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Dual Effects of Zinc Sulphate on Ethanol-induced Gastric Injury in Rats: Possibly Mediated by an Action on Mucosal Blood Flow

C. H. CHO, B. W. CHEN*†, Y. K. POON, M. M. T. NG*, W. M. HUI*, S. K. LAM* AND C. W. OGLE

*Departments of Pharmacology and *Medicine, Faculty of Medicine, University of Hong Kong, Hong Kong*

Abstract—The present study examines the protective effect of zinc sulphate against ethanol-induced gastric mucosal ulcers in rats. Absolute ethanol decreased the gastric mucosal blood flow and produced haemorrhagic lesions in the glandular mucosa. Zinc sulphate preincubation in an ex-vivo stomach chamber preparation prevented the formation of ethanol-induced lesions and attenuated the decrease of blood flow produced by ethanol. Subcutaneous injection of the same doses of the drug at 15 and 30 min before ethanol exposure, markedly reduced the blood flow and also aggravated ethanol-induced gastric injury; however, when injected at 23 and 24 h before ethanol administration, zinc sulphate protected against lesion formation but had no effect on the vascular changes induced by ethanol in the gastric glandular mucosa. These findings show that the antiulcer effect of zinc sulphate occurs only when the drug is given orally, or injected s.c. 23 and 24 h before ethanol challenge. Furthermore, this protective action is probably not entirely mediated by preservation of the gastric mucosal blood flow.

Zinc compounds have been shown to protect against experimental ulcers induced by restraint stress (Cho & Ogle 1978a,b; Cho et al 1980; Pfeiffer et al 1987), reserpine (Pfeiffer et al 1980, 1987), electrocoagulation (Mann et al 1981) and acetic acid (Lloris et al 1980). Recently, zinc compounds have also been shown to prevent gastric ulcers produced by noxious agents (Cho et al 1983; Esplugues et al 1985; Wong et al 1986). Those studies suggest that zinc ions can stabilize the cell membrane (Pfeiffer et al 1987; Cho 1989) and strengthen the mucosal defensive mechanism. In addition to this action, the antiulcer effect may be due to improved microcirculation since vascular changes have been shown to weaken the mucosal barrier and to be involved in ethanol-induced ulceration (Szabo et al 1985; Guth 1986).

The work reported in this communication examines (a) the hypothesis that the antiulcer effect of zinc sulphate is mediated by preventing the mucosal microcirculatory changes induced by ethanol, and (b) the effects of different routes and times of administration of zinc sulphate on its antiulcer action in rats.

Materials and Methods

Male Sprague-Dawley rats, 290-310 g, were fed a standard pellet diet (Ralston Purina Co.) and drank tap water. Solid food was withheld 24 h before experimentation but the animals were allowed free access to drinking tap water. All experiments were conducted in a room (temperature 22 ± 1°C, relative humidity 65–70%) where the animals were normally housed.

Correspondence to: C. H. Cho, Department of Pharmacology, Faculty of Medicine, 5 Sassoon Road, University of Hong Kong, Hong Kong.

† Present address: B. W. Chen, Department of Internal Medicine, 1st Teaching Hospital, Beijing Medical University, Beijing, People's Republic of China.

Ex-vivo chamber preparation

All the experiments were performed in an ex-vivo chamber preparation. Rats were anaesthetized with sodium pentobarbitone (Abbott) (50 mg kg^{-1} i.p.) and their tracheae cannulated. An ex-vivo chamber was prepared, as described previously (Mersereau & Hinchey 1973; Wong et al 1986). Food particles adhering to the gastric mucosa, which formed the base of the chamber, were washed out with distilled water which was also used as an incubation solution during the 30-min stabilizing period at the start of the experiment, and for the first and second 15-min preincubation periods before ethanol administration.

Drug administration and blood flow measurement

After the initial 30-min stabilizing period, the gastric mucosal blood flow was recorded by using a laser doppler flowmeter (Periflux, Sweden), with the detector placed 0·5 mm above and perpendicular to the mucosal surface. This reading was taken as the basal blood flow, and acted as the reference value for subsequent blood flow measurements during the 90 min experimental period. Each measurement was performed with a frequency of 4 kHz and a constant time of 1·5 s. The luminal bathing solution was then replaced by 38·5 or 77 μmol zinc sulphate (BDH) in 1·5 mL distilled water; this was replaced by the same solution at the end of 15 min and incubated for another 15 min. Control animals received 1·5 mL distilled water. At the end of the second 15-min preincubation period, the luminal solution was changed to 1·5 mL absolute ethanol (Merck). This solution was replaced after 15 min and replacements continued for the remaining three 15 min luminal solution changes.

In a separate experiment, ex-vivo rat stomachs were preincubated with two changes of 1·5 mL distilled water which were then replaced by four changes of absolute ethanol at 15 min intervals, as previously described. The animals were injected s.c. with zinc sulphate 38·5 or 77 μmol

at either 15 and 30 min or 23 and 24 h before ethanol exposure. This regimen of treatment was similar to the incubation experiment in which the drug was given locally in the gastric chamber twice before ethanol administration. Control animals were given a 0·9% w/v NaCl (saline) solution s.c. The mucosal blood flow in the glandular segment and in the forestomach was recorded every 15 min after the basal blood flow was determined. They were expressed as a % of the basal blood flow value. The ulcer index was determined by measuring the lesion areas (Ogle et al 1985) at 15 min intervals, immediately after the measurement of blood flow.

Statistical analysis

Statistical comparison between the zinc sulphate- and saline vehicle-treated groups, at different times, was made by Student's *t*-test. The profile analysis of drug treatment during the 90-min experimental period was performed by analysis of variance.

Results

Zinc sulphate and ethanol-induced lesion formation

Preincubation with zinc sulphate 38·5 or 77 μmol dose-dependently prevented the formation of ethanol-induced glandular mucosal injury ($F = 5{\cdot}19$, $P < 0{\cdot}05$). The higher dose of the drug significantly reduced lesion severity after ethanol exposure (*t*-test) (Fig. 1). Subcutaneous injection of the drug at 23 and 24 h before ethanol administration produced a similar protective effect ($F = 5{\cdot}31$, $P < 0{\cdot}05$), especially at the higher dose, which markedly attenuated mucosal damage at the 30th, 45th and 60th min after ethanol administration (*t*-test) (Fig. 2). However, when the drug was injected at 15 and 30 min before ethanol, the severity of lesion formation was markedly increased ($F = 6{\cdot}68$, $P < 0{\cdot}05$). Both doses of the drug significantly increased the ulcer index at each respective time after ethanol exposure (*t*-test) (Fig. 2).

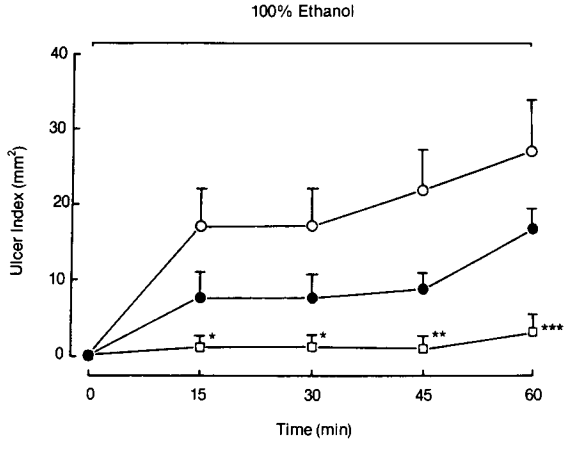

FIG. 1. Effects of zinc sulphate preincubation 15 and 30 min (○ for 1·5 mL distilled water, ● for 38·5 or □ for 77 μmole of $ZnSO_4$) before ethanol administration on ethanol-induced gastric glandular lesions. Each value represents the mean ± s.e.m. of 5 rats, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ when compared with the corresponding distilled water-preincubated control.

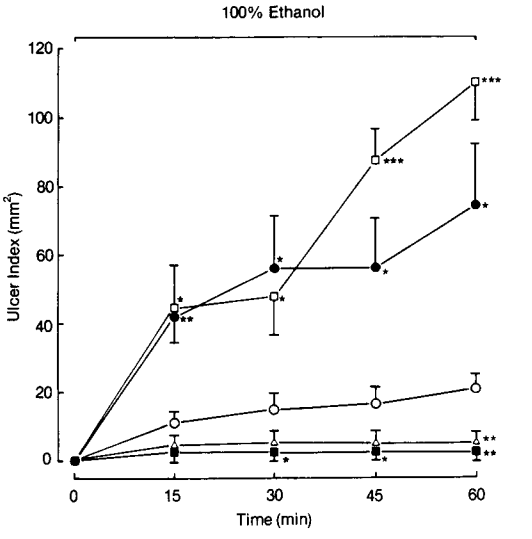

FIG. 2. Effects of zinc sulphate pretreatment injected s.c. 15 and 30 min (○ for 1 mL kg^{-1} saline, ● for 38·5 or □ for 77 μmol of $ZnSO_4$) or 23 and 24 h (△ for 38·5 or ■ for 77 μmol of $ZnSO_4$) before ethanol administration on ethanol-induced gastric glandular lesions. Each value represents the mean ± s.e.m. of 5 rats. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ when compared with the corresponding saline-pretreated control.

Zinc sulphate and ethanol-induced changes of mucosal blood flow

Ethanol significantly decreased the blood flow in the glandular portion and the forestomach at 30 and 15 min respectively, after ethanol administration (*t*-test) (Figs 3, 4). Preincubation with the higher dose of zinc sulphate tended to increase the basal mucosal blood flow of both segments of the stomach. Statistical significance was reached in the glandular mucosa at the 75th min (*t*-test) (Fig. 3). This same dose of the drug partially prevented the gastric vascular changes induced by ethanol, especially in the glandular

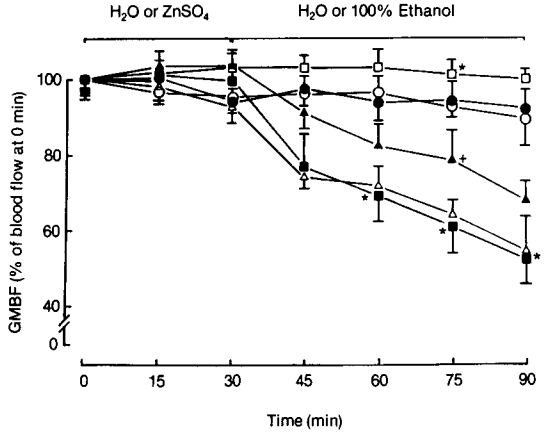

FIG. 3. Effects of zinc sulphate preincubation 15 and 30 min before distilled water (○ for 1·5 mL distilled water, ● for 38·5 or □ for 77 μmol of $ZnSO_4$) or ethanol administration (■ for 1·5 mL distilled water, △ for 38·5 or ▲ for 77 μmol of $ZnSO_4$) on glandular mucosal blood flow (GMBF). Each value represents the mean ± s.e.m. of 5 rats. * $P < 0{\cdot}01$ when compared with the corresponding distilled water-preincubated and -incubated control. + $P < 0{\cdot}05$ when compared with the distilled water-preincubated and ethanol-incubated group.

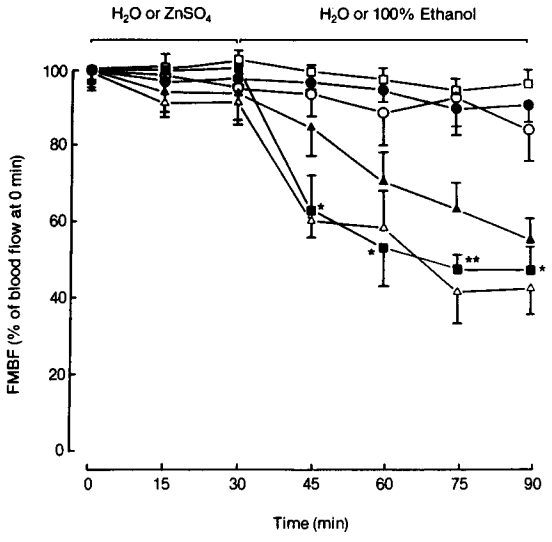

FIG. 4. Effects of zinc sulphate preincubation on forestomach mucosal blood flow (FMBF). Symbols are the same as shown in Fig. 3. Each value represents the mean ± s.e.m. of 5 rats. * $P<0.05$, ** $P<0.01$ when compared with the corresponding distilled water-preincubated and -incubated control.

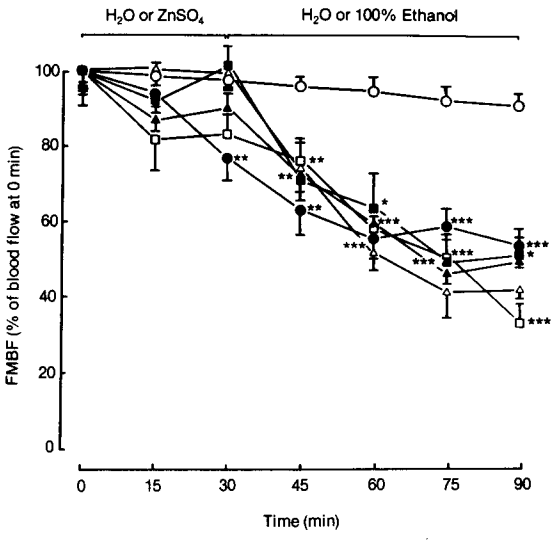

FIG. 6. Effects of zinc sulphate pretreatment injected s.c. 15 and 30 min before distilled water or ethanol administration on forestomach mucosal blood flow (FMBF). Symbols are the same as shown in Fig. 5. Each value represents the mean ± s.e.m. of 5 rats. * $P<0.05$. ** $P<0.02$, *** $P<0.001$ when compared with the corresponding saline-pretreated and distilled water-incubated control.

portion, and significantly antagonized the depressive action of ethanol at the 75 min (t-test) (Figs 3, 4). Subcutaneous injection of zinc sulphate at 15 and 30 min before experimentation markedly decreased the mucosal blood flow of both the glandular segment ($F=7.70$, $P<0.05$) (Fig. 5) and forestomach ($F=21.5$, $P<0.001$) (Fig. 6). The treatment also further reduced glandular mucosal blood flow, which was already lowered by ethanol in the glandular segment of the stomach. The higher s.c. dose of zinc sulphate significantly reduced the blood flow at the 90th min (t-test) (Fig. 5). Zinc sulphate given by the same route but at 23 and 24 h before ethanol administration did not significantly influence the reduced glandular blood flow induced by ethanol (Fig. 7). Although, the lower dose of zinc sulphate significantly

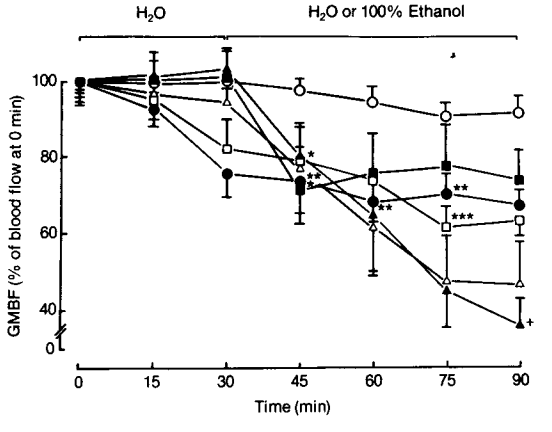

FIG. 5. Effects of zinc sulphate pretreatment injected s.c. 15 and 30 min before distilled water (○ for 1 mL kg^{-1} saline, ● for 38·5 or □ for 77 μmol of $ZnSO_4$) or ethanol administration (■ for 1 mL kg^{-1} saline, △ for 38·5 or ▲ 77 μmol of $ZnSO_4$) on glandular mucosal blood flow (GMBF). Each value represents the mean ± s.e.m. of 5 rats. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ when compared with the corresponding saline-pretreated and distilled water-incubated control. + $P<0.01$ when compared with the corresponding saline-pretreated and ethanol-incubated group.

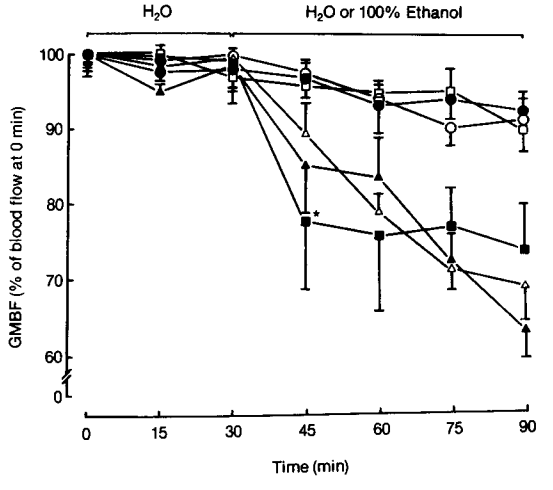

FIG. 7. Effects of zinc sulphate pretreatment injected s.c. 23 and 24 h before distilled water or ethanol administration on glandular mucosal blood flow (GMBF). Symbols are the same as shown in Fig. 5. Each value represents the mean ± s.e.m. of 5 rats. * $P<0.05$ when compared with the corresponding saline-pretreated and distilled water-incubated control.

blocked the decreased blood flow in the forestomach at the 75th min (t-test), the higher dose of the drug did not have any significant effect during the 90-min experimental period (Fig. 8).

Discussion

Marked mucosal vascular engorgement has been reported to accompany ethanol-induced gastric damage (Lacy & Ito 1982). Fluorescent microscopy studies reveal that this vascular effect is associated with the absence of blood flow in the injured area (Guth 1986) and suggest that changes in mucosal blood flow may be an important factor for ethanol-

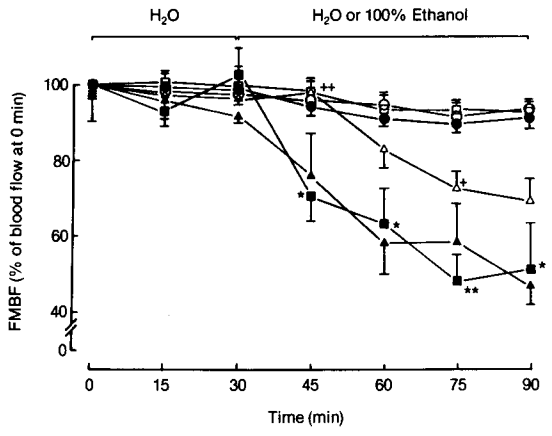

FIG. 8. Effects of zinc sulphate pretreatment injected s.c. 23 and 24 h before distilled water or ethanol administration on forestomach mucosal blood flow (FMBF). Symbols are the same as shown in Fig. 5. Each value represents the mean ± s.e.m. of 5 rats. * $P<0.02$, ** $P<0.001$ when compared with the saline pretreated and distilled water-incubated control. + $P<0.02$, + + $P<0.01$ when compared with the corresponding saline-pretreated and ethanol-incubated group.

induced gastric injury (Oates & Hakkinen 1988). Our findings, using the laser doppler technique which has been adopted for measuring human gastric blood flow (Lunde et al 1987) and is closely correlated with the H_2-clearance method (unpublished findings), point to a similar conclusion; the mucosal blood flow in the glandular segment and forestomach was reduced after ethanol administration.

Preincubation with the higher dose of zinc sulphate increased the basal blood flow and antagonised alterations in the glandular mucosal blood flow caused by ethanol. These actions may explain its protective effect against ethanol injury. Conversely, the finding that s.c. injection of the drug at 15 and 30 min before ethanol administration decreased the basal blood flow and aggravated ethanol-induced vascular changes and mucosal damage in the glandular segment, is new. The mechanisms for these adverse effects on the stomach are not known. It is likely that zinc sulphate when given by the parenteral route within a short period of time could directly affect the gastric microvasculature although the drug itself, when given in the same doses by the same route, does not significantly influence the systemic blood pressure and heart rate (unpublished findings). Subcutaneous injection of zinc sulphate at 23 and 24 h before ethanol administration neither affected the basal mucosal blood flow nor prevented the vascular changes produced by ethanol in the glandular segment, but it significantly antagonized ethanol-evoked mucosal injury. These findings suggest that the protective action of zinc sulphate may not depend entirely on its effect on mucosal blood flow. It is interesting to note that the same protective action on stress-induced ulceration has been reported when the drug is given 24 h beforehand (Ogle & Cho 1977; Cho & Ogle 1978a,b). It may be that the drug, when given by the parenteral route, needs a relatively long time before it can stabilize the mucosal barrier and produce an antiulcer effect.

It has been shown that endogenous ulcerogenic mediators are released by stress (Ogle & Cho 1977) and by noxious agents (Cho et al 1983). These mediators could participate in ethanol-induced mucosal injury either by causing vascular changes, which result in mucosal oedema and increased mucosal permeability (Szabo et al 1985), or by non-vascular effects (e.g. mucus depletion and lysosomal enzyme release) in the stomach (Cho & Ogle 1978a; Cho et al 1980, 1983; Pfeiffer & Cho 1980; Chiu et al 1983). Preincubation treatment of the stomach with zinc sulphate could prevent both vascular (Fig. 3) and non-vascular components of the ulcerogenic process after ethanol administration. On the other hand, the antiulcer effects of s.c. injection with the drug at 23 and 24 h before ethanol may be due to protection against pathological changes of a non-vascular nature (Cho & Ogle 1978b; Pfeiffer & Cho 1980; Pfeiffer et al 1980), since the vascular changes induced by ethanol were unaffected.

It is interesting that the decreased blood flow occurring in the forestomach was not accompanied by mucosal injury. These findings support the idea that depressed mucosal blood flow may not be the sole factor causing gastric ulceration. As the structure of the mucosal layers in the glandular segment and the forestomach are different, the delicate structures of the glandular portion of the stomach are probably more vulnerable to ethanol ulceration in rats. Thus, glandular mucosal lesion formation by ethanol could be the result of the combination of a decreased blood flow and a direct tissue-damaging effect.

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