

Effects of Neodymium-Doped Yttrium Aluminium Garnet (Nd:YAG) Laser Irradiation on Bone Metabolism During Tooth Movement



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Abstract

Introduction: The aim of this study is to evaluate the effects of low-level neodymium-doped yttrium aluminium garnet (Nd:YAG) laser irradiation on orthodontic tooth movement and histological examination.

Methods: Eleven male Wistar rats (aged 10 weeks) were included. To produce experimental tooth movement in rats, 10 g force was applied to maxillary first molars with nickel titanium closed coil springs. Right molars were irradiated with Nd:YAG laser on days 0, 1, 2, 3, 7, 10, 14, 17, 21 and 24, while un-irradiated left molars were used as control. Distance between mesial side of second molar and distal side of first molar was measured on μ CT image during tooth movement and the rats were sacrificed 4 weeks after the initiation of tooth movement.

Results: The amount of tooth movement was significantly greater in the irradiation group (0.20 ± 0.06) than in the control group (0.14 ± 0.03) during the first week ($P < 0.05$). However, no statistically significant difference was found afterwards. There was a tendency of higher tartrate-resistant acid phosphatase (TRAP)-positive nuclei count in the pressure zones of the laser irradiation group, but it was not statistically significant. In immuno-histological examination, expressions of alkaline phosphatase (ALP) and receptor activator of nuclear factor kappa-B ligand (RANKL) were higher at the pressure site of the laser irradiation group than the control group, whereas there was no difference in osteoprotegerin (OPG) expression.

Conclusion: The results suggest that low-level Nd:YAG laser may stimulate osteoclast and osteoblast activation and accelerate bone metabolism during tooth movement.

Keywords: Nd:YAG laser; Movement, Tooth; LLLT; RANKL.

Introduction

Orthodontic treatment involves moving tooth by force application and it requires a period of time. Prolonged orthodontic treatment can be a threat to oral hygiene and a source of trauma to oral tissue. Shortening the treatment time becomes one of the challenges for orthodontists. Several approaches have been described in the literature. Köle et al¹ reported that corticotomy accelerated experimental tooth movement in rats. Also Tuncay et al² and Young et al³ reported that fibrotomy could achieve similar results in rats. Kanzaki et al⁴ reported that local gene transfer of receptor activator of nuclear factor kappa-B ligand (RANKL) may activate osteoclast genesis and accelerate the tooth movement in periodontal tissue of rats. Also, it has been suggested that many substances such as platelet-poor plasma (PPP), platelet rich plasma (PRP), nitric oxide (NO) and prostaglandin E₂ (PGE₂) can accelerate experimental tooth movement. However, the side effect remains a major problem, making clinical application difficult. Chen et al⁵ reported that electro-

magnetic wave treatment accelerated tooth movement in guinea pigs without adverse effects. Furthermore, it is shown by Nishimura et al⁶ that by applying vibration, accelerated tooth movement can be achieved in beagle dogs without harm. Despite the fact that various methods have been shown to accelerate teeth movement, corticotomy is the only one applied in present. Nevertheless, the surgery represents a great burden for patients and there is also risk of tissue necrosis or infection. Therefore, a safer method is desired for orthodontic treatment.

The effect of laser irradiation on orthodontic tooth movement has been attracting much attention over the past few years. Although it is indicated by numerous reports that tooth movement can be accelerated by diode laser irradiation,⁷⁻⁹ there are only a few studies focused on other lasers. Ninomiya et al¹⁰ revealed that neodymium-doped yttrium aluminium garnet (Nd:YAG) laser irradiation increased bone formation in femur metaphysis. Their results have shown the possibility of altering bone metabolism by laser irradiation. Therefore, in this experiment,

we intended to examine the histological and molecular biological effects of Nd:YAG laser irradiation on experimental tooth movement.

Nd:YAG laser is a near-infrared (NIR) laser which typically has a wavelength of 1064 nm. Infrared lasers have low absorption coefficient in hemoglobin and water, hence a high penetration depth from tissue surface. Low-level laser chromophore can be found in protein complexes (cytochrome oxidase, NADH dehydrogenase) which carry out redox reactions that lead to energy release and ATP synthesis.¹¹ Absorption of specific wavelengths of low-level laser can stimulate the electron transport chain and accelerate the synthesis of ATP.¹² Due to above characteristics, low-level Nd:YAG laser can reach the sub-epithelial tissues and induce the proliferation of various cells. In the present study, the effects of low-level laser therapy (LLLT) using Nd:YAG laser on tooth movement were investigated metrically and histologically.

Methods

Animals

The animal experiment protocol in this study was approved by the Ethics Committee for Animal Experiments at the Hiroshima University, School of Dentistry. Eleven male Wistar rats (aged 10 weeks) were used (Charles river laboratories, Yokohama, Japan). All rats used in this study were handled in conformity with the rules for animal experiments, Hiroshima University. The rats were kept in the animal center using 50%-60% humidity cages in a 12-hour light/dark environment at a constant temperature of 22-24°C and provided with food and water.

Experimental Tooth Movement

The orthodontic appliance was composed of custom-ordered nickel titanium closed coil springs (SENTALLOY, Tomy International, Tokyo, Japan) linked to upper first molars by stainless steel ligature wires (0.008 in, Tomy) stretched to achieve 10 g and tied to upper incisors with second ligature wires (Figure 1). Composite resin (Unifil flow GC, Tokyo Japan) was used to cover the maximum incisors and ligature wires. The duration of tooth movement lasted 4 weeks.

Laser Irradiation

The Nd:YAG laser (1064 nm, Inpulse, Incisive, Richmond, Ca, USA) was delivered by placing a 0.32 mm diameter optical fiber tip in contact with the mesial, buccal and pal-

atal gingiva of the migrating upper first molar. Irradiation was performed for 90 seconds at three time points (a total of 270 seconds) a day, on days 0, 1, 2, 3, 7, 10, 14, 17, 21 and 24 (a total of ten times). Total energy exposure was 54 J, which is the same dosage used in a previous study.¹³ The un-irradiated left molars were used as control.

Measurement of Tooth Movement by μ CT

Computed tomography (CT) images were taken using a μ CT machine (Sky Scan 1176, Bruker microCT, Kartuizersweg kontich, Belgium). The images were taken in vivo and the pixel size of the CT was 35 μ m. Each rat was evaluated (n = 11) under general anesthesia every week up to the fourth week after commencing tooth movement. The distance between mesial side of second molar and distal side of upper first molar on the CT image was measured by using analysis data viewer software (Bruker microCT).

Tissue Processing

Maxilla was resected under deep anesthesia with diethyl ether 4 weeks after tooth movement. The specimens were fixed by formaldehyde, decalcified in 14% EDTA and embedded in paraffin. Sagittal serial sections of 5 μ m were made by rotary microtome (Microm325, Carl Zeiss, Oberkochen, Germany) and stained with hematoxylin-eosin or tartrate-resistant acid phosphatase (TRAP). TRAP staining was performed using a TRAP staining kit (Wako, Tokyo, Japan). Counting of osteoclasts nuclei was performed at tissues mesio-coronally and disto-apically to the mesial root in each selected section. The overall size of measurement area was 1175 μ m \times 815 μ m. The expression levels of RANKL, osteoprotegerin (OPG) and alkaline phosphatase (ALP) were observed by immunohistochemistry staining. In brief, primary antibody of RANKL (1:100 dilution, Santa Cruz, sc-7628, Heidelberg, Germany), OPG (1:50 dilution, Santa Cruz, sc-11383, Heidelberg, Germany) and ALP (1:500 dilution, abcam, ab108337, Tokyo, Japan) were used with Vectastain Elite ABC staining system (Vector Laboratories, Burlingame Ca, USA). Finally, the slides were counterstained with Carrazzi's Hematoxylin Solution (Wako, Tokyo, Japan). The images were captured and analyzed by image analysis software (BZ Analyzer software BZ-H1A, Keyence, Japan, Osaka).

Statistical Analyses

The difference in tooth movement between the two groups was evaluated using student *t* test. A statistically significant difference was set if resulting *P* value was less than 0.05.

Results

Amount of Tooth Movement

Space was determined between the first and second molars before and after this experimental tooth movement (Figure 2A arrows). The amount of tooth movement was significantly greater in the irradiation group (0.20 ± 0.06) than in the control group (0.14 ± 0.03) during the first

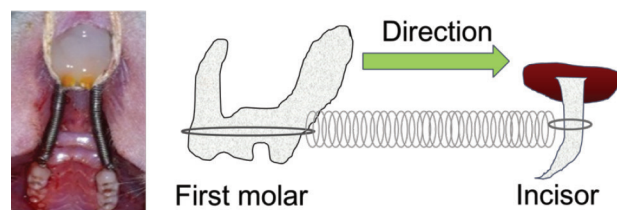


Figure 1. Shows an apparatus of tooth movement in the photograph and schematic diagram.

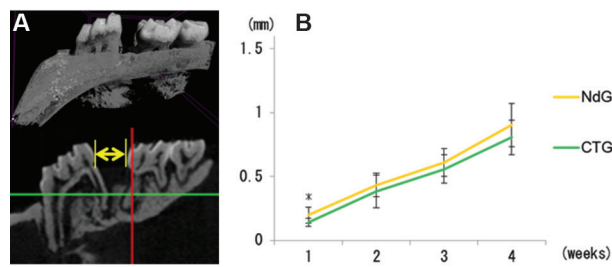


Figure 2. (A) The distance between mesial side of second molar and distal side of maxillary first molar on the CT image was measured (arrows). (B) The comparison of distance amounts in Nd:YAG laser and control groups (* $P < 0.05$ vs. control).

week (Figure 2B). However, no statistically significant difference was found in tooth movement rate afterwards.

Histo-Pathological Observation

TRAP Staining

At the fourth week of tooth movement, there were TRAP-positive nuclei present at the pressure zones in both groups (Figure 3). A tendency of higher TRAP-positive nuclei number was observed in the pressure zones of the laser irradiation group; however it was not statistically significant (Figure 4). Also, mesio-coronal area showed higher counts than disto-apical area, in both groups.

HE (Hematoxylin and eosin) Staining and IHC (Immunohistochemical) Staining

Teeth moved by crown tipping in all groups. Irregularity of periodontal ligament fibers and absorption of alveolar bone were found at the pressure site of mesio-coronal area and disto-apical area, regardless of laser irradiation (Figure 5 a1-a5). Expressions of ALP and RANKL were higher at the pressure site of the laser irradiation group compared to the control group (Figure 5 b1-b4, d1-d4). Whereas there was no difference in OPG expression between the control group and the laser irradiation group (Figure 5 c1-c4).

Discussion

Rat is in the suckling period about 3 weeks after birth, then up to 10 weeks is juvenile, and after that begins a second-order growth period until the growth is finished, approximately at the age of 14 weeks. The age of 10-14 weeks of the rats set in this study corresponds to the adolescence in human. This period encompasses the time point to perform fixed orthodontic appliances in orthodontic treatment. It is considered to be reasonable weeks of age to investigate teeth movement.

The total area of upper first permanent molar of the human is around 360 mm², which is 34 times than that of the rat. Therefore a force of 10 g used in a rat molar may correspond to 340 g in a human molar.¹⁴ According to Begg, the optimal force to move a human molar is 300–500 g.¹⁵ Gonzales et al¹⁶ reported that the result of performing the teeth retraction at 10, 25, 50, 100 g in rats showed a significantly greater amount of tooth movement

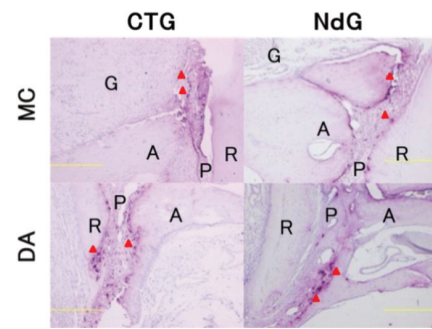


Figure 3. Shows the TRAP-stained image. G: gingiva, A: alveolar bone, R: root, P: periodontal tissue.

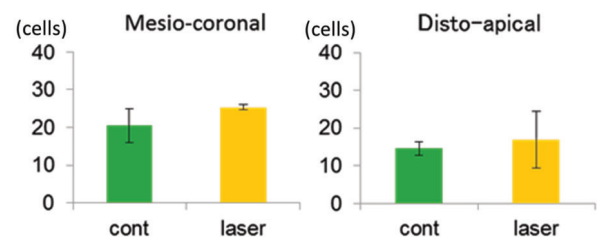


Figure 4. The TRAP-Positive Nuclei Number: Measuring Results Graph.

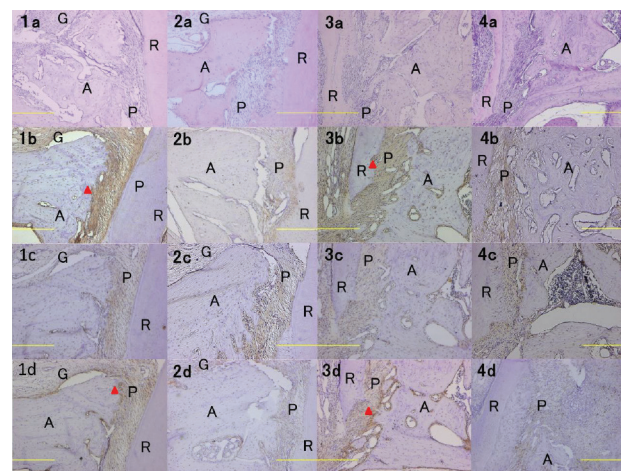


Figure 5. Shows the immunostaining images. G: gingiva, A: alveolar bone, R: root, P: periodontal tissue, a: H-E, b: RANKL, c: OPG, d: ALP, 1,2: mesio-coronal, 3,4: disto-apical.

for the group receiving the experiment for 4 weeks at 10 g, compared to the other groups. It is also reported that the amount of root resorption was the least in 10 g group. Therefore, the Ni-Ti closed coil that applies a force of 10g was used for 4 weeks in the present study.

Lasers with wavelength of 600~1200 nm are often used in biomedical treatment as it exerts optimal tissue permeability. Hamblin et al¹⁷ reported that NIR photons are absorbed in cell mitochondria, producing reactive oxygen species (ROS) and NO, which in turn activate transcrip-

tion factors (NF- κ B and AP1). Karu et al¹⁸ also reported that cellular homeostasis parameters such as intracellular pH, calcium concentration, cAMP level, and ATP concentration could be mediated by photoreceptors in the mitochondrial respiratory chain. It is shown that multi-nucleated cells have high mitochondrial activity, and cytochromes are responsible for photon energy absorption and ATP synthesis, improving the potential of cell activity.¹⁹ Therefore laser irradiation may have direct influence on osteoclast.

A variety of factors including the type of laser, the output, the area of irradiation and the animal species used could be determinant in the response of certain energy setting. Goulart et al²⁰ reported that a dosage of 5.25 J/cm² accelerated orthodontic movement but 35 J/cm² retarded the orthodontic movement in dog. Limpanichkul et al²¹ suggested that 25 J/cm² was too low to express any effect on orthodontic tooth movement. On the other hand, Altan et al²² reported that the number of osteoclasts, osteoblasts, inflammatory cells and new bone formation in rats significantly increased with a dosage of 1717.2 J/cm², compared to 477 J/cm². Nevertheless, the optimal setting for accelerating tooth movement has yet to be determined. The dosage used in this study was 249 J/cm² and we consider it to be within the median range. In terms of total energy, Kawasaki et al¹³ significantly accelerated tooth movement by using a total of 54 J. We used the same total energy setting in our experiment. Further researches are needed for examining various factors such as wavelength, the irradiation time, type of lasers, the output or the area of irradiation involved.

Fujita et al²³ indicated that on day 7, the amount of tooth movement was significantly greater in the LLLT group (1.5 fold). Kawasaki et al¹³ also reported that in laser irradiation group, the amount of tooth movement was 1.3 fold than that of the non-irradiation group. In the present study, the amount of tooth movement was significantly greater in the irradiation group at day 7, but not at day 14, 21 and 28 (Figure 2). It is shown that the Nd:YAG laser is affecting the early tooth movement in this experiment. This may have been caused by frequent irradiation at the initial period. However it may not last if we extend the irradiation interval. The irradiation methods varied among all investigations, therefore reported different results. An appropriate setting of irradiation number, energy density and irradiation time is needed for each type of laser, to facilitate the most efficient tooth movement.

Ninomiya et al¹⁰ reported that pulsed laser irradiation increased the expression of ALP, which is similar with our result. Ogasawara et al²⁴ emphasized the importance of RANKL-OPG ratio and their interaction for bone remodeling during tooth movement. Yamaguchi et al²⁵ reported that LLLT stimulated the velocity of tooth movement via M-CSF/c-fms and RANKL expression, as well as osteoclast precursor cells activation. In this study, higher RANKL expression was observed in laser irradiation side, while no change was noticed in the expression of OPG. Thus, a higher RANKL/OPG ratio is expected. It is con-

sidered possible that it lead to activation of osteoclasts. Overall, it is suggested that low power Nd:YAG laser may influence osteoclast and osteoblast activation of bone metabolism in early phase of experimental tooth movement.

Conclusion

Low-level Nd:YAG laser may stimulate osteoclasts and osteoblasts and accelerate the bone metabolism in early phase of experimental tooth movement.

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Conflicts of Interest

The author has no conflict of interest to declare.

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