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Stress-Related Effects of Low-Intensity Laser Irradiation

Anna V. Deryugina, PhD, ScD[1]; Marina N. Ivashchenko, PhD[2*]; Pavel S. Ignatyev, PhD[3]; Tatyana I. Soloveva, PhD[4]; Evgenia V. Arkhipova[4]; Michael S. Lodyanoy, PhD[2]; Vladimir A. Petrov[2]; Aleksandr G. Samodelkin, PhD, ScD[2]

[1]*Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia*
[2]*Nizhny Novgorod State Agricultural Academy, Nizhny Novgorod, Russia*
[3]*Production Association «Urals Optical & Mechanical Plant» named after E.S. Yalamov, Ekaterinburg, Russia*
[4]*Privolzhsky Research Medical University, Nizhny Novgorod, Russia*

Abstract

The purpose of this study was to investigate the effect of low-level laser (light) therapy (LLLT) on the electrokinetic properties of red blood cells (RBCs), taking into account the activity of stress-realizing systems of the body. The RBC electrophoretic mobility (RBCEM) was used as an index of stress reaction. The experiment included two series: *in vivo* and *in vitro*. Analyzing the LLLT effect on RBCEM, we can assume that the body's response to LLLT is associated with a short-term activation of the sympathoadrenal system and the subsequent longer reaction of the hypothalamic-pituitary-adrenal axis. Through activation of the hypothalamic-pituitary-adrenal axis, LLLT can indirectly cause the development of adaptation processes in the body. (**International Journal of Biomedicine. 2019;9(2):163-167.**)

Key Words: low-level laser (light) therapy • stress • red blood cells • electrophoretic mobility of red blood cells

Introduction

A growing number of reports have shown a positive outcome for low-level laser (light) therapy (LLLT), sometimes known as photobiomodulation, in restorative and rehabilitative medicine. Although the exact mechanisms are yet to be fully understood, LLLT has been used to rescue neurons from neurotoxic injuries[1-3] and help tissue repair and wound healing in animal models.[4-8] LLLT is effective in pain relief and promotes the recovery of some pathologies, including tendinopathies, osteoarthritis, wound healing, and nerve injuries.[9-15]

The fundamental mechanism of photobiomodulation[16] is proposed to involve mitochondria as the primary cellular target for the photons leading to increased cytochrome C oxidase activity,[17] release of nitric oxide,[18,19] and an increase in ATP levels.[20] Changes in intracellular signaling molecules, such as calcium ions, reactive oxygen species and redox sensitive transcription factors, like NF-κB, are also thought to mediate the effects of light.[21,22] Low-level lasers have been reported to attenuate oxidative stress.[23,24]

LLLT at low doses has been shown to enhance cell proliferation of fibroblasts, keratinocytes, endothelial cells, and lymphocytes.[25-30] The mechanism of proliferation is thought to result from photo-stimulation of the mitochondria leading to activation of signaling pathways and up regulation of transcription factors, eventually giving rise to increases in growth factors.[31] It has been observed in many studies that LLLT exhibits a biphasic dose response curve,[32,33] whereby lower doses of light are more effective than much higher doses.

Although LLLT is mostly applied to localized diseases, and its effect is often considered to be restricted to the irradiated area, there are reports of systemic effects of LLLT acting at a site distant from the illumination.[34-36]

The purpose of this study was to investigate the effect of LLLT on the electrokinetic properties of red blood cells (RBCs), taking into account the activity of stress-realizing systems of the body. Of particular interest were RBCs, due to their participation in processes related to maintaining homeostasis at the level of the whole body.

***Corresponding author:** Marina N. Ivashchenko, PhD; Nizhny Novgorod State Agricultural Academy, Nizhny Novgorod, Russia. E-mail: kafedra2577@mail.ru*

Materials and Methods

The experiment included two series: *in vivo* and *in vitro*. The first series of the experiment was carried out on 75 non-pedigree female rats at 3.5-4 months of age weighing 200±20g. The animals were divided into 5 equal groups. Group 1 (control group) included intact animals (n=15). Animals of Group 2 (n=15) were treated with LLLT. For stress modeling, the animals of Group 3 received (n=15) a single intraperitoneal injection of adrenaline hydrochloride solution (0.1 mg/kg), and animals of Group 4 (n=15) received an intraperitoneal cortisol solution (0.4 mg/kg). The animals of Group 5 (n=15) received a single intraperitoneal injection of physiological saline solution.

Animals of Group 2 were given transcutaneous LLLT at the occipital region for 10 minutes. The laser irradiation parameters: a pulse regime, 800-900 nm wavelength, pulse repetition frequency of 415Hz. The minimum value of average power density in the plane of the output window was 193 μW/cm^2. Blood samples were taken from the sublingual vein in 15 min, 30 min, 60 min, and 120 min after starting the experiment.

The second series of the experiments *in vitro* was performed on the isolated RBCs. Blood samples were taken from the sublingual vein of the animals. The effect of stress factors was studied in experiments (20 experiments in each case) *in vitro* using washed erythrocytes. RBCs were incubated with adrenaline (1×10^{-9}g/ml and 1×10^{-10}g/ml) or cortisol (5×10^{-7}g/ml), or they were treated with LLLT (for 10 minutes at 2-5 mm from the applicators). The controls for experiments with LLLT were intact cells, for experiments with adrenaline and cortisol - cells incubated with physiological saline solution.

In order to modify the cell structure, RBCs were fixed with glutaraldehyde according to Walter and Krob.[37] The erythrocyte suspension was incubated with 0.1% glutaraldehyde solution at 22–24°C for 10 min. After a triple washing in salt solution, RBCs were incubated with adrenaline/cortisol or they were treated with LLLT.

The RBC electrophoretic mobility (RBCEM) was used as an index of stress reaction. The RBCEM level was measured by the microelectrophoresis method using a cytopherometer in our modification by registering the 100 μm RBC transmission time in Tris-HCL buffer with pH of 7.4 and amperage of 12мА. The RBC electrophoretic mobility value was defined using the formula: U=S/T×H, where S – a distance to which the cells moved, T – time, H – a gradient of electric potential. The value of potential gradient was determined using the formula: H=I/g×χ, where I – amperage, g – chamber cross section, χ – electrical conductivity of the media.[38,39]

Animals were housed in keeping with the rules for good laboratory practice. Experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals (the institute of Laboratory Animal Resources, 1996) and with approval of local Ethics Committee.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA). The Shapiro-Wilk test was used in testing for normality. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired t-test was used to compare two groups for data with normal distribution. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. A value of $P<0.05$ was considered statistically significant.

Results and Discussion

The results obtained by the first series of the experiment are represented in Table 1. LLLT provoked a decrease in the level of RBCEM 15 minutes after the beginning of the experiment, and it was increased by the 120th minute by 10% relative to the control group value. It is known that LLLT acts on the body at the cell and systemic levels. We may suppose that the development of a typical stress reaction is a response to LLLT at the systemic levels. Given that the sympathoadrenal system and hypothalamic-pituitary-adrenal (HPA) axis are central stress response systems, we analyzed the effects of adrenaline and cortisol. Thus, the intraperitoneal injections of adrenaline provoked a decrease in the RBCEM level during all the experiment, making 74% of the value of the control group at the last registration point (120the min). On the contrary, the cortisol injection provoked an increase in the RBCEM level at all the observation points.

Table 1.
The RBCEM level (μm cm B^{-1}s^{-1}) during the first series of the experiment in vivo

Group	Period after the beginning of the experiment (min)			
	15	30	60	120
Group 3	1.02±0.09*	1.05±0.06*	0.99±0.08*	0.92±0.08*
Group 4	1.28±0.08*	1.49±0.05*	1.73±0.05*	1.67±0.09*
Group 5	1.17±0.02	1.19±0.02	1.19±0.02	1.23±0.04
Group 2	0.96±0.02*	0.98±0.04	1.08±0.02	1.12±0.02*
Group 1	1.02±0.02	1.00±0.03	1.04±0.02	1.02±0.02

**P<0.05 - with animals receiving physiological saline solution (Group 5).*

A registered primary decrease in the RBCEM index under stressful effects may be mediated by an increase in the level of circulating catecholamines in the blood and/or an increase in the sensitivity of cell adrenoreceptors to them. It is known that in reactions to stress, the concentration of adrenaline in the blood plasma increases tenfold in a few minutes. In the stress reaction, catecholamines, by activating the release of ACTH, stimulate an increase in the blood of the adrenal hormone level. While catecholamines reflect the onset of an immediate trigger effect, corticosteroids have a long-lasting effect.[40] Under stress, the blood content of corticosterone in rats increases after 30 minutes and reaches a maximum after 2 hours. Probably, the excretion of corticosteroids, aimed at limiting the first phase of the stress reaction, determines the increase in the RBCEM index (the second phase). Analyzing the LLLT effect on RBCEM, we can assume that the body's

response to LLLT is associated with a short-term activation of the sympathoadrenal system and the subsequent longer reaction of the HPA axis. Through activation of the HPA axis, LLLT can indirectly cause the development of adaptation processes in the body.

Based on this point, the effect of LLLT at the cellular level can also be realized through the consequent starting of processes mediated by the influence of changing concentrations of catecholamines and corticosteroids in the peripheral blood.

In the experiments *in vitro*, we found that LLLT provoked a decrease in the RBCEM index by 20% ($P<0.05$) by the 15th minute after the beginning of the experiment, but after that, the RBCEM index increased considerably and reached 188% of intact cell value by the end of the experiment. The incubation of RBCs with adrenaline in a concentration corresponding to its concentration in the blood under the conditions of the physiological norm (1×10^{-10}g/ml) did not cause changes in the RBCEM level. Concentrations corresponding to the level during stress reactions (1×10^{-9}g/ml) provoked an irreversible decrease in RBCEM ($P<0.05$). In contrast to the experiments with epinephrine, the incubation with cortisol (5×10^{-7}g/ml) led to increasing RBCEM in the range from 30 minutes to 2 hours (Table 2).

Table 2.

The RBCEM level (μm cm $B^{-1}s^{-1}$) during the second series of the experiment in vitro

Group	Before the beginning of the experiment	Period after the beginning of the experiment (min)			
		15	30	60	120
Group 2	1.26±0.08	0.92±0.09*	1.55±0.18*	1.30±0.18	2.06±0.20*
Group 1	1.26±0.08	1.15±0.10	0.96±0.07	1.09±0.12	1.09±0.10
Group 3 (AHS: 1×10^{-9}g/ml)	1.32±0.02	1.24±0.05	1.19±0.08*	1.15±0.07*	1.14±0.05*
Group 3 (AHS: 1×10^{-10}g/ml)	1.25±0.07	1.10±0.11	0.94±0.08	1.1±0.12	1.05±0.07
Group 4 (CS: 5×10^{-7}g/ml)	1.32±0.02	1.52±0.06*	1.96±0.06*	1.72±0.08*	1.72±0.08*
Group 5	1.32±0.02	1.33±0.02	1.36±0.06	1.32±0.06	1.35±0.04

*AHS - adrenaline hydrochloride solution; CS - cortisol solution; *P<0.05 - with intact animals (Group 1).*

Thus, the change in RBCEM under the effect of LLLT on isolated RBCs has the same typical picture as when it affects the whole body. The first reaction of RBCEM, reflecting a decrease in the total charge of the erythrocyte membrane, is comparable to the change in RBCEM under the adrenaline action. The second phase (an increase in RBCEM) is similar to the changes in RBCEM detected for cortisol.

Thus, the detected changes in RBCEM under LLLT *in vitro* experiments are consistent with *in vivo* experiments and associated with changes in the electrokinetic properties of RBCs in stress response, which can be realized through a modification of the receptor apparatus of cells. Taking into account that the most important structural components of this apparatus are proteins, we may conclude that they are a target for stress factors. Thus, one of the likely effects of LLLT action is associated with a nonspecific effect on biopolymers, which leads to a change in the charge of proteins, in their conformational structure, and in their functional state.[41]

To confirm the role of proteins in modifying the activity of cellular receptors, we conducted experiments with pretreatment of RBCs with glutaraldehyde fixing protein molecules due to the formation of cross-links in –NH2-groups.[37] It was shown that pretreatment of RBCs with glutaraldehyde practically nullified the first phase of the reaction of intact erythrocytes to the stress effects of LLLT and epinephrine. At the same time, in the series with cortisol, it was found that the phase of increased RBCEM detected in previous experiments was preserved (Table 3).

Table 3.

The RBCEM level (μm cm $B^{-1}s^{-1}$) in the studied animals after 10 minutes of glutaraldehyde fixation

Kind of influence	Period after the influence (min)			
	15	30	60	120
LLLT	1.64±0.10*	1.88±0.08*	1.53±0.11*	1.55±0.09*
Cortisol	1.36±0.05	1.67±0.12*	1.68±0.10*	1.65±0.08*
Adrenaline	1.32±0.06	1.36±0.10	1.39±0.08	1.44±0.10

**P<0.05 with the RBCEM index of the glutaraldehyde fixed RBC value (1.38±0.06 μm cm $B^{-1}s^{-1}$).*

The results of the study indicate that, against the background of the fixation of protein molecules by glutaraldehyde, the reaction of dropping RBCEM in response to stress is nullified. It can be argued that the mechanism of cell response is realized in the same way and is associated with changes in cell receptor responsiveness. At the same time, if the first phase of the response reaction is associated with modification of membrane adrenoreceptors, the second phase may be due to the modification of intracellular steroid receptors located in the cytosol. Apparently, the effect of LLLI *in vitro* experiments is determined by the possibility of its influence on the hormonal stress-realizing components of the cell. At the same time, the first phase can be considered as an "anxiety stage" of the system, arising by the mechanism of excitation of the sympathoadrenal regulation with an increasing adrenaline effect on the cell membranes. The second stage of stress response is characterized by the return of a reduced function to the initial state, or an increased function of the system in new conditions, which may be one of the mechanisms of LLLT action.

Competing Interests

The authors declare that they have no competing interests.

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