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Effects of zinc sulphate on gastric mucosal blood flow and gastric emptying of the rat

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Abstract—Zinc sulphate (50 mg kg^{-1} p.o.) did not modify basal gastric mucosal blood flow, as measured by [^{3}H]aniline clearance, but inhibited its reduction by noradrenaline (3·5 μg kg^{-1} min^{-1}). Zinc sulphate also influenced gastric emptying of phenol red but its effects depended upon the dose; 30 mg kg^{-1} caused no variation whereas 80 mg kg^{-1} induced a significant delay. The nature of both actions is discussed and their implications in the development and prevention of gastric ulceration have been analysed.

Zinc salts have been proposed in recent years as new therapeutic agents for human peptic ulcer (Fraser et al 1972; Alcala-Santaella et al 1985). Pretreatment with different zinc salts protects in a wide variety of experimental ulcers (Cho & Ogle 1977; Lloris et al 1980; Esplugues et al 1985; Pfeiffer et al 1987). However, the mechanisms whereby these antiulcer effects are exerted are still not completely clarified. Recent evidence points to the possibility that they may act by increasing gastric mucosal protective systems (Esplugues et al 1985; Pfeiffer et al 1987). Gastric mucosal blood flow is one of the factors involved in the defence of the mucosa against aggression. Its reduction has been proposed as one of the main changes leading to ulcer formation in different circumstances (Jacobson 1985). The purpose of the present communication was, therefore, to examine the effects of a widely used zinc salt, zinc sulphate, on the gastric mucosal blood flow of the rat. In addition, since gastric emptying might also affect the defence of the mucosa (Kelly 1981), the influence of zinc sulphate was studied.

Materials and methods

Male Wistar rats, 200–250 g, were deprived of food but allowed free access to water 24 h before the onset of the experiments. Zinc sulphate (Merks) was dissolved in 0·9% NaCl (saline) and administered orally in a volume of 0·5 mL/100 g 2 h before starting the experiments. Control animals received by the same route a similar volume of saline. During experiments animals were kept at a temperature of 22–24 °C.

Measurement of gastric mucosal blood flow. Gastric mucosal blood flow was determined by radioactive aniline clearance. Briefly, rats anaesthetized with urethane (1·6 g kg^{-1} i.m.) were continuously perfused (0·5 mL min^{-1}) through a catheter inserted into the oesophagus, with acid saline (0·01 M HCl, pH 2) to ensure aniline trapping. The perfusion fluid was collected, at 20 min intervals, from a cannula inserted into the stomach via the proximal duodenum. A stock solution of carrier aniline sulphate (3·5 mg mL^{-1}) and [^{3}H]aniline (5 μCi mL^{-1}) in sterile saline was prepared. One hour after surgery [^{3}H]aniline was injected into a jugular vein in a loading dose of 2 μCi kg^{-1} followed by a continuous infusion of 0·033 μCi kg^{-1} min^{-1} to maintain a steady plasma concentration. The [^{3}H]aniline content of arterial blood and gastric perfusate was determined in a Nuclear Chicago liquid scintillation counter. Clearance, which gives an expression for the blood flow to the gastric mucosa, was calculated as the ratio of the gastric output to blood concentration of [^{3}H]aniline and expressed as a percentage of the control value, which is the mean of the three values before infusion of noradrenaline (3·5 μg kg^{-1} min^{-1} dissolved in saline) into the jugular vein (0·2 mL min^{-1}). In preliminary studies this dose of noradrenaline had been shown to induce submaximal reduction in gastric mucosal blood flow (80% of the maximal response to noradrenaline).

Measurement of gastric emptying in conscious rats. The procedure followed was that described by Scarpignato & Calpovilla (1980). The test meal consisted of a solution of 50 mg phenol red in 100 mL of aqueous methylcellulose (1·5%) given by oral intubation as 1·5 mL per rat 2 h after the administration of zinc sulphate. The test meal was also administered to an extra control group receiving atropine (1 mg kg^{-1} i.p.). The animals were killed by an overdose of urethane (i.p.) at 20 min intervals up to a maximum of 140 min, except those in a control group which were killed immediately after the administration of the test meal. The stomach was exposed by laparotomy, quickly clamped at the pylorus and cardia and then carefully removed. The viscera and their contents were homogenized in 5 mL 0·1 M NaOH and centrifuged (5000*g* for 5 min). The colorimetric assay of phenol red was performed at 560 nm after protein precipitation (20% tricloroacetic acid) and re-alkalinization of the supernatant (borate buffer pH 10). Results are presented as percentage changes with respect to the average amount of phenol red recovered immediately after the meal from untreated animals.

Statistical analysis. Data are presented as mean ± s.e. mean. When gastric emptying was being measured, least square regression analysis was used to obtain the line of best fit through the average data points. The GE50 (time at which 50% of phenol red had been emptied) was calculated from the plot. The significance of differences was assessed by the unpaired *t*-test and a *P* value of less than 0·05 was considered to be significant.

Results and discussion

Treatment with 50 mg kg^{-1} of zinc sulphate did not modify basal gastric mucosal blood flow, as measured by [^{3}H]aniline clearance, but inhibited its reduction by noradrenaline infusion. Noradrenaline produces vasoconstriction of the abdominal vessels and changes in gastric mucosal blood flow similar to those involved in the onset of stress ulcers (Sethbhakdi et al 1970). Cho & Ogle (1978) had suggested that the protective effects of zinc sulphate on gastric ulcers were mainly due to an inhibition in the release of vasoactive agents from gastric mast cells. Our results show that this is not the only mechanism involved and that a direct inhibitory effect on vascular reactivity plays an important role. This opinion is further

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reinforced by the fact that zinc ions have been shown to prevent in-vivo vasoconstrictions induced by noradrenaline in other abdominal vessels (Manku et al 1979). Although with our data it is impossible to confirm this hypothesis, the calcium channel blocker actions of zinc ions (Cortijo et al 1985) could be held responsible for these vascular effects. Zinc salts have also been shown to be effective in ulcer models in which mucosal blood flow reductions do not play a major role, such as pylorus ligation (Cho et al 1976) and gastric distension (Lloris et al 1980). Therefore it seems unlikely that a vascular action by these compounds is the only factor responsible for their antiulcer activities.

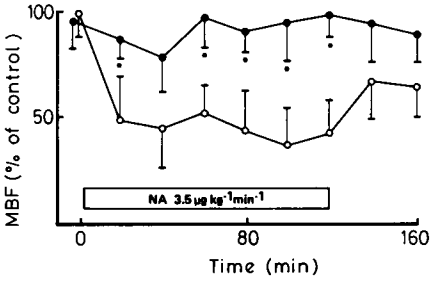

FIG. 1. Gastric mucosal blood flow, as measured by the [^{3}H]aniline clearance method in control (○) and zinc sulphate-treated (50 mg kg^{-1}) (●) rats. Results are expressed as percentage of the basal control value, which is the mean of the three values prior to infusion of noradrenaline (3·5 μg kg^{-1} min^{-1}). The results are the means ± s.e.m. of 12 animals. $^*P < 0{\cdot}05$ compared with control values.

The effects of zinc sulphate on gastric emptying of phenol red are shown in Fig. 2. When 30 mg kg^{-1} were given, no delay was observed and GE50 for control and treated animals were very similar (25·7 and 28·6 min, respectively). 80 mg kg^{-1} of zinc sulphate delayed gastric emptying (GE50 43·2 min), but this was smaller than that induced by the antiulcer and antisecretory drug atropine (GE50 73·4 min). The role that gastric emptying plays in the development of experimental ulceration is not fully understood (Kelly 1981). In previous studies (Lloris et al 1980) oral administration of similar doses of zinc sulphate was shown to inhibit ulcer formation. On this basis, delaying gastric emptying does not appear to be responsible for the antiulcer actions of zinc sulphate, since 30 mg kg^{-1} did not exert any influence on this parameter. Furthermore, oral administration of doses of zinc, in the form of other salts, much lower than those employed in the present communication have exhibited a significant antiulcer activity (Esplugues et al 1985; Pfeiffer et al 1987). The reason for the delay in gastric emptying of phenol red due to zinc sulphate is not known, but the response was similar to that induced by calcium blockers (Brage et al 1986). Nevertheless, the differences in antiulcer activity exhibited between calcium channel blocker drugs (Esplugues et al 1987) and zinc salts (Esplugues et al 1985) makes it unlikely that blocking of calcium channels would be responsible for the total antiulcer actions of zinc salts.

In conclusion, zinc sulphate inhibited the variations in gastric mucosal blood flow following noradrenaline infusion.

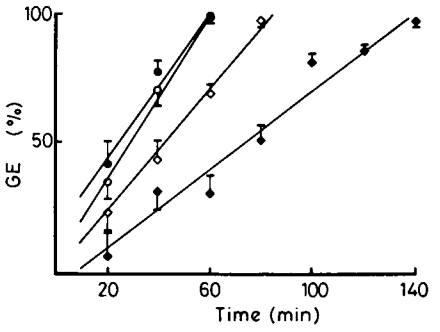

FIG. 2. Gastric emptying of phenol red in control (●), zinc sulphate 30 mg kg^{-1} (○)- and 80 mg kg^{-1} (◇)- and atropine 1 mg kg^{-1} (◆)-treated rats. Each point represents the mean ± s.e.m. of at least 7 animals. The regression lines were calculated by the method of least squares.

Pretreatment with zinc sulphate did also modify gastric emptying of phenol red but effects varied with the dose. Both actions are suggested to be the consequence, at least in part, of the calcium blocking properties of zinc.

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