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The Comparison of Effects between Pulsed and CW Lasers on Wound Healing

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ABSTRACT

***Objective:* In order to evaluate the effects of pulsed continuous wave (CW) laser and detect the role of wound healing in rats using both pulsed and CW 635-nm low-level laser therapy (LLLT), a pilot study was undertaken. *Background Data*: Some acceleration effects of wound healing on animals were found after treatment using various lasers with CW. There are other reports, however, using pulsed CW laser to evaluate the effects of wound healing in rats. *Materials and Methods:* An elliptic wound was created aseptically with a scalpel on the shaved back of the rats after anesthesia. The rats treated were restrained in a Plexiglas cage without anesthesia during the laser irradiation period. An Erchonia pulse laser (635 nm) was used in the experiment. The laser beam was delivered through an expander. The percentage of relative wound healing was calculated. *Results:* The percentage of relative wound healing was 4.32 in 100 Hz, 3.21 in 200 Hz, 3.83 in 300 Hz, 2.22 in 400 Hz, 1.73 in 500 Hz and 4.81 in CW. *Conclusion:* LLLT using pulsed, CW laser at the appropriate dosimetry and frequency can provide acceleration in wound healing in rats. The 100-Hz frequency had a better effect than other pulse frequencies used in the study. The effects of treatment using CW laser was higher than pulse frequency. The frequency of pulsed CW laser was not found to increase wound healing in rats compared with normal CW laser, as reported in our previous studies.**

INTRODUCTION

In the course of the past two decades, many studies have been conducted to investigate wound using low-level laser therapy (LLLT).[1] The basic tenet of laser therapy is that monochromatic laser light has a wavelength-dependent capability to affect cellular behavior in the absence of significant heating.

Some experiments have found that visible irradiation stimulates capillary growth and granulation tissue formation. Other reports show altered keratinocyte motility and fibroblast movement following irradiation.[2] Animal studies that show some improvement after treatment, particularly in the earliest phases of wound healing, have been reported following laser irradiation.[3] Many studies comparing the effects of lasers using visible and near-infrared wavelengths have shown the best results with visible wavelengths.[4–7] There are fewer reports, however, using pulsed continuous wave (CW) lasers on animals. The aim of this pilot study was to evaluate the effects of pulsed CW laser and detect the role of wound healing in rats using both pulsed and CW 635-nm LLLT.

MATERIALS AND METHODS

Animals

Fourteen male Sprague-Dawley rats, weighing 336–440 g (382.6 ± 31.3 g) were utilized. After anesthesia with Ketalor (up to 50 mg/kg) and Xylocaine (up to 20 mg/kg) IP, the surgical site was shaved, then a hair removal lotion was applied to cleanse the skin of excess hair, to minimize reflection losses. The site was then disinfected with an isopropyl alcohol swab. An elliptic full-thickness skin wound (mean area = 1.04 cm^2) was created aseptically with a scalpel on the shaved back of the animal in the gluteus maximus region. All animals with wounds were divided randomly into treatment group (n = 12) and control group (n = 2) on the basis of the experimental process designated. The elliptic skin wound in our earlier studies[8] showed that there was faster healing with this type than with control (Table 1). In order to shorten the time of wound healing for more experimentation, we chose the elliptic skin wound for this study.

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TABLE 1. DAYS FOR HEALING COMPLETELY IN DIFFERENT WOUND SHAPE ON CONTROL RATS

Wound shape	*Wound size (cm^2)*	*No. of rats*	*Rat age (weeks)*	*Average rat weight (g)*	*Days for healing completely*
Elliptic	0.39	4	27	430	14
Elliptic	1.26	4	27	430	16
Circular	0.79	2	27	430	16
Square	1.00	2	27	430	18

TABLE 2. TREATMENT PARAMETERS IN THE STUDY

Power (mW)	*Spot size (cm^2)*	*Wound size (cm^2)*	*Power density/dose rate (mW/cm^2)*	*Energy density/incident dose (J/cm^2)*	*Schedule (times/week)*
3.4	3.8	1.04	0.89	1.0	3

Laser system

The study was performed using an Erchonia Pulse Laser (TUCO Innovations, Inc., PL2000), at a wavelength of 635 nm. The laser beam was delivered through an expander. Peak power was measured using a laser power meter (Metrologic). Since it is a pulsed CW laser, peak power and average power are equal. The power density (PD) was calculated as follows:

$$\text{PD (W/cm}^2\text{)} = \text{ave. power (W)/spot size (cm}^2\text{)}$$

The incident dose was calculated as follows:

$$\text{Incident dose (J/cm}^2\text{)} = \text{PD (W/cm}^2\text{)} \times \text{treatment time (sec)}$$

Treatment experiments

The laser beam was aligned to cover the entire wound area including the boundaries. Pulse frequencies of 100, 200, 300, 400, and 500 Hz in the CW mode were used in the study. Every rat in the treatment group was irradiated by laser using 0.89 mW/cm^2 power density for 18.7 min to give 1.0 J/cm^2 incident dose or energy density. The treatment parameters are shown in Table 2. The rats treated were restrained in a Plexiglas cage without anesthesia during the laser irradiation period. The control group also received the same manipulation, excluding the laser exposure. The wound area of all rats was measured at regular intervals with a caliper until healing was completed. The wound area for ellipse was calculated as follows:

$$\text{Area} = L/2 \times W/2 \times \pi \text{ (cm}^2\text{)}$$

where L and W are the length and the width of the wound in centimeter, respectively. A trendline was applied on the slope chart of the wound healing rate (Fig. 1). The slope value of the healing rate in control and treatment was calculated as follows:

$$\text{Slope (cm}^2\text{/day)} = \text{Wound area/healing days}$$

The relative rate of wound healing was calculated as follows:

$$\text{Relative wound healing (\%)} = \text{(Slope treatment} - \text{slope control)/slope control} \times 100 \text{ (\%)}$$

In the equation, if the value = 0, the effect is zero biostimulation; if the value is >0, the effect is stimulation; if the value is <0, the effect is inhibition.

RESULTS

The slope mean value of healing rate was 0.081 in control, 0.0845 in 100 Hz, 0.0836 in 200 Hz, 0.0841 in 300 Hz, 0.0828 in 400 Hz, 0.0824 in 500 Hz, and 0.0849 with laser treatment. The percentage of relative wound healing is shown in Table 3 and Figure 2.

DISCUSSION

This study indicates that LLLT using pulsed CW laser at the appropriate dosimetry and frequency can provide acceleration of wound healing in rats. Our results showed that 100 Hz had a better effect on wound healing than other pulse frequencies used in the study. It is unclear, however, why 100 Hz had a better effect.

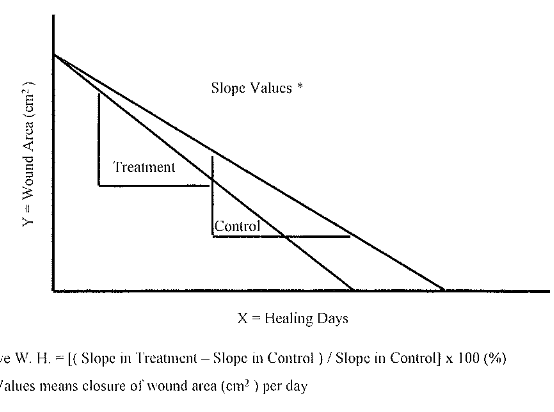

FIG. 1. Calculation of slope values and rate of relative wound healing on rats.

TABLE 3. THE SLOPE MEAN VALUE OF WOUND HEALING AND PERCENTAGE OF RELATIVE WOUND HEALING IN THE STUDY

Treatment group	*No. of rats*	*The slope mean value of wound healing*	*Percentage of relative wound healing*
Control	2	0.081	—
100 Hz	2	0.0845	4.32
200 Hz	2	0.0836	3.21
300 Hz	2	0.0841	3.83
400 Hz	2	0.0828	2.22
500 Hz	2	0.0824	1.73
C.W.	2	0.0849	4.81

A normal pulsed laser is one in which input energy is stored within the power supply of the laser. When the energy is stored, as in a capacitor, it can reach very high levels. Discharging this stored energy rapidly into the laser gives rise to high-intensity laser pulses. The peak power levels of the pulses greatly exceeds the power levels obtainable with a CW or a pulsed CW laser beam. It is common for normal pulsed lasers to produce peak power values in excess of several kilowatts of power. Each pulse may be able to deliver several joules of energy in a pulse lasting for up to 10 msec. Many normal pulsed lasers can generate pulses lasting for as short a time as 100 μsec. Depending upon the specific design features of the normal pulsed laser, the pulse repetition time can be as short as 0.01 sec. This equates to a frequency of 100 pulses per second. However, the Erchonia pulse laser (PL2000) used in our present study is a pulsed CW laser.

When a continuous laser beam is turned on and off rapidly, it gives the appearance of being pulsed. Because the top level of power in pulsed CW laser is no greater than the CW power level, the peak power and average power of the laser is equal. The beam is only "shuttered" on and off. The way this is accomplished within the laser system is to open and close a fast-acting mechanical shutter or to spin the beam with a rotating mirror device.

The effects of treatment using CW laser were higher than when using pulse frequency (Fig. 2). The mechanism of higher acceleration in the CW laser could be a result of increased photon stimulation in the cellular components of wound healing. Wound healing is a complex biologic and biochemical process that commences right after tissue injury. Low oxygen tension and the formation of platelet "plugs" highlight the coagulation phase. Macrophages, polymorphonuclear neutrophils, and lymphocytes appear during the inflammatory phase for control of debris and infection and for the secretion of growth factors for fibroplasia, angiogenesis, and re-epithelialization.

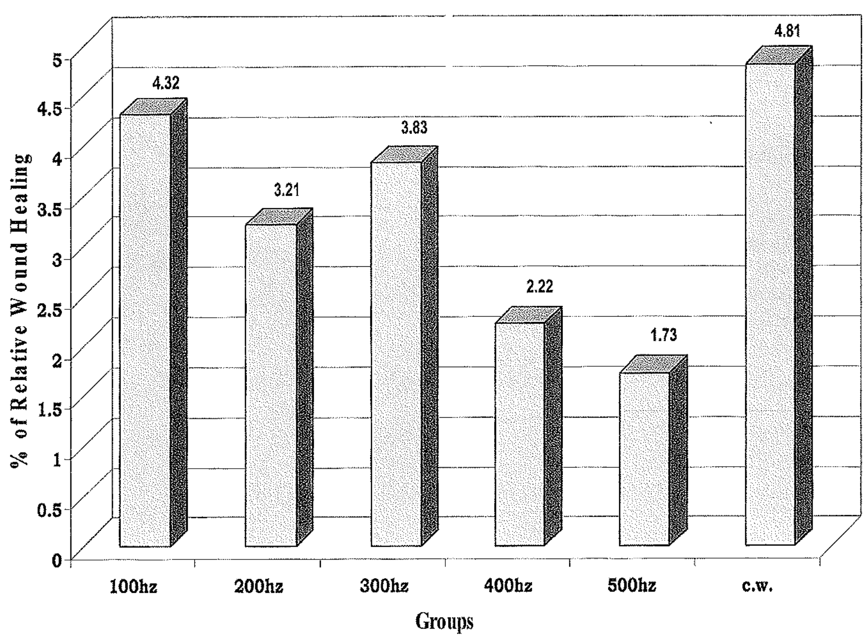

FIG. 2. Effects of wound healing on rats using pulsed and CW lasers. Power density (PD) = 0.89 mW/cm^2; incident dose = 1.0 J/cm^2.

The responsiveness of the cellular components of wound healing to photon stimulation has been studied.[9–12] The increase in cellular energy and tissue oxygenation, the enhanced micro-circulation, and the synthesis of specialized signaling proteins (such as growth factors) have been shown to be influenced by photons, which may be why wound healing is accelerated. Further experiments are necessary to test this hypothesis, and molecular biology studies should also be performed.

Many experiments have been completed in our laboratory to evaluate the effects of wound healing in animals using various normal CW laser wavelength, dose, and treatment schedules.[13,14] The results from our previous studies have shown that the treatment schedule of three times per week was appropriate, and the HeNe laser with 632.8 nm gave the best wound healing acceleration in rats using actual doses of 5 J/cm^2 (which excluded the laser loss dose from a Plexiglas cage and laser skin reflection dose). Our present study indicated that the frequency of pulsed CW laser did not improve wound healing in rats compared with the HeNe laser reported in our previous studies. A photobiological response involves the absorption of a specific wavelength of light by some unknown photoreceptor.[15] There may be more molecules that serve as photoreceptors created after irradiation with the HeNe laser. Our aim is to develop an optimum laser with treatment parameters that will be effective, safe, and cheap for clinical application. The need to find ways to enhance wound healing, especially during a state of impairment, is imperative due to the ever-increasing cost of medical care.

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