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A Proton Pump Inhibitor's Effect on Bone Metabolism Mediated by Osteoclast Action in Old Age: A Prospective Randomized Study

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Background/Aims: Proton pump inhibitors (PPIs) act by irreversibly binding to the H^+-K^+-ATPase of the proton pump in parietal cells and may possibly affect the vacuolar H^+-ATPase in osteoclasts. **Methods:** We investigated the effect of 8 weeks of PPI treatment on the parameters of bone turnover and compared PPI with revaprazan, which acts by reversibly binding to H^+-K^+-ATPase in proton pumps. This study was a parallel randomized controlled trial. For 8 weeks, either a PPI or revaprazan was randomly assigned to patients with gastric ulcers. The parameters of bone turnover were measured at the beginning of and after the 8-week treatment period. **Results:** Twenty-six patients (PPI, n=13; revaprazan, n=13) completed the intention-to-treat analysis. After the 8-week treatment period, serum calcium and urine deoxypyridinoline (DPD) were increased in the PPI group (serum calcium, p=0.046; urine DPD, p=0.046) but not in the revaprazan group. According to multivariate linear regression analysis, age ≥60 years was an independent predictor for the changes in serum calcium and urine DPD. **Conclusions:** In elderly patients, administering a PPI for 8 weeks altered bone parameters. Our study suggested that PPIs might directly alter bone metabolism via the vacuolar H^+-ATPase in osteoclasts. **(Gut Liver 2015;9:607-614)**

Key Words: Bone metabolism; Hydrogen potassium ATPase; Osteoclasts; Osteoporosis; Proton pump inhibitors

INTRODUCTION

Proton pump inhibitors (PPIs) are widely used in the treatment of gastrointestinal disorders such as peptic ulcer disease and gastroesophageal reflux disease, and they inhibit the gastric H^+-K^+-ATPase by covalent bonding which lasts long.[1] Recent studies have reported that the long-term use of PPIs has been associated with an increased risk of fracture (odds ratio [OR], 1.44 to 2.65).[2-4] The multivariable OR increase observed with high-dose long-term PPI therapy was 2.65 (95% confidence interval [CI], 1.80 to 3.90).[3] On the other hand, the long-term use of PPIs did not affect osteoporosis among patients without risk of fracture, based on data from either the hip (OR, 0.84; 95% CI, 0.55 to 1.34) or the lumbar spine (OR, 0.79; 95% CI, 0.59 to 1.06), nor did PPI use contribute to the decrease in bone mineral density.[5] According to a study that fully adjusted for age, sex, and index date (date of first-time hip fracture for cases, same date for matched controls), the relative risk (RR) for hip fracture among patients with PPI therapy was 0.9 (95% CI, 0.7 to 1.1) compared with those without PPI therapy.[6]

Although there is controversy among epidemiologic studies, long-term use of PPI treatment is thought to affect bone metabolism such as, specifically, increasing the risk of osteoporosis or fracture. Hypotheses about the association between PPI use and risk of fracture are as follows: (1) According to animal and clinical studies, PPI therapy could cause hypochlorhydria, which would lead to a decrease in calcium absorption in the small intestine, followed by a decrease in bone mineral density.[7-10] (2) As PPIs inhibit gastric H^+-K^+-ATPase, they could also inhibit the vacuolar type of H^+-ATPase of osteoclasts.[11] This process might cause aberrant osteoclast-mediated bone resorption and osteoporosis.[12,13] (3) Long-term use of PPIs could cause reduced vitamin B_{12} absorption, which would lead to an increased homocysteine concentration. A high concentration of serum homocysteine might interfere with collagen cross-linking and weaken bone, thereby increasing the risk of osteoporotic fracture.[14-17] (4) PPIs could induce hypergastrinemia, and gastrin

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causes hyperplasia of the parathyroid gland. Increased parathyroid hormone was found to induce a decrease in bone mineral density.[18,19] However, the effect of PPIs on bone metabolism remains controversial and has not been fully evaluated.

While PPIs act by irreversibly binding to H^+-K^+-ATPase, revaprazan is a novel acid pump antagonist, which acts by binding to H^+-K^+-ATPase in a reversible manner.[20-23] Thus, in order to determine whether PPIs affect H^+-ATPase on osteoclasts, we compared the outcomes of the PPIs with revaprazan, given their different pharmacologic mechanisms.

Therefore, we investigated the short-term (8-week) effect of PPIs on the biochemical parameters of bone turnover in aged patients with gastric ulcer. We also compared PPIs with revaprazan, in search of the mechanism by which PPIs impact osteoporosis or bone turnover.

MATERIALS AND METHODS

1. Study population

This study was a parallel randomized controlled trial in a single center in South Korea between February 2010 and March 2012. The subjects, aged between 55 and 85, were included based on the results of esophagogastroduodenoscopy (EGD), and satisfied the diagnostic criteria for gastric ulcer. Among the women, only postmenopausal women were enrolled. All successive patients referred with the diagnosis were eligible for inclusion in this prospective study, except those with evidence of chronic renal disease, chronic liver disease, cardiovascular disease, any endocrine disease, and those with abnormalities by routine hematology and biochemistry blood testing (total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, and calcium). We also excluded those with osteoporosis, bone fracture for 6 months prior to the study, any experience of abdominal surgery, or experience with taking PPIs, glucocorticoids, any diuretics, thyroid hormones, vitamin D, estrogen, raloxifene, or bisphosphonates within 3 months prior to the study. We enrolled 39 patients, aged between 55 and 85, and randomly divided them into two groups, then prescribed either a PPI or revaprazan to each group for 8 weeks.

The study was approved by the Institutional Review Board of the hospital (EMCIRB 09-79), and informed consent was obtained.

2. Study design

Two randomized groups received pantoprazole (40 mg daily), or revaprazan (200 mg daily). Serum calcium; osteocalcin; intact parathyroid hormone (iPTH); urinary excretion of calcium; creatinine; urine deoxypyridinoline (DPD); gastrin; and the pepsinogen I/II ratio were measured at the beginning and after 8 weeks of treatment. Initially, bone mineral densitometry (BMD) was measured in all of the patients.

3. Basic questionnaires

A survey was carried out to gather basic data on height, weight, and nutritional habits. The questionnaires also inquired about any history of osteoporosis, falls, fractures, and medication. The history-taking on medication investigated acid suppressive drugs (PPIs, H_2-receptor antagonists, or revaprazan), any diuretics, glucocorticoids, thyroid hormones, vitamin D, estrogen, raloxifene, and bisphosphonates within 1 year prior to the study.

4. Esophagogastroduodenoscopy

EGD was performed for the patients with dyspepsia or gastrointestinal hemorrhage during the study. Only those with gastric ulcer including hemorrhage were enrolled. Gastric ulcer was defined by endoscopic and histologic findings using the updated Sydney System.[24]

5. Bone turnover markers

All of the blood and urine samples were drawn in a fasting state before breakfast. These included total serum and urine calcium (PCa, UCa), iPTH, urine DPD, and osteocalcin.

Serum calcium was measured by standard calorimetric methods using the Hitachi 7600 analyzer (Hitachi High-Technologies Co., Tokyo, Japan) and corrected according to the albumin level. In addition, serum and urine creatinine (PCr, UCr) were measured and fractional excretion of calcium (Fe Ca) was calculated by this equation [Fe Ca=(UCa×PCr×100)/(PCa×UCr)].

Serum iPTH was measured by chemiluminescent immune assay (CLIA) using Immulite®2000 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The detection limit of the iPTH assay was 2.5 pg/mL (normal range, 14 to 72 pg/mL) with inter- and intra-assay coefficients of variance (CVs) of 5.8% and 5.2%, respectively.

Urine DPD, as a bone resorption marker, was measured by CLIA using Immulite®2000 with inter- and intra-assay CVs of 15.5% (normal range, 2.3 to 7.4 nMDPD/nM Cr).

Serum osteocalcin, one of the bone formation markers, was measured by electrochemiluminescent immune assay (ECLIA) (Roche Diagnostics, Mannheim, Germany), with inter- and intra-assay CVs of 1.8% and 3.3%, respectively (normal range, 14 to 46 ng/mL).

6. Bone mineral densitometry

The BMD values were measured by dual energy X-ray absorptiometry using a Lunar-iDEX (GE, Madison, WI, USA) at the lumbar spine (L2–L4) and femoral neck. The BMD was automatically calculated from the bone area (cm^2) and bone mineral content (g) and expressed absolutely in g/cm^2. The BMD at the lumbar spine was calculated by eliminating the vertebral bodies with severely degenerative changes. The CVs of the measurements of the lumbar spine and femoral neck were 1.9% and 2.5%,

respectively. Normative data were obtained from a population-based database for Koreans. Osteoporosis was defined as a T score ≤-2.5. The BMD was measured initially in all of the patients. The patients with osteoporosis though the initial BMD should be excluded in this study.

Gastrin was measured by Immulite®2000. In fasting subjects, gastrin normally circulates at levels of less than 100 pg/mL. The pepsinogen I/II ratio was calculated automatically by Toshiba-200FR (Green Cross Reference Lab., Yongin, Korea). A pepsinogen I/II ratio >3.1 indicates normal gastric mucosa.

7. Study end point

The primary end points for this study were the short-term (8-week) effect of the PPI on the biochemical parameters of bone turnover, through a comparison between the PPI and revaprazan. The secondary end points were variables affecting the change in bone turnover parameters between before and after acid suppressive medication.

8. Statistical analysis

A sample size of 60 patients (PPI group, 30; revaprazan group, 30) was estimated with reference to Mizunashi's study,[11] because no *in vivo* studies have compared PPIs to revaprazan for the effect on bone metabolism. The end point was urine hydroxyproline in the reference study. On the assumption that α=0.05, 1-β (power)=0.8, and the changes±standard deviation (SD) of urine DPD=2.5±4.7, the sample size needed for a comparison of two groups was calculated to be 30 patients in each group when considering wastage rates of 10%. By means of *post hoc* power analysis, 1-β (power) was 0.68 in urine DPD, and 0.75 in serum corrected calcium, respectively.

According to the normality test (Kolmogrov-Smirnov test), the paired t-test was appropriate as an analysis method. Therefore, the paired t-test was used to compare bone parameters before and after medication in each group.

Continuous variables, referred as mean±SD, were analyzed by the independent two-sample t-test, and categorical variables, referred as number (%), were analyzed by the chi-square test or Fisher exact test.

Variables affecting bone turnover parameters between before and after administration of acid suppressive drugs were specified by linear regression analysis.

By means of the interaction tests for age, gender, BMD, and BMI, subgroup analysis was performed to identify a tendency toward changes in bone turnover parameters even small numbers. A significance level of $p<0.05$ was adopted. Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Sixty-five patients were selected initially, and 26 of them were excluded by incongruity or personal refusal. The remaining 39 patients were randomly divided into two groups (PPI, n=20;

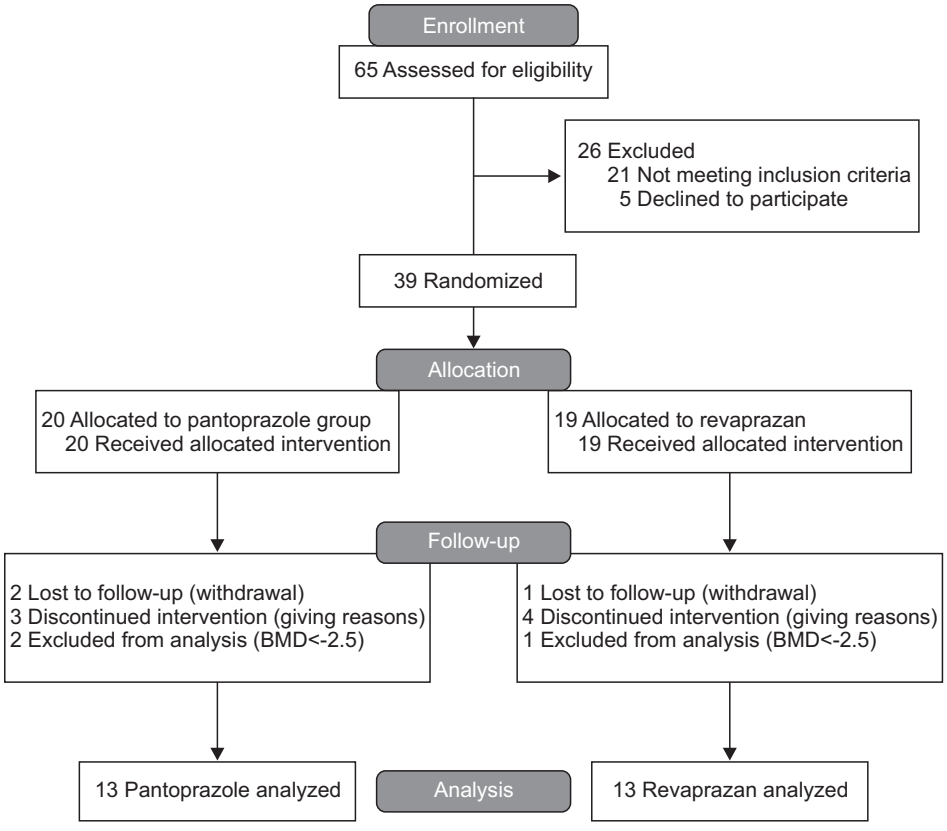

Fig. 1. Study diagram.
BMD, bone mineral densitometry.

revaprazan, n=19). Then, the PPI or revaprazan was randomly assigned to each group for 8 weeks. During the experiment, five patients from each group were excluded due to loss to follow-up or discontinued intervention. After medication, two patients from the PPI group and one patient from the revaprazan group were excluded due to osteoporosis (BMD ≤-2.5). In the end, 26 patients (PPI, n=13; revaprazan, n=13) finished undergoing the intention-to-treat analysis (Fig. 1).

The clinical characteristics of the total enrolled patients are shown in Table 1. The mean age of the patients was 63.2±9.1, and the gender distribution was 15 males and 11 females. The pepsinogen ratio, which reflects atrophy, was 4.5±1.9, showing parameters within the normal range. The comparison of basic characteristics and initial bone parameters between the PPI and revaprazan groups is shown in Table 2. The mean age of the PPI group was 62.5±11.1, and that of the revaprazan group was 63.9±6.8. The baseline serum corrected calcium, serum osteocalcin, iPTH, gastrin, pepsinogen, urinary excretion of calcium, urine DPD, and BMD of the two groups did not differ significantly.

The comparison of bone parameters between the PPI and revaprazan groups before and after treatment is shown in Table 3.

Table 1. Clinical Characteristics of the Enrolled Patients

Characteristic	Value
Age, yr	63.2±9.1
<60	8 (31)
≥60	18 (69)
Gender	
Male	15 (58)
Female	11 (42)
BMI	24.7±3.8
<25	14 (54)
≥25	12 (46)
BMD	-0.2±1.7
≥-1	14 (56)
<-1	11 (44)
Corrected calcium	8.8±0.5
Fe Ca	0.9±0.7
Urine DPD	5.7±2.4
Osteocalcin	12.0±5.8
iPTH	41.6±14.7
Gastrin	67.5±36.8
Pepsinogen I/II ratio	4.5±1.9

Data are presented as mean±SD or number (%).
BMI, body mass index; BMD, bone mineral densitometry; Fe Ca, fractional excretion of calcium; DPD, deoxypyridinoline; iPTH, intact parathyroid hormone.

Table 2. Comparison of the Basic Characteristics and Initial Bone Parameters between the Proton Pump Inhibitor and Revaprazan Groups

Variable	PPI (n=13)	Revaprazan (n=13)	p-value
Age, yr	62.5±11.1	63.9±6.8	0.689
<60	6 (46)	2 (15)	0.202
≥60	7 (54)	11 (85)	
Gender			
Male	9 (69)	6 (46)	0.234
Female	4 (31)	7 (54)	
BMI	24.1±4.0	25.3±3.7	0.454
<25	7 (54)	7 (54)	
≥25	6 (46)	6 (46)	
BMD	-0.4±1.7	-0.0±1.8	0.595
≥-1	8 (62)	6 (50)	0.562
<-1	5 (38)	6 (50)	
Corrected calcium	8.8±0.5	8.8±0.5	0.946
Fe Ca	0.9±0.7	0.9±0.6	0.883
Urine DPD	5.7±2.2	5.6±2.6	0.980
Osteocalcin	12.7±5.2	11.2±6.4	0.529
iPTH	41.1±12.6	42.2±17.0	0.842
Gastrin	58.8±20.2	76.1±47.4	0.243
Pepsinogen I/II	4.9±2.3	4.2±1.3	0.357

Data are presented as mean±SD or number (%). Continuous variables, referred to as the mean±SD, were analyzed by the independent two-sample t-test, whereas categorical variables, referred to as number (%), were analyzed using the chi-square test or Fisher exact test.
PPI, proton pump inhibitor; BMI, body mass index; BMD, bone mineral densitometry; Fe Ca, fractional excretion of calcium; DPD, deoxypyridinoline; iPTH, intact parathyroid hormone.

Table 3. Comparison of Bone Turnover Parameters before and after Proton Pump Inhibitor or Revaprazan Treatment

Variable	PPI treatment (n=13)			Revaprazan treatment (n=13)		
	Before	After	p-value	Before	After	p-value
Corrected calcium	8.8±0.5	9.2±0.6	0.046	8.8±0.5	9.0±0.3	0.326
Fe Ca	0.9±0.7	1.0±0.7	0.982	0.9±0.6	0.6±0.5	0.133
Urine DPD	5.7±2.2	6.5±2.1	0.047	5.6±2.6	6.8±2.7	0.191
Osteocalcin	12.7±5.2	13.2±5.2	0.750	11.2±6.4	13.4±7.3	0.208
iPTH	41.1±12.6	33.0±18.5	0.144	42.2±17.0	61.8±50.1	0.148

Data are presented as mean±SD. The paired t-test was used to compare bone parameters before and after medication in each group.
PPI, proton pump inhibitor; Fe Ca, fractional excretion of calcium; DPD, deoxypyridinoline; iPTH, intact parathyroid hormone.

The serum corrected calcium increased from 8.8±0.5 to 9.2±0.6 (p=0.046) and the urine DPD increased from 5.7±2.2 to 6.5±2.1 (p=0.047) in the PPI group. There were no changes in these parameters in the revaprazan group. No mobility or other side effects were present in either group.

The univariate linear regression analysis for the changes in the bone parameters over the course of treatment among all of the patients taking acid suppressive drugs is shown in Supplementary Table 1. This analysis was performed to determine whether the type of medication (PPIs or revaprazan), age, gender, BMI, or BMD affected bone parameters or not. Variables affecting bone turnover parameters between before and after administration of acid suppressive drugs were specified by linear regression analysis. The PPI, age, and BMI (p<0.1) were defined as covariates through the univariate linear regression analysis. The multivariate linear regression analysis was performed with calibrated confounders, as shown in Table 4. The multivariate analysis showed that being older (≥60 years) was an independent predictor for changes in serum corrected calcium and urine DPD.

According to an age-based subgroup analysis (Supplementary Table 2), the serum corrected calcium had increased and the iPTH had decreased among patients aged 50 to 60 years old only in the PPI group (serum calcium, p=0.016; iPTH, p=0.037). On the other hand, the urine DPD had significantly increased among patients 60 years of age or older in both the PPI and revaprazan groups. The gender-based subgroup analysis showed that the urine DPD had significantly increased among the male patients in both groups (PPI group, p=0.029; revaprazan group, p=0.05) (Supplementary Table 3). According to a BMD-based subgroup analysis (Supplementary Table 4), the serum osteocalcin had increased among the patients with a normal BMD in the PPI group, while in a BMI-based subgroup analysis (Supplementary Table 5), the urine DPD had significantly increased among the obese (BMI ≥25) in the PPI group. By means of the interaction tests for age, gender, BMD, and BMI, subgroup analysis was performed to identify a tendency toward changes in bone turnover parameters even small numbers.

DISCUSSION

We investigated whether PPIs can cause changes in bone parameters, using a comparison of revaprazan and PPI, which have different pharmacologic mechanisms. The PPIs act to irreversibly bind to H^+-K^+-ATPase on the parietal cells; on the other hand, revaprazan acts by reversibly binding to H^+-K^+-ATPase.[21] As PPIs inhibit gastric H^+-K^+-ATPase, they could also inhibit the vacuolar type of H^+-ATPase of osteoclasts.[11] It was found that, after aged patients took a PPI as an antiulcer medication just for 8 weeks, their bone parameters underwent significantly greater changes than in the equivalent group taking revaprazan. Therefore, it can be surmised that PPIs might directly alter bone metabolism mediated by osteoclast action rather than decreasing calcium absorption. The mediation of PPIs in a vacuolar type of H^+-ATPase on osteoclasts has been suggested as a possible mechanism. Recently, it was reported that bone mineral density was decreased and bone quality was reduced in H^+/K^+ ATPase β-subunit deficient mice.[25] This could be explained by a causal relationship between long-term PPI use and an increased risk of fractures.

Few studies have addressed the effect of PPIs on bone metabolism. A recent *in vivo* study by Joo *et al*.[26] aimed at evaluating the effect of long-term PPI therapy on bone turnover analyzed the signaling pathway involved in osteoclast differentiation and bone resorption/formation markers using ovariectomized rats. The expression levels of osteocalcin were decreased and the levels of serum C-terminal cross-linked telopeptides of type I collagen were increased in the group with a low-calcium diet and PPI administration. Therefore a low-calcium diet and PPI administration are thought to work in concert to alter osteoclast activity and bone resorption signaling. According to a study on the impact of short-term (2-week) administration of omeprazole for the osteoclastic H^+-pump in children, none of the levels among urinary calcium excretion, serum total alkaline phosphatase activity, collagen type 1 cross-linked C-telopeptide, orosteocalcin were altered in any age or gender group.[27] In another study considering the effect of PPIs on biochemical markers of calcium and bone metabolism, a PPI intake for 12 weeks

Table 4. Multivariate Linear Regression Analysis

Variable	Corrected calcium			Fe Ca			Urine DPD			Osteocalcin			iPTH		
	B	SE	p-value	B	SE	p-value	B	SE	p-value	B	SE	p-value	B	SE	p-value
PPI	0.19	0.27	0.491	0.01	0.26	0.973	0.36	0.94	0.703	-1.87	2.47	0.456	-27.13	15.18	0.088
Age (≥60 yr)	-0.15	0.32	0.655	-1.06	0.32	0.004	2.04	1.15	0.091	-0.65	2.99	0.831	1.72	18.41	0.926
BMI (≥25)	0.07	0.28	0.8	0.38	0.28	0.18	1.02	0.99	0.315	2.27	2.62	0.394	11.15	16.07	0.495

Variables affecting bone turnover parameters between before and after administration of acid suppressive drugs were specified by linear regression analysis. The selected covariates, PPI, age, and BMI (p<0.1), were assessed through the univariate linear regression analysis (Supplementary Table 1).

Fe Ca, fractional excretion of calcium; DPD, deoxypyridinoline; iPTH, intact parathyroid hormone; SE, standard error; PPI, proton pump inhibitor; BMI, body mass index.

showed no measurable effect on iPTH, ionized calcium, vitamin D, osteocalcin, or serum C-terminal cross-linked telopeptides of type I collagen in healthy young males (aged 18 to 50 years).[28] Through the previous research and ours, we found that the effect of PPIs on bone metabolism is more evident in the elderly than in the younger people of the previous studies. It may be that some mechanism of compensation for the effects of PPIs on bone metabolism exists and is more effective in young people.

These age-related discrepancies have been observed in large-scale epidemiological studies, and old age is a known risk factor for long-term use of PPIs. According to a study that enrolled 1,211 postmenopausal women, independent predictors of vertebral fractures were omeprazole (RR, 3.50; 95% CI, 1.14 to 8.44), age above 65 years (RR, 2.34; 95% CI, 1.02 to 5.34), prevalent vertebral fractures (RR, 3.62; 95% CI, 1.63 to 8.08), and osteoporosis (RR, 2.38; 95% CI, 1.03 to 5.49). The PPI (omeprazole) is associated with an increased risk of vertebral fractures in the elderly and postmenopausal women.[29] In a large-scale study of 15,792 cases of osteoporosis-related fractures matched with 47,289 controls, use of PPIs for 7 or more years is associated with a significantly increased risk of an osteoporosis-related fracture.[2] Therefore, the risk factors for fracture or osteoporosis based on PPI use are suggested to be at least several years of PPI use, age above 65 years, recent fracture, osteoporosis, and being a postmenopausal woman. However, long-term use of PPIs did not affect osteoporosis rates among patients without risk of fracture, based on results from either the hip (OR, 0.84; 95% CI, 0.55 to 1.34) or the lumbar spine (OR, 0.79; 95% CI, 0.59 to 1.06), and PPI use also did not contribute to a decrease in bone mineral density.[5] According to a study that fully adjusted for age, sex, and index date (date of first-time hip fracture for cases, same date for matched controls), the RR for hip fracture among patients undergoing PPI therapy was 0.9 (95% CI, 0.7 to 1.1) compared with those without PPI therapy.[6] These results from large-scale clinical studies could suggest that old age is a more important factor involved in the effect of PPIs on bone metabolism. Therefore, considering the effect of PPIs on bone metabolism according to old age, older people might be more vulnerable group. This study also showed that being older (≥60 years) was an independent predictor for changes in serum corrected calcium and urine DPD by the multivariate analysis.

In our study, a subgroup analysis of the PPI group showed that the serum calcium had increased but iPTH had decreased only in those <60 years of age. The urine DPD had increased in the male group, obese patients, and elderly patients (≥60 years). In addition, the serum osteocalcin had increased in those with a normal BMD. On the other hand, in the revaprazan group, the urine DPD had increased in the male group and elderly patients (≥60 years). This subgroup analysis showed a tendency toward changes of bone parameters by PPIs. Further large-scaled study will be helpful for elucidating on how the PPIs affect variously in the subgroup.

Among the mechanisms by which PPIs might cause secondary osteoporosis, the decreased absorption of calcium has been thought to be more important. Acid-suppressing medications were reported to cause hypochlorhydria, which leads to a decrease in calcium absorption in the small intestine, followed by decreased bone mineral density.[7-9] However, these studies had many limitations, such as enrollment (small group and patients with dialysis), only short-term use of acid-suppressing medications, and the method of detecting calcium absorption. Furthermore, short-term suppression of gastric acid was found not to modify intestinal absorption of calcium or other minerals in the other studies.[10,30] Therefore, the effect of PPIs on calcium absorption is still controversial. In our study, the urine DPD had increased after PPI use even though the serum calcium had increased. We concluded that PPI use might directly affect bone metabolism through osteoclast action, not by reduced intestinal absorption of calcium.

PPIs induce hypergastrinemia, and gastrin causes hyperplasia of the parathyroid gland. Increased PTH, in turn, was found to induce a reduction in bone mineral density.[18,19] However, an iPTH increase associated with hypergastrinemia was not shown in this study, and thus this previous hypothesis was not supported. Further long-term research will be needed regarding the potential role of PTH associated with long-term use of PPIs.

There were some limitations of our study, given that the final enrollment number was small. The causes were restriction of age and exclusion of many comorbidities, other medications, and previous osteoporosis in the elderly. Nevertheless, it is meaningful that despite the small groups, the study had statistical significance as a clinical study. We tried to overcome the limitation of the enrollment size by using statistical verification. By means of *post hoc* power analysis, 1-β (power) was 0.68 in urine DPD, and 0.75 in serum corrected calcium, respectively. Even though the power was not very high, we think this is a unique clinical study with value in suggesting further perspectives about PPIs and bone metabolism.

We were unable to collect data on dietary calcium. However, we excluded severe illness and chronic renal or endocrinologic disorders in the first step of this study. We believe the diet of the participants was likely to be fairly homogeneous because they were aged and were of the same ethnicity and culture, and residential area. There was indirect evidence of homogenous foods that intestinal microbiota of Korean populations tended to vary less between individual Koreans, unlike the results from the United States, Japan, and China.[31] Furthermore, Wright *et al*.[32] recently reported that PPI had no effect on calcium absorption and metabolism using dual-stage calcium isotope study.

In the aged, after taking a PPI as an action by irreversibly binding to H^+-K^+-ATPase just for 8 weeks, the bone parameters (urine DPD and serum corrected calcium) had changed more than in a group taking revaprazan, which acts by binding to H^+-K^+-ATPase in a reversible manner. Our study suggested that

PPIs might directly alter bone metabolism via vacuolar type-H^+-ATPase on the osteoclasts. This study could not be shown a causal relationship between PPI and bone metabolism. We hope a delicate cascade mechanism of the effect of PPIs on bone metabolism will be found out in the near future.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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