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Advancement in treating osteoporosis with traditional Chinese medicine Liuwei Dihuang pill

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Osteoporosis is becoming a prevalent disorder in the aging society, and its treatment remains challenging. The traditional Chinese medicine (TCM) Liuwei Dihuang pill (LWDHP) is widely used in treating multiple types of diseases including osteoporosis and is receiving increasing attention. Recent experimental and clinical studies have shown that LWDHP can effectively prevent and improve osteoporosis. The therapeutic mechanism of LWDHP is complicated, including the balance between osteoclasts and osteoblasts, anti-inflammation, and modulation of kinase pathways. This review summarizes recent studies of LWDHP in treating osteoporosis. Following the elucidation of its mechanism and the establishment of a modern scientific standard, LWDHP will be a promising therapeutic approach for treating osteoporosis although there is still a long way.

KEYWORDS

traditional Chinese medicine, osteoporosis, formula, Liuwei Dihuang pill, herb

Introduction

Following the increase in the aging population, osteoporosis is becoming more prevalent. Osteoporosis, a musculoskeletal disorder characterized by decreased bone mineral density (BMD) due to disturbances in the bone microstructure caused by multiple factors, can lead to fractures and life-threatening problems. Osteoporosis includes primary osteoporosis, such as postmenopausal osteoporosis (PMO) and senile osteoporosis, and secondary osteoporosis complicated with some diseases diabetic pathology and chronic kidney diseases or induced by therapeutic strategies such as glucocorticoid-induced osteoporosis (GIOP) (1). The mechanism of osteoporosis is complicated, and its treatment remains unsatisfactory (2); therefore, the outcomes of osteoporosis are different, varying from fractures, disability to osteoporotic pain (2, 3).

The therapeutic strategies for osteoporosis include prescribed exercise, specific drugs, and a combination of medications (2). Although these therapeutic strategies are effective for some patients, many patients demonstrated resistant reactions to these approaches. In addition, the currently used medication for treating osteoporosis has different side effects, which leads to an increasing number of researchers searching for effective ingredients derived from natural products, particularly Chinese herbal medicines (1). Shih et al. performed a population-based study including 16,544 patients with osteoporosis in Taiwan. They found that 70% of these patients received treatment with traditional Chinese medicine (TCM), suggesting a high level of recognition for TCM as a strategy for treating osteoporosis (4). In two cohort studies involving a large population of Chinese individuals nationwide, Drs. Wang and Cheng found that TCM displayed

significant protection against fractures in osteoporotic patients (5, 6), suggesting a potential therapeutic effect in treating osteoporosis. In addition, TCM has been included in the latest version of the International Statistical Classification of Diseases and Related Health Problems by WHO (ICD-11 version, 2019), which will greatly facilitate the research of TCM in searching for new approaches for treating osteoporosis.

Liuwei Dihuang pill (LWDHP) is a TCM formula consisting of six herbs (*Rehmannia glutinosa*, *Cornus officinalis*, *Dioscorea opposita*, *Poria cocos*, *Alismatis rhizoma*, and *Moutan cortex*, Figure 1) and has a long history in treating multiple kinds of diseases in Asia, including diabetes (5, 6), cancer (7), and osteoporosis (8, 9). Previous studies indicated that LWDHP is the most prescribed TCM in Korean patients (10) and can enhance the therapeutic effect of Western medicine in treating diabetic nephropathy (11). These studies pointed out the potential of LWDHP in treating osteoporosis. However, the wide acceptance of TCM such as LWDHP still faces a lot of problems to be resolved due to the unclear efficacy and mechanism in scientific scope like purity, dosage, and safety. Despite the barriers derived from the unclear therapeutic mechanisms of TCM and some side effects (12), increasing studies have demonstrated the potential to study LWDHP using advanced scientific approaches (13). This review aims to summarize recent advancements in TCM LWDHP in treating osteoporosis from laboratory studies to clinical studies and to find the related mechanisms, which may help us improve the treatment of osteoporosis with satisfactory effect.

Clinical practice in treating osteoporosis with LWDHP

Compared to the wide acceptance of LWDHP in practice by people in Asian countries, such as China, Japan, and Korea, based on experience-based medicine (10, 14, 15), TCM including LWDHP is not well accepted in the Western world, and there are relative fewer English publications about the treatment of osteoporosis with because of the aforementioned reasons. In traditional clinical practice, the treatment of osteoporosis with TCM is mainly based on the principle that osteoporosis is induced due to Shen (kidney) deficiency; therefore, it is proposed to "tonify Shen (kidney) and benefit marrow" from the theory that bone is controlled by Shen (kidney) and moistened by the "Marrow." This theory is somehow correlated with the Western medicine basis that the kidney modulates the mineral metabolism and therefore affects bone pathophysiology. Consistent with this theory, it is found that LWDHP which is a classic Yin and Yang tonic formula was found to induce upregulation of cardiotrophin-like cytokine factor 1 and increase BMD of the top femur of women with postmenopausal osteoporosis (PMOP) with Shen (kidney)-yin deficiency (16). In clinical practice, LWDHP is found to be helpful for the treatment of patients with diabetic nephropathy (6, 11) which is one cause of osteoporosis based both on TCM theory and modern medicine (17). Using a weighted gene coexpression network and network pharmacology, it was found that LWDHP is involved in secondary osteoporosis induced by diabetic nephropathy (8). These studies imply the potential of LWDHP in treating osteoporosis.

Laboratory evidence for LWDHP in treating osteoporosis

The antiosteoporosis effect of LWDHP is also improved in experimental animals. In secondary osteoporosis induced by diabetic nephropathy, LWDHP significantly improves bone features including BMD, bone volume, and bone microstructure of rats with osteoporosis due to diabetic nephropathy (9). In the osteoporosis model of rats induced by ovariectomy (OVX), LWDHP decreases the serum levels of alkaline phosphatase (ALP) and osteocalcin, increases the femoral BMD and the maximum loading and elastic modulus of the vertebral body of rats subjected to OVX (18).

A single herbal component of LWDHP also demonstrates protection in treating osteoporosis. The LWDHP component *Rehmannia glutinosa* increases BMD and improves the microarchitecture of the trabecula in rats with osteoporosis induced by glucocorticoid (19). In rats with OVX, *Rehmannia glutinosa* can enhance BMD and the microstructure of femurs and lumbar bones (20). In mice with OVX, the LWDHP component *Cornus officinalis* increases the BMD and improves the microstructure of the trabecula (21).

Some extracts of LWDHP or single components of LWDHP also demonstrate antiosteoporotic effects. For example, the extract of *Cornus officinalis*, *morroniside*, reduces bone resorption and improves the microstructure of bone (22, 23). The extracted protein from *Dioscorea opposita* demonstrated an antiosteoporotic effect but without stimulation of cancer cells by upregulating hormones, suggesting a possible potential for the use of *Dioscorea opposita* as a hormone replacement therapy for primary osteoporosis in postmenopausal women (24). The extract of *Poria cocos* and *Alismatis rhizoma* Aliso C 23-acetate prevents bone loss in mice with OVX (25) and rats with OVX (26).

Mechanisms of LWDHP in the treatment of osteoporosis

Because LWDHP consists of six kinds of herbs and is given to patients in the soup after long-term boiling, the mechanisms are complicated, including different signaling pathways at different levels. The bone formation is maintained by the balance between osteoclasts and osteoblasts, and any disturbance of the balance can result in dysfunction of bone and osteoporosis. LWDHP or its components can play an important role in treating osteoporosis by modulating the functions of osteoclasts and osteoblasts. It is found that the LWDHP formula can promote osteoblastic differentiation while suppressing osteoclastic differentiation *in vitro* and therefore can increase bone mass (9). In addition, LWDHP can promote the hematopoietic stem progenitor cells in the bone marrow to proliferate (27).

FIGURE 1
The herbs and productions in the formula of TCM LWDHAP.

The single component of LWDHP *Rehmannia glutinosa* also demonstrates antiosteoporotic effects by enhancing the proliferation of osteoblasts, activity of ALP, extracellular matrix mineralization, and expression of Runt-related transcription factors and osteopontin in osteoblasts. In rats with OVX, LWDHP component *Rehmannia glutinosa* can enhance the expressions of osteoprotegerin, insulin-like growth factor-1 (IGF-1), β-catenin, and Runt-related transcription factor 2 (RUNX2) while reducing the expressions of ALP, tartrate-resistant acid phosphatase (TRAP), and receptor activator of nuclear factor κ-B ligand (RANKL) (20). Another component of LWDHP *Cornus officinalis* also promotes the differentiation of osteoblasts through the upregulation of ALP and RUNX2 while inhibiting the differentiation of osteoclasts (21).

In addition, some extracts of LWDHP also demonstrated an effect in modulating the balance between osteoclasts and osteoblasts. For example, the four components (morroniside, catalpol, loganin, and acteoside) of LWDHP extraction can promote the differentiation of osteoblasts (28). The extract catalpol from *Rehmannia glutinosa* can inhibit bone resorption and osteoclast formation induced by RANKL by upregulating the activity of phosphatase and tensin homolog (PTEN) (29). On the other hand, catalpol and other extracts from *Rehmannia glutinosa* such as acteoside and echinacoside can increase the proliferation and differentiation of osteoblastic cells under high-glucose conditions, suggesting that these extracts have therapeutic potential in osteoporosis induced by diabetes. This effect is related to the signaling pathway of bone morphogenic protein (BMP)/IGF-1 phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin complex 1 (mTOR) signaling pathway (30). The extract of *Cornus officinalis*, *morroniside*, can improve the impaired differentiation of bone marrow-derived mesenchymal stem/stromal cells (BMSC) by high glucose, trigger Glo1 to attenuate the expression of receptor for advanced glycation end-products (RAGE) (22). The extract of *Poria cocos* and *Alismatis rhizoma* inhibited the differentiation of osteoclasts through the RANKL signaling pathway (25, 26).

Inflammation and oxidative stress play a critical role in the pathological development of osteoporosis. LWDHP can decrease the levels of cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, IL-1β, ALP, and TRAP (9). LWDH can upregulate NO release and endothelial nitric oxide synthase (eNOS) and activity in HUVECs, balance the levels of Bax and Bcl-2, and attenuate intracellular reactive oxygen species (ROS)

production (31). A modification of LWDHP with the addition of *Agrimonia pilosa*, nacre, *Clematis chinensis* Osbeck, and Dark Plum fruit can improve two types of diabetes (6) which is an important factor for secondary osteoporosis (6). In aging rats, LWDHP can significantly reduce inflammation in the testis through the AMPK/SIRT1/NF-κB signaling pathway (32), suggesting LWDHP may play a role in primary osteoporosis induced by aging.

In addition, LWDHP plays its role in regulating osteoporosis by other signal pathways. For example, LWDHP increases cell viability and alkaline phosphatase activity and upregulates the Wnt/β-catenin signaling pathway in rats with OVX (18). LWDHP component *Rehmannia glutinosa* also modulates the metabolites of hormone biosynthesis (19).

Taken together, the mechanisms of LWDHP in ameliorating osteoporosis include balancing the activities of osteoblasts and osteoclasts via transcriptional and translational signals of RANKL, RUNX2, PTEN, and modulation of inflammation through IL, AMPK/SIRT1/NF-κb, etc. The complex interactions of these signals remain to be further studied.

Application of advanced approaches in studying LWDHP

Recently, some advanced scientific approaches have been applied to investigate the related mechanisms of LWDHP in treating osteoporosis from multiple disciplines. For example, an ultraperformance liquid chromatography-tandem mass spectrometer is also applied to analyze the components of LWDHP, and four components (morroniside, catalpol, loganin, and acteoside) are identified to be able to modulate osteoporosis (28). Using a weighted gene coexpression network and network pharmacology analysis, 22 components of LWDHP are found to be related to diabetic nephropathy-induced osteoporosis, and the signaling pathway of mitogen-activated protein kinase (MAPK) is the main target of LWDHP. Among these components and targets, analysis of protein–protein interaction (PPI) network indicates that kaempferol and quercetin are identified as the most significant components, and MAPK1 is the potential target of miR-574. Using docking models, kaempferol and quercetin are further found to have a strong binding affinity to MAPK1 at the site of Asp 167 (8).

In addition, bioinformatics was used to study the potential target genes of LWDHP in treating primary postmenopausal osteoporosis (PMO) (33). In this study, Drs. Xu and Gao found that LWDHP reduced the expression of 52 differentially expressed genes (DEGs) which are increased in women with PMO-KY osteoporosis and that LWDHP increased the expression of 34 DEGs which were downregulated in women with PMO-KY. In addition, LWDHP increased the expression of 34 DEGs that are downregulated in women with PMO-KY, and seven TFs are predicted to regulate these DEGs. Among these LWDHP-regulated DEGs, NCOA3, TCF4, DUSP6, PELI2, and STX7 are potential therapeutic targets of osteoporosis by LWDHP. In another study with bioinformatics, three genes, namely, ATF2, FBXW7, and RDX, are identified as the therapeutic target for LWDHP to treat PMO (34).

Summary

Although LWDHP is widely prescribed to treat different bone diseases including osteoporosis in Asia, there is still difficulty for TCM LWDHP to be accepted by the Western medicine field which emphasizes purity, efficacy, dosage, and safety while TCM focuses on the compatibility of herbs and customization. The pharmacokinetics of some constitutes of LWDHP (HMFA, loganin, and paeonol) were analyzed and showed long-lasting steady properties after oral administration (35); however, considering the numerous components of the six herbs of LWDHP, there is still a big journal for fully understanding the profile of LWDHP. In addition, the toxicity of LWDHP is another concern. A series of studies of LWDHP preparations containing polysaccharides, glycosides, and oligosaccharides fraction indicate that LWDHP has no obvious toxicity on multiple systems [for review, see (36)]. Despite these difficulties ahead, however, the application of advanced research approaches including the preparation of LWDHP with modern technology (28) and the clarification of the therapeutic mechanisms of every single ingredient of LWDHP will finally help to develop novel therapeutic strategies for osteoporosis with LWDHP.

Author contributions

JW: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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