DETERMINATION OF THE DEGREE OF TISSUE HEATING UNDER THE EXPOSURE TO LASER RADIATION WITH A WAVELENGTH OF 445±40 NM

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Abstract. **This pilot study contains data on determination of the temperature indicators around the attached keratinized gingiva in the laboratory rats of the WISTAR breed, both in normal conditions and under the exposure to low-intensity laser radiation with a wavelength of 445±40 nm. The temperature was determined using the contact method by applying a DT-1200 digital thermometer. The average value of temperature indicators in he attached keratinized gingiva in the healthy laboratory animals was 29,30±0,203 °C. The procedure of exposure to low-intensity laser radiation was carried out in the non-contact way, utilizing a non-initiated fiber with a diameter of 400 µm, using the dynamic technique at a power of 0.5 W in the continuous wave mode, and with a duration of 5 minutes. The distance from the tip of the light guide to the gingival surface was 4,5–5 mm. The average value of temperature indicators in the attached keratinized gingiva during the procedure of low-level laser therapy (LLLT) was 37,68±0,043 °C. The exposure to laser radiation with a wavelength of 445±40 nm showed an increase in the temperature of gingival tissues in the laboratory animals by 8,37 °C. The threshold temperature safety index of the LLLT procedure (42 °C) has not been exceeded, allowing planning clinical trials.**

Keywords: **low-intensity laser radiation, low-level laser therapy, LLLT, blue laser, laser radiation with a wavelength of 445±40 nm, temperature indicators.**

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Introduction. Currently, diode semiconductor lasers are widely utilized in the rehabilitation of patients with periodontal diseases [1-3]. The question of the biological response of a living organism to the action of laser radiation (LR) has been studied quite well. LR has thermal, kinetic, ultrasonic, electrochemical, photochemical, and hydrodynamic effects on the living biological tissues. The LR energy is absorbed by molecules and atoms of various biological tissues, which causes an increase in their rotational and oscillatory motions. These phenomena convert laser energy into thermal energy. When tissues are heated, the rate of chemical and biochemical reactions increases, which determines the therapeutic effect of LR.

Clinically, the exposure of gingiva to low-level laser radiation is carried out by the non-contact method using a glass fiber or special scattering nozzle. When utilizing the fiberglass, it is important to dynamically deliver LR to the area of the attached keratinized gingiva. Directing the laser beam to the gingival area, the surgeon performs continuous movements, similar to drawing lace. The speed of the operator's hand movements amounts to 1 cm^2 per sec. The distance from the tip of the light guide to the gingival surface is usually 3 to 5 mm. The exposure time ranges from 1 to 5 minutes. The recommended radiation power is in the frame of 0.5 – 0.7 W. In most cases, to perform the LLLT procedure in the maxillofacial region, clinicians utilize laser devices with a wavelength of red and infrared light spectrum [4, 5].

The Scientific and Technical Association “IRE-Polyus” (Russia) has created an experimental diode semiconductor laser device with a wavelength of 445±40 nm. In the world practice, this technology is called blue laser. The presented laser device is declared as a non-contact surgical device, which implies a low invasiveness of medical manipulations and a higher safety when using this laser technology in patients of different age groups and those with concomitant general somatic pathology.

According to Joana Reichelt et al., laser-generated blue light can be used for LLLT, which was confirmed in a study showing that after the thermal exposure epithelial cells do not undergo cytoskeleton changes, do not form double-strand breaks in DNA, and do not show signs of

cell death [6]. It is known that the impact of laser radiation with a wavelength of 445±40 nm on cultured gingival fibroblasts activates their mitochondrial activity [7], which manifests itself in their increased proliferation and migration to the center of the defect in the presence of a wound surface [8]. Exposure of prechondroblasts to 445±40 nm wavelength LR causes an increase in the total amount of collagen, the level of aggrecan mRNA, type II collagen, SOX-9 (the gene encoding a protein that is a transcription factor that plays an important role in the development of musculoskeletal motor system) and DEC-1 (the gene encoding the candidate protein – tumor growth suppressor), while the activity of Ap-2alpha m-RNA, a negative transcription factor of chondrogenesis, sharply decreases [9].

In the publicly available literature, there is no information on the degree of heating of gingival tissues during prolonged exposure to LLLT. Therefore, it appeared relevant to determine the temperature indicators of the attached keratinized gingiva under the exposure to LR with a wavelength of 445±40 nm.

Materials and Methods. We have studied the temperature changes of oral mucosa in the area of exposure to the laser beam in laboratory animals during the LLLT procedure on gingiva, which has a state code A22.07.008 (Order 804n of the Ministry of Health of the Russian Federation of October 13, 2017).

This pilot study was carried out on 32 sexually mature male laboratory rats of the WISTAR breed, weighing from 170 to 200 grams. All the animals had sanitary passports. The laboratory rats were kept in a vivarium according to the rules of laboratory practice for preclinical studies adopted in the Russian Federation (GOST R50258–92, GOST 351000.3–96 and 51000.4–96). Manipulations with the laboratory animals were carried out in accordance with the standards of appropriate clinical practice [Good Clinical Practice] and the principles of the Helsinki Declaration of the World Medical Association (1964).

Determination of temperature (t^o) in the area of the attached keratinized gingiva was carried out by contact method using a digital thermometer DT-1200, manufactured by IzTeh LLC (Russia). The device is designed for deep and surface precision measurements. The digital thermometer consists of a primary temperature transducer (sensor) and an electronic digital measuring unit. Temperature measurement results are displayed on a liquid crystal display (Fig. 1). The range of temperature measurement of superficial tissues corresponds to GOST 6651–2009 (GOST R. 8.625–2006).

Fig. 1. The DT-1200 Digital Thermometer.

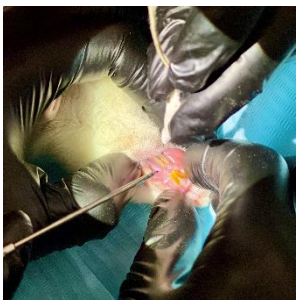

Fig. 2. Temperature measurement during the LLLT Procedure.

The probe of the digital thermometer was placed in the area of the attached keratinized gingiva of the anterior teeth of the upper jaw from the vestibular side, and the value on the display was recorded in a Data Log.

For general anesthesia, before carrying out the LLLT procedure in the area of the attached keratinized gingiva, the laboratory rats were intramuscularly injected with Zoletil (tiletamine hydrochloride and zolazepam hydrochloride produced by "Virbac", France) at the rate of 5 mg/kg of the animal weight and Xyla (xylazine hydrochloride, produced by "Interchemie", Netherlands) at the rate of 0.2 ml/kg of the animal weight. The eyes of the laboratory animals were protected from exposure to the blue light with a napkin.

The LLLT procedure was carried out utilizing a laser device with a wavelength of 445±40 nm non-invasively for 5 minutes, using a dynamic technique with a laser power of 0.5 W in the continuous wave mode (CW) and an uninitiated fiber with a diameter of 400 μm. The distance between the tip of the light guide and the gingival surface was 4,5–5 mm (Fig. 2).

The temperature measurement in the attached keratinized gingiva was performed before and during the LLLT procedure with the laser wavelength of 445±40 nm. During the 5 minutes of the procedure, in each of the cases, the value of the temperature indicator on the display of the digital thermometer changed 128 times.

The statistical processing was carried out using the R v.4.1 programming environment. The study design involved evaluating the results of the temperature measurement before the exposure of 32 laboratory animals to laser radiation, and then after the exposure of 12 laboratory animals to laser radiation, with an assessment of 128 thermometer readings (during this manipulation, 128×12=1536 thermometer readings were obtained).

For each stage, the distribution of indicators (the minimum, 1-, 2.5-, 10-, 25-, 50- (the median), 75-, 90-, 97.5-, 99-percentiles, the maximum), the average value and the standard deviation were evaluated, as well as the 95% confidence interval (CI) of the average value and the median. The threshold value correlated with the boundaries of the calculated 95% CI, as well as with the

values of the obtained distributions during the temperature measurement procedure.

Results. The average value of temperature indicators in the attached keratinized gingiva in the healthy laboratory animals without periodontal tissue diseases amounted to 29.30±0.203° C. The minimum temperature indicator of 27.8° C was recorded in 9.375% of the cases, while the maximum value – 31.1° C – was noted in 6.25% of the cases. The median amounted to 29.5° C. The results of the initial temperature measurement of 32 species are presented in the Tabl. 1.

The average value of temperature indicators in the attached keratinized gingiva during the procedure of low-level laser therapy (LLLT) was 37,68±0,043° C. The median amounted to 37.1° C. The results of the temperature measurement of 12 species are presented in the Figure 3.

Based on the obtained distribution, it can be assumed that the threshold value has not been exceeded.

Tabl. 1. The initial indicators of the distribution of temperature measurement (n = 32).

Statistics	Temperature indicator
The minimum	27.800
1%	27.800
2.5%	27.800
10%	27.900
25%	28.175
The median and the 95% CI	29.500 [28.3; 30.2]
75%	30.425
90%	30.790
97.5%	31.100
99%	31.100
The maximum	31.100
The average value and the 95% CI	29.30625 [28.89203; 29.72047]
The standard deviation	1.14889

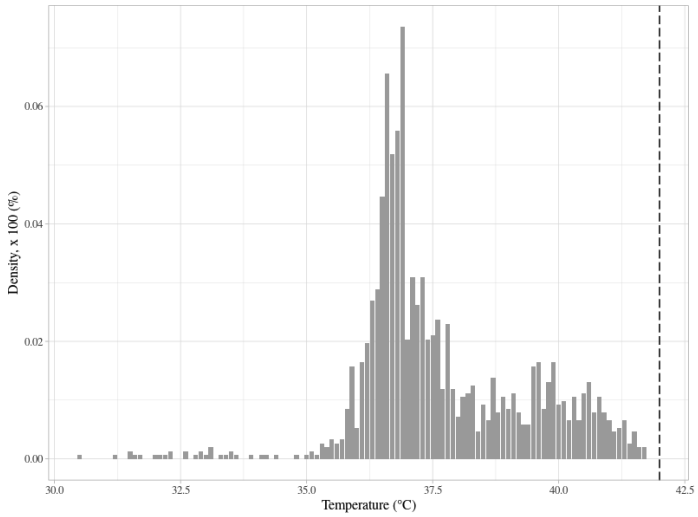

Figure 3. Histogram of temperature measurement after the exposure to laser radiation (n = 12).

Discussion. According to data provided by Niemz M.H., the negative effect of the LLLT on the tissues can be noted when the temperature level exceeds 42° C, which is manifested by the destruction of molecular bonds and changes in the structure of cell membranes. An increase in tissue temperature above 50° C causes a noticeable decrease in enzyme activity, which leads to a decrease in the usage of energy inside the cell and the immobility of the cell itself. Certain cell repair processes can also become blocked. With excessive heating of tissues, denaturation of proteins and collagen occurs, along with tissue coagulation and cell necrosis [10].

The results of a study by Nicola Alberto Valente et al. show that exceeding the temperature index of tissues by 10 degrees is possible when using a diode semiconductor laser with a static technique and at a power of 0.8 and 1.0 W in the CW mode [11]. We have chosen a power of 0.5 W and the dynamic method of delivering the laser radiation to the gingival surface.

When exposed to laser radiation with a wavelength of 445±40 nm, the temperature of the gingival tissues in the laboratory animals increased by an average of 8.37 degrees. The threshold temperature safety index for the procedure of the LLLT (42° C) has not been exceeded, which enables to plan further clinical trials.

Conflicts of Interest. The authors declare no conflict of interest.

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