J. Pharm. Pharmacol. 1990, 42: 252–256
Received November 9, 1989

Zinc Acexamate Inhibits Gastric Acid and Pepsinogen Secretion in the Rat

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Abstract—Pretreatment with zinc acexamate (25–100 mg kg^{-1} i.p.) inhibited acid and pepsinogen secretion in the pylorus-ligated rat. Zinc acexamate (5–50 mg kg^{-1} p.o.) also inhibited the increases in acid secretion induced by carbachol (10 μg kg^{-1}) and 2-deoxy-D-glucose (200 mg kg^{-1}) in the perfused stomach of the anaesthetized rat. A delayed antisecretory effect was observed with this drug on histamine induced responses. High concentrations of zinc acexamate (10^{-5}–10^{-2} M) did not modify the in-vitro activity of pepsin. Administration of zinc acexamate resulted in an increase in the presence of pepsinogen at the mucosal level. A morphological examination of the gastric mucosa confirmed an accumulation of zymogen-containing granules in the gastric chief cells of zinc acexamate-treated rats (50 mg kg^{-1} p.o.). These results indicate that zinc acexamate decreases acid and pepsinogen secretion in-vivo, and this may explain its antiulcer activity.

Zinc acexamate (ZAC) is a new zinc organic compound with antiulcer activity in man (Alcalá-Santaella et al 1985; Varas-Lorenzo 1986). Experimentally, ZAC prevents the development of gastric lesions in a wide variety of ulcer models (Escolar et al 1987a). Hitherto, the antiulcer activity of ZAC (Pfeiffer et al 1987) and that of other zinc-based compounds (Cho et al 1976; Cho & Ogle 1977; Cho & Pfeiffer 1982) has been related to their stabilizing actions on biological membranes and/or to an increase in gastric mucosal defensive factors such as mucus (Esplugues et al 1985; Escolar et al 1987b) or a local improvement of blood flow (Navarro et al 1988). Recent evidence suggests however, that these zinc compounds might also interfere with the secretion process of various secretory glands (Frederickson et al 1987). An inhibitory action on gastric acid and pepsinogen secretion would contribute to the antiulcer activity of ZAC. The present work analyses the possible inhibition by ZAC of gastric acid secretion in two in-vivo models in the rat, namely the pylorus-ligation and the perfused-stomach. The effects of ZAC on pepsinogen secretion and activity were also studied.

Materials and Methods

Measurement of gastric acid secretion in the pylorus-ligated rat

The pylorus-ligated rat model first described by Shay et al (1945) was used. Zinc acexamate †[$(CH_3CONH(CH_2)_5COO)_2$ Zn; 99·5% purity. Department of Chemical Research, Lab Viñas S.A., Barcelona] was obtained through previous hot acetylation of ε-amino caproic acid with acetic anhydride. Once the ε-acetamide caproic acid was isolated it was treated with zinc basic carbonate and, finally, the ε-zinc acetamide caproate was crystallized. The final product obtained (zinc acexamate) was given at doses equivalent to 25, 50 and 100 mg kg^{-1}, and ranitidine (12·5, 25 and 50 mg kg^{-1}) was dissolved in 0·9% NaCl (saline) and administered i.p. in a volume of 2·5 mL kg^{-1}, 15 min before starting the experiments. Control rats received a similar volume of saline by the same route. The surgical manipulation was carried out under light ether anaesthesia. Care was taken not to damage the blood supply. The animals were killed 4 h after ligation of the pylorus, the abdomen was re-opened and the stomach removed. The gastric content was collected and its volume measured. Samples (1 mL) were analysed for hydrogen ion concentration by electrometric titration (Methrom 682 titroprocessor) to pH 7 with 0·02 M NaOH.

Biochemical assay of pepsinogen content and pepsin activity

The pepsinogen activity in samples of gastric content or in homogenates of gastric mucosa was determined by the colorimetric method of Anson (1938). This method is based on the ability of pepsin to digest and liberate tyrosine from haemoglobin 2% (pH 2, 37°C, 15 min). The product of this digestion is subjected to alkaline condensation with Folin-Ciocalteu's reagent and tyrosine is spectrophotometrically measured at 580 nm. The in-vitro influence of ZAC on pepsin activity was evaluated by the modification of Rick & Fritsch (1974) on the method previously described (Anson 1938). The effects of ZAC (10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M) on the activity of a 0·1 mg mL^{-1} solution of purified pepsin (2000 units mg^{-1}, Merck, FRG) at different pH (2, 2·6 and 3·5) were analysed for 10 min.

Measurements of acid secretion in the perfused stomach of the anaesthetized rat

Rats were treated with ZAC (5 and 50 mg kg^{-1} p.o.) 1 h before the beginning of the experiments. The experimental procedure has been previously described by Ghosh & Schild (1958). Animals were anaesthetized with urethane (1 g kg^{-1} i.p.) and the trachea intubated to facilitate respiration. Two polythene cannulae were inserted so that they reached the stomach lumen, one via the oesophagus and the other via the duodenum. The antroduodenal cannula was led outside the abdominal wall. Following an initial washing of the gastric cavity with 10–15 mL of saline at 37°C, it was perfused

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† Patent: ES466544

intragastrically at a rate of 1 mL min^{-1}. After stabilization (30 min), the hydrogen ion activity of the effluent perfused from the stomach was continuously recorded by means of a glass electrode (Ingold) coupled to a pH-meter (Crison 505). Samples of the effluent, obtained every 10 min, were analysed for hydrogen ion concentration by electrometric titration (Methrom 682 titroprocessor) to pH 7 with 0·01 M NaOH. The rate of acid secretion was expressed as μ equiv H^+ min^{-1}. When the gastric acid output reached a steady state its value was recorded for 40 min and considered as basal or unstimulated acid secretion. Thereafter carbachol (10 μg kg^{-1}, Sigma, USA), pentagastrin (20 μg kg^{-1} ICI, UK), histamine (5 mg kg^{-1}, Merck, FRG) or 2-deoxy-D-glucose (2-DDG), (200 mg kg^{-1}, Merck, FRG), dissolved in 0·25 mL saline was injected as a bolus through a cannulated jugular vein and the induced response monitored for 2 h.

Histological examination

ZAC-treated (100 mg kg^{-1} p.o.) or control rats were anaesthetized and the abdomen opened. Once the duodenum and the oesophagus had been cannulated with a polythene cannula, the gastric cavity was washed with 10–15 mL of phosphate buffered saline (PBS). The stomach was filled with cold 3% glutaraldehyde in PBS, the pylorus and the oesophagus were ligated and the stomach was removed and placed in the same fixative, at 4°C. After 1 h, the stomachs were opened along the greater curvature and stored in freshly made 3% glutaraldehyde. After 24 h of fixation at 4°C, 5 mm length and 3 mm wide samples were removed from the same locations, and embedded in methacrylate (JB-4 Kit, Polysciences, Warrington). Sections (2 μm thick) were obtained, stained with Mallory and 1% toluidine blue and used for high resolution light microscopy. The study was carried out by two independent observers unaware of the treatment given.

Smaller portions (3 × 1 mm) from the same stomach were postfixed for 60 min in 1% osmium-potassium ferrocyanide at 4°C (White et al 1979), dehydrated through graded series of ethanol (30, 40, 70, 90 and 100%), infiltrated in propylene oxide/Epon mixtures and embedded in Epon 812. Contrast of ultra-thin sections cut from these plastic blocks on an ultramicrotome was enhanced with uranyl acetate and lead citrate. Studies were in parallel. One or two blocks were selected at random from each rat and seven to ten electron micrographs from parietal and chief cells were taken for each specimen.

Statistical analysis

All data were expressed as mean ± s.e.m. Comparison between groups was made with the Student's *t*-test for unpaired data and *P* values of less than 0·05 were taken as significant.

Results

Effects on the gastric secretion of the pylorus-ligated rat

Pretreatment with ZAC (25, 50 and 100 mg kg^{-1} i.p.) or with ranitidine (12·5, 25 and 50 mg kg^{-1}) significantly and dose-dependently reduced both the acid and pepsin output of the stomach (Table 1). The maximal inhibition attained with ZAC (79%) was slightly below that observed with ranitidine (90%). When dose response curves obtained in these experiments were compared, the estimated antisecretory activity of ranitidine (ED75) was twice that of ZAC.

Effects of ZAC on acid secretion in the perfused stomach of the anaesthetized rat

The results of these experiments are shown in Fig. 1. Pretreatment with 5 and 50 mg kg^{-1} of ZAC had no significant effect on unstimulated acid secretion. ZAC, however, modified the responses elicited by the different stimulants although the characteristics of these responses varied with doses and secretagogues employed. ZAC (50 mg kg^{-1}) had a delayed inhibitory effect on gastric hypersecretion induced by histamine. In contrast, the same dose inhibited by nearly 50% responses to both carbachol and pentagastrin and abolished the increase in acid output induced by 2-DDG. Administration of ZAC, 5 mg kg^{-1}, did not decrease the secretion stimulated by pentagastrin, histamine or 2-DDG. However, a significant inhibition was obtained with this low dose of ZAC when gastric acid production was stimulated with carbachol.

In all cases, the effect of ZAC in decreasing gastric total [H^+] secretion corresponded to similar increases in pH values. A correlation index of 0·86 ($P < 0·05$) was found.

Pepsinogen content. Enzymatic and microscopical study

Pre-treatment with ZAC caused an increase in the amount of pepsinogen present in the gastric mucosa. Levels of pepsinogen, measured as μmol tyrosine in control animals were found to be 66·8 ± 21·3 μmol/100 mg wet weight of mucosa. These values were statistically increased ($P < 0·01$) after treatment with 50 mg kg^{-1} or 100 mg kg^{-1} ZAC (98·5 ± 25·0

Table 1. Effects of zinc acexamate (ZAC) and ranitidine on gastric acid and pepsin production in pylorus-ligated rats (4 h).

	mg kg^{-1}	n	μequiv H^+	Inhibition (%)	Pepsin (% inhibition)
Control		19	741·8 ± 80·5		
ZAC	25	10	253·6 ± 70·0***	65·9	58·7 ± 8·4***
	50	10	170·0 ± 61·7***	77·1	78·7 ± 8·6***
	100	8	151·6 ± 91·1***	79·6	89·1 ± 8·1***
Ranitidine	12·5	8	267 ± 78***	64·0	50·3 ± 11·6***
	25	10	152·3 ± 35·4***	79·5	59·1 ± 10·3***
	50	7	61·8 ± 12·8***	90·9	74·7 ± 0·5***

Mean ± s.e.m.
*** $P < 0·001$ with respect to control animals.

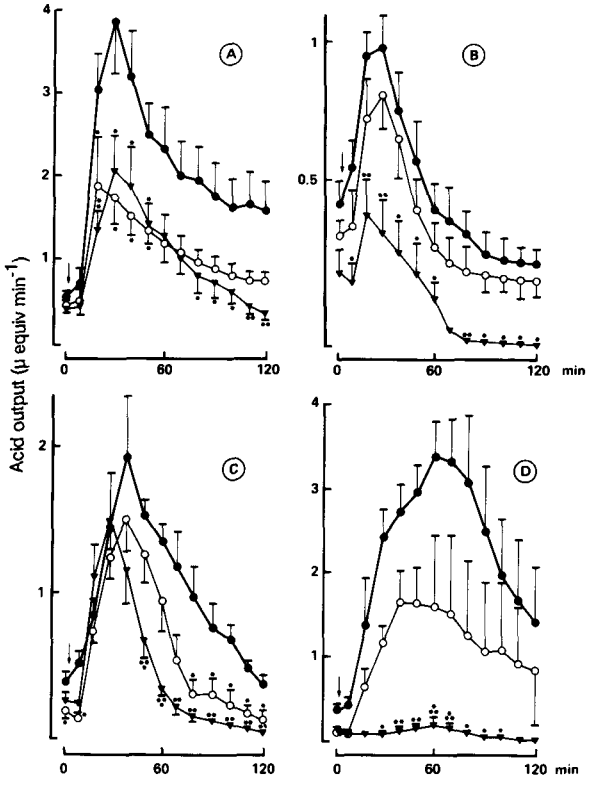

FIG. 1. Acid output [H^+] elicited by a bolus injection of (A) carbachol (10 μg kg^{-1} i.v.), (B) pentagastrin (20 μg kg^{-1} i.v.). (C) histamine (5 mg kg^{-1} i.v.) and (D) 2-deoxy-D-glucose (200 mg kg^{-1}, i.v.) in the perfused stomach of the rat. Control (●); pretreated with zinc acexamate 5 and 50 mg kg^{-1} (○, ▼). Each point represents the mean of six animals and vertical lines show s.e.m. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ versus the control group.

and 121·1 ± 30·5 μmol/100 mg wet weight of mucosa). A comparative study performed at the light microscopical level on equivalent portions of the gastric mucosa showed marked dilation of the zymogenic glands in 3 out of 4 stomachs in the group of animals receiving ZAC (100 mg kg^{-1}). These dilations only appeared in 1 out of 8 stomachs in the control group. The ultrastructural study confirmed that the number and size of zymogen secretory granules was increased in the apical portions of the glands in ZAC-treated animals (Fig. 2).

Effects on the activity of pepsin in-vitro

The enzymatic activity of a 0·1 mg mL^{-1} solution of purified pepsin, measured as ability to liberate tyrosine from haemoglobin, was not modified by ZAC in experiments in-vitro. ZAC (10^{-5}–10^{-2} M) did not significantly affect the in-vitro activity of pepsin at any of the values of pH used in our experiments (data not shown).

Discussion

Results of the present study indicate that ZAC inhibits gastric acid and pepsinogen secretion by the stomach of the rat in-vivo. This antisecretory action may help to explain the antiulcer effect displayed by this zinc compound in different experimental models. In the pylorus-ligated rat, both ZAC and ranitidine decreased acid and pepsin output in a dose-dependent manner. Although differences in dose response relationship were noticed with both drugs, the maximal percentage of inhibition attained with ZAC was basically similar to that observed with ranitidine, a classical H_2-receptor antagonist. Our results confirm previous suggestions about the antisecretory effect displayed by other zinc compounds (Cho et al 1976, 1978). This inhibitory effect on acid secretion was also shown by ZAC in the perfused stomach of the anaesthetized rat. However, various patterns of response were found in these experiments depending on the secretagogue employed for stimulating acid production.

The known activity of ZAC as an inhibitor of mast cell degranulation (Pfeiffer et al 1987) could partially account for the antisecretory effects of this compound on either the pylorus-ligated rat or when acid secretion was stimulated with 2-DDG. However, prevention of mast cell degranulation would not completely explain the inhibitory actions of ZAC on carbachol- or pentagastrin-stimulated acid secretion. Responses to histamine were only slightly modified by ZAC, which suggests that although the drug can prevent the release of this vasoactive agent from mast cells, it has little effect when the histamine is exogenously administered.

In our study the ability of ZAC to decrease gastric H^+ secretion was associated with a parallel rise in pH values in the gastric content. ZAC is known to increase mucus and PGE_2 production (Navarro et al 1988). Although these actions may contribute to modifications of the pH, they cannot explain the ability of ZAC to inhibit gastric secretion. As for the intimate mechanisms of this inhibitory action, recent in-vitro experiments have suggested that ZAC antisecretory effect can be mediated through inhibition of the secretory membrane located H^+/K^+-ATPase activity (Ray et al 1989).

The inhibition of the pepsinogen secretion after ZAC administration in the pylorus-ligated rat does not seem to be followed by changes in the activity of the enzyme. ZAC did not affect the in-vitro activity of pepsin. Furthermore, light and electron microscopical studies indicated an increased presence of secretory granules inside the cells after treatment with ZAC. Similar results have been observed with the drug at the level of the mucous gastric glands (Escolar et al 1987b). Although the decrease in acid secretion might have partly contributed to the inhibition of pepsinogen secretion through a regulatory mechanism, the increased presence of secretory granules in zymogenic glands together with an increased activity of pepsinogen at the mucosal level suggests that ZAC might interfere with the processes of zymogen granule maturation and release. We have previously described how ZAC stabilizes cell membranes and prevents mast cell degranulation (Pfeiffer et al 1987). Other authors have found similar results with other zinc compounds (Cho & Ogle 1977; Lloris et al 1980). Indeed, there is increasing evidence that zinc might be universally involved in stabilizing the structures of stored pro-protein molecules (Frederickson et al 1987).

The route of administration (i.p. and p.o.) and the doses of ZAC used in our experiments were similar to those exhibiting protective effects on a variety of models of experimental ulceration, including pylorus-ligation (Esplugues et al 1985; Escolar et al 1987a; Pfeiffer et al 1987). Therefore, the inhibitory effect on gastric secretion is likely to play a role in the antiulcer activity of ZAC. However, the fact that ZAC is active in models of gastric ulceration where acid and pepsin

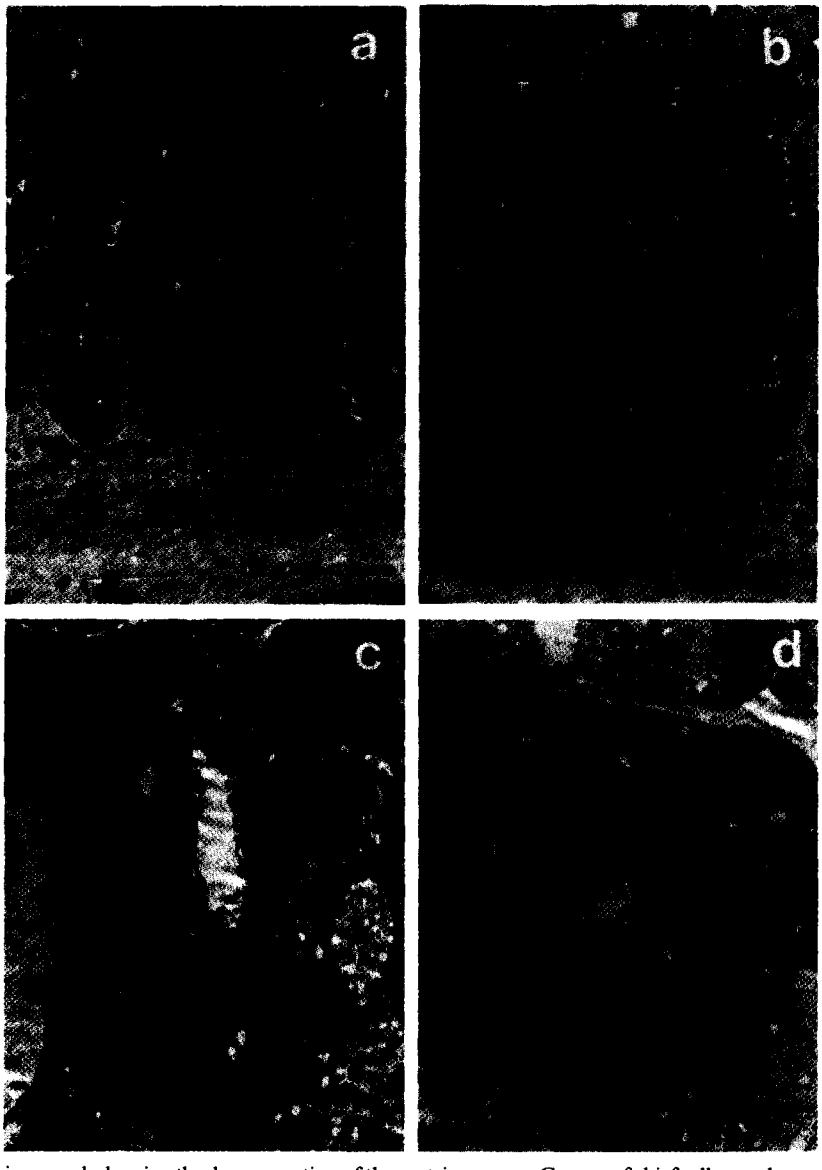

FIG. 2. Light micrograph showing the deeper portion of the gastric mucosa. Groups of chief cells are observed in a control rat (a) but those of the rat pretreated with zinc acexamate (100 mg kg^{-1}) (b), appear markedly enlarged. When observed with the electron microscope some zymogen granules appear dispersed in the cytoplasm of the chief cell in the control rats (c) but their number and size was smaller than those appearing in rats pretreated with 100 mg kg^{-1} of zinc acexamate (d).

play little or no role in the pathogenesis of mucosal damage such as ethanol (Esplugues et al 1985), suggests that other mechanisms besides gastric secretion inhibition are involved in the protective effects of ZAC. Stabilization of cell membranes might be responsible for the inhibition of pepsinogen secretion. This inhibitory action of ZAC on gastric acid and pepsinogen secretion may contribute to the antiulcer activity of this compound.

Acknowledgements

Mrs Luz Gil holds a scholarship from the Conselleria de Cultura, Educació i Ciència de Valencia.

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