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Original Article

Histological evaluation of Labrador periapical tissues following erbium-doped yttrium aluminum garnet laser irradiation

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Abstract: The aim of this study was to investigate the thermal effects of intra-canal irradiation by an erbium-doped yttrium aluminum garnet (Er:YAG) laser at different powers on *Labrador* periapical tissues as based on the antibacterial analyses of *Enterococcus faecalis* and *Escherichia coli* in root canals with an isthmus, to assess histological changes, and to select the optimal antibacterial power range for clinical applications. Two hundred root canals from 10 healthy adult *Labradors* were selected and divided into 5 groups. In addition to one as control group, root canals in 4 other groups were irradiated with an Er:YAG laser at 1.5, 2.0, 2.5, 3.0 W for 30 s. 10 *Labradors* were sacrificed at 0 (immediately after), 2 days, or 2, 4, and 8 weeks. After the preparation of pathology specimens, histological changes after laser irradiation on periapical tissues were evaluated and inflammation scores were analyzed. No significant differences were observed for the apical areas between the 1.5, 2.0, 2.5 W and control groups for most of periods ($P>0.05$), while significant differences were observed between the 3.0 W and other groups for all periods ($P<0.05$). These results suggest that at specific output powers (2.0 W~2.5 W), disinfection of root canals can be successfully completed and with minimal thermal effects on the periapical tissues.

Keywords: Animal experiments, endodontics, Er:YAG laser, histological study

Introduction

Root canal treatment (RCT) is currently an essential and primary therapy in endodontics. Although traditional RCT methods such as mechanical preparation and chemical irrigation are effective, they cannot eliminate microorganisms entirely [1]. A large amount of bacteria are stubborn, highly fecund and difficult to completely remove. Such bacteria mostly consist of gram-negative bacteria [2], survive in the lateral canals, canals with isthmus and in curved canals that are difficult to access with traditional instruments and medicines [3]. Therefore, the success of RCT is limited.

Lasers, including Er:YAG laser, have been studied extensively as a new method in many antibacterial experiments. As an infrared ray laser, Er:YAG laser has a wavelength of 2.94 μm, which is easily absorbed by water and hydroxylapatite [4]. The accepted mechanism of ablation is micro-explosion, which is caused by rapid vaporization of water in a short time along tooth structure [5]. Studies demonstrate that Er:YAG laser cannot only wipe out pathogenic microorganisms such as *Enterococcus faecalis* and *Escherichia coli* [6, 7], but also remove smear layers and remnants [8], which is regarded as a potential sterilization therapy [9].

However, the thermal damage caused by Er:YAG laser on periapical tissues has become a major concern. Studies indicate that alveolar bone is sensitive to temperatures above 47°C, and 60°C can cause obvious necrosis when vascular flow suppression [10]. Some experiments illustrate that the changes of temperatures caused by laser irradiation are related to many aspects such as different powers, frequency, irradiation time and the working tip shape [11]. Therefore, optimal laser parameters are the precondition for root canal disinfection without thermal damage and the effects of Er:YAG laser

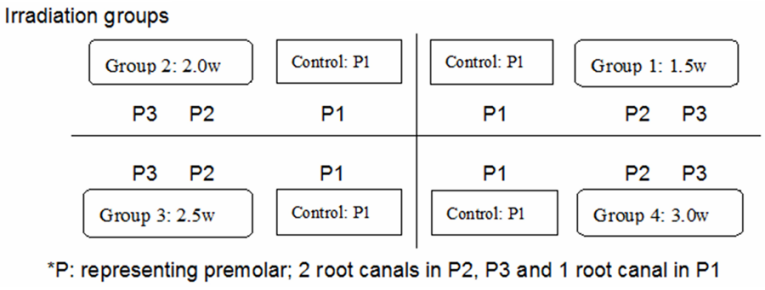

Figure 1. Illustration of groups divisions. Each group including control group consist of 4 root canals in every *Labrador*, respectively. 2 root canals in the second premolar (P2) and the third premolar (P3). 1 root canal in the first premolar (P1). P1 belonged to control group. P2 and P3 belonged to 1.5 W, 2.0 W, 2.5 W and 3.0 W group, respectively.

irradiation on periapical tissues should be assessed seriously before clinical use. The aim of this study was to investigate the thermal effects of intra-canal irradiation by Er:YAG lasers at different powers on the *Labrador* periapical tissues in regards to the antibacterial effects on *Enterococcus faecalis* and *Escherichia coli* in root canals with an isthmus, to assess histological changes, and to select the optimal power range for antibacterial clinical applications.

Material and methods

Laser system and irradiation conditions

This experiment was performed with a dental laser system (*Syneron Medical Ltd., Yokneam, Israel*). An Er:YAG laser was emitted at a wavelength of 2.94 μm from a cone tip (19 mm, tip diameter 0.4 mm) and a freely rotated handle with a spray coolant of sterile distilled water was used. The parameters used were 150, 200, 250, 300 mJ/pulse (10 Hz) and output energies at the tip were 1.5 W, 2.0 W, 2.5 W, 3.0 W, respectively, which was based on the antibacterial experiment of *Enterococcus faecalis* and *Escherichia coli* in root canals with isthmus. The laser tip was placed at the orifice of root canals and kept stationary for 30 s, with a 5 s interval after each 15 s of irradiation. The canals were filled with sterile distilled water using sterile syringes before the irradiation. The spray cooling device was able to infuse sterile distilled water into the canal continually throughout the entire procedure.

Animals

This study was approved by the Animal Care and Use Committee of the Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University. Filtrated by X-ray, 120 healthy premolar teeth (200 root canals) without immaturity and perforation in 10 healthy male *Labradors* (1.5 years old, 12-15 Kg) were selected in the experiment.

Experimental procedure

This study was performed after anesthetizing animals with intravenous administration of Nembutal (0.5 ml/Kg, *Animal Laboratories, Shanghai, China*). Then 200 root canals were divided into 5 groups (**Figure 1**). The crown and the surrounding areas of each tooth were disinfected by 1% iodine after rubber dam placement. Cavity access was prepared with a high speed turbine and a diamond bur (*TR-11, NSK, MACH-QD, Japan*) from the buccal and occlusal side, with as little destruction of occlusal surface as possible. After extirpation of the pulp with a barbed broach, the working length of root canals was established 1 mm short from the apical foramen with an apex locator. Each root canal was set up to at least 40# with K-file (*K3, Ni-Ti root canal files, 06 taper, TCM EndoIII*) and irrigated with 5.25% sodium hypochlorite (NaOCl), 3% hydrogen peroxide (H_2O_2) and 0.9% normal saline. After laser irradiation, all canals were filled with calcium hydroxide paste and cavities of crowns were filled with glass-ionomer cement. The control group was treated in the same way except without irradiation.

Pathological specimen preparation

Labradors were sacrificed at 0 (immediately after), and 2 days, or 2, 4, and 8 weeks after treatment. The maxillary and mandibular bone with teeth was excised intact and fixed in 10% formalin for 1 week. After fixation, samples were decalcified for 2 weeks, dehydrated in graded series of alcohol and embedded in paraffin. Specimens were sectioned to a thickness of 5 μm vertically (bucco-lingually) and stained with hematoxylin and eosin (H&E). The morphological changes of periapical lesions were observed under light microscope. For each canal, at least 5 serial sections and from the same sample were made the highest score of inflammation was selected.

Table 1. Criteria for degree of inflammation

Score	Criteria
0	No inflammation at the periapical region
1	Mild inflammation at the periapical region or regeneration of bone
2	Moderate inflammation (lymphocytes, polynuclear cells, and macrophages) at the periapical region, or regeneration of bone
3	Severe inflammation (including resorption of bone or root) at the periapical region, and no regeneration of bone

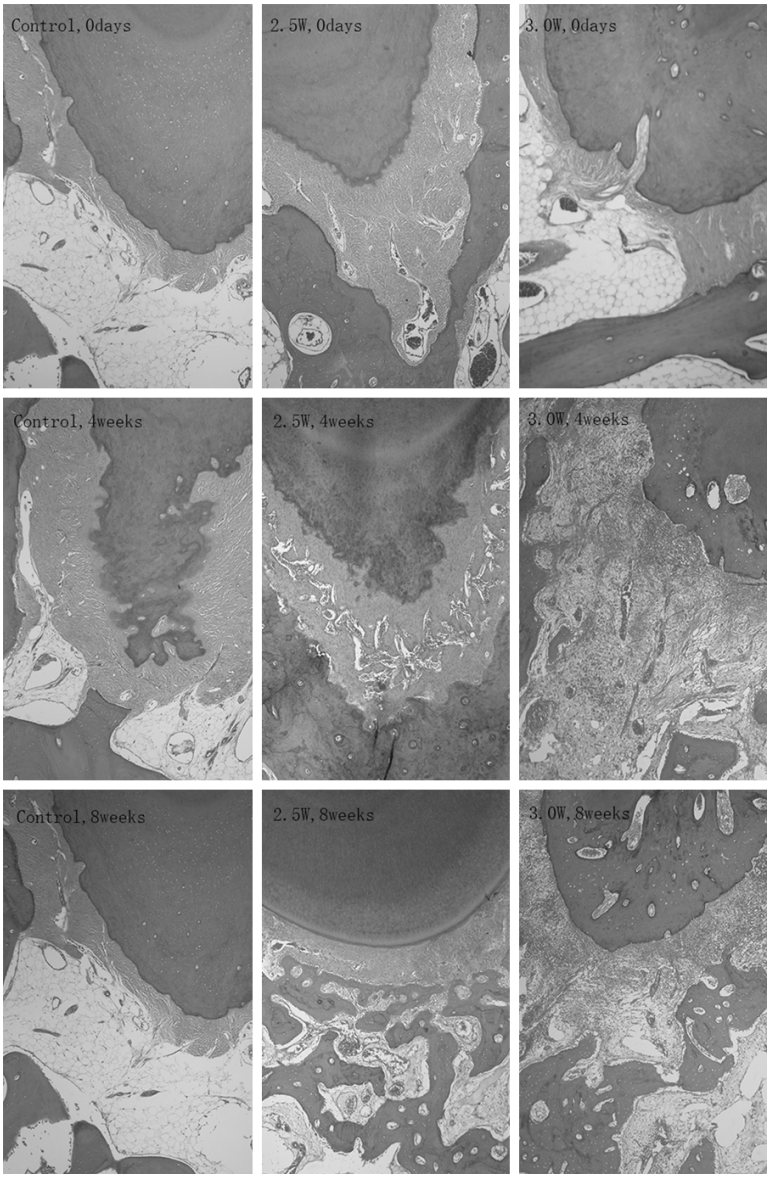

Figure 2. Representative photographs of the control, 2.5 W, and 3.0 W group in the periapical region at different periods (immediately, 4 weeks and 8 weeks) (H&E, ×4 objective). Free inflammation in the periapical region and non-resorption in the cementum or alveolar bone were observed in the control group at all periods, in 2.5 W and 3.0 W group immediately after irradiation. At 4 weeks, mild inflammation with reactive regeneration of cementum and reconstruction of fibers, vessels and alveolar bone were observed in 2.5 W group. Severe inflammation with dense infiltration of neutrophils and resorption of alveolar bone appeared in 3.0 W group. At 8 weeks, free inflammation and regeneration of the alveolar bone were observed in 2.5 W group. Moderate inflammation with infiltration of lymphocytes and regeneration of alveolar bone appeared in 3.0 W group.

Histological evaluation

Thermal effects on the periapical tissues were observed under a light microscope. The following indexes were assessed: presence or absence of inflammation and the extension at the apical area; hemorrhage of periapical tissues; resorption or regeneration of cementum and alveolar bone. The degree of inflammation of each specimen was evaluated with qualitative criteria (**Table 1**) [12, 13]. Statistical analysis were performed with the *Mann-Whitney U* test to identify differences between irradiation and control groups, and the *Kruskal-Wallis* test among all groups. A value of $P<0.05$ was considered significant.

Results

Figures 2, **3** and **Table 2** show the results of histological evaluation of periapical tissues at each time point with different laser parameters. No histological changes in periapical tissues were found in any groups after laser irradiation immediately. At day 2, less than 4 specimens displayed mild inflammation in 1.5 W and 2.0 W group, which had no differences compared with the control group. Mild to moderate inflammation were found in most cases in 2.5 W group, different from the control group *($P<0.05$)*. In 3.0 W group, moderate to severe inflammation were observed in all cases, significantly dif-

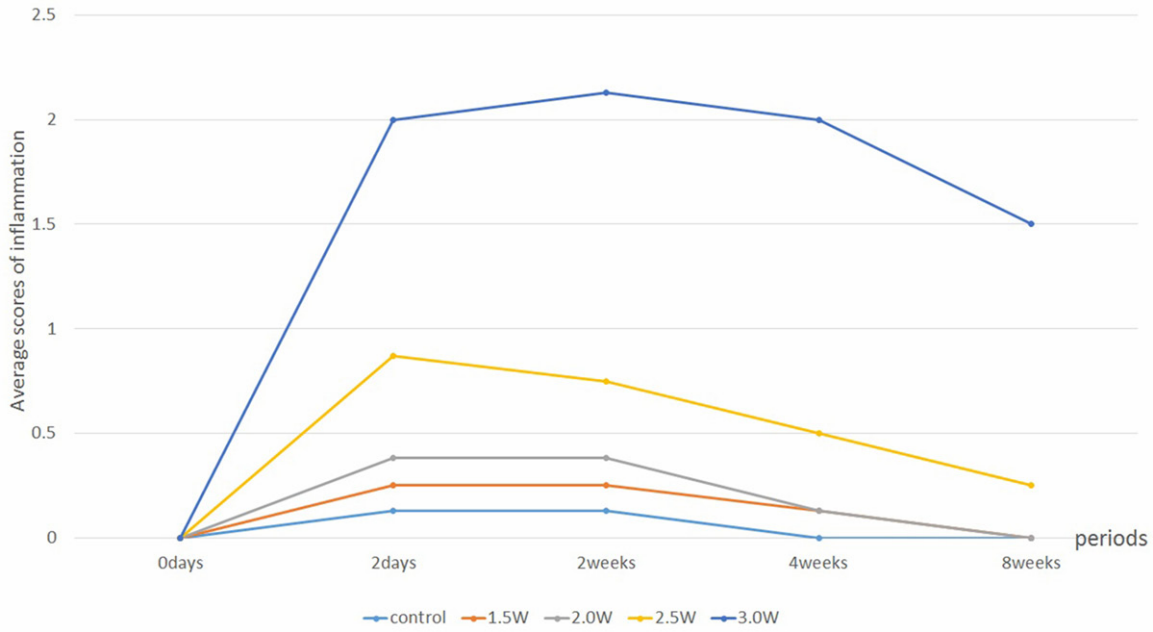

Figure 3. A tendency of average scores of inflammation on periapical region in all groups at different periods. This line chart illustrated that the average scores were all below to 1 in the control, 1.5 W, 2.0 W and 2.5 W group which represented the degree of no inflammation or mild inflammation in the periapical region. However, in 3.0 W group, average score increased to 2 at 2 days rapidly and persisted at a high score even at 8 weeks, which represented the degree of moderate to severe inflammation in the periapical region.

ferent from the control and 2.5 W group *(P<0.05)*. At 2 weeks, the score of inflammation in 2.5 W and 3.0 W groups had little changes compared at 2 days. However, only 3.0 W group had significant difference from the control group *(P<0.05)*. At 1 month, almost no inflammation was presented in the control, 1.5 W and 2.0 W group. Mild to moderate inflammation in 3 of 8 specimens were observed in 2.5 W group, which had no statistical difference from the control group. Although little changes of scores occurred in 3.0 W, it still had difference with the control group *(P<0.05)*. At 2 months, moderate inflammation in 4 of 8 specimens were still observed in 3.0 W group which differed from the control group *(P<0.05)*, while inflammation in other groups almost disappeared.

Discussion

Many studies have shown advantages of Er:YAG laser, such as significant antibacterial effects [14-21], removal of the smear layers [8], enhancing the dissolution of NaOCl [22], hemostatic effects [23] and so on. Although the features make Er:YAG laser becomes a potential method in RCT, thermal lesions resulting from energy differneces in the periapical tissues cannot be ignored. Studies illustrate that the threshold of alveolar bone survival is 47°C for 1 minute, representing approximately a 10°C increase [10], where increases of 7°C are commonly regarded as the highest biologically acceptable value in order to avoid periodontal ligament damage [24, 25]. Enhanced laser output powers can produce irreversible thermal damage to periapical tissues, while diminished power cannot guarantee the sterilization of root canals with isthmus. Therefore, optimal parameters are particularly important.

The aim of this study was to investigate the thermal effects of intra-canal irradiation with Er:YAG laser at different powers on *Labrador* periapical tissues based on the antibacterial analyses of *Enterococcus faecalis* and *Escherichia coli* in root canals with an isthmus, to assess histological changes, and to select the optimal power ranges for antibacterial and clinical applications. As the results illustrated, a laser power of 2.0 W or less caused no significant thermal damage to the periapical tissues and had no significant difference from the control group. Although mild to moderate inflammation was observed in 2.5 W group,

Table 2. Histological evaluation on the periapical regions at different periods

Period	Parameter	No. of each score				Total	Mean ± SD
		0	1	2	3		
0 days	Control	8	0	0	0	8	0.00±0.00
	1.5 W	8	0	0	0	8	0.00±0.00
	2.0 W	8	0	0	0	8	0.00±0.00
	2.5 W	8	0	0	0	8	0.00±0.00
	3.0 W	8	0	0	0	8	0.00±0.00
2 days	Control	7	1	0	0	8	0.13±0.35
	1.5 W	6	2	0	0	8	0.25±0.46
	2.0 W	5	3	0	0	8	0.38±0.52
	2.5 W	2	5	1	0	8	0.87±0.64
	3.0 W	0	2	4	2	8	2.00±0.76
2 weeks	Control	7	1	0	0	8	0.13±0.35
	1.5 W	6	2	0	0	8	0.25±0.46
	2.0 W	5	3	0	0	8	0.38±0.52
	2.5 W	3	4	1	0	8	0.75±0.71
	3.0 W	0	1	5	2	8	2.13±0.64
4 weeks	Control	8	0	0	0	8	0.00±0.00
	1.5 W	7	1	0	0	8	0.13±0.35
	2.0 W	7	1	0	0	8	0.13±0.35
	2.5 W	5	2	1	0	8	0.50±0.76
	3.0 W	0	2	4	2	8	2.00±0.76
8 weeks	Control	8	0	0	0	8	0.00±0.00
	1.5 W	8	0	0	0	8	0.00±0.00
	2.0 W	8	0	0	0	8	0.00±0.00
	2.5 W	6	2	0	0	8	0.25±0.46
	3.0 W	0	4	4	0	8	1.50±0.54

changes were reversible and only differed from the control group at 2 days. When the output energy exceeded 2.5 W, moderate to severe inflammation (lymphocytes, neutrophils and macrophages) and resorption of cementum and bone were observed at the periapical region, which differed from the control group significantly. Previously, in another study of sterilization on *Enterococcus faecalis* and *Escherichia coli* in root canals with isthmus, we found that laser irradiation was superior to mechanical preparation and chemical irrigation with higher output power. Although 3.0 W group had a better sterilization effect (99.470%, 99.878%), it had no significant difference from the 2.5 W group (99.464%, 99.579%) and damages were observed on surface and apex of root canals. For these results, we believe that laser power ranging from 2.0 W to 2.5 W can achieve bactericidal effect in root canals with isthmus without causing thermal injury on the periapical tissues of *Labradors*. Previous studies aimed to determine the thermal effects of laser irradiation on periapical tissues after sterilization of broad root canals, while our experiments aimed at to determine the thermal effects on narrow root canal system such as root canals with isthmus which supplement for the whole root canal system.

Due to difficulties in distinguishing the apical lesions from thermal damage from irradiation and original infection, we choose to examine uninfected root canals as experimental subjects in this study. Using of healthy root canals is helpful to determine apical damages by different laser parameters of sterilizing root canals with isthmus. Further, a study will be conducted on infected root canals to see if this range of laser powers can promote healing from periapical diseases.

Thermal effects on periapical tissues are not only related to laser power, but also to spray coolant, the shape of laser tips [11, 26] and time of irradiation. Meanwhile, Labradors differ from human beings in body weight, thickness of dentins, density of alveolar bone and the endurance to laser irradiation. Therefore, disinfection by Er:YAG laser irradiation of human root canals with isthmus should be considered as a potential therapy and output power ranges from 2.0 W to 2.5 W should be considered safe for human beings.

In this *in vivo* model, laser powers ranging from 2.0 W to 2.5 W not only achieved disinfection in root canals with isthmus but also decreased thermal effects on the periapical tissues to minimal. In this study, we suggest that Er:YAG laser irradiation on human root canals with isthmus should be a potential therapy. In the future, we will carry out a research of irradiation with Er:YAG laser in infected root canal *in vivo* to investigate whether the powers above can sterilize the canals while without thermal damage.

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Disclosure of conflict of interest

None.

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