Wound healing of acetic acid-induced gastric ulcer in rats and the effects of cimetidine and calcitonin, with special reference to prolylhydroxylase and collagenase enzyme activity

KATSUYA MARUYAMA,1,2 ISAO OKAZAKI,3 MASAO ARAI,2 IWAO KUROSE,2 HIROKAZU KOMATSU,2 MASAHIKO NAKAMURA,2 and MASAHARU TSUCHIYA2

1 Clinical Research Unit, National Institute on Alcoholism, Kurihama National Hospital, 5-3-1 Nobi, Yokosuka, Kanagawa, 239 Japan
2 Department of Internal Medicine, Keio University, Shinjuku-ku, Tokyo, 160 Japan
3 Department of Community Health, School of Medicine, Tokai University, Isehara, Kanagawa, 259-11 Japan

Abstract: The healing of acetic acid-induced gastric ulcer in rats and the effects of cimetidine and calcitonin were investigated with reference to the enzyme activity of both prolylhydroxylase and collagenase as related to histological findings. The rats were observed by endoscopy on the 3rd day after the subserosal injection of acetic acid; rats with ulcers were divided into three groups: non-treated, and cimetidine- and calcitonin-treated. The latter two groups were treated for 7 days. Prolylhydroxylase activity in active ulcers in the non-treated group was slightly higher on the 3rd day and significantly higher on the 10th day than the activity in control rats that had received subserosal injections of physiological saline solution on the respective days. In non-treated rats, the healed ulcer on the 10th day showed lower prolylhydroxylase activity than that in the active ulcer on the same day. Cimetidine did not affect prolylhydroxylase activity, but, with calcitonin, there was higher prolylhydroxylase activity in the healed than in the active ulcer, although the difference was not significant. Interstitial collagenase showed the highest activity on the 3rd day and decreased on the 10th day in non-treated rats. Collagenase activity was higher in the cimetidine-treated group, than that in the non-treated group, and numerous peroxidase-positive granulocytes were seen in the mucosa and submucosa. Calcitonin did not affect collagenase activity. The participation of both enzymes is indispensable in the healing process and the effects of anti-ulcer agents on these enzymes must be considered.

Key words: prolylhydroxylase, collagenase, acetic acid-induced gastric ulcer, cimetidine, calcitonin

Introduction

Gastric ulcers tend to recur, and their recurrence or relapse may be regarded as a defect in healing. Although the healing process in gastric ulcers has been investigated both clinically1,2 and experimentally,3-5 no attention has been paid to the collagen synthesizing- and degrading-enzymes which participate in the wound healing. Prolylhydroxylase (PHase; EC 1.14.11.2) is an enzyme that is necessary for the posttranslational hydroxylation of selected proline residues, a crucial process in collagen biosynthesis,6-7 and its activity has been reported to be elevated in healing wounds.6 Interstitial collagenase (collagenase; EC 3.4.24.3), a key enzyme in collagen degradation,8 plays many roles in the healing process, e.g., in the cleaning of the wound,9 in the delicate adjustment of newly synthesized collagen fibers,9,10 and in the destruction of gastric mucosal tissue.11,12 The mechanism underlying the healing process in the gastric mucosa remains unknown.

Since the use of the H2-receptor antagonist, cimetidine to treat gastric ulcer has become widespread, the recurrence of gastric ulcer after the cessation of administration of this antagonist has been a subject of much dispute.13-15 Although the effect of cimetidine on collagen metabolism16,17 has been reported, its effect on collagen metabolism in the healing process in the gastric mucosa has not been investigated.

Calcitonin, an inhibitor of gastric acid secretion through the central autonervous system,18 is also known to stimulate wound healing in the tissue,19,20 however, its effect on collagen metabolism in the healing process in gastric ulcers also remains unknown.
In this study, to clarify the mechanism of the healing process in the gastric mucosa from the aspect of collagen metabolism, we measured the activity of both above enzymes, which participate in collagen synthesis and degradation, during the formation and healing of acetic acid-induced gastric ulcers in rats. This measurement involved the histological localization of peroxidase-positive granulocytes that contain large amounts of collagenase. The effect of cimetidine and calcitonin on the activities of both enzymes was also investigated.

Materials and methods

Preparation of acetic acid-induced gastric ulcer

Male wistar rats (body weight, 200–250 g) were starved overnight but allowed free access to water. The rats were lightly anesthetized with ethylether and, after laparotomy, a gastric subserosal injection of 50 μl of 10% acetic acid was made into the fundic region of the glandular stomach according to the method of Takagi et al. Control rats received a suberosal injection of 50 μl physiological saline solution. Three days after injection, newly occurring ulcers were confirmed by endoscopy, using an Olympus SES-1717S (Olympus, Tokyo, Japan), according to the method of Fukawa et al. Forty-seven rats with ulcers confirmed by endoscopy were divided into three groups: non-treated (n = 27), cimetidine-treated (n = 10), and calcitonin-treated (n = 10). The cimetidine-treated group was treated with cimetidine (100 mg/kg per day, orally) obtained from Smith Kline and Fujisawa Co. (Tokyo, Japan), and the calcitonin-treated group was treated with calcitonin (5 units/kg per day, intraperitoneally) obtained from Toyo Jozo Co. (Tokyo, Japan). After 1 week with or without drug treatment, the rats were decapitated and the stomachs were removed for macroscopic and microscopic observations and for biochemical analysis.

Preparation of gastric tissue homogenate for enzyme assay

Immediately after the gastric tissue specimens (approximately 7 × 7 mm² in size), including the mucosa, muscle layer, and serosa, were obtained from the upper portion of the glandular stomach where the acetic acid-induced gastric ulcer occurred, they were placed in a solution of 0.05 M Tris-HCl (pH 7.4), containing 0.1 M NaCl and 0.03 M CaCl₂, and kept frozen at −80°C. Before the assay, the specimens were washed several times in the same cold buffer to eliminate small amounts of serum inhibitor. Diced gastric tissue specimens were collected on filter paper, weighed, and homogenized with ice-cold Tris buffer (0.05 M Tris-HCl, pH 7.4, containing 0.1 M NaCl, 0.1% Triton X-100, and 0.02% sodium azide) for collagenase assay and protein content determination. Aliquots of the 15,000-g supernatant of gastric tissue homogenate were used for PHase assay and protein content determination. Protein content was determined by the method of Lowry et al. using crystalline bovine serum albumin as the standard protein.

Measurement of PHase activity in gastric tissue homogenate

The assay employed is based on the stoichiometric formation of [3H]OH during the enzymatic hydroxylation of an unhydroxylated substrate rich in radio-labeled prolyl residues extracted from incubated chick embryo tibias with [3H]-proline. The substrate solution used in the present study contained 100,000 cpm/mg protein; all assays were done using the substrate from the same batch. The assay used in this study was performed according to the method of Fleckmann et al. Free [3H]OH generated in this incubation was removed and the radioactivity was determined with a liquid scintillation counter (LSC-700; Aloka, Tokyo, Japan) using PCS (phase combining system; Amersham, Buckinghamshire, England) as the liquid scintillation cocktail. PHase activity was expressed as counts per min (cpm) per mg of 15,000-g supernatant protein.

As a linear relationship between PHase activity and various amounts of enzyme protein was observed between 0.3 and 1.2 mg protein of supernatant from the gastric tissue homogenate, enzyme containing 0.7 mg protein was used in the present study.

Measurement of collagenase activity in gastric tissue homogenate

Type I collagen used as substrate for the collagenase assay was prepared by the methods of Glimcher et al. and Timpl et al., and then labeled with [3H]acetic anhydride by the method of Gisslow and McBride as described previously. The activity was determined by the release of radioactive soluble peptides from [3H]-labeled reconstituted collagen fibrils, principally based on the fibril assay of Nagai et al. In the assay, 3 mM p-chloromercuribenzoate (p-CMB; Sigma, St. Louis, Mo.) was added to inhibit cysteine proteinases and activate the latent form of collagenase, making it possible to reveal specific collagenase activity in the tissue homogenate. After incubation at 37°C for 24 h, the assay tube was centrifuged. The radioactivity of an aliquot supernatant was determined with the liquid scintillation counter, using PCS as the liquid...