Adaptive Responses of Individual Tissue Structures of Rat Gingival Mucosa to Exposure to Low-Intensity 890-nm Laser Irradiation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 3, pp. 295-297, March, 2011
Original article submitted March 15, 2010

Adaptive reactions develop in rat gingival mucosa 1 min after single exposure to low-intensity 890-nm laser: the number of mast cells, degree and index of their degranulation, the diameter of blood vessels and their total area considerably increased. These parameters returned to normal after 1 day, while on days 3-7 they were below the control.

Key Words: *low intensity laser irradiation; mast cells; blood vessels; gingival mucosa; adaptive reaction*

Laser therapy is now a widely used and efficient method for the treatment of stomatological diseases [2]. It employs low-intensity laser irradiation (LILI) in red (630 nm) and infrared (890 nm) bands [5].

It is commonly believed that the use of infrared LILI in clinical practice is determined by the spectrum of its action. Penetrating deep into the tissues, LILI of this spectral range activates physiological processes under normal and pathological conditions [2,4]. In addition, many issues concerning the morphology of adaptive processes developing in gingival mucosa (GM) in response to LILI 890 nm, remain poorly studied. Little is known about the role of various tissue structures, *e.g.* mast cells (MC), since during laser irradiation they remain to be mobile elements and play an essential role in tissue adaptation to this physical factor [3] by releasing cytokines, growth factors, mediators, which are involved in activation of microenvironment cells, chemotaxis, angiogenesis, and matrix metalloproteinases during their degranulation [4].

The objective of this study was to investigate adaptive reactions of MC and blood vessels of GM to infrared LILI with wavelength of 890 nm.

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MATERIALS AND METHODS

Experiments were carried out on 30 healthy mature laboratory rats of both sexes weighing 210-250 g. Experiments were carried out in accordance with Orders of Health Ministry of USSR No. 755 from 12.08.77 and No. 701 from 27.07.78 "Securing the Principles of Humane Treatment of Animals". The animals were divided into experimental (n=25, group 1) and control (n=5, group 2) groups. All experiments were performed after intramuscular administration of Zoletyl (2 mg/kg body weight). Group 1 animals were fixed to a table in the supine posture, the front and hind limbs were fixed with ties and special holders. Thereafter, the lower lip was pulled down with forceps and single contact continuous-wave mode irradiation of GM was performed at mandibular molar area with an ALT Uley-2KM diode laser (890 nm laser wavelength, 25 mW power, 30 sec exposure). These parameters of laser irradiation (wavelength, power, exposure, regimen) are used in stomatological practice [2,5].

Group 2 animals were not exposed to laser radiation. Biopsy specimens from the mandibular molar area were taken from group 1 rats 1 min after laser irradiation and on days 1, 3, 5, and 7 and from group

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2 rats. Biopsy specimens (0.3 mm) were taken using special biopsy forceps with pincers (Karl Storz). The above specified terms are considered to be sufficient for effects of low intensity laser therapy to appear [8]. GM biopsy samples were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Paraffin blocks were used to prepare serial sections, which were stained with hematoxylin and eosin for plain microscopy and qualitative estimation of blood vessels, or with toluidine blue pH 2.0 for detection of acid glycosaminoglycans in the granules of MC [6]. The number of MC (mm^2) was determined under a Leica light microscope (×50) and non-degranulated and degranulated MC were counted under oil immersion (×1000) in 10 fields of view using computerized system for the color image analysis and Diamorf Cito-W software. Thereafter the degranulation index was calculated using the formula: D×100%/D+N, where D and N are the numbers of degranulated and non-degranulated MC. We discriminated the following degrees of MC degranulation: weak degranulation (1, 1-2 granules outside the cell), marked degranulation (2, up to 10 exuded granules), and total degranulation (3, more than10 released granules) [7]. Proportion of the section (%) occupied by the blood vessels and vessel diameter (μ) were also calculated.

Statistical treatment of numerical data was performed using analysis of variance and Student's *t* test [1]. The differences between compared values were considered significant at $p<0.05$.

RESULTS

Examination of histological preparations stained with hematoxylin and eosin showed that GM in animals from group 1 and group 2 was lined with stratified squamous epithelium. The lamina propria was presented by loose connective tissue with formed long papillae penetrating the epithelium. The number and plethora of blood vessels in the lamina propria were considerably higher in group 1 rats (at early stages of the experiment). The number of macrophages and lymphocytes in the connective tissue of group 1 animals was also increased and toluidine blue staining revealed MC clusters of round, oval and fusiform shape along the vessels.

Morphometric study of histological GM preparations showed that 1 min after laser irradiation the studied parameters (Table 1) significantly differ from the control. Moreover, a decrease in the number of MC with the 1st and 2nd degree of degranulation was observed.

One day after laser irradiation, the studied parameters insignificantly decresed in comparison with the control, except for the count of MC with the first degree of degranulation (increase) and MC degranulation index (significant reduction). In addition, some parameters significantly decrease at this stage compared to the previous term, while other did not differ significantly (MC with the 1st, 2nd, and 3rd degree of degranulation).

TABLE. 1. Morphometric Characteristic of MC and Blood Vessels of GM after Laser Irradiation (*M*±*m*)

Parameter	Control (*n*=5)	Term of obervation				
		minute 1 (*n*=5)	day 1 (*n*=5)	day 3 (*n*=5)	day 5 (*n*=5)	day 7 (*n*=5)
Total MC content, mm^2	130.60±0.97	160.60±1.91*	129.60±1.12$^+$	121.00±1.04*$^+$	107.00±2.21*$^+$	91.60±2.03*$^+$
Non-degranulated MC, mm^2	81.80±0.58	90.00±1.58*	81.80±0.63$^+$	79.40±1.02	80.80±1.01	72.0±0.7*$^+$
Degranulated MC, mm^2	53.00±0.74	69.80±0.96*	53.20±0.66$^+$	40.60±1.51*$^+$	26.0±0.7*$^+$	20.80±0.66*$^+$
MC degranulation degree, % 1	40.6±1.5	30.40±2.01*	31.80±0.58*	24.20±0.58*$^+$	37.00±0.63$^+$	46.20±0.79*$^+$
2	31.80±0.67	29.40±0.74*	30.20±0.74	50.20±1.31*$^+$	36.60±0.86*$^+$	29.00±1.58$^+$
3	29.0±0.4	41.40±1.28*	40.0±0.4	25.40±0.51$^+$	25.70±0.51	24.20±0.58*$^+$
MC degranulation index, %	38.60±0.51	43.80±1.58*	38.20±0.66*	33.20±1.11*$^+$	27.40±0.51*$^+$	22.40±0.51*$^+$
Vessel diameter, μ	23.3±0.3	30.4±0.4*	24.1±0.4$^+$	20.20±0.68*$^+$	19.40±0.51*	16.60±0.51*$^+$
Vessel area, %	24.1±0.4	29.3±0.6*	20.2±0.66*$^+$	18.40±0.51*	18.60±0.51*	19.60±0.51*

Note. $p\leq0.05$ compared to: *control, $^+$previous term.

On day 3 after laser irradiation, significant decrease in the studied parameters in comparison with the control was generally observed, except for non-degranulated MC and their form with the 3rd degree of degranulation (insignificant reduction). Moreover, significant decrease in the majority of investigated parameters, except for non-degranulated MC and degranulation index, in comparison with the previous term was observed.

On day 5 after laser irradiation, the studied parameters were below or did not differ from the control (non-degranulated MC and their forms with degrees of degranulation 1 and 3). Moreover, some parameters were significantly lower (total MC number, degranulated MC and MC with the 2nd degree of degranulation) or higher (MC with the 1st degree of degranulation), or did not differ (non-degranulated MC and their forms with the 3rd degree of degranulation, vessel diameter and total area) from the previous term.

On day 7 after laser irradiation, the majority of the studied parameters remained significantly lower or increased (MC with the 1st degree of degranulation) in comparison with the control. Similar trend was noted for the corresponding parameters at the previous term. MC with the 1st degree of degranulation (increased in number) and degranulation index (not affected) were the exceptions.

Thus, 1 min after single exposure of rat MG to LILI (890 nm, 25 mW power, and 30 sec exposure), the development of adaptive reactions in the irradiated tissues were observed: the numbers of MC, their degranulating forms and degranulation index and the diameter of blood vessel and their total area increased in comparison with the control. The studied parameters did not differ from the control on day 1 and decreased from day 3 to day 7. These results allow regarding laser irradiation as a type of physiological stress [3].

The results of our study are in line with published data concerning sequential development of biological responses to LILI [2]: photon energy absorption by intracellular components→local heating of the tissue→changes in tissue Ca^{2+} concentration→stimulation of Ca^{2+}-dependent adaptation reactions (activation of cell metabolism and functional activity, activation of tissue microcirculation, *etc*.). On this basis, the increase in functional activity of MC and reaction of blood vessels in GM tissue after LILI are obvious.

It should be noted, that the studied adaptive reactions of MC and blood vessels of GM developing at early terms after LILI at 890 nm apparently form the basis of its effective use in dental clinics for the treatment of periodontitis.

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