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Complicated Effect of He-Ne Laser Therapy on Pro-/Anti-Inflammatory Cytokines from Serum in Rats

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Abstract: Helium-neon (He-Ne) laser is one of the most common low energy lasers used in physiotherapy for promoting wound healing. This study was about to investigate the effects of He-Ne laser therapy on the pro-/anti-inflammatory cytokines in rats up to 10 days of treatment. 16 non-pregnant female rats were divided into 4 groups randomly, 4 rats in each group. They are negative control (NC) group, positive control (PC) group, laser treatment (LA) group and erythromycin ointment (ER) treatment group. The LA group was treated with 25 mW of He-Ne laser for 30 min every day for 10 days, with 632.8 nm of wavelength immediately after three incisions were made on the mid-dorsal line of each rat. The results showed that He-Ne laser therapy could decrease the level of IL-1β compared with the PC and ER groups; while increase the level of IL-6 compared with the NC and ER groups, and the level of TNF-α compared with the PC and ER group. He-Ne laser therapy also increased the concentration of IL-10 compared with the other three groups, and the concentration of TGF-β compared with the NC and ER groups. These results indicated that laser acupuncture increased the production of IL-6, IL-10, TGF-β and TNF-α after 10 days of treatment, which was the proliferation stage of wound healing process, and the effect of He-Ne laser therapy on pro-inflammatory cytokines was not always consistent, which might be time-dependent of He-Ne laser treatment.

Keywords: He-Ne Laser Therapy, Pro-inflammatory Cytokines, Anti-inflammatory Cytokines, Wound Healing

1. Introduction

The positive effect of low level laser therapy (LLLT) on the healing process of cutaneous lesions in rats has been confirmed, in which Helium-neon (He-Ne) laser is one of the most common low energy lasers used in physiotherapy for promoting wound healing [1-2]. Inflammatory reaction is the physical response during wound healing, and anti-inflammatory effect of laser treatment has been proved [1].

The early research of He-Ne laser therapy in wound healing and inflammatory response was done in the 1980s [3-4]. Thereafter, effect of He-Ne laser on wound healing was investigated by other researchers who found that He-Ne photostimulation promotes the tissue repair process after 12 h, 24 h, 48 h, 72 h, and 5 days of treatment [5-6]. It was shown that He-Ne laser irradiation increased the wound healing process by promoting collagen formation and restoring the baseline cellularity when anti-inflammatory drugs were used after 1, 2, 3 days of He-Ne laser treatment [7]. When combined with diclofenac sodium, He-Ne laser could reduce the inflammatory response postsurgical [8]. All these investigations indicated that there were some beneficial effects of He-Ne laser on wound healing and inflammatory response after short time of treatment. Pro- and anti-inflammatory cytokines are produced and play an important role during the process of inflammatory reaction in wound healing, in which TNF-α, IL-1β and IL-6 are the most investigated pro-inflammatory cytokines, and IL-10 and TGF-β are the most investigated anti-inflammatory cytokines ([9-10]).

However, effects of He-Ne laser on the pro- and anti-inflammatory cytokines after longer period treatment remain poorly understood. Such information will assist clinicians with the application of He-Ne laser in their practice. In current studies, the effect of He-Ne laser on the pro- and anti-inflammatory cytokines from serum in the rats was investigated by making three incisions on the midline of the back, followed by stimulated with He-Ne laser. The studies showed that He-Ne laser could accelerate the healing of wound and increase the level of anti-inflammatory cytokines from serum in the rats. While the effects of He-Ne laser on pro-inflammatory cytokines were quite complicated.

2. Materials and Methods

2.1. Animal Care Ethics Statement

A total of 16 adult non-pregnant female Sprague Dawley (SD) rats (230-250 g), 10-12 weeks of age, were provided from the Central Animal House of the Xi'an Jiaotong University, Shaanxi, China, and kept in cage provided with commercial rat chow and water ad libitum with 12-h light/12-h dark cycle for 7 days prior to the study. The study was conducted according to the guidelines on animal care and with the approval of the Ethics Committee of Northwest A&F University.

2.2. Experiment Design

All of the rats were divided into 4 groups randomly, 4 rats in each group: negative control group (NC), in which the 4 rats were kept as they were kept before without any treatment, positive control group (PC), laser therapy group (LA) and erythromycin ointment treat group (ER). Each rat from groups PC, LA and ER was made three circular, full-thickness skin incisions on the midline of the dorsal area after anesthetized by diethyl ether (Bodi, Tianjin, China). Rats from PC group received neither laser nor erythromycin ointment treatment, rats from LA group received laser treatment (25 mW for 30 min, the wavelength is 632.8 nm) every day for 10 days by He-Ne Laser therapy instrument (JH35, Tianjin, China), rats from ER group received erythromycin ointment treatment, which was smear on the wounds by a swab twice a day according to the instructions.

2.3. Wound Closure Measurements

Immediately after creating the wounds, the initial wound diameter were measured using a caliper (Xigong, Wuxi, China) every day, the first day was considered the 1st d, the second day was considered the 2nd d, and so on, the 10th day was considered the 10th d. Because all of the wound diameters at the very beginning were not always the same, so uniformization of the wound diameters was carried out by expressing as the percentage of the initial wound areas by the following formula: (diameter on tested day/area on day 1st)×100. Wound area was digitally photographed using a digital camera (Canon EOS 700D, Ohta-ku, Tokyo, Japan).

2.4. Enzyme-linked Immunosorbent Analysis (ELISA)

On the 10th day, all the rats were anesthetized with diethyl ether, then blood sample was collected was collected from the eye with and without anticoagulation, blood sample with anticoagulation was used to test complete blood count, blood sample without anticoagulation was stored at room temperature for 3 h and centrifuged at 3000 × g for 15 min for the serum which was used for ELISA tests. The concentrations of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, and anti-inflammatory cytokines TGF-β and IL-10 from serum were detected by ELISA (Lufeng, Shanghai, China) in strict accordance with the manufacturer's protocols.

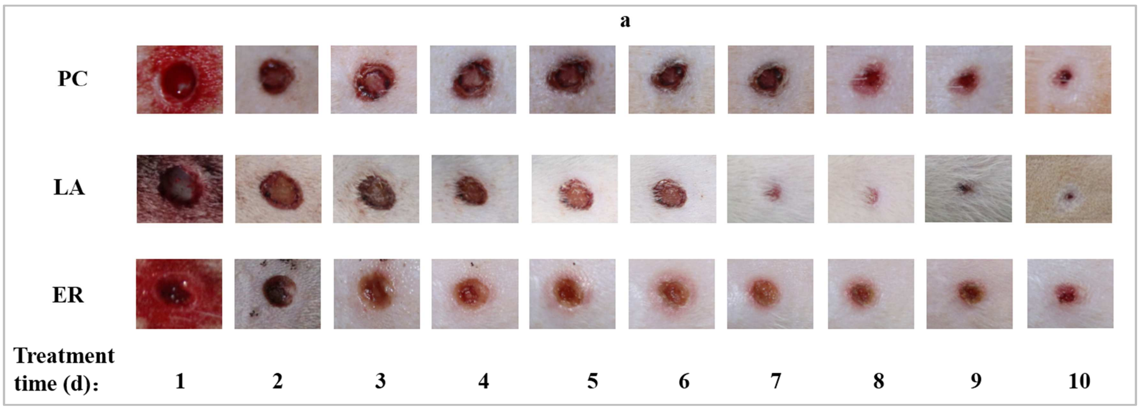
2.5. Statistical Analysis

Data presented as bar graphs are the mean±standard deviation (SD). Statistical differences were evaluated by one-way ANOVA analysis of variance, where a p-value < 0.05 was considered significant, and p-value < 0.01 was considered highly significant.

3. Result

3.1. Wound Area

Wound diameter was measured at every day immediately after creating the wounds. The closure of wound progressed more rapidly in laser treated (LA) group compared to PC and ER groups on the beginning of day 5th (Figure 1). These results indicate that laser therapy can accelerate the restoration of wound healing.



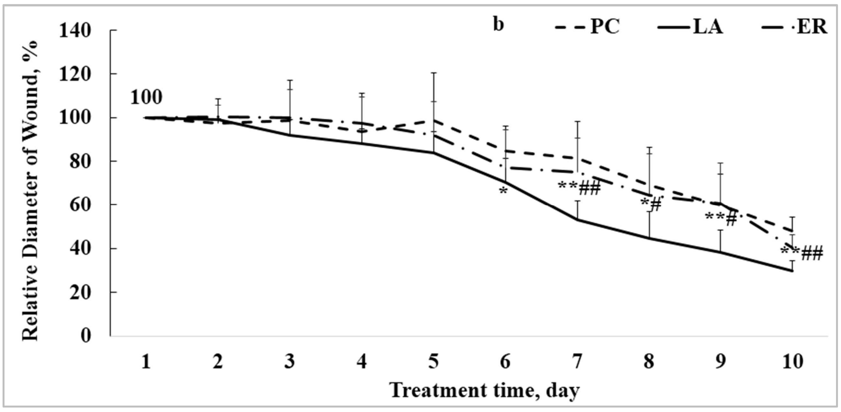


Figure 1. *Effect of He-Ne laser therapy on wound healing.*

a: Wound healing in different groups, b: Changes of wound area radio expressed by Mean ± SD.
PC: Positive Control Group, LA: Laser Treatment Group, ER: Erythromycin Ointment Treatment Group. *: Compare with PC; #: Compare with ER; */#: P<0.05; **/##: P<0.01.

3.2. Number of WBC

The number of WBC from peripheral blood was counted to evaluate the inflammatory reaction of the rats. The result showed that the number of WBC in LA group was lower than NC group, but higher than PC group, there were no significant differences between LA and ER groups (Figure 2a). Interestingly, laser acupuncture can increase the absolute number of neutrophil compared with ER group, and the percentage of granular cells significantly when compared with the NC group, but decreased the number and the percentage of lymphocytes when compared with the NC and ER groups (Figure 2b).

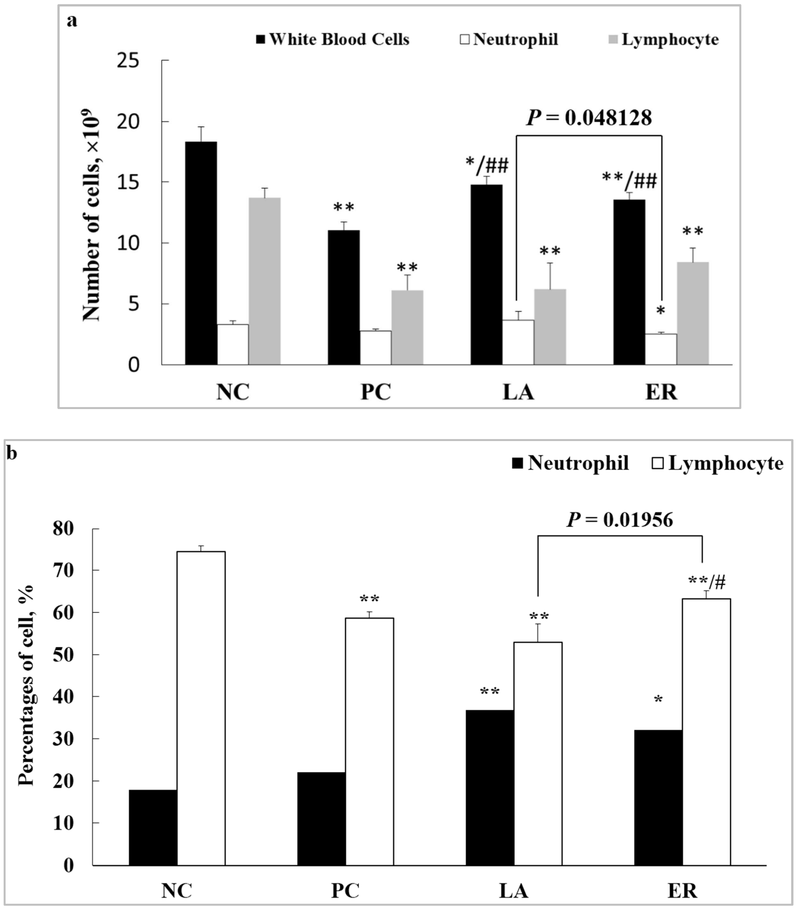
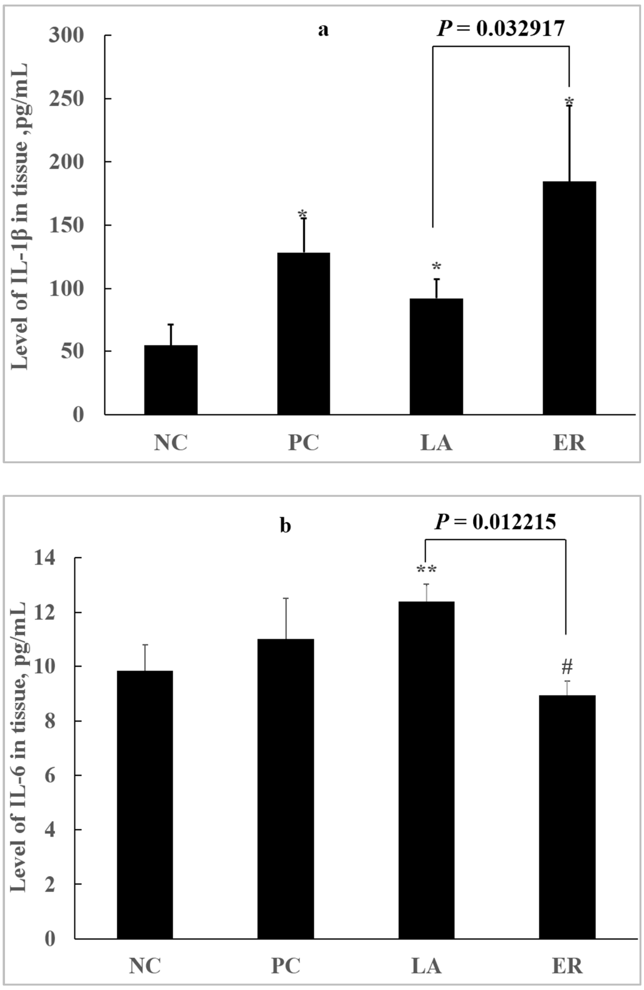


Figure 2. *Effect of He-Ne laser therapy on white blood cell, neutrophil and lymphocyte.*

a: Neutrophil and lymphocyte, b: the percentage of neutrophil and lymphocyte, NC: Negative Control Group, PC: Positive Control Group, LA: Laser Treatment Group, ER: Erythromycin Ointment Treatment Group. *: Compare with NC; #: Compare with PC; */#: *P*<0.05; **/##: *P*<0.01.

3.3. Expression of Pro-/anti-Inflammatory Cytokine

According to the progress of the wound healing (Figure 1), it is hypothesized that the laser acupuncture may protect the wound area by regulating the production of pro-inflammatory cytokines (including IL-1β, IL-6, TNF-α), and anti-inflammatory cytokines (including IL-10 and TGF-β). Therefore, the levels of the above cytokines from serum collected at the 10th day were detected by ELISA. The results shown that effects of He-Ne laser therapy on the pro-inflammatory cytokines were complicated, in which, the level of IL-1β was lower in LA and NC groups, there was no difference between them, but higher in PC and ER groups, there was significant difference between the low groups and the high groups (Figure 3). He-Ne laser acupuncture can reduce the pro-inflammatory cytokines IL-1β significantly when compared with PC and ER group, but there was no significant difference between NC group and LA groups (Figure 3a), the level of IL-6 was increased in LA group, which was significantly higher than NC and ER groups (Figure 3b), there was no difference about the level of TNF-α between NC and LA groups but higher than PC and ER groups significantly (Figure 3c).



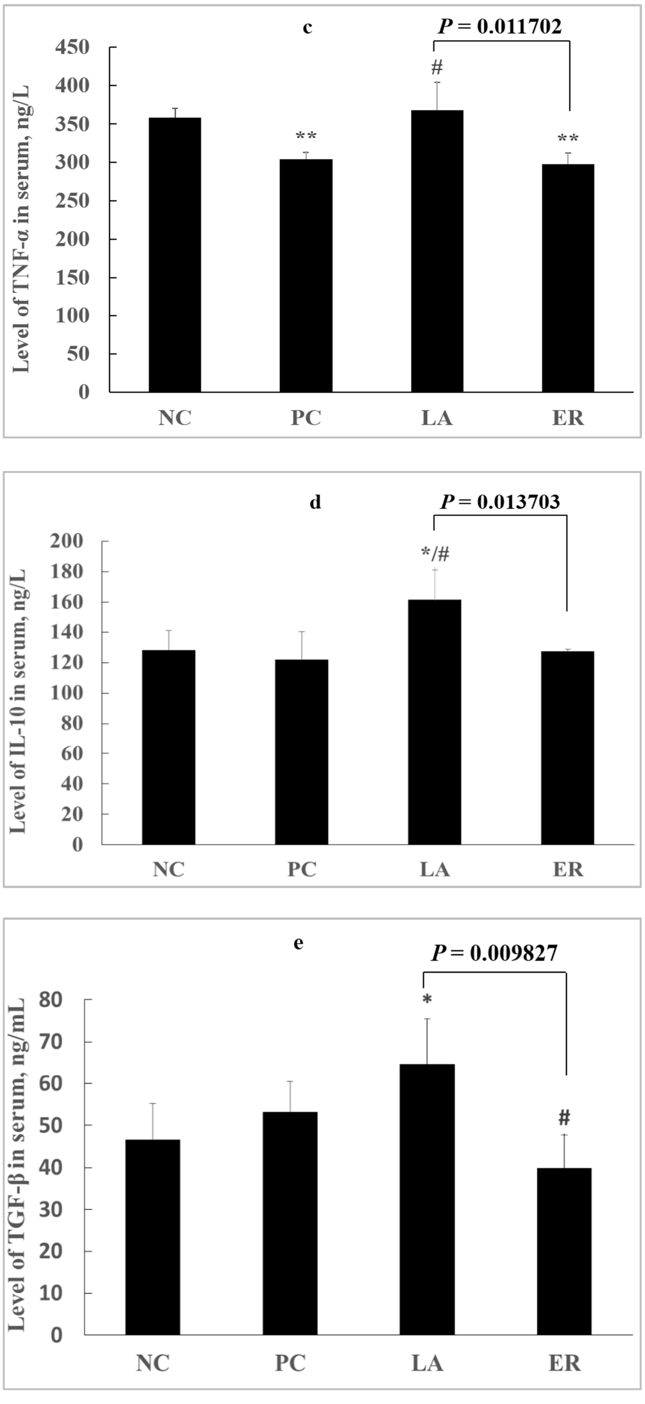


Figure 3. *Effects of He-Ne laser therapy on pro-/anti-inflammatory cytokines in serum.*

NC: Negative Control Group, PC: Positive Control Group, LA: Laser Treatment Group, ER: Erythromycin Ointment Treatment Group. *: Compare with NC; #: Compare with PC; */#: P<0.05; **/##: P<0.01.

As to anti-inflammatory cytokines, the studies showed that He-Ne laser could promote the production of IL-10 and TGF-β, both of which in LA group were higher than NC and ER groups significantly (Figure 3d and Figure 3e). The level of IL-10 was also higher in LA group than in PC group (Figure 3e). These results suggest that laser acupuncture could regulate the inflammatory reaction by controlling the release of pro- and anti-inflammatory cytokines.

4. Discussion

The current studies showed that He-Ne laser could accelerate the wound healing speed after 10 days of treatment, increased the number of WBC significantly than positive group, and increased the percentage of neutrophil. Levels of pro-inflammatory cytokines (IL-1β and TNF-α) were regulated to the level of NC group in LA group, while difference from PC and ER group, and IL-6 in LA group was higher than the other three groups, and the difference were significant when compared with NC and ER groups. Levels of anti-inflammatory cytokines (TGF-β and IL-10) from LA group were increased when compared with both of NC and ER groups, in additionally, level of IL-10 also higher in LA group than in PC group. All these results indicate that the effects of He-Ne laser on pro-/anti-inflammatory cytokines were not always consistent as reported before [11-13].

Positive effect of He-Ne laser biostimulation on wound healing in rats had been found by evaluating histopathological change and presence of polymorphonuclear leukocytes [6]. The current studies once again confirm the positive effect of He-Ne laser biostimulation by evaluating the diameter of the wounds with different treatment procedure, which was significant in the 5th day when compared the diameter of the wound with the PC and ER groups. Both of these two studies confirm that He-Ne laser is a good method to accelerate the wound healing.

The current studies showed that after 10 days of treatment, the numbers of WBC from the three treated groups were significant less than NC group, but the total number of WBC from LA group was higher than the PC group, and the difference between LA and ER groups is not significant. As to current knowledge, there are few studies about the effect of He-Ne laser on number of WBC. But there are some researches about the effect of He-Ne laser on lymphocytes. Studies carried out by E. G. Novoselova showed that short duration (less than 10 days) of laser light exposure induced an immune cell activity, while more prolonged period of treatment might even produce immunosuppression [14]. The decrease of lymphocyte number in the LA group could be due to the prolonged period of He-Ne laser stimulation.

In the present study, the effect of laser radiation on pro-inflammatory cytokines (including IL-1β, IL-6 and TNF-α) was not consistent. Significantly decreasing the level of IL-1β was observed in the LA group compared with PC and ER groups. The same effects of LLLT on IL-1β were demonstrated by other researchers using different treating conditions. Studies carried out by Anuska et al. in 2014 also demonstrated reduction of IL-1β expression post-treatment [12]. In the previous study, animals were treated with LLLT (semiconductor diode AsGaAl, continuous emission, 9 mW, 670 nm, 0.031 W/cm^2, beam with an output area of 0.28 cm^2) at a dose of 1 J/cm2, immediately following cutaneous surgery, then the tissues were collected at 1, 6 and 12 h post-surgery for detecting the pro-inflammatory cytokines by ELISA. Studies done by Pires et al. in 2011 also demonstrated reductions in IL-1β mRNA expression after treatment of LLLT on tendon [13]. In the previous study, tendonitis was induced in Wistar rats and irradiated with LLLT (λ=780 nm for 75 s at a dose of 7.7 J/cm^2) at 12 h and 7 days after the establishment of experimental tendinitis, both of the expression of IL-1β mRNA decreased at 7th day and 14th day. This experiment result also proved that LLLT would down-regulate the expression of IL-1β under different treatment time of LLLT (from 6 h to 14 days).

As to the IL-6, in this study, the level of IL-6 was increased significantly compared with the NC and ER groups, which were different from the result reported by Lima that the concentrations of IL-6 were measured at 6 h and 12 h after 31 s of LLLT treatment [12]. While the level of IL-6 after 10 days of treatment with 30 min every day were tested in this experiment, and the reason for the difference of the result could be due to the period of treatment time. As described by Song, the process of wound healing occurs in three phases: inflammation and migration, proliferation, and remodeling and maturation [15]. And the proliferation occurs in approximately 3-10 days after wounding, which mainly focus on the healing process lies in covering the wound surface, the formation of granulation tissue and restoring the vascular network [16]. Previous studies had shown that wound healing is delayed in IL-6-/- mice as a result of impaired re-epithelialization, angiogenesis, and macrophage infiltration, which indicated that IL-6 was necessary for the wound healing [17-18]. So it made easy to understand that LLLT could promote the speed of wound healing in the present study through the release of IL-6.

The effect of laser therapy on TNF-α is not always consistent. Several studies had shown that laser therapy could decrease level of TNF-α and the expression of TNF-α mRNA after 24 h and 48 h of different laser treatment, and no significant difference after 24 h and 48 h of treatment [19-21]. However, the study showed that the level of TNF-α was increased after 10 days of treatment with 30 min every day. There are some difference among these experiments, treatment period, type of laser, target animal and samples collected, among which treatment period is the key point that due to the difference. As study found that diabetes-enhanced and prolonged expression of TNF-α also contributes to impaired healing, which indicated that TNF-α is also necessary for proliferation during the wound healing process [22]. Studies also showed that level of TNF-α could be time-related expression during wounds healing process, elevating between 72 h and 168 h after wounding, which is similar to present studies [23]. In this experiment, the effect of laser therapy on TNF-α is also time-dependent, and in the proliferation stage of wound healing process, level of TNF-α is elevated, and LLLT may benefit for the physiological process by increasing concentration of TNF-α.

As described by Steven, TGF-β and IL-10 are anti-inflammatory cytokines, TGF-β are also a cytokine that promote wound healing through different pathway [24-26]. But there is no research report about effects of He-Ne laser therapy on TGF-β. Due to the acceleration effect of He-Ne laser on wound healing and the physical function of TGF-β, so there should be a positive effect of He-Ne laser therapy on the production of TGF-β. The results confirm that He-Ne laser therapy promote the release of TGF-β after 10 days of treatment during the wound healing process. However, study done by Raquel A. M. et al. found that the expression of TGF-β mRNA after treated by aluminum gallium indium phosphide (AlGaInP) diode laser was down-regulated from the 1st day of lesion to the 14th day, but with a slow increase tendency [21]. Further study should be done to identify the reason for the difference.

The expression of IL-10 in wound healing mice could be used to determine the wound age, as it began to enhance 1-3 h after injury, returned to lower level by 24 h, increased again at 48 h after injury [27]. Previous study observed a reduction of IL-10 at 6 and 12 h after laser treatment, without statistically significant [12]. Level of IL-10 produced by mesenteric and periaortic lymph node cells from mice after 6 days of laser treatment was also reduced [28]. However, the present studies showed that the level of IL-10 increases in He-Ne laser treatment group. The same result was observed by previous study, which found that the level of IL-10 from plasma increased after treated by InGaAlP 10 consecutive days in rats with heart failure [29]. The reason may be that the difference of the result is also due to laser treatment time.

5. Conclusions

In this study, the results exhibited that He-Ne laser acupuncture could improve the level of IL-6, IL-10, TGF-β and TNF-α, and accelerate the restoration of wound. These findings indicated that He-Ne laser acupuncture could be used in wound and inflammation healing process in the future. However, how does He-Ne laser acupuncture play the role in curing the wound and inflammation? After treated with He-Ne laser therapy, if the signal pathways in body show some changes? In addition, what is the mechanism of He-Ne laser acupuncture? For these questions, there are few reports, we still don't understand clearly. Therefore, the mechanism of He-Ne laser acupuncture on treating wound and inflammation will be further investigated in prospective studies.

Conflict of Interest

None of the authors has any financial or personal competing interests that could inappropriately influence or bias the content of the paper.

Authors' Contributions

Wuren Ma was responsible for protocol designing, draft editing and data analysis. Jinyang Zuo, Zhao Chen, Jun Yuan were responsible for follow-up and assessment of outcomes and data analysis. Xiaoping Song was responsible for statistical guidance. Yunpeng Fan wrote the draft and delivered the paper draft.

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