6. S. S. Gambarov and N. V. Yudina, Byull. Éksp. Biol. Med., No. 1, 70 (1980).
7. D. N. Evnin, Byull. Éksp. Biol. Med., No. 9, 61 (1970).
8. R. Ya. Meshkova, "The graft versus host reaction during reparative regeneration of the spleen," Author's Abstract of Candidate's Dissertation, Moscow (1982).
9. R. Ya. Meshkova and N. V. Yudina, in: Data on Urgent Problems in Modern Histology [in Russian], Moscow (1981), p. 104.
10. G. V. Kharlova, Regeneration of Lymphoid Organs in Mammals [in Russian], Moscow (1975).
11. N. V. Yudina, "Reaction of lymphoid tissue to injury and repair of organs with different regenerative capacity," Author's Abstract of Candidate's Dissertation, Moscow (1980).

EFFECT OF A LOW-INTENSITY CO_2 LASER ON REPARATIVE REGENERATION OF EXPERIMENTAL WOUNDS

V. N. Koshelev, A. V. Arkhangel'skii, and E. I. Glukhov

UDC 615.849.19.015.4:617-001.4-003.9-092.9+617-001.4-003.9-02:615.849.19

KEY WORDS: infrared laser; doses of wound irradiation.

Reports have recently been published on the successful use of low-intensity laser radiation in the treatment of trophic ulcers and indolent wounds [1-3, 5, 7, 8, 11]. However, parameters of laser irradiation are still determined empirically [5].

The aim of this investigation was to determine optimal power densities and exposures of low-intensity infrared laser radiation with a wavelength of 10.6 μ, which have a beneficial effect on regeneration of experimental unsterile skin wounds.

EXPERIMENTAL METHOD

Male Wistar albino rats (216) weighing 200-250 g, had a wound inflicted on the lateral surface of the thigh covering an area of 1 cm^2 bounded by a stencil. Half of the animals (18 in each series) after 1 day were irradiated on the "Klinika-2" apparatus, based on the LG-23 infrared CO_2 laser, daily. The remaining animals served as the control. The rats were decapitated on the 4th, 8th, and 15th days. When choosing the power density of irradiation (0.5, 4, and 20 mW/cm^2) and exposure (2, 6, and 10 min), experience gained in previous investigations [6] was utilized.

The time course of wound healing was monitored by the planimetric method of Hejda and Hejdova in Rusakov's modification. Material was fixed in neutral formalin. Paraffin sections were stained with hematoxylin and eosin, by the methods of Van Gieson, Weigert, Hale, and Brachet, and with Schiff's reagent. The number of vessels and of the different types of cells was counted by means of a modified Avtandilov's grid in five fields of vision in the granulation tissue. The ratio of the number of vessels filled with blood to their total number (in %) was described as the degree of filling of the granulation tissue with blood. The main components of granulation tissue, namely collagen and glycosaminoglycans, were determined as hydroxyproline and hexuronic acid. The latter were isolated by the method of Anastassiadis and Common, followed by quantitative determination of hydroxyproline by Stegemann's method and of hexuronic acid by Dische's method.

The criterion of effectiveness of laser therapy was the aggregate of factors such as wound area, thickness of scab and granulation tissue, its structure, cell composition, and filling of its vessels with blood, and also the quantity of collagen and glycosaminoglycans.

Department of Clinical Surgery and Department of Pathological Anatomy, Saratov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 338-340, March, 1985. Original article submitted May 18, 1984.

0007-4888/85/9903-0339$09.50

TABLE 1. Effect of Laser Radiation on Parameters of Reparative Regeneration of Wounds (M ± m)

Power density of radiation and exposure	Day of investigation in experiment and control	Group of animals	Biochemical parameter, μg/50 mg tissue		Morphological criterion (%)			
			hydroxyproline	hexuronic acids	filling of vessels with blood	No. of polymorphs	No. of macrophages	No. of fibroblasts
	4	Exptl.	102±6,1	11±1,6	30±3,2	55±2,8	1,6±0,8	5±1,2
		Control	142±5,4	24±1,7	61±5,1	34±3,3	4,3±2,1	27±3,6
		P	<0,001	<0,001	<0,001	<0,001	>0,5	<0,001
4 mW/cm^2, 10 min	8	Exptl.	115±4,1	13±1,4	12±3,2	56±2,6	10,4±2,1	22±2,3
		Control	171±8,1	34±1,8	66±5,3	25±2,8	4,8±2,1	34±4,2
		P	<0,001	<0,001	<0,001	<0,001	<0,01	<0,01
20 mW/cm^2, 2 min	15	Exptl.	144±5,8	30±2,3	31±2,8	33±2,2	4,3±1,6	43±3,1
		Control	160±4,4	16±0,8	25±4,1	9±2,1	4,1±1,5	16±3,6
		P	>0,25	<0,001	>0,25	<0,001	>0,5	<0,001
	4	Exptl.	159±7,7	50±2,3	66±2,7	30±2,4	9,8±3,4	58±2,6
		Control	138±6,1	26±3,3	60±4,2	35±2,6	4,9±0,9	29±2,4
			>0,25	<0,001	>0,25	>0,5	>0,05	<0,001
0,5 mW/cm^2, 2 min	8	Exptl.	253±6,4	32±3,2	72±1,6	6±2,4	7,1±2,3	86±4,1
		Control	164±7,3	30±1,6	63±4,2	29±3,1	4,3±1,6	31±2,8
		P	<0,001	>0,5	>0,25	<0,001	>0,5	<0,001
4 mW/cm^2, 2 min	15	Exptl.	176±5,7	16±1,8	50±2,6	3±1,0	4,1±1,1	93±3,3
		Control	167±5,6	14±1,1	29±2,2	9±2,2	4,8±1,3	16±2,6
		P	>0,5	>0,5	<0,001	>0,5	>0,5	<0,001

EXPERIMENTAL RESULTS

Depending on the power density and duration of exposure of the experimental wounds to laser radiation the six series of experiments could be reduced to three different versions of reparative regeneration. Irradiation of wounds by laser with a power density of radiation of 0.5 mW/cm^2 and with exposures of 6 and 10 min caused no significant differences in the principal parameters of wound healing in the irradiated and control animals.

Irradiation of wounds with a power density of 4 mW/cm^2 for 10 min and of 20 mW/cm^2 for 2 min had a similar and usually adverse effect on the principal parameters of regeneration (Table 1). Throughout the experiments the scab was 1.5-2 times thicker than the granulation tissue. The hydroxyproline level fell to two-thirds, and the filling of the vessels with blood to half of the control level. Neutrophils and macrophages were more numerous than fibroblasts. Not until the 15th day were they significantly more numerous than in the control. The hydroxyproline level had only a tendency to rise and this was accompanied by a significant fall in hexuronic acids compared with the control ($P < 0.001$).

Irradiation with a power density of 0.5 and 4 mW/cm^2 and an exposure of 2 min had the greatest effect on the course of regeneration. Under these conditions the scab was identical in thickness (300-400 μ) with the zone of granulation tissue on the 4th day. Resorption of the scab took place more intensively, for by the 15th day it was now only one-tenth as thick as the granulation tissue. Evidently on this account, the number of polymorphs on the 8th and 15th days in the granulation tissue was significantly lower. The parameters of irradiation chosen had a particularly marked effect on proliferation of fibroblasts, the number of which at all times of observation was 2-5 times greater than in the control. Correspondingly the hydroxyproline level also was raised, especially on the 8th day, and the increase in the content of hexuronic acids was less marked than in the control despite increased filling of the vessels with blood. By the 15th day, most cells in the granulation tissue were fibroblasts. The pyroninophilia of their cytoplasm was reduced, they were elongated in shape, and were arranged horizontally along the course of collagen and thin elastic fibers. The area of the wound was reduced to 0.2-0.3 cm^2 compared with 0.5-0.6 cm^2 in the control and 1.4-2.2 cm^2 after irradiation with a power density of 4 and 20 mW/cm^2 and an exposure of 10 and 2 min.

The investigations thus showed that laser irradiation with a wavelength of 10.6 μ, a radiation power density of 4 mW/cm^2 and exposure of 10 min or of 20 mW/cm^2 and an exposure of 2 min, causes inhibition of repair processes in the wound and delays healing.

Radiation with a power density of 0.5 mW/cm^2, with exposures of 6 and 10 min, has no significant effect on processes of reparative regeneration.

Boundary values of laser radiation with power density of 0.5 and 4 mW/cm^2 and an exposure of 2 min are optimal. With these parameters the wounds heal most rapidly, leukocytic infiltration is reduced (by the 8th day), and early and more intensive proliferation of fibroblasts is observed. In the early stages (4th-8th day) the most favorable effect on the course of reparative regeneration is given by laser radiation with a power density of 4 mW/cm^2 and an exposure of 2 min.

LITERATURE CITED

1. U. Ya. Bogdanovich, M. G. Karimov, and E. E. Krasnoshchekova, Lasers in Orthopedics and Traumatology [in Russian], Kazan' (1978).
2. V. N. Koshelev, O. G. Astaf'eva, A. V. Arkhangel'skii, et al., Lasers in Wound Treatment [in Russian], Saratov (1980).
3. V. N. Koshelev and E. I. Glukhov, High- and Low-Intensity CO_2 Lasers in the Treatment of Patients with Trophic Ulcers and Indolent Wounds. Technical Recommendations [in Russian], Saratov (1983).
4. A. A. Krel' and L. N. Furtseva, Vopr. Med. Khim., No. 6, 635 (1968).
5. S. D. Pletnev (ed), Lasers in Clinical Medicine [in Russian], Moscow (1981).
6. G. F. Pronchenkova, "Mechanisms of action of low-intensity infrared laser radiation on the development of experimental wounds," Candidate's Dissertation, Saratov (1983).
7. A. P. Rakcheev, "The use of laser radiation in dermatologic practice," Candidate's Dissertation, Moscow (1973).
8. O. K. Skobelkina (ed.), Current Problems in Laser Surgery [in Russian], Moscow (1982).
9. L. I. Slutskii, Biochemistry of Normal and Pathologically Changed Connective Tissue [in Russian], Leningrad (1969), pp. 78-79.
10. P. N. Sharaev, in: Modified Methods of Investigation of Parameters of Connective Tissue Metabolism [in Russian], Izhevsk (1980), pp. 4-6.
11. V. V. Shur, N. S. Makeeva, A. A. Arenberg, et al., Vest. Khir., No. 6, 85 (1972).

STATHMOKINETIC EFFECT OF CRYOPROTECTORS

E. Ya. Pankov and T. A. Glushko

UDC 541.49:576.3:57.083

KEY WORDS: cryoprotectors; stathmokinetic effect; c-mitoses; cell cultures.

Investigations have shown that the action of certain factors, notably hypothermia, hydrostatic pressure, hyperconcentrated salt solutions, and antitubulins, lead to the appearance of a stathmokinetic reaction in cell systems, which is a reversible process [1, 2, 5, 6]. Exposure to the above-mentioned factors leads to a basically similar morphological picture, expressed as delay (blocking) of mitosis at the metaphase stage, and the appearance of so-called c-mitoses, characterized by a haphazard arrangement of supercoiled chromosomes in the cells.

In the course of our work we also have observed a metaphase block and the appearance of many c-mitoses in short-term cultures of human peripheral blood and transplantable monolayer cultures of Chinese hamster fibroblast-like cells after incubation with cryoprotectors — substances used during low-temperature conservation of various cell suspensions. However, no data could be found in the accessible literature on the stathmokinetic action of cryoprotectors, notably glycerol and polyethylene oxides (PEO-400).

The aim of this investigation was to study the stathmokinetic action of glycerol and PEO-400 during contact with cell cultures.

EXPERIMENTAL METHOD

The test material was a short-term culture of human peripheral blood leukocytes stimulated by phytohemagglutinin (PHA) and a monolayer transplantable culture of Chinese hamster

Department of Histology, Khar'kov Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 340-342, March, 1985. Original article submitted April 19, 1984.