METABOLIC EFFECTS OF INFRARED LASER RADIATION IN THE ZONE OF POST-TRAUMATIC WOUND REGENERATION

N. P. Chesnokova, G. F. Pronchenkova, V. N. Koshelev, and M. P. Grudtsyna

UDC 617-001.4-003.9-02:615.849.19+615.849.19+615.849.19.015.4

KEY WORDS: infrared laser radiation; lactate; pyruvate.

Reports of the beneficial therapeutic action of low-intensity laser radiation (LILR) on the course of post-traumatic regeneration under clinical and experimental conditions have recently been published [1, 2]. Nevertheless, there are as yet no clear ideas about the mechanisms of the stimulating effect of LILR on reparative regeneration.

Characteristic features of wound healing, especially in the case of post-traumatic regeneration, are the limited vascularization and oxygenation and corresponding changes in the character of metabolic reactions. Yet no systematized information could be found in the literature on the character of glycolytic and oxidation-reduction reactions. Accordingly, in the investigation described below, the content of the end products of glycolysis (lactate and pyruvate) was determined in the course of wound healing in animals exposed in infrared laser irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 200-250 g. A wound was produced by the usual method of excision of a full-thickness piece of skin, with injury to the underlying fascia and muscular layer [5]. The diameter of the wound was 20 mm. Repeated irradiation of the wound surface was carried but by means of the LG-23 laser. The wavelength was 10.6 μ and the power density 4 mW/cm^2 and length of exposure 30 sec. The animals were decapitated on the 4th, 8th, and 15th days after wounding, which corresponded to 3, 7, and 14 sessions of laser irradiation. Comparative series of experiments were carried out at the same times of wound healing on animals not exposed to laser irradiation. In the course of wound healing the content of lactate and pyruvate in the wound was determined by an enzymic method [6], using reagents from Boehringer (West Germany); the lactate/pyruvate (L/P) ratio [7] and the redox potential (RP) [4] were determined.

EXPERIMENTAL RESULTS

By the 4th day of wound healing marked activation of glycolytic processes took place, as shown by a sharp rise in the lactate and pyruvate levels in the zone of injury (Table 1). Increased involvement of the end products of glycolysis in the tricarboxylic acid cycle also was observed at the same time, as shown by a fall in the L/P ratio and some increase in the value of RP (Table 2). On the 8th day of wound healing the lactate content in the wound remained high, but this was accompanied by a fall in the pyruvate level, an appreciable increase in the L/P ratio, and a decrease in RP. Not until the 15th day was a fall observed in the intensity of glycolysis in the control animals, as shown by a fall in the lactate and pyruvate levels, whereas RP had not yet reached normal values (Tables 1 and 2).

Analysis of the state of metabolism in the wound after three sessions of laser irradiation of the animals revealed a marked increase in the intensity of glycolysis, accompanied by activation of oxidative processes. Convincing proof of this state of affairs was given by the considerable rise in the lactate and pyruvate levels, a fall in the L/P ratio, and an increase in RP (Tables 1 and 2). Comparison of the intensity of metabolism in the wound of the irradiated and unirradiated animals on the 8th day of post-traumatic regeneration showed

---

Central Research Laboratory, Saratov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 49-51, September, 1983. Original article submitted October 26, 1982.

 0007-4888/83/9609-1248$07.50

TABLE 1. Effect of Laser Radiation on Lactate and Pyruvate Concentrations (in μmoles/g tissue) in Wound during Wound Healing in Animals (M ± m)

Experimental conditions	Day of wound healing		
	4th	8th	15th
	Lactate		
1. Control (intact animals)	3,66±0,08 (n=10)		
2. Wound without laser irradiation	6,36±0,06 (n=10) P<0,001	5,83±0,05 (n=10) P<0,001 P_2<0,001	4,03±0,07 (n=10) P<0,001 P_2<0,001
3. Wound + laser irradiation	6,87±0,09 (n=10) P<0,001 P_1<0,001	5,50±0,1 (n=10) P<0,001 P_1<0,002 P_2<0,001	3,89±0,06 (n=10) P<0,02 P_2<0,001
	Pyruvate		
1. Control (intact animals)	0,23±0,01 (n=13)		
2. Wound without laser irradiation	0,71±0,02 (n=13) P<0,001	0,34±0,01 (n=13) P<0,001 P_2<0,001	0,32±0,007 (n=13) P<0,001
3. Wound + laser irradiation	1,04±0,1 (n=13) P<0,001 P_1<0,002	0,45±0,02 (n=13) P<0,001 P_1<0,001 P_2<0,001	0,29±0,005 ((n=13) P<0,001 P_1<0,001 P_2<0,001

Legend. Here and in Table 2: n) number of animals.

TABLE 2. Effect of Infrared Laser Radiation on L/P Ratio and RP (M ± m)

Investigated character	Experimental conditions	Day of wound healing		
		4th	8th	15th
L/P	1. Control (intact animals)	14,9±0,4 (n=10)		
	2. Wound without laser irradiation	8,7±0,2 (n=10) P<0,001	16,96±0,8 (n=10) P<0,02 P_2<0,001	13,0±0,4 (n=10) P<0,002 P_2<0,001
	3. Wound + laser irradiation	7,2±0,9 (n=10) P<0,001	12,7±0,7 (n=10) P<0,01 P_1<0,001 P_2<0,001	13,7±0,4 (n=10)
RP	1. Control (intact animals)	—240±0,5 (n=10)		
	2. Wound without laser irradiation	—233±0,3 (n=10) P<0,001	—242±0,6 (n=10) P<0,02 P_2<0,001	—238±0,4 (n=10) P<0,002 P_2<0,001
	3. Wound + laser irradiation	—230±1,9 (n=10) P<0,001	—238±0,8 (n=10) P<0,05 P_1<0,001 P_2<0,001	—239±0,5 (n=10)

that laser irradiation was accompanied by a more marked decrease in the lactate concentration, a decrease in the L/P ratio, and an increase in RP. The results are evidence that seven sessions of irradiation of the wounds caused some degree of inhibition of glycolysis and stimulated involvement of the end products of glycolysis in the tricarboxylic acid cycle. By the 15th day of observation the lactate and pyruvate concentrations were still a little above normal, whereas the L/P ratio and the value of RP were indistinguishable from those in intact animals (Tables 1 and 2).

Marked intensification of glycolysis in the wound after only three sessions of irradiation, with increased involvement of the end products of glycolysis in aerobic oxidation reactions must therefore be regarded as characteristic features of the biological effect of infrared laser radiation.

LITERATURE CITED

1. U. Ya. Bogdanovich, M. G. Karimov, and E. E. Krasnoshchekova, Lasers in Traumatology and Orthopedics [in Russian], Kazan' (1978).
2. A. L. Gushcha, V. A. Vasin, V. G. Papkov, et al., in: Regulation of Inflammation and Regeneration in Surgery [in Russian], Rostov-on-Don (1976), pp. 500-520.

3. V. N. Koshelev, G. N. Tarkov, O. G. Astaf'eva, et al., in: The Use of Methods and Applicances of Laser Technology in Biology and Medicine [in Russian], Kiev (1981), pp. 37-40.
4. M. E. Raiskina, N. A. Onishchenko, B. M. Shargorodskii, et al., in: Methods of Intravital Investigation of Metabolism of the Heart [in Russian], Moscow (1970), pp. 43-44.
5. L. I. Slutskii, Biochemistry of Normal and Pathologically Changed Connective Tissue [in Russian], Leningrad (1969).
6. H. U. Bergmeyer (editor), Methoden der enzymatischen Analyse, Weinheim, Vol. 2 (1974).
7. W. E. Huckabee, J. Clin. Invest., 37, 255 (1958).

EFFECT OF NEUROTROPHIC COMPOUNDS ON TRANSMEMBRANE POTENTIAL OF SYNAPTOSOMES

E. V. Nikushkin, G. N. Kryzhanovskii,*
R. N. Glebov, O. Yu. Maloletneva,
A. P. Kaplun, N. I. Maisov,
E. M. Gankina, and E. A. Syutkin

UDC 615.21.015.44:612.815

KEY WORDS: synaptosomes; transmembrane potential; fluorescent probe; cytostatics; peptides; psychotropic drugs; depolarization.

Besides the secretory, metabolic, osmotic, and other properties of intact nerve endings [2], synaptosomes also preserve a transmembrane potential (TMP) that is closely similar to the K^+-diffusion potential [5, 6]. The very existence of the TMP, its magnitude, sign, and its other characteristics are determined by several properties of nerve endings and processes taking place in them, and they are a reflection of these properties. In particular, the TMP is determined by membrane permeability for ions (its integrity, the chemicophysical state of the membrane components, functioning of the pores and channels, and so on), by the distribution of ions relative to the membrane (the existence of gradients), the work of ion pumps (in particular, Na,K-ATPase), and metabolic activity (ATP synthesis, and so on). At the same time, it is very probable that the TMP can control activity of membrane-dependent processes and can influence the structural and functional state of the membrane, which implies also the function of nerve endings. It is also possible that the action of various neurotropic agents and drugs is determined by their ability to influence the TMP of nerve endings.

In the investigation described below the properties of TMP of the synaptosomes and the action of the various neurotropic agents and drugs on TMP were investigated.

EXPERIMENTAL METHOD

Synaptosomes were isolated from the cerebral cortex of rat weighing 180-200 g by Hajos' method [7]. The synaptosomes thus obtained were suspended in Krebs–Ringer medium of the following composition (in mM): NaCl 132, KCl 5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 1.2, glucose 10, Tris-HCl buffer 20, pH 7.6 (20°C). The synaptosomes were kept at 0°C for not more than 3-4 h. TMP of the synaptosomes was measured by the technique of fluorescent potential-sensitive probes. That this method was possible and suitable for recording and studying the properties of TMP of different cells and subcellular particles was demonstrated previously [8, 13]. Fluorometric measurements were made at 37°C on a "Hitachi-204" spectrofluorometer (Japan). Fluorescence of the 3,3-dipropylthiodicarbocyanine probe [diS-C_3-(5)] was excited

*Corresponding Member, Academy of Medical Sciences of the USSR.

Laboratory of General Pathology of the Nervous System, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. M. V. Lomonosov Institute of Fine Chemical Technology, Institute of Pharmacology, Academy of Medical Sciences of the USSR. Central Research Laboratory, Fourth Main Board, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 51-55, September, 1983. Original article submitted December 14, 1982.

 0007-4888/83/9609-1250$07.50