INTRODUCTION

Bisphosphonates are a class of compounds that have been developed as antiresorptive agents capable of treating diseases related to bone remodeling. These drugs cause a transition to bone selectively and exert an effect against bone disorders, despite their extremely low rates of absorption (<2%)(1-3). However, they can have serious side effects including bleeding, inflammation, ulceration, nausea and abdominal pain in the upper gastrointestinal tract, although the mechanism underlying these reactions remains unknown (4-7).

We recently found that alendronate, a nitrogen-containing bisphosphonate, provoked damage in the antrum of rat stomachs (8). Interestingly, the severity of these lesions was reduced by repeated treatment with allopurinol, suggesting the involvement of oxyradical production in the pathogenesis. Chen et al. (9) reported that diethyldithiocarbamate (DDC), a chelator of Cu^{2+} which is known to inhibit Cu^{2+}- dependent superoxide dismutase (SOD) activity, produced damage in the antrum and suggested the involvement of superoxide radicals in the pathogenesis. Thus, it would be reasonable to speculate that the ulcerogenic response in the antrum is causally related with the dysfunction of the anti-oxidative mechanism.

Rebamipide (2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl] propionic acid) is a mucosal protective drug that accelerates the healing of gastritis and gastric ulcers and is widely used as a prophylactic against gastric adverse effects of nonsteroidal anti-inflammatory drugs (10, 11). This agent is known to stimulate prostaglandin production and mucus secretion which play a role in the mucosal defense mechanism of the stomach (12). It has also been reported that rebamipide inhibits the infiltration of inflammatory cells, generation of free radicals and production of interleukin-8, exerting a potent anti-inflammatory effect (13-16). Furthermore, Ogino et al. (17) reported that rebamipide effectively prevented the development of antral ulcers induced in rats by DDC. However, it remains unknown whether rebamipide affords a prophylactic effect on the development of antral lesions induced by alendronate.

In the present study, we demonstrated the development of antral ulcers induced in rats by alendronate and investigated the pathogenic factors involved in this model. Animals fasted for 18 h were given alendronate p.o., and then re-fed normally and killed on various days up to 7 days later. Alendronate caused non-hemorrhagic damage in both the corpus and antrum of fasted rats, but after re-feeding for 3 days the lesions in the corpus healed completely, while those in the antrum developed into large ulcers with increased vascular permeability. The development of antral ulcers was accompanied by an increase in MPO activity and lipid peroxidation as well as a decrease in SOD activity and GSH content in the mucosa. Histologically, the damage did not penetrate the muscularis mucosa, yet severe edema and neutrophil infiltration were observed in the submucosa. Neither omeprazole nor indomethacin had any effect, while allopurinol and SOD reduced the severity of these ulcers. Rebamipide dose-dependently suppressed the ulcerogenic response to alendronate, with a concomitant reversal of the increased vascular permeability, MPO activity and lipid peroxidation as well as the reduced SOD activity and GSH content. These results suggest that alendronate did not cause gross damage in the stomach of fasted rats, yet produced large ulcers in the antrum with severe edema after refeeding. The pathogenesis of these ulcers may be explained by the impairment of the mucosal anti-oxidative system and does not involve acid/peptic digestion and deficiency of prostaglandins. Rebamipide prevents the antral ulcers, probably due to its anti-oxidative as well as anti-inflammatory actions.

Key words: alendronate, stomach, antral lesion, vascular permeability rebamipide, anti-oxidative action, superoxide dismutase

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Y. OHASHI, E. AIHARA, H. TAKASUKA, K. TAKAHASHI, K. TAKEUCHI

ANTRAL ULCERS INDUCED BY ALENDRONATE, A NITROGEN-CONTAINING BISPHOSPHONATE, IN RAT STOMACHS - PROPHYLACTIC EFFECT OF REBAMIPIDE

Division of Pathological Sciences, Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Kyoto, Japan

We demonstrated the development of antral ulcers induced in rats by alendronate and investigated the pathogenic factors involved in this model. Animals fasted for 18 h were given alendronate p.o., and then re-fed normally and killed on various days up to 7 days later. Alendronate caused non-hemorrhagic damage in both the corpus and antrum of fasted rats, but after re-feeding for 3 days the lesions in the corpus healed completely, while those in the antrum developed into large ulcers with increased vascular permeability. The development of antral ulcers was accompanied by an increase in MPO activity and lipid peroxidation as well as a decrease in SOD activity and GSH content in the mucosa. Histologically, the damage did not penetrate the muscularis mucosa, yet severe edema and neutrophil infiltration were observed in the submucosa. Neither omeprazole nor indomethacin had any effect, while allopurinol and SOD reduced the severity of these ulcers. Rebamipide dose-dependently suppressed the ulcerogenic response to alendronate, with a concomitant reversal of the increased vascular permeability, MPO activity and lipid peroxidation as well as the reduced SOD activity and GSH content. These results suggest that alendronate did not cause gross damage in the stomach of fasted rats, yet produced large ulcers in the antrum with severe edema after refeeding. The pathogenesis of these ulcers may be explained by the impairment of the mucosal anti-oxidative system and does not involve acid/peptic digestion and deficiency of prostaglandins. Rebamipide prevents the antral ulcers, probably due to its anti-oxidative as well as anti-inflammatory actions.

Key words: alendronate, stomach, antral lesion, vascular permeability rebamipide, anti-oxidative action, superoxide dismutase

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (200~260 g; Nippon Charles River, Shizuoka, Japan) were used. They were kept in individual cages
with raised mesh bottoms and deprived of food but allowed free access to tap water for 18 h before the experiments. Studies were carried out using four to eight animals per group. All experimental procedures used here were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

**Induction of antral ulcers**

The animals fasted for 18 h were given alendronate (100–600 mg/kg) orally as a single injection, then re-fed normally and killed various days (1–7 days) later under deep ether anesthesia. The stomach was excised, treated with 2% formalin for 10 min for fixation of tissue walls, and opened along the greater curvature. The area of macroscopically visible damage (mm²) was measured under a dissecting microscope with square grids (x10), summed per tissue and used as a lesion score. The effects of the following agents on the development of alendronate-induced antral lesions were examined; rebamipide, allopurinol, omeprazole, superoxide dismutase (SOD), DDC, and indomethacin. Rebamipide (3–30 mg/kg, p.o.), allopurinol (50 mg/kg, i.p.), or SOD (30000 units/kg, i.p.) was given 30 min before and 10 h after alendronate on the first day and twice daily for 2 days thereafter; while omeprazole (30 mg/kg, p.o.), indomethacin (2 mg/kg, p.o.) or DDC (750 mg/kg, i.p.) was given 30 min before and once daily for 2 days thereafter. The person measuring the lesions did not know the treatments given to the animals.

In some cases, the antral mucosa was examined with a light microscope on various days following the administration of alendronate (300 mg/kg). The tissue samples were immersed in 2% formalin-saline, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E).

**Determination of microvascular permeability**

The microvascular permeability was evaluated in the antral mucosa following the administration of alendronate, by measuring the extravasated amount of dye (Evans blue) according to a method described earlier (18). In each case, 1 ml of 1% Evans blue was injected intravenously 30 min before killing. Under deep ether anesthesia, the animals were killed by bleeding from the descending aorta, the stomachs were removed, and the amount of dye that had accumulated in the antrum mucosa in 30 min was measured. The extraction of dye was measured at 620 nm on a Hitachi spectrophotometer (U-2000, Hitachi, Ibaraki, Japan), and the amount of dye recovered from the intestinal mucosa was expressed as µg per 100 mg wet tissue. Treatment of the animals with various agents was performed as described in "Induction of antral ulcers".

**Determination of MPO activity**

Myeloperoxidase (MPO) activity was measured according to a modified version of the method of Castro et al. (19). The animals fasted for 18 h were given alendronate (300 mg/kg, p.o.), then re-fed normally, and killed 4 days later under deep ether anesthesia. All blood was withdrawn from the heart by perfusing with saline, according to a modified version of the method originally described by Kaplowitz et al. (20). The animals fasted for 18 h were given alendronate (300 mg/kg, p.o.), then re-fed normally and killed 4 days later under deep ether anesthesia. Rebamipide (30 mg/kg, p.o.) was given 30 min before and 10 h after alendronate on the first day and twice daily for 2 days thereafter. In some cases, DDC (750 mg/kg) was also given i.p. 30 min before alendronate and once daily for 2 days thereafter.

**Measurement of gastric acid secretion**

Acid secretion was measured in pylorus-ligated rats before and various days after the administration of alendronate (300 mg/kg, p.o.). The tissue samples were immersed in 2% formalin-saline, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E). Acid secretion was measured in pylorus-ligated rats before and various days after the administration of alendronate (300 mg/kg, p.o.) or SOD (30000 units/kg, i.p.) was given 30 min before and 10 h after alendronate on the 1 day and twice daily for 2 days thereafter.

**Determination of lipid peroxidation**

Lipid peroxidation in the antral mucosa was determined as thiobarbituric acid (TBA) reactants, according to the modified method of Ohkawa et al. (20). The animals fasted for 18 h were given alendronate (300 mg/kg, p.o.), then re-fed normally, and killed 4 days later under deep ether anesthesia. Then, the stomach was excised and opened along the greater curvature. After rinsing the tissue with cold saline, the antral mucosa was scraped, weighed, and homogenized in 10 ml KCl. The homogenate was supplemented with the mixture of TBA and boiled at 100°C for 1 h. The reactants were then supplemented with 5 ml of the mixture of n-butanol and pyridine, shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. Absorbance was measured at 532 nm on Hitachi spectrophotometer and the results were expressed as nmole TBA per mg protein. Rebamipide (30 mg/kg, p.o.) was given 30 min before and 10 h after alendronate on the 1 day and twice daily for 2 days thereafter.

**Determination of SOD activity and GSH content**

The animals fasted for 18 h were given alendronate (300 mg/kg, p.o.), then re-fed normally and killed 4 days later under deep ether anesthesia. Rebamipide (30 mg/kg, p.o.) was given 30 min before and 10 h after alendronate on the first day and twice daily for 2 days thereafter. In some cases, DDC (750 mg/kg) was also given i.p. 30 min before alendronate and once daily for 2 days thereafter. SOD activity was measured in the rat gastric mucosa, according to the method reported by Ikeda et al. (21). After the tissue was rinsed with cold saline, the antral mucosa was scraped with glass slides and kept cold on ice. The mucosal scrapings were weighed, minced and homogenized in a sucrose buffer solution (0.25 mol/l sucrose, 10 mmol/l Tris-HCl buffer solution pH 7.4, 1 mmol/l EDTA), the volume of which was 6–8 times the tissue weight. After centrifugation at 78000 g for 60 min, the supernatant was used for determination of SOD activity. The absorbance was measured at 450 nm on a Hitachi spectrophotometer (U1100, Mito, Ibaraki, Japan), and the results were expressed as units per ml per g tissue weight.
mg/kg, p.o.). Under ether anesthesia, the abdomen was opened and the pylorus was ligated. The animals were then allowed to recover from the anesthesia. Four hours later, the animals were sacrificed with deep ether anesthesia, and the gastric contents were collected and analyzed in terms of volume and acidity. Acidity was measured by automatic titration of the gastric contents against 0.1 mol/L NaOH to pH 7.0 with a titrator (Hiranuma; Comitite COM-550, Tokyo, Japan). Total acid output (total volume x acidity) was expressed as µEq/h.

Preparation of drugs

Drugs used were alendronate (LKT laboratories, St. Paul, ME), rebamipide (Otsuka Pharmaceutical Co. Tokushima, Japan), allopulnil, indomethacin (Sigma Chemicals, St. Louis, MO), diethylidithiocarbamate (DDC: Wako, Osaka, Japan), superoxide dismutase (SOD: Nacalai tesque, Kyoto, Japan) and omeprazole (Astra Zeneca, Möndal, Sweden). Alendronate was dissolved in saline and adjusted to pH 7.0 by adding NaOH (Kanatsu et al., 2004). Rebamipide was suspended with a carboxymethylcellulose (CMC) solution. Other agents were dissolved in saline. Each drug was prepared immediately before use and administered p.o., s.c., or i.p. in a volume of 0.5 ml per 100 g body weight. Control animals received the vehicle (saline or CMC) alone.

Statistics

Data are presented as the means±SE for 4–6 rats per group. Statistical analyses were performed by using a two-tailed Dunnett's multiple comparison test, and values of P<0.05 were regarded as significant.

RESULTS

Generation of antral ulcers by alendronate after refeeding

Orally administered alendronate (100–600 mg/kg) damaged the stomach of fasted rats, mostly generating non-hemorrhagic lesions in both the corpus and antral mucosa (data not shown).
However, when the animals were subsequently re-fed for 3 days or more after the administration of alendronate, the lesions in the corpus healed, while those in the antrum extended further and developed into severe ulcers with white cap (Fig. 1A). The severity of antral ulcers induced by re-feeding was dose-dependent for alendronate, the lesion area at 300 and 600 mg/kg being 80.2±11.3 mm² and 103.1±4.5 mm², respectively (Fig. 1B). Alendronate also dose-dependently increased vascular permeability in the antral mucosa as determined with Evans blue. The amount of dye extravasated in the mucosa at 300 and 600 mg/kg was 54.6±7.8 µg/g tissue and 92.3±18.7 µg/g tissue, respectively, both values being significantly greater than that observed in normal rats (18.7±2.1 µg/g tissue). As shown in Fig. 1C, a highly significant relationship was observed between the area of lesions and the amount of dye, the correlation coefficient (r) being 0.826.

Fig. 2A shows the time course of the development and healing of antral ulcers generated by alendronate (300 mg/kg). Ulcers were already evident 1 day after re-feeding, and the severity reached a peak (80~90 mm²) on day 4 after re-feeding, with a marked increase in vascular permeability, followed by a gradual decrease thereafter. On day 7 after re-feeding, the area of lesions was 21.1±8.7 mm², which is equivalent to that observed on day 1, with no significant increase in vascular permeability. A close relationship between lesion area and vascular permeability was also observed during the healing of these ulcers. Histologically, alendronate-induced antral ulceration was not evident deep in the mucosa on day 1 after re-feeding, yet exfoliation of epithelial cells was observed with edema in the submucosa (Fig. 2B). The severity of these lesions gradually increased with time after re-feeding, and on day 4 these ulcers were deep in the mucosa with severe edema and inflammatory cell infiltration in the submucosa. Additionally, the damaged mucosa was covered with a white cap, mainly composed of inflammatory cells and fibrin-like substances. However, on day 7 after re-feeding, the lesions had healed considerably; the area with a white cap was small and the edema had subsided.

Effects of various agents on antral ulcers generated by alendronate

Alendronate (300 mg/kg) given orally to fasted rats provoked ulcers in the antrum with a marked increase in vascular permeability after refeeding for 4 days, the lesion area and the amount of extravasated dye being 78.1±8.6 mm² and 29.1±4.8 µg/g tissue, respectively. These ulcerogenic responses were not significantly affected by repeated treatment with either omeprazole (30 mg/kg) or indomethacin (2 mg/kg) given p.o. 30 min before and once daily for 2 days after alendronate (Fig. 3). Both the lesion area and the amount of extravasated dye in these groups were equivalent to those in the control group. On the other hand, the development of antral ulcers induced by alendronate in re-fed rats was dose-dependently prevented by repeated treatment with rebamipide (3~30 mg/kg, p.o.), the value being 0.89±0.09 µmol after alendronate on the first day and twice daily for 2 days thereafter, while indomethacin (1 mg/kg, p.o.) was given 30 min before and 10 h after alendronate on the first day and twice daily for 2 days. Data are presented as the means±SE for 4-6 rats. Significant difference, from normal, at p<0.05.

Effects of various agents on changes in antral MPO activity induced by alendronate

The MPO activity in the normal antral mucosa was 0.099±0.004 µmol H₂O₂/mg protein and markedly elevated in response to alendronate (10 mg/kg), reaching 0.745±0.035 µmol H₂O₂/mg protein 3 days after re-feeding (Fig. 6). The increase in MPO activity was significantly suppressed by repeated treatment with rebamipide (30 mg/kg, p.o.), the value being 0.159±0.022 µmol H₂O₂/mg protein. Likewise, treatment with allopurinol (50 mg/kg, i.p.) or SOD (30000 units/kg, i.p.) also significantly decreased MPO activity in the antral mucosa, the inhibition being 57.4% and 59.3%, respectively.

Effects of rebamipide on changes in antral SOD activity, GSH content and lipid peroxidation induced by alendronate

SOD activity and GSH content: mucosal SOD activity in the normal rat antrum was 250±16 units/ml/g tissue. In the animals given alendronate (300 mg/kg, p.o.), however, the SOD activity in the antral mucosa 4 days later showed a significantly low value of 150±13 units/ml/g tissue, which is about 60% of that observed in the normal rat antrum (Fig. 7). The decreased mucosal SOD activity was almost totally reversed by repeated administration of rebamipide (30 mg/kg, p.o.), the activity being 234±9.8 units/ml/g tissue. On the other hand, the amount of GSH in the normal rat antrum was 1.5±0.2 µmol/g tissue. The mucosal GSH content was also significantly decreased in the alendronate-treated rats; the value was 0.38±0.08 µmol/g tissue, which is about 25% of that in normal rats. The decrease in GSH caused by alendronate was partially but significantly restored by the treatment with rebamipide, the value being 0.89±0.09 µmol/g tissue.

Lipid peroxidation: four days after the administration of alendronate (300 mg/kg) and re-feeding, the amount of TBA...
reactants in the antral mucosa was significantly increased, the values being 20.52±1.84 nmol/mg tissue, about 1.5 times greater than normal levels (15.10±1.56 nmol/mg tissue) in control rats without alendronate injection (Fig. 8). The increase in TBA reactants induced in the antral mucosa by alendronate was significantly suppressed by the repeated treatment with rebamipide (30 mg/kg), the inhibition being 31.3%.

Effect of DDC on antral ulcers generated by alendronate

It was found that alendronate decreased SOD activity and that rebamipide might prevent alendronate-induced antral ulceration partly by restoring the decreased SOD activity. To further investigate the relationship between the decrease in SOD activity and the development of antral ulcers generated by alendronate, we examined the effect of DDC, an inhibitor of SOD, on the severity of the lesions.

The SOD activity in the antral mucosa was significantly lowered by alendronate (300 mg/kg, p.o.) and further decreased by co-treatment with DDC (750 mg/kg, s.c.), the value being 48±9.7 units/ml/g tissue, which is significantly lower than that (152±10 units/ml/g tissue) observed after the treatment with alendronate alone (Fig. 9A). On the other hand, the development of antral lesions in response to alendronate was significantly exacerbated by co-treatment with DDC, the lesion area being

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**Fig. 4.** A: The effect of rebamipide on antral ulcers induced by alendronate in the rat stomach. The animals fasted 24 h were given alendronate (300 mg/kg, p.o.), re-fed, and killed 4 days later. Rebamipide (3-30 mg/kg) was given p.o. 30 min before and 10 h after alendronate on the first day and twice daily for 2 days. Figures show the lesion scores and the extravasated amount of Evans blue, and the data are presented as the means±SE for 4-6 rats. Significant difference at p<0.05, *from normal, #from vehicle. B: Gross appearance of antral lesions generated by alendronate with or without co-administration of rebamipide in rat stomachs. Note that the area of antral lesions with a white cap was apparently smaller in the animals co-treated with rebamipide.

**Fig. 5.** The effects of allopurinol and SOD on antral ulcers induced by alendronate in the rat stomach. The animals fasted 24 h were given alendronate (300 mg/kg, p.o.), re-fed, and killed 4 days later. Allopurinol (50 mg/kg) or SOD (30000 units/kg) was given i.p. 30 min before and 10 h after alendronate on the first day and twice daily for 2 days. Data are presented as the means±SE for 4-6 rats. Significant difference at p<0.05, *from normal, #from vehicle.

**Fig. 6.** The effects of rebamipide, allopurinol and SOD on the increased MPO activity in the antral mucosa of rats given alendronate. The animals fasted 24 h were given alendronate (300 mg/kg, p.o.), re-fed, and killed 4 days later. Rebamipide (30 mg/kg, p.o.), allopurinol (50 mg/kg, i.p.), or SOD (30000 units/kg, i.p.) was given 30 min before and 10 h after alendronate on the first day and twice daily for 2 days. Data are presented as the means±SE for 4-6 rats. Significant difference at p<0.05, *from normal, #from vehicle.
which is 1.6 times greater than that observed in the animals given alendronate alone (Fig. 9B). Likewise, the mucosal vascular permeability in the antrum was also increased after alendronate treatment, but this response was further enhanced by additional treatment with DDC, the value being $61.6\pm7.8 \mu g/g$ tissue, which is approximately 2.6 times greater than that observed in the control animals.

Changes in gastric acid secretion following alendronate treatment

Normal rats secreted 5~7ml of gastric juice for 4 h after pylorus ligation, the acidity and acid output being 80~100 mEq/L and 120~160 µEq/h, respectively. When the animals were given alendronate (300 mg/kg, p.o.), the volume of gastric secretion was already significantly increased on day 1 after the administration and remained increased even 3 days later, the value on day 3 being $10.2\pm0.3$ ml (Fig. 10A). In the animals given alendronate, however, the acidity was significantly decreased from 1 day after the administration to less than 40% of that observed in control animals and on day 2 was $18.4\pm43.8$ mEq/L, about 18% of the control value (Fig. 10B). Accordingly, the acid output in the animals given alendronate was not significantly changed on day 1 but markedly decreased on days 2 and 3 after the administration, the value on day 2 being $14.6\pm3.1$ µEq/h, only 8.5% of that observed in the control group (Fig. 10C).
accompanied by a marked increase in vascular permeability, and a corpus healed. Interestingly, the development of these ulcers was further to develop into ulcers with a white cap, though those in the after alendronate treatment, the lesions in the antrum extended when these animals were subsequently re-fed for 3 days or more gastric mucosa, both in the corpus and in the antrum. However, alendronate, given to fasted rats as a single injection, damaged the gastric ulcerogenic properties of bisphosphonates contained in the moiety (24). We also reported that alendronate side effects is not currently available. Previous studies showed that the gastric ulcerogenic properties of bisphosphonates depend on the pH of the drug solution and the nitrogen atom contained in the moiety (24). We also reported that alendronate caused more damage in the stomach when the pH of the drug solution was 7.0 rather than 4.0 (6). The present study confirmed that alendronate damaged both the corpus and the antral mucosa of the stomach in fasted rats and further showed that after re-feeding of these animals, the lesions in the corpus mucosa healed, while those in the antrum developed into ulcers with submucosal edema and inflammation as well as an increase in vascular permeability. In addition, the development of these ulcers was not affected by omeprazole but prevented by allopurinol and SOD, suggesting the pathogenic importance of oxyradical production but not acid digestion.

Consistent with a previous study (7), we observed that alendronate, given to fasted rats as a single injection, damaged the gastric mucosa, both in the corpus and in the antrum. However, when these animals were subsequently re-fed for 3 days or more after alendronate treatment, the lesions in the antrum extended further to develop into ulcers with a white cap, though those in the corpus healed. Interestingly, the development of these ulcers was accompanied by a marked increase in vascular permeability, and a highly significant relationship was observed between the area of lesions and the increased permeability. Histologically, the antral ulcers showed severe edema and inflammatory cell infiltration in the submucosa. It was also found that the damaged mucosa was covered with a white cap, mainly composed of inflammatory cells and fibrin-like substances. We previously reported that alendronate applied to the gastric mucosa caused a decrease in transmucosal potential difference of the stomach, suggesting a disruption of surface epithelial cells due to direct action (6). It is assumed that the development of antral ulcers is essentially due to a direct action of this agent. Indeed, extensive exfoliation of the surface epithelial cells was observed in the antral mucosa on day 1 after the administration of alendronate.

In general, it is well accepted that acid/peptic digestion or a deficiency of endogenous prostaglandins (PGs) is involved in the pathogenesis of gastric lesions as induced by various means (25-28). The gastric ulcerogenic response is prevented by antisecretory drugs such as proton pump inhibitors but aggravated by indomethacin, an inhibitor of PG production (25, 27, 28). However, the development of antral ulcers in response to alendronate was not significantly affected by either omeprazole or indomethacin, suggesting no participation of acid or PGs in the pathogenesis of these lesions. As shown in the present study, alendronate increased the volume of gastric juice but markedly decreased the acidity, resulting in a significant reduction of acid output. It is assumed that the acid output in these animals had been sufficiently decreased before the administration of omeprazole. This may be why these lesions did not respond to omeprazole. At present, however, it remains unknown why a deficiency of PGs caused by indomethacin did not affect the severity of the antral ulcers generated by alendronate. PGs are known to exert a dual role in the pathogenesis of gastric lesions, the protective and the proulcerogenic effects depending on the model (18, 29, 30). Notably, PGE₂ markedly aggravated gastric lesions that occurred accompanied by increased vascular permeability and inflammation, it is assumed that a deficiency of PGs caused by indomethacin exerts a dual effect, the suppression of increased vascular permeability and the augmentation of mucosal vulnerability, and by so doing results in no change in the severity of antral ulcers. Accordingly, alendronate-induced antral ulceration, unlike other models, is not affected by changes in

**DISCUSSION**

Bisphosphonates, potently effective against various bone diseases, are known to cause serious adverse reactions in the upper gastrointestinal tract, including esophagitis, esophageal stricture and gastric ulcer (4, 5). To minimize such side effects, patients are recommended to take these drugs with a large quantity of water and not to lie down for at least 30 min thereafter. Since the mechanism underlying these adverse reactions remains unclear, an effective means of preventing such side effects is not currently available. Previous studies showed that the gastric ulcerogenic properties of bisphosphonates depend on the pH of the drug solution and the nitrogen atom contained in the moiety (24). We also reported that alendronate caused more damage in the stomach when the pH of the drug solution was 7.0 rather than 4.0 (6). The present study confirmed that alendronate damaged both the corpus and the antral mucosa of the stomach in fasted rats and further showed that after re-feeding of these animals, the lesions in the corpus mucosa healed, while those in the antrum developed into ulcers with submucosal edema and inflammation as well as an increase in vascular permeability. In addition, the development of these ulcers was not affected by omeprazole but prevented by allopurinol and SOD, suggesting the pathogenic importance of oxyradical production but not acid digestion.

Consistent with a previous study (7), we observed that alendronate, given to fasted rats as a single injection, damaged the gastric mucosa, both in the corpus and in the antrum. However, when these animals were subsequently re-fed for 3 days or more after alendronate treatment, the lesions in the antrum extended further to develop into ulcers with a white cap, though those in the corpus healed. Interestingly, the development of these ulcers was accompanied by a marked increase in vascular permeability, and a highly significant relationship was observed between the area of lesions and the increased permeability. Histologically, the antral ulcers showed severe edema and inflammatory cell infiltration in the submucosa. It was also found that the damaged mucosa was covered with a white cap, mainly composed of inflammatory cells

![Fig. 10. The effect of alendronate on acid secretion in pylorus-ligated rats. The animals fasted 24 h were given alendronate (300 mg/kg, p.o.), and re-fed thereafter. Acid secretion was measured in pylorus-ligated stomachs for 4 h on day 1, 2, and 3 after the administration of alendronate. Data are presented as the mean±SE for 4-6 rats. *Significant difference from control, at p<0.05. Figures show: A: gastric fluid volume; B: acidity; C: acid output.](image-url)
luminal acid as an aggressive factor or PGs as a defensive factor. Satoh et al. (31,32) reported the development of antral ulcers by indomethacin in re-fed rats and showed that the solid component of food, whether nutritive or not, played an essential role in the formation of these ulcers, though the detailed mechanism remains unknown. The same might be applied to the pathogenesis of alendronate-induced antral ulcers, and the solid food component somehow contributes to the extension of mild damage into severe lesions after re-feeding. Further study should be needed to clarify this point.

Several models of antral ulcers have been established with agents that affect the mucosal anti-oxidative system. Chen et al. (9) reported an induction of antral ulceration in rats by s.c. administration of DDC, an inhibitor of SOD. The pathogenesis of these models is reportedly associated with the impairment of the mucosal anti-oxidative system, including a decrease in SOD activity or an increase of oxyradical production (9,17). Free radical scavengers such as SOD and GSH attenuate the microvascular damage observed in such models and play a role in maintaining mucosal integrity by counteracting oxygen-derived free radicals (31, 32). The increased oxidative stress may be caused not only by an accelerated production of reactive oxygen species but also by a decreased scavenging ability of those molecules. Indeed, the development of DDC-induced antral ulcers was prevented by pretreatment with SOD, a radical scavenger, and worsened by drugs that impair the anti-oxidative system (9,33). In the present study, the severity of alendronate-induced antral ulcers was also significantly reduced by repeated treatment with allopurinol, an inhibitor of oxyradical production, as well as SOD, an oxyradical scavenger. Furthermore, a marked reduction in SOD activity and GSH content was observed in the antral mucosa of alendronate-treated rat stomachs. These results suggest that the pathogenesis of the alendronate-induced antral lesions is accounted for at least partly by the impairment of the mucosal anti-oxidative system. This idea was also supported by the experiment using DDC, an inhibitor of Cu^2+-dependent SOD activity. This agent further reduced the mucosal SOD activity in the presence of alendronate and markedly worsened the severity of antral ulcers. These findings support the involvement of the impaired antioxidative system in the pathogenesis of alendronate-induced antral ulceration.

Rebamipide, a mucosal protective drug developed in Japan, is widely used as a prophylactic against gastritis and gastric ulcers (10-12). This agent exhibits the suppression of inflammatory cell infiltration and free radical generation as well as the radical scavenging action, and exerts a potent anti-inflammatory effect (13-16). As expected, rebamipide significantly reduced the severity of alendronate-induced antral ulcers, together with the suppression of increased vascular permeability. This agent also markedly suppressed the increase in MPO activity after alendronate treatment, its effect being more pronounced than that of allopurinol or SOD. These findings make sense, because rebamipide is known to inhibit the infiltration of inflammatory cells (16) and because neutrophils are one of the sources for oxyradical production (33). In addition, this agent reversed the decrease in mucosal SOD activity and GSH content as well as the increase of lipid peroxidation in the antral mucosa of alendronate-treated rats. These results strongly support the pathogenic importance of the impaired anti-oxidative system in the development of antral ulcers in response to alendronate. Certainly, the protective effect of rebamipide may be attributable, in part, to the anti-inflammatory action, because this drug significantly suppressed submucosal edema and inflammation, together with the prevention of the antral lesions.

Given the findings of the present study, we concluded that alendronate did not cause gross damage in the stomach of fasted rats but after re-feeding produced large ulcers in the antrum with increased vascular permeability and submucosal edema. The pathogenesis of these ulcers may be explained by the impairment of the mucosal anti-oxidative system and does not involve acid digestion as well as a deficiency of PGs. In addition, rebamipide prevents the alendronate-induced antral ulceration, probably due to anti-oxidative and anti-inflammatory actions. Finally, patients should avoid taking food after the administration of alendronate to prevent antral ulcers. Gastric lesions induced acutely in the corpus by alendronate might be worsened in the emptied stomach (lower pH) without food, probably because of the enhanced acid back-diffusion (6, 7). At present, we cannot make a definite suggestion about whether avoiding food intake is better for total prevention of gastric lesions in response to alendronate. To answer this inquiry, further study should be required to clarify the role of re-feeding in the pathogenic mechanism of antral ulcers.

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Author’s address: Dr Koji Takeuchi, PhD., Division of Pathological Sciences, Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan; Phone: +81-075-595-4679; Fax: +81-075-595-4774; e-mail: takeuchi@mb.kyoto-phu.ac.jp