Ranitidine, a Histamine-2 Receptor Antagonist, Ameliorates Caerulein-induced Pancreatitis in Rats

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Abstract: Histamine-2 (H2) receptor antagonists (H2RAs) are administered orally or parenterally for the treatment of gastro-duodenal ulcers, gastric hypersecretory diseases, and gastro-esophageal reflux disease. In Japan, H2RAs are frequently used in patients with acute pancreatitis to reduce the secretion of pancreatic juice. Ranitidine is a specific competitive H2RA with immunomodulatory and anti-inflammatory properties. The efficacy of ranitidine for the treatment of acute pancreatitis has not been sufficiently evaluated. In this study, we evaluated the efficacy of ranitidine for the treatment of caerulein-induced pancreatitis as a model of acute pancreatitis. The effect of ranitidine on pancreatitis was assessed by examination of serum amylase and lipase levels, pancreatic edema, and histological changes. The prophylactic administration of ranitidine significantly reduced elevated serum amylase and lipase levels, pancreatic edema, and histological changes. The prophylactic administration of ranitidine significantly reduced elevated serum amylase and lipase levels. At a histological level, ranitidine also reduces pancreatic edema, vacuole formation in pancreatic acinar cells, and inflammatory cell infiltration in the pancreas. An increase in the level of interleukin-10 in pancreatic tissue was also observed. These findings show that ranitidine accelerates the recovery of caerulein-induced pancreatitis. This effect may be due, at least in part, to increased anti-inflammatory cytokine production in pancreatic tissue and its protective role against local injury.

Key words: acute pancreatitis, histamine-2-receptor antagonist, cytokine

Introduction

Epidemiological data shows that the incidence of acute pancreatitis (AP) in Japan is increasing and ranges from 187 to 347 cases per million people. In 2003, mortality from acute pancreatitis in Japan was 0.2% for Stage 0–1 [Japan (JPN) severity score], 3.7% for Stage 2, and 35.6% for Stage 3–4 pancreatitis1). AP causes a wide range of abdominal inflammatory disorders characterized by pancreatic acinar cell injury together with local and systemic inflammatory responses. The underlying causes of AP are varied, but activation of digestive enzymes within pancreatic acinar cells is thought to be a critical initiating event2). Inflammatory cytokines play an important role in the onset of AP and in the development of severe AP3). The contribution of inflammatory cytokines to the pathogenesis of AP is...
not fully understood.

Ranitidine, a specific competitive histamine 2 (H₂) receptor antagonist (H₂RAs), is used extensively in the treatment of peptic ulcers, gastric hypersecretory diseases, and gastroesophageal reflux disease. H₂RAs alter the effect of histamine on chemotaxis, phagocytosis and superoxide anion production in phagocytes. In 1975, Nevalainen and Seppä reported that ranitidine has a beneficial effect on experimentally induced AP. Ranitidine reduces the level of elevated serum amylase, the amylase-to-creatinine-clearance ratio, and the histological damage associated with pancreatitis. Studies by Okajima et al revealed that ranitidine also prevents the release of elastase and reactive oxygen species, inhibits cell surface expression of CD11b and CD18, and increases the intracellular calcium concentration in neutrophils stimulated with formyl-methionyl-leucyl-phenylalanine (fMLP). The inhibition of neutrophil activation by ranitidine leads to a reduction in stress-induced gastric mucosal injury in rats.

The mechanisms underlying initiation of local events in the pancreas leading to AP and the systemic dissemination of inflammatory responses in AP are not fully understood. Furthermore, the role of H₂RA in AP has not been fully examined. The aim of the present study was to investigate the effect of ranitidine in caerulein-induced pancreatitis as an experimental model of acute pancreatitis that closely resembles human edematous pancreatitis.

Materials and Methods

Animals

Male Wistar rats, weighing 150–200 g, were purchased from Saitama Experimental Animal Supply Co., Ltd. (Suginomachi, Saitama, Japan) and were housed in climate controlled rooms with a 12-h light-dark cycle. All rats were fed a standard laboratory diet and were fasted overnight before the experiment.

Materials

Chemicals were purchased from the following pharmaceutical companies: disodium hydrogenphosphate 12-water (NaH₂PO₄·12H₂O) and sodium dihydrogenphosphate, anhydrous (Na₂HPO₄), from Wako Pure Chemical (Doshoumachi, Osaka, Japan); physiologic saline from Otsuka Pharmaceutical Co., Ltd. (Naruto, Tokushima, Japan); hexadecyltrimethylammonium bromide and caerulein from Sigma (St. Louis, MO, USA); sodium pentobarbital from Abbott Laboratories (Abbott Park, Illinois, U.S.A.); and ranitidine from Glaxo Smithkline Pharmaceutical Co., Ltd. (Shibuya, Tokyo, Japan).

Experimental procedures

Experimental procedures were performed as described previously. Briefly, after overnight fasting, male Wistar rats weighing 150–200 g were sacrificed 6 hours after an initial intraperitoneal (i.p.) injection of caerulein at 20 µg/kg body weight (BW) dissolved in physiologic saline (PS). Pancreatitis was induced by hourly i.p. injections (total, 4 injections) of caerulein at 20 µg/kg BW. Ranitidine at 4, 20 or 100 mg/kg BW, was administered by i.p. injection 30 min before and 2.5 hours after the initial i.p. injection of caerulein. PS administration under the same conditions served as a vehicle control (Fig. 1). Blood was collected from the abdominal aorta following administration of sodium pentobarbital (60 mg/
kg i.p.). The pancreas was removed and weighed. A portion of the pancreas was fixed in 10% phosphate-buffered formalin at room temperature for light microscopy and the remainder was frozen at −80°C for biochemical examination.

Measurement of serum amylase and lipase activity
Blood was centrifuged for 10 min at 1500 rpm. The activity of amylase and lipase in the serum was measured by the Caraway and UV-rate methods using an auto analyzer. Serum amylase and lipase were expressed as IU per liter.

Measurements of cytokine activity
The level of interleukin-10 (IL-10) in the pancreas was measured using commercially available enzyme-linked assay (ELISA) kits. Tissues were homogenized in 1.5 ml of phosphate buffer (20 mmol/L, pH 7.4). After centrifugation (14000 g for 5 min at 4°C), the concentration of IL-10 in the supernatant was measured according to the manufacturer’s instructions and expressed as pg per ml. The detection limit of the IL-10 ELISA was 31.4 pg/ml.

Histologic examination
Formalin-fixed pancreatic tissue was embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (HE). The severity of AP was evaluated in a blinded manner, by evaluation of vacuole formation within acinar cells, infiltration by inflammatory cells, hemorrhage into pancreatic tissue and necrosis of acinar cells, using Image J image management software (National Institutes of Health, USA). Photographic images of specimens stained with HE were obtained and gray-scale images were converted to black and white using the command “binary images”. This method distinguishes the black area, which is consistent with edema, vacuoles, and necrosis, from the white area. Grading of infiltration by inflammatory cells was based on the following scale: 0.absent : 1.mild : 2.moderate : 3.severe.
Fig. 2. Effect of prophylactic ranitidine administration on pancreatic edema. Pancreatic edema was measured by comparing pancreatic : body weight ratios expressed in grams / 100 grams body weight. Caerulein administration significantly increased the pancreatic : body weight ratio ( \*p < 0.01 ). This effect was inhibited by ranitidine at 20 and 100 mg / kg BW ( \*p < 0.001 ) (n = 6-10 rats).

**Statistical analysis**

Values for results are expressed as means ± SE. Data were statistically analyzed using one-way analysis of variance (ANOVA) for repeated measures, followed by the Scheffe’s F-test. Statistical comparisons between the two groups on each experimental day were performed using the unpaired t-test. P values of less than 0.05 were considered statistically significant.

**Results**

**Pancreatic tissue wet weight**

The pancreatic tissue wet weight per 100mg BW was significantly higher in the caerulein treatment group (Cae group) (0.79 ± 0.04) than in the control group (PS treatment group) (0.39 ± 0.03) (P < 0.01). By comparison, the pancreatic tissue wet weight per 100mg BW was significantly lower in the Ra (20) group (0.53 ± 0.03) and Ra (100) group (0.50 ± 0.03) than in the Cae group (P < 0.01). There was no significant difference in the pancreatic tissue wet weight per 100 mg BW between the Cae group and the Ra (5) group (0.65 ± 0.03) (P > 0.05) (Fig. 2).

**Activity of serum amylase and lipase I**

The activity of serum amylase and lipase I was significantly lower in the Ra (20) group (7,563 ± 1,137 IU / L, 1,293 ± 127 IU / L, respectively) and Ra (100) group (5,921 ± 1,859 IU / L, 1,283 ± 110 IU / L) than in the Cae group (10,353 ± 4,401 IU / L, 2,502 ± 272 IU / L) (P < 0.01). There was no significant difference in the activity of serum amylase and lipase I between the Cae group and Ra (5) group (9,695 ± 2,213 IU / L, 2,144 ± 134 IU / L) (P > 0.05) (Fig. 3, 4).

**Pancreatic tissue levels of IL-10**

The pancreatic tissue levels of IL-10 in the Control, Cae and Ra (5) groups was below
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Fig. 3. Effect of prophylactic ranitidine administration on the concentration of serum amylase. Caerulein administration significantly increased the level of serum amylase (*P < 0.01). This increase was inhibited by ranitidine at concentrations of 20 and 100 mg / kg BW (*P < 0.01) (n = 6–10 rats).

Fig. 4. Effect of prophylactic ranitidine administration on the concentration of serum lipase. Caerulein administration significantly increased the level of serum lipase (*P < 0.01). This increase was inhibited by ranitidine at concentrations of 20 and 100 mg / kg BW (*P < 0.01) (n = 6–10 rats).

31.4 pg / ml. In the Ra (20) group, the pancreatic tissue level of IL-10 reached 88.4 pg / ml (mean, n = 2) and those in the Ra (100) group reached 250 pg / ml (mean, n = 2).

**Histological staining**

Histological examination of pancreatic tissue stained with HE revealed pancreatic edema, inflammatory cell infiltration, and vacuolization of acinar cells in the Cae group. Changes in the pancreas from caerulein-induced pancreatitis, including pancreatic edema, inflammatory cell infiltration, and vacuolization of acinar cells, were markedly reduced in the Ra (20) and
Fig. 5. Microscopic section of a control pancreas showing the normal appearance of pancreatic acini. (HE; (a) ×40, (b) ×100, (c) ×200, (d) ×400.)

Ra (100) groups when compared with the Cae group. Hemorrhage or acinar cell necrosis was not observed in the pancreas in any groups in this study (Fig. 5-9). Image J analysis revealed a significant reduction in vacuolization of acinar cells in the Ra (20) and Ra (100) groups when compared with the Cae group (Fig. 10).

Fig. 11 shows infiltration of inflammatory cells in caerulein-induced pancreatitis. Infiltration of leukocytes in the pancreas was more prominent in the Cae group than in the Ra (20) and Ra (100) groups. Leukocyte infiltration in the pancreas was thus reduced by ranitidine.

Discussion

Histamine was isolated from ergot extracts in 1910 by Dale and Laidlaw.[14] Histamine is one of the most important mediators in a range of physiological and pathological conditions, including the regulation of gastrointestinal functions, such as gastric acid secretion, intestinal motility, and mucosal ion secretion and inflammatory reactions. The physiological functions of histamine are mediated through four types of membrane histamine receptors, H₁R, H₂R, H₃R and H₄R, all of which are hepta-helical G-protein-coupled receptors.[15-17] Histamine also enhances secretion of Th-helper (Th) 2 cytokines, including IL-4, IL-5, IL-10 and IL-13, by inhibiting the production of Th 1 cytokines IL-2 and IFN-γ and monokine IL-12.[18,19]

In Japan, H₂RAs are frequently used for the treatment of AP to reduce the secretion of pancreatic juice. Activation of H₂Rs is associated with a wide range of physiological actions from stimulation of gastric acid secretion to induction of human promyelocyte differentiation.[20] Histamine can also regulate cell proliferation via the H₂ receptor.[21-23] H₂Rs are expressed in many cell types, including nerve cells, vascular smooth muscle cells, hepatocytes, endothelial cells, neutrophils, eosinophils, monocytes and dendritic cells, as well as in T and
Fig. 6. Morphological changes in the pancreas 6 h after administration of caerulein. Sections of pancreas from caerulein-treated rats show interstitial edema, intracytoplasmic vacuoles and inflammatory cell infiltration. (HE; (a) × 40, (b) × 100, (c) × 200, (d) × 400.)

Fig. 7. Effect of prophylactic ranitidine administration on pancreatic histology. Sections of pancreas from caerulein-treated rats show interstitial edema, intracytoplasmic vacuoles and inflammatory cell infiltration. Caerulein + Ranitidine (5 mg/kg BW) (HE; (a) × 40, (b) × 100, (c) × 200, (d) × 400.)
Fig. 8. Effect of prophylactic ranitidine administration on pancreatic histology. Sections of pancreas from caerulein-treated rats show marked amelioration of interstitial edema, intracytoplasmic vacuoles and inflammatory cell infiltration of acinar cells. Caerulein + Ranitidine (20 mg/kg BW) (HE; (a) × 40, (b) × 100, (c) × 200, (d) × 400.)

Fig. 9. Effect of prophylactic ranitidine administration on pancreatic histology. Sections of pancreas from caerulein-treated rats show marked amelioration of interstitial edema, intracytoplasmic vacuoles and inflammatory cell infiltration of acinar cells. Caerulein + Ranitidine (100 mg/kg BW) (HE; (a) × 40, (b) × 100, (c) × 200, (d) × 400.)
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Fig. 10. Histological severity of pancreatitis (intracytoplasmic vacuoles) was evaluated using Image J image management software. Intracytoplasmic vacuoles were reduced by ranitidine at concentrations of 20 and 100 mg / kg BW (*P < 0.01) (n = 6-10 rats).

B cells. The stimulation of H2Rs inhibits a variety of immune system functions. For example, the stimulation of H2Rs in basophils and mast cells negatively regulates histamine release. Furthermore, the stimulation of H2RA in lymphocytes can inhibit antibody synthesis, T-cell proliferation, cell-mediated cytolysis, and cytokine production.

A recent study by Okajima et al demonstrated that ranitidine, a specific competitive H2RA, prevents the release of elastase and reactive oxygen species, inhibits cell surface expression of CD11b and CD18, and increases the intracellular calcium concentration in neutrophils stimulated with formyl-methionyl-leucyl-phenylalanine (fMLP). Such inhibitory activity may contribute to the ranitidine-induced reduction in stress-induced gastric mucosal injury in rats. Neutrophils play a critical role in the development of complications in AP. Animal model studies demonstrate the beneficial effect of ranitidine in experimentally induced AP.

In the present study, we showed that administration of ranitidine before and during the induction of AP attenuates the severity of pancreatic injury in caerulein-induced pancreatitis. Ranitidine is 4 to 10 times more potent than cimetidine on a molar basis in inhibiting stimulated gastric acid secretion. Ranitidine was used at 4, 20 and 100 mg / kg BW in the rat model of AP in the present study. The concentration of ranitidine typically used to treat humans is 4 mg / kg BW. At 20 and 100 mg / kg ranitidine provides high therapeutic efficacy by significantly reducing plasma amylase and lipase activity and inhibiting leukocyte infiltration into pancreatic tissue. These findings may also explain the mechanism underlying the ranitidine inhibition of neutrophil adhesion reported by Okajima et al. The improvements in vacuole formation induced by ranitidine cannot be explained by inhibition of leukocyte infiltration.

Cytokines are a family of low-molecular weight proteins (16–25 kDa) released from cells
to regulate various biological processes including cell growth, cell activation, inflammation, immunity, tissue repair, and fibrosis. Cytokines are not usually found in normal tissue but are produced in response to stimuli via receptor-induced pathways. The activation of inflammatory cells by cytokines leads to the induction of various pro- and anti-inflammatory mediators and chemokines. These inflammatory mediators play a key role in AP. Recent studies have confirmed the critical role played by inflammatory mediators such as IL-1, tumor necrosis factor α (TNF-α), IL-10, IL-6, platelet-activating factor (PAF), IL-8, IL-2, IL-4, IL-11 and IL-18. Inflammatory mediators play an important role in local tissue injury and the development of multiple organ failure in AP. The administration of inhibitors of pro-inflammatory mediators prevents local and remote tissue damage and improves survival in several animal models.

IL-10 is a major anti-inflammatory cytokine that reduces the activation of macrophages and inhibits the production of pro-inflammatory cytokines. IL-10 is thought to play a
protective role in AP\textsuperscript{35}). Administration of IL-10 in experimentally induced AP leads to a reduction in both the local inflammatory response and subsequent mortality\textsuperscript{36-38}. The present study shows that the beneficial effects of ranitidine in the treatment of AP result from an induction of IL-10 levels in pancreatic tissue. The reduction in severity of AP by ranitidine suggests that anti-inflammatory cytokines control pro-inflammatory cytokine production and play a protective role against local injury.

In conclusion, the present study shows that treatment of AP with ranitidine inhibits leukocyte infiltration of pancreatic tissue, and increases the level of IL-10 in pancreatic tissue. These findings provide evidence that ranitidine accelerates the recovery of caerulein-induced pancreatitis. This may be due, at least in part, to increases in anti-inflammatory cytokine generation in pancreatic tissue.

References

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