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Co-administration of calcium carbonate or sodium bicarbonate prevents esomeprazole-induced osteoporosis

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Long-term proton pump inhibitor (PPI) use is associated with increased risks of osteoporosis and fractures. To investigate strategies for mitigating PPI-induced bone loss, this study evaluated the preventive effects of calcium carbonate or sodium bicarbonate on bone alterations in a 12-week esomeprazole-treated Wistar rat model. Esomeprazole monotherapy exhibited compromised trabecular microarchitecture, as evidenced by a decrease in bone volume fraction and trabecular number, alongside an increase in trabecular separation. Conversely, co-administration of esomeprazole with either sodium bicarbonate or calcium carbonate preserved trabecular microarchitecture and attenuated bone resorption of osteoclast. Histological and in vitro assays showed that esomeprazole enhanced osteoclastogenesis by upregulating integrin subunit beta 3 and *cathepsin K*, which were significantly suppressed by the buffering agents. Consistently, serum levels of C-terminal cross-linked telopeptides of type I collagen were significantly lower in both combination groups. These results indicate that long-term esomeprazole administration induced bone loss, primarily by enhancing osteoclast-mediated resorption. The co-administration of calcium carbonate and sodium bicarbonate effectively attenuated these deleterious effects. Notably, the comparable efficacy of sodium bicarbonate suggests that buffering systemic acidity may be a viable strategy for mitigating PPI-induced osteoporosis.

Keywords Proton pump inhibitors (PPIs), Long-term administration, Osteoporosis, Osteoclast

Abbreviations

PPIs	Proton pump inhibitors
BMD	Bone mineral density
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
GGT	Gamma-glutamyl transferase
ALB	Albumin
BDIL	Direct bilirubin
TBIL	Total bilirubin
TP	Total protein
Ca	Calcium
Na	Sodium
K	Potassium
Cl	Chloride

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Micro-CT	Micro-computed tomography
BV/TV	Bone volume fraction
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.Th	Trabecular thickness
H&E	Hematoxylin and eosin
SO	Safranin O
IP	Inorganic phosphorus
BALP	Bone-specific alkaline phosphatase
CTX-1	C-terminal telopeptide of type I collagen
TRACP-5b	Tartrate-resistant acid phosphatase 5b
PINP	Procollagen type I N-terminal propeptide
ITGB3	Integrin subunit beta 3
CTK	Cathepsin K

Proton pump inhibitors (PPIs) are the standard of care for acid-related disorders, including gastroesophageal reflux disease (GERD), *Helicobacter pylori* infection, and peptic ulcer disease¹. By irreversibly binding to H⁺/K⁺ ATPase in parietal cells, PPIs potently and durably suppress gastric acid secretion². The prevalence of PPI use has increased sharply in recent decades. From 1999 to 2012, its usage among non-institutionalized U.S. adults almost doubled, with an estimated 20–25 million annual users³. While PPIs are indicated for specific chronic conditions, such as chronic and advanced GERD, Zollinger-Ellison syndrome, and prophylaxis against gastrointestinal bleeding, a substantial proportion of patients use them without clear clinical indications^{4–6}. Consequently, many patients are exposed to long-term therapy, raising concerns regarding adverse effects, such as intestinal dysbiosis, *Clostridium difficile* infection, chronic kidney disease, and osteoporosis^{7–9}. Numerous observational studies and meta-analyses have reported a modest, but statistically significant, association between chronic PPI use and an increased risk of hip, spine, and femoral fractures^{10,11}. Depending on the population, PPI use is associated with a 1.29- to 2.53-fold increase in fracture risk^{12–15}. Furthermore, concurrent PPI use may attenuate the fracture-reduction benefits of hormone therapy in postmenopausal women. Although some prospective studies have reported conflicting data regarding bone mineral density (BMD) loss, accumulating evidence suggests that chronic PPI use compromises trabecular bone quality^{16–18}.

Recently, pharmaceutical formulations combining PPIs with antacids, such as calcium carbonate or sodium bicarbonate, have been developed. These formulations protect acid-labile PPIs from gastric degradation by enabling their immediate release and rapid absorption^{19–21}. While calcium carbonate is a well-established supplement for bone health in postmenopausal women^{22,23}, the specific impact of sodium-bicarbonate-containing PPI formulations on bone metabolism remains underexplored.

This study evaluated the effects of long-term administration of esomeprazole, either alone or in combination with calcium carbonate or sodium bicarbonate, on bone metabolism in a Wistar rat model. We also attempted a translational evaluation using a normal animal model, such as Wistar rat, to assess the skeletal effects of long-term PPI administration in a general patient population. We hypothesized that antacid combination would substantially attenuate esomeprazole-induced alterations in bone mineral metabolism. To comprehensively characterize bone metabolism, we employed a multimodal approach to assess bone microarchitecture, serum turnover markers, histomorphometry, and gene expression profiles of key osteoclastogenic factors.

Methods

Study design and animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Hanyang University (2025–0089 A) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Twenty-four female Wistar rats (8 weeks old) were obtained from Orient Bio (Gapyeong, Korea). The animals were housed in a controlled environment (21–23 °C, 50% humidity, 12-hour light/dark cycle) with *ad libitum* access to water and a standard diet. After 1 week of acclimatization, 9-week-old rats were randomly assigned to one of four groups ($n=6$ per group): Group A (saline control), Group B (esomeprazole alone; Nexium[®], AstraZeneca, Cambridgeshire, UK), Group C (esomeprazole + calcium carbonate; Esopid[®], Yuhan, Seoul, Korea), and Group D (esomeprazole + sodium bicarbonate; Esoduo-S[®], Chong Kun Dang, Seoul, Korea). To ensure that each experimental group received an equivalent pharmacological stimulus, the administered dose was strictly calculated based on the active esomeprazole content (3 mg/kg), rather than the total weight of the processed tablets. Each drug was completely dissolved in 10 mL of saline for uniform oral administration. In accordance with stability guidelines, all prepared solutions were utilized within a hour, with any remaining portions discarded. The drugs were administered orally at a dose of 3 mg/kg/day for 12 weeks to mimic therapeutic exposure. Body weight was monitored weekly.

Gastric pH measurement

To verify pharmacological efficacy, gastric pH was measured on day 1. Gastric pH was assessed after the first dose primarily to verify the successful delivery and immediate pharmacological activity of each formulation. A gastric cannula was inserted, and gastric juice was collected prior to dosing and 3 h post-administration. The pH levels were determined using litmus paper.

Serum biochemical analysis

After 12 weeks, the rats were anesthetized with 3–5% isoflurane, and blood samples were collected via cardiac puncture. Following blood collection, the rats were immediately euthanized in a chamber using 30–70% CO₂.

Serum electrolytes (Ca, P, Na, K, and Cl) and liver function markers (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, albumin, bilirubin, total protein) were analyzed using an automated chemistry analyzer (NX700; Fujifilm, Tokyo, Japan). Bone turnover was assessed by measuring bone-specific alkaline phosphatase (BALP; MBS722033; MyBioSource, San Diego, CA, USA) for bone formation and the C-terminal telopeptide of type I collagen (CTX-1; CEA665Ra, Cloud-Clone Corp., Houston, TX, USA) for bone resorption. Additionally, the levels of tartrate-resistant acid phosphatase 5b (TRACP-5b; E-EL-R0939; Elabscience, Houston, TX, USA) and procollagen type I N-terminal propeptide (PINP; SEA957Ra; Cloud-Clone Corp.) were quantified.

BMD and bone microarchitecture

Right Femurs were fixed in 10% formalin for 1 day at room temperature. Subsequently, they were placed in 70% ethanol at 4°C and scanned using high-resolution micro-computed tomography (micro-CT; Skyscan 1272; Bruker Micro-CT, Kontich, Belgium) at 70 kV, with a 0.5 mm aluminum filter and a 15 µm pixel size. Three-dimensional (3D) reconstruction was performed using Nrecon v1.7.3.1 software (Bruker Micro-CT) and InstaRecon software (InstaRecon, Champaign, IL, USA). Data analysis was performed using CTAn v1.20.3.0 software (Bruker Micro-CT), and visualization was performed using the 3D model visualization software CTVol v2.3.2.0 (Bruker Micro-CT). For BMD, trabecular bone and cortical bone analysis, the center of the distal femoral growth plate was set as the reference plane, and starting 2.25 mm below from this reference plane and analyzing up to 5.25 mm.

Histological and immunohistochemical analysis

Right Femurs were decalcified with 15% EDTA at 4°C for at least 1 month, embedded in paraffin, and sectioned at 5 µm. Sections were stained with hematoxylin and eosin (H&E; 1.05174.0500; Sigma-Aldrich, St. Louis, MO, USA) for morphology, safranin O (SO; 146640250; ACROS Organics, Geel, Belgium) for cartilage, and tartrate-resistant acid phosphatase (TRAP; PMC-AK04F; Cosmo Bio, Tokyo, Japan) to identify osteoclasts. For immunohistochemistry (IHC), sections were deparaffinized using Neo-Clear (109843; Sigma-Aldrich) and a graded ethanol series (100% to 50%), and endogenous peroxidase activity was then blocked using BLOXALL solution (SP-6000; Vector Laboratories, Newark, CA, USA) for 10 min at RT. The sections were antigens retrieval using 1X proteinase K (VB-6009; Vitro Vivo Biotech, Rockville, USA) for 10 min at 37°C, and the sections were stained for osteocalcin (1:200; SC-365797, Santa Cruz, Dallas, TX, USA) or alkaline phosphatase (1:200; A12396, Abclonal, Woburn, MA, USA) using the Dako REAL Envision Detection System Kit (K5007; Agilent Dako, Santa Clara, CA, USA).

In vitro osteoclast differentiation

Bone marrow cells were isolated from left femurs and treated with Ammonium-Chloride-Potassium (ACK) lysis buffer (A10492-01; Thermo Fisher Scientific, Waltham, MA, USA). Cells were cultured with alpha-MEM (LM008-53; Welgene, Gyeongsan-si, Korea) containing 10 ng/mL macrophage colony-stimulating factor (M-CSF) for 24 h, and non-adherent cells were harvested and cultured in alpha-MEM containing 20 ng/mL M-CSF (400–28; Thermo Fisher Scientific) and 100 ng/mL RANKL (400–30; Thermo Fisher Scientific). On day 7, mature osteoclasts were stained for TRAP using a commercial kit (PMC-AK04-COS; Cosmo Bio, Tokyo, Japan).

RNA extraction and mRNA analysis

RNA extraction and quantitative polymerase chain reaction (qPCR) were performed as previously described²⁴. Total RNA was extracted using TRIzol reagent (15596018; Invitrogen, Carlsbad, CA, USA). One microgram of total RNA was reverse-transcribed (K1622; Thermo Fisher Scientific). Quantitative real-time PCR (qPCR) was performed using a CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). cDNAs were amplified in triplicate using IQ™ SYBR® Green Supermix (1708880; Bio-Rad Laboratories). The analysis of relative mRNA expression was performed using $\Delta\Delta C_t$ (delta-delta C_t) method, with GAPDH as the reference gene. Each value is expressed as the fold change relative to the saline. The primers used for reverse transcription (RT)-qPCR were as follows: TRAP-qF, ACCCTGGCAATGTCTCGGCACAAA; rTRAP-qR, AATTGCCACA CAGCATCACGGT; rITGB3-qF, ACTTGGCAAAAACGCGGTGAAGT; rITGB3-qR, AGAGTAGCAAGGCC AATGAGCAGA; rCTK-qF, TGACGAAACTGCGACCGTGAT; rCTK-qR, TTTGTTTCCCCAGCTTTCTC CCA; and rGAPDH-qF, TGATGGGTGTGAACACGAG, rGAPDH-qR, CCCTTCCACGATGCCAAAGT.

Statistical analysis

Data were analyzed using GraphPad Prism version 8 (GraphPad, San Diego, CA, USA). Differences were evaluated using one-way analysis of variance, followed by Tukey's post hoc test. Data are presented as the mean $\pm$ standard deviation (SD) of the mean. Statistical significance was set at $p < 0.05$.

Results

Physiological parameters and gastric acid suppression following long-term Esomeprazole and its combination with calcium carbonate or sodium bicarbonate

Nine-week-old Wistar rats were randomly assigned to one of four groups: Group A (saline control), Group B (esomeprazole alone), Group C (esomeprazole + calcium carbonate), and Group D (esomeprazole + sodium bicarbonate) (Fig. 1A). All groups exhibited steady weight gain over the 12-week period (Fig. 1B), with no significant differences in final body weight and cumulative change of body weight between groups (Fig. 1C and D), indicating that the treatments were well tolerated. Gastric pH measurements 3 h post-administration confirmed effective acid suppression in all esomeprazole-treated groups compared to the saline-treated controls

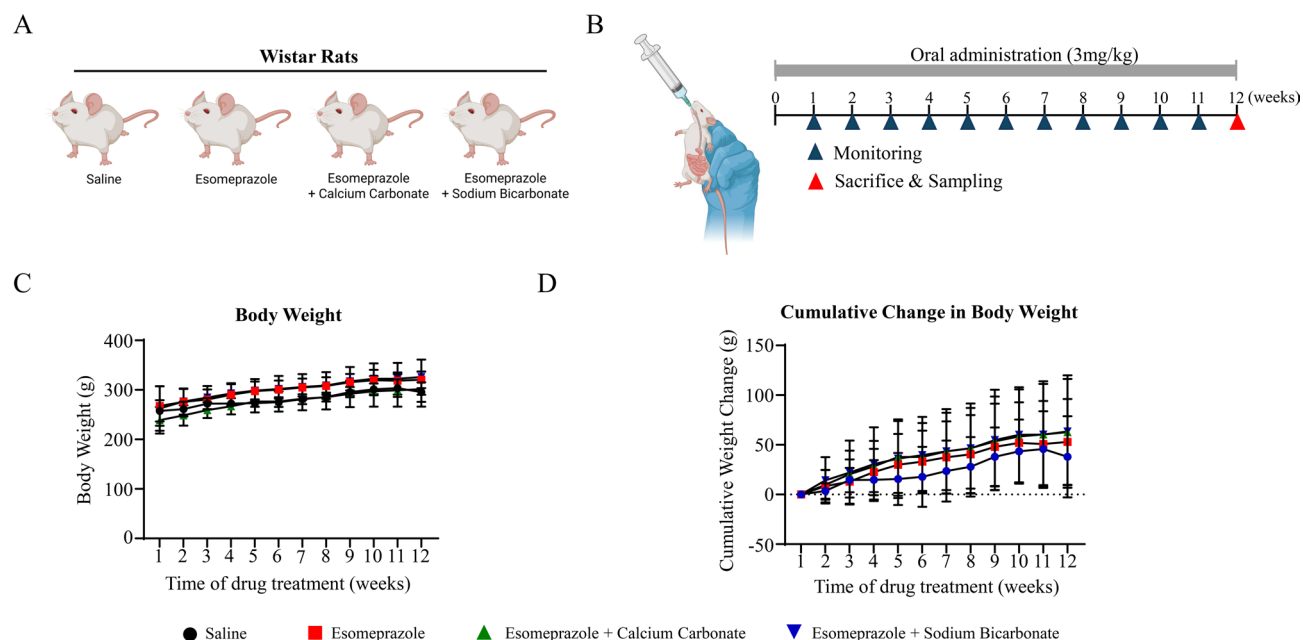


Fig. 1. Physiological parameters and gastric acid suppression following long-term Esomeprazole and its combination with calcium carbonate or sodium bicarbonate. **(A)** Experimental grouping. Nine-week-old rats were randomly divided into four groups and received oral administration of saline, esomeprazole, esomeprazole with calcium carbonate, or esomeprazole with sodium bicarbonate ($n = 6/\text{group}$). **(B)** A schematic diagram of experimental procedure. Rats were orally administered each PPI daily at a dose of 3 mg/kg for 12 weeks, and body weight was monitored weekly. **(C)** Change in body weight. **(D)** Cumulative changes in body weight. Data are expressed as the mean $\pm$ SD. **(A and B)** Created with BioRender.com.

Parameters	Saline ($n = 6$)	Esomeprazole ($n = 6$)	Esomeprazole + Calcium Carbonate ($n = 6$)	Esomeprazole + Sodium Bicarbonate ($n = 6$)
AST (U/L)	61.50 $\pm$ 9.35	58 $\pm$ 12.18	68.17 $\pm$ 8.33	65.50 $\pm$ 7.71
ALT (U/L)	40.50 $\pm$ 9.75	33.33 $\pm$ 11.79	40.67 $\pm$ 10.80	38.83 $\pm$ 6.59
ALP (U/L)	582.83 $\pm$ 118.39	500.33 $\pm$ 42.23	536.33 $\pm$ 131.33	544.17 $\pm$ 117.36
GGT (U/L)	2.67 $\pm$ 1.21	1.67 $\pm$ 0.82	2.00 $\pm$ 1.10	2.67 $\pm$ 1.37
ALB (g/dL)	4.92 $\pm$ 0.40	4.90 $\pm$ 0.22	4.68 $\pm$ 0.23	4.72 $\pm$ 0.31
DBIL (mg/dL)	< 0.1	< 0.1	< 0.1	< 0.1
TBIL (mg/dL)	0.28 $\pm$ 0.04	0.33 $\pm$ 0.15	0.28 $\pm$ 0.08	0.27 $\pm$ 0.05
TP (g/dL)	6.47 $\pm$ 0.40	6.60 $\pm$ 0.25	6.27 $\pm$ 0.16	6.52 $\pm$ 0.44
Ca (mg/dL)	10.67 $\pm$ 0.52	10.67 $\pm$ 0.52	10.50 $\pm$ 0.55	10.83 $\pm$ 0.75
Na (mEq/L)	145 $\pm$ 1.79	143.83 $\pm$ 1.94	144.50 $\pm$ 1.52	145.50 $\pm$ 2.66
K (mEq/L)	4.43 $\pm$ 0.69	4.37 $\pm$ 0.36	4.62 $\pm$ 0.46	4.60 $\pm$ 0.54
Cl (mEq/L)	94.33 $\pm$ 1.86	94.67 $\pm$ 1.97	95.67 $\pm$ 1.51	94.50 $\pm$ 1.64
IP (mEq/L)	6.10 $\pm$ 1.83	5.42 $\pm$ 1.36	5.23 $\pm$ 1.27	5.60 $\pm$ 1.02

Table 1. Biochemical analysis of Serum (mean $\pm$ SD). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase, ALB: Albumin, DBIL: Direct bilirubin, TBIL: Total bilirubin, TP: Total protein, Ca: Calcium, Na: Sodium, K: Potassium, Cl: Chloride, IP: Inorganic phosphorus.

(Supplementary Fig. 1). Serum biochemical analyses revealed no hepatic toxicity or electrolyte imbalance. Liver enzyme and electrolyte levels remained within physiological ranges across all groups. Notably, serum calcium and inorganic phosphorus levels remained similar across all groups (Table 1), suggesting that systemic mineral homeostasis was maintained despite the treatment.

Effects of esomeprazole and its combination with calcium carbonate or sodium bicarbonate on trabecular bone microarchitecture and BMD

Micro-CT analysis (Fig. 2) demonstrated that esomeprazole monotherapy significantly compromised trabecular bone microarchitecture. Figure 2A shows reconstructed images of rat femurs. The esomeprazole-only group

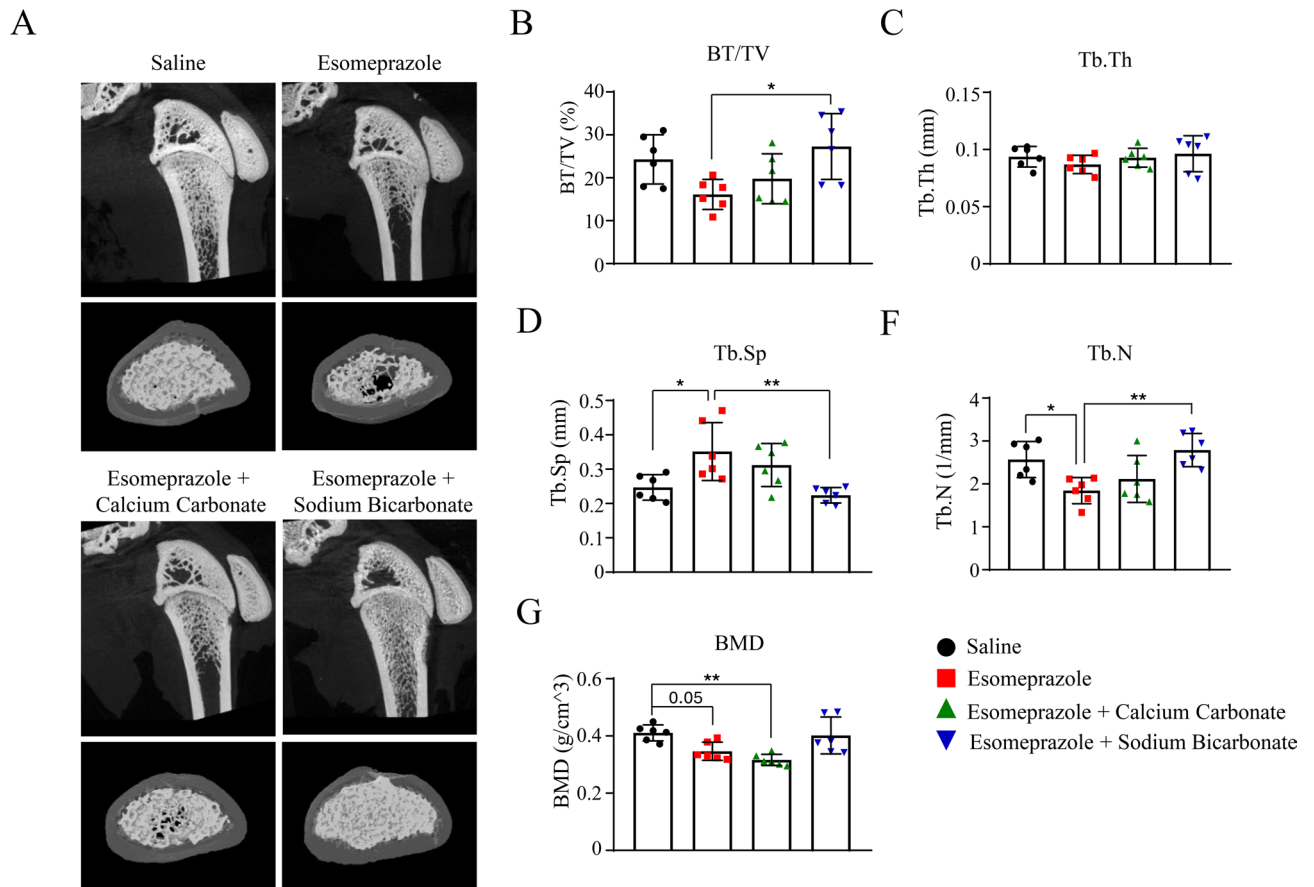


Fig. 2. Effects of Esomeprazole and its combination with calcium carbonate or sodium bicarbonate on trabecular bone microarchitecture and BMD. (A) Representative micro-CT images of the distal femur in Wistar rats administered with saline, esomeprazole alone or esomeprazole in combination with calcium carbonate or sodium bicarbonate. (B) Trabecular bone volume (BT/TV). (C) Trabecular Thickness (Tb.Th). (D) Trabecular separation (Tb.Sp). (E) Trabecular Number (Tb.N). (F) Bone mineral Density (BMD). Data are expressed as the mean $\pm$ SD. Statistical significance was determined by * $p < 0.05$, ** $p < 0.01$ based on the One-way ANOVA with Tukey's multiple comparisons test.

exhibited reduced trabecular number (Tb.N), along with increased trabecular separation (Tb.Sp) compared with the saline group, indicating a loss of connectivity. In contrast, the combination of esomeprazole with sodium bicarbonate preserved the trabecular structure, showing significantly lower Tb.Sp ($p < 0.01$) and higher Tb.N ($p < 0.01$) and BV/TV ($p < 0.05$) than esomeprazole alone (Fig. 2B, D, and F). BMD was significantly decreased in the esomeprazole-alone and esomeprazole-calcium carbonate groups compared to the controls ($p < 0.01$), whereas the sodium bicarbonate group showed a trend toward BMD preservation (Fig. 2G). Trabecular thickness (Tb.Th) did not differ significantly between groups (Fig. 2C).

Effects of esomeprazole and its combination with calcium carbonate or sodium bicarbonate on histological and immunohistochemical assessment

Figure 3 shows the histological features of the obtained rat femurs, including H&E, safranin O, and TRAP staining. H&E staining revealed trabecular bone and bone marrow structures. Safranin O staining confirmed intact cartilaginous structures in all treatment groups, indicating a preserved growth plate and articular cartilage integrity. Histological evaluation corroborated the micro-CT findings. TRAP staining revealed a marked increase in the number of osteoclasts in the esomeprazole monotherapy group ($p < 0.001$). Both calcium carbonate and sodium bicarbonate co-administration resulted in fewer TRAP-positive cells ($p < 0.01$ and $p < 0.001$; Fig. 3A and C). IHC analysis showed reduced ALP and osteocalcin expression levels in the esomeprazole alone group compared to their levels in the controls ($p < 0.001$). Conversely, both combination groups exhibited restored levels of these bone formation markers relative to those in the monotherapy group ($p < 0.05$ and $p < 0.01$; Fig. 3B, D and E).

Effects of esomeprazole and its combination with calcium carbonate or sodium bicarbonate on serum bone turnover markers

Esomeprazole treatment tended to increase the levels of bone resorption markers. However, serum CTX-1 levels were significantly lower in both the calcium carbonate and sodium bicarbonate combination groups than in the

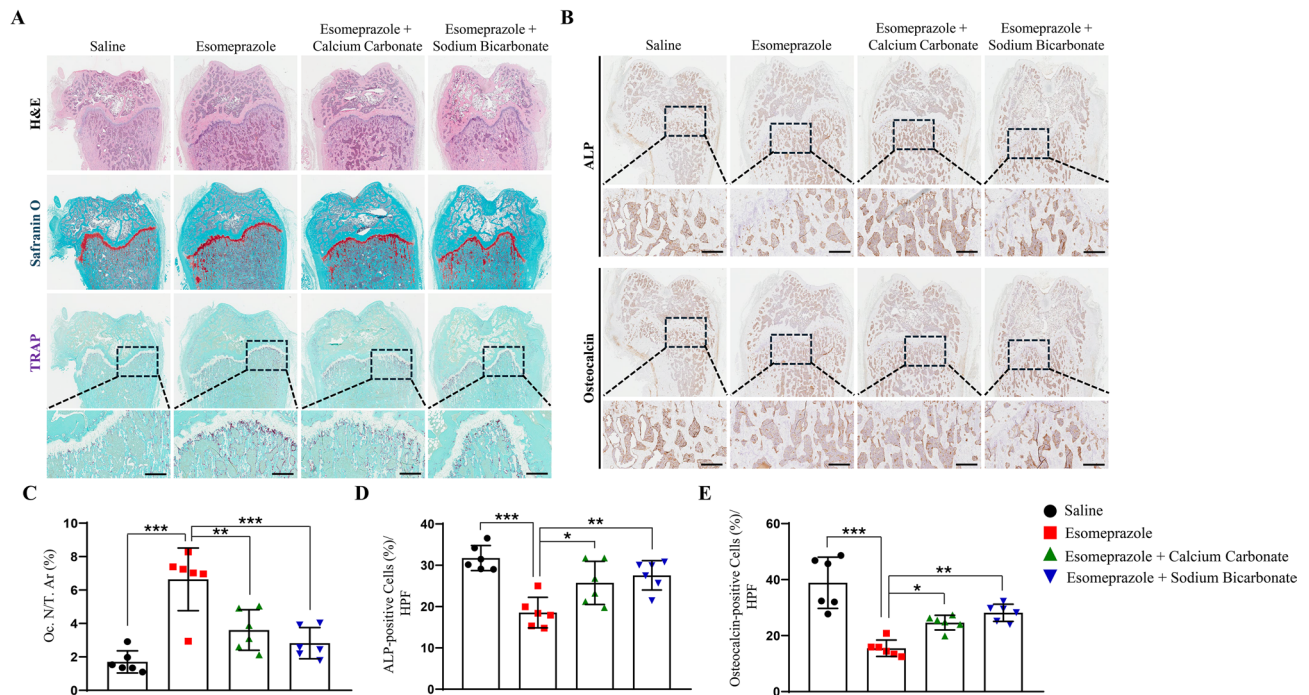


Fig. 3. Effects of Esomeprazole and its combination with calcium carbonate or sodium bicarbonate on Histological and Immunohistochemical Assessment. **(A)** Histological images of distal femur stained with H&E (upper), Safranin O (middle), and TRAP (lower). In the H&E staining, trabecular bone and bone marrow structures were observed. The Safranin O staining highlighted proteoglycans with intense red staining, a major component of cartilage. In the TRAP staining, osteoclasts were identified as characteristic purple cytoplasmic staining. **(B)** Immunohistochemical staining for ALP and osteocalcin in the distal femur of Wistar rats. The ALP- and osteocalcin-positive staining are visualized as brown deposits. **(C)** TRAP-positive cells were counted and quantified. **(D, E)** ALP- and osteocalcin-positive cells were counted and quantified. Scale bar 300 μ m. Data are expressed as the mean $\pm$ SD. Statistical significance was determined by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ based on the One-way ANOVA with Tukey's multiple comparisons test.

esomeprazole alone group ($p < 0.05$; Fig. 4A). BALP levels were significantly higher in the calcium carbonate group than in the esomeprazole alone group ($p < 0.01$; Fig. 4C). No significant differences were observed in PINP or TRACP-5b levels among the treatment groups (Fig. 4B, D).

Effects of esomeprazole and its combination with calcium carbonate or sodium bicarbonate on in vitro osteoclast differentiation and gene expression

Osteoclast precursor cells isolated from esomeprazole-treated rats exhibited enhanced differentiation potential upon stimulation with M-CSF and RANKL (Fig. 5A, B). RT-qPCR analysis of osteoclast-associated genes revealed that esomeprazole monotherapy significantly upregulated integrin subunit beta 3 (ITGB3; $p < 0.05$) and cathepsin K (CTK; $p < 0.01$) levels compared to their levels in controls (Fig. 5D, E). The expression levels of ITGB3, an essential component of the osteoclast vitronectin receptor required for bone resorption, and CTK, a marker of osteoclast proteolytic capacity, were significantly elevated in the esomeprazole monotherapy group compared to the controls. Importantly, osteoclasts from the sodium bicarbonate combination group showed significantly reduced expression levels of ITGB3 ($p < 0.05$) and CTK ($p < 0.01$) compared to those in the esomeprazole-alone group (Fig. 5D, E). The calcium carbonate group also displayed significant suppression of CTK ($p < 0.01$; Fig. 5E).

Discussion

The primary finding of this study was that long-term administration of esomeprazole in Wistar rats led to significant deterioration of trabecular bone microarchitecture, driven by increasing osteoclast formation and subsequent bone resorption. We demonstrated that the co-administration of sodium bicarbonate effectively attenuated these deleterious effects. In addition, co-administration of calcium carbonate effectively attenuated esomeprazole-induced increasing osteoclast formation and bone resorption, as well as increases in CTX-1, and decreases in ALP and osteocalcin expression. These results provide experimental evidence supporting the potential protective benefits of PPI-antacid combination formulations.

Long-term PPI use has been associated with a reduction in BMD and an increased risk of osteoporotic fractures²⁵. A previous study showed that PPI intake for more than 1 year was associated with an increased risk of fractures, particularly of the hip and femur²⁶. Although the precise mechanisms through which PPIs compromise bone metabolism remain unclear, several hypotheses have been proposed. One proposed mechanism involves

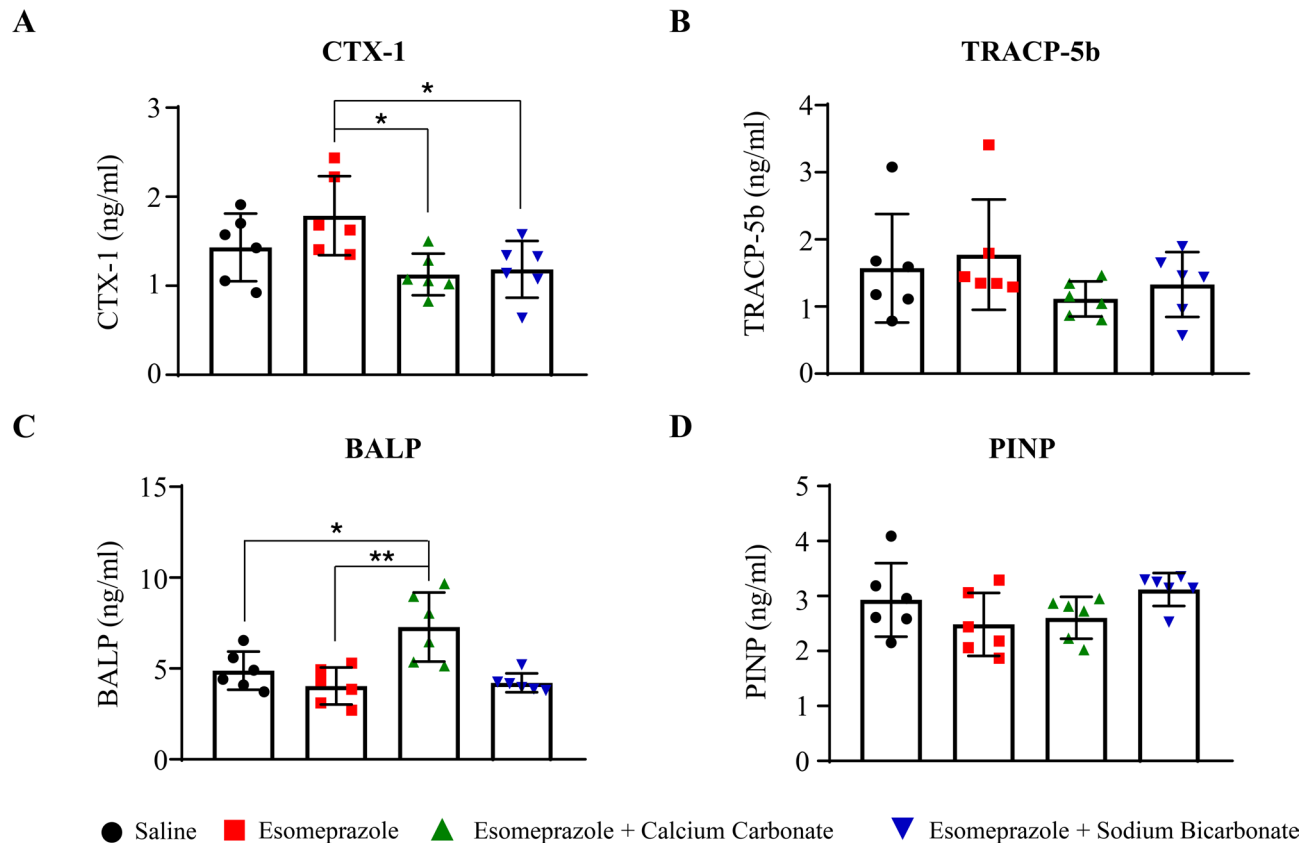


Fig. 4. Effects of Esomeprazole and its combination with calcium carbonate or sodium bicarbonate on Serum Bone Turnover Markers. (A–D) Serum levels of CTX-1, TRACP-5b, BALP, PINP measured by ELISA ($n = 6$ /group). Data are expressed as the mean $\pm$ SD. Statistical significance was determined by $*p < 0.05$, $**p < 0.01$ based on the One-way ANOVA with Tukey's multiple comparisons test.

the excess secretion of gastrin. PPIs suppress gastric acid production, leading to a compensatory increase in gastrin secretion. Elevated gastrin levels can stimulate the release of parathyroid hormone, which, in turn, stimulates osteoclasts and facilitates bone resorption²⁷. Second, PPIs reduce gastric hydrochloric acid secretion, which increases the gastric pH and may interfere with the absorption of calcium and magnesium in the small intestine^{28,29}.

Our study demonstrated that serum calcium concentrations were comparable across all groups. The maintenance of normal serum calcium levels likely resulted from compensatory bone resorption, a finding supported by the significant decreases in Tb.N in the micro-CT results of the esomeprazole-alone group (Fig. 2F). In addition to the calcium hypothesis, our *in vitro* data suggested that PPIs may induce changes in osteoclast precursors. We observed that cells from esomeprazole-treated rats exhibited elevated expression levels of CTK and ITGB3, which are critical mediators of bone resorption and osteoclast adhesion, respectively (Fig. 5D, E). This indicates that PPIs may enhance bone resorption through the direct modulation of osteoclast differentiation pathways.

The protective effect of calcium carbonate is consistent with its established role in reducing fracture risk and maintaining BMD³⁰. We observed a significant suppression of serum CTX-1 and osteoclastogenic genes (CTK) in the calcium carbonate group (Figs. 4A and 5E). Although not statistically significant, there was a downward trend in TRAP and ITGB3 expression levels (Fig. 5C, D). However, a key finding of this study was the comparable protective role of sodium bicarbonate. The sodium bicarbonate group exhibited the best preservation of the trabecular microarchitecture and significant downregulation of both ITGB3 and CTK (Fig. 5D, E). This suggests a mechanism distinct from that of calcium supplementation and acid–base buffering. Bone minerals act as buffers for systemic acidosis; as protons may stimulate osteoclastic bone resorption to release alkaline minerals that help maintain pH balance. Conversely, alkali administration has been reported to reduce bone resorption. Sodium bicarbonate provides a base that can neutralize systemic acid loads and may reduce stimuli for bone resorption. Furthermore, bicarbonate has been shown to inhibit osteoclast formation and function via a soluble adenylyl cyclase–dependent pathway³¹. However, the systemic pH-buffering capacity of orally administered sodium bicarbonate *in vivo* is generally considered to be limited. Therefore, our findings suggest that the alkaline component of PPI formulations may be associated with potential bone-protective effects, possibly through partial mitigation of low-grade metabolic acidosis or via effects on cellular signaling pathway.

This study has several limitations. First, we did not conduct experiments in elderly populations, in whom osteoporosis is known to occur frequently. In elderly, the use of PPI containing sodium bicarbonate may present

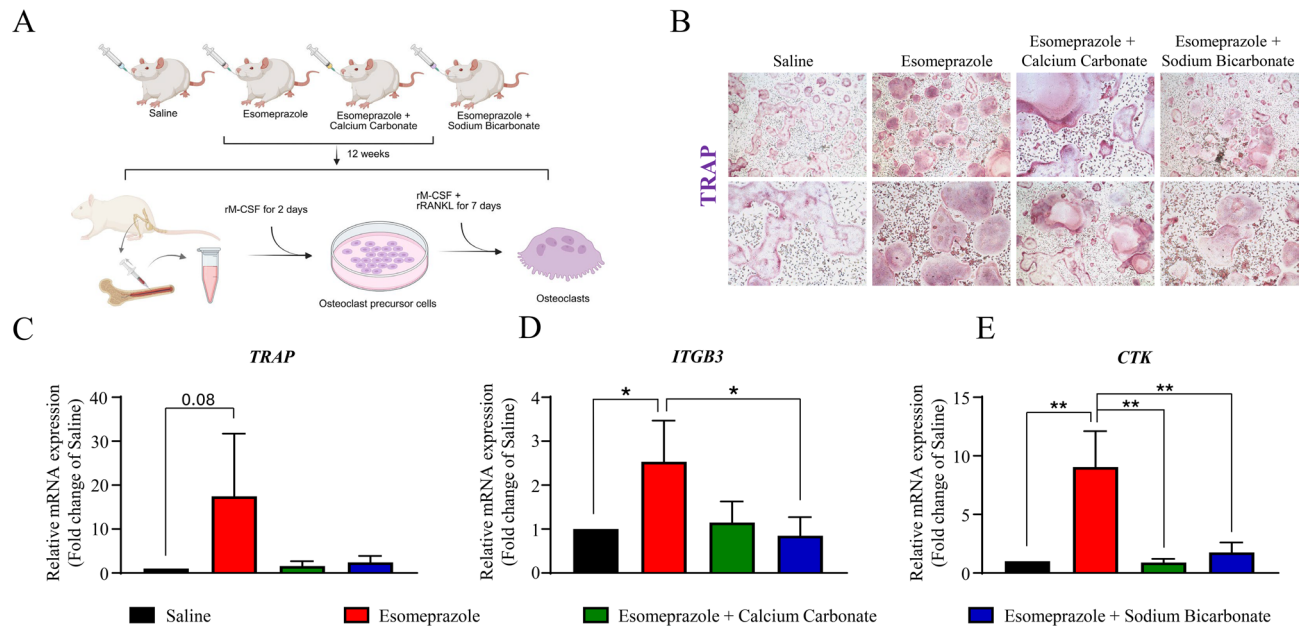


Fig. 5. Effects of Esomeprazole and its combination with calcium carbonate or sodium bicarbonate on In Vitro Osteoclast Differentiation and Gene Expression. (A) Schematic illustrating showing the isolation of bone marrow from femur of Wistar rats and its differentiation into mature osteoclast. *Created with BioRender.com.* (B) Mature osteoclasts stained with TRAP on day7. (C) RT-qPCR analysis of osteoclast markers, including TRAP, ITGB3, and CTX (normalized to expression of GAPDH; $n = 3/\text{group}$). Data are presented as the mean $\pm$ SD. Statistical significance was determined by $*p < 0.05$, $**p < 0.01$ based on the One-way ANOVA with Tukey's multiple comparisons test.

a potential risk of non-skeletal adverse effects (hypertension, arrhythmia, and hypokalemia) due to sodium overload. Further studies are needed to evaluate the use of PPIs containing sodium bicarbonate in elderly populations. Second, the 12-week duration, which was sufficient to observe bone changes in rats, may not fully replicate lifelong human exposure. Third, we did not assess serum gastrin or parathyroid hormone levels. Chronic acid suppression induces secondary hypergastrinemia, which stimulates the parathyroid glands and drives bone resorption via the gastrin-PTH axis³². The lack of such data restricts our understanding of the exact contribution of this hormonal mechanism. Finally, we did not perform pharmacokinetic analysis, such as assessing the time profile of plasma esomeprazole concentrations. Further pharmacokinetic studies are warranted.

Despite these limitations, this study provides important experimental evidence of PPI-induced bone loss and identifies potential protective strategies. Our multimodal approach, which incorporated micro-CT imaging, serum bone turnover markers, histological analysis, and in vitro osteoclastogenesis assays, provides supporting evidence that PPI-induced bone loss is primarily mediated by enhanced osteoclastic activity. Our findings demonstrate that the combination of esomeprazole with sodium bicarbonate provides protective effects on bone that are comparable to or even greater than those of calcium carbonate. This novel finding suggests that both calcium supplementation and systemic acidity buffering are essential for preserving bone health during long-term PPI therapy.

Finally, the clinical implications of our findings for long-term PPI use are substantial. Although chronic PPI use increases the risk of osteoporosis, effective preventive strategies remain undefined. Our results suggest that future interventional trials are warranted to evaluate whether combined PPI–calcium carbonate or sodium bicarbonate formulations can reduce fracture incidence in high-risk populations, such as older adults and those with preexisting osteopenia.

Conclusion

Chronic esomeprazole administration compromised trabecular microarchitecture in rats by increasing osteoclastic resorption. The co-administration of sodium bicarbonate or calcium carbonate significantly attenuated this loss. Specifically, sodium bicarbonate protected the trabecular microarchitecture, likely through mechanisms involving acid-base buffering and downregulation of osteoclastogenic genes. These findings suggest that optimizing PPI formulations with alkaline salts is a promising strategy for improving their long-term safety profiles. Future research should expand on these results by including longer treatment durations, both sexes, and postmenopausal models to comprehensively evaluate their clinical applicability.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

Dae Hyun Lim: Writing the original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. Seung Hoon Lee: Writing the original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. Dongju Kim: Methodology, Investigation. Chanhyeok Jeon: Methodology, Investigation. Da Bhin Kim: Methodology, Investigation. Hye-Ryeon Jo: Validation, Methodology, Investigation.

Jin Ah Shin: Methodology, Investigation. Sungsin Jo: Writing the original draft, Validation, Supervision, Project administration, Methodology, Data curation. Hang Lak Lee: Writing – review and editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. Tae-Hwan Kim: Writing-review and editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declarations

Ethics approval and consent to participate

All animal experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Screening Committee of Hanyang University (2025–0089 A).

Competing interests

Dae Hyun Lim, Seung Hoon Lee, Dongju Kim, Chanhyeok Jeon, Da Bhin Kim, Hye-Ryeong Jo, Jin Ah Shin, Sungsin Jo, and Tae-Hwan Kim declare they have no financial interests. Hang Lak Lee has received research support from Yuhan Corporation.

Additional information

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