Renin Inhibition Improves Ovariectomy-Induced Osteoporosis of Lumbar Vertebra in Mice

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The skeletal renin–angiotensin system (RAS) is involved in the progression of osteoporosis and the active peptide within the RAS, angiotensin II (ANG II), has deleterious effects on bones. This study was performed to investigate whether suppression of the rate-limiting step of the RAS cascade by the renin inhibitor aliskiren has a benefit on trabecular bone in osteoporotic mice. A postmenopausal osteoporosis model was induced by bilateral ovariectomy. The ovariectomized (OVX) mice were treated with a low (5 mg/kg) or high (25 mg/kg) dose of aliskiren for 6 weeks. Micro-computed tomography was performed to detect trabecular bone parameters of lumbar vertebra and to obtain 3-dimensional (3D) images. Treatment with aliskiren markedly increased bone volume over total volume (p < 0.05), trabecular bone number (p < 0.05), connectivity density (p < 0.05), and bone mineral density (p < 0.05) and reduced trabecular bone separation (p < 0.05) compared to vehicle-treated OVX mice. Similarly, the 3D images were consistent with the quantitative data that showed aliskiren could markedly reverse the ovariectomy-induced pathological changes of trabecular bone. Thus, this study indicated that the treatment of estrogen-deficient mice with aliskiren could markedly increase bone mass and improve trabecular bone structure, suggesting its potential application in treating postmenopausal osteoporosis.

Key words renin–angiotensin system; aliskiren; bone; osteoporosis; ovariectomy

The renin–angiotensin system (RAS) is a hormonal cascade that is thought to act as a master controller of blood pressure and fluid balance within the body.1 Within classical RAS, liver secreted angiotensinogen (AGT) is enzymatically cleaved to angiotensin (ANG) I by kidney-derived renin. ANG I is, hereafter, cleaved by angiotensin-converting enzyme (ACE) to the effector hormone ANG II. It is now evident that the components of RAS, in addition to the classical pathway, are expressed and act locally in multiple tissues, such as insulin secretion, glomerular sclerosis, renal inflammation, atherosclerosis, cardiac hypertrophy and brain disorders.

Recent studies showed that the components of RAS, such as renin, ACE, and ANG II receptors, are also expressed in the local milieu of bone.2,3 Functional studies revealed that ANG II could stimulate the differentiation and activity of osteoclasts in vivo and in vitro, and aggravate the loss of bone minerals in rats with osteoporosis induced by estrogen deficiency.4,5 Furthermore, the ANG II type 1 receptor knock-out mice showed high bone mass.6 In addition, we recently demonstrated that the local RAS in bone was involved in age-related osteoporosis of aging mice,7 bone deteriorations of mice with either obstructive nephropathy or type 1 diabetes,8 and others elucidated the involvement of skeletal RAS in the process of fracture healing in a mouse femur fracture model.9 Therefore, the local RAS displays important biological actions in bone tissue.

Currently, besides the applications in the prevention and treatment of anti-hypertension, inhibitors of RAS [ACE inhibitors (ACEI), ANG II receptor blockers (ARB), and renin inhibitors] are widely used in the clinic to treat tissue injury due to locally high RAS activity, such as renal and cardiovascular disease.10 The experimental studies have showed the beneficial effects of ACEI and ARB on maintaining bone health of ovariectomized (OVX) rats11,18,19 and mice,10,20 an animal model mimicking postmenopausal osteoporosis due to the decline of circulating estrogen level. While, given renin is the rate-limiting enzyme of the RAS, whether the RAS inhibition by inhibiting renin activity could show beneficial effects on postmenopausal osteoporosis is not known.

Postmenopausal osteoporosis is the most common type of osteoporosis that contributes to morbidity and mortality in millions of menopausal women worldwide, and the major clinical consequences of this disease are osteoporotic fractures of the upper extremity, spine, and hip. Thus, we recently performed an animal study to address the effects of renin inhibitor, aliskiren, on spine of mice with postmenopausal osteoporosis induced by ovariectomy. The aim of the present study is to elucidate the impact of renin inhibitor on trabecular bone of osteoporotic mice.

MATERIALS AND METHODS

Animal Study Design Eight-week-old female C57BL/6J mice (Slac Laboratory Animal, Shanghai, China) were allowed to acclimate to their environment for 1 week before surgery, during which the mice were either dorsal OVX or sham-operated (Sham) under anesthetization with a mixture of ketamine–xylazine (80:10 mg/kg). Starting from 1 week post-surgery, the mice were divided into four groups: Sham-operated mice (Sham, n = 6), OVX mice with vehicle treatment (OVX, n = 6), OVX mice with orally administration of low dose of aliskiren (OVX+LA, 5 mg/kg, n = 7) and high dose of aliskiren (OVX+HA, 25 mg/kg, n = 7). After six weeks of treatment, the lumbar vertebrae were preserved for analysis on trabecular bone properties. The animal study protocol
was reviewed and approved by the institution’s Animal Ethics Committee at the University of Shanghai for Science and Technology.

**Micro-Computed Tomography (Micro-CT) Scanning**

The lumbar vertebra without sample preparation or decalcification was fixed in a cylindrical plastic tube to prevent movement of the bone during scanning. The lumbar vertebra was scanned with a high-resolution micro vivoCT 40 system (Scanco Medical, Bassersdorf, Switzerland). The parameters for each single scan were 70kVp of the X-ray and 1000 projections per 180°. Trabecular bone was determined by a fixed threshold. After images were captured (110 µA, 100 slices) were established as the volume of interest. Trabecular bone was separated from cortical bone by free drawing regions of interests using the software provided with the scanner.

**Trabecular Bone Parameters and Structural Images**

Morphologic measurements of the trabecular bone for the 100 slices were reconstructed to obtain 3-dimensional (3D) images and quantitative parameters with µCT Evaluation Program: (1) bone volume over total volume (BV/TV); (2) bone surface over bone volume (BS/BV); (3) connectivity density (Conn.D); (4) trabecular bone number (Tb.N); (5) trabecular bone thickness (Tb.Th); (6) trabecular bone separation (Tb.Sp); (7) bone mineral density over total volume (BMD/TV); (8) the geometric degree of anisotropy (DA).

**Statistical Analysis**

The data from these experiments were reported as mean±standard error of mean (S.E.M) for each group. The statistical analysis was performed using PRISM version 4.0 (GraphPad). Inter-group differences were analyzed by one-way ANOVA, and followed by Tukey’s multiple comparison test as a post test to compare the group means if overall p<0.05. The difference with p value of less than 0.05 was considered statistically significant.

**RESULTS**

**Effects of Aliskiren on Trabecular Bone of Lumbar Vertebra**

The objective of this study was to investigate the potential beneficial effects of renin inhibition on trabecular bone of mice with postmenopausal osteoporosis due to estrogen deficiency. The OVX mice were treated with renin inhibitor, aliskiren, for 6 weeks after surgery. The biological properties of trabecular bone were determined at lumbar vertebra-5 of mice by microcomputed tomography (micro-CT) and the quantitative data were shown in Table 1. Ovariectomy significantly decreased the connectivity density (Conn.D, p<0.05), trabecular bone number (Tb.N, p<0.05), and bone mineral density (BMD, p<0.05) as well as increased trabecular bone separation (Tb.Sp, p<0.05) as compared to those of Sham group. The treatment with low dose of aliskiren (5 mg/kg) could markedly increase bone volume over total volume (BV/TV, p<0.05) and Tb.N (p<0.05), and the high dose of aliskiren (25 mg/kg) could significantly increase Conn.D (p<0.05). Additionally, both low and high dose of aliskiren could dramatically reduce the Tb.Sp by about 25% (p<0.05) and elevate BMD by about 50% (p<0.05) as compared to those of vehicle-treated OVX mice.

**Effects of Aliskiren on 3D Image of Trabecular Bone of Lumbar Vertebra**

As the quantitative micro-CT data showed the protective effects of renin inhibitor, aliskiren, against the loss of bone mass and the deteriorations of trabecular bone of lumbar vertebra-5, the 3D images of this bone site were reconstructed and captured as shown in Fig. 1. The connecting rods were well maintained in the Sham group. In the OVX group, however, the structure of trabecular bone network was markedly destroyed and many of the connecting rods were missing as well as the loss of bone mass was clearly shown. Administration of either low dose or high dose of aliskiren to the OVX mice largely prevented trabecular bone loss, and the 3D micro-architecture of trabecular bone at the lumbar vertebra-5 in these mice was maintained at a level similar to that in the Sham mice. These results were well consistent with the quantitative data (Table 1) and fully convinced

![Sham](image1.png) ![OVX](image2.png) ![OVX+LA](image3.png) ![OVX+HA](image4.png)

**Fig. 1. A Representative Microcomputed Tomography 3-Dimensional Image of the Trabecular Bone Architecture of Lumbar Vertebra-5 in Sham Mice and OVX Mice Treated with Vehicle (OVX) or Low (OVX+LA, 5 mg/kg) or High (OVX+HA, 25 mg/kg) Dose of Renin Inhibitor, Aliskiren, for 6 Weeks**

|                      | Sham    | OVX     | OVX+LA  | OVX+HA  |
|----------------------|---------|---------|---------|---------|
| BV/TV                | 0.348±0.041 | 0.260±0.036 | 0.364±0.020* | 0.358±0.038 |
| BS/BV (1/mm)         | 30.1±1.4  | 33.8±2.0 | 29.0±0.9  | 31.0±1.9  |
| Conn.D (1/mm³)       | 571.8±60.0 | 351.1±60.3* | 511.8±79.8 | 562.1±68.6* |
| Tb.N (1/mm)          | 8.78±0.89 | 6.19±0.59* | 8.25±0.33* | 8.07±0.80 |
| Tb.Th (µm)           | 69.2±1.9  | 65.4±2.5  | 70.1±1.3  | 69.5±2.3  |
| Tb.Sp (µm)           | 138.9±13.6 | 194.3±16.6* | 144.1±5.9* | 148.7±8.2* |
| BMD/TV (mg HA/cm³)   | 270.0±24.9 | 186.1±21.2* | 267.2±14.6* | 280.6±28.2* |
| DA                   | 1.35±0.02 | 1.42±0.02  | 1.34±0.03  | 1.35±0.04  |

*p<0.05, vs. Sham; *p<0.05, vs. OVX. Data are expressed as mean±S.E.M. (n=6–7).
us of the potential application of renin inhibition in treating postmenopausal osteoporosis through recovering bone mass and improving trabecular bone micro-structure.

DISCUSSION

Aliskiren, the first renin inhibitor approved for clinical use, is a small molecule competitive inhibitor that specifically inhibits the enzymatic activity of renin. It could effectively suppress the rate-limiting step within RAS cascade to reduce the production of ANG II, the active peptide with multi-activities involved in tissue injuries. The recent studies have demonstrated that aliskiren is able to attenuate the progression of nephropathy and cardiovascular diseases, and improve insulin resistance in diabetic patients and animals. Given that ANG II, the central effector of the RAS, activates multiple pathways in skeleton to induce bone deteriorations, in this report we demonstrated that inhibition of renin activity with aliskiren alleviated the damages of trabecular bone in OVX mice, confirming the critical role of the renin–angiotensin cascade in the development of postmenopausal osteoporosis.

The present study showed that the treatment with renin inhibitor, aliskiren, could effectively improve ovariectomy-induced pathological changes of micro-architecture of trabecular bone at lumbar vertebra-5, including the trabecular bone number and separation, the connectivity density and mineral density. The 3D image of the trabecular bone also consistently displayed that the renin inhibition could recover the trabecular bone network as well as raise the bone mass and bone connectivity of osteoporotic mice. These results indicated the preventive effects of inhibiting renin activity on loss of bone minerals and damages of trabecular bone structure at spine bone, while, whether there are similar effects of aliskiren on trabecular bone of long bones (likeibia and femur) and cortical bone need to be further clarified.

Renin is the rate-limiting enzyme of the RAS cascade. As such, it is considered as an ideal drug target for RAS blockade. The development of direct renin inhibitors (e.g. aliskiren), however, is much slower than that of angiotensin-converting enzyme (ACE) inhibitors (ACEI) and ANG II receptor blockers (ARB). Blockade of the RAS with all these RAS inhibitors inevitably disrupts the negative feedback loop that is critical for maintaining renin homeostasis, leading to compensatory induction of renin. This is the main cause accounting for the treatment with Losartan (belongs to ARB) alone could not exert beneficial effects on diabetes-induced osteoporosis and hyperglycemia-induced renal disease in type 1 diabetic mice. In addition, the emerging clinical data indicated that the uses of ACEI did not have beneficial effects on bones, and even led to bone loss in older American men, Chinese women, and Japanese. Thus, whether aliskiren is therapeutically better than ACEI or ARB for managing bone health remains to be determined.

Taken together, the present study demonstrated that another tissue, trabecular bone, was acted by renin inhibitor aliskiren besides its widely reported targeting tissues like heart, kidney, vascular and brain. Importantly, we reported that the treatment with aliskiren to estrogen-deficient mice could markedly increase bone mass and improve trabecular bone structure, suggesting its potential application in treating postmenopausal osteoporosis which should be further explored. In addition, there are studies showing the increased blood pressure due to estrogen deficiency in OVX rats. Thus, the demonstration on whether renin inhibitor aliskiren could improve hypertension when benefits bone tissue of OVX animals has important clinical implications for postmenopausal women with bone loss, especially those with hypertension.

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