Effects of Low Incident Energy Levels of Infrared Laser Irradiation on Healing of Infected Open Skin Wounds in Rats

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CONTENTS

Ⅰ. INTRODUCTION

Many studies, using low level laser(LLL), have been performed to investigate the influence of laser radiation on the healing process of wounds or lesions. Mester et al[1] reported that the healing of total skin defects artificially created on the back of white mice was significantly accelerated by the effect of ruby laser rays. Mester[2] was the first to hypothesise the beneficial interference of laser energy with the enzymes active in the healing process after observing the beneficial effect of laser radiation on the various stages of healing in 32 patients suffering from a variety of skin lesions. Palmieri[3] reported that an increased activity of myofibroblasts might also be responsible for a quicker closure of the wound.

It is claimed that the infra-red GaAs laser has biostimulation effect. It has been proposed that the low level laser irradiation may stimulate the protein and DNA synthesis to accelerate the proliferation of gingival fibroblast.[4]. The increasing interest for laser therapy applied in healing of post-traumatic lesions and myopathies has recently stimulated several experiments designed to investigate the molecular basis for explaining LLL therapeutic effects.[5-8]

The effect of laser irradiation on the healing of experimentally induced wounds has been investigated in animal studies.[9,10] Kim et al.[11] reported the clinical, microbiological, and histological study on the effect of LLL irradiation on treating early gingivitis. Their study demonstrated that after LLL irradiation, there was decrease in the proportion of motiles and spirochetes which are believed to cause periodontal diseases and concomittant increase in nonmotiles, and also there was decrease in the gingival infiltration of inflammatory cells. They indicated that the LLL irradiation had a favorable influence on the gingival inflammation with the change of rate in the composition of oral flora, but they did not show whether the decrease of gingival inflammation after LLL irradiation was due to the biostimulation effect of LLL irradiation in the gingival tissue or the direct effect of LLL irradiation causing the change of rate in the composition of oral flora.

Kim et al.[12] reported that *Streptococcus* *mutans* could be stimulated by low level laser therpay(LLLT) in vivo, and similar modulation could potentially occur in the other bacteria exposed to LLLT. They suggested that the acceleration of healing in the infected lesion following GaAs LLLT indicates that the cellular activity due to the biostimulation effect of LLLT in the surrounding normal tissue predominates over the tissue irritation due to the bacterial growth in the infected lesion.

So this study was undertaken to confirm the hypothesis that tissue repair due to the biostimulation effect of LLL in the lesion with infection predominates over the irriation due to the bacterial growth, comparing the differences in the size of infected wound between irradiation group and non-irradiation group.

II. Materials and Methods

A. Materials

1) Experimetal Animal

Healthy Sprague-Dawley rats, weighing 250 to 300g, were used throughout the experiment. They were given tap water and food.

2) Laser Apparatus

The laser used in this work was BIOLASER (Dong Yang Medical, KOREA) using GaAs semiconductior as diode(figure 1). It is an infrared laser apparatus with a wavelength of 904nm. peak output power is 27mW and used beams were pulse 8(1,000 Hz : 2mW average output power) laser.

3) Bacteria

Staphylococcus aureus used in this study were obtained from Dept. of Pathology, SCH CheonAn Hospital.

4) Measuring Apparatus

Reflector projector(Germany) was used to trace the wound size and Planimeter(Keuffel & Esser Co. Germany) was used to measure the wound area traced in the tracing paper(figure 2).

B. Methods

The animals were shaved on the gluteal region. Operations were performed aseptically with the animals under anesthesia with Ketamin and Xylazine. The round, full-thickness skin defects of 5-mm diameter were produced to the left and right of the gluteus superficialis of hind limb in each rat. The wounds were left open without dressing. The animals were kept in special wire mesh cages without bedding, so that the wounds remained, though not strictly sterile, in very clean conditions.

Staphylococcus aureus were cultured on BHI slant and suspended with 1ml of BHI broth, 5 ul of bacteria from BHI broth vortexed were inocluated on the wounds respectively. 10 subjects were used in this study but 3 were died during experiment. The animals were immobilzed under anesthesia with Ketamin and Xylazine during the irradiation and photographing. Energy density of experimental group was 76.43 mJ/cm^2.

The irradiation was centered on the middle of the wound which was 2 cm in diameter, then laser was applied moving around the entire wound area and adjacent normal tissue.

Experimental wounds were irradiated 1st, 3rd, 5th day, the first exposure taking place immediately after the operation and bacterial inoculation. For the control wounds, another attempt like that of experimental groups were performed without LLL irradiation : they served as contralateral controls. The wounds were examined every day and the clinical observations were recorded in a protocol. The wounds areas were determined by photographing the wounds from a constant distance (via a constant objective focus) on the

1st, 3rd, 5th, and 7th postoperative days. The slides were projected through Reflector Projector and the wound areas were traced, and measured by planimeter (Keuffel & Esser Co. Germany). In each wound, the wound areas on consecutive days were expressed as a percentage of their initial area(on the first day).

C. Statistical analysis

All measurement in each group were averaged. Statistical comparisons were then made. To determine the significance of differences among the results according to the interval and the groups two factor ANOVA was used and Scheff and Fischer's PLSD tests for multiple comparison according to the interval were applied.

To compare the difference between the incidence of swelling according to the groups, X^2-test was used.

Ⅲ. Results

The means of wound size of two groups at the 1st, 3rd, 5th, and 7th day are given in table 1, and shown in figure 5. significant differences between two groups (figure 3,4) and among all days of examination could be detected in the rate of wound contraction during total duration of examination (p=.0031, .0001). Although the rate of wound contraction was significantly decreased during experimental examination, significant difference between two groups could be detected only at the 7th day in the paired t-test(p=.0124). In other words, rapid decreased of the wound size in the irradiated group compared with the control group, especially in the late stage of healing process, could be seen in the figure 5.

The measurements of the experimental group and the control group at 3rd day, 5th day, and 7th day are compared in Table 2 and 3, using multiple comparison tests. Significant differences among the measurements at 3rd, 5th, and 7th day, which indicated that the wound size were rapidly contracted in the irradiated group, could be detected in table 2. There are, however, no differences among the measurements at 3rd, 5th, and 7th day except the difference between 3rd and 5th day in control group. These results indicate that the wound healing in the control group was delayed by bacterial infection, but that in the LLL irradiated group was accelerated by LLL irradiation although the wounds were also infected by same bacteria.

The incidences of swelling in the both groups during total examination are also compared in Table 4, using X^2 test. In general, significant differences between two groups for the duration of experiment except 2nd and 4th day (p=.0069). This result also supports that the spread of inflammation was restraned by the biostimulation of LLLT and the wound healing in irradiated group, therfore, was accelerated although the wounds were infected by bacteria.

Ⅳ. Discussion

There were clinical reports by Mester et al.[2] on the wound-healing accelerating effect of the low output laser on skin ulcers that were slow in healing. For animal studies there were reports by Mashiko et al.[13] who recognized the wound-healing effect of the laser in an incised lesion on the back of rats. A series of experiments has been arranged to examine the influence of laser irradiation on the wound healing process.[2,14-19]

However, further many studies reported that effects of LLL irradiation under normal condition, few study was undertaken by pathologic condition. It was suggested that the effect of LLL irradiation under pathologic condition is still unknown, but above of previous results, there were similarities between normal condition and pathologic condition.

Tumor cell proliferation and tumor growth have been shown to be stimulated by low-energy lasers[20-24].

K(1992) et al.[12] suggested that *Streptococcus mutans* could be stimulated by LLLT(GaAs) in vivo, and similar modulation could potentially occur in the other bacteria exposed to LLLT. Kim et al.[12] reported the bacteria cells also were increased significantly with LLL irradiation. It was assumed that low level laser radiation had same effects for all kinds of cells.

In that study, Kim also suggested the hypothesis that the healing of the lesion with infection following GaAs LLL irradiation indicates that the tissue repair due to the biostimulation effect of LLL predominates over the destructive irritation due to the bacteria growth. On the basis of the results in this study, it is indicated that the wound healing in the irradiated group was accelerated by LLL irradiation, although the wounds were also infected by same bacteria such as the wounds of control group.

In this study, *Staphylococcus aureus* was used to infect the wounds in contrast to *Streptococcus mutans* which was used in Kim's study[12] regarding the proliferation of bacteria after LLL irradiation. The reasons are that it was assumed that LLL irradiation had the same effects for all kinds of cells in Kim's study[12], and *Staphylococcus aureus* is the common cause of skin infections. In the pilot study for this research, the wounds were not infected with *Streptococcus mutans* easily. Instead, those were infected with *Staphylococcus aureus* without difficulties.

In previous Kim's study[12], the energy densities of LLLT used were 168.5, 337.0, and 505.4 mJ/cm^2 to be irradiated on to the bacteria. It was suggested that the optimal dose of LLLT for the bacterial growth is about 337, 0mJ/cm^2. The dose of LLLT used in this study was 70.mJ/cm^2. This energy density was very small compared with the optimal dose for bacteria he suggested. Nevertheless, It is not believed that small dose had an influence on the results of this study.

The structural basis of healing consists of the activation of collagen production, and a certain role should also be attributed to the vesicles with central nuclei[11]. These are assumed to contain bioactive substances that also act as catalysts of healing in the nonirradiated areas. This means that it is not necessary to fully irradiate the entire ulcer in order to achieve the stimulating effect. The possibility that the remote effect was induced by a humoral substance created in the irradiated area was raised. In this study, the wound of about 7 mm in diameter was created in the rat skin, and then LLL was irradiated on to the area of 2cm in diameter involving sound tissue around. Therfore, it was assumed that if LLL had been irradiated only on the surrounding normal tissue except the infected wound in this study, the wound healing should have been accelerated more significantly on the basis of the remote effects by humoral substance. Further study is required to confirm this assumption and positive results will be beneficial for clinicians to promote the effect of LLLT and also to treat the wound with severe infection.

Edema resorption is related in a very intensive way with the lymphatic and blood circulation

The biostimulating effect on the tissues reported in the experiments of various authors, particularly Miranda(1981)[25] showing an increase in blood and lymph flow under laser radiation.

Mester's study[26] showed that the development of blood circulation of regenerating tissues was significantly influenced by laser rays.

Nicola Passariella et al.[27] reported that laser radiation was found to have a positive effect on the microcirculation. Kovacs and Mester[28] examined the influence of laser on wound healing and more specifically on the regeneration of blood vessels. They concluded that the regeneration process was significantly increased after laser irradiation.

Liesens[29] repoted that the regeneration of the venous and lymphatic vessels began more quickly with laser therapy than in normal conditions, and also he reported that edema was disappeared quickly after laser therapy.

The incidence of swelling that indicates abscess due to bacterial infection was significantly decreased in experimental group than control group in this study.

According to these results, it was assumed that abscess formation in the experimental group could be inhibited by positive effect on the microcirculation as well as biostimulating effect after LLL irradiation.

The statistical difference in the infected wound contraction following LLL irradiation suggests, although histological assessment was not performed, that further study is warrented. Therfore, the author emphasizes the need for further research to evaluate the effects of LLL irradiation in acute and chronic infection and the effects of LLLT according to the irradiation method. Hopefully this report will stimulate further investigation and reporting in this area.

V. Summary and conclusions

Our investigation of biostimulation effects of infrared LLLT(GaAs) in the 7 samples of rats lead to the following conclusions : (1) The rate of wound closure was significantly increased in Laser-irradiation group. (2) The incidence of swelling that indicates abscess due to bacterial infection was significantly different between control and experimental group.

On the basis of above conclusions, it is confirmed that the acceleration of healing in the infected lesion following GaAs LLLT indicates that the cellular activity due to the biostimulation effect of LLLT in the surrounding normal tissue predominates over the tissue irritation due to the bacterial growth in the infected lesion.

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Table 1. Means and standard deviations of wound sizes of each group, and the results of ANOVA tests according to the interval and the group.

	1st day	3rd day	5th day	7th day	P
Exp.	1	0.556±0.188	0.443±0.180	0.257±0.107	0.0031
Ctr.	1	0.756±0.165	0.577±0.219	0.585±0.317	
P	Paired t−test	0.0561	0.2437	0.0124	
	0.0001				

Table 2. Results of Fisher's test for wound size of experimental group according to the interval

Group	3rd day 0.556	5th day 0.443	7th day 0.257
3rd day			
5th day	++		
7th day	++	+	

++ : 99% significant, + : 95% significant

Table 3. Results of Fisher's test for wound size of experimental group according to the interval

Group	3rd day 0.756	5th day 0.577	7th day 0.585
3rd day			
5th day	+		
7th day	−	−	

++ : 99% significant, + : 95% significant

Table 4. Table showing the incidence of swelling during experimental examination and the results of X2−test

Swelling	2 day	3 day	4 day	5 day	6 day	7 day
Exp.	0	0	2	0	0	0
Ctr.	3	6	6	6	6	6
P value	−	0.069	−	0.069	0.069	0.069

− : not significant

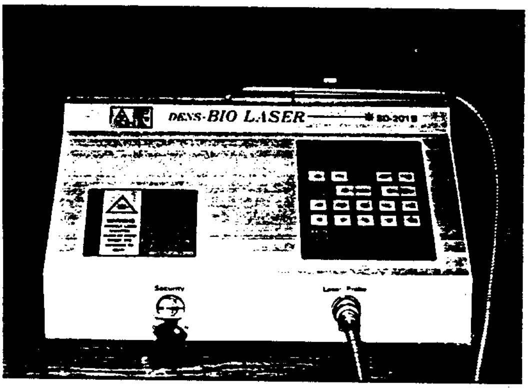

Figure 1. A set of Biolaser

Figure 3. Laser irradiation group at 7th day.

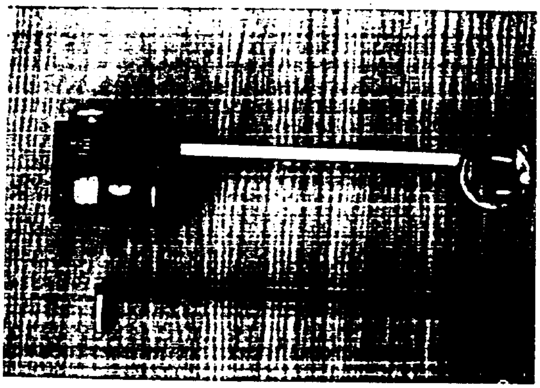

Figure 2. Planimeter(Keuffel & Esser Co. Germany)

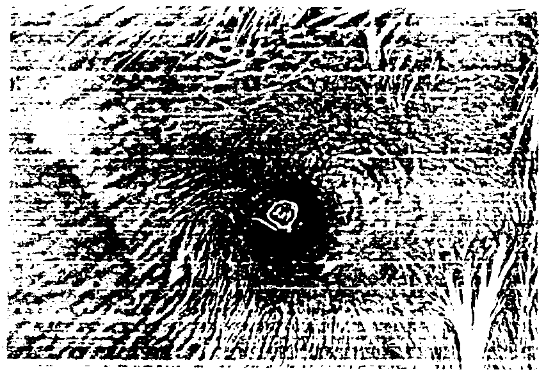

Figure 4. Control group at 7th day

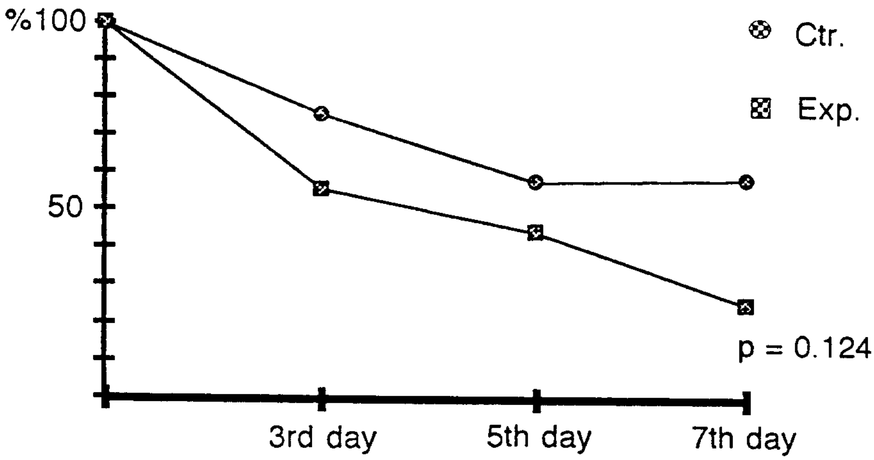

Figure 5. Linear graph showing the changes of wound size of two groups according to the interval.

백서 연조직의 감염창상에 대한 저출력레이저조사시 치유효과에 관한 실험적 연구

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[국문초록]

저출력레이저는 인체조직에 biostimulation effects를 가지므로 구강주위에 발생하는 여러가지 질병에 대한 저출력레이저광의 효과에 관하여 많은 연구가 시도되고 있으며 또한 치료에 응용되고 있다. 감염창상에 저출력레이저 조사시 조직치유의 기전이 세균성장에 의한 조직손상보다 주위 정상조직의 biostimulation effects가 우세하기 때문이라는 가설을 확인하고저 본 연구를 시행하였다.

백서 7마리를 레이저 조사군과 대조군으로 나누어 감염창상의 면적차이를 비교하여 다음과 같은 결론을 얻었다.

1. 저출력레이저 조사군에서 창상수축율이 현저히 높았다.
2. 부종의 빈도는 저출력레이저 조사시 뚜렷하게 감소하였다.

위의 사실로보아 저출력레이저조사시 감염창상의 치유촉진은 주위 정상조직의 biostimulation effect가 세균증식에 의한 조직의 손상보다 우세하기 때문이라는 가설을 확인할 수 있었다.