Dynamic assessment of oxytocin in the inhibition of deleterious osteoporosis in a rabbit model using MRS and micro-CT

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Aims and objectives

Osteoporosis (OP) is defined as a systemic skeletal disease characterized by a impairment of bone mass and microarchitecture, which increases the propensity of fragility fracture[1]. With the extension of average life expectancy and the arrival of aging society, osteoporosis in postmenopausal women has caused extensive concern of the whole society[2]. Therefore, early diagnosis and treatment of OP is becoming a hotspot for researchers. In recent years, numerous studies have indicated that the imbalance between osteoblastogenesis and adipogenesis from the mesenchymal stem cells (MSC) is one of the mechanisms leading to OP[3, 4]. In postmenopausal women, which are most likely suffered from OP, a significant decrease in circulating oxytocin was observed and OP remained significantly correlated to oxytocin(OT), regardless of age[5]. Many researches indicate that OT has positive effect on the differentiation from MSC to osteoblasts[6-8], but so far, its in vivo effects on osteogenesis/adipogenesis and that could be envisioned as a therapy for OP remain largely unknown. Moreover, the dynamic evaluation of OT function with medical imaging has rarely been studied. Our previous study[10] found that MRS can quantitatively analyse metabolism of fat and water in bone marrow of OP, providing a platform for studying its patho-physiological changes on the molecular level. Thus it was decided to dynamically evaluate variation pattern of bone quality in OP rabbit model treated by OT with both MRS and Micro-CT by comparing histopathological results of bone marrow.
Methods and materials

Animals and experimental design

Sixty 20-week-old (2.6±0.46 kg body weight), skeletally mature, female New Zealand white rabbits were included in the study. The experiment was approved by the hospital ethics committee and the guidelines for care and use of animals were followed. The animals were randomly divided into three groups (n=15 per group): Control group, OP model group#OP group# and OP model+OT-treated group#OT group#. The rabbits in control group were performed by sham operation. OP group underwent bilateral ovariectomy (OVX) \[^{[10, 11]}\]. OT group received OVX combined with a dose of subcutaneously injection of OT(1 mg/kg) \[^{[7]}\]. The rabbits underwent MRS examination of the left proximal femur at week 0, 3, 6, 10, 14 after operation. Then they were killed by intravenous administration of over-dose sodium pentobarbital (50 mg/kg) at each time point. After death, the left proximal femur were dissected for Micro-CT and histopathological examination.

MRS examination

MRS was performed on a 3.0T unit (Magneton Verio, Siemens, Germany) with a gradient strength of 40mT/m and a gradient slew rate of 200mT/ms. The body coil was used for transmitting radio frequency power, and a quadrature knee array coil was used for signal reception. MRS without water suppression was conducted using a single-voxel point-resolved spectroscopy (PRESS) sequence with the following parameters: TR/TE2000/30ms, voxel size 5 mm(AP)×6 mm(RL)×17 mm(FH) (the largest volume of left proximal femur); spectral width, 1200 Hz; excitation times,64; and number of signals averaged, 1. Field homogeneity was adjusted through an automatic procedure of localized 3D-shimming.

A commercially available imaging workstation was used for post-processing of MRS data. Spectral assignments were based on previous studies\[^{[12]}\]. Bone marrow fat content of L5 vertebra was expressed as the percentage of the fat signal intensity in respect to total signal intensity (fat fraction: FF) by formula: FF%=[I_{lipid}/(I_{lipid}+I_{water})]×100%, where I_{lipid} and I_{water} were referred to the peak amplitude of lipid and water, respectively.

Micro-CT examination

The left proximal femur was thawed completely and then put longitudinally into a sample holder for a desktop Micro-CT (Explore Locus SP, GE Health Care, Milwaukee, USA).
From the stack of cross-section images, a volume of interest (VOI) containing only cancellous bone of the left proximal femur was extracted for morphometric analysis (MicroView2.0 Advance Bone Analysis software) to calculate volumetric bone mineral density (vBMD)\textsuperscript{[13]}.

Histopathological examination

Sections of left proximal femur were stained with Oil Red O for marrow adipocytes measurement. Marrow adipocytes were quantitatively measured with a Leica Q-win Plus image analysis system according to the method as described previously\textsuperscript{[10,12]}. The bone marrow adipocyte density was determined as the number of fat cells per unit bone marrow area excluding the bone trabecula (N/mm\textsuperscript{2}), and the percentage adipocyte volume per marrow volume (Ad.V/Ma.V, %) were calculated.

Statistical analysis

All data were processed with the statistical system SPSS 17.0. Shapiro-Wilk test was performed to test the normality distribution of the data. Differences of FF, Micro-CT structural parameters, and bone marrow adipocyte number, the percentage of adipocyte volume per marrow volume among three groups were analyzed using two-way ANOVA followed by Bonferroni post-hoc test which was used to determine differences among groups at all time points.
Results

Bone marrow FF measurements

Calculated FF results of proximal femur at each time point after operation are showed in Table 1. FF values in control group displayed a gradual increase over time, but no significant differences among different time points were found (P>0.05 for all). Conversely, FF in OP group exhibited a progressive increasing trend and presented a significant differences when the data at week 6 after OVX were compared to that at baseline time (P=0.012). The difference in FF between control group and OP group was found significant from sixth week on after OVX (P<0.05 for all). FF values in OT group also exhibited a steadily and progressively increasing trend as those in OP group. However, FF in OT group was significantly lower than those in OP group(P<0.05) but higher than those in control group (P<0.05) at each time point from sixth week on after OVX Fig.1. The $^{1}$H-MRS spectra over time in three groups are demonstrated in Fig. 3.

<table>
<thead>
<tr>
<th>Table 1. Comparison of FF(%) values of proximal femur at each time point after operation in three groups</th>
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<td>Groups</td>
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<tr>
<td></td>
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<tr>
<td>Control</td>
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<tr>
<td>OP group</td>
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<td>OT group</td>
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</table>

FF, marrow fat fraction; Values are expressed as mean±SD.

*P<0.05, when compared with control group. #P<0.05 when compared with OP group.

Bone vBMD measurements

Changes in vBMD of left proximal femur calculated by Micro CT in three groups of rabbits are summarized in Table 2. The control group displayed a slight decrease trend in vBMD with no significant difference (P>0.05). OP group has shown a dramatic reduction in vBMD from week 6 after OVX when the data were compared with that at baseline time
with a significant difference (P<0.05). The difference in vBMD between control group and OP group was observed at each time point from sixth week on after OVX (P<0.05 for all). The changing trend in vBMD of proximal femur in OT group were similar to those in control group. vBMD values in OT group are signficantly higher than those in OP group from week 6 until week 14 after OVX (P<0.05 for all) Fig.2. Significant difference of vBMD was gotten between OT and control groups until week 14 after operation (P<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>baseline</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 10</th>
<th>Week 14</th>
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<tr>
<td>vBMD</td>
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<tr>
<td>Control</td>
<td>102.73±12.75</td>
<td>91.52±12.85</td>
<td>78.05±13.22</td>
<td>65.24±8.56</td>
<td>59.11±12.35</td>
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<td>OP</td>
<td>98.57±14.99</td>
<td>93.68±8.74</td>
<td>57.18±15.82</td>
<td>35.32±8.14</td>
<td>24.47±15.76</td>
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<tr>
<td>OT</td>
<td>97.58±16.25</td>
<td>95.75±13.28</td>
<td>69.66±14.57</td>
<td>58.65±16.55</td>
<td>50.58±9.63</td>
</tr>
</tbody>
</table>

vBMD, volumetric bone mineral density; OP model, osteoporosis animal model; OT, oxytocin. Values are expressed as mean±SD.

*P<0.05, when compared with control group. #P<0.05 when compared with OP model group.

Bone marrow adipocytes quantification

Both bone marrow adipocyte density and volume are increased with the rabbits aging after sham operation, but no significant difference was found (P>0.05 for all). Differences between control and OP group in bone marrow adipocyte density were statistically significant from 6 weeks on, whereas those in bone marrow adipocyte volume (diameter) were not until 10 weeks after OVX. Similar change was found between OP and OT groups as well.
Fig. 1: FF in OT group was significantly lower than those in OP group (P<0.05) but higher than those in control group (P<0.05) at each time point from sixth week on after OVX. Values are expressed as mean±SD.

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Fig. 2: vBMD values in OT group are significantly higher than those in OP group from week 6 until week 14 after OVX. Values are expressed as mean±SD.

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Fig. 3: 1H-MRS spectra of the left proximal femur of rabbits in the three groups. (a) Spectra of a rabbit in control group at week 14 after sham-operation. MRS shows that the water peak at 4.7 ppm is still higher than the lipid peak at 1.3 ppm. (b), (c), and (d) represent spectra of a rabbit in OP group at week 3, 6, and 14 after OVX, respectively. MRS shows that the amplitude of water peak decreases steadily while amplitude of lipid peak increases over time. At week 14, the increased lipid peak at 1.3-0.9 ppm is higher than the water peak at 4.7 ppm. (e) and (f) are the spectra of a rabbit at week 3 and 14 after OVX in OT group. They show that marrow fat increased gradually similar to (b) and (d) but the increasing amplitude of FF value was significantly weaker than those in OP group.

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Conclusion

The increased marrow fat content is synchronized with the deterioration of the bone mass in OP rabbit model. Early OT in vivo treatment to OP animal may slow down or inhibit bone degeneration reliably in relatively early stage, though the bone mass would not recover to its original levels. In addition, it may play an important role in inhibiting marrow fat accumulation by decreasing bone marrow adipocyte density and size. OT could be a highly promising drug for an alternative treatment of OP.

Nevertheless, our study was still defective in the following aspects: firstly, the rabbits included in the study are not large enough though no significant differences in body mass among them were observed. Some experimental errors may exist due to the difference in age of animals. Secondly, the sample number of animals at each time point was relatively small. Thirdly, individual difference might affect the final results as the pathological evidences were excessively emphasized at each time point. Finally, a separate OT control group was not made in our study, the interaction between OP development and OT intervention could not be excluded.
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References


