with the gastric wall mucus was extracted with 0·5 M magnesium chloride solution. The blue solution was then shaken vigorously with an equal volume of diethylether. The resulting emulsion was centrifuged at 3500 rev min^{-1} for 10 min and the concentration of Alcian blue in the aqueous layer measured spectrophotometrically at a wavelength of 540 nm. An appropriate standard curve of Alcian blue (which obeyed the Beer-Lambert's Law) was also prepared using a range of dye concentration closely approximating those found in the samples.

Statistical analysis of data. The results were expressed as means ± s.e. mean. Differences between the means were analysed for significance by the two-tailed Student's *t*-test.

Results

Immediately after application of acetic acid, the serosal surface of the stomach became blanched and superficial blood vessels appeared damaged. A mortality rate of < 4% was observed in all operated groups, with death usually occuring in the first 2–5 days after surgery as the result of a perforated ulcer. Body weight gain of the rats with experimental ulcers increased at a normal rate, after an initial loss during the first few post-operative days.

Effect of zinc sulphate on gastric ulcer healing process. The ulcer indices in rats treated daily with either saline (1 mL kg^{-1}), sodium sulphate (43·5 mg kg^{-1}) or zinc sulphate (22, 44 or 88 mg kg^{-1}) at 5, 10 and 15 days post-operatively are shown in Table 1. Neither the sodium sulphate nor the saline treatment affected the healing rate. However, zinc sulphate progressively accelerated the ulcer-healing process in a dose-dependent manner. Significant ($P < 0·001$) enhancement in ulcer healing rates was observed on day 15 after the operation in rats treated with 44 and 88 mg kg^{-1} of zinc sulphate.

Effect of zinc sulphate on gastric mucus. There was a gradual increase in mucus content on the stomach wall of zinc sulphate treated rats. This reached a significant value ($P < 0·05$) on day 15 after the operation, when zinc sulphate 88 mg kg^{-1} was given (Table 2).

Discussion

This study demonstrates the healing effects of zinc sulphate on acetic acid-induced chronic ulcers in rats. The present chronic ulcer model was chosen because it produces gastric lesions which

Table 1. Effect of zinc sulphate treatment on the healing rate of gastric ulcers induced by topical application of acetic acid in rat stomachs.

Daily treatment (i.p.)	Dose	Number of rats	Ulcer index (mm^2) day 5	day 10	day 15
Saline	1 mL kg^{-1}	6	36·4 ± 3·7	22·6 ± 2·7	9·90 ± 1·1
Na_2SO_4	43·5 mg kg^{-1}	7	35·4 ± 4·5	24·0 ± 2·2	10·7 ± 1·3
$ZnSO_4$	22 mg kg^{-1}	7	33·3 ± 3·9	20·6 ± 2·4	8·20 ± 1·5
$ZnSO_4$	44 mg kg^{-1}	6	36·2 ± 4·2	18·3 ± 2·6	5·30 ± 1·4*
$ZnSO_4$	88 mg kg^{-1}	7	34·0 ± 3·7	15·9 ± 2·5	2·01 ± 0·4†

Values are means ± s.e.m. i.p. = intraperitoneal injection.
* $P < 0·05$; † $P < 0·001$, when compared with the corresponding value in the saline-treated group.

Table 2. Effect of zinc sulphate treatment on gastric mucosal wall mucus content.

Daily treatment (i.p.)	Dose	Number of rats	Gastric mucosal wall mucus measured (Alcian blue μg/g glandular tissue)$^{-1}$ day 5	day 10	day 15
Saline	1 mg kg^{-1}	6	140 ± 25	160 ± 18	180 ± 18
Na_2SO_4	43·5 mg kg^{-1}	7	140 ± 28	176 ± 24	175 ± 27
$ZnSO_4$	22 mg kg^{-1}	7	134 ± 21	178 ± 19	188 ± 18
$ZnSO_4$	44 mg kg^{-1}	6	154 ± 19	180 ± 26	201 ± 24
$ZnSO_4$	88 mg kg^{-1}	7	158 ± 23	190 ± 25	238 ± 18*†

Values are means ± s.e.m. i.p. = intraperitoneal injection.
* $P < 0·02$, when compared with the 5th day value within the same treatment group.
† $P < 0·05$, when compared with the corresponding value in saline-treated group.

are similar to human chronic ulcers. Zinc treatment progressively accelerated the ulcer healing process in a dose-dependent manner with a significant increase in healing rates using 44 and 88 mg kg^{-1} of zinc sulphate. The healing effect was due to the zinc and not to the sulphate ions because sodium sulphate containing the equivalent amount of sulphate ion as zinc sulphate (88 mg kg^{-1}) had no significant effect on the healing rate. The present observation is supported by previous findings by Mann et al (1981). Also, Frommer (1975) presented clinical data of patients treated with zinc showing a significant reduction in ulcer size. The present study (Table 2) also confirms that zinc treatment increases adherent mucus in rat stomachs (Cho & Ogle 1978). It is possible that the healing effects of zinc ion on gastric ulcers could be multifactorial. It has been shown that increased back-diffusion of secreted H^+ through damaged gastric mucosa aggravates ulcer formation (Oner et al 1981). This effect is accompanied by an increase in the flux of Na^+ into the lumen, which is considered a good indication of failure of the gastric barrier (Davenport & Barr 1973). Treatment with zinc also results in a reduction of H^+ back-diffusion into the gastric mucosa of the ex-vivo stomach preparation (Wong et al 1986). Thus, it is reasonable to postulate that zinc ion can stimulate mucus secretion, as observed in this study (Table 2), and promote ulcer healing by strengthening the gastric mucosal barrier.

There is evidence that oxygen-derived free radicals may play an important role in endothelial and epithelial damage. Zinc has been postulated to stabilize membrane protein by forming stable mercaptides (Chvapil 1973). This may protect against the damaging action of highly reactive radicals on the gastric mucosa. It is unlikely that the healing effects of zinc sulphate were due to an action on gastric acid or pepsin because it has been shown that inhibition of gastric acid or pepsin does not accelerate ulcer healing (Takagi et al 1969).

As previous and present studies suggest that zinc ions promote the gastric ulcer healing process primarily through reduction of H^+ back-diffusion into gastric mucosa, by increased mucus synthesis, stabilization of biomembranes, and a direct action on the enzyme systems of damaged tissues, further investigation on its effects on other pathological conditions in the gastrointestinal tract would seem to be worthwhile.

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