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Effect of Chlorpromazine on Ulcer Formation by Indomethacin in Histamine- and Insulin-Stimulated Rats

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Summary

The effect of chlorpromazine on ulcer formation by indomethacin and on total gastric secretion and gastric acid secretion was studied in rats. Secretion and ulceration were evaluated under basal conditions and after the administration of histamine or insulin, i.e. substances stimulating gastric acid secretion. The authors confirmed that chlorpromazine inhibits basal secretion and found that it also inhibits histamine- and insulin-stimulated gastric secretion, in correlation to the dose. It also strongly inhibits the formation of stomach lesions caused by indomethacin under basal conditions and after pretreatment with histamine (3 and 10 mg/kg) and insulin (0.3 IU/kg). Chlorpromazine did not inhibit lesions formed after combining indomethacin with insulin in a dose of 3 IU/kg. The results show that although chlorpromazine inhibits both basal and centrally or peripherally stimulated gastric secretion, its effect on stomach lesions caused by indomethacin is not uniform. Pretreatment with insulin in a dose of 3 IU/kg demonstrates that indomethacin-induced stomach lesions are markedly potentiated by this dose of insulin and are not dependent on gastric secretion only. The inability of chlorpromazine to inhibit these lesions gives the evidence that other – probably central – mechanisms play a role in their development.

Key words
Indomethacin – Chlorpromazine – Histamine – Insulin – Gastric secretion – Stomach lesions

Introduction

Gastrointestinal lesions and haemorrhage are a frequent undesirable consequence of treatment with non-steroidal anti-inflammatory drugs (Barbour and Dickerson 1938, Grossman *et al.* 1961, Rainsford 1975, Rainsford and Willis 1982). Numerous data indicate that various factors (prostaglandins, histamine, serotonin, leukotrienes, neuropeptides, endothelin, hypersecretion of gastric acid and others) may participate in the development of these lesions.

There are many substances capable of protecting the gastric mucosa from experimental lesions caused by ulcerogens. Two types of protection are known. Lesions induced by substances which are ulcerogenic only in an acid medium in the stomach lumen (e.g. non-steroidal anti-inflammatory drugs) are inhibited by

substances which depress HCl secretion. Necrotic damage of direct origin (e.g. exposure of the mucosa to absolute ethanol), which is not dependent on gastric pH, is inhibited by "cytoprotective" substances without gastric secretion being affected (Robert *et al.* 1984). These include (for example) prostaglandins, which have a cytoprotective effect in doses which do not inhibit gastric acid secretion (Robert 1984).

In a previous paper, we described the protective effect of chlorpromazine on ulcer formation by indomethacin (Mirossay *et al.* 1987). The mechanism of this anti-ulcerogenic effect of chlorpromazine has not been unequivocally explained. Though its inhibition of gastric acid secretion was described in 1959, it was presumed to be mediated by a central mechanism. In the present study we investigated the effect of chlorpromazine on ulcer formation by indomethacin in relation to centrally and peripherally influenced gastric secretion. We therefore used the above substances combined with histamine (peripheral stimulation of gastric acid secretion) and insulin (central vagus-mediated stimulation) (Arai *et al.* 1985, 1987).

Material and Methods

The experiments were performed in male Wistar rats (Velaz, Prague) weighing 180–220 g, which were deprived of food 24 h before the experiment, but were allowed free access to water. Gastric secretion was measured after ligation of the pylorus under ether anaesthesia. The animals were killed 4 h after the operation. The contents of the stomach were centrifuged and their volume, pH and acidity were measured. Total acid formation in every sample was quantified by titration with 0.1 N NaOH. using phenol red as the indicator (Ohtsuki *et al.* 1985). The total amount of acids was expressed in μmol . $(100\ g\ .\ h)^{-1}$.

Stomach lesions were induced by administering indomethacin (Sigma, St. Louis) dissolved in 2 % $NaHCO_3$ solution (Assouline and Danon 1985). Four hours after the subcutaneous administration of indomethacin in a dose of 20 mg/kg the animals were killed and their stomach was removed and cut along the major curvature. The number and length of the lesions (in mm) were evaluated, taking the length of spot lesions as 1 mm (Glavin 1985).

Histamine in doses of 3 and 10 mg/kg (Histamin ad diagnosim, Spofa) and insulin in doses of 0.3 and 3 IU/kg (Neutral Pur Insulin, Spofa) were injected subcutaneously 30 min before ligating the pylorus or administering indomethacin. Chlorpromazine in doses of 5, 10 and 15 mg/kg (Plegomazin, Egyt, Hungary), or 0.9 % NaCl solution (in the control groups) was administered intraperitoneally 30 min before injecting histamine or insulin.

The gastric secretion results are expressed as the mean values ± S.E.M from eight animals. Statistical significance of differences between the individual groups was evaluated by means of Student's unpaired t-test and is expressed as $p<0.05$ and $p<0.01$. For the evaluation of ulceration we used Wilcoxon's non-parametric test, taking the values d_5 and d_1 to be significant.

Results

Effect of chlorpromazine on basal and on histamine- and insulin-stimulated gastric secretion

In a dose of 5 mg/kg, chlorpromazine mildly reduced gastric juice volume compared with the control group. In doses of 10 and 15 mg/kg it inhibited total gastric secretion ($p<0.01$). In a dose of 3 mg/kg, histamine mildly stimulated gastric juice production compared with the control group, whereas insulin in a dose of 0.3 IU/kg significantly increased total secretion volume. The stimulant effect of

both substances was significantly inhibited by chlorpromazine in doses of 5, 10 and 15 mg/kg ($p<0.01$). Larger doses of both histamine (10 mg/kg) and insulin (3 IU/kg) raised gastric juice production significantly in the control group, while chlorpromazine, in all the given doses, inhibited this stimulant effect ($p<0.01$) (Fig. 1).

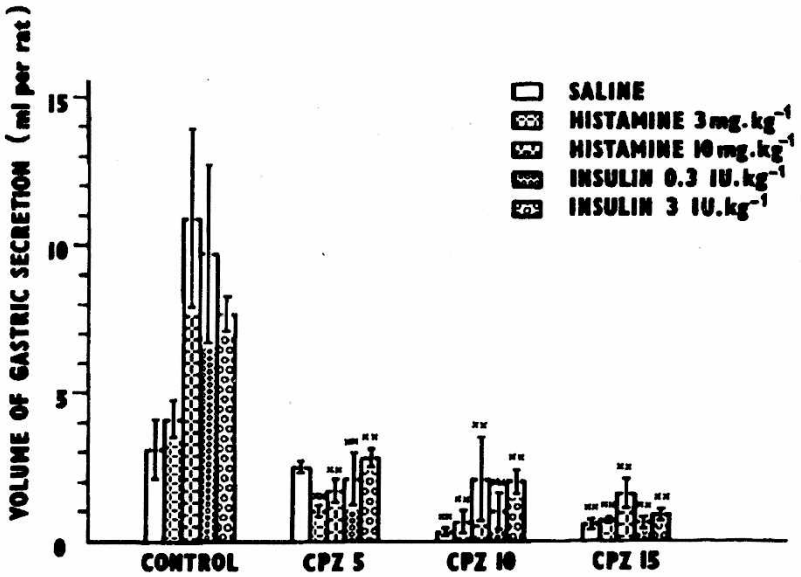

Fig. 1
Effects of i.p. injection of chlorpromazine (CPZ – 5, 10 and 15 mg/kg) on total gastric secretion under basal conditions (saline) and after s.c. injection of histamine (3 and 10 mg/kg) and insulin (0.3 and 3 IU/kg). The pylorus was ligated one hour after administration of chlorpromazine. Results are the means ± S.E.M. of 8 experiments (xx $p<0.01$, t-test versus control).

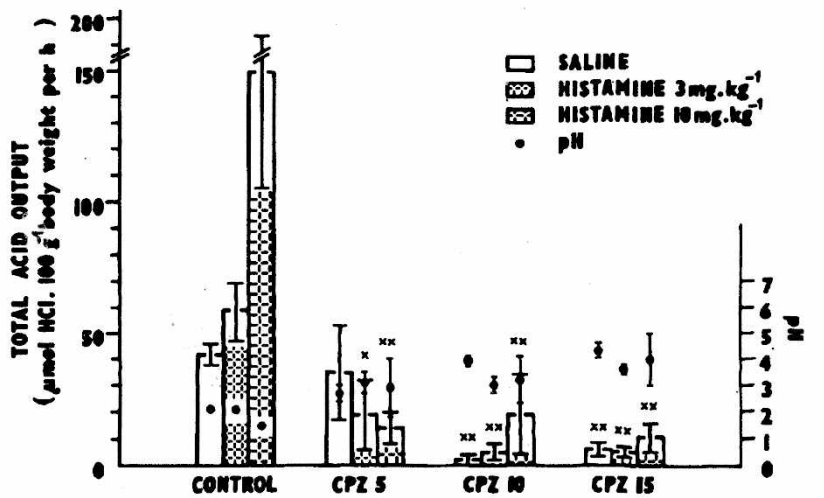

Fig. 2
Effect of i.p. injection of chlorpromazine (CPZ – 5, 10 and 15 mg/kg) on gastric acid secretion and pH in control (saline injected s.c.) and after stimulation by histamine (3 and 10 mg/kg). The values are expressed as means ± S.E.M. of 8 experiments (x $p<0.05$, xx $p<0.01$).

Table 1
Incidence of gastric lesions and gastric bleeding

Chlorpromazine mg/kg	Pretreatment mg/kg or IU/kg	No. of rats with lesions / %	gastric bleeding
Control	–	8/100	1
5.0	–	5/ 63	0
10.0	–	2/ 25	0
15.0	–	4/ 50	0
Control	Histamine 3.0	8/100	1
5.0	Histamine 3.0	5/ 63	0
10.0	Histamine 3.0	2/ 25	0
15.0	Histamine 3.0	4/ 50	0
Control	Histamine 10.0	8/100	0
5.0	Histamine 10.0	8/100	0
10.0	Histamine 10.0	5/ 63	0
15.0	Histamine 10.0	4/ 50	0
Control	Insulin 0.3	8/100	3
5.0	Insulin 0.3	8/100	0
10.0	Insulin 0.3	5/ 63	2
15.0	Insulin 0.3	8/100	3
Control	Insulin 3.0	8/100	8
5.0	Insulin 3.0	8/100	8
10.0	Insulin 3.0	8/100	0
15.0	Insulin 3.0	8/100	8

Indomethacin (20 mg/kg) was applied 1 h after administration of chlorpromazine, histamine or insulin.

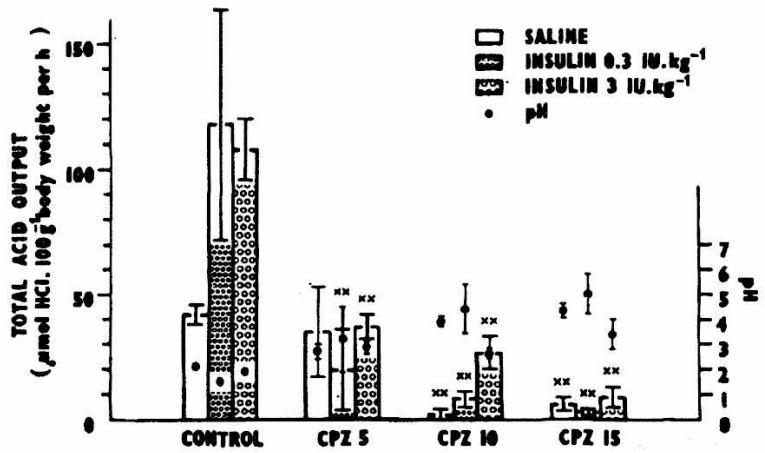

Fig. 3
Effect of i.p. injection of chlorpromazine (CPZ – 5, 10 and 15 mg/kg) on gastric acid secretion and pH control (saline injected s.c.) and after s.c. stimulation by insulin (0.3 and 3 IU/kg). The values are expressed as means ± S.E.M. of 8 experiments (xx $p<0.01$).

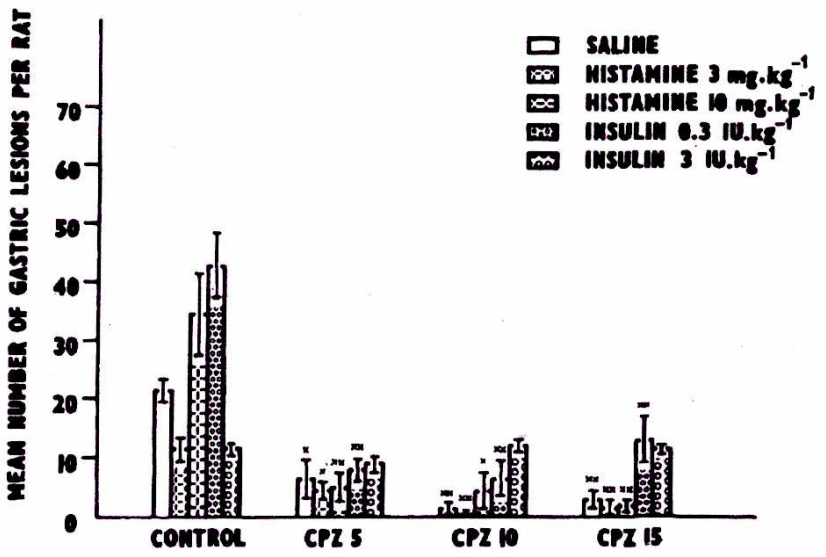

Fig. 4
Mean number of gastric lesions for chlorpromazine-treated rats (CPZ, i.p.) after s.c. administration of saline, histamine and insulin in combination with indomethacin (20 mg/kg). The significance of the change is shown as x $d_5 < 0.05$ xx or $d_1 < 0.01$ (non-parametric Wilcoxon's test).

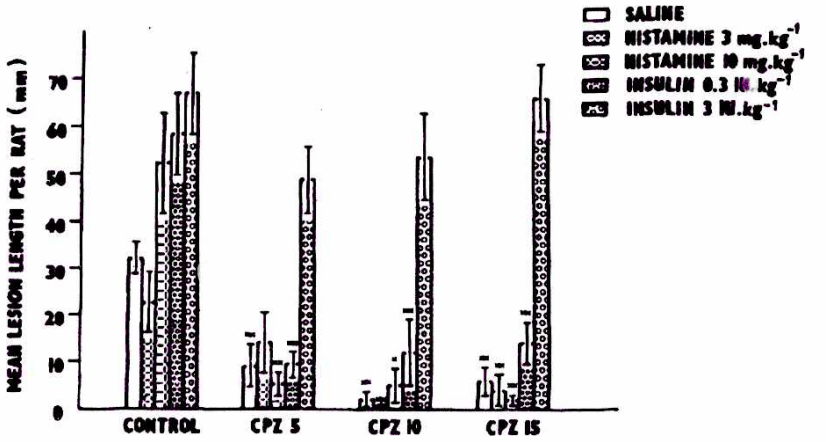

Fig. 5
Total length of gastric lesions expressed as a mean per rat for chlorpromazine-treated rats (CPZ, i.p.) after s.c. administration of saline, histamine and insulin in combination with indomethacin (20 mg/kg). The significance of the change is shown as x $d_5 < 0.05$ or xx $d_1 < 0.01$ (non-parametric Wilcoxon's test).

After histamine and insulin pretreatment, total gastric acid secretion also rose significantly compared with the values in the control rats. After chlorpromazine doses of 10 and 15 mg/kg this was significantly inhibited. A significant decrease in acid secretion after all three doses of chlorpromazine was also observed in the groups pretreated with histamine (in doses of 3 and 10 mg/kg) and insulin (0.3 and 3 IU/kg) ($p < 0.05$ and $p < 0.01$ respectively). Compared with the control group, pH rose after all three doses of chlorpromazine (Fig. 2 and 3).

Effect of chlorpromazine on the formation of stomach lesions induced by indomethacin in rats pretreated with 0.9 % NaCl solution, histamine and insulin

The incidence of stomach lesions after administration of the above mentioned test substances combined with indomethacin (20 mg/kg) is shown in Tab. 1 and Fig. 4 and 5. The results show a significant decrease in the number and size of lesions in the glandular part of the stomach after the administration of chlorpromazine in doses of 5, 10 and 15 mg/kg compared with the control group ($d_1 < 0.01$).

Pretreatment with histamine in a dose of 3 mg/kg did not influence the number of rats with stomach damage, but mildly reduced the number and size of the lesions compared with the control group (Tab. 1, Fig. 4 and 5). The larger dose of histamine (10 mg/kg) raised the number and length of the stomach lesions ($d_1 < 0.01$). The simultaneous administration of chlorpromazine and histamine inhibited the formation of stomach lesions ($d_5 < 0.05$, $d_1 < 0.01$) in every case except in the combination 5 mg chlorpromazine/kg and 3 mg histamine/kg.

The most striking damage to the gastric mucosa, among all experimental animals, was observed after combining insulin with indomethacin. Ulceration after indomethacin with insulin pretreatment (0.3 IU/kg) was inhibited, in regards to both the number and length of lesions, by all three doses of chlorpromazine ($d_1 < 0.01$). Inhibition of lesion formation after the larger dose of insulin (3 IU/kg) was weaker. In this case, chlorpromazine in doses of 5, 10 and 15 mg/kg did not influence the number and extent of stomach lesions. At the same time, massive haemorrhage into the lumen of the stomach was present in this group (Tab. 1).

Discussion

The results indicate that under basal conditions chlorpromazine inhibits gastric secretion and total acid formation in rats, thereby raising the pH of the stomach contents. It also has the same effect after peripheral stimulation of secretion by histamine or after central stimulation by insulin. This inhibitory effect of chlorpromazine on gastric secretion was compared with its effects on the ulcerogenic activity of indomethacin. In the control groups pretreated with 0.9 % NaCl solution, the ulcerogenic activity of indomethacin fell as the dose of chlorpromazine rose, i.e. correlated directly with inhibition of gastric secretion. This finding supports the theory that substances which do not irritate the gastric mucosa directly (as is evident mainly after the s.c. administration of indomethacin), but lower its defense, require an acid environment in the gastric lumen to be able to induce mucosal lesions (Guth *et al.* 1979, Robert 1984).

Similar results were obtained after administering chlorpromazine to animals pretreated with histamine. The mild decrease which occurred in lesion formation after the administration of histamine in a dose of 3 mg/kg and which does not fit into the anticipated pattern, can be attributed to "adaptive cytoprotection". This is a phenomenon in which weak irritants or mild stress situations raise the resistance of the gastric mucosa to subsequently administered strongly aggressive stimuli (Glavin *et al.* 1987, Takeuchi *et al.* 1987a). A different effect was observed after administering indomethacin with histamine in a dose of 10 mg/kg, when the extent of damage to the gastric mucosa was significantly greater. Similar results were

obtained by Hansen *et al.* (1980), who found that histamine pretreatment (i.v.) worsened gastric lesions induced in cats by the intravenous infusion of aspirin. In the combination of indomethacin with histamine chlorpromazine also significantly inhibited the formation of lesions.

Insulin-stimulated secretion was inhibited by chlorpromazine in correlation to the dose, but indomethacin-induced gastric lesions were inhibited only in the case of the smaller dose of insulin (0.3 IU/kg). The combination with the larger dose of insulin is at variance with the theory of the need for an acid environment in the stomach for ulcer formation after indomethacin.

It is known from the literature that large doses (5 IU/kg) of insulin alone induce stomach lesions in rats (Axelson *et al.* 1987). Since the formation of gastric lesions after insulin was inhibited by antrectomy and vagotomy, and also after the administration of omeprazole and ranitidin, the authors considered that an acid environment in the stomach was necessary for the formation of ulcers. On the other hand, they demonstrated that this dose of insulin – as distinct from lower doses – inhibited acid secretion. At the end, they therefore attributed the formation of lesions to a combination of acid with activity of the vagus and of circulating gastrin (Axelson *et al.* 1987). As far as the protective effect of chlorpromazine is concerned, it can be concluded from the results that this may be mediated by inhibition of acid secretion. Chlorpromazine, however, belongs to a group of drugs with a very wide range of action on the structure of membranes and the function of cells. It inhibits the flow of calcium ions across cell membranes, the activity of both membrane-bound and intracellular enzyme systems (Farber *et al.* 1977) and calmodulin (Cheung 1980) and influences the stabilization of cell membranes (Rabkin 1987). Many of these properties are considered to participate in the protective effect of chlorpromazine on liver cells (Farber *et al.* 1977, Chien *et al.* 1977) and myocardial cells (Rabkin 1987) and of trifluoperazine on the survival of dog kidneys conserved for transplantation (Asari *et al.* 1987). All these protective effects on different organs were described *in vivo* and *in vitro*.

Another possible mechanism of protection of the gastric mucosa is the ability of large doses of chlorpromazine to raise the blood sugar level (Ginestet *et al.* 1989). It has been demonstrated that diabetics have a lower incidence of duodenal ulcers (Dotewall 1959) and that hyperglycaemia protects animals from stress- and aspirin-induced gastric ulcers (MacDonald 1977). A correlation between the motility of the stomach, the blood sugar level and raised ulcerogenicity of indomethacin has likewise been described and it may be modified by chlorpromazine (Takeuchi *et al.* 1986, 1987b). It is also considered that the antihistaminic effect of chlorpromazine should be taken into account; this effect is particularly manifest after large doses and could thus serve as evidence for the peripheral action of chlorpromazine. Promethazine, another phenothiazine derivative with an antihistaminic effect, inhibits phenylbutazone-induced stomach ulcers (Kohút and Nicák 1972).

The inability of chlorpromazine to prevent the formation of ulcers after the administration of indomethacin together with a large dose of insulin is indicative of a multifactorial aetiology. The ulcerogenicity of indomethacin (due to its effect on motility and prostaglandin synthesis, for instance) is multiplied by further effects of insulin, such as its catabolically proteolytic effect in the tissues of fasting rats and the lack of sources of energy and glucose (Takeuchi *et al.* 1989). Potentiation of the

effect of indomethacin by these factors could be responsible for the ineffectiveness of chlorpromazine despite inhibition of acid secretion.

We can conclude that chlorpromazine influences indomethacin-induced lesions by both a peripheral and a central route.

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