Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Zuogui Wan alleviates ovariectomy-induced osteoporosis by maintaining FoxO3 and increasing NK1R

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ARTICLE INFO

Original paper

Article history:
Received: August 17, 2023
Accepted: September 19, 2023
Published: October 31, 2023

Keywords:

ZGW, postmenopausal osteoporosis, FOXO3, NK1R, ovariectomy

ABSTRACT

Sixty Sprague-Dawley female rats were randomly divided into sham-operated groups and five ovariectomy (OVX) subgroups. Rats subjected to sham and OVX were treated with the vehicle, alendronate, and Zuogui Wan (ZGW) at the doses of low, medium and high lyophilized powder daily for 3 months, respectively. The gene or protein expression of NK1R, PPAR γ, and OSX were assayed by either quantitative polymerase chain reaction or Western blot analysis. The results showed that compared with the OVX group, ZGW could reduce the level of PPARγ and increase the levels of OSX and. Meanwhile, ZGW could prevent bone loss. In addition, we found ZGW upregulated for the NK1R mRNA or protein expression by promoting the expression level of transcription factor FoxO3 and increasing its binding to the NK1R promoter region -700/-200 sequence. These results suggest that the regulation of FoxO3 and NK1R played a role and contributed to the mechanism of ZGW underlying the increase in bone mass in the OVX rat model.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.10.28 Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

Postmenopausal osteoporosis (PMOP) is a kind of common disease associated with aging, mainly in postmenopausal women. Bone loss caused by the lack of estrogen further changes the bone structure aggravates osteoporosis, is easy to fracture, seriously affects the health and quality of life of the elderly, and even shortens life expectancy(1-3). For long-term osteoporosis treatment, sequential use of a bone-forming agent and an antiresorptive agent has been shown to provide better clinical outcomes(4). Osteogenic drugs include parathyroid hormone, prostaglandin E2, calcium and vitamin D. Drugs that inhibit osteoclasts include estrogen replacement therapy and bisphosphonates(5, 6). These drugs act directly on the processes of bone formation and absorption and mainly inhibit osteoclasts. Of course, these drugs play an important role in the prevention and treatment of osteoporosis, but their use is limited by high treatment costs and side effects such as cancer, stroke and heart disease. Therefore, the search for drugs with superior efficacy and fewer side effects is still a problem to be solved.

Traditional Chinese medicine (TCM) has been used to treat various diseases for thousands of years because of its stable efficacy, few side effects and low price. Zuoguiwan (ZGW), a typical Chinese herbal medicine, which was formulated by Zhang Jingyue, a famous physician in Ming Dynasty and first recorded in Jingyue Quanshu, could be used to strengthen bone(7, 8). With the development of modern medicine, the mechanism of ZGW for bone strengthening has been gradually recognized. ZGW plays critical roles in chronic kidney disease-mineral and bone disorders by adjusting the metabolism of calcium and phosphorus(9, 10). In addition, a number of studies have shown that ZGW has no acute, chronic toxicity and other side effects on the liver and kidney, demonstrating the therapeutic safety of ZGW in clinical application(11, 12). In our previous experiments, a series of experiments proved that ZGW up-regulated the level of OPG, down-regulated the expression of RANKL, increased the expression of Runx2 mRNA, and promoted the proliferation of BMSCs in vitro(13). In terms of mechanism exploration, our previous study also showed that ZGW has a bone protective effect and regulates bone metabolism imbalance in oophorectomized rats through β2AR mediated RANKL/OPG(13). However, the mechanism of ZGW in osteoporosis has not been adequately study.

Neurokinin-1 receptor (NK1R) is a member of the Neurokinin receptors family(14, 15).As the receptor of Tachykinins, it is a member of the family that interacts most closely with SP(16). When SP binds to the NK1R ligand, they internalize the nucleating endosome and induce the grid-dependent internalization of the receptor. After that, SP is degraded and NK1R is recovered to the cell surface(17). NK1R is expressed by osteoblast, osteoclasts, and their precursors. Neuropeptides such as SP can bind to receptors and activate NK1R to control the function of osteoclasts and osteoblasts and participate in bone repair, reconstruction and growth by binding to receptors and activating NK1R(18). Over the last few years, the func-

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tion of SP has been demonstrated in different osteoporosis models. Reduction in SP was observed in both ovariectomized female rats and in sciatic nerve-resected rats(17, 19). In addition, SP blocked bone microstructure damage and bone mineral density reduction induced by ovariectomy in rats, suggesting its therapeutic effect on osteoporosis. However, whether ZGW has an effect on SP/NK1R and its mechanism have not been explored

We observed low NK1R expression in BMSC cells extracted from OVX rats. The level of NK1R in BMSC cells from OVX rats treated with ZGW was rescued. Furthermore, ZGW also increased the level of FoxO3 in cells, where FoxO3 was identified as a transcription factor of NK1R. Our findings indicate that FoxO3/NK1R is vital for Signaling pathways in the treatment of osteoporosis. ZGW is also a potential drug for the treatment of osteoporosis.

Materials and Methods

Reagents

Primary antibodies against NK1R (ab183713), OSX (ab209484) and PPAR γ (ab272718) were purchased from Abcam (USA); FoxO3 (9532S), as well as secondary antibody (12829s) were purchased from Cell Signalling Technology (USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, GB11002) was acquired from Servicebio (Wuhan, China). The Dual Luciferase Assay Kit was acquired from Promega (E1910, Promega, US). All reagents were used according to the instructions.

Animal models and drug administration

Sixty Sprague-Dawley female rats were randomly divided into six groups and given normal saline, OVX group (1mL/100g/d, OVX), alendronate treatment group (63mg/kg/d, OVX-A), ZGW low dose [2.42g/kg/d freeze-dried powder, OVX-ZGW(L)], ZGW medium dose [4.84g/kg/d freeze-dried powder, OVX-ZGW(M)] and ZGW high dose [9.68g/kg/d freeze-dried powder, OVX-ZGW(H)], Sham operation rats were given normal saline placebo (1mL/100g/d, Sham). All the animals were raised in the Animal Center of Nanjing University of Chinese Medicine. The conditions were as follows: four in each cage, free feeding and drinking, 12h day-night cycle, room temperature 22.6±0.4℃, After 12 weeks of administration, the rats were anesthetized with 3% pentobarbital solution (0.2mL/100g). After blood collection, the rats were sacrificed and the bilateral femurs and tibia of the hippocampus were taken for further study. All animal experimental designs and plans have been approved and approved by the Animal Ethics Committee of Nanjing University of Chinese Medicine (Ethics No. 201901A011).

Quantitative real-time PCR assay

Total RNA was extracted according to the commercial instructions for the Tissue/Cell RNA Rapid Extraction Kit. According to the commercial protocol of HifairTM 1 Strand cDNA Synthesis SuperMix, cDNA was synthesized for qPCR. QPCR was performed on the cDNA samples by real-time PCR (ABI 7500, Applied Biosystems, Inc., Waltham, MA, USA) in accordance with commercial instructions HieffTM qPCR SYBR Green master mix, with the amplification procedure of 1 cycle for 95 ℃ for 5 min, followed by 40 cycles of 10 s of denaturation at 95 ℃, 20 s of annealing at 60 ℃, 20 s of extension at 72 ℃, and then followed 1 cycle, with the three stages of dissolution curve for 15 s at 95 ℃, 60 s at 60 ℃, and 15 s at 95 ℃. Primer sequences were purchased from Realgene, Nanjing. GAPDH was used as an internal control. Primers are purchased from China Shenggong Co., LTD. Data were analyzed and calculated using the $2^{-\Delta\Delta Ct}$ technique.

Western blot

Total protein was extracted with a protein extraction buffer. Each sample was separated by 8%-10% SDS-PAGE for about 20 μg. The sample was then transferred to a 0.45 mm polyvinylidene fluoride membrane. After blocking with 5% BSA solution for 1 h, the bands were incubated with the first antibody overnight. The bands were cultured with secondary antibodies and were Goat anti-mouse IgG (H+L) (1:10000) or Goat anti-rabbit IgG (H+L) (1:10000). The films are visualized using Enhanced ECL Chemiluminescent Substrate Kit and then exposed to X films by gel recording system (Image Quant LAS 4000, GE Co., Ltd, Boston, MA, USA). Image J software (National Institutes of Health, Bethesda, MD, USA) detects and analyzes the total density of the membrane.

Alizarin Red staining

BMSCs were immobilized in 4% PFA for 20 min at room temperature, then rinsed with 1 PBS and ddH2O. Then it was stained with 2% alizarin red staining (Sigma-Aldrich) for 10 minutes, washed with water, fixed with xylene and imaged. For quantification, ImageJ 1.55(National Institutes of Health) was used to calculate the alizarin red reaction area (shown in arbitrary units) for each amplification field, and the average was repeated for individual organisms.

Luciferase reporter gene assays

A dual-luciferase reporter assay was employed to examine the interaction between NK1R and FOXO3. The NK1R promoter (−2000 to +100, −700 to +100, −200 to +100) was amplified and cloned into the pGL3 basic vector (Promega, USA). HEK 293T cells (5 × 104 cells per well in 96-well plates) were co-transfected with NK1R promoter construct plasmids and FOXO3 overexpression plasmids. After 48 h of transfection, the HEK 293T cells were lysed and subjected to luciferase activity measurement with a Dual-Luciferase Assay Kit (E1910, Promega, US) according to the manufacturer's instructions.

CHIP-PCR

The cells were treated with formaldehyde, and the cells were collected and broken by ultrasound. Antibodies to FOXO3 were added, and the target protein-DNA complex was bound overnight. Protein A was added to bind the antibody-DNA complex and precipitated. The precipitated complexes were washed to remove some non-specific binding. After elution, an enriched target protein-DNA complex was obtained. Finally, the enriched DNA fragments were purified for PCR analysis

Statistical analyses

All data were expressed as means ± standard deviation. One-way analysis of variance followed by post hoc analysis using the Student-Newman-Keuls test (more than two groups), and Student's t-test (two-group comparison) were used to calculate and analyze all results. Statistical ana-

lysis was performed with the SPSS 22.0 software (IBM SPSS, Armonk, NY, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

NK1R decreased in BMSCs derived from OVX rats

In the previous literature and our study, it has been proved that ovariectomized rats can lead to secondary osteoporosis. We assayed the endogenous expression level of relative protein and mRNA in BMSCs from OVX rats. Compared with the sham group, BMSCs from OVX rats showed a marked decreased in NK1R expression (Fig. 1A). At the same time, OSX, the osteogenic differentiation-specific factor, was significantly decreased, while PPAR γ, the lipid-specific factor, was significantly increased. The quantification of each protein is shown in Figure 1B. RT-PCR was also used to detect mRNA levels of NK1R, OSX and PPAR γ, and the results were consistent with the protein results (Fig. 1C).

ZGW eliminated the decrease in NK1R induced by OVX

In order to explore the effect of ZGW on OVX-induced osteoporosis and the underlining mechanisms, we respectively used positive drug alendronate, low, medium and high dose ZGW to feed OVX-induced rats. As shown in Figure 2A, ovarian removal leads to a reduction in NK1R, which can be ameliorated by the positive drug Alendronate, and the recovery of NK1R by ZGW is also dose-dependent. The same trend was observed in OSX, but the effect was not as obvious as in NK1R. PPAR increased by OVX can also be inhibited by high doses of ZGW. The quantitative analysis of each protein is shown in Figure 2B.

ZGW ameliorated osteoporosis caused by OVX

In previous studies, micro-CT was used to analyze the structural thickness of the distal femur of rats. In two-dimensional reconstruction sections, ZGW can significantly prevent OVX-induced bone loss and improve the microstructure of bone trabecular (13). In this experiment, BMDS were co-cultured with a drug-containing serum of experimental rats, and the effect of serum on cell calcification was observed under the induction of osteogenic medium for 2 weeks. Alizarin red staining results are shown in Figure 3A. The calcification alleviation effects of OVX were reversed by ZGW, as well as Alendronate. The higher the concentration of the drug, the more pronounced the effect. The quantitative analysis is shown in Figure 3B.

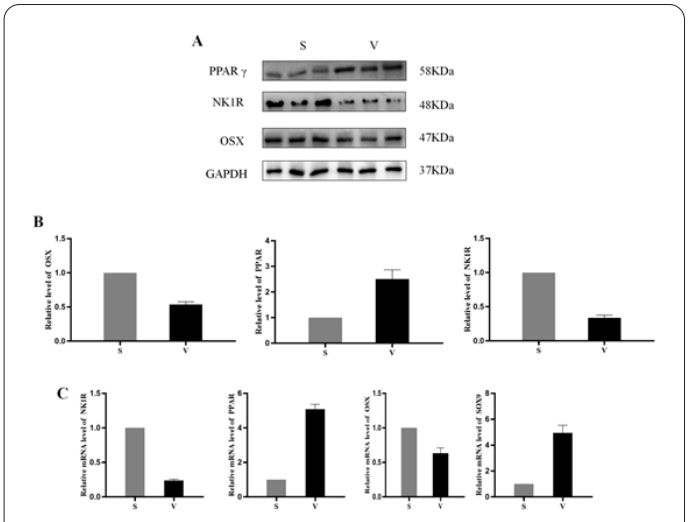

Figure 1. NK1R decreased in BMSCs derived from OVX rats. One month after model construction, BMSCs were extracted from rats in the OVX group or sham group. A. Protein levels of NK1R, OSX and PPAR were detected by western blot; B. Quantification of protein level in both groups; C. mRNA levels of NK1R, OSX and PPAR γ were detected by PCR. N=3, Notes: **P < 0.01 vs. Sham; ***P < 0.001 vs. Sham.

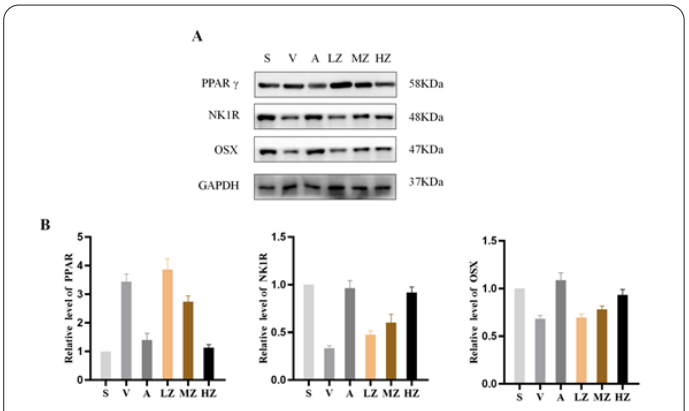

Figure 2. ZGW eliminated the decrease in NK1R induced by OVX. One month after the establishment of the model, three administration groups were given different doses of drugs, the Sham group and OVX group were given the same amount of comfort, and group A was given alendronate sodium. After 12 weeks, BMSCs were extracted. A. Protein levels of NK1R, OSX and PPAR were detected by western blot; B. Quantification of protein level in both groups. N≥3, Notes: *P < 0.05 vs. Sham; **P < 0.01 vs. Sham; #P < 0.05 vs. OVX; ##P < 0.01 vs. OVX.

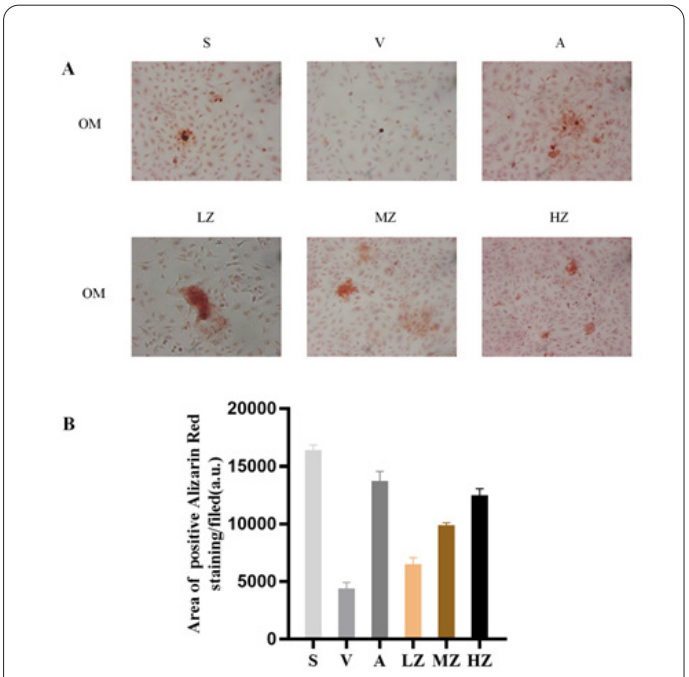

Figure 3. ZGW ameliorated osteoporosis caused by OVX. A. BMSCs from each group were cultured with osteogenic induction conditional medium for 2 weeks, Calcium nodules were stained with alizarin red (10×). B. Quantification of the positive area of Alizarin red. N≥3, Notes: **P < 0.01 vs. Sham; #P < 0.05 vs. OVX; ##P < 0.01 vs. OVX.

FOXO3 binds to the promoter of NK1R to increase its expression

To determine the presence of conserved regulatory elements in the NK1R promoter, we sequenced the promoter regions of these genes using Clustal Omega software and determined the predicted binding site for FOXO3 using Genomatix software. This analysis revealed three FOXO3 sites consistent with the NK1R promoter (Fig. 4A). To identify the NK1R promoter region that mediates FOXO3 activation of NK1R expression, we generated plasmids expressing luciferase reporter genes driven by full-length NK1R promoter and NK1R promoter fragments (-2000 /+100 bp, -700 /+100 bp, and -200 /+100 bp). FOXO3 increased luciferase activity of the first two fragments (Fig. 4B). Chip qPCR further demonstrated that the −700/+100 bp fragment of the NK1R promoter contained a strong regulatory element that mediated FOXO3 activation (FIG. 4C). Subsequently, we found that OVX induced a decrease in FOXO3 levels, but this was offset by high dose of ZGW (Figure 4D). The mRNA level of FOXO3 induced by OVX was also recovered with ZGW (Figure 4E).

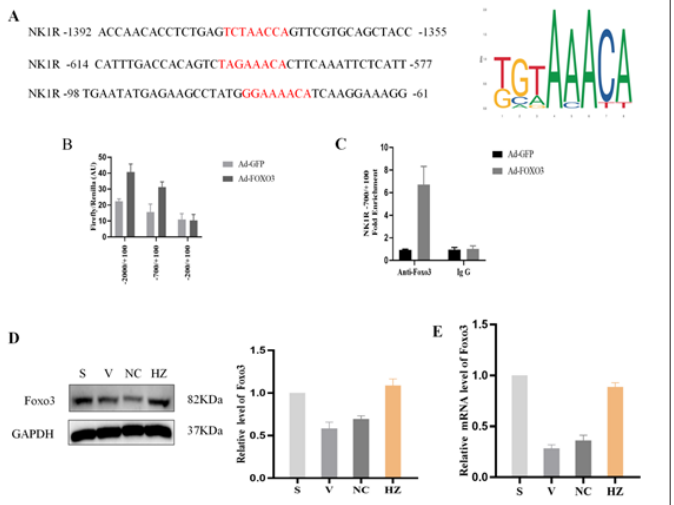

Figure 4. FOXO3 binds to the promoter of NK1R to increase its expression. Left: three FOXO3 sites consistent with the NK1R promoter; Right: prediction of transcription factor binding sites in the NRP2 promoter using the JASPAR CORE database., Calcium nodules were stained with alizarin red. B. co-transfected with NK1R promoter construct plasmids (-2000/+100, -700/+100, -200/+100bp) and FOXO3 overexpression plasmids. After 48 hours, luciferase activity was detected. N≥3, Notes: **P < 0.01 vs. Ad-GFP; ***P < 0.001 vs. Ad-GFP; C. CHIP-PCR was to verify −700/+100 bp fragment of the NK1R promoter contained a strong regulatory element that mediated FOXO3 activation. D. FOXO3 was detected by Western blot in BMSCs; mRNA levels of FOXO3 were detected by PCR in BMSCs **P < 0.01 vs. Sham ##P < 0.01 vs. OVX.

FOXO3 overexpression increased the expression of NK1R

To verify whether FOXO3 affects the expression of NK1R, we overexpressed exogenous NK1R alone in vitro. The results showed that OVX induced a decrease in FOXO3 and NK1R levels, and FOXO3 overexpression could significantly improve the expression of NK1R. The same results were obtained for both protein and mRNA relative levels (Figure 5A, 5B). Alizarin red staining suggested that OVX-induced reduced calcification levels were also inhibited by FOXO3 overexpression (Figure 5C). FOXO3 binds to the NK1R promoter region and increases the transcription level of NK1R (Figure 5D).

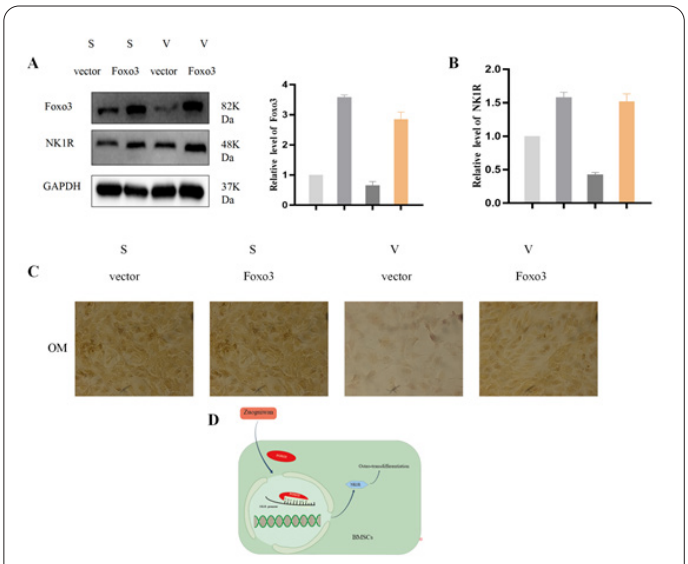

Figure 5. FOXO3 overexpression increased the expression of NK1R. A. Left: FOXO3 was overexpressed in BMSCs, and the levels of FOXO3 and NK1R were detected. B. The mRNA level of NK1R was detected. N≥3, Notes: **P < 0.01 vs. Ad-GFP; ***P < 0.001 vs. Ad-GFP; C. Alizarin red staining shown the Osteogenic differentiation ability with FOXO3 over-expression (10×). D. Graphical Summary. NK1R expression was decreased in the OVX-induced osteoporosis model. Its decrease is related to FOXO3.ZGW increases the transcription level of NK1R by increasing the expression level of transcription factor FOXO3 and promoting FOXO3 binding to the promoter region of NK1R. Finally, the osteogenic differentiation ability of BMSCs was restored.

Discussion

Pain and loss of joint function caused by postmenopausal OA are one of the main causes of motor dysfunction and chronic disability in postmenopausal women(20, 21). The prevalence of OA in postmenopausal women is greatly increased, and the secretion of estrogen is closely related to the severity of OA, suggesting that estrogen is involved in the occurrence and development of postmenopausal OA. Preclinical studies and clinical trials of estrogen therapy for OA have reported inconsistent results. Several experiments have proved the role of ZGW in the prevention and treatment of PMOP diseases, but the specific active components and the potential mechanism of action are poorly understood.

In our lab, a comorbidity model of PMOP and postmenopausal OA rats was established by OVX. It was found that the femur trabecular bone of the model rats was sparse, disorganized and spaced, the number of trabecular bones decreased, the separation degree of trabecular bone increased, and the bone density decreased significantly, indicating that severe PMOP occurred in this animal model. Therefore, in this study, the PMOP disease model was established by the OVX. Rats were fed with positive drugs and ZGW of different doses, and their serum, femur and tibia were collected. We found that the reduced expression of NK1R was related to the transcription factor FOXO3 in the extracted BMSC. In further experiments, it was found that the therapeutic effect of ZGW on PMOP might be due to the increase of FOXO3.

NK1R is expressed by osteoblast osteoclasts and their

progenitors(22). SP and other neuropeptides by binding to receptors to control the function of osteoclasts and osteoblasts and participate in bone repair, reconstruction and growth. The accelerated effect of NK1R-related signaling pathway on bone resorption of osteoclasts has been previously reported. On the other hand, the effect of NK1-related signaling pathways on bone formation is not fully understood. However, in animal models of osteoporosis, many animal studies have demonstrated the role of NK1R-related signaling pathways in bone formation and the pathogenesis of osteoporosis(19, 23). All of the abovementioned studies are limited in that they only used animal models. Nevertheless, it seems that the pathophysiology of bone-related diseases such as osteoporosis is associated with the SP-NK1R pathway. In our experiment, the changes of NK1R in osteoporosis were first verified in animal models, and the results obtained were consistent with previous studies. In the model of OVX-induced osteoporosis, the NK1R signaling pathway is inhibited. At the same time, the extracted primary cells showed higher lipid differentiation ability and lower osteogenic differentiation ability. Interestingly, we also found that ZGW had a significant patient effect on NK1R reduction. Subsequently, we predicted the transcription factor of NK1R and found that the transcription factor FOXO3 might be its effective transcription factor.

FoxOs represent a subclass of the large family of forkhead proteins characterized by the presence of a wing-helix DNA binding domain called forkhead box. FoxOs are major targets of the insulin-IGF1 signaling pathway, which inhibits FoxOs activity through Akt-mediated phosphorylation(24-26). Mouse models of loss and acquisition of FoxOs function have demonstrated that FoxOs is an important regulator of osteoclast differentiation and bone resorption(27-29). Specifically, the combined loss of FoxO1, 3, and 4 in the myeloid system promotes cell proliferation, osteoclast formation, and bone resorption, resulting in reduced trabecular and cortical bone mass(30). In contrast, FoxO3 overexpression attenuates osteoclast formation and bone resorption and increases bone mass(31). FoxOs family has shown its unique role in the reverse of osteoporosis, and we found that it can be protected by ZGW and act as a transcription factor to increase the transcription of NK1R.

The pathological mechanism of postmenopausal osteoporosis is complex, affecting a variety of growth factors and hormones, and involving many signaling pathways, and our study is just one of them. ZGW was considered to have a good effect on the prevention and treatment of PMOP in the experimental reports. Although our experiment revealed that the mechanism of ZGW in the treatment of osteoporosis may be through the regulation of NK1R and its transcription factor FOXO3, the specific components of ZGW still need to be further explored and studied. ZGW was a compound with complex components and it was difficult to identify the active components. We have identified 11 potential anti-osteoporosis compounds in ZGW by ULTRA-performance liquid chromatography-high resolution mass spectrometry(13). Perhaps we should conduct more in-depth research into the effects and mechanisms of the various ingredients to identify the most effective ones.

In conclusion, the results of this study illustrated that the regulation of FoxO3 and NK1R played a role and contributed to the mechanism of ZGW underlying the increase in the bone mass in the OVX rat model. One or more of the principal components of ZGW may be a potential herbal alternative for PMOP treatment.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No.81573874 and 81873229).

References

1. Srivastava M, Deal C. Osteoporosis in elderly: prevention and treatment. Clin Geriatr Med 2002; 18(3):529-555. doi:10.1016/s0749-0690(02)00022-8
2. Ma Z, Li X, Chen Y, et al. Comprehensive evaluation of the combined extracts of Epimedii Folium and Ligustri Lucidi Fructus for PMOP in ovariectomized rats based on MLP-ANN methods. J Ethnopharmacol 2021; 268:113563. doi:10.1016/j.jep.2020.113563
3. Ren ZQ, Wang YF, Ao GF, et al. Overall adjustment acupuncture for postmenopausal osteoporosis (PMOP): a study protocol for a randomized sham-controlled trial. Trials 2020; 21(1):465. doi:10.1186/s13063-020-04435-7
4. Lorentzon M. Treating osteoporosis to prevent fractures: current concepts and future developments. J Intern Med 2019; 285(4):381-394. doi:10.1111/joim.12873
5. Kabasawa Y, Asahina I, Gunji A, Omura K. Administration of parathyroid hormone, prostaglandin E2, or 1-alpha, 25-dihydroxyvitamin D3 restores the bone inductive activity of rhBMP-2 in aged rats. DNA Cell Biol 2003; 22(9):541-546. doi:10.1089/104454903322405428
6. Qaseem A, Forciea MA, McLean RM, et al. Treatment of Low Bone Density or Osteoporosis to Prevent Fractures in Men and Women: A Clinical Practice Guideline Update From the American College of Physicians. Ann Intern Med 2017; 166(11):818-839. doi:10.7326/M15-1361
7. Liu H, Zhong L, Dai Q, et al. Zuoguiwan Ameliorates Cognitive Deficits and Neuro-Inflammation in Streptozotocin-Induced Alzheimer's Disease Rats. Neuroimmunomodulation 2022; 29(1):63-69. doi:10.1159/000516396
8. Zhang JH, Xin J, Fan LX, Yin H. [Intervention effects of Zuoguiwan containing serum on osteoblast through ERK1/2 and Wnt/beta-catenin signaling pathway in models with kidney-Yang-deficiency, kidney-Yin-deficiency osteoporosis syndromes]. Zhongguo Zhong Yao Za Zhi 2017; 42(20):3983-3989. doi:10.19540/j.cnki.cjcmm.20170907.002
9. Ma X, He L. The intervention effect of zuogui pill on chronic kidney disease-mineral and bone disorder regulatory factor. Biomed Pharmacother 2018; 106:54-60. doi:10.1016/j.biopha.2018.06.092
10. Lia X, Wang L, Guo YQ, et al. [Effect of modified zuoguiwan

on Th17/Treg subpopulation of estrogen deficiency induced bone loss mice]. Zhongguo Zhong Xi Yi Jie He Za Zhi 2014; 34(11):1359-1364.
11. Liu S, Xu L. Clinical Research on the Treatment of Menopausal Syndromes of Liver and Kidney Yin Deficiency with Modified Kidney-yin Reinforcing Pills. Chin. Med 2012:13-19.
12. Wei Z, Ma T. Combined treatment with Zuoguiwan and ingenious agent, improves the syndromes of Yin deficiency of liver and kidney. Chengdu Univ. Tradit. Chin. Med 2015:17-24.
13. Liu F, Tan F, Tong W, et al. Effect of Zuoguiwan on osteoporosis in ovariectomized rats through RANKL/OPG pathway mediated by beta2AR. Biomed Pharmacother 2018; 103:1052-1060. doi:10.1016/j.biopha.2018.04.102
14. Ebrahimi S, Alalikhan A, Aghaee-Bakhtiari SH, Hashemy SI. The redox modulatory effects of SP/NK1R system: Implications for oxidative stress-associated disorders. Life Sci 2022; 296:120448. doi:10.1016/j.lfs.2022.120448
15. Matalinska J, Kosinska K, Halik PK, et al. Novel NK1R-Targeted (68)Ga-/(177)Lu-Radioconjugates with Potential Application against Glioblastoma Multiforme: Preliminary Exploration of Structure-Activity Relationships. Int J Mol Sci 2022; 23(3). doi:10.3390/ijms23031214
16. Schank JR. The neurokinin-1 receptor in addictive processes. J Pharmacol Exp Ther 2014; 351(1):2-8. doi:10.1124/jpet.113.210799
17. Kingery WS, Offley SC, Guo TZ, Davies MF, Clark JD, Jacobs CR. A substance P receptor (NK1) antagonist enhances the widespread osteoporotic effects of sciatic nerve section. Bone 2003; 33(6):927-936. doi:10.1016/j.bone.2003.07.003
18. Li FX, Xu F, Lin X, et al. The Role of Substance P in the Regulation of Bone and Cartilage Metabolic Activity. Front Endocrinol (Lausanne) 2020; 11:77. doi:10.3389/fendo.2020.00077
19. Liu X, Liu H, Xiong Y, et al. Postmenopausal osteoporosis is associated with the regulation of SP, CGRP, VIP, and NPY. Biomed Pharmacother 2018; 104:742-750. doi:10.1016/j.biopha.2018.04.044
20. Advani AS, Moseley A, O'Dwyer KM, et al. SWOG 1318: A Phase II Trial of Blinatumomab Followed by POMP Maintenance in Older Patients With Newly Diagnosed Philadelphia Chromosome-Negative B-Cell Acute Lymphoblastic Leukemia. J Clin Oncol 2022; 40(14):1574-1582. doi:10.1200/JCO.21.01766
21. Meinhardt A, Ramos PC, Dohmen RJ, et al. Curative Treatment of POMP-Related Autoinflammation and Immune Dysregulation (PRAID) by Hematopoietic Stem Cell Transplantation. J Clin Immunol 2021; 41(7):1664-1667. doi:10.1007/s10875-021-01067-7
22. Zheng Y, Wang N, Chen Z, Shi L, Xu X. Blocking SP/NK1R signaling improves spinal cord hemisection by inhibiting the release of pro-inflammatory cytokines in rabbits. J Spinal Cord Med 2022:1-11. doi:10.1080/10790268.2021.2024029
23. Piao J, Park JS, Hwang DY, Son Y, Hong HS. Substance P blocks ovariectomy-induced bone loss by modulating inflammation and potentiating stem cell function. Aging (Albany NY) 2020; 12(20):20753-20777. doi:10.18632/aging.104008
24. Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene 2005; 24(50):7410-7425. doi:10.1038/sj.onc.1209086
25. Izquierdo MC, Shanmugarajah N, Lee SX, et al. Hepatic FoxOs link insulin signaling with plasma lipoprotein metabolism through an apolipoprotein M/sphingosine-1-phosphate pathway. J Clin Invest 2022; 132(7). doi:10.1172/JCI146219
26. Jalgaonkar MP, Parmar UM, Kulkarni YA, Oza MJ. SIRT1-FOXOs activity regulates diabetic complications. Pharmacol Res 2022; 175:106014. doi:10.1016/j.phrs.2021.106014
27. Sergi C, Shen F, Liu SM. Insulin/IGF-1R, SIRT1, and FOXOs Pathways-An Intriguing Interaction Platform for Bone and Osteosarcoma. Front Endocrinol (Lausanne) 2019; 10:93. doi:10.3389/fendo.2019.00093
28. Kim HN, Iyer S, Ring R, Almeida M. The Role of FoxOs in Bone Health and Disease. Curr Top Dev Biol 2018; 127:149-163. doi:10.1016/bs.ctdb.2017.10.004
29. Galli C, Passeri G, Macaluso GM. FoxOs, Wnts and oxidative stress-induced bone loss: new players in the periodontitis arena? J Periodontal Res 2011; 46(4):397-406. doi:10.1111/j.1600-0765.2011.01354.x
30. Bartell SM, Kim HN, Ambrogini E, et al. FoxO proteins restrain osteoclastogenesis and bone resorption by attenuating H2O2 accumulation. Nat Commun 2014; 5:3773. doi:10.1038/ncomms4773
31. Gusakov AV, Sinitsyn AP, Klesov AA. [Enzymatic hydrolysis of cellulose. Inactivation and stabilization of the enzymes of the cellulose complex]. Biokhimiia 1982; 47(8):1322-1331.