INTRODUCTION

Bisphosphonates (BPPs) are a class of compounds that have been developed as antiresorptive drugs capable of treating diseases related to bone remodeling (1, 2); however, they can have serious side effects including bleeding, inflammation, ulceration, nausea and abdominal pain in the upper gastrointestinal tract (3-5). Indeed, many studies confirmed in experimental animals that BPPs caused lesions or ulcers in the gastric mucosa and impaired the healing of preexisting gastric ulcers (6-8). We also reported that alendronate (Fig. 1A) decreased transmucosal potential difference when applied topically to anesthetized rat stomachs (8) and produced ulcers in the antrum when administered to fasted rats followed by refeeding (9). In addition, Elliott et al. (10) reported that BPPs at relatively low doses delayed the healing of gastric ulcers and worsened the healing impairment action of nonsteroidal anti-inflammatory drugs, frequently prescribed together with BPPs in patients with arthritis or osteoporosis. We also reported that alendronate delayed the healing of gastric ulcers in rats, with concomitant impairment of angiogenesis at the ulcer base (11).

Risedronate is a third-generation BPP with a nitrogen atom that forms part of a pyridine ring (Fig. 1B). In most clinical studies, risedronate caused untoward effects similar to a placebo (12, 13). Lanza et al. (14), however, reported that the incidence of gastrointestinal side effects was similar after treatment with the therapeutic dose of risedronate and alendronate in postmenopausal women with existing vertebral fractures. Although we found that both alendronate and risedronate showed mucosal irritative and healing impairing effects in rat stomachs, the effects of risedronate were much less pronounced than alendronate (8); however, there has been controversy concerning the less ulcerogenic properties of risedronate. Thus, it is worth re-investigating whether risedronate shows fewer adverse effects in the gastric mucosa than other BPPs.

In the present study, we examined the adverse effects of risedronate on rat stomachs, especially ulcerogenic action in the antrum as well as the healing impairment action on preexisting gastric ulcers, and compared these with the effects of other BPPs frequently used in patients in Japan, such as alendronate and minodronate (Fig. 1C).

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 24 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 24 h were administered risedronate (10-300 mg/kg), alendronate (10-300 mg/kg) or minodronate (10-100 mg/kg) orally as a single injection, then re-fed normally, and killed 3 days later under deep ether anesthesia (9). 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 (10), summed per tissue and used as a lesion score. 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 3 days after the administration of risedronate (300 mg/kg), alendronate (300 mg/kg) or minodronate (100 mg/kg). The tissue samples were immersed in 10% buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin.

Determination of myeloperoxidase activity

Myeloperoxidase (MPO) activity was measured according to a modified version of the method of Castro et al. (15). After sacrificing the animals, all blood was withdrawn from the heart by perfusing with saline, and the stomach was excised and opened along the greater curvature. Then, the tissue was rinsed with cold saline, and the antral mucosa was scraped with glass slides, weighed, and homogenized in 50 mmol phosphate buffer containing 0.5% hexadecyl-trimethyl-ammonium bromide (pH 6.0; Sigma Chemicals, St. Louis, MO). The homogenized samples were then subjected to freezing and thawing 3 times and centrifuged at 1600 g for 10 min. After an aliquot (5 µl) of each supernatant was mixed with 145 µl phosphate buffer containing 0.17 mg/ml 0-dianisidine dihydrochloride (Sigma) and 0.0005% H₂O₂, the change in the rate of absorbance at 450 nm was measured with a microplate reader (Thermo Max; Molecular Devices, Sunnyvale, CA). Sample protein content was estimated by spectrophotometric assay (Protein Assay Kit, Pierce, IL), and MPO activity is expressed as µmol H₂O₂/min/mg protein.

Determination of lipid peroxidation

Lipid peroxidation in the antral mucosa was determined as thiobarbituric acid (TBA) reagents, according to the modified method of Ohkawa et al. (16). After sacrificing the animals, the stomach was excised and opened along the greater curvature. The tissue was rinsed with cold saline, and the antral mucosa was scraped, weighed, and homogenized in RIPA buffer (1 M Tris-HCl, pH 7.4, 50 mM EDTA, SDS). The homogenate was supplemented with the mixture of TBA reagents, boiled at 100°C for 1 h, and the reagents were 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 with a microplate reader, and the results are expressed as µmole malondialdehyde (MDA) per mg protein.

Determination of superoxide dismutase activity and glutathione content

Superoxide dismutase (SOD) activity was measured in the rat gastric mucosa according to the method reported by Ikeda et al. (17). After sacrificing the animals, the stomach was excised and opened along the greater curvature. The tissue was rinsed with cold saline, and 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 mol/l EDTA), the volume of which was 6-8 times the tissue weight. After centrifugation at 78,000 g for 1 h, the supernatant was used for determination of SOD activity. The absorbance was measured at 450 nm with a microplate reader, and the results are expressed as units per ml per mg protein. The amount of glutathione (GSH) was measured in the antral mucosa of the stomach, according to a modified version of the method originally described by Kaplowitz et al. (18, 19). 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, homogenized in 2 ml phosphate buffer (0.1 M NaH₂PO₄, plus 0.25 M sucrose, pH 7.4) and centrifuged at 4000 rpm for 15 min at 4°C. A 0.5-ml aliquot of 25% trichloroacetic acid was added to 1 ml of the supernatant of each sample, and the sample was kept for 30 min at 4°C. After centrifugation at 4000 rpm for 15 min, the supernatant was used to determine GSH using DTNB ([5,5-dithiobis (2-nitrobenzoic acid)]. Absorbance was measured at 412 nm with a microplate reader, and the results are expressed as micromoles per gram tissue.

Induction of chronic gastric ulcers

Chronic gastric ulcers were induced by thermal cauterization, according to a method described previously (20). Under ether anesthesia, the stomach was exposed through a midline incision, the electric probe (Fuchigami, Kyoto, Japan; diameter: 8 mm²) was attached to the mid-corpus mucosa, and a gastric ulcer was induced by heating the tip at 70°C for 30 s. Risedronate (30 and 60 mg/kg), alendronate (30 and 60 mg/kg) or minodronate (10 and 30 mg/kg) was given p.o. once daily for 7 days, starting 3 days after ulceration. Control animals received the vehicle alone. Ten days after ulceration, the animals were killed under deep ether anesthesia, the stomach was removed, inflated by injecting 8 ml of 2% formalin for 10 min to fix the inner wall, and opened along the greater curvature. The ulcer area (mm²) of the stomach was measured under a dissecting microscope with a square grid (x10). The person measuring the ulcer area did not know the treatments given to the animals.

Histological observations and evaluation of angiogenesis

At the time of autopsy of rats with chronic gastric ulcers, 12 µm frozen sections of the ulcerated stomachs were prepared. For evaluation of angiogenesis, sections were incubated with an antibody for von Willebrand factor (factor VIII-related endothelial antigen; DAKO, Glostrup, Denmark) after the deactivation of endogenous peroxidase with 0.3% H₂O₂, and nonspecific binding sites were blocked. The microvessel was visualized by the avidin-biotin-peroxidase complex method using a vectastain ABC-peroxidase kit (Vector, Burlingame, CA). Sections were successively stained with hematoxylin. The degree of microvasculature in the ulcer base granulation tissue was determined in three randomly chosen 1 mm² fields. The density of microvasculature was expressed as the number of vessels per mm² of ulcer base. For light microscope studies, rat stomachs were placed into 10% buffered formalin and small pieces of tissue containing ulcers were excised, embedded in paraffin, and sectioned at a thickness of 4 µm. Hematoxylin and eosin staining was subsequently performed.
Western blot analyses for vascular endothelium-derived growth factor and basic fibroblast growth factor

Tissues from the ulcerated mucosa were minced with scissors, collected and weighed. Samples were homogenized with protease inhibitor cocktail tablets (Complete; Roche, Penzberg, Germany) and centrifuged at 20,000 g for 30 min at 4°C, and the supernatant was collected as protein samples. The protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). To analyze the expression of vascular endothelium-derived growth factor (VEGF) and basic fibroblast growth factor (bFGF), the protein samples (20 µg) were electro-phoresed on sodium lauryl sulfate (SDS)-14% polyacrylamide slab gels as described by Laemmli (21) and electrically transferred to a nitrocellulose membrane (Protran; Schleicher&Schuell, Dassel, Germany). Sequential immunoblotting was performed using a monoclonal anti-VEGF (Santa Cruz Biotechnology, CA) or anti-bFGF (Santa Cruz Biotechnology, CA) antibody as the primary antibody. The membrane was then reacted with horseradish peroxidase-conjugated goat anti rabbit-IgG antibody (Santa Cruz Biotechnology, CA) for 1 h at room temperature. Western blots were visualized by an enhanced chemiluminescence system (Western blot chemi-luminescence Reagent Plus; NEN, Boston).

Preparation of drugs

Drugs used were risedronate (Ajinomoto Pharmaceutical Co., Ltd, Tokyo, Japan), alendronate (LKT laboratories, St. Paul, ME) and minodronate (Chengdu D-Innovation Pharmaceutical Co., Ltd, China). These BPPs were dissolved in saline and adjusted to 7.0 by adding NaOH, since previous studies showed that the gastric ulcerogenic properties of BPPs depend on the pH of the drug solution and that the action is more potent at pH 7 than pH 4 (8). Each drug was prepared immediately before use and administered p.o. in a volume of 5 ml/kg. Control animals received the vehicle (saline) alone.

Statistical analyses

Data are presented as the means ±S.E. of 4-8 rats per group. Statistical analyses were performed using the two-tailed Dunnett’s multiple comparison test, and P<0.05 was considered significant.

RESULTS

Generation of antral lesions by bisphosphonates after refeeding

When the animals fasted for 18 h were given BPPs p.o., such as risedronate (10-300 mg/kg), alendronate (10-300 mg/kg) or minodronate (10-100 mg/kg), and then re-fed for 3 days, large lesions developed in the antrum. However, the dose required to provoke damage was different in these BPPs: 300 mg/kg for risedronate, 100 mg/kg for alendronate, and 10 mg/kg for minodronate (Fig. 2). In addition, the severity of lesions varied depending on BPPs. The lesion score induced by minodronate at 100 mg/kg was 88.8±17.7 mm², which was almost equivalent to that (78.8±6.9 mm²) induced by alendronate at 300 mg/kg, respectively. On the other hand, the lesion score induced by risedronate even at 300 mg/kg was less than 20 mm². The damaged mucosa was covered with a white cap, mainly composed of inflammatory cells and fibrin-like substances, and the area of the white cap nicely paralleled the lesion score (Fig. 3A-3C). Histologically, these lesions showed severe edema and inflammatory cell infiltration in the submucosa (Fig. 3D-3F). Likewise, the histological severity of damage was also less pronounced in the animal treated with risedronate (300 mg/kg) when compared to alendronate (300 mg/kg) or minodronate (100 mg/kg).

Changes in antral myeloperoxidase activity induced by bisphosphonates

Meyloperoxidase (MPO) activity in the normal antral mucosa was less than 0.01 µmol H₂O₂/min/mg protein, and significantly elevated after p.o. administration of risedronate (100 and 300 mg/kg), alendronate (100 and 300 mg/kg) or minodronate (30 and 100 mg/kg), although the degree of increase differed according to the BPP (Fig. 4). MPO activity was increased to 0.80±0.19 µmol H₂O₂/min/mg protein by 30 mg/kg of minodronate and 0.42±0.07 µmol H₂O₂/min/mg protein by 100 mg/kg of alendronate, while the activity was reached 0.25±0.03 µmol H₂O₂/min/mg protein by risedronate, even at 300 mg/kg.

Changes in antral superoxide dismutase activity, glutathione content and lipid peroxidation induced by bisphosphonates

Fig. 1. Chemical structures of alendronate (A), risedronate (B) and minodronate (C).
1. Superoxide dismutase activity and glutathione content

Mucosal SOD activity in the control rat antrum was 61.1±4.7 units/g protein. In the animals treated with alendronate (300 mg/kg) or minodronate (100 mg/kg) p.o., SOD activity in the antral mucosa 3 days later showed a significantly low value of 35.0±2.6 units/mg protein or 37.3±3.3 units/mg protein, respectively, both of which were approximately 60% of the value observed in the control rat antrum (Fig. 5). By contrast, risedronate even at 300 mg/kg did not significantly affect SOD activity, the value being 54.6±4.0 units/mg protein, about 94.6% in the activity of control rats. On the other hand, the amount of GSH in the control rat antrum was 2.3±0.2 µmol/g tissue. The mucosal GSH content was also significantly decreased in the antrum 3 days after p.o. administration of alendronate (300 mg/kg) or minodronate (100 mg/kg); the value was 1.6±0.3 µmol/g tissue or 0.9±0.1 µmol/g tissue, respectively, which was about 70% or 45% of that in control rats (Fig. 5). Risedronate (300 mg/kg) did not significantly affect GSH content in the antral mucosa, the value being 2.1±0.6 µmol/g tissue.

2. Lipid peroxidation

The amount of TBA reactants in the antral mucosa of control rats was 0.57±0.07 µmol MDA/mg protein. Three days after p.o. administration of alendronate (300 mg/kg) or minodronate (100 mg/kg), the amount of TBA reactants slightly increased to 124.6% and 129.8% of control values, respectively (Fig. 6).
However, risedronate (300 mg/kg) had no influence on the amount TBA reactants, and the value observed 3 days after administration was 0.58±0.04 µmol MDA/mg protein, which remained in the range of control values.

**Effect of bisphosphonates on healing of chronic gastric ulcers**

Three days after thermal cauterization (70°C for 30 s), well-defined gastric ulcers were observed in all animals, the ulcerated area being 17.1±1.1 mm² on day 3 after ulceration. These ulcers showed spontaneous healing, and the ulcerated area on day 10 became smaller, the value being 3.3±0.4 mm², about 19.3% of the ulcer area observed initially (3 days after ulceration). Alendronate (30 and 60 mg/kg) given p.o. once daily for 7 days dose-dependently and significantly impaired the healing of gastric ulcers, and at 60 mg/kg the ulcerated area on day 10 was approximately 2.2 times greater than that of the control (Fig. 7A). Likewise, minodronate (10 and 30 mg/kg) given p.o. once daily for 7 days also significantly delayed ulcer healing in a dose-related fashion, and at 30 mg/kg the ulcerated area on day 10 was 10.1±1.7 mm², approximately 3 times greater than control values. However, repeated treatment of risedronate once daily for 7 days did not significantly affect the spontaneous healing of gastric ulcers, even at 60 mg/kg, and the ulcerated area on day 10 was 4.4±1.1 mm², which was not significantly different from that (3.3±0.4 mm²) of control animals. Histological observation confirmed the presence of extensive deep damage in the gastric mucosa of control rats, similar to that commonly seen in chronic-type ulcers. On day 10 after ulceration, a control rat had a partially healed ulcer, the base of which was covered with regenerating mucosa (Fig. 7Ba), whereas rats treated with alendronate or minodronate, but not risedronate, still had deep open ulcers without much covering by regenerating mucosa (Fig. 7Bb,c,d).
Angiogenesis in ulcerated mucosa

On day 10 after ulceration, the ulcer base was spontaneously reconstructed by the growth of granulation tissue and newly formed microvasculature, as represented by Factor VIII-positive cells, the number of microvessels at the ulcerated mucosa being 34.5±2.9 counts/mm² (Table 1). Alendronate (60 mg/kg) given once daily for 7 days prevented the growth of granulation tissue (angiogenesis) in the ulcer base; the degree of vascularization was 15.0±0.6 microvessels/mm², which is significantly less than that in control rats. Likewise, repeated treatment with minodronate (30 mg/kg) also significantly hampered the angiogenic response at the ulcer base, the degree of vascularization being 15.2±2.4 microvessels/mm². Histologically, irregular fibrosis was observed in the ulcer base of the animals treated with either alendronate or minodronate (Fig. 7Bc,d); however, the angiogenesis of the ulcerated mucosa was not significantly affected by repeated administration of risedronate (60 mg/kg), the degree of vascularization being similar to control rats.

Expressions of vascular endothelium-derived growth factor and basic fibroblast growth factor in ulcerated mucosa

On conventional Western blot analysis, VEGF and bFGF proteins were slightly expressed in the normal mucosa, yet these expressions were markedly up-regulated in the ulcerated mucosa 10 days after ulceration by thermal cauterization (Fig. 8A). When the animals were treated with alendronate (60 mg/kg) or minodronate (30 mg/kg) once daily for the last 7 days, the expressions of these growth factors were apparently down-regulated; in particular, the expression of VEGF was markedly suppressed, while that of bFGF was slightly attenuated (Fig. 8B and 8C); however, repeated administration of risedronate (60 mg/kg)
mg/kg) did not have much influence on the expression of VEGF and bFGF in the ulcerated mucosa.

**DISCUSSION**

BPPs, 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 (3-5). 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. In experimental animals, several studies have demonstrated that alendronate, a nitrogen-containing BPP, caused damage to the gastric mucosa and impaired the healing of chronic gastric ulcers (6-11). The present study confirmed that other nitrogen-containing BPPs, such as minodronate, also showed ulcerogenic and healing impairing effects in the rat stomach, similar to alendronate, yet these adverse effects of risedronate were much less pronounced than those of other BPPs.

Consistent with a previous study (9), we found that alendronate, given to fasted rats, produced ulcers in the antrum after re-feeding for 3 days. These ulcers were accompanied by a marked increase in vascular permeability, and a highly significant relationship was observed between the area of lesions and the increased permeability (9, 22). Indeed, histological observation revealed that these ulcers were accompanied with 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 further found that another BPP, minodronate, similarly provoked severe antral ulcers, but risedronate hardly damaged the antral mucosa. Thus, the dose required to produce antral ulcers differed depending on BPPs; minodronate at >10 mg/kg, alendronate at >100 mg/kg, and risedronate at >300 mg/kg. These results suggest that the ulcerogenic property of risedronate is much less potent than that of other BPPs.

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The mechanism underlying the ulcerogenic action of BPPs in the antrum remains unknown. Lichtenberger et al. (23) proposed that BPPs caused epithelial injury through an interaction with the layer of surface-active phospholipids within the adherent mucus gel. We showed that BPPs 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, 24). It is possible that antral ulcers are essentially induced by a direct action of BPPs. Satoh et al. (25, 26) reported the development of antral ulcers by indomethacin in re-fed rats and showed that the solid component of food played an essential role in the formation of these ulcers, although the detailed mechanism remains unknown. The same might be applied to the pathogenesis of BPP-induced antral lesions, and the solid food component somehow contributes to the extension of mild damage to severe lesions after re-feeding.

In general, acid/peptic digestion or a deficiency of endogenous prostaglandins (PGs) is involved in the pathogenesis of gastric lesions as induced by various means (27-29). However, it has been reported that alendronate-induced antral lesions were not significantly affected by either omeprazole or indomethacin (9), excluding the possibility that gastric acid or PG deficiency is not involved in the pathogenesis of these ulcers.

On the other hand, several models of antral ulcers were induced by drugs that negatively affected the mucosal anti-oxidative system (30-32). Chen et al. (30) reported the induction of antral ulceration in rats by diethyl-dithiocarbamate, an inhibitor of SOD, and the pathogenesis of these ulcers was associated with the impairment of the mucosal anti-oxidative system, including a decrease in SOD activity or an increase of oxyradical production. Consistent with the previous study (9), we confirmed a decrease in both SOD activity and GSH content in the antral mucosa after the administration of alendronate and minodronate. Furthermore, these BPPs slightly increased mucosal lipid peroxidation, suggesting the generation of oxidative stress. A previous study also showed that the severity of alendronate-induced antral ulcers was significantly reduced by pretreatment with SOD as well as allopurinol, an inhibitor of oxyradical production (9, 22). These findings support the involvement of the impaired antioxidative system in the pathogenesis of BPP-induced antral ulceration. It should be noted that risedronate did not significantly affect any of these parameters such as SOD activity, GSH content and lipid peroxidation. These results are understandable, because treatment with risedronate slightly damaged the antral mucosa, causing small ulcers (<15 mm²) even at 300 mg/kg.

Consistent with the previous observation (11), we found that alendronate (30 mg/kg or greater) dose-dependently and significantly impaired the healing of gastric ulcers. Similarly, the healing of gastric ulcers was markedly delayed by minodronate, at a much lower dose of 10 mg/kg or greater. Although risedronate slightly delayed healing at 60 mg/kg, the effect was not statistically significant. It is known that endogenous PGs derived from cyclooxygenase (COX)-2 play a crucial role in the healing of gastric ulcers (33-35); however, we previously reported that alendronate had no effect on the up-regulation of COX-2 expression and PGE₂ production in the ulcerated mucosa (11). Thus, it is assumed that other factors may be involved in the mechanism by which BPPs impair the healing of gastric ulcers.

We found that both alendronate and minodronate significantly mitigated the angiogenic response in the ulcerated mucosa, as evidenced from immuno-histochemical staining with factor VIII. These results were consistent with the findings of Wood et al. (36), who demonstrated that one BPP, zolendronic acid, possessed significant antiangiogenic activity in several different in vitro and in vivo models. In addition, many studies have demonstrated an important role of growth factors such as VEGF and bFGF in the healing of gastrointestinal ulcers (37-40). As expected, we found that both alendronate and minodronate down-regulated the expression of these growth factors, particularly VEGF expression. Again, risedronate even at 60 mg/kg hardly affected the expression of either VEGF or bFGF, as this BPP had no effect on ulcer healing. These results suggest that these BPPs impaired the healing of gastric ulcers, at least partly, through dysregulation of the expression of bFGF and VEGF, the growth factors essential for angiogenesis. The mechanism by which BPPs dysregulated the expression of such growth factors remains unknown. The expression of these growth factors is known to be mediated by COX-2/PGE₂ production (33); however, because BPPs had no effect on COX-2 expression and PGE₂ production in the ulcerated mucosa (11), it is unlikely that these agents caused the dysregulation of growth factor expression by down-regulating COX-2/PGE₂ production.

At present, why the gastric adverse effects of risedronate, a nitrogen-containing BPP, are much less pronounced than those of other nitrogen-containing BPPs remains unknown. The synthesis and biological evaluation of a large number of nitrogen-containing BPPs took place in the 1980s, but still with an incomplete understanding of their structure-activity relationships (41). In general, the suppressive effect on bone resorption is increased by inclusion of nitrogen in the chemical structure, yet the gastric adverse effects are also strengthened. Thus, the presence of nitrogen may positively affect the gastric adverse effects but not contributes to the less harmful influence on the gastric mucosa. Several studies suggest that the nitrogen-containing BPPs can impair the stomach through topical irritant action (7, 8, 10). We previously found a decrease in gastric transmucosal potential difference by both alendronate and risedronate, when applied topically to the stomach, yet the degree of this response was significantly less in case of risedronate (8). The difference in their irritating actions was also supported by the histological observation that the mucosa exhibited widespread exfoliation of surface epithelial cells following topical application of alendronate while no apparent damage was observed after risedronate. So, it is assumed that the chemical moieties other than nitrogen might account for the difference in gastric adverse effects of BPPs, including topical irritancy.

Given the findings of the present study, we concluded that nitrogen-containing BPPs produced antral ulcers in rat stomachs after re-feeding and impaired the healing of chronic gastric ulcers, yet these adverse effects varied in potency, depending on the BPPs; in the order of minodronate >> alendronate >> risedronate. In addition, it is also suggested that the pathogenesis of BPP-induced antral ulcers may be partly accounted for by impairment of the mucosal anti-oxidative system, while the healing impairment action may be due to the dysregulation of growth factors/angiogenesis in the ulcerated mucosa. Although further studies are needed to understand why risedronate shows fewer adverse effects on the stomach, the present study suggests that risedronate may be used more safely than other BPPs as an antiresorptive drug in patients.

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