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The effect of low-intensity pulsed ultrasound on rib fracture: An experimental study

Düşük yoğunluklu kesikli ultrasonun kaburga kırığı üzerindeki etkisi: Deneysel çalışma

Saniye Göknil Çalık[1], Mustafa Çalık[2], Zümrüt Ela Arslan Kasdoğan[3], Halim Yılmaz[4], Gülten Karaca[4], Halil Ekrem Akkurt[4], Hacı Hasan Esen[5], Mustafa Cihat Avunduk[5], Hıdır Esme[2], İsmet Tolu[6]

Institution where the research was done:
Necmettin Erbakan University Kombassan Experimental Medicine Research And Application Center

Author Affiliations:
[1]Emergency and First Aid Program, KTO Karatay University, Vocational School of Health Services, Konya, Turkey
[2]Department of Thoracic Surgery, Health Sciences University, Konya Training and Research Hospital, Konya, Turkey
[3]Department of Department of Anesthesiology and Reanimation, Health Sciences University, Konya Training and Research Hospital, Konya, Turkey
[4]Department of Physical Medicine and Rehabilitation, Health Sciences University, Konya Training and Research Hospital, Konya, Turkey
[5]Department of Pathology, Necmettin Erbakan University Meram Faculty of Medicine, Konya, Turkey
[6]Department of Radiology, Health Sciences University, Konya Training and Research Hospital, Konya, Turkey

ABSTRACT

Background: In this study, we aimed to investigate the effects of low-intensity pulsed ultrasound on rib fracture healing in a rat model.

Methods: A total of 72 male Wistar-Albino rats were randomly divided into three equal groups. To induce a rib fracture, right thoracotomy was performed under general anesthesia and a 0.5-cm segment was removed from the fourth and fifth ribs. After 24 h of surgery, low-intensity pulsed ultrasound was implemented according to the groups. Group 1 served as the control group for the observation of normal bone healing. Low-intensity pulsed ultrasound was applied at a dose of 20% (2 msn pulse-8 msn pause) 100 mW/cm^2 and 50% (5 msn pulse-5 msn pause) 200 mW/cm^2 for six min, respectively in Group 2 and Group 3. All subjects were followed for six weeks. Eight animals from each group were sacrificed at two, four, and six weeks for further assessment. Histological alterations in the bone were examined.

Results: Although there was no statistically significant difference in osteoblasts, osteoclasts, new bone formation, and lymphocyte count among the groups, histological consolidation was significantly increased by low-intensity pulsed ultrasound. While low-intensity pulsed ultrasound induced osteoblastic, osteoclastic, and new bone formation, it inhibited lymphocyte infiltration.

Conclusion: Low-intensity pulsed ultrasound, either at low or high doses, induced the histological consolidation of rib fractures and inhibited lymphocyte infiltration. This effect was more prominent in the long-term and at higher dose with increased daily and total administration time. We, therefore, believe that accelerating the natural healing process in patients with rib fractures would enable to treat more effectively in short-term.

Keywords: Experimental, low-intensity pulsed ultrasound, rat, rib fracture.

ÖZ

Amaç: Bu çalışmada, bir sıçan modelinde düşük yoğunluklu kesikli ultrasonun kaburga kırığı iyileşmesi üzerindeki etkileri araştırıldı.

Çalışma planı: Toplam 72 erkek Wistar-Albino sıçan rastgele üç gruba ayrıldı. Kaburga kırığını oluşturmak için genel anestezi altında sağ torakotomi yapıldı ve dördüncü ve beşinci kaburgadan 0.5 cm'lik segment çıkarıldı. Ameliyattan 24 saat sonra gruplara göre düşük yoğunluklu kesikli ultrason uygulandı. Grup 1 kontrol grubu olup, normal kemik iyileşmesi gözlendi. Grup 2 ve Grup 3'e altı dakika boyunca sırasıyla %20 (2 msn pulse-8 msn pause) 100 mW/cm^2 ve %50 (5 msn pulse-5 msn pause) 200 mW/cm^2 düşük yoğunluklu kesikli ultrason uygulandı. Denekler altı hafta boyunca takip edildi. Her gruptan sekizer sıçan iki, dört ve altıncı haftalarda ileri tetkik için sakrifiye edildi. Kemikteki histolojik değişiklikler incelendi.

Bulgular: Osteoblast, osteoklast, yeni kemik oluşumu ve lenfosit sayısında gruplar arasında istatistiksel olarak anlamlı bir fark olmamasına rağmen, düşük yoğunluklu kesikli ultrason ile histolojik konsolidasyon anlamlı düzeyde arttı. Düşük yoğunluklu kesikli ultrason osteoblastik, osteoklastik ve yeni kemik oluşumunu uyarırken, lenfosit infiltrasyonunu inhibe etti.

Sonuç: Düşük veya yüksek dozlarda düşük yoğunluklu kesikli ultrason, kaburga kırıklarının histolojik konsolidasyonunu uyardı ve lenfosit infiltrasyonunu azalttı. Bu etki, günlük ve toplam uygulama süresinin artırılmasıyla uzun dönem ve yüksek dozda daha belirgindi. Bu nedenle, kaburga kırığı olan hastalarda doğal iyileşme sürecinin hızlandırılmasının daha etkili ve kısa sürede tedavi sağlayabileceği kanısındayız.

Anahtar sözcükler: Deneysel, düşük yoğunluklu kesikli ultrason, sıçan, kaburga kırığı.

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Correspondence: Mustafa Çalık, MD. SBÜ Konya Eğitim ve Araştırma Hastanesi, Göğüs Cerrahisi Kliniği, 42090 Meram, Konya, Türkiye.
Tel: +90 332 - 323 67 09/1837 e-mail: drmcalik@hotmail.com

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Trauma, which is one of the leading causes of death in young adults, is the third leading cause of mortality in all age groups.[1] Rib fractures (RFs) are detected in 4 to 12% of cases admitted to the health care providers due to trauma.[2,3] Pain associated with RFs increases with respiratory movements and coughing. The mortality rate is around 10% and has been increasing with the increased age and number of RFs.[4] Current treatment of RFs is symptomatic for pain and accompanying injuries and/or possible complications. Traditionally, clinicians consider that pain and disability would improve within six to eight weeks.[5] However, patients are usually able to return to their daily work and social life about 70 days later.[6,7] Persistent pain and deformity, the most common cause of morbidity, is up to 60%.[4] About 20 to 60% can never be fully healed.[8]

The sound is a linearly propagating mechanical wave in the material environment. A total of 20 to 20,000 Hz frequencies can be heard by the human ear. The sound waves under this frequency are called infrasound and above are called ultrasound (US), the name given to the higher frequency sound waves that the human ear cannot hear.[9,10] While US is commonly used for imaging purposes, low-intensity pulsed ultrasound (LIPUS) has been used in physiotherapy for the treatment of pain, musculoskeletal system injuries, and soft tissue lesions for about six decades.[11]

Rib fractures may be painful and potentially injurious. In chest traumas, particularly in RFs, mortality and morbidity can be reduced with the control of pain. Pain is reduced by the provision of bone stabilization.[12] In the literature, LIPUS has been shown to increase osteogenesis in animal models.[13] In the present study, we hypothesized that LIPUS would accelerate RB healing and aimed to investigate the effects of LIPUS on RF healing in a rat model.

MATERIALS AND METHODS

This experimental study was carried out at the Experimental Medicine and Research Center of Necmettin Erbakan University. A total of 72 Wistar-Albino rats from the same generation weighing 250 to 300 g selected randomly were used in this study. Test animals were kept in room 12 h dark, 12 h light, at standard room temperature (21±10°C) and were fed ad libitum with standard diet and tap water through feeding bottles in steel cages cleaned daily by washing. Each subject received humanitarian care in accordance with the European Convention on the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes, prepared in 1986 (86/609/EEC) and updated in 2003 (2003/65/EC). This study was approved by the local Animal Ethics Committee (2011/091) and was approved and funded by the Health Sciences University School of Medicine, Animal Care and Investigational Committee of Konya Training and Research Hospital.

a) Study groups

The subjects were divided into three equal groups including 24 (n=24) male rats in each group. Prior to the operation, the rats were numbered separately by drawing a line on their tails, and their weights (g) were measured and recorded. To induce the experimental RF model, a 0.5-cm rib was removed from the fourth and fifth ribs of the right hemithorax. Group 1 served as the control group and the course of natural healing was followed. Group 2 and Group 3 were administered LIPUS.

b) Application of LIPUS

The application of LIPUS with BTL-4000 Sono (BTL Hertfordshire, UK) (Figure 1) pulse US was initiated 24 h after the operation. Each subject underwent daily LIPUS administration at a frequency of 1 MHz daily under general anesthesia following manual restriction. A total of 20% of Group 2 received 100 mW/cm^2 (2 msec pulse-8 msec pause) and 50% of Group 3 received 200 mW/cm^2 (5 msec pulse-5 msec pause). Low-intensity pulse ultrasound was applied

Figure 1. A photograph of BTL-4000 Sono (BTL, Hertfordshire, UK).

to each animal for six min at a single session daily between 09:00 AM and 05:00 PM for a total of six consecutive weeks.

c) Anesthesia protocol

Induction was provided with ketamine hydrochloride (Ketanest, Pfizer Pharma GmbH, Karlsruhe, Germany) with 15 to 20 mg/kg intravenous (IV) or 20 to 25 mg/kg intramuscular (IM). General anesthesia with xylazine (Rampun®, 2% 50 cc Bayer Türk İlaç A.Ş., Istanbul, Turkey) at a dose of 0.5 to 1 mg/kg IV or 1 to 2 mg/kg IM, and anesthesia was maintained with the same doses. The mean duration of anesthesia was 10 to 15 min for each rat. The subjects were administrated 50 mg/kg IM of ceftazidime pentahydrate twice daily (Fortum®, GlaxoSmithKline Inc., Brentford, UK) for five days for prophylaxis.

d) Operation technique

After proper field cleaning and antisepsis for the rats under general anesthesia, a lateral thoracotomy incision was performed through the right fifth lateral intercostal space while lying on the left side position. The muscles were dissected, and the chest wall was reached. The rats underwent double subperiosteal half (0.5)-cm costal resection starting from the fourth rib in the right hemithorax. The layers were closed with continuous sutures from the muscles according to the procedure. Pneumothorax control of each subject was performed after the operation.

e) Postoperative care and follow-up

Tramadol hydrochloride (Contramal, 100 mg 2 mL, Abdi Ibrahim İlaç Sanayi ve Tic. A.Ş., Istanbul, Turkey) at a dose of 1 to 2 mg/kg IM was used for five days to control pain in the postoperative period. A total of 24 subjects including eight from each group were euthanized with a lethal IV dose of non-barbiturate anesthetic (ketamine/xylazine) painlessly according to the existing instructions established by the latest report of the American Veterinary Medical Association Panel on Euthanasia.[14] For three times, the anesthetic dose was used for euthanasia at two, four, and six weeks after surgery. The total follow-up was six weeks.

f) Pathological examination

All materials were decalcified in 10% buffered formaldehyde for 48 h after the fixation period, until they were tempered enough to be cut with a microtome. Tissue specimens from appropriate sites were, then, taken for the Autotecnicon (Autotecnicon-Shandon, Cheshire, UK) follow-up, embedded in paraffin, stained with hematoxylin-eosin (H-E), and sectioned with a microtome to calculate the osteoclast, osteoblast, lymphocyte count, and the area of new bone formation (Figure 2). All stained preparations were examined with Nikon Eclipse E400 light microscope (Nikon Corp., Minato-ku, Tokyo, Japan). Care was given to select as possible as the same areas for each case during the assessment. The selected areas were scanned with a Nikon Coolpix 5000 digital camera (Nikon Corp., Minato-ku, Tokyo, Japan) with a microscope mounted at the same microscope magnification. At the same time, the Nikon Stage Micrometer (MBM11100, Nikon Corp., Minato-ku, Tokyo, Japan) images were also taken for calibration with the same microscope magnification. All images were transferred to a PC environment for analysis using the Clemex Vision Lite 3.5 (Clemex Technologies Inc., Longueuil, Quebec, Canada) (Figure 3). First, the length was calibrated with the Nikon Stage Micrometer (MBM11100, Nikon Corp., Minato-ku, Tokyo, Japan). After the calibration, the area to be examined was determined as 38732.7 μm^2. The osteoblasts, osteoclasts, and lymphocytes on the 38,732.7 μm^2 areas selected on the digital images of H-E stained preparations were marked and automatically counted by the aforementioned image analysis program. The damaged cells were excluded from the analysis.

Statistical analysis

Statistical analysis was performed using the PASW version 18.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean ± standard deviation or number and frequency. The Kruskal-Wallis and Mann-Whitney U test were used to analyze significant differences among the groups. A p value of <0.05 was considered statistically significant.

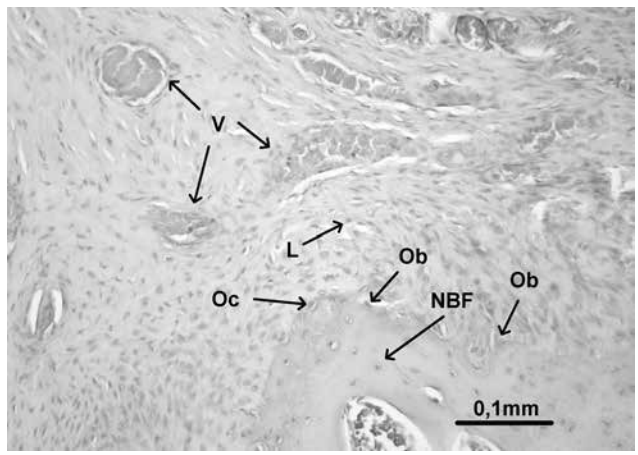

Figure 2. Osteoblast (OA), osteoclast (Oc), vessel (V), lymphocyte (L), and new bone formation area (NBF) (H-E ×0.1 mm).

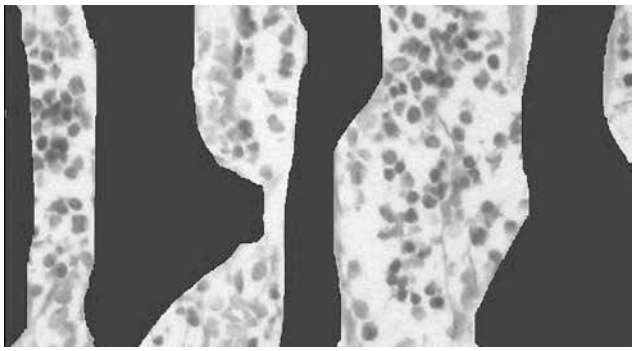

Figure 3. Calculation of new bone formation (NBF) area in PC environment with Clemex Vision Lite version 3.5 program (Clemex Technologies Inc., Longueuil, Quebec, Canada).

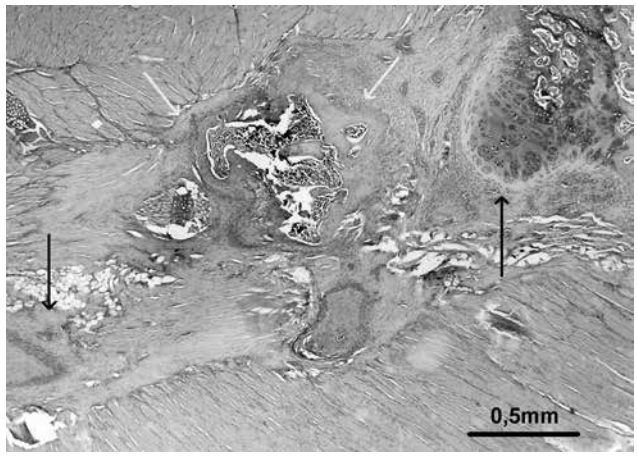

Figure 4. New bone formation areas are shown with black arrows and broken ends of ribs are shown with yellow arrows (H-E ×0.5 mm).

RESULTS

There was no statistically significant difference in osteoblasts (p=1.0), osteoclasts (p=1.0), new bone formation (p=1.0), and lymphocyte count (p=0.128) among the groups (Figure 4).

Osteoblast, osteoclast, and new bone formation were not detected in all groups at the end of the second week. However, lymphocytes from inflammatory cells were higher in LIPUS groups.

Osteoblast, osteoclast, and new bone formation were not detected in the control group during the fourth week. However, osteoblast, osteoclast, and new bone formation were higher in LIPUS groups compared to the control group. Group 2 stimulated osteoblasts more than group 3. However, new bone formation was higher in group 3. Although lymphocytes decreased in all groups; In the LIPUS groups, this reduction was proportionally higher.

In the sixth week, osteoblast osteoclast and new bone formation were encountered for the first time in the control group. Osteoblast, osteoclast, and new bone formation were higher in the LIPUS groups compared to the control group. The opposite situation was also observed in lymphocytes. However, histological consolidation was significantly increased by LIPUS at any dose. This effect was more long-lasting and pronounced at higher doses (Table 1).

DISCUSSION

Currently, RFs are the signs of high energy serious injury, high mortality, and accompanying multiple injuries.[15,16] Even if new causes have been blamed over time, former ones have never lost their importance. Today, chest traumas including motor vehicle accidents and high fall-related RF injuries range the second after head traumas among the causes of trauma-related deaths.[16] Blunt traumas which may cause thoracic RFs are accompanied by secondary injuries at a rate of 85 to 90%.[17] It is estimated that RFs affect nearly 145,000 individuals annually, at least a third of whom require hospitalization in the United States alone.[8] The actual figures and costs are probably much higher than expected, considering that only one-third of these patients are referred to the emergency services.

Chest pain, which is the most common symptom of RFs, arises from broken bones, damaged soft tissues, and muscles. It is usually exacerbated by movements of the chest wall, including deep breathing and coughing, even in normal breathing movements.[6] Traditionally, the treatment of RFs is symptomatic based on the control of pain, which is provided with analgesics including narcotic and non-steroidal anti-inflammatory drugs, respiratory physiotherapy, and specific treatment approaches of related complications. Despite all treatment options, 31% of cases experience complications such as nosocomial pneumonia, prolonged respiratory failure, long-term hospitalization, or death due to advanced age, and accompanying comorbid diseases and traumas.[6,8,18] Although the number of RFs and the age of the patient are designated as the factors which increase mortality and morbidity in the literature, this issue is still controversial.[15] While high morbidity is defined in patients older than 65 years, a similar high mortality and morbidity tendency has been described in younger patients, as well.[19] The number of RFs is similar. Whitson et al.[20]

showed that high mortality and morbidity rates were not associated with the number of RFs in a total of 35,467 study cohort. However, the authors were unable to assess whether RFs worsened or exacerbated existing comorbidities or had any effect on the severity of accompanying injuries. On the other hand, Jones et al.[16] identified five and more RFs as independent causes of mortality in a total of 98,836 patients. Mortality and morbidity are reduced with the control of pain in chest traumas, particularly in RFs and reduced by the provision of pain and bone stability.[12]

The biological effects of the US were first noticed in 1917.[9] It is used for the treatment of pain, traumatic musculoskeletal traumas, and soft tissue lesions. The US, which is acoustic radiation, is a form of mechanical energy which can be transmitted into the body as a high-frequency pressure wave.[21] This energy can be divided into two categories: high-intensity (5,000 to 15,000 W/cm^2) and low-intensity (0.5 to 3,000 mW/cm^2). Low-intensity US is used in physiotherapy and have micro-massage effect on the interstitial fluid movement in tissues. This effect is utilized in edematous tissues, and wound healing is accelerated. Intracellular calcium concentration increases the cell membrane permeability, mast cell degranulation, chemotactic factor and histamine release, macrophage response, and protein synthesis from fibroblasts. Ultrasound is known as a modality which accelerates tissue healing, as cellular events are indispensable components of tissue healing.[22,23] The healing effect of US on bones and soft tissue is well-known and widely used.[24]

The factors which increase bone healing have been extensively researched in the literature in the last six decades. It has been shown extensively in animal experiments that US which is widely used in diagnosis and treatment augments osteogenesis. In these experiments, extreme fractures such as radius and femur bones have been studied.[13,18,21,22,24] In the literature, there is only one study investigating the treatment of RFs with US.[17] In this study, the subjects were divided into three groups of 10% (50 mW/cm^2), 20% (100 mW/cm^2), and 50% (250 mW/cm^2) and LIPUS was applied for three minutes daily for 28 days. Finally, 50 mW/cm^2 was determined as the most effective dose available. In our study, we determined the application period as six min which was 15 to 20 min in the literature for lower extremity fractures. As we aimed to investigate the effects of LIPUS on RFs in the longer application, daily application time was six min,

Table 1. A comparison of parameters analyzed among the rat groups

	Week 2						Week 4						Week 6						
	Group 1		Group 2		Group 3		Group 1		Group 2		Group 3		Group 1		Group 2		Group 3		
	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	*p*
Osteoblast							0	0-16.0	18	0-26.5	8	0-31.5	22	16-26	26	23.5-31.5	29	25.5-32.5	1.0
Osteoclast							0	0-1.0	0.5	0-1.7	0.5	0-2.0	1.0	1.0-2.0	1.5	1.0-2.2	1.0	0.5-2.5	1.0
New bone formation							0	0-4621.3	6788.5	0-11077.3	8291.2	0-19203.5	13554.8	8829.6-15412.3	18504	16237.7-21347.7	20333.2	18434.3-21887.4	1.0
Lymphocyte	15.0	13.0-21.5	22.0	20.0-23.0	22.0	21.0-24.0	10.0	8.0-11.0	12.0	11.3-13.8	13.5	11.5-15.3	8.0	6.0-9.0	8.0	6.8-9.0	7.0	5.0-8.5	0.128

Min: Minimum; Max: Maximum; Based on Mann-Whitney U test.

rather than three min and the total period was six weeks, rather than four weeks in our study. In the first two weeks, no osteoblast, osteoclast, and new bone formation were detected. However, at four weeks, osteoblast, osteoclast, and new bone formation were detected, and bone formation was more evident in the LIPUS-treated groups. The results were similar at six weeks (Table 1). We also found that osteoblasts were similarly affected by new bone formation; however, at four weeks, the osteoblast count was 20% (100 mW/cm^2) higher in the LIPUS group. This finding is consistent with the findings of Santana-Rodríguez et al.[18]

Nonetheless, there are some limitations to this study. First, we studied only major ribs or bones, although soft tissue is worth investigating in further studies. Second, we found no significant difference among the groups in terms of the osteoblast, osteoclast, new bone formation, and lymphocyte infiltration. Third, as an experimental animal study, our study might have yielded different results in human tissues. Therefore, these findings should be clarified with further experimental and clinical studies. Finally, further large-scale, long-term studies are needed to gain a better understanding of the effects of LIPUS on RF healing.

In conclusion, rib fractures are painful and can be potentially disabling. This may result in social and economic costs, both to the national healthcare system and to individuals in the form of lost productivity and impaired quality of life. Any improvements of its treatment would have evenly great benefit on not only on individual, but also on society. Clinical and experimental investigations have demonstrated that low-intensity pulse ultrasound is effective for bone metabolism and new bone development. Histological examination has shown that osteoblast, osteoclast, chondrocyte, and mesenchymal stem cells are responsible for new bone development and healing. In accordance with the literature, although there was no significant difference among the groups in terms of osteoblast, osteoclast, new bone formation, and lymphocyte infiltration, histological consolidation was significantly increased and lymphocyte infiltration decreased in the rat groups treated with low-intensity pulse ultrasound. This effect was more prominent in the long-term and at higher doses with increased daily and total administration time. We, therefore, believe that accelerating the natural healing process in patients with rib fractures would enable to treat more effectively in short-term.

Declaration of conflicting interests

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