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Review

Potential of natural medicines for treatment of osteoporosis: a narrative review

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Abstract

OBJECTIVE: To organize the fragmented information available in the literature to describe and summarize the extracts used by natural medicines in the treatment of bone loss, and to provide evidence and support for the potential use of natural medicines in the treatment of osteoporosis.

METHODS: A literature survey for relevant information regarding the osteogenesis of Dongkuiguo (*Fructus Malvae Verticillatae*), Machixian (*Herba Portulacae Oleraceae*). etc., was conducted using PubMed, ScienceDirect, MEDLINE, Springer LINK and Google Scholar electronic databases from the years 2000-2020.

RESULTS: Dongkuiguo (*Fructus Malvae Verticillatae*), Machixian (*Herba Portulacae Oleraceae*). etc., both inhibit the activity of osteoclasts and reduce bone resorption by regulation of signaling pathways through interacting with signaling molecules.

CONCLUSIONS: In this review, the current knowledge of the novel medicines with osteogenesis properties were summarized and their potential in the treatment of bone loss were demonstrated, but the lack of research on the regulation of the signaling pathway's mechanism of action, and the corresponding theoretical basis for the application of natural medicines in clinical osteoporosis, made it difficult to be widely applied and promoted in clinical practice. Further experiments with some of the medicines and the mechanisms is needed to realize their potential as osteoporosis treatments.

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Keywords: osteoporosis; natural medicines; biological products; molecular mechanisms of pharmacological action; review

1. INTRODUCTION

The bones of a normal human body are continually ablated by osteoclasts and replaced by osteoblasts, thus maintaining bone homeostasis. Bone mass of an average human increases as they age, peaking at around age 35. After age 35, the balance of bone formation and resorption can be significantly affected by aging and unhealthy lifestyles.[1] This is especially true in postmenopausal women due to the reduced secretion of estrogen in the body, which leading to more significant bone loss and eventually osteoporosis (OP) and increased risk of bone fractures.[2-4] As the population ages, OP becomes increasingly common. It is a major public health problem worldwide which urgently requires effective medicine or approaches for prevention and treatment.[5] Currently, drugs that inhibit bone resorption, including bisphosphonates, calcitonin, and estrogen, are used to treat osteoporosis. However, recent studies also show that they can also cause breast cancer, ovarian cancer, osteonecrosis, and cardiovascular disease.[6,7] Compared to synthetic drugs, the prevention and treatment of osteoporosis by natural medicines has been gradually accepted and recognized by doctors and patients due to their lower chance of side effects. This article reviews the therapeutic effects of several natural medicines extracts on osteoporosis, to provide evidence and support for the potential application of natural medicines in the treatment of osteoporosis.

2. NATURAL MEDICINES RELATED TO BONE FORMATION SIGNALING PATHWAYS

2.1. Wnt/β-catenin signal pathway

Wnt is a large family of extracellular cysteine-rich glycoproteins. Wnt signaling contributes to the

reconstruction of embryonic bone and participates in the process of cell growth, as well as regulation of many life processes.[8] Three Wnt signaling pathways are currently known: the Wnt/β-catenin signaling pathway, also known as the classic Wnt signaling pathway; the Wnt/calcium (Wnt/Ca^{2+}) signaling pathway; and the Wnt cell polarity (planar cell polarity, PCP) signaling pathway.[9] Bone formation and bone reconstruction have been demonstrated to be associated with the classical Wnt signaling pathway.[10] Wnt protein binds to the LRP5/6 receptor, which activates downstream *β*-catenin to enter the nucleus and induce the expression of key osteogenic transcription factors, such as Runx2 and Osterix, to promote osteoblast differentiation and bone formation.[11]

Muli (*Concha Ostreae*) are one of the world's most popular edible shellfish because they are nutrient-rich, with up to 60% protein.[12] Recent studies have shown that the components of Muli (*Concha Ostreae*) shells promote bone tissue regeneration due to their high calcium content.[13,14] Muli (*Concha Ostreae*) extracts could up-regulate the specific osteogenic differentiation gene in MC3T3-E1 cells and the activity of alkaline phosphatase (ALP). In addition, Muli (*Concha Ostreae*) extracts have been shown to increases the expression of osteoblast-related proteins and mineralization/ calcification in osteosarcoma MG-63 human osteoblasts,[12] as well as the promotion of vertebrae formation in young zebrafish and the tail fin regeneration of adult zebrafish.[15] By increasing the expression of β-catenin, Muli (*Concha Ostreae*) extract inhibits the degradation of β-catenin, enhances the motivation of β-catenin to enter the nucleus, activates the transcription of the targeted gene, protects the bone tissue, and relieving the symptoms of osteoporosis.[12]

Roucongrong (*Herba Cistanches Deserticolae)* is a parasitic plant, which is mainly distributed in the deserts in Northwest China, known as “Ginseng of the deserts”.[16,17] Rich in echinacoside and acteoside, it has various pharmacological activities such as bone protection, anti-aging, neuroprotection, antioxidant and improvement of learning and memory ability.[18,19] Studies showed that both water and alcohol extracts of Roucongrong (*Herba Cistanches Deserticolae*) could improve serum ALP, osteocalcin and calcium ion levels, as well as promoting the expression of bone morphogenetic protein 2 (BMP2) in rat osteoblasts.[20,21] Total glycosides and polysaccharide active components in total extract of Roucongrong (*Herba Cistanches Deserticolae*) could significantly reduce the expression of receptor activator NF-*κ*B ligand (RANKL) and P-β-catenin, while simultaneously upregulate the expression of BMP2, Osteocalcin (OCN), Osteoprotegerin (OPG) and P-GSK-3 *β* (Ser9). The total extract of Roucongrong (*Herba Cistanches Deserticolae*) can also promote senescence accelerated mouse prone 6 (SAMP6) mice osteoblast bone formation and improve bone microstructure injury healing.[22] Therefore, Roucongrong (*Herba Cistanches Deserticolae*) can regulate osteoporosis *via* activating Wnt/β-catenin signaling pathway.

3. NATURAL MEDICINES RELATED TO BONE RESORPTION SIGNALING PATHWAYS

3.1. RANKL/RANK/OPG signaling pathway

The OPG/RANKL/RANK system plays an important role in regulating the balance of osteoblasts and osteoclasts, and is empirical in the prevention of bone loss and ensuring normal bone regeneration.[23] RANKL is a member of the TNF ligand superfamily and is expressed by bone stromal cells in osteoblasts.[24, 25] The receptor activator of nuclear factor *κ*B (RANK) is a homologous tumor necrosis factor receptor (TNFR) superfamily receptor member expressed by a precursor of osteoclasts in the hematopoietic myeloid system. Bone stimulating factor stimulates osteoblasts to secrete RANKL and macrophage colony-stimulating factor (M-CSF); both will then bind to the RANK surface of osteoclast precursor cells and macrophage colony-stimulating factor receptor (M-CSFR).[26] The RANK specific site domain binds to the tumor necrosis factor receptor-associated factor (TRAF) in osteoclasts, resulting in downstream signal transduction in osteoclasts. The latest research shows that the pathway may be involved in the following: (a) the NF-*κ*B signaling pathways: After RANK binds with TRAF6, the nuclear transcription factor *κ*B induced kinase is activated, and the NF-*κ*B complex enters the nucleus, and promotes the expression of immediate early genes in the nucleus, binds with the activated T nuclear factor, and induces the transcription of osteoclast genes, so that osteoclasts reach maturity.[27] (b) JNK signaling pathway: After binding with TRAF6, RANK activates extracellular signal-regulated kinase (ERK), mitogen activated protein kinase (MKK), and c-Jun N-terminal Kinase (JNK).[21] Activated JNK induces activation of c-Jcun/Fos activated protein-1 (AP-1), which then phosphorylates c-Jun, increases c-Fox expression, and activates osteoclast precursor cells to differentiate into osteoclasts.[28] (c) Akt signaling pathway: RANK binds to TRAF6, activates phosphatidylinositol, and activates Protein Kinase B (PKB), which then actives NF-*κ*B and promotes osteoclast maturation.[29] OPG, another member of the TNFR receptor superfamily, is primarily expressed in bone marrow stromal cells and can be induced in B lymphocytes, dendritic cells, and follicular dendritic cells. OPG competitively binds RANK with RANKL to inhibit osteoclast differentiation and maturation. The increase of OPG can inhibit the differentiation of osteoclasts, whereas the increase of RANKL can promote the proliferation and differentiation of osteoclasts; therefore, the expression level of RANKL/OPG reflects the degree of bone resorption.

Dongkuiguo (*Fructus Malvae Verticillatae*) a popular ingredient in East Asia for herbal tea and medicine. Its seeds contain a variety of polysaccharides and flavonoids

that have a variety of pharmacological activities, [30, 31] and it is often used in the treatment of kidney stones, chronic mastitis, and lactation. Water extract of Dongkuiguo (*Fructus Malvae Verticillatae*) seeds has been shown to inhibits the RANKL-induced osteoclast differentiation in mouse bone marrow macrophages (BMMs).[32] However, Water extract of Dongkuiguo (*Fructus Malvae Verticillatae*) WEMV did not increase BMP-2 induced ALP activity, decrease the viability of C2C12 cells, or affect the differentiation of osteoblasts or the viability of calvarial cells.[33] These results suggested that WEMV does not regulate the early, middle, or late stage of osteoblast differentiation and proliferation. Furthermore, WEMV has also been shown to suppressed the nuclear factor of activated T cells (NFATc1) and c-Fos expression, and inhibites MAPK, nuclear factor-kappa B (NF-κB), and PLCγ2 activation in BMMs.[34]

Machixian (*Herba Portulacae Oleraceae*) is a common weedy species that is widely distributed, and is very popular in Europe, Asia, and other regions as a wild vegetable.[35] Machixian (*Herba Portulacae Oleraceae*) is rich in potassium, magnesium, and calcium, and its extracts contain flavonoids, named luteolin, kaempferol and quercetin,[36] which have anti-inflammatory,[37] antioxidant,[38] and anti-aging[39] pharmacological activities. By inhibiting RANKL-induced c-Fos expression, NFATc1, and the phosphorylation of RANKL-induced glycogen synthase kinase 3 (GSK3 kinase), purslane has been shown to have anti-osteoclast activity under both *in vitro* and *in vivo* conditions.[40] Additionally, purslane also inhibits the formation of actin rings, inhibits the bone resorption activity of mature osteoclasts, inhibits the mRNA expression of RANKL-induced osteoclast specific gene, and prevents lipopolysaccharide induced bone loss *in vivo.*[39]

Huaihua (*Flos Sophorae*), is the flower and flower-bud of *Sophora japonica* L. SF. Studies have reported that extracts of Huaihua (*Flos Sophorae*) are rich in flavonoids and flavonoid glycosides such as sophorabioside, quercitrin and rutin, which have a variety of pharmacological activities including anti-obesity, anti-allergy, anti-inflammation, anti-tumor, and anti-cancer properties.[41-43] Treatment with Huaihua (*Flos Sophorae*) extract (SFE) markedly inhibited RANKL-induced osteoclast formation from BMMs in a dose-dependent manner. SFE significantly inhibits the expression of genes related to osteoclast differentiation, such as Acp5 (TRAP), Oscar, Ctsk, Tm7sf4 (dendritic cell-specific transmembrane protein, DC-STAMP), and Atp6v0d2, as well as the expression of NFATC1. However, treatment with SFE did not change the expression of c-Fox.[44-47] Studies found that SEF regulated RANKL-induced osteoclast differentiation by affecting the NF-κB pathway at an early stage of osteoclast differentiation.[45] During which there were no effect on the activation of MAPKs.[48]

Duzhong (*Cortex Eucommiae*), is widely distributed in Asia, Europe, and North America.[49] This species has long been used as tonic in China[50] due to its antihypertensive, diuretic, anti-aging, anti-tumor, anti-inflammatory, analgesic, and other pharmacological activities.[51] The iridoids and lignans are the major active constituents of *E. ulmoides.*[52] In addition, Duzhong (*Cortex Eucommiae*) is one of the most commonly used formulas in traditional Chinese medicine for the treatment of osteoporosis.[53] Extracts from Duzhong (*Cortex Eucommiae*) (EEUO) inhibits ovariectomy (OVX)-induced bone mass decline and trabecular microarch degradation without affecting uterine hyperplasia, maintaining bone structural integrity and biomechanical quality.[54,55] Studies does on rats have demonstrated that EEUO can promote the proliferation of primary osteoblasts in rats, regulate the OPG/RANKL system of osteoblasts, and improve the expression rate of OPG/RANKL of primary osteoblasts, thus promoting the differentiation and maturation of osteoblasts and inhibiting the occurrence of osteoclasts.[56]

Shuweizao (*Sargassum Thunbergii*) is a widely distributed brown algae in coastal areas between South Korea and Japan, and it is a delicacy.[57] *In vivo* and *in vitro* studies have shown that it has a strong antioxidant, anti-inflammatory, and anti-allergic properties.[58,59] In addition, The effects of sargaquinoic and sargahydroquinoic acid extracts from Shuweizao (*Sargassum Thunbergii*) on osteoblast differentiation were also investigated.[57] Brown algae significantly reduced the expression of lipid accumulation and lipid differentiation markers, such as peroxisome proliferators activated receptor γ, CCAAT/enhanced binding protein, as well as sterol regulatory element binding protein 1c. Additionally, brown algae has been shown to inhibit the activity of tartrate-resistant acid phosphatase (TRAP) and F-actin ring structure formation, thus reducing the osteoclastic differentiation of RAW 264.7 induced by RANKL.[60] The increase in RANKL-induced osteoclast-related genes expression in RAW264.7 cells, which were dose-dependently decreased (*e.g*, activated T cell cytoplasmic 1 (NFATC1), TRAP, cathepsin K (CTSK) and matrix metalloproteinase 9 (MMP-9) and the production of reactive oxygen species.[61] The expression of Nrf2 and HO-1 in RAW264.7 cells was enhanced.[61]

Zhouyeyangti (*Radix Rumicis Crispi*) is a perennial that has been reported to have functional activity in its roots and leaves which can be used to treat a variety of diseases.[62] The roots of Zhouyeyangti (*Radix Rumicis Crispi*) inhibit arachidonic acid-induced inflammation in mice[63] and may be used to treat hemoptysis, scabies, hematochezia, neurodermatitis, as well as protecting the liver from damage.[64] In recent years, Zhouyeyangti (*Radix Rumicis Crispi*) has also been shown to be able to scavenge free radicals, inhibit cancer cell proliferation, suppress phytopathogenic fungi, and treat arthritis.[65-67] *R. crispus* water extract (WERC) emodin, chrysophanol, and physcion promote osteoblastic mineralization by increasing the expression of transcription factors (Runx2, osterix, ATF-4, SATB2, Fra-1, and JunB) and phosphorylation of ERK.[64] Studies have shown that WERC activity occurs at the end of the osteoblast

differentiation process through ERK/Runx2 signaling.[64] During osteocyte differentiation or proliferation, WERC partly stimulated ALP activity, bone nodule formation, and proliferation of MG-63 osteosarcoma cells.[68] Additionally, WERC has also been shown to inhibit RANKL-induced osteoclast differentiation and trabecular bone loss in mice.[69] In terms of the inhibition mechanism of osteoclast differentiation, WERC not only inhibits the key factors of RANKL/NFATc1, but also increases the inhibitors of NFATc1, such as ID2, regulating the initiation of differentiation.[64]

Yizhi (*Fructus Alpiniae Oxyphyllae*) is a famous medicinal plant[70] that is used commonly in China, Japan, and South Korea for its fruit "Yi-zhi-ren," which is widely used to treat diarrhea and enuresis. Studies have shown that "Yi-zhi-ren" has a wide range of biological activities and can be used to treat tumors,[71] allergies,[72] ulcers,[73] and demonstrates a neuroprotective effect.[74,75] Recent studies have found that it also has a certain effect on bone metabolism. Water extract of the fruits of *Yizhi* (*Fructus Alpiniae Oxyphyllae*) (WEAO) inhibits the differentiation of RANKL-induced BMMs to osteoclasts in a dose-dependent manner. WEAO inhibits the expression of RANKL-induced c-Fos, an upstream activator of NFATC1,[76] through the inhibition of the classical NF-*κ*B signaling pathway. WEAO can also inhibit the downregulation of NFATc1 negative regulators Id2 and MafB induced by RANKL through the JNK and p38 MAPK pathways.[77] However, WEAO was not shown to directly affect the bone resorption activity of mature osteoclasts.[78] Results of *in vitro* experiments suggest that WEAO alleviates RANKL-induced bone destruction in mice by inhibiting osteoclast differentiation.

3.2. TGF-β1/Smads signaling pathway

The TGF-*β*1/Smads signaling pathway plays an important role in osteoblast differentiation and bone formation.[79,80] TGF-*β*1 is the most abundant growth factor in the human skeleton. It functions to enhance bone formation, promotes the proliferation and differentiation of mesenchymal cells *via* recruiting pre-osteoblast cells, and promotes osteoblast differentiation and increases production of the bone matrix.[81,82] Smad is the main substrate protein of TGF-*β*1.[83,84] The conduction of the TGF-*β*1 signal starts from the heterodimer formed by the type I and type II receptors of TGF-*β*1.[79] The activated TGF-*β*1 receptor phosphorylates Smad2 and Smad3, which then forms a heterotrimer with Smad4, and localizes to the nucleus to complete the transduction of the TGF-*β*1 signal, stimulating bone formation.[81,85] TGF-*β*1 also activates the inhibitory protein Smad7, which blocks the TGF-*β*1/Smads signaling pathway by competitively binding smad2/3 to the TGF-β1 receptor, inhibiting its phosphorylation and interfering with the formation of functional Smad DNA in the nucleus.

Fengru (*Lac Regis Apis,* LRA) is a food secreted by the hypopharyngeal and mandibular glands of worker bees for the queen bee and queen bee larvae to eat.[86] It contains a large number of proteins, amino acids, lipids, vitamins, minerals, sugars, and other components.[87] LRA is pharmacologically active and has anti-allergy,[88] anti-tumor,[89] anti-fatigue,[90] anti-hypercholesterolemia,[91] and anti-hypertension[92] properties. Recent studies have found that LRA also regulates bone metabolism. LRA stimulated the proliferation and collagen production of MC3T3-E1 mouse osteoblastic cells.[68] A study found that the estrogenic effect of LRA on the proliferation of MC3T3-E1 cells was mediated by the estrogen receptor system.[93] However, the effect on collagen production is not due to the intrinsic estrogen activity of LRA,[93] instead, LRA is reported to be rich in 10-hudroxy-2-decenoic acid. This induces collagen production in human skin fibroblasts. This is demonstrated by the production of TGF-1,[94] while there is no direct evidence, LRA may promote collagen synthesis through TGF-1 in MC3T3-E1 cells and mouse osteocytes. *In vivo*, LRA can increase the number of osteoblasts, capillaries, and the formation of new bone in rats undergoing maxillary dilatation.[68] LRA can also reduce the number of osteoclasts and promote bone regeneration. LRA could maintain the ratio of Ca%/P% in lumbar vertebrae and the femur of ovariectomized rats and inhibit the change of trabecular bone structure.[95]

4. NATURAL MEDICINES RELATED TO OTHER BONE RESORPTION SINGNALING PATHWAYS

Huangfenchong (*Tenebrio molitor,* TM; Coleoptera), also known as yellow mealworm, is a new food source.[96] Dried mealworm larvae contain considerable amounts of protein, giving them the nickname of the "treasure house of protein feed." Recent studies have shown that mealworm larvae extract can promote bone health and offer protection from neurodegenerative sexual dysfunction.[97,98] In the OVX model of osteoporosis, estrogen deficiency leads to the inhibition of cell proliferation and neurogenesis in the hippocampus,[99] chronic oral TM extract can significantly increase the number of DG Ki67 (cell division) and DCX (the number of nerve cells and their dendritic branches) in the hippocampus. The impairment of the regulatory function of HPA axis and the decline of hippocampal neurotrophic status under the condition of chronic estrogen deficiency can be alleviated by oral administration of TM.[96]

5. CONCLUSIONS

Natural medicines have shown promise in treating a wide range of diseases due to their wide availability, limited side effects, pharmacological activity, and long history of use. Previous studies have demonstrated that natural medicines, such as Dongkui (*Malvae Verticillatae*) seeds, Machixian (*Herba Portulacae Oleraceae*), Roucongrong (*Herba Cistanches Deserticolae*), Huaihua (*Flos*

Sophorae), Fengru (*Lac Regis Apis*), *Yizhi (Fructus Alpiniae Oxyphyllae)*, *Duzhong (Cortex Eucommiae)*, Zhouyeyangti (*Radix Rumicis Crispi*), Shuweizao (*Sargassum thunbergii*), and Muli (*Concha Ostreae*), have anti-osteoporosis effects. With the development of anti-osteoporosis drug research, numerous recent studies have been conducted on natural anti-osteoporosis medicines. The pathogenesis of osteoporosis includes many factors such as heredity, as well as deficiency of calcium, vitamins, and estrogen. These anti-osteoporosis medicines can relieve the symptoms of osteoporosis to different degrees. The anti-osteoporosis mechanisms include promoting the formation and reconstruction of new bone, increasing the expression levels of BMP2 and the transcription factors Runx2, osterix, and JunB, decreasing the expression levels of RANKL-induced osteoclast-related genes, NFATC1, TRAP, cathepsin K, and matrix metalloproteinase 9, and the production of reactive oxygen species.

Natural medicines show promise in treating various diseases due to their wide availability, limited side effects, pharmacological activity and long history of use. Although medical researchers have done a lot of research on the application of natural medicines in the prevention and treatment of osteoporosis, and have made some progress. However, there are still many problems to be solved in clinical application. At present, there is a lack of research on the molecular mechanism of natural medicines regulating signal pathways, resulting in the lack of relevant theoretical basis for the application of natural medicines in clinical osteoporosis. It is suggested that based on the research on the prevention and treatment effect of natural medicines on osteoporosis in the future, the effect of drugs and the mechanism of action should be determined to lay a theoretical foundation for the wide clinical application. China is rich in natural medicine resources. Combined with modern pharmacology, molecular biology and other emerging disciplines, it is of good development value and great social and health significance to explore the effect and mechanism of natural medicines with anti-aging effect and intervention of rhythm gene function in preventive intervention of osteoporosis.

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