Journal of Dental Sciences 21 (2026) 184–190

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Original Article

Zoledronate enhances lipopolysaccharide-induced inflammation via toll-like receptor 4 upregulation

Kanan Bando [a*], Yasuo Endo [b]

[a] Division of Orthodontics and Dentofacial Orthopedics, Tohoku University Graduate School of Dentistry, Sendai, Japan
[b] Division of Oral and Maxillofacial Surgery, Tohoku University Graduate School of Dentistry, Sendai, Japan

Received 20 May 2025; Final revision received 5 June 2025
Available online 23 June 2025

KEYWORDS
Bisphosphonates;
TLR4;
Neutrophils;
Keratinocytes

Abstract *Background/Purpose:* Bisphosphonates (BPs) are commonly used to treat osteoporosis and bone metastases, but their use is associated with bisphosphonate-related osteonecrosis of the jaw (BRONJ), a condition characterized by chronic inflammation and impaired healing. Since bacterial infection is a major risk factor for BRONJ, this study investigated whether zoledronate (Zol), a nitrogen-containing BP, modulates Toll-like receptor 4 (TLR4) expression and enhances lipopolysaccharide (LPS)-induced inflammation.
Materials and methods: Zol or other BPs were injected into the ear pinnae of mice. Five days later, tissues were harvested and analyzed by flow cytometry and ELISA to assess TLR4 expression and cytokine production. TLR4 expression in immune cells and keratinocytes was evaluated, and inflammatory cytokine production (IL-1β, TNF-α) was measured after LPS stimulation.
Results: TLR4 expression was significantly increased in keratinocytes and neutrophils after Zol administration, whereas it was decreased in macrophages due to an increased proportion of inflammatory macrophage subsets with intrinsically low TLR4 expression. Zol-treated neutrophils exhibited elevated IL-1β and TNF-α production following LPS stimulation. Co-administration of Zol and LPS markedly enhanced local inflammation compared to either agent alone.
Conclusion: Zoledronate upregulates TLR4 expression in neutrophils and keratinocytes and augments LPS-induced inflammation, suggesting a mechanism by which BPs exacerbate oral inflammatory responses. These findings provide new insight into the pathogenesis of BRONJ and suggest that targeting TLR4 signaling may be a potential strategy to mitigate BP-associated oral complications.

* Corresponding author. Division of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan.
E-mail address: kanan.bando.a5@tohoku.ac.jp (K. Bando).

https://doi.org/10.1016/j.jds.2025.06.004

Introduction

Bisphosphonates (BPs) are widely used for the management of osteoporosis and bone metastases due to their potent antiresorptive effects mediated through inhibition of osteoclast activity and induction of apoptosis.[1,2] Although BPs have significantly improved outcomes in bone-related diseases, prolonged use has been linked to bisphosphonate-related osteonecrosis of the jaw (BRONJ) have increased greatly since the 1st report of this condition in 2003,[3] a debilitating condition characterized by necrotic bone exposure, pain, and delayed mucosal healing, most often following dental procedures.[4] Nitrogen-containing bisphosphonates (NBPs) possess stronger antiresorptive activity than non-nitrogen-containing BPs (non-NBPs) and are also effective in managing bone metastases.[5,6] However, NBPs are uniquely associated with BRONJ, whereas such complications are rarely reported with non-NBPs.[7]

The pathogenesis of BRONJ is multifactorial and incompletely understood, but bacterial infection is recognized as a major contributing factor.[8] Clinical and microbiological studies have consistently demonstrated that BRONJ lesions are frequently colonized by oral pathogens, including Gram-negative bacteria.[9] Lipopolysaccharide (LPS), a key component of Gram-negative bacterial outer membranes, is a potent activator of innate immunity via toll-like receptor 4 (TLR4) signaling. Activation of TLR4 triggers downstream pro-inflammatory pathways, including NF-κB-mediated production of cytokines such as IL-1β and TNF-α.[10] Recent studies suggest that BPs may enhance the inflammatory response to LPS, potentially exacerbating tissue damage[11,12] and ameliorated in TLR4 knockout mice.[13]

Macrophages, neutrophils, keratinocytes, and endothelial cells are abundant in the oral mucosa and play essential roles in inflammation and wound repair; they may also contribute to BRONJ development.[12—14] In macrophages, zoledronate (Zol) does not significantly alter TLR4 expression but enhances SOCS1 expression, a downstream regulator of TLR4 signaling, leading to increased IL-1β and TNF-α production upon LPS stimulation.[15] Conversely, other studies have reported that Zol directly upregulates TLR4 expression at the transcriptional level.[13] Thus, the mechanisms underlying this interaction remain poorly defined.

In the present study, we investigated the effects of Zol, a typical NBP, on TLR4 expression and inflammatory signaling in a murine model. We aimed to determine whether Zol modulates the cellular response to LPS by enhancing TLR4 expression in neutrophils and keratinocytes, thereby promoting inflammation and impairing epithelial regeneration. Understanding this interaction may provide mechanistic insight into BRONJ pathogenesis and suggest potential targets for prevention or treatment.

Materials and methods

Animals

C57BL/6N, C3H/HeN and C3H/HeJ mice were purchased from SLC (Shizuoka, Japan).All animal procedures were approved by the Institutional Animal Care and Use Committee of Tohoku University (approval number: 2019DnA-044 and 2023DnA-017). All experiments complied with Regulations for Animal Experiments and Related Activities at Tohoku University. Males (8—10 weeks old) were used.

Reagents

Zol, Alendronate (Ale), etidronate (Eti), and clodronate (Clo) were purchased from TCI (Tokyo, Japan) and dissolved in saline, with the pH of the solution being adjusted to 7 using NaOH. *E coli* O55:B5 LPS) was purchased from Sigma—Aldrich (St. Louis, MO, USA). All other reagents were purchased from Nacalai Tesque (Kyoto, Japan), unless otherwise indicated.

Measurement of toll-like receptor 4 and cytokines by enzyme-linked immunosorbent assay

In in vivo experiments, cytokines in ear-pinnae were determined as described previously.[12] A volume of 20 μl of each test substance was injected into the ear pinnae of mice. At designated time points, the entire ear tissue was collected and homogenized in RPMI 1640 medium supplemented with 5 μl/ml Triton X-100, 10 μmol/ml HEPES, 100 μg/ml bovine serum albumin, 50 μg/ml gentamicin sulfate, and 10 μl/ml protease inhibitor cocktail. The homogenates were centrifuged at 10,000×*g* for 10 min at 4 °C, and the resulting supernatants were used to assess TLR4 levels and cytokine concentrations. Enzyme-linked immunosorbent assay (ELISA) kits were employed to quantify IL-1β and TNF-α (BioLegend, San Diego, CA, USA) as well as TLR4 (Abbexa, Cambridge, UK).

Induction of ear-swelling

Zol (1 mM) was i.d. injected into the ear-pinnae (20 μl/ear) of both ears. Five days later, LPS (50 μg/ml) Ear-swelling was measured by means of a Peacock dial thickness gauge (Ozaki MFG Co. Ltd, Tokyo, Japan).

Histology and immunohistochemistry

At 5 days after injection of mice with Ale, ear-pinnas were fixed in 4% paraformaldehyde-phosphate-buffer solution and embedded in paraffin. Sections (5 μm thick) were incubated

in heated antigen-retrieval solution with HistoVT One (Nacalai Tesque). Sections were blocked with Blocking One (Nacalai Tesque) for 1 h at room temperature, and staining was performed with a primary antibody [rabbit polyclonal anti-TLR4, 1:50, Santa Cruz Biotechnology (Santa Cruz, CA, USA)] overnight at 4 °C, followed by staining with a secondary antibody [Alexa 488-conjugated anti-rabbit, 1:1000, (Abcam, Cambridge, UK)] for 60 min at room temperature. After the sections had been covered with mountant [Prolong Glass Antifade Mountant with NucBlue (DAPI) Stain, (Invitrogen, Waltham, MA)], images were acquired using a BZ-9000 fluorescence microscope (Keyence, Tokyo, Japan).

Bone marrow-derived macrophages and stimulated by zoledronate

Bone marrow cells obtained from non-stimulated mice were cultured with 50 ng/ml M-SCF (BioLegend) for 7 days in DMEM high glucose supplemented with 10 % serum and antibiotics. Purity of macrophage (CD11b + F4/80+ cells) was confirmed by flow cytometry, which revealed purity >99 %. Bone marrow-derived macrophages (BMDMs) were stimulated with Zol (10 μg/ml) for 24 h.

Neutrophil isolation and stimulation by zoledronate

To isolate bone marrow neutrophils, bone marrow was harvested from C57BL/6N male mice tibias. Red blood cells were lysed using lysis buffer (155 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA), and the cells were incubated with 10 μg/mL Fc Block (clone 2.4G2) for 5 min at RT. They were then stained with PE-labeled anti-Ly6G antibody for 15 min at RT and Ly6G-positive cells were purified using an EasySep PE selection kit (StemCell Technologies, Vancouver, Canada). Purification of neutrophils was confirmed by FACS analysis, which showed purity >95 %. Isolated neutrophils were stimulated with Zol (10 μg/ml) for 24 h.

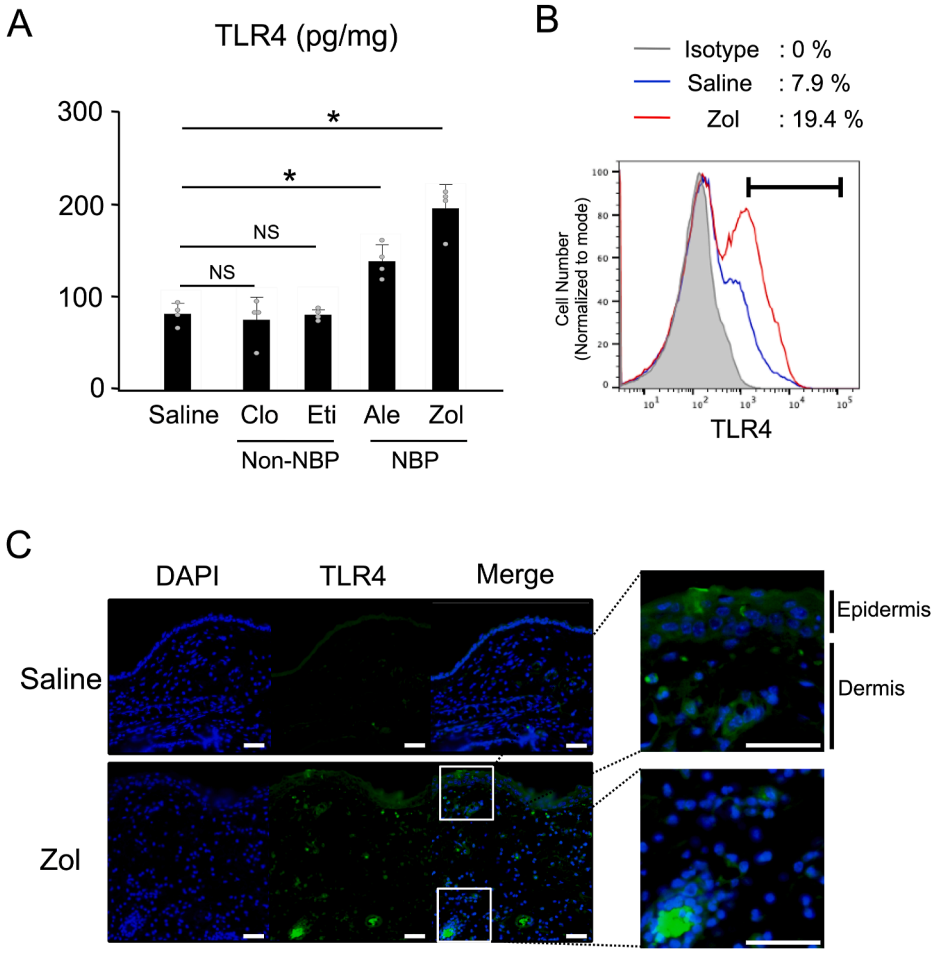

Figure 1 NBPs induce upregulation of TLR4 expression. Saline, Clo (50 mM), Eti (50 mM), Ale (8 mM), or Zol (1 mM) was injected into ear of C57BL/6N mice. (**A**) At day 5, the ear pinnae were analyzed by ELISA. *$P < 0.01$. (n = 4). (**B**) Representative flow cytometry. Values given for TLR4$^+$ cells among live cells in the ear-pinnas 5 days after injection of Zol or saline. Blue-line histogram represents injection of saline, red-line histogram represents injection of Zol, while gray-shaded histogram represents isotype controls. Each value is the mean (n = 4). (**C**) TLR4-expressing cells in the ear-pinnas 5 days after injection of Zol or saline. Results are representative of four independent experiments. Ear-pinnas were stained with DAPI (blue), and TLR4 (green). (scale bar = 25 μm). TLR4, Toll-like receptor 4; Clo, Clodronate; Eti, Etidronate; Ale, Alendronate; Zol, Zoledronate; NBP, Nitrogen-containing bisphosphonates; Non-NBP, non nitrogen-containing bisphosphonates: DAPI, 4′,6-diamidino-2-phenylindole.

Flow cytometry

Each ear-pinna was minced into small fragments and incubated in RPMI 1640 medium supplemented with 10 % fetal bovine serum and 1 mg/ml collagenase IV (Sigma–Aldrich) for 2 h at 37 °C in a shaking water bath. Bone marrow cells were harvested from tibiae, rinsed with PBS, and passed through a 70 μm cell strainer. Red blood cells were lysed using a standard lysis buffer. For blocking Fc receptors, cells were treated with 10 μg/ml TruStain FcX (BioLegend) on ice for 15 min. Subsequently, surface staining was performed for 30 min on ice using the following fluorophore-conjugated antibodies (all from BioLegend): PE-anti-TLR4, Pacific Blue-anti-CD45, APC/Cy7-anti-CD11b, PE/Cy7-anti-CD31, FITC-anti-F4/80, Alexa647-anti-EpCAM, PerCP/Cy5.5-anti-Ly6G, and APC-anti-Ly6C. Dead cells were excluded by DAPI staining (Dojindo, Hamamatsu, Japan). Flow cytometry was carried out using an LSRFortessa analyzer (BD Biosciences, San Diego, CA, USA), and data were analyzed using FlowJo software (BD Biosciences). In histogram plots where cell numbers differed substantially across groups, vertical axis values were normalized to mode and labeled as "Cell number (normalized to mode)." Fluorescence intensity is shown on the horizontal axis.

Statistical analysis

Experimental values are given as the mean ± SD. Sample size was based on an α error of 0.05 and a β error of 0.2 using power analysis. The statistical significances were analyzed using a Bonferroni multiple comparison test after ANOVA with the aid of JMP software (SAS Institute, Tokyo, Japan).

Results

Nitrogen-containing bisphosphonates, but not non-nitrogen-containing bisphosphonates, induce toll-like receptor 4 expression

To assess the impact of BPs on TLR4 expression, we compared non-nitrogen-containing BPs (Clo and Eti) with nitrogen-containing BPs (Ale and Zol). Mice were administered each BP, and ear tissue was collected five days later.

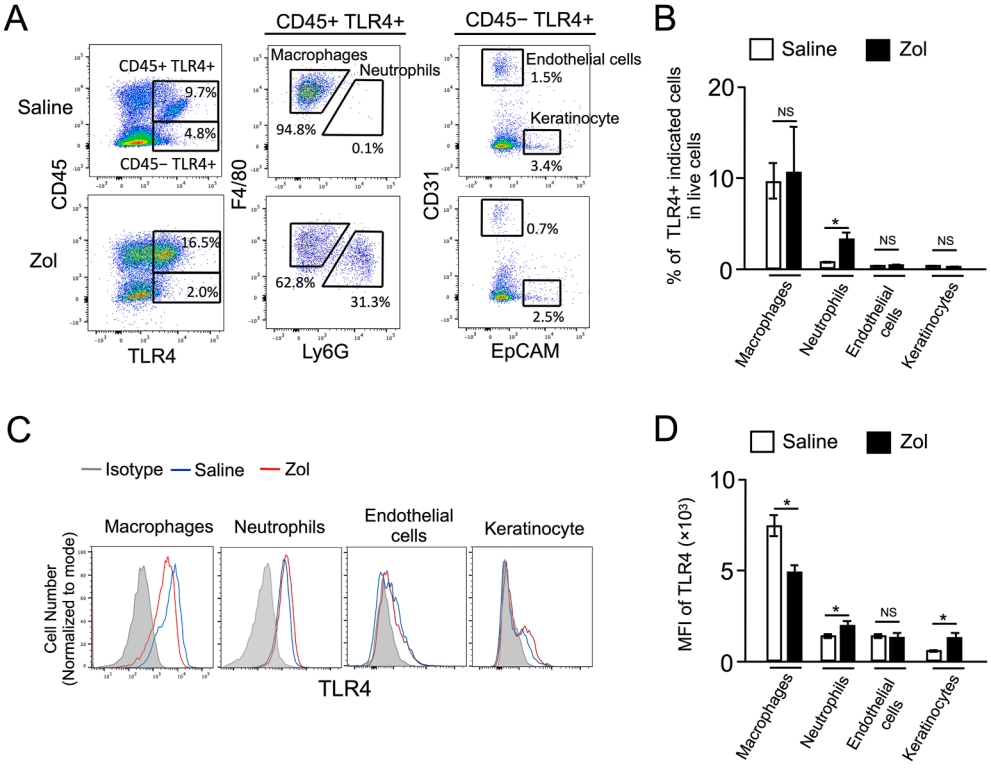

Figure 2 TLR4 expression cells in Zol-stimulated ear-pinnas. Ear-pinnas of C57BL/6N mice were injected with Zol or saline. At day 5, TLR4 expression in the ear-pinnas was analyzed by flow cytometry. (**A**) A representative flow cytometry. Each cell subset defined as follows: Macrophage ($CD45^+F4/80^+$), Neutrophil ($CD45^+Ly6G^+$), Endothelial cell ($CD45^-CD31^+EpCAM^-$), Keratinocyte ($CD45^-CD31^-EpCAM^+$). (Left) Values indicate the percentage of $CD45^+TLR4^+$ cells and $CD45^-TLR4^+$ cells among live cells. (middle) Values indicate the percentage of macrophages and neutrophils among $CD45^+TLR4^+$ cells. (right) Values indicate the percentage of endothelial cells and keratinocytes among $CD45^-TLR4^+$ cells. Results are representative of four independent experiments. (**B**) The percentage of $TLR4^+$ cells in the indicated cell subsets among live cells. $^*P < 0.05$. (n = 4). (**C**) Line histograms represent $TLR4^+$ staining.Blue-line histogram represents injection of saline, red-line histogram represents injection of Zol, while gray-shaded histogram represents isotype controls. (**D**) Mean fluorescence intensity (MFI) of $TLR4^+$ cells in the indicated cell subsets. $^*P < 0.05$. (n = 4). Zol, Zoledronate; TLR4, Toll-like receptor 4; MFI, Mean fluorescence intensity.

TLR4 expression was unchanged following treatment with non-NBPs but was markedly upregulated in response to NBPs (Fig. 1A). The proportion of TLR4-positive cells was significantly increased in both the epidermis and dermis (Fig. 1B and C).

Zoledronate decreases toll-like receptor 4 expression in macrophages but increases it in neutrophils and keratinocyte

To identify the cellular sources of TLR4 expression, we performed flow cytometric analysis. The proportion of $CD45^+$ immune cells was increased in the Zol-treated group, with a marked increase in neutrophils (Fig. 2A and B). We next assessed TLR4 expression in individual cell types. TLR4 expression was decreased in macrophages, whereas it was significantly upregulated in both neutrophils and keratinocytes (Fig. 2C and D).

Inflammatory macrophages exhibit low toll-like receptor 4 expression

As shown in Fig. 2, although the proportion of $TLR4^+$ macrophages remained unchanged, the overall expression level of TLR4 in macrophages was reduced. To investigate this discrepancy, we analyzed macrophage subsets and found that the proportion of inflammatory macrophages was increased in the Zol-treated group (Fig. 3A). Further analysis revealed that inflammatory macrophages expressed lower levels of TLR4 compared to tissue-resident macrophages (Fig. 3B). Notably, Zol did not directly affect TLR4 expression in macrophages.

We previously reported that neutrophils are Zol recruits inflammatory neutrophils.[12] Neutrophils are rarely present in the skin under normal conditions; however, we observed an accumulation of inflammatory neutrophils following Zol administration. As shown in Fig. 2C, inflammatory neutrophils exhibited higher TLR4 expression than tissue-resident neutrophils. Furthermore, Fig. 3D demonstrates that tissue-resident neutrophils express higher levels of TLR4 compared to immature neutrophils in the bone marrow. Lastly, direct stimulation of neutrophils with Zol resulted in increased TLR4 expression (Fig. 3E).

Zoledronate enhances lipopolysaccharide-induced inflammation

We previously showed that Zol upregulates TLR4 expression. To determine whether Zol influences the inflammatory response to LPS, ligand of TLR4. Zol was first injected into the ear pinnae, followed by LPS administration at the same site five days later (Fig. 4A). Zol pretreatment significantly enhanced LPS-induced ear swelling (Fig. 4B). Moreover, the production of pro-inflammatory cytokines, including IL-1β and TNF-α, was markedly increased (Fig. 4C). As shown in Fig. 4D, this enhancement of ear swelling was not observed in C3H/HeJ mice, which lack functional TLR4. These results indicate that TLR4 is essential for the augmentation of LPS-induced inflammation by Zol.

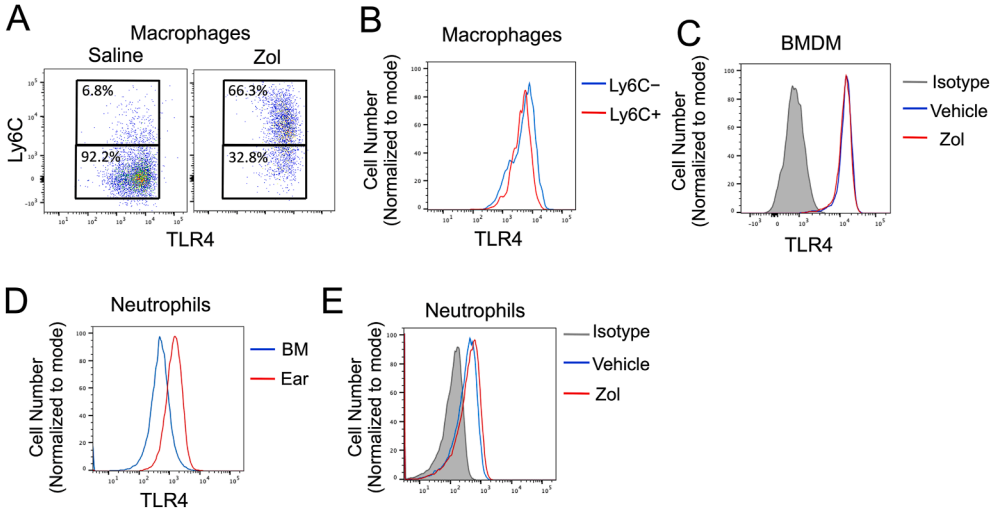

Figure 3 TLR4 expression in macrophages and neutrophils by Zol. Ear-pinnas of C57BL/6N mice were injected with Zol. At day5, macrophages in the ear-pinnas was analyzed by flow cytometry. (**A**) A representative flow cytometry. Values indicate the percentage of $Ly6C^+$ and $Ly6C^-$ cells among macrophages. (**B**) Line histograms represent $TLR4^+$ staining. Blue-line histogram represents $Ly6C^+$ macrophages, red-line histogram represents $Ly6C^-$ macrophages. (**C**) Effects of Zol on TLR4 expression in BMDMs. BMDMs were stimulated with Zol (10 μg/ml) for 24 h and TLR4 expression was analyzed. Blue-line histogram represents vehicle, red-line histogram represents Zol, while gray-shaded histogram represents isotype controls.(**D**) Line histograms represent $TLR4^+$ staining. Blue-line histogram represents neutrophils in bone marrow (BM), red-line histogram represents neutrophils in ear-pinnas. (**E**) Effects of Zol on TLR4 expression in neutrophils. Bone marrow-derived neutrophils were stimulated with Zol (10 μg/ml) for 4 h and TLR4 expression was analyzed. Blue-line histogram represents vehicle, red-line histogram represents Zol, while gray-shaded histogram represents isotype controls. Results are representative of four independent experiments.*$P < 0.01$. (n = 4). Zol, Zoledronate; TLR4, Toll-like receptor 4; BMDM, Bone marrow-derived macrophages; BM, Bone marrow.

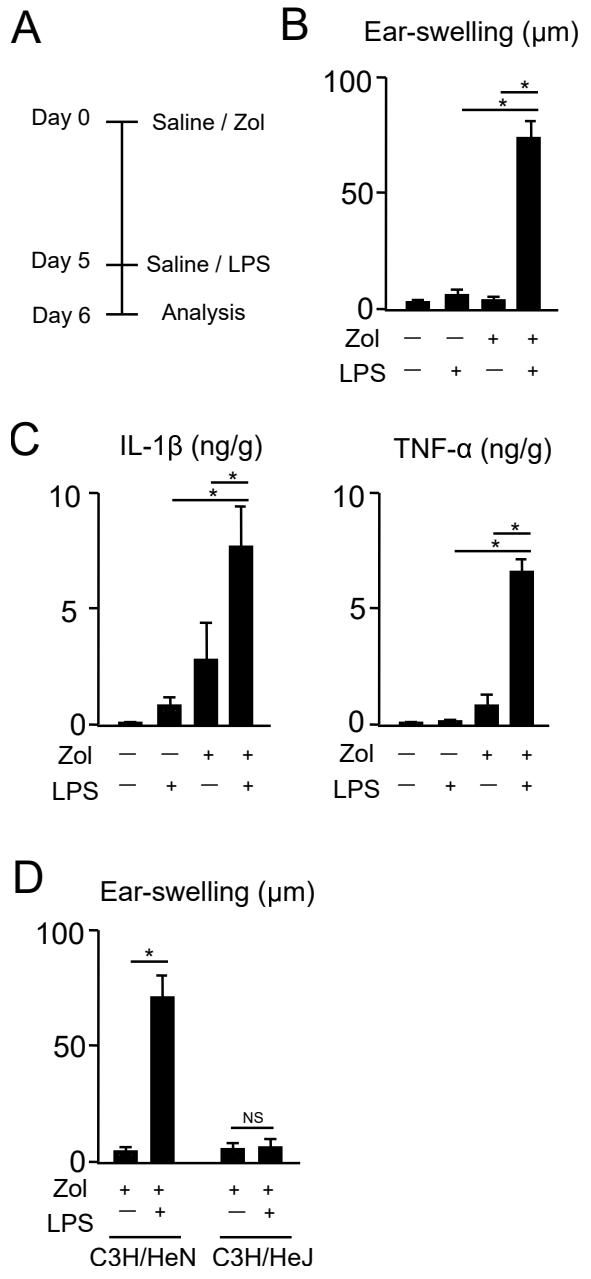

Figure 4 Effect of Zol-stimulated TLR4 upregulation in vivo. (**A**) Schematic protocol. Zol-stimulated TLR4 augmented inflammation. Ear-pinnas of C57BL/6N mice were injected with Zol. At day5, LPS (100 μg/ml) was injected into ear-pinnas. At day6, (**B**) ear-swelling and (**C**) the indicated cytokines in ear-pinnas were measured. (**D**) Ear-pinnas of C3H/HeN and C3H/HeJ mice were injected with Zol. At day5, LPS (100 μg/ml) or saline was injected into ear-pinnas. At day6, ear-swelling were measured.*$P < 0.01$. (n = 4). Zol, Zoledronate; LPS, lipopolysaccharide; IL-1β, Interleukin 1-β; TNF-α, Tumor necrosis factor-α.

Discussion

The present findings may be summarized as follows: (i) NBPs, such as Ale and Zol, significantly increased TLR4 expression in skin tissue, unlike non-NBPs, such as Eti and Clo. (ii) Zol specifically enhanced TLR4 expression in neutrophils and keratinocytes, while decreasing it in macrophages due to a shift toward inflammatory macrophage subsets with intrinsically low TLR4 expression. (iii) Functionally, Zol pretreatment amplified LPS-induced skin inflammation and cytokine production, indicating heightened TLR4-mediated inflammatory responses. These findings are discussed in detail below.

In the murine macrophage cell line, RAW264.7, Zol has been shown to increase the expression of SOCS1, a negative regulator of TLR4 signaling, thereby amplifying LPS-induced cytokine production (IL-1β, IL-6, TNF-α) without altering TLR4 surface expression.[15] Thus, Zol may potentiate inflammation in macrophages through downstream modulation of TLR4 signaling rather than receptor upregulation. On the other hand, Zol enhances TLR4 gene expression in BMDM.[13] We observed that Zol did not increase TLR4 protein expression in BMDM. Instead, the proportion of TLR4-expressing cells remained unchanged, while the overall TLR4 protein expression level was reduced. Subset analysis revealed an increase in inflammatory macrophages, which express lower levels of TLR4 compared to tissue-resident macrophages. These results suggest that the decrease in TLR4 expression reflects a shift in macrophage phenotype rather than direct inhibition of TLR4 transcription. The mechanisms governing this cell-type-specific regulation warrant further investigation.

TLR4 plays a key role in neutrophil activation, microbial recognition, and inflammation resolution,[16–18] yet the impact of BPs on TLR4 expression in neutrophils has not been previously reported. In this study, we observed that Zol administration induced the accumulation of inflammatory neutrophils with elevated TLR4 expression. Furthermore, Zol directly or indirectly upregulated TLR4 expression in neutrophils. Upregulation of TLR4 may amplify their pro-inflammatory functions, as evidenced by the elevated production of IL-1β and TNF-α following LPS stimulation in Zol-treated neutrophils. These findings are in line with previous reports implicating neutrophils in chronic inflammation and tissue injury.[12,19] Considering their prevalence in oral mucosal lesions, Zol-induced TLR4 elevation in neutrophils may contribute to persistent mucosal inflammation and impaired wound healing, key features in the pathogenesis of BRONJ.

Keratinocytes, key components of the epithelial barrier, are increasingly recognized as active participants in immune signaling. Our data demonstrated that Zol increased TLR4 expression in keratinocytes in vivo, suggesting that Zol may prime these cells to respond more robustly to microbial stimuli. LPS recognition by keratinocyte TLR4 can induce the secretion of cytokines and chemokines, exacerbating local inflammation and impeding epithelial regeneration.[20,21] Given that epithelial integrity is often compromised in BRONJ, Zol-induced TLR4 upregulation in keratinocytes may play a central role in sustaining inflammation and impairing mucosal healing.

Despite these novel insights, certain limitations must be acknowledged. This study employed a murine skin model, which may not fully recapitulate the complex immune microenvironment of the oral mucosa. Nevertheless, under inflammatory conditions, it is well established that

neutrophils, macrophages, and IL-1β play central roles in both skin and oral tissues.[22] Furthermore, given that bisphosphonate-related osteonecrosis of the external auditory canal has been reported as an adverse effect, findings derived from skin-based models are also considered clinically relevant.[23] While our findings suggest a cell-type-specific regulation of TLR4 by Zol, the precise molecular mechanisms—such as transcriptional regulation, epigenetic modifications, or cytokine-mediated feedback loops—remain to be elucidated. It also remains unclear whether Zol modulates other pattern recognition receptors, such as TLR2, which may functionally interact with TLR4 during inflammation in oral mucosa.[24]

In conclusion, this study provides novel evidence that Zol enhances TLR4-mediated inflammatory responses in neutrophils and keratinocytes, potentially contributing to the pathogenesis of BRONJ. These findings improve our understanding of BP-associated inflammation and suggest new therapeutic strategies targeting the TLR4 pathway to mitigate adverse effects in the oral mucosa.

Declaration of competing interest

The authors have declared that there are no competing interests.

Acknowledgments

This work was supported by Grants from the Japan Society for the Promotion of Science [21K10157 and 24K13168 (Bando Kanan)]. We thank the Biomedical Research Core of Tohoku University Graduate School of Medicine for the use of its equipment.

References

1. Russell RG. Bisphosphonates: the first 40 years. *Bone* 2011;49:2—19.
2. Billington E, Aghajafari F, Skulsky E, Kline GA. Bisphosphonates. *BMJ* 2024;21:e076898.
3. Marx RE. Pamidronate (aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. *J Oral Maxillofac Surg* 2003;61:1115—7.
4. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American association of oral and maxillofacial surgeons' position paper on medication related osteonecrosis of the jaws-2022 update. *J Oral Maxillofac Surg* 2022;80:920—43.
5. Rogers MJ, Crockett JC, Coxon FP, Mönkkönen J. Biochemical and molecular mechanisms of action of bisphosphonates. *Bone* 2011;49:34—41.
6. Caraglia M, Santini D, Marra M, Vincenzi B, Tonini G, Budillon A. Emerging anti-cancer molecular mechanisms of aminobisphospho-nates. *Endocr Relat Cancer* 2006;13:7—26.
7. Fliefel R, Tröltzsch M, Kühnisch J, Ehrenfeld M, Oto S. Treatment strategies and outcomes of bisphosphonate-related osteonecrosis of the jaw (BRONJ) with characterization of patients: a systematic review. *Int J Oral Maxillofac Surg* 2015;44:568—85.
8. Migliorati CA, Siegel MA, Elting LS. Bisphosphonate-associated osteonecrosis: a long-term complication of bisphosphonate treatment. *Lancet Oncol* 2006;7:508—14.
9. Katsarelis H, Shah NP, Dhariwal DK, Pazianas M. Infection and medication related osteonecrosis of the jaw. *J Dent Res* 2015;94:534—9.
10. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004;4:499—511.
11. Sakaguchi O, Kokuryo S, Tsurushima H, et al. Lipopolysaccharide aggravates bisphosphonate-induced osteonecrosis in rats. *Int J Oral Maxillofac Surg* 2015;44:528—34.
12. Bando K, Kuroishi T, Tada H, et al. Nitrogen-containing bisphosphonates and lipopolysaccharide mutually augment inflammation via adenosine triphosphate (ATP)-mediated and interleukin 1β (IL-1β)-mediated production of neutrophil extracellular traps (NETs). *J Bone Miner Res* 2021;36:1866—78.
13. Zhu W, Xu R, Du J, et al. Zoledronic acid promotes TLR-4-mediated M1 macrophage polarization in bisphosphonate-related osteonecrosis of the jaw. *FASEB J* 2019;33:5208—19.
14. Mawardi H, Giro G, Kajiya M, et al. A role of oral bacteria in bisphosphonate-induced osteonecrosis of the jaw. *J Dent Res* 2011;90:1339—45.
15. Muratsu D, Yoshiga D, Taketomi T, et al. Zoledronic acid enhances lipopolysaccharide- stimulated proinflammatory reactions through controlled expression of SOCS1 in macrophages. *PLoS One* 2013;8:e67906.
16. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010;140:805—20.
17. Sabroe I, Jones EC, Usher LR, Whyte MK, Dower SK. Toll-like reptor (TLR)2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. *J Immunol* 2002;168:4701—10.
18. Bugl S, Wirths S, Radsak MP, et al. Steady-states neutrophil homeostasis is dependent on TLR4/TRIF signaling. *Blood* 2013;121:723—33.
19. Okawa H, Kondo T, Hokugo A, et al. Mechanism of bisphosphonate-related osteonecrosis of the jaw (BRONJ) revealed by targeted removal of legacy bisphosphonate from jawbone using competing inert hydroxymethylene diphosphonate. *eLife* 2022;22:e76207.
20. Pivarcsi A, Bodai L, Réthi B, et al. Expression and function of toll-like receptors 2 and 4 in human keratinocytes. *Int Immunol* 2003;15:721—30.
21. Or C, Cui J, Matsubara J, Forooghian F. Pro-inflammatory and anti-angiogenic effects of bisphosphonates on human cultured retinal pigment epithelial cells. *Br J Ophthalmol* 2013;97:1074—8.
22. Iglesias-Bartolome R, Uchiyama A, Molinolo AA, et al. Transcriptional signature primes human oral mucosa for rapid wound healing. *Sci Transl Med* 2018;10:eaap8798.
23. Kharazmi M, Hallberg P, Persson U, Warfvinge G. Bisphosphonate-associated osteonecrosis of the auditory canal. *Br J Oral Maxillofac Surg* 2013;51:e285—7.
24. Allam JP, Duan Y, Winter J, et al. Tolerogenic T cells, Th1/Th17 cytokines and TLR2/TLR4 expressing dendritic cells predominate the microenvironment within distinct oral mucosal sites. *Allergy* 2011;66:532—9.