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Biological Effects of Skeletal Renin-Angiotensin System in Osteoporosis

Yan Zhang and Liang Wang

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1. Introduction

1.1. Classical renin-angiotensin system

The renin-angiotensin system (RAS) is an endocrine system that governs body fluid, electrolyte balance and blood pressure. Within the classical RAS, angiotensinogen (AGT) secreted by liver is enzymatically cleaved to angiotensin (ANG) I by kidney-derived renin. ANG I is, hereafter, cleaved by angiotensin-converting enzyme (ACE) to generate the effector hormone ANG II, which exerts various biological actions through its receptors, ANG II type 1 receptor (AT1) and ANG II type 2 receptor (AT2). The initial reaction between the enzyme renin and the substrate AGT is the rate-limiting step of the RAS [1].

As the RAS is a hormonal cascade that is thought to act as a master controller of blood pressure and fluid balance within the body [2], the systematic RAS has been an important target of antihypertensive medications. There are several groups of drugs in this category that affect different parts of the RAS axis, including ACE inhibitors (ACEI) and angiotensin receptor blockers (ARB), both of which are widely used for anti-hypertension treatment [3]. Additionally, Aliskiren, the first orally active direct renin inhibitor approved for clinical use, is a small molecule competitive inhibitor that specifically inhibits the enzymatic activity of renin [4, 5], consequently it could effectively suppress the rate-limiting step within RAS cascade to reduce the production of ANG II. The recent evidences have shown the effective blood pressure control of Aliskiren, generally well tolerated as monotherapy or in combination with other antihypertensive drugs [3, 6].

2. Tissue renin–angiotensin system

From an evolutionary point of view, it is cost-effective to have a common system to potentiate and effect the actions of the regulating hormones. Interestingly, the RAS is also found in primitive animals without a closed circulatory system, which indicates that the system is far more than a mediator of vasoconstriction [7]. It is now evident that the components of RAS, in addition to the classical pathway, are produced and acting locally in multiple tissues, a concept known as tissue RAS [7]. The local effects of tissue RAS are diverse and depend on the specific tissues involved.

The functional tissue RAS is postulated to participate in various physiological and pathological processes such as insulin secretion [8], glomerular sclerosis [9], renal inflammation [10], atherosclerosis [11], cardiac hypertrophy [12], brain ischemia [13] and follicular development and endometrial cancer in female reproductive tract [14]. A growing body of studies has demonstrated that the diabetic complications, such as cardiovascular disease [15], nephropathy [16] and retinopathy [17], are caused by the high activity of tissue RAS and the increased production of ANG II in local tissues, and the clinical practice has revealed that these pathological alterations associated with diabetes were significantly improved in response to the treatment with RAS inhibitors [15-17]. Additionally, hyperglycemia, obesity, hypertension, and cortisol, well-known risk factors of metabolic disease, are all stimulators on tissue RAS, whereas glucagon-like peptide-1, vitamin D, and aerobic exercise could, to some extent, prevent metabolic disease through inhibiting tissue RAS [7]. Thus, the factors and drugs suppressing tissue RAS activity have potential in improving RAS-involved tissue injuries.

Recent *in vivo* studies showed that the components of RAS, such as renin, ACE, and ANG II receptors, were expressed in the local milieu of bone [1, 18-20], and *in vitro* study identified the expression of ANG II receptors in primary osteoblasts derived from newborn mouse calvaria [1], indicating the components of RAS are expressed locally in bone microenvironment. Our further animal studies demonstrated that the local RAS in bone was involved in age-related osteoporosis of aging mice [21], and bone deteriorations of mice with either obstructive nephropathy [22] or type 1 diabetes [23]. Other groups elucidated the involvement of skeletal RAS in the process of fracture healing in a mouse femur fracture model [24], and the steroid-induced osteonecrosis in rabbits [20] as well as the development of postmenopausal osteoporosis in ovariectomized (OVX) animal models [25, 26] and glucocorticoid-induced osteoporosis [19]. Therefore, it concludes that the local RAS exists in bone tissue and plays an important role in local bone metabolism.

3. Action of angiotensin II on bone

ANG II has been postulated to be able to act upon the cells involved in bone metabolism through receptors located in osteoblasts and osteoclasts or regulate blood flow in bone marrow capillaries. At the end of last century, the studies showed that ANG II stimulat-

ed DNA and collagen synthesis and decreased alkaline phosphatase (ALP) activity in bone cell populations derived from calvariae of fetal rat [27] and newborn rat [28]. Similar effects of ANG II were observed in osteoblastic ROS17/2.8 cells [29] and human adult bone cells obtained by collagenase digestion from trabecular bone [27]. The clonal cell analysis, autoradiographic studies, and receptor subtype analysis suggested that ANG II might be intimately involved in the proliferation of the osteoblast-rich populations of cells through the AT1 receptor [27, 28], which also plays one of the essential roles in bone metabolism as a mechanoreceptor of osteoblasts [30].

When investigating the direct effects of ANG II on matured osteoblasts, the results revealed that ANG II inhibited the expression of mRNA for osteocalcin, which is a protein that is specifically expressed during maturation of osteoblastic cells, decreased the activity of ALP, the number and the total area of mineralized nodules as well as reduced the accumulation of calcium in cells and the matrix layer [31]. Besides, the ANG II-involved impairment of bone formation may be attributed that it altered the expression of Cbfa1 by activating the cAMP signaling pathway and subsequently reduced osteoblast number and osteoblastic function [32]. SOST, which encodes sclerostin, is a secretory product of osteocytes that counters Wnt signaling, thereby negatively regulates bone formation [33]. The AT1-involved inhibition on bone formation was highly correlated with its regulation on downstream factor SOST, as the decreased SOST expression in osteocytes was observed in AT1-deficient mice [34]. Furthermore, the treatment with ANG II strikingly increased the expressions of matrix metalloproteinase (MMP)-3 and -13 through MAPK signaling pathways via the AT1 in osteoblastic ROS17/2.8 cells, suggesting that ANG II stimulated the degradation process that occurs during extracellular matrix (ECM) turnover in osteoid by increasing the production of MMP-3 and -13 in osteoblasts [29]. Additionally, ANG II induced mitochondrial dysfunction and promoted apoptosis via JNK signaling pathway in primary mouse calvaria osteoblast [35]. Taken together, the target genes including Cbfa1, SOST, MMP-3 and MMP-13, and the signaling pathways like MAPK and JNK are involved in the mediation of ANG II on osteoblastic function and bone formation.

Osteoblast modulates osteoclast differentiation by producing both positive and negative regulators, most notably receptor activator of NF- κ B ligand (RANKL) and osteoprotegerin (OPG), respectively [36]. Of note, it has been recently found that ANG II could induce the differentiation of bone marrow mononuclear cells to multinuclear cells and the number of multinuclear cells in osteoclasts as well as increase tartrate-resistant acid phosphatase (TRAP)-positive multinuclear osteoclasts due to its stimulation on the expression of osteoclastogenesis-supporting cytokine, RANKL in osteoblasts, leading to the activation of osteoclasts [1, 25], whereas these effects were completely blocked by either ANG II type 1 receptor blockade (olmesartan) or mitogen-activated protein kinase inhibitors (U0126) [25]. Importantly, ANG II itself had no capacity to induce osteoclast differentiation and did not potentiate osteoclast formation triggered by RANKL, while it stimulated the formation of osteoclasts in the co-culture system of primary osteoblasts and bone marrow macrophages in a dose-dependent manner [1, 32]. Taken together, these results suggested that ANG II stimulates

osteoclastogenesis by acting on osteoblastic cells (i.e., 'the soil cells'), but not through a direct action on hematopoietic 'seed cells' [1].

It was found that the TRAP activity and the TRAP-positive stained area were significantly increased in the tibia of OVX rats with systemic administration of ANG II at a subpressor dose (200 ng/kg/min), and the treatment with ANG II significantly induced the ovariectomy-induced increase in urinary level of deoxypyridinoline [25]. The ratio of ALP to TRAP was significantly decreased in the tibia of OVX rats upon to ANG II treatment. These results suggested that ANG II accelerated the turnover of bone metabolism, which is similar to the typical pattern in elderly postmenopausal women who are at high risk for osteoporosis [25]. Of importance, the bone density as assessed by double energy X ray absorptiometry (DEXA) was significantly decreased in the tibia of OVX rats by ANG II. These results suggested that ANG II directly accelerated estrogen deficiency-induced osteoporosis.

4. Interaction of AT1 and AT2 on bone

Previous studies have focused on the ANG II-AT1 interactions since these are the best described and considered the most important. However, the system is complex and several other components probably play significant roles as well [7]. Several publications raise the possibility that AT1 and AT2 carry out negative cross-talk within fibroblasts and vascular endothelial cells with respect to each other's signaling pathways and responses [37]. This may be of particular importance when the AT1 are pharmacologically blocked.

Asaba et al. determined the relative contribution of the two receptors for transducing the osteoclastogenesis-supporting function of ANG II in osteoblasts by knocking down the expression of each of the receptors with siRNA in primary osteoblasts in culture [1]. In AT1-knockdown osteoblasts, the stimulatory effect of ANG II on osteoclast formation was somewhat enhanced. In AT2-knockdown osteoblasts, in contrast, the osteoclastogenic potential was markedly attenuated [1], which was consistent with that AT2 deficiency increased bone mass of distal metaphyseal regions of femoral in mice as well as the treatment with AT2 blocker PD123319 suppressed ANG II-induced increase in the number of osteoclasts in organ cultures of bone [18]. *Asaba* et al.'s study suggested that the action of ANG II on osteoblasts in terms of stimulating osteoclastogenesis was mainly mediated through the AT2 and AT1 might exert an inhibitory effect on AT2. These findings in osteoblasts are consistent with the notion that the functions of AT1 and AT2 are in many cases counter-regulatory to each other [7]. However, they are contrary to the conclusion that AT2 is the protective arm of RAS and counterbalances pathological processes and enable recovery from disease [38]. Thus, further studies are needed to dissect the signaling pathways downstream of each receptor in osteoblasts.

5. ACE inhibitors and osteoporosis

Osteoporosis, hypertension, diabetes are major chronic diseases in older subjects and the latter two are well known to be high risk factors for osteoporosis. As ACE inhibitors are usually prescribed for hypertension, cardiac failure, and diabetic nephropathy [23, 39], it is important to know the prospective effects of ACEI on bones of these patients taking ACEI treatment.

Previously, most of clinical studies demonstrated that patients treated with ACEI showed an increased bone mineral density (BMD) and a reduced fracture risk [40-45]. The menopausal and hypertensive women who followed treatment with ACEI fosinopril did not present the physiological loss of bone mass that affected to menopausal women without treatment [43]. A large case-control analysis carried out in the UK, suggested a possibly decreased fracture risk associated with longer-term use of ACE inhibitors [40], and in an open prospective study including 134 patients with low to moderate hypertension and stable BMD, the plasma calcium and 25-hydroxyvitamin D levels were both increased in patients treated with the ACEI quinapril [41]. It also significantly increased BMD of lumbar spine in female subjects with ACE DD genotype, which could induce a higher level of ANG II [41]. The research group from Hong Kong performed two large scale cohort studies which investigated the risk factors for osteoporotic fractures in Hong Kong-dwelling elderly Chinese, and their data concluded that male ACEI users had higher BMD at the total hip, female neck and lumbar spine than non-users. Likewise, female ACEI users also had higher BMD than non-users, although only significant at the femoral neck [42].

While, in the contrary to the above mentioned beneficial effects of ACEI on bone health, the recent emerging evidences indicated that ACEI use did not change the rate and risk of fracture [46], and even led to greater bone loss [39, 47, 48]. The same research group from Hong Kong recently also stated that female continuous users of ACEI had increased bone loss both in total hip and femur neck [47]. A large sample size study in American men also supported this theory by showing that ACEI use was associated with increased bone loss [39], moreover, another prospective study-a cohort study of atomic bomb survivors in Japan, demonstrated that ACEI use was associated with increased bone loss of femoral neck in older Japanese [48].

Similarly in animal studies, the use of ACEI enalapril (10-20 mg/kg, i.g.) did not show positive effects on bone function of OVX mice [49] or OVX spontaneously hypertensive rats (SHR) [50], and the administration of enalapril (0.4 mg/kg, i.p.) in a dose recommended for the treatment of hypertension did not cause significant changes in bone density, the ash and mineral content or morphometric parameters of the femur in female Wistar rats [51]. Another ACEI moexipril, when given alone at oral dose of 10 mg/kg, had no effect on the cancellous bone site in either OVX or sham-operated rats and did not hamper the osteoprotective effects of 17 β -estradiol [52]. Even though the treatment of Tsukuba hypertensive mouse with enalapril improved osteoporosis [1] as well as the OVX rats in response to the treatment with ACEI captopril (1 or 5 mg/kg) showed the increased trabecular area of lumbar vertebrae (L4) and the improved biomechanical properties by increasing L5 break stress and elastic modulus [26], our recent published article elucidated that the treatment with captopril (10 mg/kg, i.g.) significantly elevated serum level of TRAP 5b, and had a trend to decrease BMD of trabecular bone and

damage micro-architecture of proximal tibial head and distal femoral end in type 1 diabetic mice [23].

Based on the facts that ANG II locally in bone tissue has detrimental effects to bone function and ACE is the major enzyme producing ANG II, it is surprising that ACEI could not improve even accelerate bone loss in both humans and animals. Since the modest changes in ACE level affect the levels of its substrates much more than its products, indicating that relatively small changes in the levels of ACE affect kinin level more than ANG II level [53], a possible reason comes from the regulation of ACE on kinin-kallikrein system within which bradykinin can stimulate bone resorption and reduce BMD [47]. Another possible explanation we should consider is that although short-term ACEI therapy was associated with decreased ANG II level, there were some evidences that long-term ACE inhibition resulted in a return of ANG II towards baseline level, so-called 'ACE escape' [47]. The complete mechanisms have not yet been fully demonstrated. More research needs to be carried out to clarify the influence of the ACEI treatment on bone health as this might be of clinical relevance when antihypertensive therapy is initiated, particularly in hypertensive women who typically suffer from a concomitant rapid onset of osteoporosis after menopause [52].

6. Angiotensin receptor blockers and osteoporosis

The clinical profiles of users of ACEI and ARB were very similar. In the USA, ARB was usually prescribed when ACEI was not tolerated, thus explaining the smaller number of ARB users [39] and limited human studies of ARB and BMD or fracture risk in the literatures [46]. The completed studies on the affections of ARB on bone function in human and animals have shown contradictory results.

The recent population-based, retrospective cohort study with propensity score-matching using administrative databases in Ontario, Canada to examine the risk of osteoporosis-related fractures in hypertensive elderly patients treated with ARBs versus ACEIs, showed that there was no significant difference between the effects of ARBs and ACE inhibitors on hip and other osteoporotic fractures [54]. A large cohort study on Medicare beneficiaries with a diagnosis of hypertension initiating single-drug therapy for anti-hypertension treatment suggested the increasingly protective effect of ARB on relative fracture risk over time [46]. While, the study with large sample size of community-dwelling older adults from six different geographic regions demonstrated that the use of ARBs did not have any significant overall effect on bone loss in older men [39].

The contradictory results about the actions of ARBs on bone metabolism were also shown among animal studies. The treatment with telmisartan, olmesartan, and losartan, could reduce bone loss of OVX mice [49], attenuate the ovariectomy-induced decrease in BMD [25], and increase bone strength, mass and trabecular connections of OVX rats femur [55, 56], respectively. Moreover, telmisartan partially protected from thiazolidinedione-induced bone loss by actively blocking thiazolidinedione-induced anti-osteoblastic activity via maintaining PPAR γ serine 112 phosphorylation [57], and promoted fracture healing in

a mice model [58]. However, some studies reported ARBs did not cause significant changes of bone properties in normal female rats [51], type 2 diabetic mice [57], OVX rats [59] or orchiectomized rats [60]. Importantly, it was noted that in some animal models ARBs may lead to more bone injuries [1, 61]. The treatment of transgenic Tsukuba hypertensive mouse with losartan resulted in exacerbation of the low bone mass phenotype [1]. The study in our group demonstrated a trend of losartan to promote the loss of bone mass and the deteriorations of trabecular bone micro-architecture in type 1 diabetic mice due to the compensatory stimulation of bone RAS activation as shown by the up-regulation of renin and ANG II expression in bone tissue [61].

7. Perspective

It has been argued that neither ACEIs nor ARBs completely block the RAS cascade due to the disruption of the feedback inhibition of renin production [62]. The increase in renin activity stimulates the conversion of ANG I and ultimately ANG II, which largely limits the efficacy of RAS inhibition [63]. The increased renin can also act through the prorenin/renin receptor, which may cause tissue damages independent of ANG II [64]. Thus, as compared to single treatment with RAS inhibitors, whether combining renin inhibitor, like Aliskiren, with ARB or ACEI could generate better therapeutic effects on tissue injuries, such as osteoporosis, should be further clarified.

As discussed in this chapter, RAS locally plays a key role in the modulation of bone metabolism. However, over the past 10 years, several studies have presented evidences for the existence of a new arm of the RAS, namely the ACE2/ANG-(1-7)/Mas axis [65]. The identification of the ACE homolog, ACE2 as a key ANG-(1-7)-forming enzyme, unravels the existence of a distinct enzymatic pathway for the production of ANG-(1-7), which has a broad range of effects in different organs and tissues that goes beyond its initially described cardiovascular and renal actions [66]. This heptapeptide exerts its actions through binding to a G protein-coupled receptor Mas, distinct from AT1 and AT2 [67]. It is now accepted that the ACE2/ANG-(1-7)/Mas axis is able to counteract most of the deleterious actions of the ACE/ANG II/AT1 axis, especially in pathological conditions [68] such as cardiac dysfunction, increased blood pressure, decreased baroreflex function, endothelial dysfunction, reduced reproductive function, increased thrombogenesis [66]. Thus, how the cross-talk and the interaction between the dual axis systems of RAS contribute to the maintenance of bone metabolism needs to be further investigated and elucidated for better understanding the molecular mechanism of bone metabolic diseases.

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Author details

Yan Zhang^{1*} and Liang Wang²

*Address all correspondence to: medicineyan@aliyun.com

1 Center for Systems Biomedical Sciences, University of Shanghai for Science and Technology, Shanghai, China

2 Department of Orthopaedics, The 309th hospital of Chinese People's Liberation Army, Beijing, China

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