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EFFECTS OF He-Ne LASER BEAM ON MECHANICAL, HEAT, CHEMICAL AND SUPERFICIAL WOUNDS *

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ABSTRACT

This study summarizes the effects of low-doses of He-Ne laser radiation ($\lambda = 6328$ Å), on healing of four types of wounds, including mechanical, heat, chemical and superficial wounds. The results revealed that variations between complete wound-closure in irradiated samples and that of control groups were statistically significant. Moreover, the results suggest that the stimulative action of laser is an accumulative phenomenon, that affects factors involved in the course of wound healing. The results also indicate that the skin epithelium is a highly responsive tissue towards this sort of radiation, which suggests that the stimulative action of He-Ne laser could be assayed easily by using such tissues as a test target.

1. INTRODUCTION

Einstein theories in 1917 made the first contribution to the principles and the essentials of the lasers. But the first working device for such highly coherent light was not produced until 1960 by Theodore Maiman (Maiman, 1960). The causes for such delay could be understood. Since Einstein made no mention of coherence in the case of stimulated emission. This event was followed by the construction of the first continuous wave laser in 1960. Successful attempts have been made to obtain lasers from other sources.

Lasers found applications in different fields of science and technology, including biology and medicine. The development of such diverse applications proceeded very fast in comparison with any sources of energy. Argon lasers were used for the first time in 1965, for treating diabetic retinopathy. The ways that biological tissues can interact with laser beams is described in terms of reflection, transmission, scattering and adsorption properties in such systems (Absten et al., 1985).

The effects of such beams on the human body come from four main functions: Heat function, Pressure function, Photochemical function and electromagnetic function (Yoshino et al., 1982). Such light can be used as stimulative (constructive) or destructive tool in biosubstances because of its ability to interact with such substances at various molecular and atomic level (Letokov, 1985). In general high energy laser beam is used as a destructor, while low energy beam is used as a stimulator, specially in the living systems. Although the biological effects of low level radiation suggests serious problems (biological effects of low level radiation, 1983), such problems - to our knowledge - with low doses of laser beam have not been firmly reported, and the possibility remains for low doses damage of laser beam. The stimulative effects appeared in the mid-sixties (Wolbarsht, 1974). Although the exact mechanism of such stimulation in living systems is not known precisely, many postulates were put forward to achieve a solution for such an obscure problem (Fröhlich, 1985; Karu et al, 1983).

There have been reports of biostimulation ability of the red laser light, including anti-inflammatory, stimulation of regenerative power of tissues, indolent wounds and severe burns treatment. This is well accepted in medicine by some groups (Karu et al., 1983). The indirect effect of red light on nucleic-acid synthesis rates, and the changes in the permeability of the cell membrane has also been reported (Karu, et al., 1982). No significant difference is known to exist between laser and incoherent red

light stimulation effect on the growth of E-Coli (Karu et al., 1983). Many works have been carried out in skin injuries of laboratory animals and wounds (Wolbarsht, 1977).

In a trial for further investigations concerning the effects of He-Ne laser (Alsenawi, 1985) on different classes of wounds, four types of wounds were chosen and made by us, two of which at least have been put into test for the first time.

Connective tissue elements attracted previous workers, in this present work, the attention is devoted to the epithelia as well, since they are greatly responsive tissue to high doses of radiation and injuries. They are stratified into known layers with well established ways of quantitatively, assaying its cellularity, regeneration, proliferation and differentiation.

2. EXPERIMENTAL TECHNIQUE

Following hair plucking, mechanical, epidermal tape-stripping, temperature and chemical (nitric acid) wounds were inflicted on the dorsal areas of swiss white mice. The radiation source used was He-Ne gas laser (PHYWE, Göttingen, West Germany), with a power of 1.0 mW, $\lambda = 6328$ Å and a beam diameter of 0.6 - 1.00 mm at the source. Special irradiation technique used, which is summarized as follows: the animals were kept at a distance of 25cm from laser source throughout all the experiments; the wounds peripherally irradiated at three points (focussed beam) using three such instruments simultaneously to form a semi-equilateral triangle. Actually, this is one of the methods which we are trying in our laboratory. The reason for peripherical irradiation arose from the remark that the epithelial cells are absent in the central region of the wound. Mechanical and stripping wounds were exposed to doses between 0.36 - 3.6 Joule/day. A fractionation experiment for the greatest stimulating dose (0.9 Joule/day) in stripping wounds was also carried out. Like mechanical wounds, the temperature and chemical burns involve killing of both the epiderms and dermis, and hence, only the most stimulating dose for the former experiment was experienced here. The wound-healing in irradiated, and in unirradiated groups of mice, was followed by daily measuring the wound diameter. This was done at four different directions and then obtaining the surface area of each wound. The data were averaged for each group (4 mice per group). This procedure was followed daily until the phase of complete wound-closure was achieved. The results were subjected to proper statistics, and revised using ANOVA programme - SPSS, at University of Trieste Computer Centre.

In addition, several other observations were carried out during the course of wound healing. The healing for the most stimulatory dose in both mechanical wounds and epidermal stripping was subjected to histolical study. Moreover, gross observations were made on the irradiated and unirradiated animals for the period of four months, in search of the development of any abnormalities in the skin, although we are still carrying out our investigations on possible latent effects.

3. RESULTS AND DISCUSSIONS

The results revealed that low doses of laser radiation stimulated several aspects of wound-healing. Some data were liable to be assayed quantitatively, others were in the form of qualitative phenomena. However, they are all interrelated and as a whole produce the end results of their significant effect over the control (unirradiated) in wound-healing. We think that the stimulation factor, despite the diverse aspects, could arise as a result of a single primary motivation, somewhere and somehow in the living system.

Although the He-Ne laser is known to be noncoagulative radiation, blood clotting in mechanical wounds was obvious soon after irradiation, whereas in control wounds it was completed only a day later, a point which puzzled us, and is left for further investigations. The promotion effect of laser here is likely to be due to elevation in temperature at the wound surface. The skin colour in the irradiated stripped wounds changed from normally pink to reddish before the unirradiated ones, probably as a result of dilation in the dermal blood vessels. The re-establishment of normal colour in the irradiated area also occurred before the unirradiated one. Skin contraction was also promoted by laser, especially in mechanically wounded animals. While the mechanism responsible for this action has been attributed to dermal elements, it is suggested herein that fibrin may also play a role. This is achieved by connecting the cut margins and then withdrawing them towards each other, which obviously should result in the contraction of the skin at a very early stage after its wounding. Appearance of an elevated areola around the mechanical wound constitutes another promotion by the laser. At an early stage this is apparently due to the skin contraction. The occurrence of hyperplasia at the wound margins is common in various sorts of skin injuries. But usually it occurs at the time of maximum reaction. This acts as a reservoir for the epidermal cell proliferation and migration into the inside of the wounded region. The scars covering the irradiated wounds appeared earlier; their size were smaller and peeled prior to those covering the unirradiated wounds. The stimulation of new hair growth in areas

surrounding the mechanical and tape-stripped wounds was enhanced by laser irradiation. In the other types, it appears that conduction of temperature, or diffusion of acid from the treated areas into areas surrounding the wounds, have partially masked such an action.

This study was devoted to assaying the complete wound-closure, which as mentioned above previously, comes from complex factors, which may have originated from one single factor. The assay is liable to be carried out in living animals and, therefore, should be more suitable for application to human wounds.

Fractionation of the laser's most stimulating dose suggests that the action exerted by the laser is an accumulative mode, or that several phases of wound-healing process are targets for such stimulation. Since stripping involves only the epidermis, the results of this experiment confirmed that the skin epithelium is a highly responsive tissue. This fact verifies that the maximum stimulatory dose for stripping was lower than that for the mechanical wounds (i.e. 0.9 vs 2.7 Joule/day), and that a dose of 3.6 Joule/day which was stimulating in mechanical experiment was inhibitary in the stripping one. Other doses ignored till it is verified, as indicated in Table 1.

In epidermal stripping exposed to 0.9 Joule/day, the histological study revealed a significant increase in the number of basal cells per unit area. The cells were scored in epidermal sheets between days 3-9 post-wounding, at comparable distances from wound margins for irradiated and control specimens. A similar promotion was seen in mechanical wounds, where exposures to 2.7 Joule/day produced a significant increase in the number of epidermal nucleated cell layers and their thickness, as well as the thickness of the whole epiderms. These parameters were determined in the skin sections between days 3-13 post-wounding, at various comparable distances from the wound margins. No abnormalities in cells were detected. This could add something in favour of such stimulation. Another indication was that the epidermis, as a whole, started to return to its normal state, as a result of a decrease in the above mentioned parameters. Four months of results suggested no abnormalities in epidermis. The histological examination also revealed that the hair-follicles, which are sited at the margins of mechanical wounds, and inside as well as at the margins of stripped wounds, were enlarged in size. The role of follicular cells in wound-healing was appreciated.

In most of the works in the field of laser stimulation of wound healing, energies and wave-lengths were chosen so that (hopefully) they produce no serious damage to the skin of species used in the experiment. Since other low level radiation could cause biological damages, these possibilities should not be ruled out in case of low power laser effects. Factors such as pigmentation, keratinization, hair and its growth cycle, site variation and tissue composition and other macroscopic and microscopic factors should be taken into consideration.

Finally, if we consider a general exponential decay for the original wound area with time, and using the term $S = S_0 e^{-\alpha t}$ where

S_0 = percentage surface area of the wound at $t = 0$

S = percentage area of the wound after time t during the process of the healing

t = time in days

α = decay constant for each type

then the average values' qα derived for each case are indicated in Table 2, which shows how close some of the cases are from a smooth expontential decay and indicates the sharpness of each decay until the phase of complete wound-closure.

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TABLE CAPTIONS

Table 1 . The healing days for all types of wounds for unirradiated and irradiated samples with different doses with a significant of F over the control for effective doses F = 0.001.

Table 2 The average α values for each wound type in all cases.

TABLE 1

Type of the Wound	D = 0.0 (control)	D = 0.36	D = 0.9	D = 1.8	D = 2.7	D = 3.6	D = 7.2
1 - Mechanical	18	16	15	15	14	15	-
2 - Heat	17	-	-	-	13	-	-
3 - Chemical	26	-	-	-	21	-	-
4 - Superficial	13	12	10	11	15	15	11

D = exposure doses in Joule/day

TABLE 2

Average Values of α for each case

Wound Type	D = 0	D = 0.36	D = 0.9	D = 1.8	D = 2.7	D = 3.6	D = 7.2
1 - Mechanical	9.73×10^{-2}	2.14×10^{-1}	1.52×10^{-1}	1.40×10^{-1}	2.56×10^{-1}	2.44×10^{-1}	-
2 - Heat	6.55×10^{-2}	-	-	-	8.33×10^{-2}	-	-
3 - Chemical	8.04×10^{-2}	-	-	-	1.05×10^{-1}	-	-
4 - Superficial	1.88×10^{-1}	2.09×10^{-1}	2.63×10^{-1}	2.32×10^{-1}	2.11×10^{-1}	1.86×10^{-1}	2.65×10^{-1}

D = exposure doses in Joule/day.