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ORIGINAL ARTICLE

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Action of low-level laser therapy on living fatty tissue of rats

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Abstract Little is known about the action of laser rays on normal adipose cells. The present study attempts to observe the behavior of fatty cells submitted to laser therapy. Dorsal fat pads of normal adult rats were submitted to low-level laser irradiation applied locally through intact skin, with four different dose schedules (4, 8, 12, and 16 J/cm^2), with a further group being sham-irradiated. Histology, morphometry, immunofluorescence, and electron microscopy were all used to analyze irradiated tissues. Changes were restricted to the brown fatty tissue, in which a tendency was shown for multivacuolar cells to be transformed into the unilocular type. The number of cells which exhibited enlargement and fusion of small vacuoles was greater in the 4- and 16-J/cm^2 groups ($p<0.05$). Increased vascular proliferation and congestion was another more evident finding in laser-treated animals compared to nontreated animals. Low-level laser rays cause brown adipose fat droplets to coalesce and fuse. Additionally, they stimulated proliferation and congestion of capillaries in the extracellular matrix.

Keywords Low level laser · Adipocyte · Lipolysis

Introduction

Progress in endocrinology and plastic surgery has resulted in having a great deal of new data on adipose tissue biology available, and it is becoming clear that the structure and dynamics of this type of tissue is much more complex than was previously thought, to the point that it has even been considered to be a true autocrine, paracrine, and endocrine organ [1–3]. Consequently, there is an urgent need for more information on agents that can affect fatty cells, among them, laser rays.

In an attempt to characterize the action of low-level laser rays on adipocytes, Neira et al. [4] added a 10-mW potency diode laser to the lipoplasty procedure and observed that pores were produced on the fat cell membrane, followed by the emptying of its contents.

However, biological activity induced by low-level laser rays presents a wide spectrum. Its influence on pain, chronic inflammatory diseases, and wound healing has already been documented [5–7]. Therapeutic laser is able to influence the functional activities of several cellular types, including fibroblasts, myofibroblasts, endothelial cells, pericytes, osteoblasts, and epithelial cells [8, 9]. However, little is known about the changes that laser therapy can induce on other cellular types in vivo, especially on adipocytes.

In the present study, the behavior of normal fatty tissue in response to low-level laser therapy, with different degrees of energy densities and time intervals, was investigated on the dorsal fatty pads of normal rats, by means of histology, morphometry, immunofluorescence, and electron microscopy.

Materials and methods

Experimental groups

Twenty Wistar rats of both sexes, weighing between 150 and 250 g and kept in wide metal cages under similar conditions of temperature and light, were maintained with free access to a commercially balanced diet and water. The animals were randomly separated into five groups of four animals each. Group I represented sham-irradiated controls, treated in the same way as for the other groups, but with the laser apparatus off. Groups II, III, IV, and V represented animals

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treated with 1 J/cm^2 every other day, performing total doses of 4, 8, 12, and 16 J/cm^2, respectively.

Surgical procedure

With the animals under general anesthesia, the dorsal region was shaved, followed by the delimitation of an area of approximately 2.0 cm in the largest diameter, reserved for the laser applications through normal skin. An apparatus (Laser VR-KC-610 Dentoflex, Brazil) generating continuous emission by a semiconductor diode Ga–As–Al, with 9 mW, 670 nm wavelength, was used.

The time schedule for each application followed the equation proposed by Tunér and Hode [10], such being 31, 62, 124, and 248 s for the groups receiving 4, 8, 12, and 16 J/cm^2, respectively. The area reached by the active tip of the apparatus was automatically calculated (Mitutoyo, digimatic caliper 500-144B, Brazil).

The rat's dorsal fat pad was removed immediately after the total dose was completed. The total pad was carefully removed by dissection and fixed in 10% buffered formalin, followed by paraffin embedding. Histological sections were routinely stained with hematoxylin and eosin, Weigert's orcein for elastic fibers and Sirius-red for collagen.

Morphometry

Five 46,100.02-μm^2 areas of the brown fatty tissue from each section stained by hematoxylin and eosin were randomly selected for counting the large adipose cells present. A 400× magnification was used (AxioVision 2.0, Zeiss).

Immunofluorescence

Fragments of fatty tissue were immediately embedded in Tissue-teck (OCT compound, Miles, Inc., Diagnostic Division, Elkhart, USA) and immersed in liquid nitrogen for a few minutes and then kept frozen at −70 °C in airtight boxes, until the moment they were sectioned in a cryostat at −30 °C. The sections were submitted to the indirect immunofluorescence technique to demonstrate the collagen isotypes (I, III, and IV; dilution 1:80), laminin (1:100), and fibronectin (1:800). Specific polyclonal antihuman antisera were obtained in rabbits (Institute Pasteur, France). They were used in dilutions varying from 1:40 to 1:100. Details concerning their preparation and specificity tests appear elsewhere (Andrade and Grimaud 1988). Secondary fluoresceinated antirabbit-IgG was commercially obtained from Sigma (St. Louis, MO, USA).

All procedures were performed according to the manufacturers' instructions. Primary antibodies were incubated from 30 min at 37°C. The fluorescent antibody was also incubated using the same pattern. The slides were seen through a microscope provided with UV light and appropriate filters. Control of nonspecific staining was done by substituting the primary antibody with phosphate buffer solution (PBS) and bovine serum albumin (BSA).

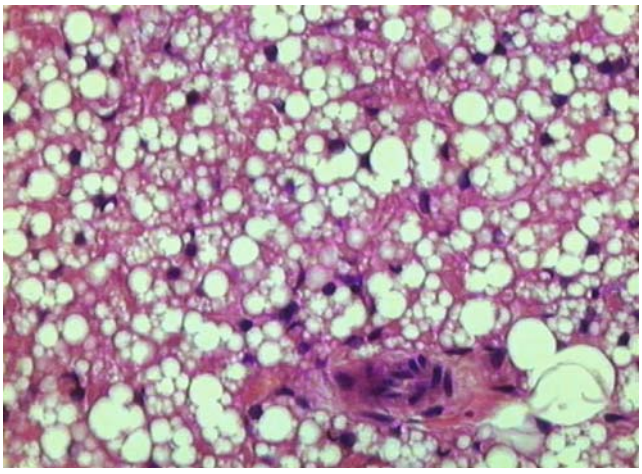

Fig. 1 Normal appearance of the multilocular fat tissue present in the dorsal fat pad of the rat. H & E, ×400

Electron microscopy

Tiny fragments of tissue were fixed for 1 h at room temperature in 2% glutharaldehyde in 0.1 M cacodylate buffer, postfixed in 2% OsO_4, dehydrated in graded acetone, and embedded in Epon 812. Semithin sections were cut and stained with methylene blue. Representative areas selected for ultrathin sections were collected on copper grids, double-stained with uranyl acetate and lead citrate, and examined with a Zeiss EM-109 electron microscope at 50 kV.

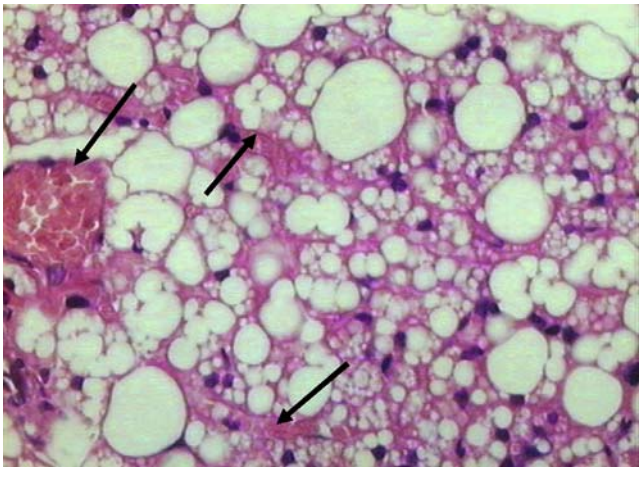

Fig. 2 After laser radiation the fat goblets of the dorsal fat pad appear enlarged (*fused*) and the blood vessels congested (*arrows*). H & E, ×400

Table 1 Distribution of adipose cells which exhibit enlargement and fusion of small vacuoles in brown adipose tissue

Experimental groups	Mean±SD	*p* value
Control	28.15±7.05*	0.02
4 J/cm^2	42.3±5.4	
Control	28.15±6	0.24
8 J/cm^2	39.25±5.2	
Control	34.72±6.3	0.78
12 J/cm^2	39.2±4	
Control	30.73±3.2*	0.001
16 J/cm^2	45.50±4	

*$p<0.05$

Statistical analysis

Parametric versions of one-way ANOVA and the Kruskal–Wallis test were used, the former for variance analysis and the latter for differences between groups. Results showing $p<0.05$ were considered significant.

Results

All routine histological sections, including those stained with hematoxylin and eosin, Weigert's orcein and Sirius-red, showed the presence of the two types of adipose tissue found in the dorsal pad: the uni- and multilocular types. The yellow fat was predominant, represented by focal collections of unilocular fat cells delimited by loose bands of connective fibrous tissue. With regard to morphometrical measurements, the brown fatty tissue represented 35% of the fat pad. It exhibited a characteristic appearance, with a central nucleus and a microvacuolated cytoplasm. The fibrous tissue had isolated clusters of cells and was rich in blood vessels. A marked expression of fibronectin and collagen types I and IV was also observed in connective tissue by immunofluorescence. Elastic fibers were rare and within normal limits in all sections examined with the orcein stain.

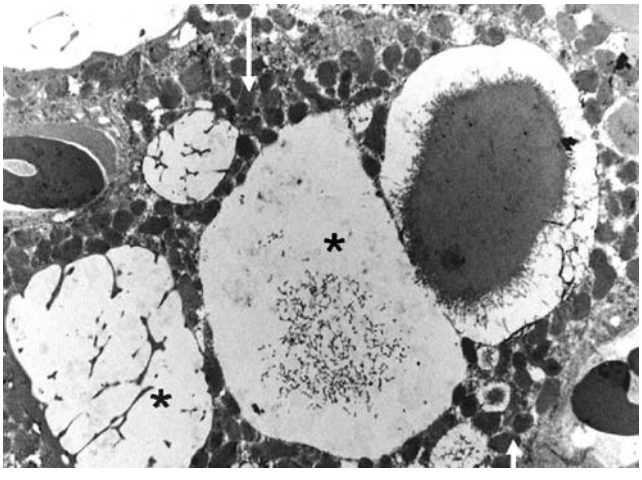

Fig. 3 Ultrastructural changes observed in the multilocular fat tissue of the dorsal fat pad of rats following laser radiation. Surrounded by numerous mitochondria (*arrows*), three vacuoles are seen (*asterisks*), probably representing different stages of progressive dissolution of fat. Electron micrograph. ×7,000

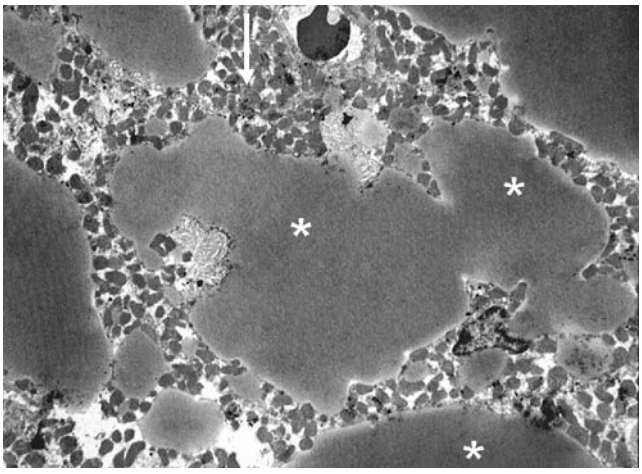

Fig. 4 A large and convolute-shaped fat droplet appears encircled by mitochondria (*arrows*) inside a multilocular fat cell from the dorsal fat pad of the rat which had been irradiated by laser rays. Electron micrograph. ×7,000

The groups submitted to laser radiation presented only mild alterations and exclusively for the microvacuolar brown fatty tissue. These were represented by variable degrees of enlargement and fusion of vacuoles that resulted in the transformation of the microvacuolar cells into the unilocular type (Figs. 1 and 2). Islets of large unilocular fatty cells, with nuclear displacement toward the cell periphery, appeared more frequently and markedly in the group of animals treated with 16 J/cm^2 compared to the other groups or the control group (Table 1). Ultrastructural findings confirmed this tendency of the multivacuolar cells to be transformed into the unilocular type in the group treated with 16 J/cm^2 (Figs. 3 and 4). However, the external cell membrane preserved its normal appearance, never presenting ruptures or pores, in spite of the disposition of its

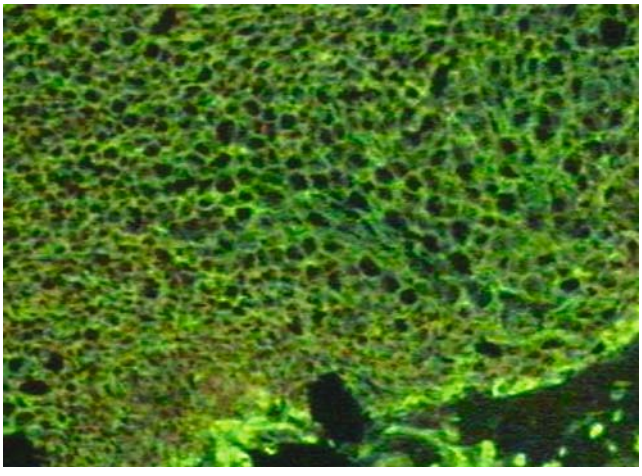

Fig. 5 Immunofluorescence staining for laminine in the unilocular fat of the dorsal fat pad of the rat discloses the integrity of the interstitium. ×200

fused fatty vacuoles. On the other hand, the yellow fatty tissue always preserved its normal appearance. During the ultrastructural analysis, no evidence of lipolysis was ever found in any of the groups. Demonstration of collagen IV and laminin helped to confirm basement membrane integrity (Fig. 5).

A rather constant finding, particularly for the groups treated with 4, 8, and 12 J/cm^2, was vascular congestion, which was marked in capillaries and veins.

Discussion

The advent of laser treatment has stimulated the study of the mechanisms involved with the transformations living tissues and cells undergo following exposure to this form of monochromatic light. The majority of in vivo and particularly in vitro studies have dealt with transformations affecting the fibroblasts [11, 12]. The fibroblast is the central member of the so-called "fibroblast-family", which includes osteoblasts, condroblasts, pericytes, and adipocytes, among other cells [13]. However, although the influence of laser treatment on these cells has become clear, its effects upon several other cell types, such as the adipocytes, still remain poorly understood. According to Neira et al. [4], a low-power laser of the semiconductor diode type, with a 635-nm wavelength, is able to disrupt the external membrane of the fat cell, inducing lipolysis. This effect was even more evident when laser beam exposure was combined with the tumescent technique, which seemed to enhance the action of the laser rays. The present investigation showed that the action of different radiances from a gallium–aluminum arsenide type of laser, applied through the intact skin to the dorsal fat pad of rats, caused definite changes in the brown fat cells. The yellow macrolocular fatty tissue seemed more resistant. No signs of lipolysis were observed. Such data are at variance with that of Neira et al. [4], but this divergence has to be considered with caution since the type of laser used and its mode of application, doses, schedules, etc., were not the same for the two experiments. For example, it has been known that the photon density falls during light penetration in soft tissues [14]. The skin can reflect, absorb, or transmit light rays. Furthermore, there are also other variables that influence laser light, like the density of microvascular structures, and in human skin, the percentage of melanocytes.

Embryologically, the multilocular fat cells appear to be the precursors of the unilocular type. Apparently, the laser rays have the potential to accelerate or provoke this transformation in an adult tissue. Furthermore, one may assume that multilocular fat is unstable, and that its fat droplets tend to coalesce under the slightest stimulus. As a matter of fact, laser light may cause changes at the biochemical level that may not be detected by morphological techniques [15–17]. Additionally, in our preparations, mitochondria were numerous in fat cells and did present some variations in diameter, although we did not notice any presence of megamitochondria as described by Karu [15].

It is interesting to note that congestion was a more evident finding in laser-treated animals than in nontreated animals. Moreover, the groups submitted to irradiance of 4, 8, and 12 J/cm^2 presented more congested vessels when compared to the other groups, revealing a dose-dependent effect. Congestion can be a transient change, but may be at the root of the potential for laser rays to induce biological changes in living tissues. This vascular factor may be crucial during wound healing under laser treatment. These data are in accordance with reports from other studies. Garavello et al. [18] observed endothelial cell proliferation in animals submitted to laser therapy. Schindl et al. [19, 20] described an increased circulation in local areas of patients treated with laser rays.

The effectiveness of low-level lasers in producing biological changes in different cells and tissues, even in low doses, has been well documented. This study contributes to the understanding of what happens to the adipocyte when it interacts with this type of light, particularly the alterations observed in brown adipose tissue. Further investigations are necessary to establish the optimal dose, the ideal modality of application, and all other parameters that are crucial for reliable results.

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