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Effects of two wattages of low-level laser therapy on orthodontic tooth movement

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ABSTRACT

Introduction: Mixed outcomes have been found in animal and clinical studies with regard to the use of low-level laser therapy (LLLT) as a modality to accelerate orthodontic tooth movement (OTM). One major reason for the variable findings is the different methodologies and protocols for laser therapy use.
Objective: The aim of this study was to determine whether orthodontically moved molars exposed to two different wattages at the same energy density of LLLT exhibited differences in the amount of tooth movement and molecular and histological changes in the adjacent periodontal areas.
Methods: An orthodontic force was applied to rat upper first molars exposed to 500 mW (EX-500) and 1000 mW (EX-1000) of laser application, with a control group (CT) with no laser application. Gene expression in the periodontal ligament (PDL) and histology of the palatal gingiva of the molars were analyzed.
Results: There was a statistically significant difference for OTM between EX-500 but not between EX-1000 and CT groups. *RANKL* and *MMP-13* expression levels in the PDL of orthodontically moved molars, however, were increased significantly in laser-exposed groups compared to CT. Early signs of dysplasia were observed in over half of the animals in the EX-1000 group.
Conclusions: Our results provide evidence for molecular changes and the potential dysplastic effects of laser on the surrounding soft tissues. Further studies are needed to better identify an optimum laser protocol to maximize the desired effect.

1. Introduction and literature review

Various strategies have been used in attempts to increase the rate of orthodontic tooth movement (OTM). Several surgical methods, chemical agents and physical stimuli have been reported to increase the rate of OTM, including corticotomy, micro-perforation, micro-piezocision and use of vibrational forces, magnetic fields, and electric current (Huang, Williams, & Kyrkanides, 2014; Kim et al., 2010; Marquezan, Bolognese, & Araújo, 2010). Although some surgical techniques have been shown to be effective and predictable, they are invasive and can cause postsurgical discomfort and possible postoperative complications. The difficulties with chemical agents are possible negative systemic effects and routes of administration which are algesic (Torri & Weber, 2013). One potential therapy that avoids harmful systemic or local effects on the PDL is the use of laser (light amplification by stimulated emission of radiation) with its greater accuracy for specific and local applications.

Photobiostimulation is a term used to describe changes in tissues upon laser application with energy densities less than 500 mW, a level below those required for ablation, cutting, and thermally coagulating tissue (Reza, Katayoun, Farzaneh, & Tadayon, 2011). Described under numerous terminologies, e.g., low-level laser therapy (LLLT), cold or soft laser, photobiostimulation is used extensively in a number of clinical applications, producing changes in physico-chemical properties with little or no temperature change in target tissues (Cernavin, Pugatschew, de Boer, & Tyas, 1994). The most common light sources for photobiostimulation are the He-Ne, argon, and diode lasers (Kravitz & Kusnoto, 2008). Important factors to consider when using phototherapy are adequate wavelength (to ensure energy absorption of target tissue), wattage and duration of laser exposure, both of which influence energy and power densities (Niemz, 2007; Reza et al., 2011). Laser wavelengths used in dentistry fall within the range of 488 to 10,600 nm (Nalcaci & Cokakoglu, 2013) and tissue

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interactions occur within the energy range of 1–1000 J/cm^2 (Niemz, 2007). Laser therapy has been used in dentistry, e.g., caries detection, tooth desensitization, composite resin curing, sterilization of infected root canals, and in soft tissue procedures (Cernavin et al., 1994; Walsh, 2003). In the field of orthodontics, LLLT has been used in the treatment of pain reduction after orthodontic appliance placement and for temporomandibular joint disorders, bone regeneration after rapid palatal expansion, and in increasing the rate of OTM (Cepera et al., 2012; Reza et al., 2011; Torri & Weber, 2013 Torri & Weber, 2013).

A number of animal and human studies have been performed with diode lasers to alter the rate of OTM (reviewed in Chung, Milligan, & Gong, 2015). Overall, conflicting results of LLLT's effects on OTM in both animal and human studies exist, reflecting the extensive variability in the experimental parameters used between studies. Thus, while the diode laser has the benefits of being non-invasive, safe, painless and cost effective, its true value as a modality to increase tooth movement remains controversial in the literature (Torri & Weber, 2013). The aim of this study, therefore, was to test whether there was a difference in the effects of LLLT therapy delivered by two different wattages at the same energy density on the amount of tooth movement, expression level changes of genes involved in bone remodelling in the PDL, and histological changes in the adjacent gingiva.

2. Methods and materials

2.1. Animals

The animal experimental protocol in this study was approved by the University Animal Care Committee at the University of Toronto (approval no. 20010570). A total of 27 male Wistar rats (150–175 g) were used. Mashed food and water *ad libitum* were provided, with no chew toys to reduce the risk of appliance failure. The weights of all 27 rats were recorded at the initial time-point and every other day before irradiation up to the day 14 end-point (Table 1).

2.2. Orthodontic tooth movement

The 27 rats were divided into three groups, control (CT; n = 8) and two experimental groups subjected to 500 (EX-500; n = 10) and 1000 (EX-1000; n = 9) mW of LLLT irradiation. To allow unlimited access to the oral cavity during placement of an orthodontic coil spring, the rats were placed under intraperitoneal general anesthesia (GA) with 90 mg/kg Ketamine and 5 mg/kg Xylazine. In all three animal groups, a 25 g NiTi coil spring (GAC Dentsply) was measured with a dynamometer to generate a force of 10 g (equivalent to 11 mm islet to islet) and checked intra-orally to ensure adequate activation. The coil was placed according to previous reports (Fujita, Yamaguchi, Utsunomiya, Yamamoto, & Kasai, 2008; Yamaguchi et al., 2010; Yoshida et al., 2009) for 14 days between the first molar and the incisors on the left side to induce mesial movement of the molar (Fig. 1I). Proper activation of coil was checked every other day. The lower incisors were trimmed in all groups at the start and throughout the experimental period to relieve occlusion.

2.3. Laser irradiation and parameters

The Picasso Lite diode laser (a gallium-aluminium-arsenide diode laser, wavelength of 810 nm; AMD Lasers, Dentsply, GAC) was used in a continuous mode at 500 mW (lowest possible setting) and 1000 mW with a 0.4 mm diameter beam. This laser was chosen because it is one of the most readily available and widely used diode lasers in Canada (e.g., for soft tissue procedures), thus increasing the clinical applicability of this study. The determination of the parameter of interest, i.e., energy density (J/cm^2 or W × s/cm^2) was calculated based on four published studies that showed increased OTM in rats with LLLT (Fujita et al., 2008; Kawasaki & Shimizu, 2000; Yamaguchi et al., 2010; Yoshida et al., 2009). Therefore, the exposure times of laser application to generate 19098.6 J/cm^2/tooth at 500 and 1000 mW was calculated to be 24 and 12 s each for the buccal and palatal gingiva, respectively.

2.4. Laser exposure

The laser beam was applied through a 0.4 mm diameter optical fiber. The fiber tip was held within 1 mm from the gingiva and moved in a circular motion over the palatal and buccal root surfaces for a total of 24 s and 48 s for EX-1000 and EX-500, respectively. No irradiation was applied to the control groups subjected to the same orthodontic forces. The experiment was run for 14 days with 7 LLLT sessions (every other day starting on the day of coil spring placement and activation). LLLT was performed under inhalational GA with isoflurane at 4% for induction and 2–2.5% for maintenance at 1L/min oxygen.

2.5. Measurement of orthodontic tooth movement

To measure tooth movement amount, polyvinylsiloxane (PVS) impressions, taken at appliance activation and end of the experiment, were viewed under a dissecting microscope (Fig. 1II). The midpoints of the gingival margin between the buccal and lingual surfaces of the first and second molars in the final impressions were marked and the distance was measured with an electronic caliper (Fig. 1II). All measurements were performed under the dissecting microscope by two different observers at two different times (MM and another independent investigator blinded to the study).

2.6. Tissue preparation

After 14 days, the rats were euthanized, the coil spring removed and the left first molar extracted using a half Hollenback instrument. Extracted molars were immediately stored in liquid nitrogen and then transferred to −80 °C for subsequent collection of PDL cells for RT-qPCR. After extraction, the palatal tissue adjacent to the extracted tooth was excised and stored in Bouin's fixative solution (Sigma-Aldrich) for histological analysis.

Table 1
Mean body weights and total weight gain of control and experimental groups at initial (Day 1) and final (Day 14) (mean weight gain ± standard deviations).

	Mean Initial Weight (±SD) (g)	Mean Final Weight (±SD) (g)	Mean Weight Gain (±SD) (g)
CT	211.9 (±10.2)	306.0 (±12.6)	94.1 (±10.1)
EX-1000	212.3 (±9.2)	312.0 (±18.9)	99.7 (±12.7)
EX-500	221.4 (±10.1)	317.0 (±16.1)	95.6 (±12.4)