Biomedical Research 2025; 36 (4): 1-8

ISSN 0970-938X
www.biomedres.info

Inhibitory effects of magnesium hydroxide nanoparticles on osteoclast differentiation *via* suppression of RANKL-induced MAPK signaling.

Soichiro Hikita[1,2], Ayaka Koga[2,3], Yoshie Nagai-Yoshioka[2], Ryota Yamasaki[2], Daisuke Kudo[4], Dao Nguyen Duy Phuong[4], Yoshihito Iwamoto[4], Osamu Takahashi[1], Masaaki Sasaguri[1], Manabu Habu[1], Wataru Ariyoshi[2*]

[1]Division of Oral and Maxillofacial Surgery, Department of Oral and Maxillofacial Disease Control, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan

[2]Division of Infections and Molecular Biology, Department of Advanced Pathophysiological Science, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan

[3]Department of Health Sciences, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan

[4]R&D Center, SETOLAS Holdings Inc., 4285 Hayashida-cho, Sakaide, Kagawa 762-0012, Japan

Abstract

Objectives: Magnesium hydroxide has been reported to promote bone formation; however, its specific effects on osteoclast differentiation remain unclear. This study aimed to investigate the inhibitory effects of magnesium hydroxide nanoparticles (NM80) on osteoclast formation and elucidate the underlying molecular mechanisms.

Methods: Osteoclast differentiation was induced in RAW264.7 cells and mouse bone marrow cells using Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL). The effects of NM80 on Tartrate-Resistant Acid Phosphatase (TRAP)-positive multinucleated cell formation, osteoclast-related gene expression, and MAPK signaling pathway activation were evaluated using TRAP staining, quantitative RT-PCR, and Western blot analysis, respectively.

Results: NM80 significantly reduced RANKL-induced osteoclast formation in both RAW264.7 and bone marrow cells without cytotoxic effects. The mRNA expression of osteoclast-related markers, including cathepsin K, Acp5, Dc-stamp, and Nfatc1, was downregulated. Furthermore, NM80 inhibited the phosphorylation of p38 MAPK and ERK but did not affect JNK activation or IκBα degradation, indicating selective suppression of the MAPK pathway.

Conclusion: Magnesium hydroxide nanoparticles suppress osteoclast differentiation *via* inhibition of the RANKL-induced MAPK signaling pathway. These findings suggest NM80 as a potential therapeutic agent for bone metabolic diseases.

Keywords: Magnesium hydroxide, Osteoclast, Differentiation

Accepted on December 10, 2025

Introduction

Bones are hard tissues that are rich in calcium phosphate and collagen; they play crucial roles in maintaining the skeletal structure, protecting internal organs, hematopoiesis, and serving as reservoirs for calcium and minerals. Similar to other organs, bones maintain homeostasis *via* metabolism, and old bones are constantly replaced by new bones. In this remodeling process, osteoblast-mediated bone formation and osteoclast-driven bone resorption are tightly regulated [1]. However, an imbalance in bone remodeling and predominance of bone resorption leads to development of metabolic bone diseases such as osteoporosis along with inflammatory bone destruction including Rheumatoid Arthritis (RA) and periodontitis.

Osteoclasts are large multinucleated cells that originate from the fusion of precursors belonging to the monocyte/macrophage lineage under the influence of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) [2]. In osteoporosis, bone resorption increases throughout the body, particularly in the spine, femoral neck, lumbar vertebrae, and wrists. This progressive bone loss leads to a decrease in bone density and increase in fracture risk [3].

RA is an inflammatory disease that causes hyperplastic synovitis and destruction of bone and cartilage caused by activated osteoclasts. This is mediated *via* the action of inflammatory cytokines produced in the joint and synovial tissues [4,5]. Therefore, targeting osteoclasts has been recognized as a crucial therapeutic approach for managing these bone-related diseases. Osteoclast precursors interact with RANKL, which is mainly expressed on the plasma membrane of osteoblasts, through Receptor Activator of Nuclear Factor Kappa-B (RANK), triggering the activation of intracellular signaling cascades, such as the Mitogen-Activated Protein Kinase (MAPK) and NF-κB pathways. This signaling cascade leads to the upregulation of Nuclear Factor of Activated T-cells, cytoplasmic 1 (NFATc1), a key transcription factor that governs osteoclast differentiation [6,7]. Furthermore, inflammatory cytokines such as Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-1β, and IL-6 have been shown to enhance osteoclast differentiation and activation, ultimately contributing to bone resorption [8,9].

Bisphosphonates and the human RANKL monoclonal antibody denosumab are currently used as therapies for bone diseases including osteoporosis and RA by controlling osteoclastogenesis [7]. However, since Marx reported the occurrence of Medication-Related Osteonecrosis of the Jaw (MRONJ) in patients with malignant tumors and osteoporosis under bisphosphonate treatment in 2003 [10], it has been established that MRONJ can also occur with the use of bisphosphonates and human RANKL monoclonal antibodies [11]. Therefore, there is a need for alternative drug therapies with fewer side effects. Magnesium hydroxide is widely used in pharmaceuticals such as antacids [12] and laxatives [13] owing to its low cost, low toxicity, and environmental friendliness. We previously showed that magnesium hydroxide nanoparticles prepared by a continuous reaction system of aqueous magnesium chloride and sodium hydroxide solutions can suppress the inflammatory response triggered by periodontopathic bacteria [14] and eliminate both logarithmic-growing and persistent *Escherichia coli via* physical damage [15]. However, the specific roles of magnesium hydroxide nanoparticles in osteoclast differentiation and function remain unknown. Therefore, we aimed to elucidate the effects of magnesium hydroxide nanoparticles on osteoclast differentiation and their molecular mechanisms.

Materials and Methods

Reagents and antibodies

Recombinant human soluble RANKL was obtained from ORIENTAL YEAST (Tokyo, Japan). The magnesium hydroxide nanoparticle slurry NM80 was provided by SETOLAS Holdings Inc. (Kagawa, Japan). Anti-phosphorylated (phospho)-p38 MAPK rabbit monoclonal (D3F9), anti-p38 MAPK rabbit polyclonal, anti-phospho c-Jun N-terminal Kinase (JNK) rabbit monoclonal (81E11), anti-JNK rabbit polyclonal, anti-phospho-extracellular signal-regulated kinase 1/2 (ERK1/2) rabbit monoclonal (D13.14.4E), anti-ERK1/2 (rabbit) monoclonal (137F5), and anti-inhibitor of NFκB (IκBα) rabbit polyclonal antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-β-actin (mouse) monoclonal antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

The mouse monocyte/macrophage-derived cell lineage RAW264.7 was purchased from RIKEN Cell Bank (Ibaraki, Japan) and cultured in Alpha-Minimum Essential Medium (αMEM, FUJIFILM Wako Pure Chemical, Osaka, Japan) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS, Sigma-Aldrich) and 1% penicillin-streptomycin (FUJIFILM Wako pure chemical) at 37°C in a 5% CO_2 atmosphere.

WST-8 assay

RAW264.7 cells (4×10^4 cells/well) were cultured in a 96-well plate in the presence of NM80 (0 to 1 mg/ml) for 48 h at 37°C in a 5% CO_2 atmosphere. Cell viability was assessed using the Cell Counting Kit-8 (Dojindo Molecular Technologies, Rockville, MD, USA). Absorbance was measured at 450 nm using a microplate reader (Multiskan FC; Thermo Fisher Scientific, Rockford, IL, USA). The absorbance of the culture medium incubated with NM80 at different concentrations in the absence of cells for 48 h was subtracted from the absorbance to serve as an indicator of the number of viable cells.

TRAP staining

RAW264.7 cells (4×10^3 cells/well) were cultured in a 96-well plate in the presence of 100 ng/mL RANKL and NM80 (25, 50, and 100 μg/ml) for 5 d at 37°C in a 5% CO_2 atmosphere. The culture medium was changed every 2 d. Cultured cells were fixed for 2 min with a fixative solution prepared using acetone (FUJIFILM Wako Pure Chemical), formaldehyde (FUJIFILM Wako Pure Chemical), and citrate (Sigma-Aldrich). Tartrate-Resistant Acid Phosphatase (TRAP) staining was performed using a TRAP kit (Sigma-Aldrich) according to the manufacturer's protocol to detect osteoclast differentiation. Multinucleated cells with three or more nuclei were counted as osteoclast-like cells under bright-field microscopy using an OLYMPUS IX71 microscope (OLYMPUS, Tokyo, Japan).

RNA extraction and real-time quantitative RT- PCR

RAW264.7 cells (5×10^5 cells/well) were cultured in a 96-well plate and incubated with NM80 100 ng/ml RANKL for 48 h at 37°C in a 5% CO_2 atmosphere. Total RNA extraction, Reverse Transcription (RT) reactions and analysis for Polymerase Chain Reaction (PCR) were performed according to the previous

reported protocol [14]. The relative mRNA expression levels were calculated using the $2^{-\Delta Ct}$ method with Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) as the control. The primers used in this study were as follows: Gapdh, 5′-GACGGCCGCATCTTCTTGA-3′ (forward) and 5′-CACACACCGACCTTCACCATTTT-3′ (reverse); Nfatc1, 5′-ACCACCTTTCCGCAACCA-3′ (forward) and 5′-GGTACTGGCTTCTCTTCCGTTTC-3′ (reverse); tartrate-resistant acid phosphatase (Acp5), 5′-GCAGTATCTTCAGGACGAGAAC-3′ (forward) and 5′-TCCATAGTGAAACCGCAAGTAG-3′ (reverse); dendritic cell-specific transmembrane protein (Dc-stamp), 5′-CCGCAGCCTGACATTTGAG-3′ (forward) and 5′-CGGCCAGAATGAAAGCA-3′ (reverse); and Cathepsin K (Ctsk), 5′-TATGACCACTGCCTTCCAATAC-3′ (forward) and 5′-GCCGTGGCGTTATACATACA-3′ (reverse).

Animal experiments

Eight-week-old male ddY mice (Japan SLC, Shizuoka, Japan) were used in the experiments. Bone marrow cells were flushed from the femurs and tibias using αMEM (FUJIFILM Wako Pure Chemical) containing 1% penicillin-streptomycin (FUJIFILM Wako Pure Chemical). The isolated bone marrow cells were cultured in αMEM supplemented with 10% FBS and recombinant human macrophage Colony-Stimulating Factor (rhM-CSF) (REPROTEC, Gunma, Japan) at 37°C in a 5% CO_2 atmosphere. After 72 h, the cells were harvested and used as osteoclast precursor cells. Osteoclast precursor cells were seeded at a density of 4×10^5 cells/ml in 96-well plates and cultured with rhM-CSF, RANKL (ORIENTAL YEAST), and NM80 (100 or 250 μg/ml) at 37°C in a 5% CO_2 atmosphere for 72 h. TRAP staining was performed as described above and the stained cells were observed under an OLYMPUS IX71 microscope. The experiments performed in this study were approved by the Kyushu Dental University Experimental Animal Care and Use Committee (approval number: 24-016).

Western blotting

RAW264.7 cells (6×10^5 cells/well) were cultured in 6-well plates for 24 h at 37°C in a 5% CO_2 atmosphere. The cells were stimulated with 100 ng/ml RANKL and 100 μg/ml NM80 for 15 and 30 min. Preparation of cell lysates, measurement of total protein concentration, separation of equal amounts of protein *via* sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 12.5% polyacrylamide gel (SuperSep TM Ace; Fujifilm Wako Pure Chemical) and transfer to a polyvinylidene difluoride membrane (Pall Life Sciences, Port Washington, DC, USA) were performed according to the previous reported protocol [14]. Band detection was also performed according to a previously reported protocol [16] by using primary antibodies against IκBα, phospho-JNK, JNK, phospho-p38 MAPK, p38 MAPK, phospho-ERK, ERK, and β-actin.

Statistical analysis

Data were analyzed using Excel (Microsoft, Redmond, WA, USA) and EZR software [17]. Statistical differences were determined using one-way analysis of variance followed by Tukey's or Dunnett's post-hoc test. A p-value of <0.05 was considered significant.

Results and Discussion

NM80 suppressed osteoclast differentiation induced by RANKL

Extracellular magnesium ions in the concentration range of 1.46-6.08 mM have been reported to modulate the differentiation and function of osteoclasts [18]. Additionally, magnesium hydroxide is widely used in medical applications and can promote bone formation when adapted for use in scaffolds [19]. However, studies on its specific role in osteoclast differentiation and function remain limited. In this study, we evaluated the bioactivity of magnesium hydroxide nanoparticles in RANKL-induced osteoclast differentiation using the mouse osteoclast precursor cell line RAW264.7 and mouse bone marrow cells.

First, the cytotoxicity of NM80 was assessed. A significant decrease in optical density values was observed after treatment with 1,000 μg/ml NM80 (Figure 1A). Next, we evaluated the effect of NM80 on osteoclast differentiation and found that NM80 suppressed the formation of RANKL-induced TRAP-positive multinucleated cells. The inhibitory effect on osteoclast formation by NM80 was dose-dependent, and a significant reduction was observed at treatment with 100 μg/ml (Figure 1B-1C).

In addition to RAW264.7 cells, we examined the impact of NM80 on osteoclast differentiation in bone marrow cells using TRAP staining. A notable reduction in the number of TRAP-positive multinucleated cells was observed by the addition of 100 μg/ml NM80, compared to the RANKL-stimulated control cells. This inhibitory effect of NM80 was dose-dependent, with maximum inhibition observed at treatment with 200 μg/ml (Figure 2).

NM80 negatively regulated osteoclast differentiation in both RAW264.7 cells and mouse bone marrow cells. NM80 was previously synthesized *via* a sequential reaction of magnesium chloride and sodium hydroxide (at 20°C and 500 rpm), with an average particle size of approximately 81.0 nm [15]. Magnesium hydroxide nanoparticles with an average particle size of less than 10 nm, which were synthesized using rosehip extract, have been shown to suppress TRAP activity and osteoclast differentiation markers expression in THP-1 cells in the presence of RANKL and M-CSF. In contrast, magnesium hydroxide nanoparticles (average particle size of 90 nm) synthesized using pure water exhibit only minimal modulation of osteoclast differentiation [20]. In the present study, magnesium hydroxide nanoparticles synthesized using magnesium nitrate and potassium hydroxide were used,

suggesting that their bioactivity may depend on particle size and synthesis method.

NM80 suppressed the osteoclast differentiation-related genes expression

To further demonstrate the effect of NM80 on osteoclast differentiation, we examined the mRNA expression levels of osteoclast differentiation-related markers (Nfatc1, Dc-stamp, Ctsk, and Acp5) were evaluated *via* RT-qPCR. The RANKL-increased mRNA expression of these markers was significantly suppressed after administration of NM80 (Figure 3).

NM80 inhibited the activation of p38 MAPK and ERK-mediated signaling

We performed western blotting to determine whether NM80 affected the MAPK and NF-κB pathways. RANKL stimulation induced the phosphorylation of p38 MAPK, ERK, and JNK and the degradation of IκBα protein. NM80 administration inhibited the RANKL-induced p38 MAPK and ERK phosphorylation (Figure 4A). In contrast, no inhibitory effect of NM80 was observed on JNK phosphorylation (Figure 4A) or IκBα degradation (Figure 4B).

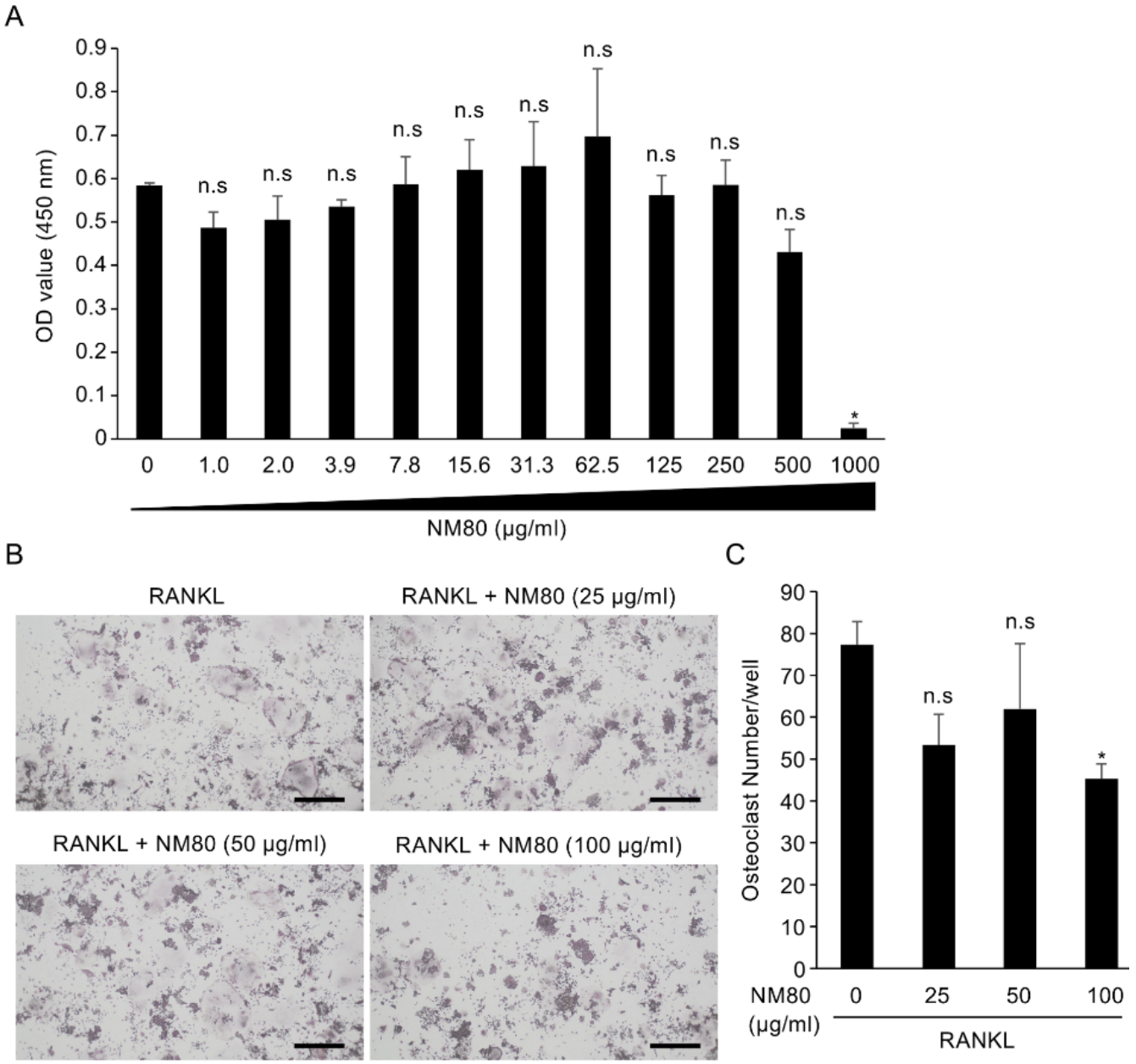

Figure 1. *Effect of NM80 on RAW264.7 cells, (A): Cytotoxicity of NM80 was determined using the WST-8 assay; (B): Osteoclasts were detected using TRAP staining. Scale bar=500 μm; (C): TRAP-positive cells with three or more nuclei were counted; Note: Data are presented as the mean ± standard deviation from at least three independent experiments (*P<0.05). n.s indicates not significant compared with (A) non-treated or (B) RANKL-treated cells. RANKL: Receptor Activator of Nuclear Factor kappa-B Ligand; TRAP: Tartrate-Resistant Acid Phosphatase.*

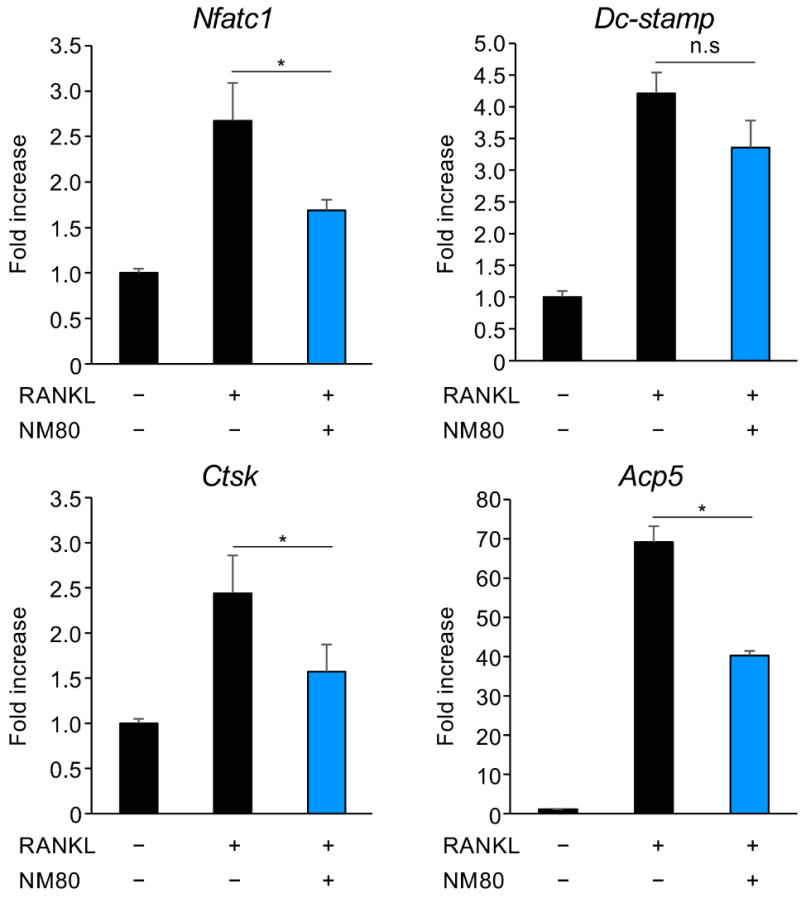

Figure 2. *Effect of NM80 on bone marrow cells, (A): Osteoclasts were detected using TRAP staining. Scale bar = 500 μm; (B): TRAP-positive cells with three or more nuclei were counted. Data are presented as the mean ± standard deviation from at least three independent experiments. *P<0.05, **P<0.005 compared with RANKL-treated cells. RANKL: Receptor Activator of Nuclear Factor kappa-B Ligand; TRAP: Tartrate-Resistant Acid Phosphatase; M-CSF: Macrophage Colony-Stimulating Factor.*

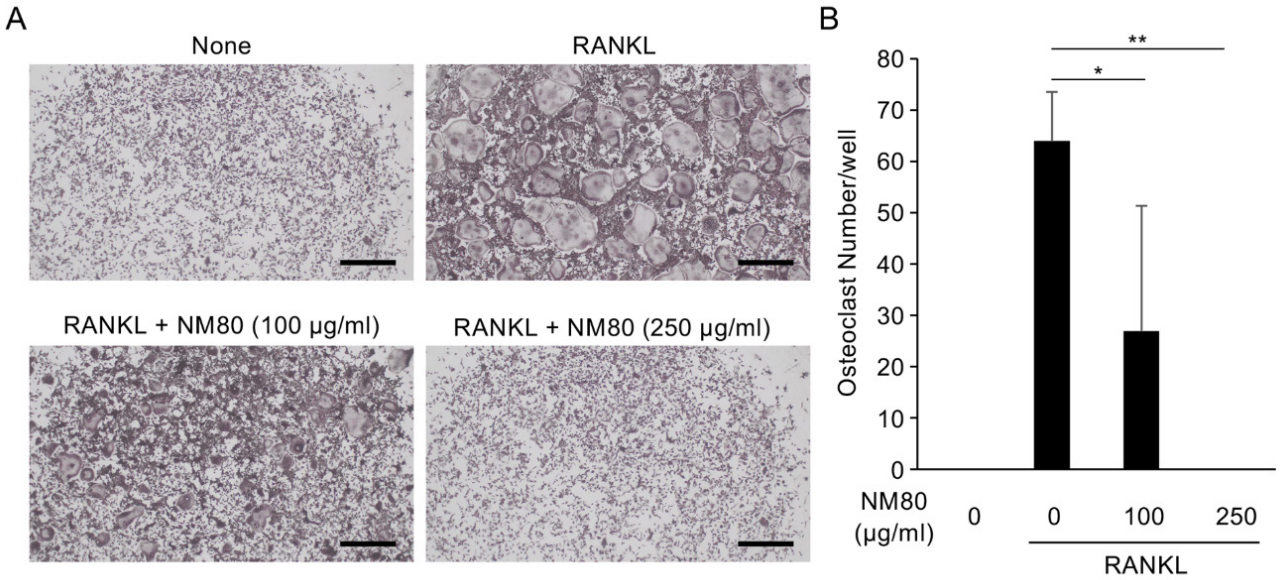

Figure 3. *Effect of NM80 on RANKL-induced expression of osteoclastogenic genes in RAW264.7 cells. Real-time quantitative RT-PCR was performed to measure the expression levels of osteoclast-related genes. Data represent the mean ± standard deviation from at least three independent experiments (*P<0.05). n.s indicates not significant compared with RANKL-treated cells. RANKL: Receptor Activator of Nuclear Factor kappa-B Ligand; Nfatc1: Nuclear factor of activated T-cells, cytoplasmic 1; Dc-stamp: Dendritic cell-specific transmembrane protein; Ctsk: cathepsin K; Acp5: tartrate-resistant acid ATPase.*

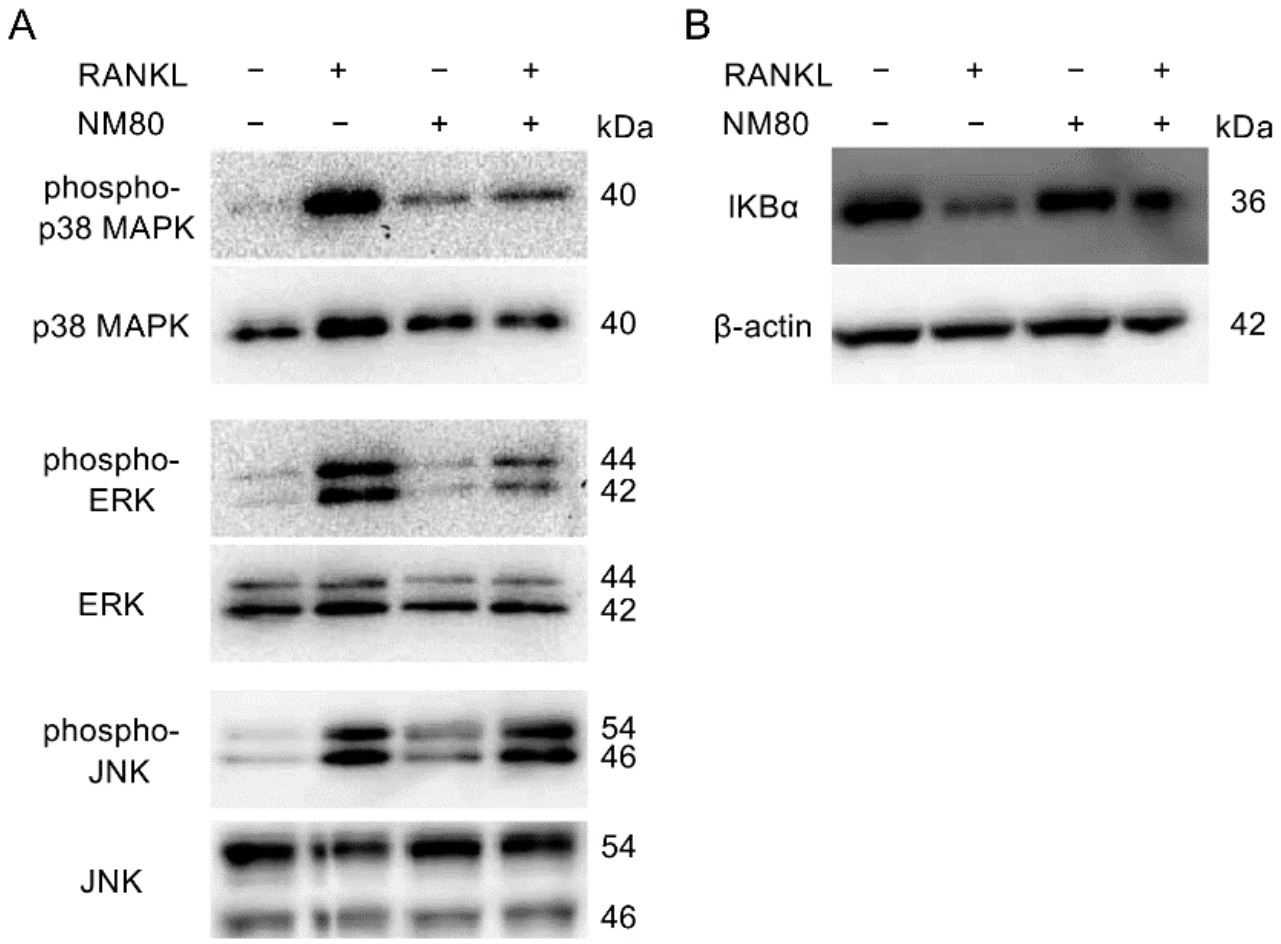

Figure 4. *Effects of NM80 on the MAPK and NF-κB pathways. The protein levels of phospho-p38 MAPK, p38 MAPK, phospho-ERK, ERK, phospho-JNK, JNK (A) and IκBα (B) in cultured RAW264.7 cells were detected using western blot analysis. MAPK: Mitogen-Activated Protein Kinase; phospho-, phosphorylated; NF-κB: Nuclear Factor kappa B; ERK: Extracellular signal-Regulated Kinase; JNK: c-Jun N-terminal kinase; IκBα.*

Investigation of the intracellular signaling pathways associated with RANKL-activated osteoclast differentiation revealed that NM80 negatively regulated osteoclast differentiation by suppressing the MAPK signaling pathway mediated by p38 MAPK and ERK, without affecting the NF-κB pathways. In contrast, NM80 did not affect the activation levels of JNK, another MAPK component. We previously found that NM80 negatively regulates the IL-1β production stimulated by periodontal pathogenic bacterium-derived lipopolysaccharide in J774.1 macrophage-like cells by inhibiting NF-κB activation and the phosphorylation of JNK, ERK1/2, and p38 MAPK [14]. These results suggest that the intracellular signaling targets regulated by NM80 may depend on cell type, culture conditions, and stimulatory factors. The activation of p38 MAPK, ERK, and JNK involves their upstream molecules MKK3/6, MEK1/2, and MKK4/7, respectively [21]. To elucidate the selective inhibitory mechanisms of NM80 on p38 MAPK and ERK activity observed in RAW264.7 cells, the interactions of NM80 with these upstream molecules need to be demonstrated.

The concentration of extracellular magnesium ions can affect the differentiation and activation of osteoclasts [18]. Therefore, NM80 may potentially modulate osteoclast differentiation around precursor cells. However, the amount of magnesium ions released in a 100 μg/ml aqueous solution of NM80 is relatively low (approximately 0.3 mmol/L). Lipid nanoparticles with a particle size of less than 200 nm [22] and iron oxide nanoparticles with a particle size of less than 30 nm [23] are internalized into RAW264.7 cells. Therefore, it is also plausible that NM80 exerts its bioactivity intracellularly. Ongoing studies are currently investigating the uptake and intracellular trafficking of NM80 in RAW264.7 cells.

This study demonstrates for the first time that newly synthesized magnesium hydroxide nanoparticles with a particle size of 80 nm have the ability to target the MAPK signaling pathway and inhibit osteoclast differentiation. A limitation of this study is that the evidence for bioactivity of NM80 to suppress osteoclast differentiation and its molecular mechanisms is confined to the cellular level. For enabling future clinical applications, the functionality and potential side effects of NM80 should be evaluated in vivo along with experiments using osteoporotic animal models, such as ovariectomized mice. Nevertheless, the findings of this study highlight the potential for applying magnesium

hydroxide nanoparticles as a novel and promising therapeutic approach for bone metabolic diseases.

Conclusion

This study demonstrates that magnesium hydroxide nanoparticles suppress osteoclast differentiation in vitro without apparent cytotoxicity. These findings provide basic evidence that may contribute to understanding the potential role of magnesium-based nanomaterials in bone biology.

Acknowledgement

We would like to thank Editage for English language editing.

Funding

This work was partially supported by JSPS KAKENHI (grant numbers 21H03145 and 23K21495).

Author's Contribution

Soichiro Hikita: Writing-original draft, Writing-review and editing, Visualization, Investigation, Data curation, Funding acquisition, Ayaka Koga: Visualization, Investigation, Data curation, Yoshie Nagai-Yoshioka: Visualization, Investigation, Data curation, Daisuke Kudo: Methodology, Resources, Dao Nguyen Duy Phuong: Methodology, Resources, Yoshihito Iwamoto: Methodology, Resources, Ryota Yamasaki: Visualization, Methodology, Osamu Takahashi: Supervision, Masaaki Sasaguri: Supervision, Manabu Habu: Supervision, Wataru Ariyoshi: Writing-original draft, Writing-review and editing, Visualization, Funding acquisition, Methodology, Project administration, supervision.

Declaration of Competing Interest

D.K., D.N.D.P., and Y.I. are employees of SETOLAS Holdings Inc. and were involved in the development of the magnesium hydroxide nanoparticles used in this study. SETOLAS Holdings Inc. had no role in the preparation of the manuscript, or in the decision to publish the results.

References

1. Feng X, McDonald JM. Disorders of bone remodeling. Annu Rev Pathol 2011; 6: 121-145.
2. Roodman GD. Osteoclast differentiation. Crit Rev Oral Biol Med 1991;2(3):389-409.
3. Li HL, Shen Y, Tan LH, Fu SB, Guan CH, Zhen DH, Lv HH, Wu XY, Tang XL. Association between BMI and osteoporotic fractures at different sites in Chinese women: A case-control retrospective study in Changsha. BMC Musculoskelet Disord 2024; 25: 187.
4. Radu AF, Bungau SG. Management of rheumatoid arthritis: An overview. Cells 2021; 10: 2857.
5. Zheng H, Liu Y, Deng Y, Li Y, Liu S, Yang Y, Qiu Y, Li B, Sheng W, Liu J, Peng C. Recent advances of NFATc1 in rheumatoid arthritis-related bone destruction: Mechanisms and potential therapeutic targets. Mol Med 2024; 30: 20.
6. Zeng XZ, He LG, Wang S, Wang K, Zhang YY, Tao L, Li XJ, Liu SW. Aconine inhibits RANKL-induced osteoclast differentiation in RAW264. 7 cells by suppressing NF-κB and NFATc1 activation and DC-STAMP expression. Acta Pharmacol Sin 2016; 37: 255-263.
7. Yao Z, Getting SJ, Locke IC. Regulation of TNF-induced osteoclast differentiation. Cells 2021; 11: 132.
8. Zhao B. Intrinsic restriction of TNF-mediated inflammatory osteoclastogenesis and bone resorption. Front Endocrinol 2020; 11: 583561.
9. Nishida M, Saegusa J, Tanaka S, Morinobu A. S100A12 facilitates osteoclast differentiation from human monocytes. PloS one 2018; 13: e0204140.
10. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg 2003; 61: 1115-1117.
11. AlRowis R, Aldawood A, AlOtaibi M, Alnasser E, AlSaif I, Aljaber A, Natto Z. Medication-Related Osteonecrosis of the Jaw (MRONJ): A review of pathophysiology, risk factors, preventive measures and treatment strategies. The Saudi Dent J 2022; 34: 202-210.
12. Decktor DL, Robinson M, Maton PN, Lanza FL, Gottlieb S. Effects of aluminum/magnesium hydroxide and calcium carbonate on esophageal and gastric pH in subjects with heartburn. Am J Ther 1995; 2: 546-552.
13. Kinnunen O, Salokannel J. Constipation in elderly long-stay patients: Its treatment by magnesium hydroxide and bulk-laxative. Ann Clin Res 1987; 19: 321-323. [Google Scholar] [PubMed]
14. Koga A, Thongsiri C, Kudo D, Phuong DN, Iwamoto Y, Fujii W, Nagai-Yoshioka Y, Yamasaki R, Ariyoshi W. Mechanisms underlying the suppression of IL-1β expression by magnesium hydroxide nanoparticles. Biomedicines 2023; 11: 1291.
15. Nakamura Y, Okita K, Kudo D, Phuong DN, Iwamoto Y, Yoshioka Y, Ariyoshi W, Yamasaki R. Magnesium hydroxide nanoparticles kill exponentially growing and persister Escherichia coli cells by causing physical damage. Nanomaterials 2021; 11: 1584.
16. Chaweewannakorn W, Ariyoshi W, Okinaga T, Fujita Y, Maki K, Nishihara T. Ameloblastin attenuates RANKL-mediated osteoclastogenesis by suppressing activation of Nuclear Factor of Activated T-cell cytoplasmic 1 (NFATc1). J Cell Physiol 2019; 234: 1745-1757.
17. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013; 48: 452-458.
18. Wu L, Luthringer BJ, Feyerabend F, Schilling AF, Willumeit R. Effects of extracellular magnesium on the differentiation and function of human osteoclasts. Acta Biomater 2014; 10: 2843-2854.
19. Bedair TM, Lee CK, Kim DS, Baek SW, Bedair HM, Joshi HP, Choi UY, Park KH, Park W, Han I, Han DK. Magnesium hydroxide-incorporated PLGA composite attenuates inflammation and promotes BMP2-induced bone formation in spinal fusion. J Tissue Eng 2020; 11: 2041731420967591.
20. Pinho LC, Garbieri TF, Grenho L, Alves MM, Sousa Gomes P, Santos CF, Fernandes MH, Santos C, Colaço B. Rosehip extract-functionalized magnesium hydroxide nanoparticles and its effect on osteoblastic and osteoclastic cells. Materials

2021; 14: 4172.

21. Lee JO, Hwang SH, Shen T, Kim JH, You L, Hu W, Cho JY. Enhancement of skin barrier and hydration-related molecules by protopanaxatriol in human keratinocytes. J Ginseng Res 2021; 45: 354-360.
22. Zhao G, Xue L, Geisler HC, Xu J, Li X, Mitchell MJ, Vaughan AE. Precision treatment of viral pneumonia through macrophage-targeted lipid nanoparticle delivery. Proc Natl Acad Sci U S A 2024; 121: e2314747121.
23. Guo J, Xu S, Majeed U, Ye J, Zhang H, Xue W, Luo Y. Size-related pathway flux analysis of ultrasmall iron oxide nanoparticles in macrophage cell RAW264. 7 for safety evaluation. ACS Omega 2024; 9: 3480-3490.

Correspondence to:

Wataru Ariyoshi
Division of Infections and Molecular Biology
Department of Advanced Pathophysiological Science
Kyushu Dental University
2-6-1 Manazuru
Kokurakita-ku
Kitakyushu
Fukuoka 803-8580
Japan