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Review

Identifying the effective combination of acupuncture and traditional Chinese medicinal herbs for postmenopausal osteoporosis therapy through studies of their molecular regulation of bone homeostasis

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Abstract

Worldwide, as the population age, osteoporosis is becoming increasingly common, and osteoporotic fractures have a significant economic burden. Postmenopausal women are the most susceptible to developing osteoporosis and the most critical time to prevent it is during the perimenopausal and early menopausal years. In this regard, we hypothesize rational combination of acupuncture and Traditional Chinese Medicine (TCM) in the form of herbal extract could prevent osteoporosis in women. Estrogen deficiency during menopause causes low-level inflammation that stimulates the formation of osteoclasts, the bone-resorbing cells, and simultaneously inhibits the viability and function of osteoblasts, the bone-forming cells. The most potent inflammatory cytokine in skeletal homeostasis is the receptor activator of nuclear factor kappa B ligand (RANKL) that stimulates osteoclast function. Conversely, the canonical Wnt pathway is essential for osteoblastogenesis and bone formation, and estrogen deficiency leads to diminished functioning of this pathway. TCM and acupuncture could target the RANKL and the Wnt pathway in favorable ways to prevent the accelerated bone loss experienced during the early menopausal stage and promote the gain in bone mass in postmenopausal women. In this review, we propose a rational combination of specific TCM and acupuncture targeting those signaling molecules/pathways by the drugs that are in clinical use for the treatment of postmenopausal osteoporosis. Our rational approach revealed that Danshen (*Radix Salviae Miltiorrhizae*) could exert a synergistic effect with acupuncture. We then propose a translational path for developing the putative combination in women with postmenopausal osteoporosis to curtail the risk of osteoporotic fractures.

Keywords: medicine, traditional; osteogenic; anti-resorptive; review

1. INTRODUCTION

The Study of Women's Health Across the Nation (SWAN) conducted on middle-aged American women of various ethnic backgrounds observed that bone loss is significantly increased during late perimenopause and early menopause.[1] This is due to the sharp falls in the levels of estrogen, a hormone that is a key regulator of skeletal homeostasis. Estrogen limits the activity of the bone-resorbing cells, and osteoclasts by directly inhibiting their formation and inducing apoptosis of differentiated osteoclasts. On the other hand, estrogen promotes osteoblast differentiation by stimulating the production of bone morphogenetic protein subfamily (BMPs) by osteoprogenitor and bone marrow stromal cells by a paracrine mechanism. Additionally, the estrogen-induced differentiation of bone marrow-derived Master of Science to osteogenic lineage over the adipogenic lineage gets reversed in menopause resulting in bone marrow adipogenesis enhancement. The resultant increase in the adipocyte pool in the bone marrow provides a cytokine milieu that becomes favorable for increased bone resorption and reduced bone formation. Finally, estrogen regulates several feeding-related neuropeptides in the arcuate nucleus and ventromedial nucleus of the hypothalamus that has

multifactorial regulation in bone homeostasis. A fall in circulating estrogen level could impair the hypothalamic regulation of bone homeostasis resulting in bone loss.[2]

The preclinical and clinical assessment of these Traditional Chinese Medicine (TCM) revealed that they preserve bone mass in the postmenopausal osteoporosis condition. Acupuncture also displays beneficial effects on bone metabolism and to this effect, various acupoints have been identified.[3] Since bone metabolism has a complex regulation with the interplay of various signaling pathways, a rational combination of TCM and acupuncture types by considering the clinically established targets for bone metabolism is likely to be effective in increasing bone mass in postmenopausal osteoporosis.

The overarching objective of this review was to consider the molecular pathways that have been pharmacologically targeted to successfully treat postmenopausal osteoporosis and to identify TCMs and acupoints that may be able to favourably influence these pathways in order to suggest a combination to prevent bone loss and promote bone formation.

2. BONE REMODELING (BR) CYCLE AND MENOPAUSE

BR cycle is critical for the removal of damaged bones and replacement with new materials. BR cycle consists of quiescent, resorptive, reversal and formation phases. Quiescent bone surface requiring first undergoes bone resorption by the action of osteoclasts which takes between 2-3 weeks. Resorption is followed by a reversal phase in which the precursors of bone-forming cells, osteoblasts populate the resorption pits in preparation for filling up the pits with newly synthesized bone materials. BR cycle is a coupled process until the third decade of life, as the amount of bone resorbed is replaced by an equal amount of new bone.[4]

However, the BR cycle uncouples around the fourth decade of life because there is a net loss of bone mass as more bone is lost than is formed. Since, estrogen is a major regulator in the coupling of the BR cycle, in perimenopausal and early postmenopausal women, declining estrogen levels worsen the uncoupling as bone resorption much exceeds bone formation due to exuberant osteoclastic activation and simultaneous inhibition in bone formation due to the reduction of osteoblast viability.[5] A major outcome of increased osteoclast activity is the initiation of the BR cycle at many more bone surfaces than would normally be required, increasing bone turnover which is linked to a loss of Bacheor of Medical Sciences (BMD) and an increased risk of fracture. Another method of treating osteoporosis involves restoring the coupling process, which enables the stimulation of osteoblast activity to correspond with the increased osteoclast function.[6] Various pharmacologic approaches that are in use to prevent these altered events in BR are described subsequently.

3. PHARMACOLOGICALLY VALIDATED TARGETS FOR OSTEOPOROSIS TREATMENT

3.1. Anti-resorptives

Selective estrogen receptor modulators (SERM) that function as either an estrogen agonist or an antagonist, depending on the target tissue have been developed. The chemical structures of many plant constituents resemble estrogen and are called phytoestrogens, which could act as weak estrogens in some conditions. So far, a vast number of literature has accrued on the salutary effects of phytoestrogen on the skeleton.[7] Although these phytoestrogens have a variety of reported mechanisms of action, the predominant mechanism is reminiscent of estrogen in that it inhibits osteoclast function.

Since the advent of bisphosphonates (BPs) in the 1990s, this class of drug has become the first line of anti-osteoporosis therapy. BPs have a high affinity to hydroxyapatite and are preferentially incorporated into active BR sites. Moreover, a subset of BPs that contain nitrogen (alendronate, ibandronate, pamidronate, risedronate, and zoledronic acid) inhibit farnesyl pyrophosphate synthase (FPPS). Inhibition of FPPS prevents the production of FPP and its downstream metabolite, geranylgeranyl pyrophosphate (GGPP).[8] The function and survival of osteoclasts are critically dependent on GGPP as it is necessary for the prenylation of small GTPases like the Ras, Rho, and Rab family proteins.[9] Suppressing osteoclast function by this mechanism, nitrogen-containing BPs protect against osteoporosis, and establish FPPS as a clinically validated target.

Receptor-activator of tumor necrosis factor ligand (RANKL) is a most potent osteoclastogenic cytokine produced by osteoblastic cells, and serves as a clinically validated target for osteoporosis treatment.[10] The lack of biologically active RANKL causes autosomal recessive osteopetrosis which is characterized by an increased bone mass due to a failure in bone resorption.[11] Osteoprotegerin (OPG) is also secreted from the osteoblastic cells, belongs to the tumor necrosis factor (TNF) receptor family, and acts as a decoy receptor for RANKL. A homozygous insertion/deletion in exon 5 of the OPG gene results in truncation of the protein at amino acid 325 and causes a form of juvenile Paget's disease[12] characterized by rapid bone turnover, osteopenia, and increased fracture incidence. The stoichiometry of RANKL and OPG in bone is a critical regulator of osteoclast function and the BR cycle. Estrogen is a strong suppressor of RANKL production, and postmenopausal women have significantly higher levels of circulating RANKL. A neutralizing antibody against RANKL (denosumab) is used for the treatment of postmenopausal osteoporosis.[13]

Cathepsin K is a cysteine protease produced by mature osteoclasts and is responsible for the degradation of type I collagen in bone matrix. Serum cathepsin K level is increased in postmenopausal osteoporosis and BP treatment suppressed it.[14] Loss-of-function mutations of

cathepsin K result in pycnodysostosis, a high bone mass condition.[15,16] These reports were sufficiently compelling for the development of small molecule inhibitors of cathepsin K for the treatment of osteoporosis.[17] Indeed odanacatib, an active site inhibitor of the enzyme completed a phase 3 trial with a positive effect on bone mass in postmenopausal women.[18] Presently, there is no approved drug targeting cathepsin K. However, adverse cerebrovascular effects in a few patients prevented the launching of this drug. It is thus possible that a milder inhibitor from the natural source could be beneficial for bone without the detrimental cerebrovascular effects. Figure 1 shows the schematic representation of the molecular targets that the hormones, cytokines, and drugs regulate in osteoclasts.

3.2. Bone anabolics

Whereas the anti-resorptives address the aspect of heightened bone resorption the bone-forming drugs, also known as bone anabolics address that of reduced bone formation in osteoporosis. The mainstream anabolic strategy is the activation of PTH receptor-1 by teriparatide and abaloparatide that leads to the downstream activation of the canonical Wnt pathway and induces osteogenesis.[19] The low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) is a co-receptor of the canonical Wnt receptor, Frizzled. In humans, recessive loss-of-function mutations in LRP5 causes osteoporosis whereas dominant missense mutations in LRP5 causes disorders of high bone mass. Moreover, sclerostin, a gene product of SOST and a bone-specific antagonist of Wnt signaling also has profound skeletal effects when the SOST gene is mutated. Loss-of-function mutations in the SOST gene give rise to a high bone mass conditions such as sclerosteosis and van Buchem disease.[20-22] These findings firmly establish LRP5 and SOST as clinically validated targets for osteoporosis. Indeed, sclerostin levels are increased in postmenopausal women, and estrogen and PTH suppress it.[23] Taking into account the strong regulatory role of Wnt signaling in skeletal homeostasis, the U.S. FDA recently approved the use of a monoclonal antibody to neutralize the action of sclerostin as the new bone anabolic treatment of osteoporosis. Figure 2 shows the schematic representation of the molecular targets that the hormones and drugs regulate in osteoblasts.

In the following sections, we discuss the efficacy of some acupuncture techniques and TCM that could favorably modulate the molecular targets discussed in the foregoing sections potentially leading to mitigation of postmenopausal osteoporosis.

4. ACUPUNCTURE AND OSTEOPOROSIS

In the TCM, since the kidneys are connected to the generation of marrow, osteoporosis therefore, is primarily attributed to kidney deficiencies. Besides, the spleen, stomach, and liver are also considered important organs in regulating bone function. There are numerous published clinical trials and meta-analyses on the use of acupuncture for osteoporosis yet acupuncturists still have difficulties in identifying the evidence from these studies because the effect is not robust.[24]

Acupoint catgut embedding therapy (ACET) is a

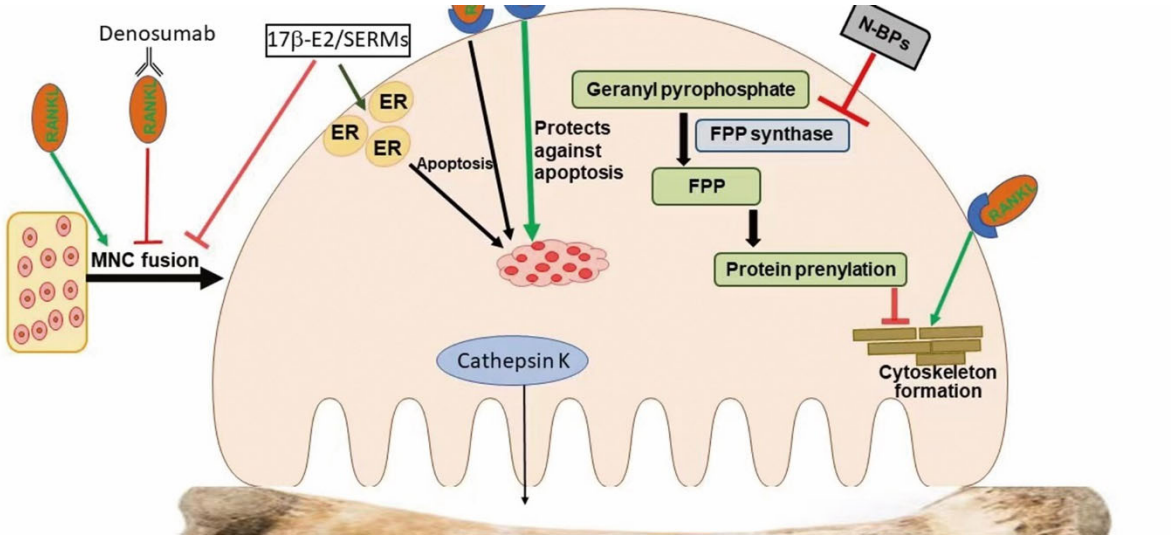

Figure 1 Molecular regulation of osteoclast targets by drugs, hormones, and cytokines

The schematic illustration showing various molecular targets in osteoclasts that are regulated by drugs, hormones and cytokines. Osteoblast produced RANKL stimulates and 17 -E2 or SERMs inhibits the fusion of MNC in the blood that are precursor cells for the formation of osteoclasts. RANKL is a pro-survival factor for mature osteoclasts whereas E2 or SERM induces their apoptosis. Denosumab by depriving osteoclasts of RANKL induces osteoclast apoptosis. N-BPs by inhibiting protein prenylation inhibit the cytoskeleton formation of osteoclasts which is necessary for their adherence to bone surface for resorption. Cathepsin K, a cysteine protease and the major collagenase produced by mature osteoclasts is secreted to the resorption site and degrades bone matrix by digesting type I collagen. RANKL: receptor-activator of nuclear kappaB ligand; MNC: mononuclear cells; N-BPs: nitrogen-containing bisphosphonates; 17β-E2: estradiol; SERM: selective estrogen receptor modulators (raloxifene); FPP: farnesyl pyrophosphate synthase. Refer to table 1 for possible intervention points by acupuncture and Traditional Chinese Medicine discussed in the text.

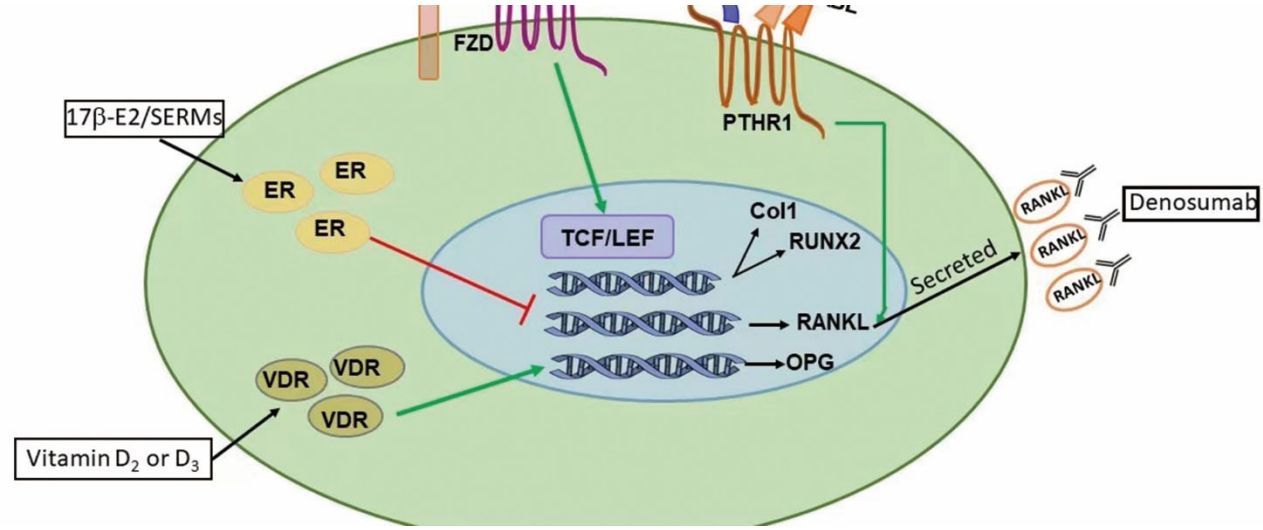

Figure 2 Molecular targets in osteoblasts regulated by drugs and hormones
The schematic illustration showing various molecular targets in osteoblasts that are regulated by drugs and hormones. The bone anabolic drugs TPTD or ABL via the PTHR1 activates Wnt signaling by increasing the production of Wnt agonists Wnt3a and Wnt10B. Activation of Wnt pathway elicits a nuclear event which via the transcription factor TCF/LEF upregulates transcription of osteogenic genes (Runx2 and Col1). TPTD or ABL on the other hand upregulates the production of RANKL. E2 or SERMs *via* the cognate ERs downregulate the transcription of RANKL and vitamin D *via* the VDR upregulates OPG. Denosumab, the neutralizing antibody against the secreted RANKL suppresses RANKL action by neutralizing this osteoclastogenic cytokine. Green arrow: upregulation; red arrow: downregulation. TPTD: teriparatide (1-34 PTH); ABL: abaloparatide (1-36 PTHrP); TCF/LEF: T-cell factor/lymphoid enhancer factor; Col 1: type I collagen; Runx2: runt-related transcription factor 2; OPG: osteoprotegerin (decoy receptor of RANKL); FZD: frizzled receptor (Wnt receptor); LRP: low-density lipoprotein receptor-related protein (Wnt co-receptor). Refer to Table 1 for possible intervention points by acupuncture and Traditional Chinese Medicine discussed in the text.

specialized form of acupuncture therapy that can cause continuous needling effects at specific points through the insertion of the catgut. ACET is a useful therapy for weight management and is widely applied in China, and has similar therapeutic effects as estrogen. ACET has also been tested on postmenopausal women for the treatment of osteoporosis. A meta-analysis of twelve RCTs studied the effect of ACET on postmenopausal osteoporosis. These studies used a variety of acupoints, but the five most frequently used ones were Shenshu (BL23), Pishu (BL20), Zusanli (ST36), Gansu (BL18), and Sanyinjiao (SP6) in accordance to TCM hypothesis that deficiencies in the function of kidney, liver, and spleen are the causes of osteoporosis. To strengthen bone and tendon, the ACET strategy was aimed at "tonifying" these organs. The Back-shu acupoints, Shenshu (BL23) and Gansu, into which the *Qi* of the respective viscera and bowels transports and infuses, are used to treat the diseases of those organs. The liver, spleen, and kidney meridians are connected at Sanyinjiao (SP6), which allows for simultaneous adjustment of all three meridians. The spleen and stomach, which are the foundation of acquired constitution were strengthened by Pishu (BL20) and Zusanli (ST36) acupoints. Combination of these acupoints "harmonized" the effectiveness of these organs towards the improvement of skeletal function. The meta-analysis concluded that ACET although increased serum estradiol, however, alone was not effective in increasing BMD at the lumbar spine and femur neck over those receiving Ca and vitamin D supplementation. When combined with Ca supplementation, however, ACET showed an increased effect on BMD.[25]

Bilateral ovariectomy (OVX) of sexually mature rats or mice serves as the most widely used preclinical model of postmenopausal osteoporosis that are used for understanding mechanism of action of acupuncture. In one study, electropuncture (EA) treatment to OVX rats suppressed OVX-induced increase in body weight, increased body weight-adjusted BMD, decreased urinary resorption marker [deoxypyridinoline or uridine diphosphate glucose (uDPD)] and increased serum bone formation marker [bone-specific alkaline phosphatase (BAP)] over the sham-treated control rats.[26] When acupuncture by needling on Pishu (BL20) and Shenshu (BL23) was performed in OVX rats daily for 15 mins for 16 weeks, trabecular bone restoration along with the restoration of femur strength was observed. The anti-resorptive effect of acupuncture was apparent from the suppression of OVX-induced rise in urinary DPD compared to control OVX rats.[27] In OVX rats, EA stimulation at Guanyuan (CV4) improved bone mass, strength, and turnover markers compared with the OVX rats. Activation of Wnt pathway leading to osteogenic response appeared to be induced by EA as Wnt agonist, Wnt 3a was and the osteoblast transcription factor, Runx2 were upregulated in the bones of OVX rats that received EA in comparison to OVX rats that served as the control.[28] EA treatment at the acupoints of Governor Vessel and Taiyang Bladder Meridian of Foot of OVX rats for 90 maintained bone mass that was accompanied by OPG/RANKL ratio as well as femoral expression of recombinant low density lipoprotein receptor related protein 5 and Runx2 compared with control.[29] Similar effects were also reported in studies with OVX rabbits.[30]

Since OPG is a decoy receptor of RANKL, the trend of increased bone mass appears to be mediated by an anti-resorptive mechanism.

5. MEDICINAL PLANTS FROM TCM AND OSTEOPOROSIS

A recent thorough analysis of the extensive body of literature on herbal TCM's role in bone health and osteoporosis revealed 30 herbs with salutary effects on bone.[31] For the sake of rapid clinical translation, it is crucial to select candidates that have undergone some clinical studies in postmenopausal women and whose action mechanisms resemble those of the clinically used drugs. Based on these criteria, we selected the following three medicinal herbs from TCM.

5.1. Yinyanghuo (*Herba Epimedii Brevicornus*)

Yinyanghuo (*Herba Epimedii Brevicornus*) or horny goat weed is a well-known TCM and is called Yinyanghuo in Chinese. The usage of Yinyanghuo (*Herba Epimedii Brevicornus*) for erectile dysfunction, sexual dysfunction, weak and brittle bones, health issues during menopause, and other ailments is widespread, although none of these claims are well-supported by scientific evidence. In a randomized and placebo-controlled clinical study, Yinyanghuo (*Herba Epimedii Brevicornus*) water extract (25.3% icariin) given to postmenopausal women for 6 months significantly increased serum estradiol but not progesterone and testosterone.[32]

In OVX rats, 28% icariin-rich extract of Yinyanghuo (*Herba Epimedii Brevicornus*) preserved bone mass and increased bone mRNA levels of Runx2, the osteoblast transcription factor. The increase in Runx2 was mediated by the increased production of BMP-2 in osteoblasts, which also increased the synthesis of OPG.[33,34] Icariin had an endoplasmic reticulum (ER)-dependent effect on the osteoblastic OPG/RANKL ratio, and it also upregulates estrogen receptor alpha (ERα) levels, which presumably favoured the prevention of osteoblast transdifferentiation to adipocytes, which are a source of osteoclastogenic cytokines.[35,36] Since icariin is the major constituent of Yinyanghuo (*Herba Epimedii Brevicornus*) extract, and it favorably modulates several clinical targets for osteoporosis, as described above, and this extract has already been assessed in postmenopausal women which attested its safety, osteoporosis trial in these women should thus be conducted.

5.2. Drynariae Rhizoma (DR) from Guanzhong (*Rhizoma Dryopteridis Crassirhizomatis*)

DR is the dried rhizome of Guanzhong (*Rhizoma Dryopteridis Crassirhizomatis*) and is known in Chinese as "Gusuibu," which translates to "bone fracture healer".[37] In TCM, DR is used in postmenopausal diseases including osteoporosis. According to the Chinese pharmacopeia, naringin is a frequently used marker for the authenticity of DR extract, which is also rich in neoeriocitrin.[38] Guanzhong (*Rhizoma Dryopteridis Crassirhizomatis*) improved the lipid profile and increased BMD in postmenopausal women with dyslipidemia. Guanzhong (*Rhizoma Dryopteridis Crassirhizomatis*) inhibited Recombinant NLR Family, Pyrin Domain Containing Protein 3 (NLRP3) inflammasome and inflammatory cytokines by upregulating Sirt1 or decreasing Notch in PBMCs of postmenopausal women. Guanzhong (*Rhizoma Dryopteridis Crassirhizomatis*) inhibited inflammatory cytokines including interleukin (IL)-1, IL-18, tumour necrosis factor (TNF)-, IL-6, and IL-8, all of which are important stimulators of RANKL.[39] The suppression of inflammation and improvement of lipid profile by Guanzhong (*Rhizoma Dryopteridis Crassirhizomatis*) are reminiscent of the actions of estrogen and SERM.

In preclinical studies, DR mitigated the increased bone turnover rate assessed by urinary and serum markers including uDPD, and serum type I collagen cross-linked C-telopeptide (CTX-1), osteocalcin, and carboxy-terminal propeptide of type 1 procollagen (PICP). Furthermore, BMD, trabecular microarchitecture, and bone strength were improved by the extract to the levels comparable to E2 although, unlike E2, DR had no uterotrophic effect. In vitro, DR increased osteoblast proliferation, and differentiation, and increased the expression of ERα and ERβ. It appears that naringin[40] and its glycosylated analog[41] present in DR have bone protective effects in OVX animals through their ER-mediated effects in osteoblasts.

Moreover, increased OPG/RANKL ratio in the osteoblasts by DR appeared to contribute to the inhibition of the osteoclastic function in vivo which resulted in mitigation of bone turnover rate.[42] An ER-dependent enhancement of OPG/RNAKL ratio by flavonoid-enriched extract has also been reported in rat UMR-106 osteoblastic cells, and this effect likely contributed to the maintenance of bone mass and strength in OVX rats.[43] Despite these two studies reporting *in vitro* increase in OPG/RANKL ratio by DR, however, a flavonoid-enriched extract of DR did not increase the OPG/RANKL ratio in long bones of OVX rats but significantly decreased the OVX-induced RANK levels. Because DR activated the Wnt3a/β-catenin pathway that has reciprocal effects on bone cells-stimulates osteoblast function and inhibits osteoclast function, the bone conserving effect is likely mediated by the favorable modulation of the Wnt pathway by the extract.[44] $CaCO_3$ had an additive effect on DR in improving the bone mass in OVX rats which suggested a similar outcome can be anticipated in clinical trials where the experimental drugs are administered with Ca and vitamin D. Estrogen deficient state is marked by inhibition of Ca absorption which leads to hyperparathyroidism and the later condition results in osteoclast activation and bone resorption. Therefore, a synergistic action of DR and Ca supplement is clinically relevant in the treatment of phosphorodiamidate morpholino oligomer (PMO). Through a bioinformatics analysis and computer

simulation approach, the molecular mechanism of bioactive compounds of DR has been predicted that consist of a large number of targets in addition to RANKL and ER, however, their biochemical validation is required.[45]

5.3. Danshen (*Radix Salviae Miltiorrhizae*)

Danshen (*Radix Salviae Miltiorrhizae*) is frequently used in TCM for the prevention and treatment of cardio-cerebral vascular disorders[46] and osteoporosis.[47] Danshen (*Radix Salviae Miltiorrhizae*) is found to have anticoagulant, vasodilatory, anti-inflammatory, free radical scavenging, and mitochondrial protective effects. Twenty-five clinical trials reported the effect of Danshen (*Radix Salviae Miltiorrhizae*) on primary osteoporosis of which 13 were on postmenopausal osteoporosis and 2 on senile osteoporosis. Even though the majority of studies show promising efficacy in enhancing BMD and decreasing pain, their significance is constrained by the small patient sample size (generally >100), the brief duration of the majority of the studies (3 to 6 months), and the heterogeneous efficacy outcome measures used as indicators of treatment success. To ascertain a final therapeutic efficacy, longer-term trials that take fracture rates into account are required.

Phytochemical analysis of Danshen (*Radix Salviae Miltiorrhizae*) found the presence of a tanshinone group of compounds that potently inhibit cathepsin K (29278432). Tanshinone IIA sulfonic sodium, a synthetic derivative of tanshinone, by specific inhibition of cathepsin K and without affecting osteoclastogenesis increased bone mass in OVX mice.[48] Cryptotanshinone, a natural analog of tanshinone present in Danshen (*Radix Salviae Miltiorrhizae*) suppressed the RANKL-induced synthesis of cathepsin K in osteoclast precursors and suppressed bone loss in OVX rats.[49] An aqueous extract of Danshen (*Radix Salviae Miltiorrhizae*) consisting of puerarin analogs, tanshinone analogs, and diadzin analogs protected bone mass in OVX rats by inhibiting osteoclastogenesis but had no effect on bone formation. The aqueous extract however modulated the OPG to RANKL ratio in favor of inhibition of osteoclastogenesis and inhibited cathepsin K-positive area in bones.[50] The effects of the aqueous extract of Danshen (*Radix Salviae Miltiorrhizae*) were replicated in OVX mice with bone mass protection achieved by a pure anti-osteoclastogenic mechanism likely mediated by an increase in OPG and suppression of RANKL production, and decrease in cathepsin K. The extract significantly attenuated the OVX-induced increase in body weight without having an uterotrophic effect which suggested a SERM-like action.[51] Taken together from these reports, the effects of Danshen (*Radix Salviae Miltiorrhizae*) include (a) suppressing cathepsin K expression and function, and (b) suppression of osteoclastogenesis directly and via the osteoblastic increase in OPG/RANKL ratio.

6. PROPOSED COMBINATION

Acupuncture and TCM in combination aimed at suppressing bone resorption and promoting bone formation by considering molecular targets modulated by the individual intervention could yield an effective anti-osteoporosis therapy.

In preclinical studies on OVX animals, EA activated the osteogenic Wnt pathway and OPG, the potent anti-resorptive cytokine. Moreover, in clinical studies on postmenopausal women, acupoint catgut embedding for 3 months increased serum estradiol levels, decreased serum PTH and suppressed bone turnover. These changes are favorable for gain in bone mass if continued for a longer period such as one year or more as observed in cases of clinically used drugs. Moreover, acupuncture combined with icariin-rich extract of Yinyanghuo (*Herba Epimedii Brevicornus*) beneficially modulates several clinically validated targets including the Wnt pathway, neutralization of RANKL via OPG, and increase in estradiol levels. As an icariin-rich extract of Yinyanghuo (*Herba Epimedii Brevicornus*) enhanced serum estradiol without the rise in the compensatory progesterone,[32] this therapy could pose a long-term risk of uterine carcinogenicity. On the other hand, Guanzhong (*Rhizoma Dryopteridis Crassirhizomatis*) extract increased BMD in postmenopausal women and suppressed the inflammatory response of peripheral blood mononuclear cells that are precursors of osteoclasts, thus suggesting its anti-resorptive effect.[39] Therefore, DF extract in combination with acupuncture could have an additive effect on bone mass gain in PMO.

However, Danshen (*Radix Salviae Miltiorrhizae*) appears to be the most suitable candidate for combining with acupuncture due to the following reasons; (a) the safety and tolerability of this extract have been strongly established through 25 clinical studies, (b) 15 studies showed efficacy in postmenopausal osteoporosis and c) the phytochemical contributing to the extract's anti-osteoporosis effect is mediated by tanshinone-II that inhibits cathepsin K (for review[47]). Given this unique mode of action of Danshen (*Radix Salviae Miltiorrhizae*), it could exert a synergistic effect with acupuncture.

7. TRANSLATIONAL APPROACH

Based on the foregoing discussion, double-blind and randomized clinical trials on women with postmenopausal osteoporosis with the intention-to-treat can be conducted using two different combinations; (a) acupuncture and DF and (b) acupuncture and SM. Several study designs could be used to determine the therapeutic efficacy of the combinations at fixed or escalated dosing of the extract with a fixed acupuncture protocol. Women with high-risk of fracture should be recruited for the trials. High risk is determined by the guidelines of the World Health Organization (WHO) or National Osteoporosis Foundation (NOF).[52] WHO guideline recommends that postmenopausal women with BMD values > 2.5 standard deviations below the mean BMD are having osteoporosis and require anti-osteoporosis treatment. To identify women for treatment

the NOF recommends a *t* score cut off −2.0 for women with no risk factors and −1.5 for women with prior fragility fractures. Ideally, women who have not taken any prior anti-osteoporosis therapy should be included. Patients with a history of metabolic or bone diseases (except osteoporosis), current hyper- or hypocalcemia, current uncontrolled hyper- or hypothyroidism, current uncontrolled hyper- or hypoparathyroidism, and secondary osteoporosis should be excluded from the study. All participants in the placebo and treatment groups should receive 1200 mg calcium and 800 IU vitamin D daily during the entire study period.

For a proof of concept, change in total hip BMD between month 0 and month 12 should be considered as the primary outcome measures. The percentage of participants with new vertebral fractures through 12 months could be taken as a secondary outcome measure. Initially, comparative efficacy and safety studies have to be conducted in which the number of participants with adverse events for the entire duration of the study has to be recorded.

8. SUMMARY AND CONCLUSION

This review describes the action mechanisms of acupuncture and three TCMs that target clinically relevant molecules for the treatment of postmenopausal osteoporosis. Table 1 shows the list of clinically validated targets of osteoporosis for pharmacologic manipulation. These targets are the osteogenic Wnt pathway and anti-osteoclastogenic RANKL-OPG and cathepsin K pathways. The Wnt pathway is stimulated by teriparatide, the peptide that promotes bone formation, and RNAKL is neutralized by denosumab, the antibody that inhibits bone formation. Teriparatide while stimulating bone formation also triggers bone resorption and denosumab suppresses both bone resorption and formation. Acupuncture and TCM discussed here has the dual effect of stimulating bone formation and inhibiting bone resorption, which is a novel mechanism for treating PMO. These treatments achieve the dual role by stimulating Wnt signaling and inhibiting RANKL and cathepsin K and have the potential to act synergistically when combined e.g. acupuncture and MS. Sclerostin, a Wnt antagonist produced by osteocytes is another clinically validated target against which romosozumab, neutralizaing antibody has been developed. It would be interesting to test acupuncture that activates Wnt pathway could simultaneously suppress sclerostin. Since postmenopausal women have higher circulating levels of sclerostin, this Wnt antagonist could be conveniently measured to compare its levels between the placebo and treatment arms.

Since acupuncture and the three TCMs discussed here have all been tested in postmenopausal women for evaluating their efficacy in preventing osteoporosis or ameliorating menopausal symptoms, the critical discussion on their action mechanisms will help in designing rationale combination of these traditional therapies for the treatment of PMO. Given that acupuncture and TCMs have been reported to increase the estradiol levels in postmenopausal women, it is important to pay particular attention to uterine safety of these treatments.

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Table 1 Differential molecular targets of acupuncture and Traditional Chinese Medicine used in postmenopausal women concerning their skeletal effects

Treatment type	Wnt pathway	/receptor activator of NF-KB ligand/osteoprotegerin ratio	Serum Estradiol levels	Cathepsin K
Acupuncture	Stimulates Wnt 3a	Suppresses the ratio	Increases	-
Yinyanghuo (*Herba Epimedii Brevicornus*)	-	Suppresses the ratio	Increases	-
Guanzhong (Rhizoma Dryopteridis Crassirhizomatis)	Stimulates Wnt 3a	Suppresses the ratio	-	-
Danshen (*Radix Salviae Miltiorrhizae*)	-	Suppresses the ratio	-	Suppresses expression and function

Notes: -: data not available.

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