Osteoporotic Bone showed Enhanced Fracture Healing Response with Low Intensity Pulsed Ultrasound
+ 1Chin, W C; 1Leung, K S; 1Qin, L; 1Cheung, W H
+ 1The Chinese University of Hong Kong, Shatin, Hong Kong, SAR, China
louis@urt.cuhk.edu.hk

Introduction:
Most fractures in elderly are fragility fractures occurring in osteoporotic bones. Delayed in treatment may cause morbidity and mortality with many complications and social burdens. Our previous clinical study had shown that the use of low intensity pulsed ultrasound (LIPUS) could provide a beneficial effect on complex fracture. This promising efficacy was further consolidated through in vitro findings in which LIPUS stimulated the osteogenic activity of periosteal cell. With a poorer repair potential in osteoporotic bone fracture, a similar beneficial effect of LIPUS is, therefore, expected. In this study, the effects of LIPUS on fracture healings, and most importantly on the osteoporotic condition, were investigated.

Methods:
In this study, a total of 120 female Sprague-Dawley (SD) rats, in which 60 of them were randomly chosen for the ovarietomy procedures, were used. Closed femoral fractures were performed on the left femurs. The assignment of rats was shown in the table below.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Sham</th>
<th>OVX</th>
<th>Sham</th>
<th>OVX</th>
<th>Sham</th>
<th>OVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6*</td>
<td>6*</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Fractured rats, both normal (Sham) and osteoporotic (OVX), were divided into the LIPUS treatment group (T) and the control group (C). Each group contained 6 rats. Treatment was given 20 minutes a day, 5 days a week over three different time periods of 2, 4 and 8 weeks. Radiographic analysis was performed weekly. Mechanical testing (at 4 weeks and 8 weeks groups only), micro-computed tomography and histomorphometry were performed at the end of each time point after euthanasia. For all the statistical calculations, one-way ANOVA was used.

Results:
For the radiology analyses, the callus width and the callus area of each sample was measured (Figure 1).

![Figure 1: Measurement on Callus Width (A) and callus Area (B)](image)

On callus width, significant differences were found between OVX-T and OVX-C at week 2 (p<0.001), week 3 (p=0.001) and week 4 (p=0.002). Between Sham-T and Sham-C at week 2 (p=0.0015), and between OVX-T and Sham-T at week 4 (p=0.0015). On callus area, significant differences were found between OVX-T and OVX-C at week 2 (p<0.001), week 3 (p=0.001) and week 4 (p=0.001). And between Sham-T and Sham-C at week 2 (p=0.009).

On mechanical test, the parameters of ultimate load (N), stiffness (N/mm) and energy-to-failure (J) were measured (Figure 2).

![Figure 2: The Mechanical Test analyses on Ultimate Load (A), Stiffness (B and Energy-to-Failure (C ))](image)

At week 4, under the ultimate load assessment, significantly better Sham-T (p=0.019) and OVX-T groups (p=0.015) than the Sham-C and OVX-C groups respectively were observed. A similar trend (treatment groups were better than the control groups) was observed without significant in stiffness measurement at week 4. At week 8, the stiffness value from the OVX-T was significantly higher than the OVX-C (p=0.018). On energy-to-failure, the Sham-T and OVX-T were always higher than their corresponding control groups at week 4 and week 8.

Qualitatively, both the Sham-T and OVX-T were shown to have the better healing responses than their corresponding control groups. And the OVX-T was shown to have the best. Quantitatively, on the measurement of BV/TV, overall increases in the BV/TV values among all the groups were observed. Moreover, the OVX-T was showed with the highest increment (increased by 36.8%) between week 2 and week 4 than all other groups.

For histological analysis, Safranin O / Fast Green staining was performed which indicates the presence of proteoglycan. The mean areas were measured and were shown in the table below.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Sham</th>
<th>OVX</th>
<th>Sham</th>
<th>OVX</th>
<th>Sham</th>
<th>OVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.72 ± 0.06</td>
<td>0.72 ± 0.08</td>
<td>0.72 ± 0.09</td>
<td>0.72 ± 0.11</td>
<td>0.72 ± 0.06</td>
<td>0.72 ± 0.08</td>
</tr>
</tbody>
</table>

Overall decreasing trends were observed among all the groups with the OVX-T showed the greatest decrease by 15.8% from week 2 to week 4.

Discussion:
All the results showed the stimulatory effects of LIPUS in both the normal and osteoporotic bone healings, which was in accord with our previous study on complex fracture. Based from the aspects on callus formation, mineralization and bone remodeling in this study, moreover, an even stronger enhanced healing effect was observed on the osteoporotic bone than on the normal bone. On the results related to bone formation, a significantly higher callus width measurement was found in the osteoporotic bone. The quantitative micro-CT analysis also showed the OVX-T group with the highest increment on bone formation (BV/TV). On mineralization, the qualitative micro-CT result showed that the narrowing of the fracture gap was the fastest in the OVX-T group. This similar result was also observed in histomorphometry analysis in which the OVX-T was shown to have the biggest decrease in the amount of cartilage around the fracture site from week 2 to week 4. This indicated an earlier response of endochondral ossification and resulting in a faster callus bridging process. On remodeling, although the mechanical test parameters of ultimate load and energy-to-failure did not show too much differences between the Sham-T and OVX-T, the stiffness measurement showed that at both week 4 and week 8, the OVX-T was higher than that of the Sham-T. A better remodeling process of OVX-T under the influence of LIPUS was observed.

With in-vitro study showing the use of LIPUS could accelerate the bone nodule formation, enhancing alkaline phosphatase activity and resulting in the acceleration of osteogenesis, together with another study showing the osteoporotic condition could sensitize the cortical bone to cyclic mechanical stimulation, these were concurred with the results of our study in showing osteoporotic condition resulting in a faster and better fracture healing process under the use of LIPUS. With study showing that estrogen acts as a negative modulator of the mechanotransduction process at the periosteal surface via ERβ signaling, the investigation on the response of estrogen receptor under the effect of LIPUS in the estrogen deficiency environment might provide a further insight on explaining this stronger enhanced fracture healing on osteoporotic bone than on the normal bone.

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