In-vivo Effects of Low Intensity Ultrasound Stimulation for Prevention of Osteoporotic Bone Fracture

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Introduction

Ultrasound (US) is a form of mechanical energy that is transmitted through and into biological tissues as acoustical pressure waves. The acoustical pressure waves generally generate micromechanical strains of the bone, resulting in promotion of bone formation activation. Based on the fact, a low intensity US has been recently used to treat effectively bone fractures by increasing artificially bone formation activation. It has been, however, still controversial whether or not the low intensity US is effective for prevention of osteoporotic bone fracture. The current study is, therefore, performed to identify quantitatively if the low intensity US stimulation is effective on prevention of osteoporotic bone fracture.

Materials and Methods

Eight virgin ICR mice (14-week-old, approximate weight 25g) were housed in individually ventilated cages. All mice were ovariectomized (OVX) to induce osteoporosis and a degree of occurrence of the osteoporosis for each mouse was confirmed morphologically through in-vivo micro-CT images with at a resolution of 18μm. One right hindlimb for each mouse was then stimulated with the low-intensity US (assigned to US Group), but the left hindlimb was not stimulated and served as an internal control (assigned to CON Group) (Fig. 1A). Here, US stimulation was applied to the proximal tibia with following parameters: 1.0 kHz frequency, 30mW/cm^2 intensity, 200μs pulse width, and stimulation for 20 minutes/day and 5 days/week over a 6-week period.

Trabecular bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), trabecular number (Tb.N, 1/mm), trabecualr bone pattern factor (Tb.Pf, 1/mm) and structure model index (SMI) were analyzed to identify variations of the morphological characteristics for the entire trabecular bone of the tibia due to the application of the US stimulation, based on in-vivo micro-CT images acquired at both 0 week (before stimulation) and 6th week (after stimulation). BV/TV was primary analyzed to identify detailed variations of the morphological characteristics for the five regions of interest (ROIs) of the trabecular bone. Here, the ROIs were selected at 0.54mm under growth plate (Fig 1B) and the morphological characteristics were calculated by CT-AN 1.8.1.2 (Skyscan 1076, Belgium).

A paired wilcoxon signed rank test was carried out to identify the differences of the morphological characteristics between CON and US Groups. Here, the significance level for the wilcoxon signed rank test (p) was set at 0.05.

All procedures for specimen preparation were in accordance with the approved National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals under a protocol approved by the Yonsei University School of Animal Care and Ethics Committee.

Results

The relative variations of BV/TV and Tb.N in US Group resulted from the application of the US stimulation were significantly bigger than those in CON Group, while the relative variation of Tb.Pf in US Group was significantly smaller than that in CON Group ($p<0.05$, Fig. 2). The relative variations of other morphological characteristics in US Group were not significantly occurred compared with those in CON Group ($p>0.05$).

The relative variation of BV/TV at the R5 (ROI 5) in US Group was significantly bigger than that in CON Group ($p<0.05$, Fig. 3), while the relative variations BV/TV at others ROIs (R1, R2, R3 and R4) in US Group were not significantly occurred compared with those in CON Group ($p>0.05$).

All results for the alterations of the morphological characteristics on the entire region and each ROI are not depicted here due to page limitations.

Discussions and Conclusions

In the current study, positive effects of US stimulation on the osteoporotic bone were identified. The results of the morphological characteristics for the entire trabeular bone region indicated that losses of bone quantity were suppressed and the trabecular connections were kept. In addition, the results of BV/TV for the ROIs showed that the losses of BV/TV at the R5, which was stimulated directly by US (Fig. 1B), were suppressed. These results may indicate that the low intensity US may prevent effectively the osteoporotic bone fracture. However, the findings and the results in the current study require further confirmation using concepts incorporated in micro-mechanics and molecular biology. We are, therefore, currently investigating those in an ongoing study, the results of which may increase the confidence levels of the results presented here.

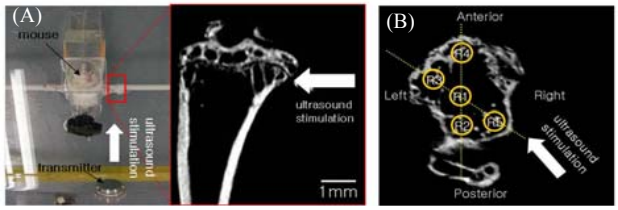

Fig 1. (A) Ultrasound stimulation and its site, (B) Five regions of interests (ROIs) for evaluation of effects of ultrasound; R1: ROI 1, R2: ROI 2, R3: ROI 3, R4: ROI 4, R5: ROI 5

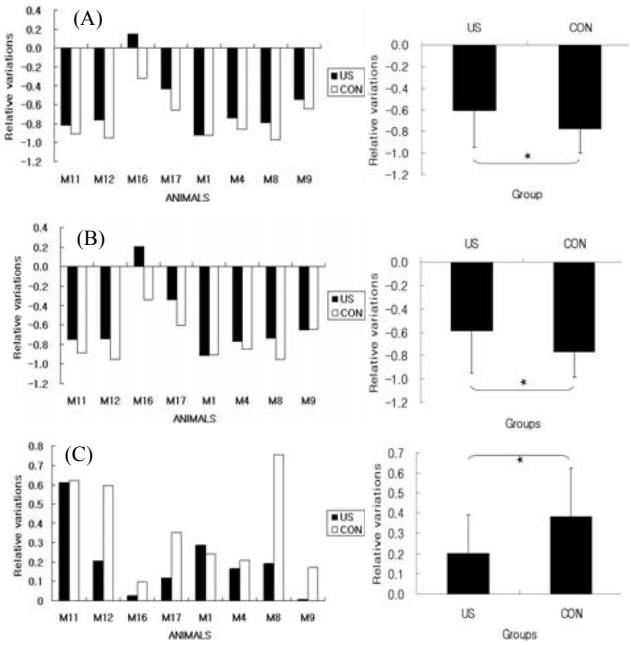

Fig 2. The relative variation of (A) BV/TV, (B) Tb.N, and (C) Tb.Pf; US: hindlimb stimulated by ultrasound, CON: hindlimb not stimulated by ultrasound, *: significant difference between US and CON Groups ($p<0.05$)

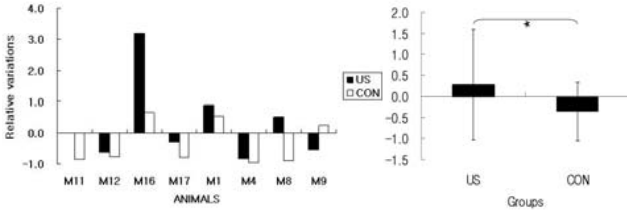

Fig 3. The relative variation of BV/TV at the ROI 5; US: hindlimb stimulated by ultrasound, CON: hindlimb at which was not stimulated by ultrasound, *: significant difference between US and CON ($p<0.05$)