Front. Med. 2014, 8(3): 389–393
DOI 10.1007/s11684-014-0362-y

COMMENTARY

Mechanisms of "kidney governing bones" theory in traditional Chinese medicine

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Abstract **Studies conducted by our group on the mechanism of "kidney governing bones" theory in traditional Chinese medicine (TCM) are reviewed in this paper. Conclusions can be summarized as follows. (1) Neuroendocrine-immune network (NIN)-osteoclast regulatory pathway OPG-RANKL-RANK is one of the mechanisms of "kidney governing bones." Although kidney-reinforcing therapy is regarded as one of the holistic regulatory mechanisms of the body, characteristic holistic regulation in TCM can be reflected through nonselective regulation of the NIN during kidney reinforcement therapy, which can be used to treat osteoporosis through microadjustments in the microenvironment of the bone marrow. (2) Marrow exhaustion in TCM, which is the state wherein lipocytes in the bone marrow increase whereas other cells decrease, serves as the pathogenesis of osteoporosis brought about by failure of the "kidney governing bones." (3) The kidney in TCM can be regarded as a complex system comprising multiple functional units in the body, including the unit "governing bones." Kidney deficiency refers to a deficiency in only one or more units of the kidney system and not the whole system itself, which explains the kidney-reinforcing effect of many herbs; some herbs can treat osteoporosis, but some cannot. Although both classified as kidney-reinforcing agents, the former can resolve failure of the "kidney governing bones" unit while the latter regulates the failure of other units in the kidney system. Despite the current understanding on "kidney governing bones" theory, the mechanism of "kidney governing bones" remains complicated and unresolved. Thus, further studies in this area are warranted.**

Keywords kidney governing bones; kidney deficiency; marrow; osteoporosis; neuroendocrine-immune network; osteoclast regulatory pathway

Introduction

"Kidney governing bones," one of the most important theories in traditional Chinese medicine (TCM), has been used effectively as a guide in clinical practice for thousands of years. This theory introduces one of the physiological functions of the Zang viscera kidney, that is, the essential kidney qi, which promotes and regulates bone growth and development. Thus, the modern scientific mechanism of "kidney governing bones" theory has gained significant attention in medical research. For decades, researchers have conducted thorough studies on this theory, and the main achievements can be summarized into four aspects. Kidneys can govern bones by: (1) affecting vitamin D absorption; (2) converting the human growth hormone into somatomedin; (3) regulating the metabolism of calcium, phosphorus, and other trace elements; and (4) regulating the hormone secretion of the gonad, thyroid, and parathyroid glands.

Different from previous studies, the focus of our research group on this theory is the neuroendocrine-immune network (NIN) and osteoclast regulatory pathway OPG-RANKL-RANK. Given that holistic regulation is one of the key features in TCM, a systemic disease as target was first selected. In this case, osteoporosis was used to explore the effect of NIN on governing bones. Results show that the kidney can govern the bones by NIN regulation. Bone marrow microenvironment plays a key role in bone metabolism, osteoblasts, marrow stromal cells, and osteoclasts. In this microenvironment, all key cells directly participate in bone metabolism. Therefore, the effects of NIN regulation on "kidney governing bones" may also affect these cells. Given the crucial role of some cytokines, such as

Received April 29, 2014; accepted August 6, 2014
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interleukin-1 (IL-1) and interleukin-6 (IL-6), in NIN and their regulatory effect on bone metabolism, levels of IL-1 and IL-6 in the marrow microenvironment were also observed. OPG-RANKL-RANK is another important regulatory pathway for osteoclasts. IL-1 and IL-6 have indirect effects on the activity of osteoclasts via this pathway; therefore, studies on the OPG-RANKL-RANK pathway were conducted to investigate the mechanism of "kidney governing bones." Presented in this paper is a summary of our previous studies on the mechanism of "kidney governing bones," along with some further discussions.

Neuroendocrine-immune network and "kidney governing bones"

According to TCM, the kidney stores essence, governs bones, and generates marrow. Sufficient kidney essence ensures powerful bones, whereas a deficiency of kidney essence results in brittleness of the bones. Thus, the deficient or excessive status of kidney determines bone mass. This correlation has been verified by some studies using modern techniques [1–4]. A survey conducted in 13 provinces/cities in China measured the bone mineral density (BMD) of the radius, ulna, lumbar spine, and proximal femur of 36 879 people aged 2–100 years old. Results demonstrated three periods for the growth and development of bone mass: the epacme at ages 2–20 years old, the acrophase at ages 20–40 years old, and the senile decrement phase at ages beyond 40 years old; the BMDs of patients with kidney deficiencies were also lower than those in patients who did not demonstrate this pattern of bone mass growth and development [5]. Chi *et al.* tested the BMDs of 189 postmenopausal women diagnosed with kidney deficiency and observed that the BMDs of women diagnosed with kidney deficiency are significantly lower than those of healthy control groups of women younger than 45 years and those older than 45 years [6]. The correlation between kidney deficiency and low BMD values has also been observed in other studies [7]. Thus, high scores of kidney deficiency can result in greater risk of senile bone loss [8].

Different from the view of modern medicine, the kidney in TCM can be regarded as a complex system comprising multiple functional units in the body, including the partial function of modern kidney.

Long-term studies have proven that the kidney in TCM is closely associated with the endocrine system, including, at least, the functions of the hypothalamic-pituitary-gonad, hypothalamus-pituitary-thyroid, and hypothalamus-pituitary-adrenal (HPA) axes [9].

In our previous study, the mechanism of "kidney governing bones" was explored under the context of NIN using rats with osteoporosis. Kidney deficiency was induced by ovariectomy, and results showed that NIN plays a crucial role in the maintenance of normal bone metabolism. Lack of estrogen causes disorders in NIN, which can lead to osteoporosis. The mechanisms of the effect of estrogen deficiency on osteoporosis include: (1) direct reduction of bone resorption inhibition, resulting in increased bone resorption; (2) inhibition of potent bone-resorption-stimulating cytokines, which promotes IL-1 and IL-6 activity, indirectly leading to an increase in bone resorption; (3) stimulation of the HPA axis and decreases in the turnover rate of hypothalamic 5-hydroxyindoleacetic acid and 5-hydroxytryptamine, as well as concentrations of adrenocorticotropic hormone (ACTH) and β-endorphin (β-END) in the peripheral blood; these actions reduce inhibition of ACTH and CORT on IL-1 and IL-6, thereby increasing bone resorption. Decreases in CORT consequently enhance bone formation because of direct inhibition of bone formation. β-END can enhance the activity of IL-1; thus, decreases in β-END prevent the excessive increase of IL-1; (4) direct or indirect decreases in the concentration of calcitonin (CT), which directly inhibits bone resorption, resulting in increased bone resorption; and (5) changes in the concentration and activity of the hormones mentioned above, the normal balance of which is disrupted by cytokines.

A negative feedback regulation loop between IL-1, IL-6, and HPA axis occurs. Increases in IL-1 and IL-6 could enhance the function of the HPA axis, but not to normal levels, because of the absence of the synergetic effect of estrogen. In normal conditions, CT promotes the release of ACTH, β-END, and CORT. Thus, the decrease in CT results in low release of ACTH, β-END, and CORT. In summary, the lack of estrogen disturbs the NIN and changes the levels or activities of various hormones and cytokines, which directly or indirectly affect bone metabolism and promote bone resorption and formation.

Higher rates of bone resorption than bone formation leads to osteoporosis. Zuogui Pill, a classical herbal formula with kidney-reinforcing effects, is used for the prevention and treatment of osteoporosis through regulation of the disordered NIN. After the disturbed NIN has been restored, abnormal increases in bone resorption and bone formation caused by ovariectomy are significantly decreased, resulting in balance between the two processes and prevention of bone mass loss.

How can kidney reinforcement regulate the disturbed NIN of rats with osteoporosis and kidney deficiency? The possibility of increasing estrogen levels to regulate the NIN can be excluded because kidney reinforcement does not affect the level of serum estradiol (E2) in ovariectomized rats. The main mechanisms may be as follows. (1) Regulation of the HPA axis prompts the release of ACTH, CORT, and β-END. Increases in ACTH and CORT inhibit the activities of IL-1 and IL-6. Although the increase in β-END can resist the inhibitory effects of ACTH and CORT on IL-1 activity to some extent, the overall inhibitory effect of ACTH and CORT is greater than the stimulatory effect of β-END. (2) Direct or indirect stimulation thyroid C cells promotes the release of CT. Increasing levels of CT directly inhibit the activity of

osteoclasts and activate the HPA axis, which inhibits the activities of IL-1 and IL-6. (3) Inhibition of the activities of IL-1 and IL-6 directly prevents bone resorption [10–12].

Therefore, besides direct promotion of bone resorption, the lack of estrogen disturbs the NIN, resulting in changes in the levels or activities of hormones and cytokines, which can directly or indirectly affect bone metabolism. Although these changes promote both bone resorption and formation, the factors affecting bone resorption are predominant, such that higher rates of bone resorption compared with those of bone formation lead to osteoporosis. Kidney reinforcement regulates the unstable NIN to prevent and treat osteoporosis. These findings indicate that the characteristics of TCM kidney can be reflected to some extent by the some of the functions of the NIN and that kidney deficiency is one of the manifestations of instability in the functions of NIN. A stable and organized NIN in a healthy body ensures normal bone metabolism; by contrast, a body with kidney deficiency indicates instability in some of the functions of NIN, which leads to imbalances in bone resorption and bone formation and, eventually, abnormal bone metabolism. Thus, TCM kidney achieves its bone-governing role through the NIN.

Osteoclast regulatory pathway OPG-RANKL-RANK and "kidney governing bones"

As mentioned above, IL-1 and IL-6 are powerful bone resorption-stimulating factors in the NIN. As their activities can be greatly increased and decreased by ovariectomy, bone resorption by kidney reinforcement can be inhibited in rats with osteoporosis. Hence, do IL-1 and IL-6 directly or indirectly promote bone resorption? The indirect effects of IL-1 and IL-6 on the secretion of the receptor activator of NF-κB ligand (RANKL) by regulating the osteoblasts and marrow stromal cells have been proven [13]. Osteoprotegerin (OPG), RANKL, and the receptor activator of NF-κB (RANK) play significant roles in osteoclast growth, development, activation, and maturation. OPG-RANKL-RANK is an important pathway in osteoclastic regulation. Bone resorption-stimulating factors, such as IL-1 and IL-6, stimulate osteoblasts and marrow stromal cells to express RANKL, which binds RANK on the surface of osteoclast precursor cells and osteoclasts and transmits signals into cells, thereby promoting osteoclastic differentiation, maturation, and activation. Osteoblasts and marrow stromal cells also express OPG, which competes with the binding of RANK to RANKL and inhibits osteoclastic differentiation and maturation. Based on this pathway, excessive bone resorption can be avoided [14,15]. Given the actions of IL-1 and IL-6 on the OPG-RANKL-RANK pathway, as well as inhibition of abnormally elevated activities of IL-1 and IL-6 through kidney reinforcement in rats with osteoporosis, further studies on the mechanism of "kidney governing bones" based on the OPG-RANKL-RANK pathway were conducted. Results showed that percent trabecular bone volume, as an important indicator of bone mass, is significantly reduced in ovariectomized rats. The expressions of OPG mRNA and protein in tibia osteoblasts and marrow stromal cells were also observed to decrease significantly, whereas RANKL mRNA and protein increased dramatically. Kidney reinforcement could reverse the above changes to a certain extent. On the one hand, kidney reinforcement could inhibit secretion of RNAKL in osteoblasts and marrow stromal cells, thereby decreasing osteoclastic activation. On the other hand, kidney reinforcement can also promote secretion of OPG, which combines with more RANKL and further reduces osteoclastic activation. As a result, the OPG-RANKL-RANK pathway can be accessed to treat osteoporosis [16,17] through direct or indirect inhibition of IL-1 and IL-6 activities. As such, the OPG-RANKL-RANK pathway is considered one of the mechanisms of "kidney governing bones." Although reinforcement therapy is regarded as a holistic regulatory mechanism in the body, characteristic holistic regulation in TCM can be reflected by the nonselective regulation of the NIN during kidney reinforcement therapy, which can be used to treat osteoporosis by micro-adjustment of the microenvironment of the bone marrow.

Pathomechanism of osteoporosis caused by dysfunction of "kidney governing bones"

From the viewpoint of TCM, the kidney can govern bones because the organ is responsible for storing essence. Kidney essence generates marrow, which is stored in the bone and nourishes it. This physiological process has been described in the ancient TCM book "*Suwen-Liujie Zangxiang Lun"* as follows, "The kidneys are responsible for hibernation; they are the basis of seclusion and storage. They are the location of the essence. Their effulgence is in the hair on the head. Their fullness [manifests itself] in the bones. They are the minor yin in the yin. They communicate with the qi of winter" [18]. Sufficient kidney essence ensures generation of marrow, which provides nutrition to the bones and makes them strong. Insufficient essence leads to brittle bones as well as lumbar and back pains, similar to symptoms of osteoporosis. This pathological process is described in *Suwen-Wei Lun*, "When the kidney qi is hot, then the lower back and the spine cannot be raised. The bones dry and the marrow decreases. This develops into bone limpness" [19]. Thus, the bone marrow plays a crucial role in the mechanism of action of "kidney governing bones" and maintains the growth and development of bone.

Insufficient marrow could lead to bone limpness (osteoporosis) because of failure of the kidney to govern bones. As observed in ovariectomized rats, tibial trabecular bones significantly decrease in number and thickness, with radio-

graphic trabecular patterns occurring in rats with osteoporosis compared with healthy rats. In addition, the number of adipocytes in the marrow cavity increases, whereas those of other types of cells, including osteoblasts and bone marrow stromal cells involved in bone metabolism, decrease significantly compared with findings in healthy rats. Thus, marrow exhaustion in TCM reflects a condition of increased lipocytes and reduction of other cells in the bone marrow, which is the pathogenesis of osteoporosis caused by failure of the kidney to govern bones.

Bone remodeling is an active metabolic bone process occurring throughout a person's life. As the bones experience physical stress everyday, they are remodeled; old bones are replaced, and minor injuries are repaired to accommodate the changing ways of the body. During remodeling, osteoclasts and osteoblasts work together at the same sites and achieve dynamic balance. Osteoclasts remove old bone, whereas osteoblasts replace this bone with new tissue.

At the beginning of each bone remodeling cycle, bone lining cells retreat and expose the bone surface. Then, prosomatic osteoclasts migrate to the exposed surface to form osteoclasts and absorb the bone matrix. After bone absorption, osteoclasts are replaced by osteoblasts, which can form new bone matrix to fill up the concavities left by osteoclast absorption [20]. Abnormally active osteoclasts result in larger concavities. Unfilled concavities caused by insufficient osteoid secretion from the osteoblasts may cause bone mass loss, followed by development of postmenopausal osteoporosis. Therefore, the balance between bone resorption by osteoclasts and bone formation by osteoblasts plays the key role in maintaining normal bone metabolism. Bone resorption and bone formation can be understood as "yin" and "yang" in TCM, respectively, because of their contradictory aspects, and the balance between these elements is important for bone health. Therefore, the balance of "yin and yang" in the bone marrow microenvironment contributes to the maintenance of normal bone metabolism; conversely, an imbalance of "yin and yang" can cause bone diseases, such as osteoporosis. Whether or not an herb with kidney-reinforcement effects can treat osteoporosis depends on its effect on restoring the balance between the "yin" and "yang" in the bone marrow microenvironment. Formulations that affect kidney reinforcement, such as Zuogui Pill and Yougui Pill, can treat osteoporosis by restoring balance in the bone marrow microenvironment, as confirmed in our previous studies [10–12].

Screening and exploration of herbs with kidney reinforcement functions for treating osteoporosis based on "kidney governing bones" theory

Given that kidney deficiency can lead to osteoporosis based on "kidney governing bones" theory, whether or not all herbs with the function of kidney reinforcement can treat osteoporosis is unknown. Thus, 34 herbs, which are commonly used for kidney reinforcement, were selected to treat osteoporosis in model rats with ovariectomy-induced kidney deficiency. Histomorphometric analysis of bone tissue was adopted for efficacy evaluations. Herbs with curative effects on rats with osteoporosis were screened and analyzed for their characteristic mechanisms of action. Among 34 herbs, 21 showed curative effects and 13 failed to treat the animal models. Effective herbs include Shanyao (Rhizoma Dioscoreae), Wuweizi (*Schisandra chinensis*), Gouqizi (Fructus Lycii), Sangjisheng (*Taxillus sutchuenensis*), Shayuanzi (*Astragali complanati*), Gouji (Rhizoma Cibotii), Xuduan (Herba Pterocephali), and Luxiancao (Herba Pyrolae). Ineffective herbs include Sangshen (Fructus Mori), Roucongrong (*Cistanche deserticola*), Jiucaizi (Tuber Onion Seed), and Suoyang (*Cynomorium songaricum*), among others [21–25].

The effective herbs demonstrate various mechanisms for treating osteoporosis model rats. For instance, Shayuanzi promotes OPG protein and its mRNA expression in osteoblasts and marrow stromal cells and inhibits RANKL protein and its mRNA expression. Gouji only inhibits RANKL protein and its mRNA expression, showing no influence on OPG protein and its mRNA expression [24]. These findings could provide solid bases for herbal prescriptions in clinical practice and drug research and development to treat osteoporosis and improve herbal efficacy. These findings also reveal that not all herbs with kidney-reinforcing effects can treat osteoporosis.

Based on our studies on "kidney governing bones" theory, the underlying mechanism of "kidney governing bones" can be regarded as very complicated and further scientific studies in this area are warranted.

Compliance with ethics guidelines

Dahong Ju, Meijie Liu, Hongyan Zhao, and Jun Wang declare that they have no conflicts of interest in this study. This manuscript is a commentary article and does not involve a research protocol, which requires the approval of a relevant institutional review board or ethics committee.

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