Journal of Dental Sciences 20 (2025) 764–769

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Original Article

Exacerbating orthodontic tooth movement in mice with salt-sensitive hypertension

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Received 25 September 2024; Final revision received 16 October 2024
Available online 24 October 2024

KEYWORDS
Hypertension;
Orthodontics;
Osteoclast;
Salt-sensitive hypertensive mouse model;
Tooth movement

Abstract *Background/purpose:* Orthodontic tooth movement (OTM) is a critical aspect of dental treatment that requires the precise control of bone remodeling processes. Hypertension (HTN) can affect the effectiveness of OTM. Salt-sensitive hypertension (SSHTN) is of particular concern due to its detrimental effects on bone health, potentially altering orthodontic outcomes. This study aimed to investigate the effects of SSHTN on OTM using a mouse model.
Materials and methods: Male mice were divided into a normal and an SSHTN group. The SSHTN model was generated by administering N(ω)-nitro-L-arginine methyl ester (L-NAME) followed by a high-salt diet. The OTM was performed using a nickel-titanium (Ni-Ti) closed-coil spring, and the tooth movement was measured after 12 days. Silicone imprinting was used to estimate the OTM distance. Osteoclast activity was assessed using tartrate-resistant acid phosphatase (TRAP) staining of decalcified maxillary sections.
Results: SSHTN mice exhibited significantly increased tooth movement compared to normal mice. This enhanced movement was associated with more osteoclasts in the SSHTN group than in the control group. These findings suggest that SSHTN increases OTM levels by promoting bone resorption.
Conclusion: SSHTN significantly affected OTM by enhancing osteoclast activity and increasing tooth movement. These results underscore the importance of considering hypertensive conditions in orthodontic treatment planning as they may require adjustments in force application to prevent potential adverse effects.

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https://doi.org/10.1016/j.jds.2024.10.020

Introduction

Orthodontic tooth movement (OTM) is a fundamental procedure in dental treatment used to correct malocclusion and improve dental function and aesthetics. This process relies on the controlled application of force to teeth, resulting in bone remodeling facilitated by the resorption and deposition of bone within the periodontal ligament (PDL).[1–3] While OTM is widely practiced, the duration required to achieve the desired results varies significantly among patients and is influenced by factors such as age, systemic health, and individual biological responses. Age-related changes in bone metabolism, such as reduced bone density and slower cellular activity, could significantly affect the speed and efficiency of OTM in older adults, especially in those with underlying hypertensive conditions. Similarly, systemic health factors like diabetes or osteoporosis, which often co-exist with HTN, may further compromise bone remodeling processes during orthodontic treatments. Genetic predispositions also play a role in determining individual biological responses to orthodontic forces, with some patients exhibiting faster or slower rates of tooth movement due to variations in gene expression that regulate bone remodeling. Incorporating these individual factors into treatment plans could lead to more personalized and effective orthodontic care.[4–6]

One notable systemic condition that may affect the rate of OTM is hypertension (HTN).[7] In recent years, the number of HTN patients receiving orthodontic treatment has increased.[8–10] Our previous study demonstrated the detrimental effects of salt-sensitive hypertension (SSHTN) on bone health.[11] The degradation of bone microstructure observed in these hypertensive animals may have resulted from elevated production of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) and excessive bone renin-angiotensin system (RAS) activation.[11–14] Furthermore, we found that antihypertensive treatments may reduce inflammation-related bone tissue damage.[15] HTN not only exacerbates cardiovascular risks but also influences the microenvironment of periodontal tissues,[16,17] potentially altering their response to orthodontic forces.

While hypertension can present in various forms, such as essential (primary) and secondary hypertension, the focus of this study is on SSHTN due to its unique pathophysiological impact on bone remodeling processes that closely align with the objectives of investigating OTM. The SSHTN model provides a relevant basis for understanding how the body's response to increased salt intake directly influences periodontal tissue dynamics and orthodontic treatment outcomes. Few studies have investigated OTM in hypertensive mice due to the lack of standard animal models. However, a recent study established a hypertensive mouse model.[18] SSHTN mouse model is particularly relevant as it mirrors the pathophysiological changes seen in humans who exhibit heightened blood pressure responses to salt.[19,20] Investigating OTM in these models allows us to examine the cellular and molecular mechanisms driving the alterations in bone remodeling processes. This knowledge could enable more tailored orthodontic treatments for individuals with varying susceptibilities to HTN.

Understanding the acceleration of OTM under these conditions can lead to the development of more efficient orthodontic strategies, potentially reducing treatment time and improving outcomes in patients with HTN. Such advancements would enhance orthodontic care and provide insights into the broader implications of HTN on skeletal dynamics.

This study aimed to investigate these complex interactions by assessing the OTM rate in SSHTN mice to uncover the underlying mechanisms that could inform orthodontic and hypertensive management strategies.

Materials and methods

SSHTN mouse model generation

Male C57BL6/J mice (CLEA, Tokyo, Japan), aged 5 weeks were housed in an animal facility with a 12-h light/12-h dark cycle at temperatures ranging from 21 to 24 °C. Mice were administered L-NAME (0.5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) in their water for 2 weeks to inhibit nitric oxide synthesis. This was followed by a washout period of 2 weeks and a 3-week exposure to a high-salt diet (CE-2 containing 4 % NaCl; CLEA) to create the SSHTN model (Fig. 1 A).[11,18] Normal diet (ND) mice received tap water and a standard diet for 7 weeks. Water and all other food were provided ad libitum. In previously trained mice, systolic blood pressure (SBP) was measured at the end of each period using tail-cuff plethysmography with a blood pressure monitor (MK-1030; Muromachi Kikai Co., Tokyo, Japan). Blood pressure was taken in an incubator after it had warmed up for 15 min to 37 °C. This allowed for relatively stress-free blood measurements. For every animal, an average of 10 readings were recorded (Fig. 1 B). After 2 weeks of L-NAME treatment, the SBP of mice increased to 120 $\pm$ 3 mmHg, compared to 87 $\pm$ 7 mmHg in untreated mice ($P < 0.01$). Following a 2-week washout period, the SBP of mice treated with L-NAME recovered to normal levels (85 $\pm$ 3 mmHg). SSHTN was observed in L-NAME-treated mice after a 3-week salt loading period (126 $\pm$ 9 mmHg vs. 88 $\pm$ 4 mmHg, $P < 0.01$) (Fig. 1C). The Tohoku University of Science Animal Care and Use Committee approved all animal care practices and experiments.

Experimental orthodontic tooth movement

Mice were anesthetized before OTM was performed. A small hole was carefully drilled into the maxillary alveolar bone located just below the incisors using a slow-speed handpiece and a tungsten carbide bur. After the hole was created, a nickel-titanium (Ni-Ti) closed coil spring (Tomy, Fukushima, Japan) was attached to a 0.1 mm stainless steel wire. One end of the biocompatible spring was connected to a hole in the alveolar bone, while the other was affixed to the left maxillary first molar. The spring applied a constant and gentle orthodontic force of 10 g, as directed by the manufacturer,[21] moving the first molar in the mesial direction (Fig. 2A). OTM was performed for 12 days with four mice per group.

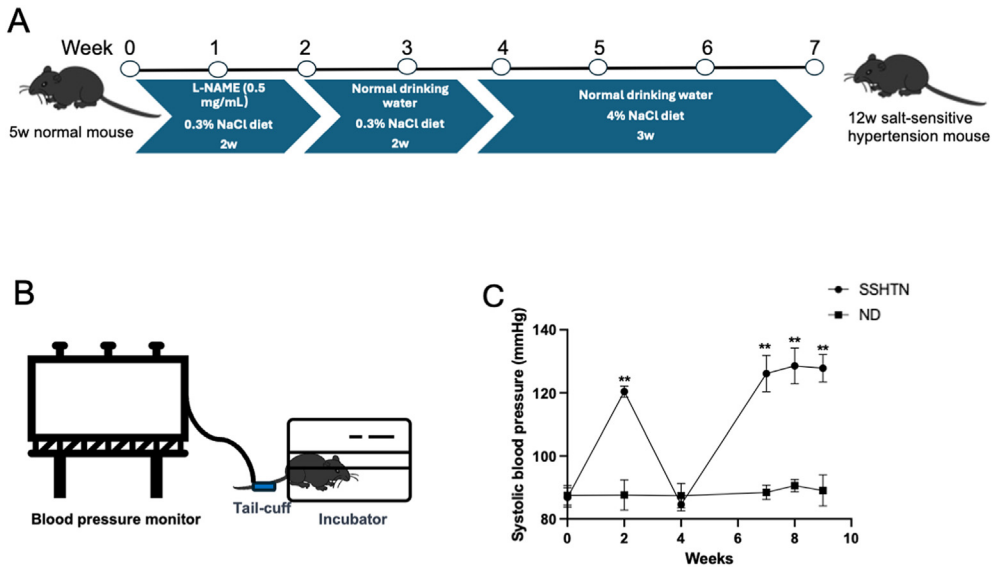

Figure 1 Systolic blood pressure of SSHTN mice and normal mice. (A) Three sequential phases of the experimental technique used to create the SSHTN mouse model were as follows: a 2-week L-NAME-induced nitric oxide production suppression period, a 2-week washout period, and a final 3-week high-salt diet. (B) Schematic diagram of the blood pressure measurements in mice. The SBP was measured at the end of each period using tail-cuff plethysmography and blood pressure monitoring. Blood pressure was taken in an incubator after it had warmed up for 15 min to 37 °C. (C) SBP was assessed in normal and SSHTN mice. n = 4/group. $^{**}P < 0.01$. ND, normal diet; SBP, Systolic blood pressure; SSHTN, Salt-sensitive hypertension.

Measurement of tooth movement

After 12 days of OTM, mice were euthanized using excess 5 % isoflurane. The maxillary region was removed from the skull, teeth, and maxillary impressions were obtained using separate trays filled with a hydrophilic vinyl polysiloxane impression material (EXAFAST Injection Type; GC Co., Tokyo, Japan). The closest distance between the first and second molars in the imprint was measured using a stereoscopic microscope (VH-7000; Keyence, Osaka, Japan) to ascertain the extent of tooth movement (Fig. 2B).[21]

Micro-computed tomography examination of changes in tooth movement

The maxillae were subsequently subjected to Micro-computed tomography (μCT) (ScanXmate-E090; Comscan, Kanagawa, Japan) to evaluate the level of tooth movement by generating reconstructed images with the TRI/3DBON64 software (RATOC Systems, Inc., Tokyo, Japan).[22]

Histological analysis

The maxillae were cut off from the skull and fixed in 4 % phosphate-buffered saline (PBS)-buffered formaldehyde at 4 °C. After a 3-day fixation, the maxillae were decalcified in 14 % ethylenediaminetetraacetic acid (EDTA) for 30 days at room temperature, with the solution changed the solution every 3—4 days. After decalcification, samples were dehydrated using graded ethanol in a tissue processor (TP1020; Leica, Wetzlar, Germany) and embedded in paraffin. Subsequently, they were sectioned into 4- μm sections in the horizontal plane with a microtome (Leica EG1160; Leica). To assess osteoclasts in each slice, sections at 100, 140, 180, 220, and 260 μm apical to the maxillary left first molar bifurcation area were stained for tartrate-resistant acid phosphatase (TRAP) staining. The sections were first stained with a TRAP staining kit (FUJIFILM, Osaka, Japan) following the manufacturer's instructions and then counterstained with hematoxylin. Multinucleated TRAP-positive cells on the bone surface are referred to as osteoclasts.[22]

Statistical analysis

Mean values and their standard deviations are presented. The Tukey-Kramer test was used to assess the significance of group differences, with the threshold set at $P < 0.05$.

Results

Salt-sensitive hypertension increased the distance of tooth movement

After 12 days of mechanical loading, the normal mice's tooth movement measured 127 ± 40 μm. In the SSHTN mice, the distance of tooth movement was dramatically increased to 163 ± 27 μm. The differences between the two groups were statistically significant. No significant difference was observed between SSHTN mice without OTM and those with OTM (Fig. 2C and D).

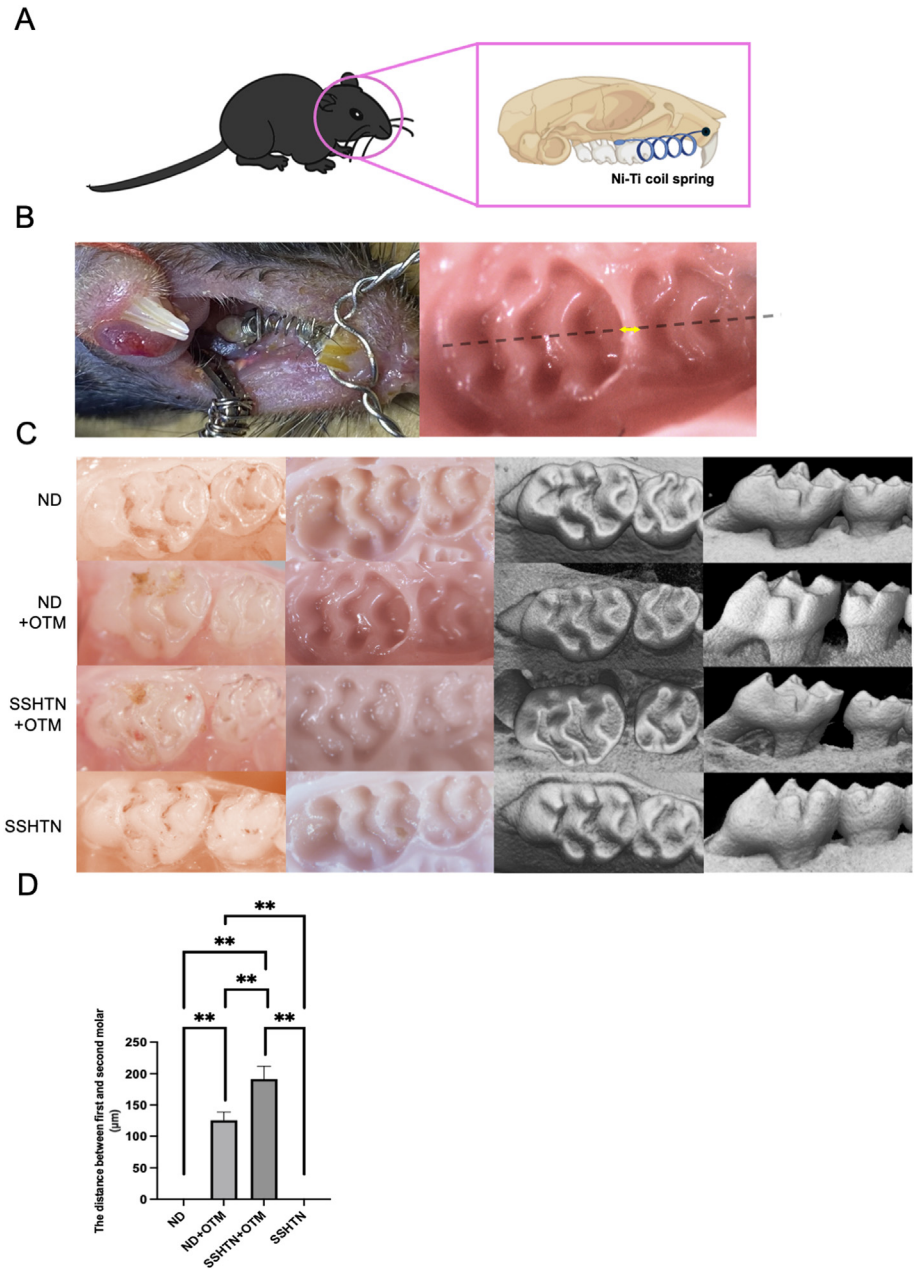

Figure 2 SSHTN increased the distance of tooth movement. (A) Schematic illustration of OTM in mice. (B) Intraoral image of a nickel-titanium closed-coil spring positioned between the upper-left first molar and the maxillary alveolar bone beneath the incisors. A 0.1 mm stainless steel wire was used to bind the appliance to the first molar at its posterior end and through holes drilled into the alveolar bone. The maxillary left first molar moved mesially due to the resulting stress. (C) Images captured intraorally of the upper left molars after the 12-day experimental loading period. Photograph of the silicone impression after tooth movement. Typical μCT pictures that make the experimental tooth movement for every group more understandable. Sagittal (right) and occlusal (left) images were acquired. (D) Comparison of tooth movement among the four groups. n = 4/group. $^{**}P < 0.01$. μCT, micro-computed tomography; ND, normal diet; OTM, orthodontic tooth movement; SSHTN, salt-sensitive hypertension.

Salt-sensitive hypertension enhances osteoclast formation during tooth movement

No TRAP-positive osteoclasts were observed along the alveolar bone on the mesial side of the roots in the control group. In mice on a regular diet, osteoclasts were found along the alveolar bone on the pressure side of the teeth, while SSHTN mice exhibited notably more osteoclasts in the same area. On Day 12, a noticeable difference was found between the groups that received SSHTN without OTM and those that did not (Fig. 3A and B).

Discussion

In this study, we explored the effects of SSHTN on OTM in a mouse model. These key findings suggest that SSHTN

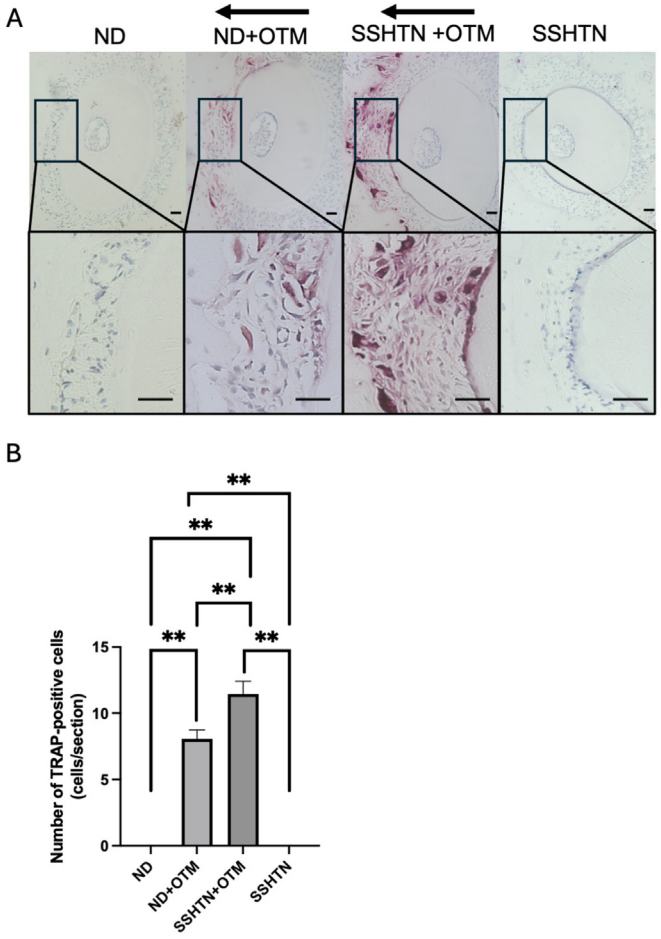

Figure 3 Assessment of transverse histological sections osteoclast activity. **(A)** Histological sections of the distal root of the left maxillary first molar stained with TRAP. The direction of movement of the teeth in orthodontics is indicated by the arrows. Scale bars = 20 mm. **(B)** Number of TRAP-positive multinuclear cells on the mesial side of the distal upper left first molar. n = 4/group. $^{**}P < 0.01$. ND, normal diet; OTM, orthodontic tooth movement; SSHTN, salt-sensitive hypertension; TRAP, tartrate-resistant acid phosphatase.

significantly influences the rate of OTM, potentially through enhanced osteoclast activity and bone remodeling processes.

The results showed that SSHTN mice exhibited a significantly greater distance of tooth movement than normal mice. This suggests that HTN may accelerate the bone remodeling process that underlies OTM. Previous research has linked HTN to changes in bone metabolism, particularly through the activation of the RAS and the increased production of pro-inflammatory cytokines such as TNF-α. These cytokines, particularly TNF-α, are known to play a crucial role in bone resorption processes by promoting osteoclast differentiation and activity. In the context of hypertension, the heightened inflammatory state may thus contribute to a more reactive periodontal microenvironment, enhancing the response to orthodontic forces.[11,23–26] The elevated osteoclast activity observed in hypertensive conditions suggests that inflammatory pathways are potentially upregulated, accelerating bone remodeling essential for effective OTM.

We also observed increased osteoclasts in SSHTN mice during the 12-day OTM period. Osteoclasts play a critical role in bone resorption, which is essential for tooth movement.[27,28] The elevated osteoclast activity in SSHTN mice suggests that HTN enhances bone resorption and accelerates OTM. This aligns with previous studies showing that hypertensive conditions can increase osteoclastogenesis, possibly through the upregulation of inflammatory pathways, particularly those involving key cytokines like TNF-α and interleukins that are known to promote osteoclast differentiation and function.[11,15] The potential upregulation of these inflammatory pathways under hypertensive conditions provides a plausible mechanism through which hypertension could influence the microenvironment of the periodontal ligament, thereby enhancing the response to orthodontic forces.

The epidemiology of SSHTN patients in Japan and its influence on orthodontic care could also play a significant role in the clinical translation of our findings. With an increasing number of patients with HTN undergoing orthodontic treatment, understanding how HTN affects OTM is crucial for tailoring treatment plans. The accelerated tooth movement observed in the SSHTN mice suggests that patients with HTN may experience faster OTM, leading to shorter treatment times. However, this rapid tooth movement may also increase the risk of unwanted side effects,

such as root resorption or periodontal damage. Clinicians should consider these risks when planning treatment for patients with HTN and may need to adjust force application strategies accordingly.

This study highlighted the impact of SSHTN on OTM, demonstrating that hypertensive conditions can enhance tooth movement, likely through increased osteoclast activity. These findings suggest that HTN should be carefully considered in orthodontic treatment planning, and further research is needed to develop optimized strategies for managing orthodontic care in patients with HTN. While the study provides valuable insights, certain limitations should be noted. Although informative, using a mouse model may not fully capture the complexities of human physiology, and the focus on SSHTN represents only one of the many types of HTN. Future research should explore other forms of HTN and investigate the underlying molecular mechanisms in detail, including human subjects, to confirm the translational relevance of these findings.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

This work was supported in part by JSPS KAKENHI grants from the Japan Society for the Promotion of Science (No. 22K10236 to HK) and JST SPRING (No. JPMJSP2114 to ZF).

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