Evaluation of the Use of Low Level Laser and Photosensitizer Drugs in Healing

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Background and Objectives: In the last decade, many different kinds of therapies have emerged as a consequence of advances in the field of applied technology. It is known that low level laser therapy contributes to tissue healing; however, the use of photodynamic therapy (PDT) in healing and the scar formation processes has not been fully explored. The present study analyses the effect of low level laser InGaAlP (685 nm), radiation, either alone or combined with a phthalocyanine-derived photosensitizer (PS) in a gel base delivery (GB) system, on the healing process of cutaneous wounds in rats.

Study Design/Materials and Methods: The rats were divided into six groups: control (untreated) (CG), gel base (GB), photosensitizer (PS), laser (LG), laser + photosensitizer (LPS), and laser + photosensitizer in a GB (LPSG). Standardized circular wounds were made on the dorsum of each rat with a skin punch biopsy instrument. After wounding, treatment was performed once daily and the animals were killed at day 8. Tissue specimens containing the whole wound area were removed and processed for histological analysis using conventional techniques. Serial cross-sections were analyzed to evaluate the organization of the dermis and epidermis as well as collagen deposition.

Results: The animals of groups LG, PS, LPS, and LPSG presented higher collagen content and enhanced re-epithelialization as compared to CG (control) and GB rats. Connective tissue remodeling was more evident in groups LPS and LPSG.


Key words: laser therapy; photodynamic therapy; photosensitizer; wound healing

INTRODUCTION

The ability of the body to restore its integrity subsequent to many different kinds of injury is essential for the maintenance of life. Any living organism, in its constant interaction with the environment and with other organisms, eventually faces challenging situations, such as harsh climatic conditions, infections diseases or accidents about the need to repair or reconstitute its body structures in order to preserve function.

Wound healing is an organized and complex response of the organism to tissue injury and subsequent loss of integrity. In open wounds that heal by secondary intention, several factors come into play and invariably contribute to delay in healing, almost always resulting in inflammation, edema, and scars. Several therapeutic approaches have been used in order to obtain better healing results. Research on treatment of skin loss has basically followed three main directions: the improvement of healing by factors that speed up the process and reduce scar formation, the development of skin substitutes or functional equivalents, and the identification and characterization of factors that induce the regeneration of the skin [1].

Studies on the effects of low level laser irradiation on the skin are essential and serve as a research model for the effects of laser light on the epidermis (epithelium) and dermis [2]. Medical, odontological, and rehabilitative specialties use low level laser therapy in some therapeutic applications, including those which use its analgesic and anti-inflammatory effects, tissue repair action [3–9], and also those which rely on substances that reduce the risk of self contamination and speed up the healing process [10]. Photodynamic therapy (PDT) has been explored in the last three decades as a new therapy that involves the activation by light (visible and near infrared) of different exogenous dyes (called photosensitizers (PSs)) which are easily taken up by the target tissue [11,12]. This process takes place in the presence of molecular oxygen at normal levels.

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PDT is mainly used in the treatment of neoplastic and non-neoplastic diseases [12,13], and appears to be a promising clinical tool for treating tumors and a considerable number of other conditions, such as arthritis, skin disorders, and many other non-oncological diseases [14]. The technique is based on the injection, ingestion, or topical application of PS drugs (usually in the presence of a specific drug delivery system) followed by light activation [14]. These PSs are chemical compounds that absorb light of a specific wavelength and are able to use this energy to induce excited state reactions on PS molecules. The reactions follow complex mechanistic pathways through classical photochemical reactions [12], leading to the production of several reactive oxygen species (ROS) that are active in photodynamic processes.

Trends in PDT research, since it has been discovered in the 1970s [15], have shifted towards the development of new PSs with improved characteristics. The extraordinary advances are due to its relatively simple concept—the combination of an innocuous PS, light of adjustable wavelength, and molecular oxygen—allied with the high degree of interdisciplinarity achieved in research studies of this therapeutic modality [12]. Some studies with PDT systemically mediated PS can inhibit the healing process [16,17]. However, other studies show that it does not cause any inhibition in animals [10], and may even accelerate the wound healing process [18].

The purpose of this study is to histologically analyze the healing process of standardized cutaneous wound in rats, using low level laser radiation, alone or combined with a phthalocyanine-derived PS delivered in a gel base (GB).

MATERIALS AND METHODS

Animals and Surgical Procedure

All the experiments performed in animals were approved by the “Committee for Animal Research Ethics Committee of the São Lucas Hospital (Brazilian Animal Protection Act, Federal Law No 6638 from 05/08/1979 COBEA July 1991, Protocol SL0139) of the Brazilian Federal Ministry of Science and Research.”

Sixty male adult Wistar rats (Ratus norvegicus albinus), from 3 to 3½ months of age, weighing between 200–250 g, were obtained from the animal facilities of Salesinas College of Lins, São Paulo, Brazil. The animals were kept in individual cages and were provided with standard ration and water “ad libitum.” Sixty (n = 60) rats were divided into six groups: control group (CG); gel base (GB); photosensitizer (PS); laser (LG); laser + photosensitizer (PS); and laser + photosensitizer + gel base (LPSG). The rats were placed under anesthesia with Zoletil® 50. (0.1 ml/100 g/i.p.) and shaved on the back, with adequate disinfection of the shaved area. Standardized circular wounds were then created on the dorsum of each animal with a skin punch biopsy instrument (8 mm diameter). No dressing was applied in the wounds during the experimental period.

Laser Therapy and Topical Agents

Irradiations were carried out at 685 nm using an InGaAlP diode laser (Thera Lase-DMC, São Carlos, SP, Brazil) and were performed transcutaneously at four sites on the edge of the wound (fluence of 2.5 J/cm² and 35 mW power density). In the experimental groups LPS and LPSG, laser light exposure was done after 10 minutes of topical application of the dye, either with or without the GB. Other studies involving different PSs, used in topical application for skin cancer treatment, have shown that the time range of 10–15 minutes after application is enough to reach superficial basal cell carcinoma and squamous cell carcinoma [19].

The topical agents used were a Chloro-Aluminium phthalocyanine-derived PS and a GB delivery drug 23% w/w of Poloxamer 407 with Polyetilenoglicols PEG 400 in a buffer solution with pH = 7.4. PS (30 mg/ml) and GB (1.5 mg/ml) were applied at volumes of 0.1 and 0.2 ml, respectively. When associated with other treatments, the PS was always the first to be applied. The laser irradiations and the application of topical agents were performed (alone or in combination) 1 day after wounding and once daily thereafter for 7 days, with the animals immobilized, but without anesthesia.

RESULTS AND DISCUSSION

After the experimental period, the results showed that healing of excisional skin wounds in the CG evolved with the conventional features of healing by secondary intention. On the other hand, improved healing was observed in the animals submitted to treatment with low level laser radiation and the topical agents (except in the cases of those treated only with the GB). The wounds in the controls presented a mild inflammatory infiltrate, discrete granulation tissue, and low and rather unexpressive, proliferation of fusiform fibroblasts compared to the treated wounds (Fig. 1). A crust of clotted fibrous exudates, with an acidophilic appearance, was observed in all the groups.

Rats treated with the GB alone were histologically similar to the CG. Their wounds contained a scabby population of fusiform fibroblasts, vascularized granulation tissue with an edematous appearance, and discrete deposition of an extracellular matrix. Inipient epithelial recovery was also observed in all the animals, together with areas of acantholysis. There was no indication of remodeling of the connective tissue (Fig. 2).

The inflammatory process was similar in all the groups treated with laser radiation. Rats of the laser group (LG) presented a mild to moderate inflammatory infiltrate with predominance of mononuclear cells. This feature agrees with the histological findings of other authors, who observed cell proliferation, with the presence of inflammatory cells, until the tenth day of healing [20]. Spongioid dermatitis and edema in the intercellular spaces of the epidermis were observed in two animals. Re-epithelialization was generally mild to moderate but intense in two animals, where more than half of the wound area re-
covered with epithelium. Only one animal presented adipocytes, an indication of connective tissue remodeling. On the other hand, there was stronger deposition of an extracellular matrix, and the proliferation of fusiform fibroblasts was characterized by moderate collagen deposition, slightly more marked compared with the CG (Fig. 3). The observation is in accordance with previous studies showing a greater proliferation of fibroblasts associated with the use of laser both in vitro and in vivo [21,22].

Fibroblast proliferation might be taken as a manifestation of the laser light’s effect on the endothelium, which could result from an increased release of chemical mediators involved in cellular proliferation, already cited in other studies [20,23–25]. The presence of young fibroblasts is another indication of the acceleration of the healing process, and even an indication of regeneration of an epithelial pavement, the last stage of healing.

Low level laser therapy (at a fluency of 2.0 J/cm² and short time of exposition to radiation) has been shown [21] to induce proliferation in cultured human gingival fibroblasts. Studies [26] performed on normal rats submitted to several laser wavelengths and drugs showed that the effects of laser exposition depend on the dose used and may cause either stimulation (2–16 J/cm²), zero bioactivation (20 J/cm²), or inhibition (24–28 J/cm²).

The results corroborate the positive biomodulator effect of laser therapy on wound healing described in the literature [22,26]. The use of laser radiation of low intensity to stimulate the proliferative phase of healing has been previously reported [4], although, in general, such studies have focused on the isolated progress of specific healing periods [20].

The proposed mechanism for the stimulation of healing by low level laser light is the absorption of light energy by mitochondria, which increases the energy of the cell and stimulates the release of chemical mediators [4,27]. The bio-stimulating effects of several lasers emitting within the visible region were recently associated with the quantity of radiated light which leads to the generation of minimum levels of ROS. High concentrations of ROS cause cell death (by ATP depletion and lipid peroxidation), but recent
evidence demonstrates that controlled and relatively low concentrations of ROS play an important role in the triggering of many cellular processes, including the proliferation of fibroblasts [28]. Therefore, ROS should be viewed not only as agents that cause cell damage, but also as mediators of physiological processes [28].

Rats of the PS group presented a mild to moderate inflammatory infiltrate with predominance of mononuclear cells and some exudate was observed around the blood vessels. Proliferation of fusiform fibroblasts and collagen deposition were moderate to intense and collagen content was generally more pronounced than that observed in the CG. Remodeling of the connective tissue as indicated by the presence of adipocytes was evident in three animals. Re-epithelialization was moderate to intense, generally with more than half the wound area re-covered by epithelium, and with complete re-epithelialization in two animals (Fig. 4).

Rats of the LPS group presented mild to moderate inflammatory infiltrate, being intense in two animals and with predominance of mononuclear cells. The proliferation of fusiform fibroblasts and deposition of extracellular matrix was characterized by moderate collagen content, which was slightly more marked when compared with the controls. Re-epithelialization was evident in all of the animals of this experimental group, with more than half the wound area re-covered by epithelium. In two animals, re-epithelialization was complete. Spongioid dermatitis was identified in four animals, with light acantholysis. There were indications of connective tissue remodeling in eight rats, mainly through the presence of adipocytes (Fig. 5). Glandular tissue and hair follicles were also observed in this group, a feature already described in other histological studies on photodynamic modulation of wound healing with PS [14].

In the search for new strategies against microorganisms, studies using PDT have been done routinely against bacteria [29]. A number of methods have been developed to control bacterial proliferation within the mouth specific studies, using laser light irradiation, indicate that certain oral bacteria including *S. mutans* and *S. sobrinus* can be efficiently inactivated after PDT with dyes such as toluidine blue and methylene blue [30–32] or even with more complex molecules, such as dissulphonated phthalocyanine aluminum [33].

The main focus of this research is the treatment of wounds with PDT. The anti-microbiotic effect of dye photosensitization action is a positive point in the wound healing process.
healing process and our studies detected no inhibition in the wound healing process, reinforcing the main idea that PDT could be used not only as a procedure to speed up the wound treatment, but also another way to avoid local infections during the cicatrization process.

In rats of the LPSG group, histology revealed improved healing to a greater degree than that observed in the LPS group. Mild to moderate inflammatory infiltrate with pre-dominance of mononuclear cells was observed. There was more deposition of extracellular matrix and the proliferation of fusiform fibroblasts was also characterized by a greater collagen content, which was more pronounced when compared with the controls and was particularly marked in five animals. Re-epithelialization was moderate to intense in practically all of the animals, with more than half the wound area re-covered by epithelium; one rat presented complete re-epithelialization. Remodeling of the connective tissue as indicated by the presence of adipocytes was observed in eight animals (Fig. 6). Glandular tissue and hair follicles were also evident in this group, as already observed by other authors [14].

When compared with the CG, the histological analysis of the groups with PS indicated faster healing, with stronger collagen deposition in a more extensive extracellular matrix (i.e., enhanced remodeling of the connective tissue) occurring in the groups combining photosensitizer and laser. Re-epithelialization was more marked in treated animals. It varied from moderate to intense, with half the wound area re-covered by epithelium. Complete re-epithelialization was observed in two animals in each of the PS and LPS groups and in one rat of the LPSG group. Re-epithelialization in animals treated with low level laser only was not as pronounced as in those treated with a combination of laser and the topical agents or with the PS alone, but was, nonetheless, more marked than in the controls or the rats treated with the GB only. Re-epithelialization was more efficient in the LPSG group and remodeling of the connective tissue was more pronounced in the LPS and LPSG groups.

Irradiation with monochromatic red light can stimulate cellular metabolism and the photobiological effects of such stimulation depend on the light's wavelength, dose, and intensity [5]. The results showed that the delivery of laser energy at an appropriate power density could act within the local environment of a wound and modify cellular activities and tissue components thereby promoting increased deposition of collagen and faster wound closure.

Ongoing studies involve the analysis of the effect of bio-stimulation, induced by low energy laser radiation, in a biomimetic system, such as a protoliposome reconstructed in the presence of kidney Na-K-ATPase, in the presence and absence of PS dyes. Preliminary data show that most of the bio-stimulation processes observed are directly related to the activation of the Na-K-ATPase and other ATPase enzymes and laser therapy is able to photo-stimulate the injured tissue and positively affect the healing process.

Other studies utilizing phthalocyanines (34–37) indicate that the final concentration of the photosensitizers used in present study is completely atoxic to the wound, or to the wound recovery process in association with neoplastic diseases.

This is a field with great potential for exploring the treatment not only of healing, but also of scars and other cutaneous conditions, associated with hindered healing. The potential application of PDT in healing and scar formation seems poorly explored to this date.

The study clearly indicates that the association of light and a PS applied with an appropriate delivery agent does not inhibit any stage of healing but, on the contrary, can improve the process, lending support to other positive findings on PDT and wound healing [10,18,22]. Multiple doses of PDT with variations in the time intervals between doses may speed up the positive effects of this treatment on healing and scar formation.
The results of this study support the use of low level laser alone or combined with a phthalocyanine-derived PS for the rapid control of wounds and possibly other local injuries. Table 1.

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