Comparison of the effects of laser and ultrasound treatments on experimental wound healing in rats

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Abstract—A randomized controlled study investigated the effects of ultrasound and laser treatments on wound healing in rats. The duration of the inflammatory phase decreased with both laser and ultrasound treatments; however, laser was more effective than ultrasound, with more significant results. The proliferation phase showed, for both treatments, an increase in the level of hydroxyproline and the number of fibroblasts, as well as stimulation of the collagen synthesis and the composition. Laser treatment was again more effective than ultrasound. The wound breaking strength was significantly higher with both treatments, and no statistically significant difference emerged between the laser and ultrasound groups, although laser treatment provided a much greater increase in the wound breaking strength than ultrasound. Both treatments have beneficial effects on the inflammatory, proliferation, and maturation phases of wound healing. Both can be used successfully for decubitis ulcers and chronic wounds, in conjunction with conventional therapies such as debridement and daily wound caring. However, laser treatment was more effective than ultrasound in the first two phases of wound healing.

Key words: laser, physical medicine, physical therapy, rat, rehabilitation, ultrasound, wound healing.

INTRODUCTION

Wound healing is a complex process, and recently, rapid developments in the knowledge of its basic principles have been reported. Natural healing takes time, and humans quickly become impatient. As a result, open sores have been treated with medicines and a range of natural and synthetic materials in an attempt to speed healing.

However, problems related to wound healing are still the cause of significant morbidity and mortality [1,2]. In spinal cord injury and immobilization, one of the main problems is decubitis, or pressure ulcers. This condition causes not only morbidity but also mortality. Much time and money are spent treating this problem. Studies on wound healing have increased our knowledge and understanding of these pressure ulcers, which constitute an important clinical problem in rehabilitation medicine [3].

Most studies concerning wound healing focus on accelerating wound and soft tissue healing, obtaining normal wound breaking strength, and preventing keloid and scar formation [2]. Recently, some researchers concluded that some physical methods, including therapeutic ultrasound and laser treatments, accelerate and facilitate wound healing and increase scar quality [4–17].

Abbreviations: GaAs = gallium arsenide, KCl = potassium chloride, PNL = polymorphonuclear leukocyte.

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Therapeutic ultrasound has been widely used over the past 50 years to treat many musculoskeletal complaints, including tendon injuries, pressure sores, venous ulcers, poor wound healing, lateral epicondylitis, herpes zoster, muscle damage, Dupuytren’s contractures, and others. Since the 1960s, the effects and benefits of ultrasound on wound healing have been investigated by various studies [4–7,16]. Because many studies have produced conflicting findings, the clinical use of ultrasound for wound healing is still under investigation [18,19]. Although some investigators saw no acceleration in the repair of wounds with ultrasound treatment [19–21], positive results and treatment effects have been reported in studies of ultrasound treatment on tendon injuries, lateral epicondylitis, and wound healing [4–7,22,23]. In addition, the beneficial effects of laser treatment on wound healing and qualified scar formation have been reported by numerous studies [10–15,17,24,25], including our previous study [26].

In the literature, no comparison exists of ultrasound and laser treatments on the acceleration of wound healing and quality of scar formation. In this study, we investigated and compared the effects of ultrasound and laser treatments on the wound healing process through a randomized controlled trial.

METHODS

We used 124 healthy female Swiss-Albino rats, each 200 to 240 g and 8 to 10 months of age. The study was carried out at Erciyes University Experimental and Clinical Research Center. All rats were housed in metal cages maintained at 15 °C to 18 °C and fed standard rat chow and water. After making a 6 cm linear incision at the dorsal skin, we divided the rats randomly into 4 groups of 30 rats each. Four rats were used for initial trials. The treatment protocols used included ultrasound in Group I, control ultrasound in Group II, laser in Group III, and control laser in Group IV. In all groups, we performed biochemical and histopathologic evaluations on the 4th and 10th days, and a biomechanical test on the 25th day.

Surgical Procedure

After local preparation of the dorsal skin and general anesthesia of rats by ketamine (60 mg/kg, intraperitoneally), a 6 cm full-thickness linear incision was made 2 cm away from the dorsal midline including the panniculus carnosus. The incision was sutured with 5.0 prolene intradermally. The same investigator (SY) performed all surgical procedures.

Treatment Methods and Group Formation

The treatment was started in each group within 2 hours of the surgical procedure.

Group I: Ultrasound Treatment

An ultrasound device (model Sonopuls 463, Enraf-Nonius Co., The Netherlands) was used with a probe 8 mm in diameter and sterile Sonogel (Enraf-Nonius Co.). We applied a pulsed ultrasound (2 ms on, 8 ms off, and 0.5 W/cm² intensity) by a moving applicator technique, 5 minutes daily, for 10 days. An ultrasound intensity of 0.5 W/cm² minimized its thermal effect.

Group II: Sham Ultrasound Treatment

A procedure similar to the ultrasound treatment was applied with no current (sham method).

Group III: Laser Treatment

A gallium-arsenide (GaAs) laser device (model Laserpet 100, Petas Co., Turkey) with a probe 1 cm in diameter and 15° emission angle, at 904 nm wavelength, 6 mW average power, 1 J/cm² dosage, 16 Hz frequency, 10 minutes duration, was used continuously for 10 days by a stroking method.

Group IV: Sham Laser Treatment

A procedure similar to the laser treatment was applied with no current (sham method).

Evaluations

All wounds were cleaned with povidon-iodine solution every day, and all rats were housed in their metal cages.

Ten rats in each group were killed with a 2 cc intracardiac potassium chloride (KCl) injection on the 4th, 10th, and 25th days. We marked the incision as parts a, b, c, d, e, and f (Figure). Full-thickness samples were obtained after the surgical process. Then parts a and c were used for histopathologic evaluation, parts b and d for biochemical evaluation, and parts e and f for biomechanical evaluation. Histopathologic and biochemical evaluations on the 4th and 10th days and a biomechanical evaluation on the 25th day were performed.

Slides were stained with hematoxylin and eosin for polymorphonuclear leukocytes (PNL), fibroblasts, and macrophages with the Young and Dyson method [6],...
Masson’s trichrome for collagen density and arrangement with the Brown method, and toluidine blue for mast cells with the Weiss et al. method [27] for histopathologic analysis. Using the double-blind method, we measured the level of tissue hydroxyproline for biochemical analysis using the method of Reddy and Enveneka in parts b and d [28].

After sacrificing the rats with a KCl injection and removing the sutures, to evaluate the biomechanical parameter on the 25th day, we used a parallel surgical blade to excise two 10 mm strips, 6 cm long, in parts e and f of the wound, according to the method of Mustoe et al. [29]. We measured the wound breaking strength with a tensiometer (model 4411, Instron Inc., England) to get data to identify the maturation phase of the wound for our biomechanical evaluation with the blind method.

Statistical Analysis

We used a Mann-Whitney U-test for statistical analysis of all the parameters, except the collagen density and arrangement. A chi-square test was used to compare the collagen density and the arrangement of the groups.

RESULTS

A small amount of serohemorrhagic leakage was observed on wound edges within the first few days in the ultrasound, sham ultrasound, and sham laser treatment groups. The hairs were observed to grow on the 7th day in the ultrasound, sham ultrasound, and sham laser treatment groups, and on the 5th day in the laser treatment group.

The duration of inflammatory phase decreased in the laser and ultrasound groups compared with the control groups ($p < 0.05$). Compared with ultrasound treatment, the laser treatment was found to be more effective, with more significant results ($p < 0.05$).

The proliferation phase was positively affected in the treatment groups by an increase in the level of hydroxyproline and the number of fibroblasts, as well as stimulation of the collagen synthesis and the composition, compared with the control groups ($p < 0.05$). The laser treatment proved more effective than the ultrasound treatment in the proliferation phase ($p < 0.05$).

The laser treatment increased the mast cell count much more than the ultrasound treatment in the inflammatory phase ($p < 0.05$), although no statistically significant difference emerged in mast cell count between the laser and ultrasound groups in the proliferation phase ($p > 0.05$).

The wound breaking strength was significantly higher in the treatment groups than in the control groups ($p < 0.05$). No statistically significant difference was observed between the treatment groups ($p > 0.05$), although the laser treatment provided much more increase in the wound breaking strength than the ultrasound treatment.

The collagen density and the arrangement were significantly better in the treatment groups than in the control groups ($p < 0.05$), but no statistically significant difference existed between the treatment groups ($p > 0.05$). Overall results are summarized in Tables 1 to 4.

DISCUSSION

Recently, some physical methods, including therapeutic ultrasound and laser treatments, were found to accelerate and facilitate wound healing and increase scar quality [4–17]. However, conflicting findings have been reported in some studies, and some investigators found no treatment effect on accelerating the repair of wounds [18–21].

Because treatment methods vary and some studies are poorly controlled or provide little detail on their protocols, attempts to determine efficacy are confounded. The possible mechanism through which ultrasound helps tissue
Table 1.
Analysis of histopathologic and biochemical results on 4th day.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>US* (X ± SD)</th>
<th>SUS* (X ± SD)</th>
<th>L* (X ± SD)</th>
<th>SL* (X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNL</td>
<td>37.4 ± 1.1</td>
<td>62.2 ± 1.7</td>
<td>17.4 ± 1.1</td>
<td>61.0 ± 5.2</td>
</tr>
<tr>
<td>Macrophages</td>
<td>27.4 ± 1.1</td>
<td>27.0 ± 1.6</td>
<td>25.6 ± 1.1</td>
<td>35.6 ± 0.9</td>
</tr>
<tr>
<td>Mast Cells</td>
<td>16.0 ± 1.2</td>
<td>16.2 ± 1.3</td>
<td>24.2 ± 0.8</td>
<td>17.6 ± 0.6</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>66.2 ± 1.3</td>
<td>37.0 ± 1.6</td>
<td>82.6 ± 2.1</td>
<td>32.8 ± 2.2</td>
</tr>
<tr>
<td>Hydroxyproline (mg/g)</td>
<td>0.38 ± 0.12</td>
<td>0.08 ± 0.03</td>
<td>0.62 ± 0.02</td>
<td>0.14 ± 0.04</td>
</tr>
</tbody>
</table>

*_{n = 10} p < 0.05 except for ultrasound treatment and sham ultrasound treatment macrophages and mast cells, where p > 0.05.

Table 2.
Analysis of histopathologic and biochemical results on 10th day.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>US* (X ± SD)</th>
<th>SUS* (X ± SD)</th>
<th>L* (X ± SD)</th>
<th>SL* (X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNL</td>
<td>12.8 ± 0.8</td>
<td>66.0 ± 1.0</td>
<td>8.6 ± 1.5</td>
<td>43.2 ± 1.8</td>
</tr>
<tr>
<td>Macrophages</td>
<td>17.2 ± 0.8</td>
<td>27.0 ± 1.6</td>
<td>17.4 ± 1.1</td>
<td>27.4 ± 0.9</td>
</tr>
<tr>
<td>Mast Cells</td>
<td>6.8 ± 0.8</td>
<td>15.6 ± 1.1</td>
<td>6.2 ± 0.8</td>
<td>22.8 ± 0.8</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>82.0 ± 1.6</td>
<td>51.8 ± 3.5</td>
<td>113.2 ± 4.1</td>
<td>53.0 ± 2.8</td>
</tr>
<tr>
<td>Hydroxyproline (mg/g)</td>
<td>0.67 ± 0.08</td>
<td>0.23 ± 0.03</td>
<td>1.01 ± 0.13</td>
<td>0.22 ± 0.01</td>
</tr>
</tbody>
</table>

*_{n = 10} p < 0.05

Table 3.
Comparison of data on 4th and 10th days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4th Day (n = 10)</th>
<th>10th Day (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>US (X ± SD)</td>
<td>L (X ± SD)</td>
</tr>
<tr>
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<td>Fibroblasts</td>
<td>66.2 ± 1.3</td>
<td>82.6 ± 2.1</td>
</tr>
<tr>
<td>Hydroxyproline (mg/g)</td>
<td>0.38 ± 0.12</td>
<td>0.62 ± 0.02</td>
</tr>
</tbody>
</table>

*_{p < 0.05 except for macrophages and mast cells, where p > 0.05.}

Table 4.
Comparison of wound breaking strength on 25th day.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>US* (X ± SD)</th>
<th>SUS* (X ± SD)</th>
<th>L* (X ± SD)</th>
<th>SL* (X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breaking Strength (N)</td>
<td>5.1 ± 1.6</td>
<td>1.9 ± 0.7</td>
<td>6.8 ± 1.3</td>
<td>1.8 ± 0.6</td>
</tr>
</tbody>
</table>

*_{n = 10} p < 0.05

repair is likely related to its mechanical effects, as opposed to the thermal effect [30,31]. Micromassage or ultrasound of the tissue produces a change in membrane permeability and stimulates second messenger substance, such as calcium, across the cell membrane [31,32]. Then the proliferation of myogenic cells may be stimulated by these second messengers [33]. The anti-inflammatory effects of ultrasound have been shown in some studies [6,8]. This mechanism could be explained by the reduction of the macrophage number [8,34]. In our study, the macrophage number was found to be significantly decreased in the ultrasound group compared with its control group on the 10th day, which is consistent with the reported anti-inflammatory effect of ultrasound treatment.

Ultrasound has also been shown to effectively stimulate fibroblasts [34]. In some animal studies, researchers found that ultrasound at the intensities of 0.1 W/cm\(^2\) and 0.5 W/cm\(^2\) accelerates the inflammatory phase of repair [6,9,35]. This reported accelerated repair agrees with the findings from several other suggestions that low-dose ultrasound of approximately 0.5 W/cm\(^2\) pulsed with a frequency of 1 or 3 MHz promotes wound healing [34–37]. Our treatment protocol included pulsed ultrasound with a dose of 0.5 W/cm\(^2\) at a frequency of 1 MHz for 5 days in the ultrasound group, a protocol that was applied in most studies. In the ultrasound group of our study, the duration of inflammatory phase decreased and the proliferation phase was positively affected by the increase in the level of hydroxyproline and the number of fibroblasts, as well as stimulation of the collagen synthesis and the composition, compared with its control group. These findings are consistent with the studies reporting the positive effects of ultrasound on wound healing [4–7,30].

Laser treatment has also been studied in wound healing [10–15,17,24–26]. Currently, laser treatment is used for decubitus and diabetic ulcers, open wounds, venous ulcers, graft ulcers, incisions, lacerations, and burns. Studies in vivo and in vitro showed that laser treatment accelerated biochemical reactions, fibroblast activity, collagen metabolism, neovascularization, qualified scar formation, and wound formation [10–15,17,24–26]. The issue of “significant” thermal change is controversial, although some studies conclude that the low-energy laser does not produce significant tissue temperature changes [17,38]. Therefore, no unanimous agreement exists on the thermal effects and the treatment protocol of laser treatment on wound healing, and more studies are required in this field. A wide variation exists in recommendations for the optimal energy for different conditions; the usual ranges are from 0.5 to 10 J/cm\(^2\) [17]. Generally, a laser wavelength of 600 to 984 nm is used in physical medicine, and a laser wavelength of 632.8 nm helium neon and 904 nm GaAs are most frequently used in wound healing [10–15,17,26]. Therefore, we used a GaAs laser with a wavelength of 904 nm and power of 1 J/cm\(^2\). With the laser treatment, the duration of inflammatory phase decreased and the level of hydroxyproline and the number of fibroblast increased, and also the collagen synthesis and the composition were stimulated in the proliferation phase compared with its control group. These findings are consistent with the literature.

Laser treatment has an antibacterial effect as well. Laser treatment has been reported to inhibit the proliferation of bacteria in cultures. We found a decreased number of macrophages in the laser treatment group compared with the sham laser treatment group, consistent with the literature. However, we did not find any previous study in the literature regarding the effects of laser treatment on the number of macrophages. Laser treatment also stimulates the phagocytic activity of leukocytes in vitro [39]. In our study, the PNL number increased in the laser treatment group compared with its control group. This finding indicates suppressed inflammation, which is desired in clean wound healing. Some researchers have reported that low-energy laser treatment decreased the duration of the inflammatory phase [15,40].

When we compared these two modalities in the inflammatory phase, the laser treatment was more effective, with more significant results than the ultrasound treatment. The numbers of PNL and macrophages were decreased in the laser group compared with the ultrasound group, indicating that the laser treatment decreased the duration of inflammatory phase significantly more than the ultrasound treatment. We could not find any similar study comparing the effect of ultrasound and laser treatment on wound healing in rats in the literature. Mechanical stress is known to affect the collagen arrangement [41]. Tissue hydroxyproline level is accepted as an important parameter in the evaluation of collagen metabolism [42]. In our study, the proliferation phase was positively affected in the treatment groups by the increase in the level of hydroxyproline and the number of fibroblasts, as well as stimulation of the collagen synthesis and the composition, compared with their control groups. Compared with the ultrasound treatment, the laser treatment proved more effective in the proliferation phase.
The maturation or remodeling phase is the last and longest phase of wound healing. The most important development is the remodeling and maturation of collagen during this phase. The wound breaking strength can be used for the biomechanical evaluation of wound in this phase. Breaking strength increases significantly after the third week of wound healing, so we measured the breaking strength on the 25th day. The wound breaking strength was significantly higher in the treatment groups than in the control groups, which is also consistent with the literature [9,10,12,14]. No statistically significant difference existed between the treatment groups, although the laser treatment provided much more increase in the wound breaking strength than the ultrasound treatment.

Our study showed that both ultrasound treatment and laser treatment have beneficial effects in the inflammatory, proliferation, and maturation phases of wound healing, compared with their control groups.

CONCLUSION

We conclude that both laser and ultrasound treatments can be used successfully for decubitis ulcers and chronic wounds, when used with conventional therapies such as debridment and daily wound caring. The laser treatment, however, was considered more effective than the ultrasound treatment in the first two phases of wound healing.

REFERENCES


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