Effects of Endurance Exercise Training and Zoledronic Acid Treatment on Biomaterial Properties in Femoral of Ovariectomized Rats

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ABSTRACT

PURPOSE: To determine the effects of endurance exercise training (EXE) and zoledronic acid treatment (ZOL) on bone loss induced by ovariectomized rats. METHODS: Sixty female Wistar rats (5 weeks old) were assigned into one sham-operated group (SHAM) and four postmenopausal groups with different treatments, which were OVX, EXE, ZOL, and EXE+ZOL, respectively. A single injection of zoledronic acid (20 μg/kg body weight) was done on the ZOL and EXE+ZOL groups. For the EXE and EXE+ZOL groups, animals were trained on a treadmill for 5 min/day, 5 days/week for 10 weeks. Methods of biomechanical properties analysis were included in the current study. Statistical analysis was done using one-way ANOVA. Pair wise comparisons were used the Fisher's LSD method when statistical significance was shown (p<0.05).

RESULTS: In body weight gain, the EXE, SHAM, EXE+ZOL groups were significantly lower than the OVX and ZOL groups (p<0.05). In biomechanical properties, treatment with ZOL resulted in significant increases in fracture load in the EXE+ZOL and ZOL groups as compared to the SHAM and SHAM groups (p<0.05). A single injection of zoledronic acid (20 μg/kg body weight) resulted in significant increases in bone mineral density (BMD), cortical bone mineral density (BMC), and bone mineral content (BMC) in both the SHAM and OVX groups (p<0.05).

CONCLUSION: Exercise training and zoledronic acid treatment respectively showed protective effects on bone degradation induced by ovariectomy. The current study did not show convincible evidence regarding the combination or antagonistic effects of exercise and zoledronic acid treatment.

Introduction

Exercise as one of the most comprehensive strategies for counteracting aging related disease, is also a suggestive way for patience of osteoporosis. The effects of exercise favor the bone formation activity, which protect bone loss via promoting more bone osteoblastic activity (Li et al., 2003; Lespessailles et al., 2009).

Zoledronic acid, as one of the newest bisphosphonates, has been approved as a yearly infusion for treatment of osteoporosis which reduced number of vertebral fractures and improved bone mineral density (Frith et al., 1997; Fleisch, 2002). However, since bisphosphonate protected bone through an anti-osteoclastogenic effect, exercise will favor the bone formation activity, which protect bone loss via promoting more bone osteoblastic activity.

It would be worthy to investigate the effects as well as potential interactions of endurance training and zoledronic acid treatment on bone quality related parameters.

Methods

Animals : Sixty female Wistar rats (5 weeks-old).

Experimental design
• Groups: a sham operated group (SHAM, n=12), an ovariectomized group (OVX, n=12), a zoledronic acid (20 μg/kg body weight) treated group (ZOL, n=12), a endurance training group (EXE, n=12), and a zoledronic acid treatment combined endurance training group (ZOL+EXE, n=12).
• Experimental period were 10 weeks which was begun three days after ovariectomy.

Three days before surgery, animals of two zoledonic acid treated groups were respectively pretreated with zoledronic acid (ZOMETA®; Novartis International AG, Basel, Switzerland) via single subcutaneous (s.c.) injection.

Exercise training protocol
The training protocol for two exercise trained groups modified the protocol of previous study (Lespessailles et al., 2009). The training protocol was as followed: for 10 weeks, animals of the exercise trained groups were trained on a motorized treadmill for 5 min/day, 5 days/week at a speed of 16m/min, 60 min/day.

Biomechanical properties measurement
Three-point bending test was performed in mediolateral direction of femoral cross-section. The span of the two support points was 20mm, and the deformation rate was 1mm/min.

Histomorphometric analysis
Right tibiae were fixed with 4% neutral paraformaldehyde PBS solution (pH 7.4) for 24 hours, then dehydrated in gradient alcohol, cleared by xylene, finally, embedded in methymethacrylate (MMA). Tissue section (5 μm) was done by a motorized microtome (Microm HM 355S, Microm International GmbH - Walldorf, Germany). Sections samples were subjected to Masson’s trichrome stain. Histomorphometric parameters were measured including bone volume ratio (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, 1/mm) and trabecular separation (Tb.Sp, μm).

Statistical analysis
Data were presented as mean ± SEM. One-way (Gender x Exercise) analysis of variance (ANOVA) was used to compare the data among groups.

When significant levels (p<0.05) were revealed, pair-wise comparisons between groups were made using the Fisher’s least significant difference (LSD) method.

Results

Table 1. Biomechanical properties

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>OVX</th>
<th>EXE</th>
<th>ZOL</th>
<th>EXE+ZOL</th>
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<tbody>
<tr>
<td>YL (N)</td>
<td>36.5 ± 2.1</td>
<td>37.4 ± 3.8</td>
<td>41.0 ± 3.7</td>
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<td>38.5 ± 2.5</td>
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<td>P value</td>
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<td>0.08</td>
<td>0.03</td>
<td>0.03</td>
<td>0.09</td>
</tr>
</tbody>
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Key: YL: yield load; PL: fracture load; PF: energy; pT: post-yield energy; YS: yield stress; YE: yield energy. Mean±SEM values within a group not sharing a common superscript are significantly different (p < 0.05).

Conclusions

Endurance training as well as zoledronic acid treatment respectively showed protective effects on bone degradation induced by ovariectomy. The current study did not show convincible evidence regarding the combination or antagonistic interaction between bisphosphonate and exercise.

Figure 1: Body weights. After 12 weeks of experimental period, two exercise groups and the SHAM group showed significant lower body weight gain as compared to the OVX and ZOL groups (p<0.05). Mean±SEM values within a group not sharing a common superscript are significantly different (p < 0.05).

Figure 2: Histomorphometric analysis, BV/TV (%) values in different treatment groups showed significantly higher bone volume ratio. Tb.Th: Trabecular thickness, Tb.N: Trabecular number. Mean±SEM values within a group not sharing a common superscript are significantly different (p < 0.05).