Tonifying Shen-Yin and -Yang Principles in Treating Osteoporosis: All Roads Lead to Rome

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ABSTRACT

Tonifying Shen strategy works as an important alternative and complementary method that is widely used to treat osteoporosis in Traditional Chinese Medicine (TCM) based on the proper identification of Zheng. Shen deficiency Zheng is one of the main types of osteoporosis while Shen-Yin and Shen-Yang deficiencies represent two basic principles/types of Shen deficiency. Currently, Tonifying Shen strategy, in particular, the Tonifying Shen-Yin and Shen-Yang principles, has been demonstrated to exert osteoprotective effects. However, the mechanisms by which Tonifying Shen strategy and/or Tonifying Shen-Yin and Shen-Yang principles function in treating osteoporosis are still not clearly understood. Here we first briefly explore current understanding of Tonifying Shen strategy, such as Tonifying Shen prescriptions, herbs, and effective components, in treating osteoporosis. Furthermore, the mechanism of Tonifying Shen-Yang principle in treating osteoporosis is reviewed from the clinical experience, animal, and mechanistic investigation. Moreover, the mechanisms by which Tonifying Shen-Yin principle in treating osteoporosis are established. Finally, we compare the similarity and difference between Tonifying Shen-Yang and Shen-Yin principles in treating osteoporosis. Our findings indicate that both principles exert bone-protective effects in treating osteoporosis by simultaneously stimulating the differentiation of mesenchymal stem cells (MSCs) into osteoblast and the differentiation of hematopoietic stem cells (HSCs) into osteoclast. However, Tonifying Shen-Yin principle is in favor of increasing bone mineral density (BMD) while Tonifying Shen-Yang principle is in favor of osteoporosis-related syndromes. Our findings indicate that Tonifying Shen-Yin and Shen-Yang principles not only share some similarity but also obtain some difference in treating osteoporosis.

Key words: Osteoporosis, Shen-Yin and -Yang Deficiency, Tonifying Shen-Yin and -Yang, Osteoclast, Osteoblast

Abbreviations: 1,25(OH)\textsubscript{2}D: 1, 25-dihydroxy Vitamin D\textsubscript{3}; ALP: Alkaline phosphatase; Beta 2-MG: Beta 2-microglobulin; BGP: Bone gla protein; BGSSC: Bugushengsu capsule; BMD: Bone mineral density; BMP: Bone morphogetic protein; BSA: Bone sialoprotein; BSF: Bushenshengfufang; BSJG: Bushenjiangujiaonang; BSG: Bushenfang; BSJG: Bushenjiangujiaonang; Col-1: Collagen type I; CORT: Corticosterone; DM: Dexamethasone; DRG: Dried root of Rehmanniaglutinosa; FLL: Fructus Ligustri Lucidi, one effective ingredient form herb; GLP: Gulin Pill; HSC: Hematoepoietic stem cell; IGF-1: Insulin-likefactor-1; IKSBC: Bushenjiangujiaonang; IL-1,6,7: Interleuikin (IL)-1,6,7; LPS: Lipopo polysaccharide; LWDHP: Liuweidihuangpills; MAFK: Mitogen-activated protein kinase; MSC: Mesenchymal stem cell; M-CSF: Macrophage Colony-Stimulating Factor; NEIC: Neuron-Endocrine-Immune-Circulation (NEIC) system; NFATc1: Nuclear factor of activated T-cells, cytoplasmic 1; OA: Oleanolic acid; OCN: Osteocalcin; OXV: Ovariectomized; OPG: Osteoprotegerin; PLC\textsubscript{a}ι: Phospholipase C\textsubscript{α}ι; PTH: Parathyroid hormone; RANK: Receptor activator of NF-κB; RANKL: Receptor activator of NF-κB ligand; Runx 2: Runx-related transcription factor 2; TCM: Traditional Chinese Medicine; TKEBP: Bushenjiangujiaonang; TKS: Bushenjiangujiaonang; TKYD: Bushenfang; LWDHP: Liuweidihuang pills; TNF-α: Tumor necrosis factor α; TRAP: Tartrate-resistant acid phosphate; YGP: Yougui Pill; ZGP: Zuogui Pill.

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INTRODUCTION

Tonifying Shen (Including the functions of anatomic Kidney and partially the function of neuro-endocrino-immune-circulation (NEIC) system) strategy works as an important alternative and complementary method that is widely used in Traditional Chinese Medicine (TCM) based on the proper identification of Zheng. Zheng is the summarization of the complexes pathological syndromes that occur during the different stages of disease(s), and both the pathogenic and healthy factors are included\textsuperscript{[11]}. The correct identification of Zheng is the requirement for clinical physicians to perform proper treatments. Shen deficiency Zheng is a basic pathological identification for osteoporosis according to TCM theory which holds the idea that the deficiency of Shen always leading to deteriorated bone structure and function\textsuperscript{[2–3]}. Therefore, Tonifying Shen strategy is intensively used in the management of osteoporosis. Furthermore, Shen deficiency Zheng can be divided into Shen-Yin and Shen-Yang deficiency Zheng bases on osteoporosis-induced symptoms. Briefly, patients with flaccid waist and knees, dysphoria with smothery sensation, and night sweat are regarded as Shen-Yin deficiency Zheng.
and are treated with Tonifying Shen-Yin principle. Patients with weak waist and knees, feeling of chilliness, and frequent micturition can be considered as the Shen-Yang deficiency Zheng and are treated with Tonifying Shen-Yang principle[4]. Therefore, Tonifying Shen strategy is divided into Tonifying Shen-Yin and -Yang principles. Currently, many prescriptions are constructed to treat osteoporosis based on the exact identification of Shen-Yang and -Yin deficiency Zheng in TCM.

Osteoporosis is one bone disturbance that is characterized by reduced bone mineral density (BMD) and deteriorated bone structure[5]. In osteoporosis, the balanced bone remodeling processes are disturbed by osteoclast-dominated bone resorption that overpasses osteoblast-dominated bone formation[6–7]. Osteoporosis always leading to the impairment of vital skeletal functions: (a) providing support and site for the muscle to maintain the movement, (b) protecting organs such as bone marrow and brain, and (c) acting as one metabolic organ that regulates the homeostasis of minerals and hormones[6]. Therefore, patients who are diagnosed with osteoporosis often suffer from skeleton pains, limitation of movement, soreness of joints, weakness of the waist and knees and so on[6]. According to the theory of TCM, osteoporosis-induced syndromes can be regarded as the Shen-Yin or Shen-Yang deficiency Zheng (Fig. 1). However, the mechanism by which Tonifying Shen strategy, particular two principles of Shen-Yang and Shen-Yin principles, exerts bone-protective effects in regulating bone remodeling are still rarely investigated.

Osteoporosis reflects the imbalance of bone formation by osteoblast and bone resorption by osteoclast in favor of bone resorption[6,7]. Therefore, drugs that inhibit osteoclast formation/activity or stimulate osteoblast formation/activity are considered as effective methods for treating osteoporosis[9–13]. However, currently marketed drugs in treating osteoporosis have caused heavy economic burdens or side effects[14–16]. Tonifying Shen strategy, including Tonifying Shen-Yin or Shen-Yang principle, has been established to exert bone-protective effects as well as rescuing bone loss in osteoporosis patients and in vivo investigations[17–19]. Till now, TCM prescriptions, herbs and effective components are three mainly forms that are used in treating osteoporosis. Usually, a Tonifying Shen prescription is constituted by many Tonifying Shen herbs and the prescription often exerts more predominant effect than a single herb. Meanwhile, the effective components are extracted from herb(s), and they are very convenient to perform the molecular mechanistic investigations. All these three forms have been established to exert bone-protective effects and treat osteoporosis by the instruction of Tonifying Shen-Yin and Shen-Yang principles. However, many questions still need to be addressed: 1) the exact molecular mechanism of Tonifying-Shen strategy in treating osteoporosis is still not fully understood. 2) Underlying mechanism processes of Tonifying Shen-Yin or Shen-Yang principles in treating osteoporosis are still obscure. 3) Tonifying Shen-Yin or Shen-Yang principles do exert bone-protective effects. However, what is the similarity and difference between Tonifying Shen-Yin and Shen-Yang? Therefore, it is necessary to address these important issues in search of better strategies for treating osteoporosis.

In this paper, we select the typical Tonifying Shen prescriptions (herbs, effective components) which play bone-protective to address the following points: 1) first explore the bone-protective effects and molecular mechanisms of Tonifying Shen strategy, particular their effects in regulating the differentiation of MSCs into osteoblasts, and the differentiation of HSCs into osteoclast. 2) we further explore the bone-protective effects and molecular mechanisms of Tonifying Shen-Yang and -Yin principles in treating osteoporosis are systematically analyzed. 3) we explore the similarities between Tonifying Shen-Yang and -Yin principles in treating osteoporosis are identified. 4) more important, the difference between Tonifying Shen-Yin and Shen-Yang principles in treating osteoporosis are established.

We hope that this paper will not only help gain new insights into Tonifying Shen-Yin or Shen-Yang in treating osteoporosis, but also broaden our strategies in the treatment of osteoporosis. In addition, our findings will promote new drug discoveries in treating osteoporosis.

1 TONIFYING SHEN STRATEGY IN TREATING OSTEOPOROSIS

1.1 Clinical investigations

In the late 1980s, clinical studies observed the bone-protective effects of Tonifying-Shen prescriptions and herbs in treating patients who were diagnosed with osteoporosis[18–20]. One clinical investigation reported that Bushenfang (BSF, the ingredients are seen in the Table1/2) in TCM both improved osteoporosis-induced symptoms and delayed the developing process of osteoporosis[18]. Furthermore, one subsequent study found that Tonifying Shen prescription named Bushenjiangufang (BSJG, table 1&2) capsule might alleviate bone loss and symptoms in postmenopausal osteoporosis[19]. This study found that the syndromes in nearly 90% osteoporosis patients (Totally 51 cases) treated with BSJG capsule for 3 months were improved in comparison with calcium group (P<0.05). Moreover, BMD of the lumbar vertebra in 68.8% patients was

![Figure 1. Shen-Yin deficiency and Shen-Yang deficiency Zheng in osteoporosis. A. Patient who were diagnosed with osteoporosis that can be identified as the Shen-Yin deficiency Zheng. B. Patient who were diagnosed with osteoporosis that could be identified as the Shen-Yin deficiency Zheng. C. Patient who were diagnosed with osteoporosis that can be considered as both the Shen-Yin and -Yang deficiency Zheng. D. Patient who were diagnosed with osteoporosis but not regarded as the Shen-Yin or Shen-Yang deficiency Zheng in TCM.](image)
largely increased in contrast to calcium supplementation after the treatment for 6 months. Mechanistic findings indicated that the increased bone formation and depressed bone absorption were attributed to bone-protective effect[19]. In addition, one clinical study with 52 osteoporosis patients was performed to investigate the bone-protective effects of Bushenjiangugufang (TKEBP, table1&2). After the treatment for 3 months, patients treated with TKEBP showed significant higher levels of blood alkaline phosphates (ALP), beta 2-microglobulin (beta 2-MG) level, urinary beta 2-MG, calcium and phosphorus. Moreover, the symptoms of lumbar dorsal pain, general fatigue, palpitation and vertigo were improved after the treatment[20]. Taken together, these studies established that Tonifying Shen strategy is effective in preventing bone loss and alleviating osteoporosis-induced symptoms.

From then on, series of studies were carried out with larger numbers of patients, and more representative bone biological markers in osteoporosis[21–22]. These studies confirmed that Tonifying Shen prescriptions/herbs administration significantly increased BMD and relieved the symptoms in treating osteoporosis[23]. Mechanistically, one study indicates the expressions of bone gla protein (BGP), pyridinoline, estradiol, testosterone, and blood urea nitrogen were greatly increased after the treatment with Bushenshenggufang (BSSF, table1/2)[24]. Moreover, one Five-year-follow up study observed the long-term bone-protective effects with BSSF[25]. 194 patients participated, and the results showed that BSSF greatly increased BMD from baseline (0.211 ± 0.022 g/cm) at the end of (3 year) the study (0.284 ± 0.015 g/cm), whereas the control group decreased significantly from baseline (0.212 ± 0.023 g/cm) to one higher level (0.187 ± 0.022 g/cm).

### Table 1. Formulae for Tonifying Shen strategy

<table>
<thead>
<tr>
<th>Formula</th>
<th>Ingredients</th>
<th>Tonifying Shen &amp; Yin &amp; Yang category</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGSSC:</td>
<td>6 herbs: yin yang huo (Epimedi Herba); bú gú zhí (Psoralea corylifolia L); shèng huáng qì (Astragali Radix Cruda); guī bān (tortoise plastron); bìjiā (turtle shell); dàng guì (Angelicae Sinensis Radix), etc.</td>
<td>Shen</td>
<td>[51]</td>
</tr>
<tr>
<td>Bugushensuipian</td>
<td>5 herbs: dū zhòng (Eucommae Cortex); lú róng (Cervi Cornu Pantoricticum); yin yang huo (Epimedi Herba), bú gú zhí (Psoralea corylifolia L); Di Huáng (Rehmannia glutinosa), etc.</td>
<td>Shen</td>
<td>[18]</td>
</tr>
<tr>
<td>BSF:</td>
<td>5 herbs: dū zhòng (Eucommae Cortex); lú róng (Cervi Cornu Pantoricticum); yin yang huo (Epimedi Herba), bú gú zhí (Psoralea corylifolia L); Di Huáng (Rehmannia glutinosa), etc.</td>
<td>Shen</td>
<td>[18]</td>
</tr>
<tr>
<td>Bushenjiangugufang</td>
<td>8 herbs: dī huáng (Rehmannia glutinosa); bù gú zhí (Psoralea corylifolia L); gòu qí zī (Wolfberry Fruit); dū zhòng (Eucommae Cortex), shān yào (Dioscoreae Rhizoma); shān zhù yù (Fructus corni); lú jiāo (Deer horn); dāng guì (Angelica sinensis), etc.</td>
<td>Shen</td>
<td>[19]</td>
</tr>
<tr>
<td>BSJG:</td>
<td>6 herbs: yin yang huo (Epimedi Herba); bú gú zhí (Psoralea corylifolia L); guī bān (tortoise plastron); tū sī zhí (semenscucutae), etc.</td>
<td>Shen</td>
<td>[21–23]</td>
</tr>
<tr>
<td>Busheshenggufang</td>
<td>6 herbs: yin yang huo (Epimedi Herba); bú gú zhí (Psoralea corylifolia L); guī bān (tortoise plastron); tū sī zhí (semenscucutae), etc.</td>
<td>Shen</td>
<td>[49]</td>
</tr>
<tr>
<td>BSYG:</td>
<td>6 herbs: yin yang huo (Epimedi Herba); bú gú zhí (Psoralea corylifolia L); dà huáng (Rhei Radix et Rhizoma); shān yào (Dioscoreae Rhizoma), etc.</td>
<td>Shen</td>
<td>[29]</td>
</tr>
<tr>
<td>NSFSC:</td>
<td>8 herbs: dī huáng (Rehmannia glutinosa); bù gú zhí (Psoralea corylifolia L); gòu qí zī (Wolfberry Fruit); dū zhòng (Eucommae Cortex), shān yào (Dioscoreae Rhizoma); shān zhù yù (Fructus corni); lú jiāo (Deer horn); dāng guì (Angelica sinensis), etc.</td>
<td>Shen</td>
<td>[20]</td>
</tr>
<tr>
<td>TKEBP:</td>
<td>5 herbs: guī bān (tortoise plastron); bìjiā (turtle shell); dà huáng (Rhei Radix et Rhizoma), etc.</td>
<td>Shen</td>
<td>[20]</td>
</tr>
</tbody>
</table>
One in vivo study evaluated the bone-protective effect of *Fructus Ligustri Lucidi* (FLL), a Tonifying Shen-Yin herb, in ovariectomized (OVX) rat. The result showed that FLL could reverse the OVX-induced bone loss and increase BMD. Particularly, FLL intervention alleviated OVX-induced calcium loss in rats by increasing intestinal calcium absorption and suppressing urinary Ca++ excretion. Further mechanistic investigations established that FLL could modulate bone remodeling by affecting calcium homeostasis in OVX rat.[28]

Recently studies with one Tonifying Shen prescription named Bushenjiangjuaoaang (IKSBC, table1&2) showed that IKSBC attenuated bone loss in OVX-induced osteoporosis rats. The concentrations of calcium and phosphorus in serum from OVX rat was increased after treatment with IKSBC. Meanwhile, the expression of ALP was increased, and the trabecular bone volume was significantly increased in contrast to the pretreatment[29]. Therefore, the bone-protective effect of Tonifying Shen strategy may partly be due to their regulatory effects on the homeostasis of calcium and phosphorus (Fig. 2A&2B).

### 1.2.2 RANKL/RANK/OPG axis

RANKL/RANK/OPG (Osteoprotegerin) axis was discovered in the late 1990s, and has been shown to play a critical role in coordinating bone remodeling. Both osteoblast and osteoclast are regulated by this axis,[30] and studies have shown that Tonifying Shen strategy may regulate bone remodeling by affecting RANKL/RANK/OPG axis. One Tonifying Shen prescription, Gulin Pill (GLP), has been indicated exerting bone-protective effects by stimulating osteoblast differentiation in MG-63 cells. The mechanistic investigation indicated that GLP treatment significantly promotes the proliferation and differentiation of MG-63 cells by stimulating the expression of OPG, and down-regulating the expression of RANK at the protein and mRNA levels. Furthermore, GLP-treated

![Figure 2](image.png)

**Figure 2.** (A) Mechanism of Tonifying Shen strategy in regulating osteoblastogenesis. During normal osteoblastogenesis, Tonifying Shen strategy, including Tonifying Shen prescription, herbs, effective components, stimulates the expression of estrogen signaling, Wnt/β-catenin, Vitamin D, Notch, and BMPs to stimulate osteoblastogenesis. Meanwhile, they play bidirectional effects on osteoprotegerin (OPG) and regulating osteoblastogenesis. In addition, they also regulated the transportation of calcium and phosphors that necessary for normal osteoblastogenesis. (B) Mechanism of Tonifying Shen strategy in controlling osteoclastogenesis. During osteoclastogenesis, Tonifying Shen strategy, including Tonifying Shen prescriptions, herbs, effective components, stimulates the expression of estrogen signaling down-regulated interlunkin-1, 6, 7, TNFs and RANKL, osteoclastogenesis and the transportation of calcium and phosphors.
Bone formation. Moreover, osthole exerted the stimulatory effect on the expression of Wnt/β-catenin and BMP signaling. This finding indicates that osthole may be one potential to stimulate new bone formation to prevent estrogen deficiency-induced bone loss (Fig. 2A&2B).

Psoralen, one effective component from Tonifying-Shen herb, was found to promote osteoblast differentiation by up-regulating the expressions of osteoblast-specific genes, including type I collagen (Col-1), osteocalcin (OCN), bone sialoprotein (BSA). One further study indicated that psoralen stimulates the expression of BMP-2 and BMP-4, and increase the level of phospho-Smad1/5/8 as well as the expression of osteonectin.

1.2.5 Notch signaling

Notch signaling is necessary for the differentiation of osteoblasts by working together with BMP, and Wnt[45], and is considered as one important factor for osteoblast differentiation in MSCs[45–46]. Oleanolic acid (OA) and its glycosides have been reported to prevent bone loss in OVX-induced mice according to Micro-CT assessment. Subsequently, one animal investigation indicated that OA-treated (10mg/kg/day, ip.) mice displayed increased osteoblast numbers, higher expression of OCN and Runt-related transcription factor 2 (Runx2). Furthermore, in vitro studies indicated that OA exerts an osteoprotective effect in OVX-induced osteoporotic mice by enhancing osteoblastic differentiation of MSCs. Molecular investigations indicated that this effect was due to their regulatory effect on Notch signaling (Fig. 2A&2B)[49].

1.2.6 Vitamin D3

Vitamin D3 and its active ingredients supplementation, 1, 25-dihydroxy Vitamin D3 (1, 25(OH) D3), have been reported to play critical roles in determining the bone formation and structure[48]. One animal study discovered that the level of 1, 25(OH) D3 in blood serum, liver and Kidney was significantly elevated after the treatment of Bushengyanggupian (BSYG, table1&2) in OVX-induced (Lower level of estrogen model) rats. Meanwhile, BSYG-treated group rats showed an increase of BMD in femoral heads compared with saline control. Moreover, bone maximal load and stress index was improved after the intervention of BSYG[49]. These studies indicate that Tonifying Shen prescriptions/herbs might stimulate bone formation through up-regulating the level of 1, 25(OH) 2D3 (Fig. 2A&2B).

1.3 Tonifying Shen strategy in regulating homeostasis

Therefore, Tonifying Shen strategy, including the applications of Tonifying Shen prescriptions, herbs and effective components, exerts bone-protective effects in osteoporosis by affecting bone formation and resorption. At the cellular level, they exert bone-protective effects by promoting MSCs into osteoblast and bone formation (Fig. 3). Meanwhile, Tonifying Shen strategy may also promote MSCs into more osteoblasts instead of adipocytes[47]. On the other hand, Tonifying Shen strategy has been established to exert inhibitory effect on HSCs-originated osteoclastogenesis and bone resorption[49]. Next, further studies have been performed to investigate...
the underlying processes of how these two principles in Tonifying Shen strategy, Tonifying Shen-Yin and -Yang, in treating osteoporosis.

2 TONIFYING SHEN-YANG PRINCIPLES IN TREATING OSTEOPOROSIS

2.1 Clinical Studies

More than 20 years ago, Tonifying Shen-Yang principle has been selected in the management of osteoporosis with Shen-Yang deficiency Zheng in TCM to investigate the bone-protective effect and the mechanisms\[51\]. One Tonifying Shen-Yang prescription, Bugushengsi capsule (BGSSC), was investigated in treating primary osteoporosis. The clinical effects in patients treated with BGSSC were significantly higher than control (Vitamin D plus calcium). Moreover, BGSSC intervention improved BMD according to the test with double energy X-ray as well as increasing the level of serum calcitonin, hormone and calcium. This clinical finding indicates that the bone-protective effects of BGSSC on primary osteoporosis including the improvement of BMD, and suppressed effect on bone resorption. Moreover, one randomized, double-blind, multicenter, and placebo-controlled study revealed that Tonifying Shen-Yang prescription, Yougui Pill (YGP, table1&2), may largely relieve the syndromes in patients with osteoporosis. Moreover, a total of 200 patients, aged from 55-75, diagnosed with lower BMD were enrolled. Moreover, YGP may significantly relieve the osteoporosis related syndromes, such as weak waist/knee, and extreme chilliness\[4\].

2.2 Mechanism of Tonifying Shen-Yang principle in treating osteoporosis

Animal models with Shen-Yang deficiency Zheng and osteoporosis phenotype were successfully investigated by the usage of the high dose of corticosteroids (CORT) \textit{in vivo} (52). Shen-Yang deficiency zheng with osteoporosis was treated with Tonifying Shen-Yang herbs, effective component from Tonifying Shen-Yang prescription or herbs to assess their bone-protective effect. Icariin is an active component from \textit{Herbal Epimedium}, one Tonifying Shen-Yang herb in TCM. One investigation induced osteoporosis rats by CORT injection and OVX, and both were treated with icariin for 2 weeks and 3 months. This result indicated that icariin may promote MSC differentiation from CORT and OVX-induced rat by stimulating the secretion of OCN, Col-1, and Runx2. Meanwhile, gene profiles screen revealed a significant shift of osteoblast-related gene expression after the intervention of icariin, which were more significance in CORT-treated rat\[52\]. Dexamethasone (DM) was always used to induce Shen-Yang deficiency Zheng model in animals. One study investigated the efficacy of Bushengyangfang (TKYR, table1&2) in osteoporotic rats with DM injection. In TKYR treated group, the BMD, femur bending strength, serum-BGP, serum-PTH and serum-E2 in the TKYR-treated group were significantly higher than DM-treated group (P<0.01). Meanwhile, compared with DM group, the levels of serum-T in TKYR prevention or TKYR treatment group were significant increased (P<0.05)\[54\]. One investigation established the underlying mechanism of the Bushenwenyangfang (TKYDP, table1&2) in regulating osteoblast formation/activity. Human osteoblastic cell line (hFOB 1.19) was treated with 10% TKYDP administrated pattern-serum from postmenopausal women (30 cases, aged 60-70). The cell viability, expression of ALP and numbers of calcified nodules in TKYD treated-serum were remarkably increased in hFOB1.19 cells as well as decreasing the expression of RANKL. In addition, the concentration of estradiol (E2), and insulin-like factor-1 (IGF-1) in the TKYDP treated-serum was higher in patients than calcium control. Therefore, these findings indicate that the down-regulation of estradiol E2, IGF-1, RANKL in TKYD-treated serum may contribute to the increased bone formation as well as decreasing bone resorption in patients\[53\].

Osthole is one effective component from \textit{Fructus Cnidii}, a traditional herb of Tonifying Shen-Yang (Table 3). One investigation indicated that osthole could promote the activity of ALP and the formation of bone nodules by enhancing osteoblastic proliferation and differentiation\[56\]. Moreover, osthole intervention can greatly prevent OVX caused bone loss in rats by improving bone micro architecture. Further molecular assays demonstrated that osthole activated the expression of Wnt/β-catenin and BMP-2\[43\]. On the other hand, following studies indicated that osthole inhibited osteoclast formation and bone resorption pits on bone slices. Further mechanistic studies indicated that osthole inhibited the activity of osteoclast and bone resorption through suppressing the RANKL/TRAF6/Mkk/JNK signaling cascade\[57\].

In addition, one study found that icariin dose-dependently inhibited the growth and differentiation of HSCs, and less TRAP positive cells formation were observed in icariin-treated group. Meanwhile, bone resorbed pits formation was also

<table>
<thead>
<tr>
<th>Effective component</th>
<th>Herbs</th>
<th>Tonifying Shen &amp; Yin &amp; Yang category</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleanolic acid</td>
<td>mò hàn lián (Yerbadetajo Herb)</td>
<td>Yin</td>
<td>[47,66–68]</td>
</tr>
<tr>
<td></td>
<td>nǔ zhén zǐ (Fructus Ligustri Lucidi)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Icariin</td>
<td>yín yáng huò (Herbal Epimedium; \textit{Epimedium koreanum Nakai})</td>
<td>Yang</td>
<td>[43,50,53,58,59,60]</td>
</tr>
<tr>
<td>Osthole</td>
<td>shè chuáng zǐ (Fructus Cnidii)</td>
<td>Yang</td>
<td>[43,56,57]</td>
</tr>
<tr>
<td>Psoralen</td>
<td>bū gǔ zǐ (Psoralea corylifolia L)</td>
<td>Yang</td>
<td>[44,69–71]</td>
</tr>
<tr>
<td></td>
<td>nǔ zhén zǐ (Fructus Ligustri Lucidi)</td>
<td>Yin</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Dì Huáng (Rehmannia glutinosa)</td>
<td>Yin</td>
<td>[64]</td>
</tr>
</tbody>
</table>
dramatically decreased than saline control. RT-PCR analysis indicated that the gene expression of TRAP and RANK was greatly down-regulated in bone marrow macrophages (BMMs)\(^5\). Moreover, the study found that icariin reduced the size of lipopolysaccharide (LPS)-induced osteoclast formation, and the expression of TRAP. On the other hand, icariin inhibited ALP activity without inhibiting cell viability. Meanwhile, icariin also inhibited LPS-induced bone resorption and the expression of IL-6 and TNF-α in BMMs. Gene expression of OPG was up-regulated, and the expression of RANKL was suppressed. Mechanistic findings indicated that icariin inhibited LPS-induced osteoclast formation by suppressing the expression of p38 and JNK\(^4\). Later on, one study finding indicated that bone loss was attenuated by the treatment with icariin in OVX-induced osteoporotic rats\(^3\). In addition, icariin intervention could completely correct the decreased serum concentration of calcium, and phosphorus in OVX rats, and biomechanical strength in icariin treated rats was also largely enhanced in comparison to sham. Moreover, icariin intervention could stimulate new bone formation in OPG knockout mice after local injection in calvarias, and reversed OPG Knockout-induced bone loss. Mechanistic assessments indicated that icariin up-regulated the expression of BMP-2, BMP-4, Runx2, OC, Wnt1, and Wnt3a in OPG Knockout mice. Furthermore, icariin intervention increased the expression of Axin2, DKK1, TCF1, and Lef1, which are the direct targeted genes of β-catenin signaling pathway. These findings indicated that icariin reversed the phenotypes of OPG-deficient mice through enhancing the activation of Wnt/β-catenin or BMP signaling\(^6\). In vitro assays found that icariin promoted MC3T3-E1 osteoblastic differentiation and mineralization by enhancing the expression of ALP, Col-I, and bone nodule formation. Molecular investigations indicated that the expression of mitogen-activated protein kinase (MAPK) -activated signaling, such as ERK and JNK, contributed to the stimulatory effect of icariin on osteoblastogenesis\(^7\). DM has been routinely used as a classical inducer for osteoblastogenesis, as well as Alleviating the level of ACTH, cAMP, cGMP, and TSH. The Shen-Yin deficiency Zheng model was stable and reliable for the integration of disease and syndrome in TCM\(^8\). Molecular studies have shown that the level of ACTH, cAMP, cGMP and TSH in serum was greatly decreased in this Shen-Yin deficiency Zheng model. Meanwhile, BMD of lumbar vertebra and femur was decreased according to HE staining. However, Liuweidihuang pills (LWDHP, table1&2), one famous Tonifying Shen-Yin prescription, could partly reverse the bone loss phenotype and as well as Alleviating the level of ACTH, cAMP, cGMP, and TSH.

OA, one effective component from Tonifying Shen-Yin herb (Table 3), has been established to exert bone-protective effects by inhibiting the formation of osteoclast, and bone resorptive activity of osteoclast in RAW264.7 cell line\(^9\). Meanwhile, OA selectively induced the apoptosis at an early stage of mature osteoclast via increasing the caspase-3 activity. Furthermore, OA intervention significantly prevented bone loss in OVX mice without any hormone-like adverse effects, and different OA derivatives may exert different effects on osteoclast\(^{10}\). One molecular investigation indicated that OA inhibited RANKL-induced osteoclast formation by suppressing the phosphorylated expression of Btk and phospholipase Cγ2 (PLCγ2), calcium (Ca\(^{2+}\)) oscillation, and the expression of nuclear factor of activated T-cells, cytoplasm 1 (NFATc1). Furthermore, OA-intervention attenuated LPS-induced bone erosion based on μ-CT and histological analysis in femur in vivo\(^{11}\) as well as rescuing OVX-induced osteoporosis rat model via stimulating the differentiation of bone MSCs by suppressing Notch signaling\(^{12}\).

3 TONIFYING SHEN-YIN PRINCIPLES IN TREATING OSTEOPOROSIS

3.1 Clinical Studies

One clinical study indicated that the administrations of ZGP, one famous Tonifying Shen-Yin prescription, did not significantly relieve the symptoms of Shen-Yin deficiency Zheng in patients who were diagnosed with osteoporosis. However, the results indicated that ZGP intervention remarkably improved BMD at lumbar spine than placebo after the treatment for 6 months. Moreover, BMD in the lumbar spine of ZGP group was increased by 4.1% and 4.7% after another 6 months follow-up study\(^{13}\). Another clinical investigation found that FLL could alleviate bone loss in osteoporosis patients. Subsequently, one study indicated that FLL enhanced BMD and mechanical properties in growing male rats\(^{14}\). Mechanistic data demonstrated that FLL could alter bone turnover by increasing bone formation biomarkers, such as the expression of OCN and CTX-I, and both calcium absorption and retention rates were elevated after FLL treatment. Mechanistic studies indicated that the expression of 1α-hydroxylase, duodenum, and calcium absorption-related genes were increased to activate the 1, 25(OH) 2D3-dependent calcium transportation in stimulating bone formation.

Dried root of rehmanniaglutinosa (DRG) is a Tonifying Shen-Yin herb in TCM for the treatment of osteoporosis. One study indicated that treatment with DRG caused the decrease of BMD in the femur and lumbar in OVX-induced bone loss in mice without affecting the body, organs, and uterus weights. Further mechanistic investigation indicates that serum ALP level in the DRG-treated group was increased. This study indicated that DRG is able to prevent the OVX-induced bone loss in influencing the level hormones, such as estrogen\(^{15}\).
Psoralen, one effective component from Tonifying Shen-Yin herb (Table 3), has been reported to exert bone-protective effects. Psoralen-treated osteoblastogenesis and ultra-structural assessment showed that the effect of psoralen on osteoblast formation ranged from negligible to unambiguous structural alterations[69]. One animal investigation indicated that there were more new bone presented after the treatment with psoralen than the normal control (4.5:1). Meanwhile, cell culture assays indicated that the osteoblast number was also predominantly increased in the psoralen-treated group[70]. Subsequently, investigations indicated that psoralen dose-dependently promoted osteoblast differentiation from primary mouse calvarias. The mechanistic assessment indicated that the stimulatory effects may be due to enhanced expressions of osteoblast-specific marker genes[44]. Interestingly, osteoblastogenesis assessment indicated that the intervention of psoralen elevated the expression of ALP in osteoporotic rats, and the formation of unit osteoblast[71].

4 COMPARISON OF TONIFYING SHEN-YIN AND -YANG PRINCIPLE IN TREATING OSTEOPOROSIS

Current studies have established the common mechanisms of Tonifying Shen-Yin and Shen-Yang principles in treating osteoporosis, and both bone formation and bone resorption were affected. Molecular investigations have demonstrated that a variety of signaling pathways in osteoblastogenesis and osteoclastogenesis were involved in. In order to clearly address the similarity as well as the difference between these two principles, we compared the effects of Tonifying Shen-Yin and -Yang principles in treating osteoporosis as the bellowed:

(1) Tonifying Shen-Yin and -Yang principles both are effective methods for treating osteoporosis, such as increasing BMD, alleviating osteoporosis-induced symptoms, and modulating series of bone metabolic markers. Mechanistic studies indicated that Tonifying Shen-Yin or -Yang principle exerts stimulatory effects on enhancing osteoblast differentiation/activity and bone formation. On the other hand, both principles exert inhibitory effects on osteoclast differentiation/activity and bone resorption (Fig. 4).

(2) Tonifying Shen-Yin principle exerts the favorable effect on increasing BMD by stimulating the activity of osteoblast and bone formation. Therefore, the bone-protective effect of Tonifying Shen-Yin principle exerts bone-protective effects due to their stimulatory effect on osteoblast-mediated bone formation and inhibitory effect on bone resorption (Osteoclast) in favor of the former (Fig. 4).

(3) Tonifying Shen-Yang exerts a more remarkable effect on alleviating the osteoporosis-induced symptoms.

Therefore, this effect is mainly due to their preferable inhibitory effects on bone resorption (Osteoclast) and stimulatory effect on bone formation (Osteoblast) in favor of the former (Fig. 4). This finding is in consistent with results of one previous clinical investigation carried out with ZGP and YGP[44].

For this study, 200 patients who were diagnosed with osteoporosis aged from 55-75, were enrolled. All patients were treated with ZGP or YGP after the proper identification of Shen-Yin or -Yang deficiency Zheng. Patients with Shen-Yang deficiency Zheng were treated with YGP, while those with Shen-Yin deficiency Zheng were treated with ZGP. The result showed that YGP significantly reduced pains according to VSA and ECOS-16 questionnaire scores than placebo at 9, and 12 months. ZGP could significantly increase BMD in lumbar spine in patients. Therefore, ZGP significantly increased lumbar spine BMD, whereas YGP significantly reduced symptoms such as the severe of pain, and improved the life quality in patients[44].

5 DISCUSSIONS

Shen deficiency is the pathogenesis in osteoporosis in TCM, and Tonifying Shen strategy is the basic rule in the management of osteoporosis[48]. Currently, Tonifying Shen prescriptions/herbs are the most common agents used in treating osteoporosis in clinic practice of TCM[8,39,72]. One study reviewed that herbal prescriptions have been used in treating osteoporosis throughout TCM history. Totally 389 prescriptions were found, and 238 herbs were involved with a total frequency of appearance at 4236. According to the result, Tonifying Shen herbs are the most commonly used in treating osteoporosis[72]. Furthermore, the most common categories were Tonifying Shen-Yang medicinal, and nearly account for 37.8% of the total frequency. Therefore, a proper selection of Tonifying Shen-Yang and -Yin principles with proper herbs/prescriptions is necessary for the treatment of osteoporosis in order to obtain better clinic outcomes[73]. Although both Tonifying Shen-Yin and -Yang principles are effective in treating osteoporosis, but underlying process are quite different. Our finding first implies that Tonifying Shen-Yin principle significantly increased BMD in bone. In contrast, Tonifying Shen-Yang principle largely improved osteoporosis related symptoms, and life qualities.
Bone remodeling is maintained through the coupling mechanisms between bone formation and bone resorption. Therefore, the purpose of osteoporosis treatment is to restore the balance of bone formation and bone resorption. Therefore, the coupling mechanisms between osteoblast formation/activity and osteoclast formation/activity represent two independent but united aspects in bone remodeling. Given previous investigations have proposed that osteoblast mediated-bone formation and osteoclast-mediated bone resorption should be regarded as Shen-Yin and -Yang in bone[21]. Therefore, the pathogenesis of Shen-Yin deficiency Zheng in osteoporosis is mainly accompanied by impaired osteoblast-determined bone formation, whereas Shen-Yang deficiency Zheng is mainly due to more osteoclast activated bone resorption[24]. More recent studies have proved that osteoclast activated bone resorption (Yang) are the main cause of pains in osteoporosis[25]. Therefore, the inhibitory effect of Tonifying Shen-Yang principle on osteoclasts (Bone resorption) may account for clinical effects of alleviating osteoporosis-induced pains. On the other hand, Tonifying Shen-Yin principle may increase BMD by exerting the stimulatory effect on osteoblastogenesis and bone formation (Yin).

Targeting osteoclasts and osteoblasts have long been regarded as critical strategies not only in treating osteoporosis, but also many other diseases with osteoporotic phenotype[76-78], such as diabetes, osteoarthritis, and cancers induced bone metastasis. Both Tonifying Shen-Yang and Tonifying Shen-Yin principles have been established to suppress osteoclast-mediated bone resorption or stimulate osteoblast-activated bone formation. However, Tonifying Shen-Yang and -Yin principles exert similar but different effects in treating osteoporosis: Tonifying Shen-Yang may mainly alleviate the osteoporosis-induced symptoms, such as pains; Meanwhile, Tonifying Shen-Yin principle may mainly increase BMD. Our findings indicate that Tonifying Shen strategy, including the prescriptions, herbs and effective components with bone-protective effects, are potential resources of the new drugs discovery in treating bone osteoporotic diseases. However, their bone-protective effects may be better if based on the proper selection of Tonifying Shen-Yang or -Yin principle. Our studies partly explore the potential mechanisms of how Tonifying Shen strategy, in particular Tonifying Shen-Yang or -Yin principles, exerts bone-protective effects on osteoporosis. More importantly, we try to propose the similarity and difference of Tonifying Shen-Yang and -Yin principles in treating osteoporosis in bone remodeling.

However, some questions are still necessary to be addressed: 1) there are hundreds of Tonifying Shen-Yin and -Yang prescriptions, herbs, effective components with bone-protective effects in treating osteoporosis, it is necessary to perform more investigations to investigate their effect. 2) More effective ingredients and their derivatives with fewer side effects from Tonifying Shen-Yang and -Yin prescriptions or herbs with bone-protective effects should be extracted for more molecular investigations. 3) Bone homeostasis is regulated by neuron-endocrine-immune-circulation (NEIC)[79-83] axis, it is necessary to investigate the exact effect of Tonifying Shen-Yang and -Yin principle on NECI axis in osteoporosis. 4) As Tonifying Shen-Yang and Shen-Yin principles exert bone-protective effects simultaneously by affecting different signaling pathways during osteoblastogenesis and osteoclastogenesis, it is necessary to examine the cross-talk signaling pathways. Therefore, future research need to focus on in gaining more insights into the molecular mechanism of Tonifying Shen-Yin or -Yang principles in treating osteoporosis.

COMPETING INTERESTS
All the authors state that they have no conflicts of interest or disclosure.

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