The Influence of Photodynamic Therapy on the Wound Healing Process in Rats

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ABSTRACT: In photodynamic therapy (PDT), photosensitisers (PS) are used along with lasers for the treatment of tumors. The combined effect of photosensitisers and lasers on the wound healing process is studied using δ-aminolevulinic acid (ALA) (5 mg/kg) and hematoporphyrin derivative (HPD) (5 mg/kg) as photosensitisers in the open excision wounds of rats. The lasers used were He-Ne laser (3 J/cm^2) and Nd: YAG laser (30 J/cm^2). This study is important for understanding the healing process involved after PDT. Open excision wounds treated with He-Ne lasers in animals that received ALA as photosensitiser showed complete wound closure at the earliest by 13 ± 1 days, and with results obtained for HPD and the combination of lasers with complete closing by 14 ± 1 days. However, the control group of animals that received ALS or HPD with no laser treatment showed wound healing on the twentieth and eighteenth days with a deviation of one day and two days, respectively. ALA with

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the combination of Nd: YAG and He-Ne lasers and HPD with He-Ne laser alone does not show quicker wound healing effects. Histopathological results also gave similar results. Tensile strength measurements do not vary significantly from control group to the test group.

ALA along with He-Ne laser of HPD along with the combination of He-Ne and low power Nd-YAG lasers are found to be ideal methods for quickening the wound healing process in rat.

KEY WORDS: He-Ne laser, Nd:YAG laser, photosensitiser, δ-aminolevulinic acid, hematoporphyrin derivative, tensile strength, wound contracture.

INTRODUCTION

Wound healing is a natural process for maintaining the integrity of skin and epithelium of those who underwent surgery, those with diabetes and those who need accident and emergency care. Wound management in medicine and surgery is one of the paramount facts of therapeutic measures. Many methods have been adopted to enhance the wound healing process in patients affected with different types of wounds. There have been numerous reports indicating the potential use of low-energy laser irradiation in the enhancement of the wound healing process in open wounds of animals and in clinical trials [1–3]. A wide variety of animal models have been used in experimental studies of laser photobiomodulation of wound healing [4–6]. Experimental studies in animals and clinical observations by Kana et al. and Kovacs [7,8] provided adequate evidence that He-Ne laser treatment of cutaneous and peritoneal lesions would accelerate the process of wound healing. Laser irradiation of open wounds is also claimed to stimulate fibroblast replication [9]. It has been suggested that low energy laser radiation stimulates skin regeneration by inducing the mitotic activity of epithelial cells [7]. Because no rise in temperature is observed during He-Ne laser irradiation, effects at the cellular level may be considered more biochemical than thermal. Hence, no stress is expected during irradiation.

Here, an attempt is made to study the influence of photosensitisers like δ-animolevulinic acid and hematoporphyrin derivative with lasers He-Ne and Nd:YAG on the wound healing pattern in rats.

MATERIALS AND METHODS

Wistar rats of both sex with 160–200 gm of body weight were used for the study. Xylaxin (Indian Immunologicals, B No. XLN 1/97) and Ketamine hydrochloride injection (Neon Laboratories Ltd., B No. 70624) were used for anesthetising the animals. The photosensitising

agents used were δ-aminolevulinic acid and hematoporphyrin derivative (Sigma Chem. Co., USA). He-Ne laser (5 mW) and Nd:YAG laser (1 W) (Cooper Lasersonics) were used for irradiating the wound bed and wound edges.

The animals were divided into seven groups of six animals as shown in Table 1. The first group served as control-1 and did not receive photosensitiser or laser treatment. The second and third groups received ALA and HPD, respectively, without laser and served as control-2 and control-3. The fourth and sixth groups, respectively, received ALA and HPD with He-Ne laser (tests 1 and 3). The fifth and seventh groups received ALA or HPD, respectively, along with the combination of both lasers (tests 2 and 4).

All of the animals except control 1 group were given photosensitisers 24 hours prior to making open excision wounds. ALA was administered intraperitonially, while HPD was administered orally, both at a dose of 5 mg/kg of body weight. Animals were anesthetised (Xylaxin 0.005 mg/kg and Ketamine 50 mg/kg), clipped and wounds of 2 × 2 cm in dimension were marked dorsally on either side of the midspine. After the excision of the wound, animals in the test group were treated with laser (He-Ne 3 J/cm^2 and Nd:YAG 30 J/cm^2), while the control groups were left undisturbed. Irradiation of the wound area was done through a focusing lens from a distance of 1 mm from the skin. Animals of groups 4 and 6 (tests 1 and 3) were irradiated with He-Ne (3 J/cm^2) laser. Groups 5 and 7 (tests 2 and 4) received an additional amount of 30 J/cm^2 of Nd:YAG laser in addition to 3 J/cm^2 of He-Ne laser, simultaneously. After one hour, the wound area of all the animals, including the control groups, were bandaged with sterile bandages, and the animals were caged in hygienic conditions. Laser treatment continued for the next two days for the test groups of animals. The animals were housed in wire mesh cages without

Table 1. Wound closure patterns of different groups of animals.

Sl No.	Sample	Wound Healing Period (days)*
1	Control-1	19 ± 0
2	Control-2 (ALA only)	20 ± 1
3	Control-3 (HPD only)	18 ± 2
4	Test-1 (ALA + He-Ne)	13 ± 1
5	Test-2 (ALA + He-Ne + Nd:YAG)	17 ± 1
6	Test-3 (HPD + He-Ne)	16 ± 2
7	Test-4 (HPD + He-Ne + Nd:YAG)	14 ± 1

*The values given are the mean of 12 wound sites.

bedding, so that the wounds remained dry, though not sterile. Animals were inspected daily for their normal health, and the body weight changes were recorded on alternate days. The animals were fed with chow and water ad libitum. The areas of the wounds were measured daily, and the wounds were photographed periodically.

HISTOLOGICAL ANALYSIS

On the fifth, eighth, fifteenth and twenty-first days, animals from each group were necropsied, and the open wound along with the surrounding newly formed skin and the inner wound beds were removed and kept in formalin until examination. The specimens were fixed in 10% buffered formalin. Parallel sections of tissue across the wound were dehydrated in alcohol, cleared and embedded in paraffin. 5 μm thick sections were stained with haemotoxylin and eosin and examined under the light microscope. The histological features of wound healing were investigated in each section. Final comparisons between the healing process in all the groups were made.

TENSILE STRENGTH MEASUREMENTS

On the twenty-first day, the animals were sacrificed, and a skin sample of size 3 × 1 cm perpendicular to the axis of the wound edges, with the wound site at the center, were excised for tensile strength measurements. The samples were stored in saline, and the tensile strength measurements were carried out within 2 hours of the excision of the skin. After measuring the thickness and width of the skin, the samples were placed in the grip of the instrument. The specimens were distended with a constant speed of 5.0 mm/min up to the point of rupture. The tensile strength was calculated using the following formula:

$$\text{Tensile Strength} = \frac{\text{Load at breaking point}}{\text{Area}}$$

All measurements were done following ASTM specification D882-1981 [10].

RESULTS

The wound healing pattern of the two groups treated with HPD and

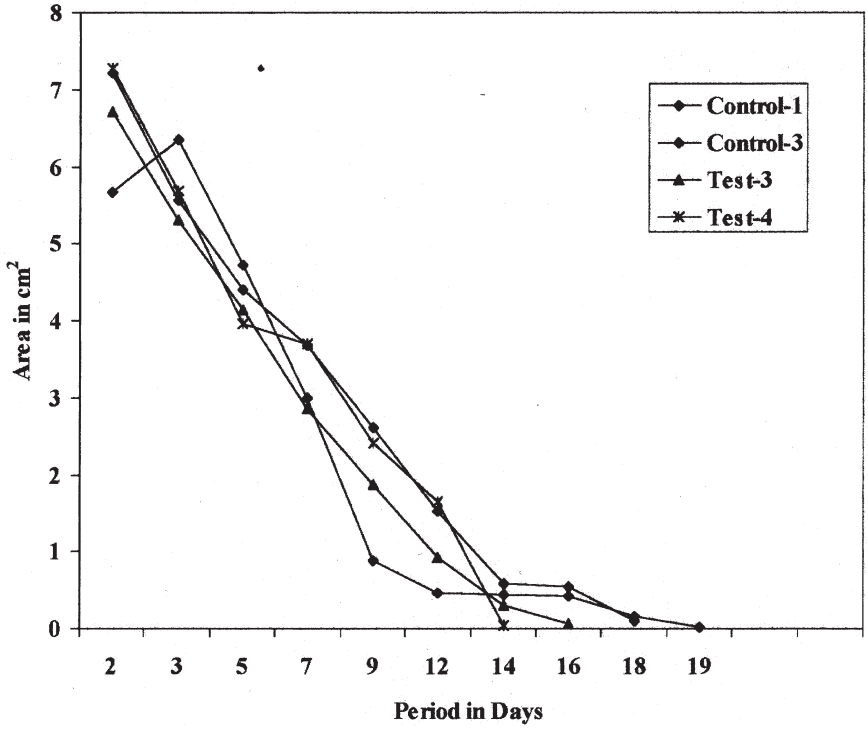

Figure 1. Wound healing pattern of animals administered HPD.

ALA are shown in Figure 1 and Figure 2, respectively. The test-1 group of animals that received ALA and He-Ne laser showed the quickest wound healing pattern with a complete closure of the wound on the 13 ± 1 day. Complete wound closure took place on the 17 ± 1 day among the test-2 group of animals, whereas the control-1 group showed complete closing on the nineteenth day. Among the HPD-treated animals, the quickest wound healing occurred among the test-4 group of animals treated with the combination of He-Ne and Nd:YAG lasers by 14 ± 1 day, whereas the animals treated with only He-Ne laser (test 3) showed complete healing on the 16 ± 2 day. The wound-healing pattern of the control-2 and control-3 groups showed complete healing on the 20 ± 1 day and 18 ± 2 day, respectively (Table 1). Based on the percentage reduction factor of each wound from the previous day to the next day, no significant effect of laser irradiation on the rate of wound healing was observed in this study. A steady increase in the body weight of the

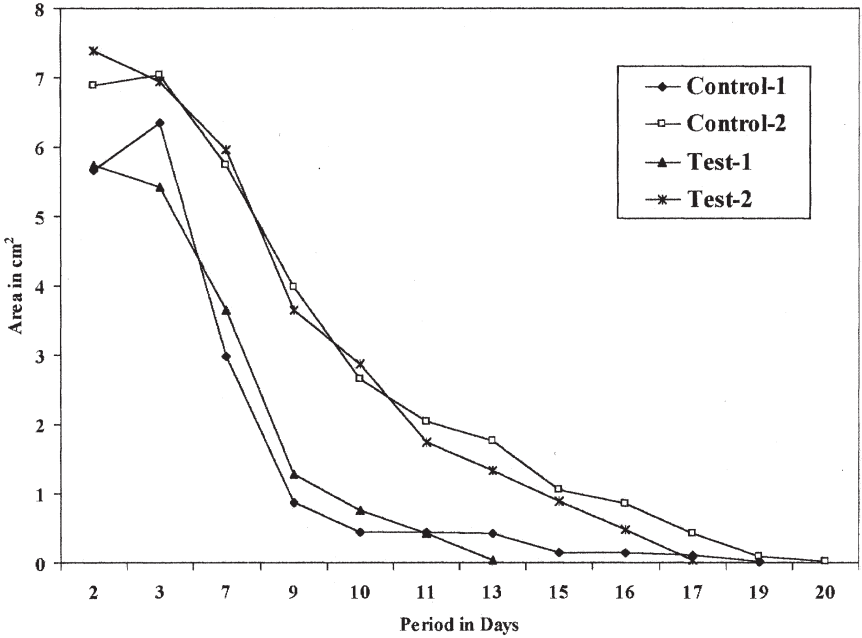

Figure 2. Wound healing pattern of animals administered ALA.

animals was observed irrespective of the groups. No mortality was observed in any of the groups.

Histopathological reports give similar patterns for all of the samples on the fifth day with marked necrosis and inflammation of the epidermis extending into the dermis. On the eighth day, the picture was similar to that of the fifth day with a lesser degree of necrosis and inflammation. Sections from all groups except the control groups that received only ALA (control-2) showed almost complete re-epithelization on the fifteenth day, though mild subepidermal necrosis and inflammation were present. Collagen deposition was present in all groups on the fifteenth day. On the twenty-first day, the histopathological studies showed complete healing of sections from all groups with dense collageneous scar. Only the test-4 group partially showed maturization of this collagen, while in all other cases, it was immature. In this group, more reduction in wound width was also observed. Typical histopathological pictures are shown in Figures 3, 4 and 5.

The tensile strength measurement results of all of the samples are

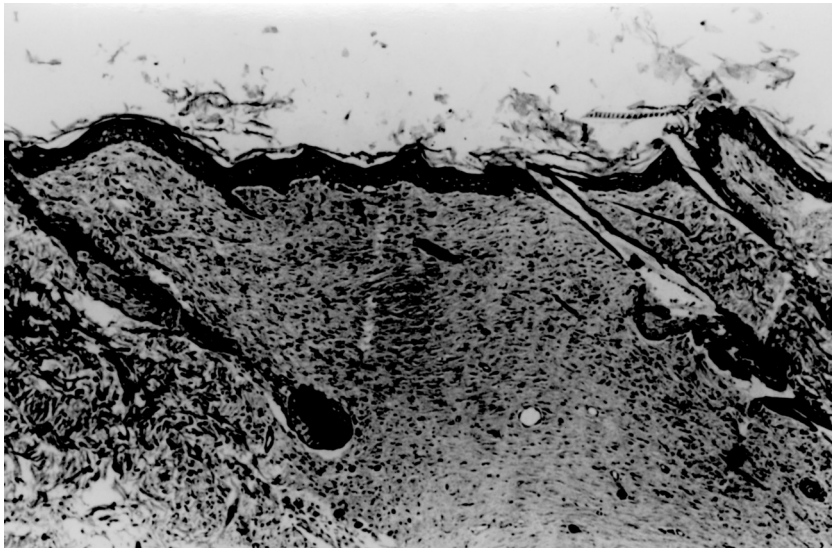

Figure 3. Photomicrograph of histological section of skin showing wound healing pattern on twenty-first day: Bare control.

Figure 4. Photomicrograph of histological section of skin showing wound healing pattern on twenty-first day: ALA + He-Ne laser.

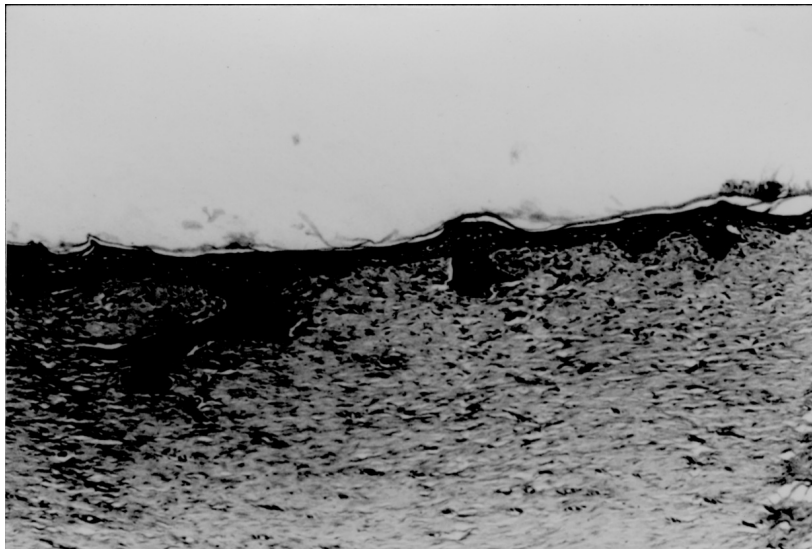

Figure 5. Photomicrograph of histological section of skin showing wound healing pattern on twenty-first day: HPD + He-Ne + Nd:YAG laser.

given in Table 2. The tensile strength measurements were not significantly different for the test and control samples. Of the test samples, the test-1 group showed better results compared to the other groups. However, the values for all groups were significantly less than the values for the normal skin.

Table 2. Tensile strength results of different samples on the twenty-first day. (In all cases, N = *6.)*

Sl No.	Sample	Tensile Strength (gm/cm^2)	% Elongation
1	Normal skin	1296 ± 17.00	110 ± 10.00
2	Control-1	435 ± 9.00	96.66 ± 9.18
3	Control-2 (ALA only)	450 ± 28.00	74.56 ± 7.39
4	Control-3 (HPD only)	418 ± 14.00	67.25 ± 6.50
5	Test-1	620 ± 7.20	73.33 ± 8.54
6	Test-2	420 ± 10.00	83.33 ± 7.82
7	Test-3	510 ± 18.00	71.28 ± 6.09
8	Test-4	640 ± 20.00	69.81 ± 7.32

DISCUSSION

In photodynamic therapy, the photosensitiser is retained in malignant cells at a higher rate than in the surrounding normal tissues, which enables selective tumor therapy. In this study, the effect of PDT on the wound healing effect was evaluated utilizing lasers that are reported to be effective for biostimulation. Only one energy level for both lasers has been evaluated. The methods used in this study to evaluate the effect of PDT are wound contracture measurements, histopathological evaluation and tensile strength measurements. Wound contracture measurements showed good results among animals administered ALA, along with He-Ne laser and HPD along with the combination of both lasers. Histopathological results favour the test-4 group that received HPD along with the combination of both lasers because wound reduction was more than that found in the other groups. With regard to epidermal closure and dermal healing, the pattern of healing in animals administered ALA was better than that of the animals administered HPD. Even though tumor destruction is effective using PDT, its effect on wound healing after surgery has not attracted the attention of many studies. In this context, a study on the effect of wound healing patterns using photosensitisers may be considered essential for those who are using PDT for tumor treatment. Preliminary studies by Stern et al. [11] using phthalocyanines and by Jeffers et al. [12] using hematoporphyrin derivatives in animal models have demonstrated that PDT has a significant role in wound healing including enhancement of early granulation tissue formation, quickened re-epithelization and reduction in the necrosis of muscle, mucosal tissue and inflammation.

Most of the studies in which a nonirradiated wound site in the same animal has been used as the control have met with negative results. Here, tissue factors released from irradiated wounds into circulation may have an effect on the opposite, nonirradiated wound site. This systemic effect could explain the failure in observing significant effects when comparing lased tissue to the opposite side in the same animal. On account of this reason, we have selected a different group of animals that served as control.

Efforts to find significant effects of laser irradiation on wound healing have met with mixed results. Kana et al. [7] observed a significant increase in circular wound closure between the third and twelfth postoperative day in wounds exposed to 4 J/cm^2 of He-Ne laser and a slight deceleration at 20 J/cm^2 of He-Ne laser, without any photosensitising agents. But, Berton Braverman et al. [13] reported that there was no significant increase in the rate of wound closure in the case of rabbit

wounds exposed to 1.65 J/cm^2 of He-Ne laser. They observed a significant increase in the tensile strength of the laser-treated groups compared to the control group. But, Kana et al. failed to observe this result. Our study contradicts Berton's results of wound contracture and tensile strength measurements and is consistent with Kana's findings. The differences between the results of various studies may be due to the factors of wavelength, energy output, exposure time, the effect of photosensitisers or possibly of the species tested. In the present study, the addition of Nd:YAG laser to He-Ne laser slows the healing process from 13 to 17 days in the groups where ALA was used as the photosensitiser, while it appears to speed it when added to the group where HPD was used as the photosensitiser. Further studies on the wound healing property of Nd:YAG laser may be required to explain this situation. Studies with various lasers and photosensitisers suitable for this purpose are also recommended to standardise the procedure.

CONCLUSION

The results of this study suggest that photodynamic therapy quickens the wound healing process of rats. δ-aminolevulinic acid along with He-Ne laser was found to be more suitable in this regard. Hematoporphyrin derivatives along with the combination of He-Ne and 1 W Nd-YAG laser are also a promising step towards wound healing.

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