Synergistic action of famotidine and chlorpheniramine on acetic acid-induced chronic gastric ulcer in rats

Zhen Qin, Chao Chen

AIM: To assess the synergistic action of famotidine (FMD) and chlorpheniramine (CPA) on acetic acid-induced chronic gastric ulcer in rats.

METHODS: Chronic gastric lesions were induced in male Sprague-Dawley (SD) rats by serosal application of the acetic acid. Forty SD rats were randomly divided into blank group (n = 8), control group (n = 8), FMD group (n = 8), CPA group (n = 8), and FMD+CPA group (n = 8). Each group was given intraperitoneally (i.p.) 0.5 mL/100 g distilled water, 9 g/L NaCl saline, 4 mg/kg FMD, 10 mg/kg CPA, 4 mg/kg FMD+10 mg/kg CPA, respectively, daily for 10 d. On d 10, ulcer area was determined by planimetry. The level of myeloperoxidase (MPO) in the liver homogenate was determined by biochemical methods and the plasma levels of 6-ketoprostaglandin F1 alpha (6-keto-PGF1α) and IL-8 were determined by radioimmunoassay.

RESULTS: The synergistic effects of FMD+CPA group on the lesion, IL-8, 6-keto-PGF1α and MPO were confirmed. The effect of FMD+CPA group was significantly different as compared to the control and FMD groups. The lesion (mm²) was reduced from 40.18±2.6 in control group to 2.83±2.97 in FMD+CPA group, P<0.01, and from 3.29±3.27 in FMD group to 6.83±2.97 in FMD+CPA group, P<0.01. The level of 6-keto-PGF1α increased from 7.55±1.65 ng/L in control group to 16.62±0.97 ng/L in FMD+CPA group, P<0.01, and from 3.29±3.27 in FMD group to 6.83±2.97 in FMD+CPA group, P<0.01. The levels of MPO in the liver homogenate decreased from 9.12±2.05 u/L in control group to 4.33±0.95 u/L in FMD+CPA group, P<0.01, and from 8.3±1.29 u/L in FMD group to 4.33±0.95 u/L, P<0.01.

CONCLUSION: The synergistic action of FMD and CPA on acetic acid-induced chronic gastric ulcer in rats decreases the incidence of ulcer and also enhances the healing of ulcer.

Key words: Gastric ulcer; Acetic acid; Famotidine; Chlorpheniramine; Interleukin-8; 6-Ketoprostaglandin F1 alpha; Myeloperoxidase

Qin Z, Chen C. Synergistic action of famotidine and chlorpheniramine on acetic acid-induced chronic gastric ulcer in rats. World J Gastroenterol 2005; 11(45): 7203-7207

INTRODUCTION

Peptic ulcer is a common disorder of the gastrointestinal system and the pathogenesis of peptic ulcer disease is multifactorial, including *Helicobacter pylori*, gastric acid, pepsin, gastroduodenal motility, smoking, use of nicotine, and complex interaction between so-called aggressive and protective factors[1]. Mast cells are initiators and regulators of inflammation. After mast cell degranulation, histamine causes the secretion of gastric acid by triggering H₂-receptors, markers of infiltration of inflammatory cells into the gastric mucosa, and expression of cytokines by triggering the H₂-receptors. Consequently, mast cells are considered as important effector cells in the pathogenesis of gastritis, especially in *H pylori*-associated peptic ulcer[2]. Histamine H₂-receptor antagonists that possess a potent antiresecretory activity can greatly enhance the healing rate of peptic ulcers. However, after H₂-receptor antagonist therapy peptic ulcer recurs rapidly and frequently. The possible reasons why the recurrence rate is high after H₂-antagonist therapy are acid rebound after the cessation of treatment, deficiency in gastric defensive factors such as gastric prostaglandin levels, and low maturity of regenerated mucosa. Recently, H₂-receptor antagonists are potent anti-inflammatory compounds. Erwin[3] reported that H₂-receptor antagonists have anti-inflammatory activity, including their effects on eicosanoid production.
and cytokine release and their influence on the release rate of proinflammatory mediators. Cetirizine reduces the attraction of inflammatory cells to the inflammatory focus after the antigen challenge and inhibits the expression of the intercellular adhesion molecule 1 (ICAM-1) on the surface of epithelial cells[4]. It also might be able to block the antigen-induced production of leukotrienes (LTC4)[7]. Loratadine and its metabolite decarboethoxyloratadine inhibit the release of tryptase and a-macroglobulin[8], interleukins (IL) 6 and 8[7], leukotrienes and prostaglandin D2 (PGD2)[8], and suppress the expression of ICAM-1 and of HLA-II antigens on the surface of epithelial cells[8]. Therefore, H1 and H2 blockers have not only antisecretory activity but also anti-inflammatory activity when they are used in the treatment of gastric ulcer. The aim of the present study was to assess the synergistic action of famotidine (FMD) and chlorpheniramine (CPA) on acetic acid-induced chronic gastric ulcer in rats.

MATERIALS AND METHODS

Materials

IL-8 and 6-ketoprostaglandin F1 alpha (6-keto-PGF1a) kits were obtained from Beijing North Institute of Biological Technology. Myeloperoxidase (MPO) kit was obtained from Nanjing Jiancheng Bioengineering Institute.

Animals

Male Sprague-Dawley (SD) rats (180-220 g) were provided by the Medical Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology (No: TJLA-2004-159). Animals were fed with standard laboratory chow and tap water, and kept in a room with constant humidity and temperature (25 °C) in a 12-h light-dark cycle for 1 wk before the experiments.

Induction of gastric ulcer

Gastric ulcers were induced using the method described by Takagi et al[9] with some modifications. Gastric ulcers were produced by the application of round filter paper (diameter: 5 mm) immersed in a 100% acetic acid on the serosal surface of anterior wall of the stomach approximately at the center of the corpus for 30 s and the process was repeated twice. This produced an immediate necrosis of the entire mucosa and submucosa (but not serosa) within the area (20 mm²), where the acetic acid was applied. The excess of acetic acid was then removed and the serosa was gently washed with saline. Control animals received no surgery. The abdomen was then sutured, and the animals were allowed to recover and returned to their cages with free access to food and water.

Experimental groups

Two days after the ulcer induction, 40 male SD rats, weighing 180-220 g, were randomly divided into blank group (n = 8), control group (n = 8), FMD group (n = 8), CPA group (n = 8), FMD+CPA group (n = 8). Rats were given intraperitoneally (i.p.) 0.5 mL/100 g distilled water, 0.5 mL/100 g, 9 g/L NaCl saline, 4 mg/kg FMD, 10 mg/kg CPA, 4 mg/kg FMD+10 mg/kg CPA for 10 d. On d 8, the rats were deprived of food for 48 h prior to the experiment but allowed free access to water. After the last dose, each animal was anesthetized with 10 g/L sodium thiopental. Five milliliters of blood was collected by intracardiac puncture. Each sample contained 0.22 mL 6-keto EDTA, and was centrifuged for 15 min at 5 500 r/min. The plasma from each sample was stored at -80 °C for the determination of the level of interleukin IL-8 and 6-keto-PGF1a by RIA using IL-8 and 6-keto PGF1a kits, respectively. Subsequently, the abdomen was opened and the stomach was exposed after the esophagus and pyloric were ligated, and then 5 mL 40 g/L neutral buffered formalin was instilled into the stomach via the incision for pathological examination.

Ulcerated area (mm²) determination

After being fixed in formalin overnight, the stomach was opened along the greater curvature and spread out with pins on a cork board, and then photographed. The ulcerated area (mm²)[10] was computed using the following equation: S = π(d1/2) × (d2/2), where S represents the ulcerated area (mm²), d1 and d2 represent the longest longitudinal and transverse diameters of the ulcer respectively.

Light microscopy

After fixation, the stomach was divided into two parts and each part was subdivided into four tissue strips by cutting along the whole width of the half stomach. The blocks were embedded in paraffin wax. Five micrometer thick sections were cut in a standard fashion and stained with hematoxylin and eosin.

Radioimmunoassay (RIA) for IL-8 and 6-keto-PGF1a

All the blood samples for IL-8 and 6-keto-PGF1a determination were stored in the tubes. Serum level of IL-8 and 6-keto-PGF1a was determined by RIA with the IL-8 and 6-keto-PGF1a kits according to their manufacturer's instructions.

MPO activity

The level of MPO in the liver homogenate was determined by biochemical methods with the MPO kits according to its manufacturer's instructions.

Statistical analysis

All the values were expressed as mean±SD. One-way ANOVA was used to analyze the differences among them. Student’s t-test was applied to comparisons between the two groups. P<0.05 was considered statistically significant. Software SPSS10.0 was used in all statistical analyses.

RESULTS

Ulcerated area (mm²)

The lesion (mm²) decreased from 40.18±2.6 in control
group to 0 in the blank group. The lesion (mm$^2$) was reduced from 40.18±2.6 in the control group to 6.83±2.97 in the PMD+CPA group ($P<0.01$), and from 32.9±3.27 in FMD group to 6.83±2.97 in PMD+CPA group ($P<0.01$) (Table 1).

**Light microscopy**

Microscopic findings on the gastric mucosal scar showed that the mucosal architecture of the scar healed by FMD+CPA group (Figure 1D) was better restored than that of the scar in the control group or in the case of treatment with FMD alone which exhibited much lymphocyte infiltration, and surface epithelial lesions (Figures 1A and B).

**Plasma level of 6-keto-PGF$_{1a}$**
The level of 6-keto-PGF$_{1a}$ in the FMD+CPA group was significantly different from that in the control and the FMD groups, which increased from 7.55±1.65 ng/L in the control group to 16.62±0.97 ng/L in the PMD+CPA group ($P<0.01$) ng/L, and from 13.15±1.48 ng/L in the FMD group to 16.62±0.97 ng/L in the PMD+CPA group ($P<0.01$) (Table 1).

**MPO level in liver homogenate**

In the liver homogenate, the level of MPO activity decreased from 9.12±2.05 µ/L in the control group to 4.33±1.29 µ/L in the PMD+CPA group, $P<0.01$, and from 8.3±1.29 µ/L in the FMD group to 4.33±1.29 µ/L ($P<0.01$) (Table 1).

**The plasma levels of IL-8**
The plasma level of IL-8 decreased from 0.69±0.11 ng/L in the control group to 0.4±0.04 ng/L, $P<0.01$, and from 0.51±0.08 ng/L in the FMD group to 0.4±0.04 ng/L in the PMD+CPA group ($P<0.05$) (Table 1).

**DISCUSSION**
The acetic acid injection-induced ulcer model is a mature classical model. The drawback of the model is that the dose of injection is not rigorous. We used a round filter paper (diameter: 5 mm) immersed in a 100% acetic acid.
on the serosal surface to establish the model, which is characterized by high success rate, small coefficient of variation, low rate of perforation.

Prostaglandins (PGs) are well-known mucosal defense factors, protecting the gastric mucosa against injury caused by a variety of toxic stimuli\cite{2,3,4}. PGE2 stimulates the secretion of gastric mucus and bicarbonate, increases mucosal blood flow, inhibits acid secretion, and reduces gastric motility. In addition, downregulation of proinflammatory cytokine expression by PGs is also likely to be important for mucosal protection against \textit{H. pylori} infection. 6-Keto PGF\(_{1\alpha}\) is a PGI2 metabolite, and PGI2 plays an important role in gastric cytoprotection. This study also approved that the ulcerated area decreased with the increase in the level of 6-Keto PGF\(_{1\alpha}\). This supports that prostaglandins possess gastric cytoprotection function.

Gastric mucosal integrity is maintained by an interplay of some aggressive and defensive factors controlling cell apoptosis and proliferation. Disturbing this balance leads to ulcer. Proinflammatory cytokines play an important role. IL-8 is an important cytokine in the host inflammatory response to \textit{H. pylori}\cite{2,4,5,6,7,8}, which correlates with its induction in gastric epithelial cells co-cultured with \textit{H. pylori} \textit{in vitro}\cite{2,4,5,6,7,8}. Upregulation of IL-8 by \textit{H. pylori} may lead to free radical generation and the release of proteolytic enzymes from activated neutrophils, affecting mucosal integrity\cite{9,10}. Eradication of \textit{H. pylori} in ulcer patients results in a reduction in antral IL-8 mRNA expression, neutrophil infiltration, and surface epithelial lesions\cite{9,10}, suggesting that inflammatory cytokines may play an important role in mucosal damage due to \textit{H. pylori} infection. Leukocyte infiltration in gastric mucosa is assessed by determining tissue activity of MPO\cite{11}, an enzyme used as a marker for leukocyte infiltration in a variety of tissues including the rat gastric mucosa. Recently, experimental data on \textit{H1} receptor antagonists demonstrate potentially anti-inflammatory effects in addition to their anti-allergic effects on histamine production, the histamine-induced secretion by the \textit{H1}-antagonist decarboethoxy-loratadine and 7-methoxy-loratadine\cite{12,13,14,15,16,17}.

The present experimental study demonstrated that \textit{H1}-antagonists reduced the release of proinflammatory mediators (IL-8 and MPO) from mast cells in comparison with the controls. The synergistic effect indicates that \textit{H1} antagonists may reduce the incidence rate of carcinoids.

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