MICRO-ARCHITECTURE AND NANO-MECHANICS OF LUMBAR VERTEBRA WITH STRONTIUM TREATMENT IN LARGE ANIMAL MODEL OF OSTEOPOROSIS

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Introduction: Strontium (Sr) is one of the most exciting concepts in field of osteoporosis treatment by concomitantly inhibiting bone resorption and enhancing bone formation. Previously, a novel Sr fortified calcium (Ca) compound was developed. We hypothesized that, with the supplementation of this compound, Ca will supply nutrition for bone while Sr will display synergistic effects for enhancing bone formation activity.

In preventing and treating osteoporosis, one of the major objectives is to improve bone mass and mechanical properties, thus reduce the risk of fractures. In this study, the micro-architecture and nano-mechanical property of lumbar vertebra were evaluated after co-administration of Sr and Ca.

Materials and Methods: 18 aged goats (range of 6–8 years) were ovarioctomized to establish an osteoporosis model. One (1) year post-ovarioctomy, animals were randomly assigned to four (4) different groups and treated as follows: control group (3 goats); normal Ca diet (Ca), normal Ca plus low Sr diet (Ca+LSr) and normal Ca plus high Sr diet (Ca+HSr) (5 goats per group) respectively for 16 weeks.

All animals were sacrificed 16 weeks after onset of treatment. Lumbar vertebra (L1-L4) were harvested from the fresh goat cadaver and freed of soft tissues. L4 was frozen at -30 centigrade for compression test and L3 was fixed, dehydrated and embedded in poly methylmethacrylate (PMMA).

PMMA embedded lumbar vertebra (L3) were cut to give 3-mm midshaft cross-section for 3D data and image acquisition with a desktop μCT system. The apparent bone mineral density (BMD) and material BMD were determined. Bone volume fraction (bone volume/total volume (BV/TV)), trabecular thickness (Tb.Th*), and separation (Tb.Sp*) were also calculated. After μCT reconstructing, the same sections were metallographically polished for nanoindentation and five different areas were selected for nanoindentation to reduce random errors. In each region, five indents were made for calculating the elastic modulus (E) and hardness (H) of nanoindentation.

Data were expressed as mean ± SD. The significance of difference was determined using one-way analysis of variance (ANOVA). Difference with p value <0.05 was considered statistically significant.

Results: A representative 3D visualization of lumbar vertebra section in control and Ca+HSr groups is shown in Fig.1. μCT of vertebra demonstrated that all treatments increased apparent BMD. Significant improvement by 8.59% was only observed between control and Ca+LSr treated groups (p=0.028). In contrast, material BMD of bone was slightly decreased in all treated groups (p=0.05). Bone content, as measured by the ratio BV/TV, was increased in all groups and significance was only observed between control and Ca+LSr groups (+12.22%, p=0.010). Trabecular thickness (Tb.Th*) was increased by 13.41% (p=0.088) in Ca alone treatment. Sr combined with Ca treatment significantly increased trabecular thickness (Tb.Th*) by 14.63% (p=0.015) and 15.85% (p=0.038), respectively. The decrease in Trabecular number (Tb.N*) was accompanied with an increase in trabecular separation (Tb.Sp*) for Ca alone treatment. In contrast, for Ca combined with Sr treatments, trabecular number (Tb.N*) increased while trabecular separation (Tb.Sp*) decreased.

The elastic modulus (E) and hardness (H) of single trabecula is shown in Table 1. The mean modulus of these four groups was comparable and no significant difference could be observed. The hardness of trabecula also decreased by 11.03% (p=0.231), 6.68% (p=0.416) and 6.38% (p=0.180) in Ca alone, Ca+LSr and Ca+HSr groups, respectively. However, neither of them caused significant changes compared with controls. Although there was a tendency for both Ca alone and Ca combined with Sr treatment to increase the elastic modulus to hardness ratio (E/H), this was not found to be statistically significant. The E/H increased by 0.86% (p=0.746) in Ca alone treatment. And in Ca+LSr and Ca+HSr groups, the E/H increased by 7.64% (p=0.378) and 6.72% (p=0.122), respectively.

From indentation data, the indentation modulus of both the treatment and control groups showed no significant difference between each other, which was consistent with Ammann P et al. Theoretically, in the bone mineral phase, Sr is mainly located in the hydrated layer, and could potentially modify the bone matrix structure by altering the hydration state of the bone tissue, to cause an overall increase in Young’s modulus of bone containing water. E/H is useful in evaluation of the overall deformation characteristics during indentation and is proportional to the fracture toughness of bone. In the treatment with Sr administration, a considerable increase in Young’s modulus of bone containing water. E/H is useful in evaluation of the overall deformation characteristics during indentation and is proportional to the fracture toughness of bone. In the treatment with Sr administration, a considerably higher E/H ratio, without statistical significance, was observed. The result might provide evidences for fracture risks reduction as observed in postmenopausal women after treatment with Sr containing drug.

Discussion: This study described the results of treatment on OVX goats with osteoporosis-like bone loss using Sr fortified Ca salt. The stimulatory effects of Sr treatment were confirmed by the micro-architecture parameters derived from μCT determination. Apparent BMD of the bones as measured by μCT increased slightly under anabolic effect of Sr. The increase in apparent BMD and BV/TV in Ca combined with Sr treatment seemed to result from increased bone formation rate while the increase in apparent BMD and BV/TV of Ca alone treatment may result from the decreased bone remodeling rate.

The reduction in fracture of vertebræ is most pronounced, among other skeletal sites, after Sr treatment in humans. Mechanical assessment of the biomechanical properties of individual trabecular would provide additional information on Sr treatment. Improvement in trabecular bone parameters, such as apparent BMD, BV/TV and Tb.Th* would be expected to increase bone strength, while assessment of the biomechanical properties of individual trabecular did not support this notion.

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Figure 1. Representative μCT image of cross-section of lumbar vertebra