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"Incorporation of [^{3}H]-Proline in the Dermis of Mice Following He-Ne Polarized Laser Radiation in the Wound Healing Process. A Preliminar Study"

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INTRODUCTION

Low-level laser therapy (LLL) has been used in many experiments since the 60 decade to examine the influence of laser radiation on the healing process of wounds. However, despite of a large number of studies published in the literature, results are many times conflicting and only a few has presented scientific argument (karu, 1989; karu, 1995; Fenyo, 1984; Kamikawa and Ohnishi, 1992). Some papers have suggested the increase in the wound healing rate of closure alters LLL exposure in rats (Becker, 1990; Kana et al., 1981; Rochkind et al., 1989) while others found no change (Kass, 1990; Anneroth et al., 1988). In these experiments the polarization effects were not considered. A more recent paper showed that the coherence and polarization of laser light play an important role in wound repair but the effect of the polarization component was not considered (Nicola et al., 1994). According to the Maxwell's equations to optical properties of surfaces, the energy deposition efficiency in a microroughness interface depends on the electrical field polarization component (Ribeiro, 1991). Considering a linearly polarized beam, this efficiency will depend of the roughness parameters to p-polarized light and it will not depend of such parameters to s-polarized light. For this reason, a radioautographical study on ^{3}H-proline incorporation by mice dermis was performed in which the effects of the s- and p- polarization components of laser light on wounds healing artificially created in mice skin were investigated.

PROCEDURES

It was used a group of 10 male adult mice weighing about 30 grs. The source of light was a He-Ne laser (UNIPHASE, USA) emitting a wavelength of λ=632.8 nm, 10 mW in output power and beam diameter about 2 mm, mounted in a convenient set up. The emission from the probe was modified to ensure an uniform exposure of the wound by inserting optical components: a Glan-Thompson polarizing prism with a precision disk used as holder to rotate it in 90° and thus to get s- and p-polarized light; a convergent lens (f= 7 cm) and a neutral density filter 0.04 for λ= 632.8 nm. To obtain an expanded beam of 6 mm an objective was used with f= 5 cm and ratio 2:1.

Five experimental groups of 2 animals each were used. The animals were anesthetized by ether inhalation. After anesthesia the back of mice was shaved. Two round burning measuring about 6 mm in diameter were produced at the end of the spinal column of each animal during three consecutive days using a cylindrical brass rod cooled to 77 K. The contact was kept for five seconds. The application was made twice a day with an interval of five minutes for a total of three days (Ribeiro, 1991). After the last application, the lesion # 1 was illuminated by either He-Ne s-polarized or p-polarized laser and the lesion # 2 was not irradiated (control). The total dose was 1 J/cm^2 per irradiation corresponding to an exposition time of 3 minutes. The lesions were irradiated on the 3rd, 7th, 10th and 14th day. After each irradiation one group of mice was injected intraperitoneally with 5 μCi/g of body weight of ^{3}H proline and killed by cervical dislocation after 1 hour. On the 17th day the last animals were killed. After sacrifice, the wounds were removed and fixed in Bouin's liquid overnight. They were then dehydrated in graded ethanol followed by clearing in xylem. The specimens were then embedded in paraffin and cut at 5 μm. After, sections of tissue were submitted to liquid emulsion technique. The specimens were mounted on glass slides and coated in dark chamber with K2 radioautographical emulsion by a dipping method (Oliveira, 1995). Following this procedure, they were kept in a black box for 30 days. Afterwards, the autoradiographs were developed in D19b developer and stained with hematoxylin and eosin. The quantitative analysis was evaluated by counting silver granules on a selected area of tissue. An eyepiece containing a grid which outlined squares measuring 81 μm^2 was used. Silver granules were counted within 20 squares of each region (wounded and non-wounded) of each animal dermis.

7759

1178

RESULTS

The curve of incorporation of ^3H proline is showed in figure 1. It was observed that: a-) both irradiated groups showed a very similar pattern of incorporation; b-) the incorporation in p-polarized dermis was slightly higher than in s-polarized during the first 7 days; c-) both groups showed a higher incorporation on day 3 than the control group; d-) in the irradiated groups, however, the incorporation decreases on day 7 and increases again up to a peak on day 14; e-) the incorporation in control group starts lower but progressively increases up to day 10 when it decreases below the level of irradiated group. On the 17th day, however, both control and irradiated dermis had similar levels of incorporation.

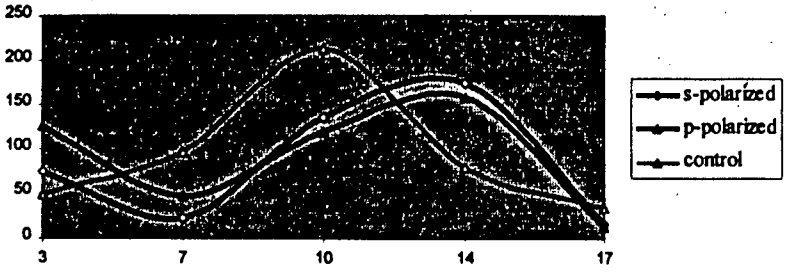

Figure 1: Incorporation Curve of [^3H]-Proline In the Dermis of Mice

DISCUSSION

There is a rich literature about the biostimulating effects of low power light, but information is quite dispersed. Lubart et al., 1993 reported that the coherent irradiation is not essential on fibroblast proliferation. Rigau et al., 1991 showed no increase in the number of fibroblasts following LLL exposure, but revealed significant changes in metabolic rates when compared with unirradiated controls. Loevschall and A-Bindslev, 1994 demonstrated an increased incorporation on tritiated thymidine in cultured human oral fibroblasts after LLL irradiation. These experiments did not consider the polarization effects. Mester et al., 1978 were the first to compare the effects of monochromatic polarized and non-polarized normal light with those of laser light with respect to the immunosuppressive effect of human lymphocytes. They found that the effect of incoherent light was 0.74% when compared to that of the laser. With plano-polarization of corresponding plane, an efficiency of 80% was achieved. Bolton et al., 1992 reported that the proliferate response of fibroblasts was greatest in the cultures exposed to supernatants from macrophages treated with the 95% polarized light source when compared to 14% polarized light irradiation.

Although non polarized and/or non coherent light are made responsible for many biological effects, Nicola et al., 1994 showed that coherent and polarized light plays an important role in the wound healing. We suggest that the polarization component of radiation is essential factor too, if the exposure is visible light. This argument is in according with the Maxwell's theory for the optical properties of surfaces.

Our results indicated an accelerated healing process of inflammatory lesions created in the end of the spinal column of mice rats by p-polarized low energy He-Ne laser. We propose that this electrical field component polarization enhances fibroblast collagen production. Quantitative autoradiography with ^3H proline indicates that laser radiation induces waves of proline incorporation that start earlier in the wounded dermis and thus could be related with the improvement of wound healing.

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