HISTAMINE IN STRESS ULCER PROPHYLAXIS IN RATS

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Background: Gastrin and its analogues increase the gastric acid secretion, but also enhance mucosal defense mechanisms. On the other hand, increased formation of histamine leading to an increase in gastric acid secretion is accompanied with gastroprotection and acceleration of gastric ulcer healing. Aim of this study was to examine the effect of histamine on stress induced gastric ulcers in rats. Methods: Male Wistar rats were exposed to water immersion and restrain stress (WRS) for 3.5 h at 23°C. Before WRS rats were pretreated with saline, histamine, ranitidine or omeprazole. Results: WRS produces gastric lesions which were strongly reduced by ranitidine or omeprazole. Also treatment with histamine markedly reduced ulcer area evoked by WRS. Addition of histamine to ranitidine or omeprazole caused an additional reduction in ulcer area. Gastroprotective effect of histamine was accompanied with the increase in gastric blood flow (GBF). Administration of omeprazole or ranitidine alone was without significant effect on GBF. Histamine caused a slight decrease in gastric luminal pH, whereas ranitidine or omeprazole significantly increased gastric luminal pH. Plasma interleukin-1β was significantly reduced after administration of omeprazole, ranitidine, or histamine, however, the effect of histamine was less pronounced. DNA synthesis was increased after administration of omeprazole, ranitidine or histamine when compared with WRS alone. Administration of histamine in combination with ranitidine or omeprazole caused an additional increase in DNA synthesis. Conclusions: Histamine exhibits protective effect and increases gastroprotective effect of ranitidine and omeprazole against stress-induced gastric lesions. This effect of histamine seems to be independent on gastric acid secretion but related to the increase in gastric blood flow and the reduction in activation of cytokine cascade.

Key words: stress ulcer, gastroprotection, histamine, gastric blood flow, interleukin-1β, DNA synthesis.

INTRODUCTION

Stress gastric ulcer is a serious complication which may occur in patient exposed to intensive stress. The development of such ulcer is observed usually within a few hours after burns, polytrauma, central nervous system lesions,
shock, big operations or severe infection (1). Several mechanisms have been considered to play a role in the pathogenesis of stress induced gastric lesions such as an increase in gastric acid and pepsin secretion, a decrease in gastric blood flow and bicarbonate secretion, a suppression of mucosal generation of prostaglandins, an inhibition of mucosal growth and cell proliferation and an alteration of gastric motility (1—3).

Peptic ulcer has been attributed to an imbalance between the aggressive factors and mucosal resistance (4). For many years it was assumed that an overproduction of gastric acid due to excessive gastrin-histamine stimulation was the most important factor in the development of peptic ulcer, and for this reason the treatment of this disease was based mainly on an inhibition of gastric acid secretion (5, 6).

On the second hand, it has been demonstrated that gastric defense mechanisms, which prevent mucosal injury, are enhanced by the same factors that increase acid secretion such as central vagal stimulation and administration of gastrin or its analogues (7—9). Gastrin and its analogues exhibit the protective effect against ulcers evoked by HCl (10, 11), acidified aspirin (12), ethanol (8, 13, 14) and stress (15, 16).

Gastrin stimulates enterochromaffin-like cells (ECL cells) to enhance histamine synthesis and release in the oxyntic mucosa (17). Histamine is the predominant chemostimulator of hydrogen ion secretion in the stomach. It activates H₂ receptors of parietal cells leading to an increase in gastric acid secretion (17). Under certain condition such as pregnancy, histamine formation is increased (18, 19) leading to the rise in gastric acid secretion and continuous mucosal exposure to high concentration of hydrogen ions (20, 21). However, pregnancy is accompanied with remission of peptic ulcer disease in humans (22, 23) and gastroprotection (24, 25), and acceleration of gastric ulcer healing (26) in animal experimental studies. Also, studies with administration of exogenous histamine or its analogs have shown gastroprotective effect against experimental ulcers evoked by HCl (10, 11), ethanol (27) or ammonia (28), whereas administration of H₂ receptor antagonists to block the gastric acid secretion may result in aggravation of experimental gastric ulcers evoked by ethanol (29), acidified aspirin (12) or ammonia (28, 30).

The aim of the present study was to examine the effect of histamine and gastric acid secretion on stress induced gastric lesions and to evaluate the factors involved in histamine evoked effects on gastric mucosal integrity.

**MATERIALS AND METHODS**

Studies were performed on Wistar male rats weighing 200—250 g fasted for 24 h prior to the experiment with the free access to water. Studies were conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University.
Production of gastric lesions

Stress induced gastric lesions were provoked by placing the animals in individual cages, causing immobilization and immersing them in water at 23°C up to the xyphoid process for 3.5 h (water and restraint stress — WRS) as described previously (2). Studies included the following groups of rats: [1] animals treated with vehicle before WRS (control); [2] animals treated ranitidine (1 h before WRS, 40 mg/kg subcutaneously (s.c.)); [3] animals treated with omeprazole (1 h before WRS, 20 mg/kg s.c.); [4] animals treated with histamine (30 min before WRS, 2 mg/kg s.c.); [5] animals treated with the combination of ranitidine and histamine (at the dose and time as above); [6] animals treated with the combination of omeprazole and histamine (at the dose and time as above).

Determination of gastric blood flow

After 3.5 h of WRS animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland) and the abdomen was opened by a midline incision. The stomach was exposed and the gastric blood flow (GBF) was measured using laser Doppler flowmeter (PeriFlux 4001 Master monitor, Perimed AB, Järfläa, Sweden). GBF was measured in five areas of the oxyntic portion of the stomach and the mean value of five recordings presented as percent of the flow recorded in rats treated with saline.

Determination of gastric luminal pH, and gastric mucosal lesions

After measurement of GBF, the pH of gastric content was determined using Digital pH meter PM1 (Polimed, Wroclaw, Poland). The number and the area of necrotic lesions in the oxyntic mucosa were measured using computerized planimeter (Morphomat, Carl Zeiss, Berlin, Germany) as described previously (31). The measurement was made by person blinded to the origin of coded specimens. The stress lesion was defined as a round or linear mucosal black or red defect of at least 0.1 mm in diameter. After determination of the number and area of gastric lesions, biopsy samples (100—150 mg) of the oxyntic mucosa were taken and placed either in 10% formalin for histology or weighed and used for assessment of mucosal DNA synthesis.

Determination of mucosal DNA synthesis

The rate of DNA synthesis in the mucosa scraped from the oxyntic gland area was determined as described previously (32). Briefly, the mucosa was incubated at 37°C for 45 min in 2 ml of medium containing 8 μCi/ml of [3H]thymidine [6-3H]-thymidine, 20—30 Ci/mmol, Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic). The reaction was stopped with 0.4 N perchloric acid. DNA content of the samples was determined by Giles and Myers procedure (33). The incorporation of [3H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as disintegrations per minute [3H]thymidine per microgram DNA (dpm/μg DNA).

Determination of plasma gastrin and interleukin-1β concentration

After measurement of gastric lesion area the venous blood was taken from vena cava, the plasma was separated and collected for later determination of plasma gastrin and interleukin-1β (IL-1β) concentrations. Plasma gastrin level was measured by radioimmunoassay (RIA) as
described previously (32). The sensitivity of assay was 1 pmol/l. Plasma IL-1β was measured in duplicate using the BioSource Cytoscreen rat IL-1β kit based on a solid phase sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) (BioSource International, Camarillo, California, USA). Concentration of IL-1β was determined from a standard curve of recombinant IL-1β and concentrations was expressed as pg/ml. The ELISA detection limit of IL-1β was 3 pg/ml.

**Statistical analysis**

Comparison of the differences between the mean values of various groups of experiments was made by analysis of variance and the Student’s T test for unpaired data. A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as means (±S.E.M.).

**RESULTS**

The exposure to stress for 3.5 h resulted in appearance of multiple erosions in oxyntic mucosa and the mean lesion area reached 8.75 ± 0.75 mm² (Fig. 1). Administration of ranitidine or omeprazole prior to stress caused a marked decrease in area of gastric lesions by 93 or 95%, respectively. Treatment with histamine before stress reduced significantly ulcer area by 70%. Addition of histamine to ranitidine or omeprazole caused further significant reduction in ulcer area evoked by WRS below that observed after pretreatment with ranitidine or omeprazole given alone.

![Fig. 1. Area of gastric lesions in rats exposed to stress and treated with saline, ranitidine, omeprazole or histamine given alone or in combination. Mean ± S.E.M. of 8—14 observations. *P* < 0.05 compared with saline (control), †P < 0.05 compared with ranitidine given alone, ‡P < 0.05 compared with animals treated with histamine, ‡‡P < 0.05 compared with animals treated with omeprazole.](image-url)
Histological appearance of intact gastric oxyntic mucosa is shown in Fig. 2. Histological examination of the oxyntic mucosa after 3.5 h stress revealed damage to the surface epithelium with many cells sloughed off into the gastric lumen and necrosis occupying about 10—20% of the mucosal strip length (Fig. 3). Administration of histamine before exposure to stress resulted in the reduction of gastric mucosal damage in morphological examination (Fig. 4). The gastric mucosal damage or necrosis were limited to less than 5% of mucosal strip length. Administration of ranitidine or omeprazole completely prevented gastric mucosal damage in morphological features.

Fig. 2. Histological appearance of intact gastric mucosa. Hematoxylin and eosin stain, magnification ×260.

Figure 5 demonstrates gastric mucosal DNA synthesis in animals receiving saline (control), ranitidine, omeprazole or histamine and exposed to stress for 3.5 h. In control group of animals exposed to stress mucosal DNA synthesis reached value 27.11 ± 1.6 dpm/μg DNA. Treatment with ranitidine alone resulted in a significant increase in DNA synthesis to 155% of control value. Similar effect was observed after administration of omeprazole. Histamine alone significantly increased the gastric mucosal DNA synthesis by 26%, but this effect was less pronounced than that in group treated with ranitidine or omeprazole. Administration of histamine in the combination with ranitidine or omeprazole caused a further significant increase in DNA synthesis.
**Fig. 3.** Histological appearance of typical erosion in the oxyntic mucosa after 3.5 exposure to water immersion and restraint stress. Hematoxylin and eosin stain, magnification ×260.

**Fig. 4.** Histological appearance of gastric mucosa obtained from animals treated with histamine and exposed to water immersion and restraint stress for 3.5 h. Hematoxylin and eosin stain, magnification ×260.
Fig. 5. Effect of saline, ranitidine, omeprazole or histamine given alone or in combination on gastric mucosa DNA synthesis in animals exposed to water immersion and restraint stress for 3.5 h. Mean ± S.E.M. of 8–14 observations. *P < 0.05 compared with saline (control), †P < 0.05 compared with ranitidine given alone, ‡P < 0.05 compared with animals treated with histamine.

After 3.5 h of stress the gastric luminal pH was 2.2 ± 0.2 (Fig. 6) in control group of animals. Administration of histamine decreased luminal pH to 1.8 ± 0.3 but this effect was not statistically significant. Ranitidine, administrated before stress, markedly enhanced gastric luminal pH to 3.8 ± 0.4. Effect of omeprazole administration on luminal hydrogen ion concentration was more pronounced with pH reaching value of 5.6 ± 0.4. Treatment with histamine slightly reduced effect of ranitidine on luminal pH and abolished significant difference between ranitidine treated and control group. Administration of histamine in animals treated with omeprazole failed to affect significantly the effect of omeprazole on gastric luminal pH.

Mucosal GBF in control group exposed to stress and treated with saline reached 26.2 ± 2.5 ml/100 g tissue/min and was recognized as 100 ± 9.4% (Fig. 7). Ranitidine given alone was without significant effect on GBF and the same result was observed after administration of omeprazole. Treatment with histamine increased GBF by 48.8%. Addition of histamine to ranitidine or omeprazole caused the significant increase in GBF when compared to ranitidine or omeprazole given alone and this value was similar as in animals treated with histamine alone.
Fig. 6. Gastric luminal pH in animals in groups as in Fig. 5. Mean ± S.E.M. of 8—14 observations. *P < 0.05 compared with saline (control).

Fig. 7. Gastric blood flow in animals in groups as in Fig. 5. Mean ± S.E.M. of 8—14 observations. *P < 0.05 compared with saline (control), *bP < 0.05 compared with ranitidine given alone, *cP < 0.05 compared with animals treated with omeprazole.
Fig. 8. Plasma gastrin concentration in animals in groups as in Fig. 5. Mean ± S.E.M. of 8—14 observations. *P < 0.05 compared with saline (control).

Fig. 9. Plasma interleukin 1β in animals in groups as in Fig. 5. Mean ± S.E.M. of 8—14 observations. *P < 0.05 compared with saline (control).
Plasma gastrin concentration in animals treated with saline and exposed to stress (control group) reached value $136.9 \pm 10.6$ pmol/l (Fig. 8). Administration of ranitidine caused an increase in plasma gastrin level by 160%. Plasma gastrin concentration in animals treated with omeprazole and exposed to stress was four fold higher than in control group. Treatment with histamine alone was without any significant effect on plasma gastrin level in animals exposed to stress. Administration of histamine in the combination with ranitidine reduced ranitidine evoked increase in plasma gastrin level, but this effect was not statistically significant. The same effect of histamine was observed when histamine was added to omeprazole treated group of animals.

Plasma IL-1β levels (Fig. 9) in control group treated with saline and exposed to stress reached $132.6 \pm 8.0$ pg/ml. Treatment with ranitidine, omeprazole or histamine alone significantly reduced plasma IL-1β concentration by 27, 33 or 16%, respectively. Administration of histamine in the combination with ranitidine or omeprazole additionally reduced plasma IL-1β, but this effect was not statistically significant.

DISCUSSION

The incidence of stress ulcers ranged between 80 to 100% in burns, polytrauma and sepsis before the era of routine prophylaxis (1). Treatment with antacids (1), histamine H₂ receptor antagonists (1, 34) or omeprazole (34, 35) caused a significant reduction in the frequency of stress ulcer bleeding in burn or mechanically ventilated trauma patients. Similar gastroprotective effect of ranitidine administration on stress-induced ulcer was observed in our present study. Ranitidine caused an increase in gastric luminal pH and this effect was accompanied by a reduction in gastric ulcer area. Also, the treatment with ranitidine before stress led to an increase in gastric mucosal DNA synthesis and plasma gastrin concentration and, a decrease in plasma interleukin-1β concentration. Administration of omeprazole before stress resulted in similar beneficial effect on gastric mucosa as treatment with ranitidine.

The major finding of the present study is the observation that histamine administration reduces the development of stress-induced gastric lesions. It is assumed that an imbalance between the aggressive factors and the protective mechanisms of the stomach causes acute ulceration. Since Schwartz's dictum in 1910 "no acid — no ulcer", excessive gastric acid secretion was considered to be the most important factor in the pathogenesis of peptic ulcer (36) and treatment of this disease was based mainly on the inhibition of gastric secretion. In our present study, we have found, for the first time, that histamine administration attenuates gastric mucosal damage evoked by stress. This gastroprotective
effect of histamine administration was observed despite low pH in gastric lumen. Several mechanisms may be involved in gastroprotective effect of histamine. Administration of histamine was accompanied with the increase in gastric blood flow and DNA synthesis, as well as, the reduction in plasma interleukin-1β concentration.

An increase in, or maintenance of mucosal blood flow play an important role in the prevention of gastric acute injury (37). The role of blood is not only attributed to the transport of oxygen and nutritive substrates to the mucosa, but also to the removal of carbon dioxide and hydrogen ions and bring bicarbonate to the mucosa. The importance of blood flow for acid-base balance was clearly demonstrated in experiments performed by Starlinger et al. (38). At the same time, they measured pH of lamina propria and mucosal blood flow. Perfusion of the gastric lumen with acid, caused the drop of intramural pH for short time, and after them pH returned to baseline. Simultaneously, during this period, mucosal blood flow increased substantially. On the second hand, when the increase in gastric blood flow was blocked by administration of vasopressin, gross ulceration of the stomach was visible. Moreover, they investigated the effect of shock on the gastric mucosa (39). When the rat stomach was filled with acid and rats were subjected to shock, ulceration appeared in 100% of mucosal surface. Recovery of gastric mucosal blood flow to control level and treatment parenteral bicarbonate abolished gastric ulceration (39). In our present study, gastroprotective effect of histamine administration was accompanied by an increase in gastric blood flow. This circulatory effect of histamine seems to be unrelated to H₂ receptor activation because treatment with H₂ receptor antagonist — ranitidine did not reduce but significantly enhanced histamine evoked increase in GBF.

Another mechanism of histamine evoked gastroprotection and gastric hyperaemia may be related to stimulation of sensory nerves. Histamine has been shown to stimulate nociceptive afferent fibers in gastrointestinal tract (40, 41) On the second hand, the stimulation of sensory fibers by capsaicin or administration of their mediator — CGRP increase gastric mucosal blood flow and exert a protective effect in different experimental models of gastric ulcers (37, 42), whereas the deactivation of sensory nerves aggravates gastric mucosal lesions induced by various ulcerogenic factors (43, 44), as well as, prolongs the gastric ulcer healing (45).

The exposure to stress results in inhibition of DNA synthesis and cell renewal in gastric mucosa (2, 46, 47). In our present study, administration of ranitidine, omeprazole or histamine enhanced mucosal DNA synthesis. According to previous studies (48, 49), ranitidine and omeprazole increased plasma gastrin concentration. Gastrin exhibits gastroprotective effect (10—16), and stimulates gastric mucosal growth (50). In contrast to ranitidine or omeprazole, administration of histamine showed tendency to reduce plasma
gastrin concentration. However, mucosal DNA synthesis in histamine treated animals was also increased. This effect seems to be related to the attenuation of gastric mucosal damage.

In the present study, administration of ranitidine, omeprazole or histamine before WRS caused the reduction in plasma interleukin 1β concentration. Interleukin-1β is a well known component of acute inflammation and plays a crucial role in the induction of release other members of the cytokine cascade (51). Interleukin 1 is a stimulus of its own gene expression and synthesis (52) as well as induces production of tumor necrosis factor platelet activating factor, prostaglandins and pro-inflammatory interleukins such as IL-2, IL-3, IL-4, IL-5, IL-6, IL-7 and IL-8 (51, 53). On the other hand, blocking the action of interleukin 1 by interleukin 1 receptor antagonist prevents the synthesis and release of pro-inflammatory mediators and reduces the severity of inflammation (53). Our observation that treatment with ranitidine, omeprazole or histamine decreases plasma interleukin-1 concentration in animals exposed to stress indicates their beneficial influence on gastric mucosa.

Another interesting finding of our present study is that histamine increases gastroprotective effect of ranitidine and omeprazole against stress-induced gastric lesions causing maximal reduction in gastric lesions area. These combinations causes also a maximal increase in the gastric mucosal blood flow and DNA synthesis. This observation suggests that addition of histamine to treatment with ranitidine or omeprazole may be useful in stress ulcer prophylaxis, however its clinical application requires further studies.

In summary, our present results demonstrate that histamine exhibits the protective effect against stress-induced gastric ulcer and increases the gastroprotective effect of ranitidine and omeprazole against stress-induced gastric lesions. The improvement of gastric blood flow and the decrease in activation of cytokine cascade seem to be a major mechanisms involved in the histamine evoked gastroprotection against ulcers induced by stress.

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