Journal of Lasers in Medical Sciences

J Lasers Med Sci 2021;12:e43

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2021.43

Morphological Assessment of the Effect of Nanosecond Diode Laser Radiation With a Wavelength of 1265 nm on Periodontal Tissue in the Treatment of Apical Periodontitis: An Experimental Study

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Received: January 7, 2021
Accepted: April 12, 2021
Published online August 7, 2021

Abstract

Introduction: There is a morphological assessment of the damaged area and the inflammatory response of periodontal tissues after therapy. It seems relevant to evaluate the reaction of the periapical tissues of the teeth of experimental animals after the treatment of simulated apical periodontitis using laser photodynamic therapy (PDT) and non-pigment laser transcanal microablation using morphological research.

Methods: The lower incisors of 15 rabbits were used to simulate acute apical periodontitis of pulpal origin. Subsequently, the traditional treatment of teeth canals using mechanical cleaning and irrigation with antiseptic solution started. Then, three groups underwent different therapies - using PDT (n = 5; photosensitizer chlorin e6, [l = 660 nm, 60 mW/cm^2] for 2 minutes), using a new technology of transcanal laser ablation (n = 5; diode laser [l = 1265 nm, 180 mW/cm^2] for 3 minutes), and without additional treatment (n = 5). Laser therapy in two groups was carried out for 10 days once in 2 days. The animals were euthanized 10 days after the treatment started, decapitation was performed, and morphological specimens were studied under a microscope. Descriptive analysis (study of inflammatory apical infiltrate, bone regeneration of the alveoli and periodontal ligament) and quantitative analysis were performed. The data were statistically processed using the Kruskal-Wallis test.

Results: The group treated with the new laser technology showed the best results with moderate lymph-macrophage infiltration and congested vessels, moderate neoangiogenesis and fibrogenesis. The size of the lesion was 506.4 mm^2, compared with the PDT group - 872.0 mm^2 ($P \leq 0.05$).

Conclusion: A decrease in the focus of inflammation, moderate neoangiogenesis and fibrogenesis in the periapical region when using transcanal laser microablation indicate the prospects of using the new technology in clinical practice in the complex therapy of apical periodontitis.

Keywords: Photodynamic therapy, Apical periodontitis, Singlet oxygen, Laser microablation, Morphological study

Introduction

An essential role in the development of periodontitis is played by a bacterial infection, which has a polymicrobial nature with a predominance of anaerobic gram-negative microorganisms. Microorganisms in the periodontium are inaccessible for the mechanical and medicament treatment of root canals, which leads to low rates of treatment success in the long term.[1] For the eradication of the foci of infection in the periodontium, alternative methods of treatment are used, particularly methods of photodynamic laser therapy. Photodynamic therapy (PDT) is based on the activation of photons of a light source of light-sensitive substances (photosensitizers) that accumulate in target cells, leading to the formation of singlet oxygen, which, in turn, becomes an oxidizing agent and triggers free radical reactions.[2] Due to the increased permeability of the pathogenic microorganisms, the photosensitizer selectively accumulates in their membranes and contributes to its destruction upon the activation of photodynamic reactions and the death of a microbial cell. This technique is called antimicrobial photodynamic therapy (aPDT). Singlet oxygen affects not only the cell membrane of microorganisms but also other cellular structures containing nucleic acid,

Please cite this article as follows: Chunikhin NA, Bazikyan EA, Chunikhin AA, Klinovskaya AS. Morphological assessment of the effect of nanosecond diode laser radiation with a wavelength of 1265 nm on periodontal tissue in the treatment of apical periodontitis: an experimental study. *J Lasers Med Sci.* 2021;12:e43. doi:10.34172/jlms.2021.43

lipids, and proteins, which reduces the chances of developing microorganism strains resistant to aPDT therapy in contrast to antibacterial drugs.[3] To activate photosensitizers, laser radiation of the visible spectrum 400-660 nm is used, which is monochromatic and promotes a high generation of singlet oxygen from the photosensitizer at a certain wavelength.[4,5] Dyes are used as photosensitizers - phenothiazine, methylene blue, chlorins, porphyrins, which also have high systemic toxicity and low selectivity to microorganisms. The concentration of the photosensitizer, the time of its exposure in tissues, as well as the time and power of exposure to laser radiation are important for planning optimal treatment using PDT.[6,7] Many in vitro and in vivo studies in recent years have shown the high antibacterial efficacy of various laser technologies in the treatment of apical periodontitis, including the use of a diode laser with a wavelength of 810 nm. However, PDT is a better alternative for use in the treatment of periapical lesions due to its lower heat effect.[8] At the same time traditional methods of transcanal therapy and methods of PDT have low efficiency when they affect the regenerative processes of periodontal tissues.[9] The search for new minimally invasive methods for the treatment of acute apical periodontitis of pulpal origin which effectively remove pathogenic microorganisms and granulation tissue from the lesion and promote the regeneration of periodontal tissues is an urgent scientific task that ensures the optimal level of dental health and the quality of life for patients.[10] The development and improvement of laser technologies made it possible to expand the horizons for the design of new laser medical devices with unique radiation parameters.[11] Effective generation of singlet oxygen without the use of photosensitizers in model biochemical media and blood plasma in laboratory studies in vitro under the action of laser radiation with a wavelength of 1265 ± 5 nm at the maximum oxygen absorption by tissues has been proven.[12,13] Ultrashort pulsed radiation with a given wavelength allows producing the ablation of granulations with simultaneous stimulation of healthy tissue repair. There are not enough studies to investigate the response of periodontal tissues to the effects of various types of laser radiation in the treatment of apical periodontitis.[14]

The aim of this study was a comparative morphological assessment of the in vivo response of the periapical tissues of rabbit teeth with experimentally induced K 04.4 acute apical periodontitis of pulpal origin after courses of photodynamic laser therapy and laser photoablation of periodontal tissues with a wavelength of 1265 nm to reduce surgical invasion in the treatment of periodontitis.

Materials and Methods

The study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments)[15] guidelines and the rules for working with animals based on the provisions of the Declaration of Helsinki and the recommendations contained in the EU Directive 86/609/ECC and the Council of Europe Convention on the Protection of Vertebrate Animals used for experimental and other scientific purposes with the permission of the Interuniversity Ethics Committee of the Moscow State University of Medicine and Dentistry (Minutes N 07-19 dated 18.07.2019). Fifteen mature male rabbits of the “Soviet Chinchilla” breed weighing 2.5-3.0 kg were used in the study. The protocol for modeling apical periodontitis was reproduced by the method of spontaneous infection of the periodontium with the flora of the oral cavity after opening the tooth cavity described by Silva et al.[16] In accordance with the international classification of diseases ICD-10, we performed modeling of K04.4 Acute apical periodontitis of pulpal origin. Under general anesthesia the introduction of xylazine (4-6 mg/kg) intramuscularly and Zoletil-100 (5-10 mg/kg) were used to provide analgesia and relaxation for 30-40 minutes, the pulp chamber of the central incisor was opened, then the pulp was removed from the tooth canal. The teeth were left open for 28 days for microbial invasion and the appearance of granulations (Figure 1).

The study was carried out in accordance with the protocol recommended by ISO (ISO 7405: 1997).[17] After the induction of chronic apical periodontitis, after 28 days, all animals underwent mechanical and drug treatment of the canals using K-files up to N 40 (Dentsply Maillefer, Ballaigues, Switzerland) and irrigation with 2.5% NaOCl solution. The canals were dried using paper pins. Further, in accordance with the conditions of the experiment, 3 groups were formed:

Group I (n = 5) - after mechanical and medical treatment, laser ablation of the periapical focus was performed using a new laser device with a wavelength of 1265 nm in a nanosecond pulsed radiation mode. Laser

Figure 1. Modeling of periodontitis - the cavity of the tooth was opened, the pulp was removed, and the canal was mechanically processed to the apex using the K-file.

radiation was delivered to the inflammation focus using a flexible fiber-optic light guide with a diameter of 400 µm, which was guided to the entire depth of the canal up to the physiological apex and then removed from the canal with slow rotational movements for 3 minutes. The optical fiber design allows exposing laser radiation in all directions evenly. Irradiation was performed with an average laser power of 1.8 W. Thus, the total exposure dose was 324 J/cm^2. After the laser ablation procedure, the canal was washed with saline and dried with sterile absorbers, and the tooth cavity was closed with temporary cement to fill the cavities. The procedure was repeated once in 2 days with a total of 5 therapy sessions for 10 days.

Group II (n = 5) - in this group, after mechanical and drug treatment, PDT was performed using a photosensitizer based on chlorin e6, which was injected into the canal for 7 minutes, and then irradiation with a laser with a wavelength of 660 nm was performed in continuous mode radiation for 2 minutes through an optical fiber with a diameter of 400 µm with an average radiation power of 0.2 W. The number of therapy sessions and sealing the tooth cavity between sessions were identical to group I.

Group III (n = 5) - after mechanical and drug therapy, the canals were left empty, the tooth cavities were closed with cement for temporary filling, and no additional therapy was received.

All animals were euthanized 10 days after starting the treatment with an intravenous lethal dose of Zoletil-100 (50 mg/kg). The jaws were decapitated, fixed in 10% buffered formalin solution and decalcificated according to the standard technique (reagent Biodec R, Italy), then embedded in paraffin blocks. Histological sections, 4 µm thick, obtained with an HM355S microtome (Thermo Scientific, Germany), were stained with hematoxylin and eosin. Descriptive and quantitative microscopic analyses were performed using an AxioLab A1 microscope (Carl Zeiss Microscopy, Germany) with the participation of 3 laboratory technicians and the samples were distributed between them in a blinded manner.

The description of the nature of the state of the tissues of the periapical region in all groups was carried out according to the following parameters: the presence or absence of newly formed bone trabeculae, periapical inflammatory infiltrate, thickness and restorative nature of changes in the periodontal ligament, and resorption of tooth root cement. Quantitative analysis was performed according to the size of the periapical lesion in mm^2 and the number of inflammatory cells in the periapical area using Axiovision Rel 4.6 software - automatically by the measurement program (Carl Zeiss). Quantitative data were analyzed by the statistically non-parametric Kruskal-Wallis test.[18] The significance level was set at = 0.05.

Results

Descriptive Microscopic Analysis

In group I, in the periapical region of the incisor root, growths of connective tissue with the formation (neoplasm) of periodontal ligament structures and moderate diffuse-focal lymph-macrophage infiltration were found. The connective tissue was edematous, with plentiful vessels. The periapical area showed up without inflammatory cells with moderate neoangiogenesis and fibrogenesis. In the surrounding cancellous bone tissue, newly formed bone trabeculae surrounded by a shaft of osteoblasts were obtained. Periodontal congestion indicates the presence of reactive inflammation that stimulates reparative osteogenesis. It is important to note that Malassez rests in this group in the periapical region were not detected when evaluating the results of morphological examination (Figure 2).

In group II, the periodontal ligamentous space is enlarged, represented by a diffuse mixed, predominantly mononuclear inflammatory infiltrate, concentrated near the apex of the tooth. In more distant layers, the connective

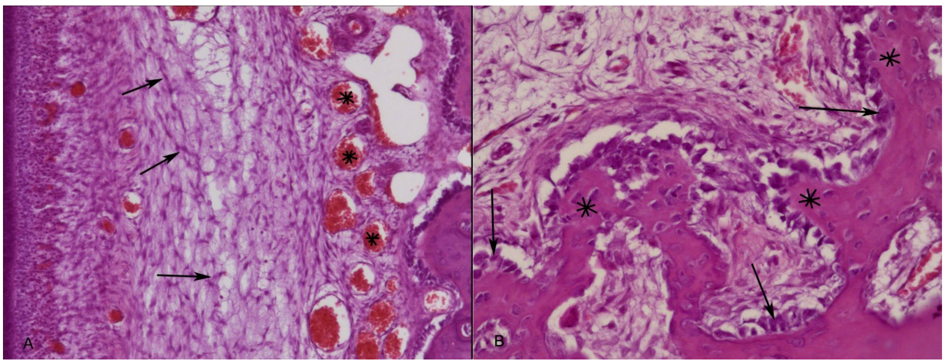

Figure 2. Group I (A) Overview of the periapical region with the proliferation of connective tissue with the formation (neoplasm) of the structures of the periodontal ligament. The connective tissue is edematous, with congested vessels (*) and a large number of fibers (arrow) (hematoxylin-eosin, Zeiss, ×120). (B) In the cancellous bone surrounding the incisor root, newly formed bone trabeculae (*) surrounded by a shaft of osteoblasts (arrows). (hematoxylin-eosin, Zeiss, ×250).

tissue is edematous with local initial fibrogenesis, moderate diffuse-focal lymph-macrophage infiltration, and congested vessels. The expressed growth of maturing granulation tissue with a violation of the structure of the periodontal ligament, generalized edema and fibrillar dissociation, diffuse mixed predominantly mononuclear inflammation was found. In the surrounding cancellous bone tissue, newly formed bone trabeculae surrounded by a shaft of osteoblasts were obtained. No Malassez rests were found in the periodontal ligament, as well as the main group (Figure 3).

In group III, the periodontal tissues are edematous and contain remnants of necrotic tissues and inflammatory cells, and there are extensive areas of bone resorption in the periapical region. The strongly expanded space of the periodontal ligament is represented by generalized edema, fibrillary dissociation, areas of necrosis, and dense mixed, predominantly mononuclear inflammatory infiltrate that spreads over the entire periapical region. The peripheral part of the infiltrate is represented by a wide shaft of leukocytes, and the central part is presented by tissue detritus with lysed leukocytes and bacterial colonies. In this group, Malassez rests and remnants of the epithelial sheath were easily detected at the border of the inflammatory infiltrate. This suggests that in the future in this area, the inflammatory process can lead to the development of a radicular cyst for the treatment of which it will be necessary to use invasive surgical methods of treatment (Figure 4).

Quantitative Microscopic Analysis

The results of quantitative microscopic analysis of the size of the periapical inflammation focus (in mm^2) are presented in Table 1. In the main group, in the root canals in which the laser radiation with a wavelength of 1265 nm was performed, the smallest foci of periapical lesions were assessed on morphological images. In the comparison group, with using traditional PDT for the treatment of tooth canals, medium-sized lesions were found, and the largest lesions were observed in the control group with traditional single mechanical and drug therapy of root canals. A statistically significant difference (P = 0.00334) was noted among all observation groups.

The quantitative assessment of inflammatory cells in the periapical region is shown in Table 2. In the main group, the lowest number of inflammatory cells was noted, in

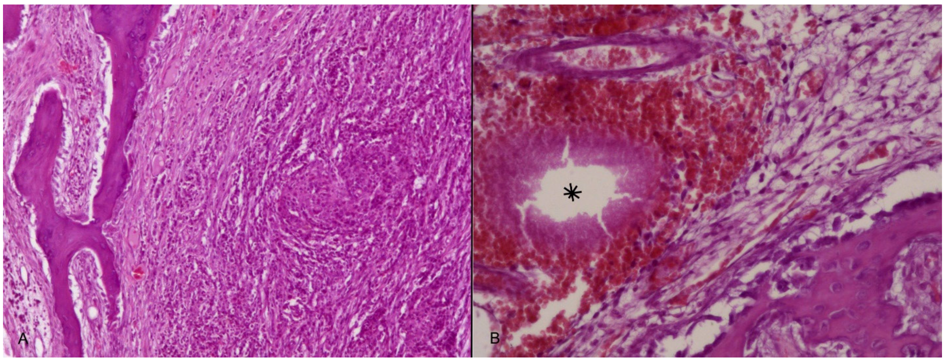

Figure 3. Group II (A) Overview of the periapical region - pronounced growth of maturing granulation tissue with a violation of the structure of the periodontal ligament (hematoxylin-eosin, Zeiss, x120). (B) In the periapical region of the incisor root, a small focus of tissue detritus (*) with lysed leukocytes remains, surrounded by a zone of hemorrhage. (hematoxylin-eosin, Zeiss, ×250).

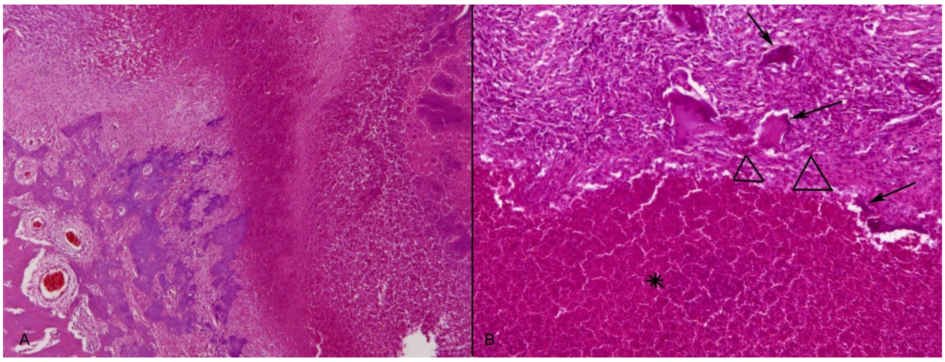

Figure 4. Group III (A) Periapical view - encapsulated periapical abscess (hematoxylin-eosin, Zeiss, ×60). (B) Peripheral part of the abscess with a wide shaft of leukocytes, central tissue detritus with lysed leukocytes and bacterial colonies (*). The connective tissue contains necrotic bone trabeculae (arrows) and Malassez rests (Δ) (hematoxylin-eosin, Zeiss, ×120).

Table 1 Sizes of Periapical Foci of Inflammation (in mm^2) in Different Groups

Group	n	Median	Min-Max
I (main)	5	506.4	120.0-930.0
II (comparisons)	5	872.0	580.0-1100.0
III (control)	5	1552.0	1190.0-1950.0

Notes: Kruskal-Wallis test: H = 23.08643. The difference is statistically significant (P = 0.00334) between all indicators in the main group, the comparison group and the control group.

Table 2 Number of Inflammatory Cells in the Periapical Region

Group	n	Median	Min-Max
I (main)	5	377,8	205.0-703.0
II (comparisons)	5	685,8	368.0-1072.0
III (control)	5	4603,0	3078.0-7860.0

Notes: Kruskal-Wallis test: H = 22.16516. The difference is statistically significant (P = 0.00083) between all indicators in the main group, the comparison group and the control group.

the comparison group, the number of inflammatory cells was slightly higher than in the main group, while the control group showed a significantly greater number of inflammatory cells when studying morphological pictures compared with the main group and the comparison group. The statistical difference in all groups was significant (P = 0.00083).

Discussion

Effective treatment of periodontitis with possible minimal surgical invasion through the root canal using traditional and innovative techniques is an important task of modern dentistry. Many researchers point to a significant increase in efficiency with the use of intracanal PDT therapy together with SRP.[4,19] The studies were carried out to indicate a high antibacterial activity of PDT against various microorganisms, including those contained in the microbial focus of periapical inflammation.[20,21] At the same time, there are studies proving the harmful effect of photosensitizers on osteoblasts and fibroblasts.[7,22] The toxic effect of PDT is variable depending on the type of photosensitizer, its concentration, the exposure time of the photosensitizer and laser radiation, and the exposure dose of laser radiation.

In the research by Silva et al,[23] the analysis of the morphological response of apical and periapical tissues using PDT in the treatment of periodontitis was performed. It was shown that using PDT stimulates fibroblastogenesis and vascular growth in periodontal tissues.

The development of laser medical technologies has led to the proof of the possibility of PDT without exogenous photosensitizers with simultaneous laser ablation and stimulation of regenerative processes in tissues. The presented in vivo study with modeling of the inflammatory process in periapical tissues in animals shows the morphological response of periodontal tissues to treatment with the use of a new laser PDT technology with laser ablation of periodontal tissues in comparison with traditional PDT. Obviously, induced apical lesions in experimental studies on animals do not always have the same degree of damage to periodontal tissues and the severity of the course. The extent to which the apical tissue is affected depends on correct pulp exposure and sufficient root canal exposure. Stochastic differences in the behavior of animals and the susceptibility of an animal to bacteria are also important.[24] In rodents, the anatomy of the teeth differs from that of humans, and the importance of these differences is known. At the same time, the study of the main mechanisms and phenomena in model periodontitis and exposure to the treatment of various factors is possible by following the protocol for modeling apical periodontitis. Accurate adherence to the protocol allows achieving accurate modeling of apical periodontitis in 85% of cases, which is a high result in achieving the method.[25] We followed all the protocols when modeling apical periodontitis in rabbits, which made it possible to minimize the difference in both the size of the lesions and the degree of damage to the periodontal tissues.

Research by modern scientists proves the effectiveness of the impact of various physical factors, including high-intensity laser radiation on pathological foci of periapical tissues in the induction of apical periodontitis in animals. In a study by Matsui et al, it was proved that under the action of high-frequency wave irradiation there is an increase in the expression of fibroblast growth factor-2 and transformation of growth factor No. 1, which promotes healing of periapical lesions and may be useful as an adjuvant non-surgical treatment of apical periodontitis.[26]

Morphological studies of the periapical region after treatment with PDT-induced periodontitis in dogs demonstrated a decrease in periapical lesions, a decrease in the number of inflammatory cells, moderate neoangiogenesis and fibrogenesis compared to the group without PDT with root canal treatment in one session.[27] Studies of modern scientists using X-ray and morphological studies show that the use of laser radiation in the treatment of apical periodontitis does not contribute to significant tissue repair, but at the same time contributes to a significant decrease in inflammatory cells, which indicates a significant anti-inflammatory effect of laser radiation and PDT. Laser radiation helps to optimize the restoration of periodontal tissues by modulating the inflammatory process in the tissues. PDT cannot be an independent type of treatment, but it is only regarded as an addition to the main drug therapy with the endodontic treatment of teeth canals with apical periodontitis.[28]

Conclusion

An important conclusion of this study is the proof of the high efficiency of laser photodynamic ablation of periodontal tissues without the use of photosensitizers in the treatment of periodontitis. A comparative morphological assessment of traditional PDT and new laser technology after a course of therapy for acute apical periodontitis of pulpal origin showed that using laser photodynamic ablation reduce the number of inflammatory cells in the lesion compared to the traditional PDT and the area of the lesion itself is smaller. The stimulating effect of laser radiation, expressed in neoangiogenesis and fibrogenesis, is higher than that with traditional PDT. In addition, morphological evidence showed the complete absence of granulation tissue in the lesion area after treatment with non-pigmented laser ablation, as well as Malassez rests in the periodontal tissues which indicates a multifactorial orientation of the effects - antibacterial, anti-inflammatory, stimulating regeneration, as well as a surgical component that promotes removing granulations from the lesion and preventing the development of radicular cysts. The data obtained allow us to designate this new method of treating periodontitis as transcanal laser microsurgery.

The data obtained in this study indicate a high perspective of the use of a new technique of laser transcanal microsurgery in the treatment of acute apical periodontitis of pulpal origin for use in clinical practice.

Conflicts of Interests

The authors have declared that no competing interests exist.

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