Stimulatory Effects of Low Magnitude High Frequency Vibration on Blood Flow and Angiogenesis at Fracture Site in Rat Model

INTRODUCTION:
A good blood supply is a prerequisite for initiating the fracture repair, and angiogenic response is reported crucial yet impaired during the osteoporotic fracture healing. Our previous studies confirmed low magnitude high frequency vibration (LMHFV) could promote both normal and osteoporotic fracture healing in rats. Therefore, we hypothesized LMHFV could enhance the blood flow of hind limb and promote the angiogenesis at fracture site in both normal and osteoporotic rats, hence to accelerate the healing process.

METHODS:
9-month-old ovariectomy-induced (OVX) and sham-ovariectomized (Sham) rats were created closed femoral fractures and were randomized into LMHFV (Sham-V, OVX-V) and control (Sham-C, OVX-C) groups (N=6). At weeks 2, 4 and 8 post fracture, pulsed-wave Doppler ultrasound was utilized to measure the blood flow velocity of fractured femoral artery. 3-dimensional (3D) high frequency power Doppler sonography was adopted for assessing the microcirculation at the fracture site. After that, the vascular system of each animal was perfused with Microfil and the fractured femur was subjected to microCT scanning for microvasculature analysis. Immunohistochemistry was performed to evaluate the vascular endothelial growth factor (VEGF) signals in bone tissues. ANOVA and Bonferonni multiple comparison test were used. Significance level was set at p<0.05.

RESULTS:
(1) Pulsed-wave Doppler showed an increasing blood flow velocity of femoral artery from weeks 2 to 8. It indicated an increasing blood flow velocity in vibration groups than control groups (Week 2: OVX-V>OVX-C, p=0.030; Week 4: Sham-V>Sham-C, p=0.020; OVX-V>OVX-C, p=0.012). A lower flow velocity was found in osteoporotic rats as compared with normal ones (Week 8: Sham-V> OVX-V, p=0.006; Sham-C>OVX-C, p=0.005). (Fig. 1)

(2) 3D high frequency power Doppler demonstrated an enhanced blood volume at fracture site by LMHFV treatment compared to the controls during the early phase of fracture healing (Week 2: Sham-V>Sham-C, p=0.021). The microvasculature of OVX group was inferior to the corresponding Sham group. (Fig. 2)

(3) Microfil perfusion & microCT analysis also confirmed increased vascular volume (VV) within callus in vibration groups (Week 2: OVX-V>OVX-C, p=0.009; Week 4: OVX-V>OVX-C, p=0.077), and an inferior level of angiogenesis was found in osteoporotic fractures as compared with normal fractures (Week 2: Sham-V> OVX-V, p=0.014; Sham-C>OVX-C, p=0.014). A higher percentage of increase in microvasculature was observed in OVX groups (Week2: 20.33%; Week 4: 19.84%) than corresponding Sham groups (Week2: 12.10%; Week 4: 2.78%). (Fig. 3) The ratio of vascular volume to total tissue volume (TV) showed a similar trend as above. (Fig. 4)

(4) Immunohistochemistry assessment also indicated a higher level of VEGF expression in vibration groups than controls in early phase of fracture healing. (Fig. 6a, 6b)

DISCUSSION:
Osteoporotic rats had suboptimal femoral blood supply than normal rats because estrogen deficiency would increase blood viscosity, thus decreased the blood flow velocity. LMHFV could reduce the peripheral resistance by widening small vessels in muscles, which resulted in an increase of blood flow velocity. Vibration also promoted angiogenesis in both normal and osteoporotic fractures. This might be vibration increased the blood flow shear forces at vascular endothelium, which augmented the functions of VEGF by up-regulating VEGFR-2. The percentage of increase in angiogenesis by LMHFV in osteoporotic groups was higher than normal ones, which suggested osteoporotic rats might have higher sensitivity of angiogenic response to mechanical stimulation. In conclusion, LMHFV can enhance blood flow of hind limb and angiogenesis at fracture site with different extent in normal and osteoporotic rats, which indicates the promotion of both systemic and local blood circulation is one of the mechanisms for LMHFV to accelerate the fracture healing.

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REFERENCES: