한국전문물리치료학회지 제6권 제4호
KAUTPT Vol. 6 No. 4 1999.

# Effect of Low-Intensity Ultrasound on Bone Repair in Rat Model

Yi Chung-hwi, Ph.D., P.T.
Dept. of Rehabilitation Therapy, College of Health Science, Yonsei University Institute of Health Science, Yonsei University
Kim Jong-man, MPH, P.T., O.T.
Dept. of Physical Therapy, Division of Health, Seonam University
Hwang Tae-sun, M.D., Ph.D.
Dept. of Anatomy, College of Medicine, Pochon CHA University

## 국문요약

### 토끼모델에서 저강도 초음파치료의 골절치유 효과

**이충휘**
연세대학교 보건과학대학 재활학과 및 보건과학연구소
**김종만**
서남대학교 물리치료학과
**황태선**
포천중문의과대학 해부학 교실

이 연구에서는 24마리의 Japanese White 토끼를 대상으로 양쪽 비골을 인위적으로 골절시킨 후 초음파치료가 골절의 치유에 효과가 있는지를 알아보았다. 초음파 치료 후 대조군의 비골과 실험군의 비골에서의 골소주 비율은 차이가 없었으며 초음파 주파수를 0.875 ㎒로 하였을 때와 3 ㎒로 하였을 때의 골소주 비율도 차이가 없었다. 따라서 초음파 치료는 토끼의 비골 골절의 치유 효과가 없었다. 그러나 다양한 주파수와 초음파 전달양식을 변화시켰을 때 골절치유 효과가 있는지에 대해서는 지속적인 연구가 필요하다.

**핵심단어:** 저강도 초음파; 골절치유.

## Introduction

This study was designed to determine the effect of ultrasound on bone repair. There have been conflicting reports on the use of ultrasound at fracture site, but ultrasound does not interfere with the healing process or with callus formation, except perhaps in the very early stages when the blood clot proceeds to form granulation tissue. But ultrasound may interfere with the healing process, particularly if high doses are given

(Wadsworth and Chanmugam, 1983). When the fracture site is exposed to ultrasound, the possibility of disruption of the osteogenic process in healing fractures suggests that extreme caution in use of ultrasound in and around recent fracture sites is necessary (Baldes et al, 1958). Especially if the treatment is applied on the epiphyseal plate, special care or precautions are required to avoid the accumulation of ultrasound energy. Some practioners, however, claim that ultrasound is a stimulant to osteogenesis (Nyborg and Ziskin, 1985).

However, as the ultrasound treatment units have been developed and improved, new, different functions are introduced by their manufacturers. These machines have different frequencies, intensities, and transmission mode. So physical therapists can expect that various treatment effects can be obtained by varying these various parameters.

Clinicians should study the professional literature pertaining to this subject carefully before making a decision regarding applications of ultrasound in and around healing fractures (Kahn, 1994). Therefore, the purposes of this study were to determine whether the application of ultrasound has an effect on bone repair and to compare bone healing effect by two ultrasound frequencies in Japanese White rabbits.

## Methods

### Animal Model and Procedure

Twenty-four skeletally mature male Japanese White rabbits weighing 2.37±31 kg were used. All rabbits were individually separated and fed standard rabbit chow throughout the experiment. Anesthesia was achieved by ketamine hydrochloride (6 ㎎/㎏) and 0.1 mg of atropine intraperitoneally. After preparation, the hindlimb placed in the flexed position. A 3 cm linear incision was made on the lateral aspect of the limb and the fibular osteotomy was performed. A small pair of scissors was used to make an experimental fracture in the middle of the fibula bilaterally. And then we did ultrasound treatment by the protocol. Bilateral closed fibular fractures were made in skeletally mature male Japanese White rabbits. The twenty-four rabbits were randomly divided into 2 groups: experimental group 1 (n=12), and experimental group 2 (n=12). Experimental group 1 received 0.875 ㎒ continuous ultrasound (Fig. 1) and experimental group 2 was treated with 3 ㎒ continuous ultrasound (Fig. 2). Aquasonic gel was used as a coupling medium. The ultrasound intensity was 50 ㎽/㎠ and treatment time was 10 minutes for every session in both groups. In each abbit, one fibula served as a control and the other was subjected to ultrasound treatment 5 times per week for 3 weeks. Sham ultrasound (control) hindlimb selection was done by flipping a coin. After three weeks, rabbits were sacrificed and the ratios of the area between the trabeculae and bone marrow of the fibulae were calculated. All rabbits were killed by injection with air in the veins of their ears and their fibulae were obtained. After fixation with 4% paraformaldehyde (in 0.1M phosphate buffer, pH 7.4, 4℃) for 4 days, the fibulae were decalcified with a decalcification solution over 7 days. After complete decalcification, they were incubated in 5% sodium sulfate solution overnight washed with running water, dehydrated with graded alcohol, embedded in paraffin and then cut serially in sections of 5 ㎛ thickness. Every 20th section was stained with hematoxylin-eosin dye. We were able to obtain about 5 slide sections per specimen.

### Measurement

Using a microscope with a camera (Nikon optiphot), the photo of each stained section was taken (×100). The mid-portion of the newly formed callus in the bone marrow was photographed (Fig. 3). Tracing paper was placed on the photograph and the margins of the trabecula were traced with a 0.1 ㎜ thickness pen (Fig. 4). Using a scanner (Scanjet 3c, Hewlett-Packard) and Photoshop 3.0 program, these drawings were input in our computer as the BMP files. These files were then opened in image-analyzing program (KS 400, Kontron Electronik) and the ratios of the area between trabeculae and bone marrow were calculated (Fig. 5). In fig. 5, black areas shows trabecular, and white areas shows bone marrow. To calculate the ratios of the area between the fibular trabeculae and bone marrow, we used this formula. Black area was divided by black plus white, and then multiplied by 100.

### Statistical Analysis

One-sample Kolmogorov-Sminorv test was used to determine whether the distribution of dependent variables fit the normal distribution or not. Statistical analysis was done using paired t-test and independent t-test. A confidence level of 95% ($p<.05$) was chosen for significance.

## Results

At the end of the experimental period, 12 of the 24 rabbits were excluded due to complications from surgery or inadequate fracture status for this study. Two more cases were excluded due to inappropriate handling for the decalcification and image analyzing procedure. During the procedure, even if only one hind limb of the animal had a problem, we had to exclude the case. The Table 1 shows distribution of subjects by frequency and treated side.

**Table 1.** Distribution of subjects by ultrasound frequency and treated side

| Frequency (㎒) | Treated side | | Total |
|---|---|---|---|
| | Left | Right | |
| 0.875 | 2 | 3 | 5 |
| 3 | 3 | 4 | 7 |
| Total | 5 | 7 | 12 |

The Table 2 shows the ratios of the are a between the trabeculae and bone marrow of the fibulae. In the treated limb, the mean of the ratios of the area between the trabeculae and bone marrow of the fibulae was 56.68±0.08. And In the untreated limb, the mean of the ratios of the area between the trabeculae and bone marrow of the fibulae was 52.83±6.68. We applied the paired t-test and the there is no significant difference between treated limb and untreated limb.

Table 3 shows the ratios of the area between fibular trabeculae and bone marrow by frequency. In the group treated with 0.875 ㎒ ultrasound, the mean of the ratios of the area between the trabeculae and bone marrow of the fibulae was 57.96±8.21. In the group treated with 3 ㎒ ultrasound, the mean of the ratios of the area between the trabeculae and bone marrow of the fibulae was 55.40±6.63. We applied the independent t-test and the there was no significant statistical difference in the ratios of the area between the trabeculae and bone marrow of the fibulae by frequency.

**Table 2.** The ratios of the area between the trabeculae and bone marrow of the fibulae

| | Mean±SD | t-Value | p |
|---|---|---|---|
| Treated limb ($n_1$=10) | 56.68±0.06 | 1.573 | .150 |
| Untreated limb ($n_2$=10) | 52.83±6.68 | | |

**Table 3.** The ratios of the area between fibular trabeculae and bone marrow by frequency

| Frequency (㎒) | Mean±SD | t-Value | p |
|---|---|---|---|
| 0.875 ($n_1$=5) | 57.96±8.21 | .478 | .645 |
| 3 ($n_2$=5) | 55.40±6.63 | | |

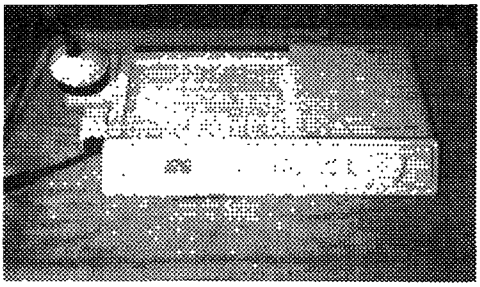

Fig. 1. Ultrasound unit
(0.875 ㎒, ERA: 1 ㎠)

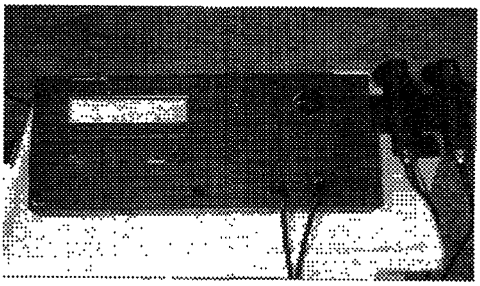

Fig. 2. Ultrasound unit
(3 ㎒, ERA: 1 ㎠)

Fig. 3. Light micrograph of hematoxylin - eosin showing the distribution of bony trabeculae (in the control)×100

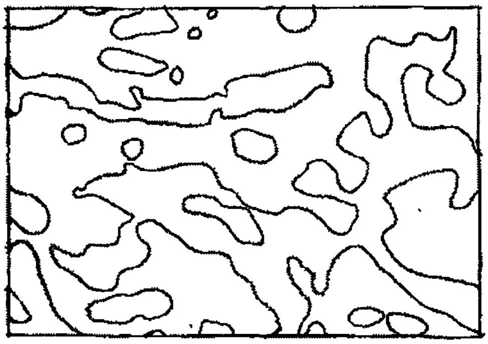

Fig. 4. Tracing

Fig. 5. Black and white contrast coloring of trabecular area. Calculating formula of the area is ([black/(black +white)]×100).

## Discussion

Ultrasound has been recently recognized of its potential in promoting fracture healing. However the use of ultrasound in the acceleration of fracture healing has been controversial and its effects on bone healing remain unclarified. Many factors are involved in bone fracture healing. The present study was initiated to determine the effort of ultrasound stimulation on bone repair and to compare the effects of frequency.

Careful investigations of the action of ultrasonic energy on bone showed that if therapeutic dosage is applied, no detrimental effects are observed in either growing or adult bone (Janes et al, 1960). In animal experiments, Corradi and Cozzolino (1953) found that healing of experimental fractures in rabbits was accelerated on the treated side compared with that of the untreated side. Intensities of 1.5 watts per ㎠ were applied daily for five minutes over a period of 15 days. And Tsai and associates (1992) found that ultrasound at 0.5 W/㎠ were stimulatory to fracture repair, if given for 15 min/day, and were recommended for future clinical trials. However, ultrasounds at the intensity of 1.0 W/㎠ were deleterious to the treated fracture and not recommended for clinical trials. Maintz (1950), on the other hand, found no improvement of callus formation as a result of ultrasound treatment. Hippe and Uhlmann (1959) clinically described 181 cases of delayed fracture healing that they treated with 15 watts and intensities of 1.5 watts per ㎠ for five minutes every second to every third day, and observed improvement. On the other hand, whereas improved healing of fractures may be explained by a moderate increase in temperature, pathological fractures may be due to excessive heating. The length of time of application may also have an influence. In addition, Cochran and co-workers (1985) showed experimental evidence that the application of ultrasound can produce a piezoelectric effect in bone that, in turn, may produce osteogenesis and an increased rate of fracture healing, which is especially useful in delayed union. Excessive dosage led to pathological fracture (Ardan et al, 1954).

The data in Tables 1 and 2 show that There was no statistically significant difference in the trabeculae area between experimental leg and control leg in experimental group 1 and experimental group 2 ($p>.05$). And there was also no statistically significant difference between experimental group 1 and experimental group 2 according to ultrasound treatment frequencies, 0.875 ㎒ and 3 ㎒ ($p>.05$). These data suggest that in Japanese White rabbits, low intensity ultrasound stimulation does not facilitate fracture repair nor is there any difference in fracture repair results between ultrasound frequencies, 0.875 ㎒ and 3 ㎒. Even though we failed to prove the effect of low intensity ultrasound to stimulate bone healing in this study, there are a lot of possibilities that many stimulation parameters for facilitation of healing appear to vary between tissues with different cellular compositions. Therefore more research is needed to identify the specific ultrasound parameters to accelerate fracture healing.

## References

Ardan NI, Janes JM, Herrick JF. Ultrasonic energy and surgically produced defects in bone. J Bone Joint Surg. 1957;39A: 394-402.

Baldes EJ, Herrick JF, Stroebel CF. Biologic effects of US. Am J Phys Med. 1958; 37:111.

Cochran GVB, Johnson MW, Kadaba MP, et al. Piezoelectric internal fixation devices: A new approach to electrical augmentation of osteogenesis. J Orth Res. 1985;3:508-513.

Corradi C, Cozzolino A. Gli ultrasuoni (U.S.) e l'evoluzione del callo osseo nei focolai di frattura. Arch Orthop. 1953;66:77-98.

Hippe H, Uhlmann J. Die Anwendung des Ultraschalls bei schlecht heilenden Fracturen. Zentralblatt Chirurg. 1959;28:1105

-1110.

Janes JM, Herrick JF, Kelly PJ, Peterson LFA. Long-term effect of ultrasonic energy on femora of the dog. Proc. Staff Meet. Mayo Clin. 1960;35:663-671.

Kahn J. Principles and Practice of Electrotherapy. 3rd ed., New York, Churchill Livingstone, 1994:57.

Maintz G. Tierexperimentelle Untersuchungen über die Wirkung der Ultraschallwellen auf die Knochenregeneration. Strahlentherapie 1950;82:631-638.

Nyborg WL, Ziskin MC. Biological Effects of Ultrasound. New York, Churchill Livingstone, 1985:9.

Tsai CL, Chang WH, Liu TK. Preliminary studies of duration and intensity of ultrasonic treatments on fracture repair. Chin J Physiol. 1992;35(2):168.

Wadsworth H, Chanmugam APP. Electrophysical Agents in Physiotherapy. Science Press, 1983:108.