INTRODUCTION

Bone remodeling occurs throughout life via synthesis of bone matrix through the action of two major bone cells: osteoblasts and osteoclasts [1-3]. Osteoclasts are responsible for bone resorption, while osteoblasts are responsible for bone formation [4,5]. The proper functioning of these cells is necessary for the maintenance of bone mass as well as bone mineral density (BMD). During old age and especially postmenopause there is excessive bone resorption relative to bone formation due to hormone deficiencies, which reduces bone mass and ultimately causes bone diseases and osteoporosis [6,7].

Osteoporosis is a widespread health dilemma and its occurrence is projected to rise in the upcoming decades due to the aging of many societies [8]. It is a bone disease that is thought to cause stumpy bone mass and microarchitectural weakening of bone materials, enhance bone brittleness, decrease bone strength, and subsequently increase the threat of fracture [9]. Osteoporosis causes distress throughout the United States, Europe, China, Japan and the rest of the world [10]. According to the World Health Organization, almost 75 million people in the United States, Europe, and Japan are affected by osteoporosis [11].

TYPES OF OSTEOPOROSIS

There are two main types of osteoporosis: primary osteoporosis and secondary osteoporosis. Primary osteoporosis is usually associated with aging and low levels of reproductive hormones, especially estrogen, which leads
to reduced gonadal activity in the elderly. Secondary osteoporosis has other causes, including deficiencies of calcium, vitamin D, and parathyroid hormone [12].

**PATHOPHYSIOLOGY OF OSTEOPOROSIS**

An understanding of the molecular mechanisms and anabolic and catabolic activity of bone is important in the development of new drugs for treating bone diseases, especially osteoporosis. For osteoporosis, the search for new therapeutic agents is mostly concerned with the control of bone resorption and the induction of bone formation by osteoclasts and osteoblasts, respectively [12]. The main molecular factors involved in osteoporosis are the receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) and the receptor activator of nuclear factor kappa-B (RANK).

**OSTEOCLASTOGENESIS/OSTEOCLAST DIFFERENTIATION**

Osteoclasts are multinucleate and highly specialized giant cells that have the ability to work in perfect synchronization with osteoblasts to retain the skeletal system. They are produced from monocytic precursors. On completion of bone formation, they migrate into the bone marrow [13,14].

The discoveries of the RANKL and OPG have altered our understanding of the primary mechanisms involved in osteoclast differentiation and activity. RANKL, a transmembrane protein, belongs to the tumor necrosis factor superfamily member 11, and a ligand for OPG is produced by osteoblast and stromal cells [15–17]. RANKL binds with the membrane bound protein RANK that is expressed by osteoclasts (OC) with the combination of tumor necrosis factor (TNF)-receptor-associated

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Fig. 1. Osteoclastogenesis. Proposed model of a ginsenoside inhibiting the binding of receptor activator of nuclear factor kappa-B ligand (RANKL) to receptor activator of nuclear factor kappa-B (RANK) and melatonin receptor type 1A, matrix metallopeptidase (MMP) by binding with RANK or RANKL. This decreases the response of RANKL, reduces the induction of MMP-9, and blocks the RANKL, RANK signaling pathway. RANKL binds to RANK, so tumor necrosis factor-receptor-associated factor-6 (TRAF6) binds to RANK and plays a key role in osteoclast differentiation by regulating and activating downstream signaling pathways, such as the nuclear factor-kappa B (NF-κB) pathway, the inhibitor of NF-κB kinase (IKK) pathway and the p38 pathway. These pathways ultimately prop up osteoclast differentiation and bone resorption by stimulating different transcriptional factors such as activator protein-1 (AP1) and NF-κB pathways. It is not clear how these factors are activated by TRAF6 and cause bone resorption by activating osteoclast specific markers, such as tartrate-resistant acid phosphatase (TRAP), cathepsin (CTSK), β3 integrin and calcitonin receptors (CTR). MMPs, and in particular MMP-9, are responsible for bone resorption, which is extremely articulated in osteoclasts, are stimulated by the action of RANKL signaling pathways and influence osteoclast differentiation and bone resorption [18]. C-K, compound K; PNS, Panax notoginseng saponins; OPG, osteoprotegerin; NIK, NF-kappa-B-inducing kinase; MEK, mitogen-activated protein kinase kinase; AMPK, AMP-activated protein kinase; IKK, inhibitor of nuclear factor kappa-B kinase; PKD, protein kinase D; NFAT, nuclear factor of activated T-cell.
factor-6 (TRAF-6) and activates different downstream signaling pathways (Fig. 1) [18]. Consequently, it plays an important role in osteoclast differentiation and bone resorption, which leads to osteoporosis [19].

Osteoblasts secrete a natural soluble decoy receptor for the RANKL. OPG belongs to tumor necrosis factor receptor superfamily member 11B, which has the ability to restrain osteoclast differentiation/formation by preventing RANKL from binding to RANK. Hence, stability and balance between RANKL and OPG is necessary for osteoclast activity [20,21]. The most important factor in osteoclast activation is a protein known as RANKL, which brings these osteoclasts and osteoblasts in contact by inserting itself into the external membrane of osteoclasts, thereby triggering its receptor RANK, which is then responsible for bone resorption. The coupling of RANKL to RANK is facilitated by the nuclear factor (NF)-κB signaling pathway [19].

The TRAF family of proteins (TRAF, 2-6) comprises the most significant factors involved in osteoclastogenesis when RANKL binds to RANK. A pivotal role is played by TRAF6 via the regulation and activation of downstream signaling pathways, such as the NF-κB pathway, the inhibitor of NF-κB kinase pathway, the c-Jun N-terminal kinase (JNK) pathway, and the p38 pathway. These pathways ultimately prop-up osteoclast differentiation and bone resorption by stimulating different transcriptional factors such as activator protein-1 and the NF-κB pathways. However, it is not clear how these factors are activated by TRAF6 or how they lead to bone resorption by activating osteoclast-specific markers such as tartrate-resistant acid phosphatase (TRAP), cathepsin, β3 integrin, and calcitonin receptors [21-23]. Recently it has been suggested that, among the proteases, matrix metalloproteinases (MMPs) are responsible for the deprivation of bone extracellular matrix. In particular, MMP-9 is strongly expressed in osteoclasts since it is stimulated by the action of the RANKL signaling pathway and it plays a vital role in bone resorption. At the molecular level, however, little is known about how RANKL induces MMP-9 gene stimulation [24].

**OSTEOBLASTOGENESIS/OSTEOBLAST DIFFERENTIATION**

Osteoblasts are mononucleate cells that are derived from mesenchymal stem cells, which have the ability to suppress osteoclast activity and increase bone formation by secreting a bone mineralization organic matrix such as collagen-I (Col-I) in the osteoid [25]. In osteoblast differentiation, the Wnt canonical signaling pathway and the bone morphogenetic protein (BMPs) pathways are
Table 1. Effects of ginseng on different molecular pathways related to osteoporosis in cell line and animal studies

<table>
<thead>
<tr>
<th>Ginsenosides</th>
<th>In vitro/in vivo</th>
<th>Molecular mechanism</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Rh2 (S)</td>
<td>MC3T3-E1</td>
<td>↑ mRNA expression ALP, OCN, OPN</td>
<td>[36]</td>
</tr>
<tr>
<td>Rh2 (R)</td>
<td>RAW264.7</td>
<td>↓ OC activity and bone resorption</td>
<td>[37]</td>
</tr>
<tr>
<td>Rb1</td>
<td>RAW264.7</td>
<td>↓ RANKL, NF-κB, JNK and p38 MAPK, specific transcription factors (c-Fos and NFATc1), reduce OCs and bone resorption</td>
<td>[38]</td>
</tr>
<tr>
<td>Rd</td>
<td>MC3T3-E1</td>
<td>↑ BMP-2 secretion, AMPK phosphorylation, ALP, OCN &amp; Col-I</td>
<td>[39]</td>
</tr>
<tr>
<td>Rh2(S)</td>
<td>MC3T3-E1</td>
<td>↑ mRNA expression ALP, OCN, Oex &amp; Col-I</td>
<td>[40]</td>
</tr>
</tbody>
</table>

- Mouse bone marrow cells: ↑ c-Fos, NFATc1, Bone resorption
- Bone marrow stromal cells: ↑ ALP, OPG, BSN, cbfa1
- Bone marrow stromal cells: ↑ mRNA level of ALP, BSN, cbfa1,ERK, p38 phosphorylation
- Bone marrow stromal cells: ↓ mRNA level PPARγ2
- Mouse bone marrow cells: ↑ Osteoestrogenesis by blocking RANKL activity
- Bone marrow stromal cells: ↑ Bone formation
- Bone marrow stromal cells: ↑ Bone loss
- Bone marrow stromal cells: ↑ Secretion of PPARγ2
- Bone marrow stromal cells: ↑ BMSCs proliferation, ALP, Runx2, OC, and BSP

- Bone marrow stromal cells: ↑ BMP-2 secretion, AMPK phosphorylation, ALP, OCN & Col-I
- Bone marrow stromal cells: ↑ mRNA expression ALP, OCN, Oex & Col-I
- Bone marrow stromal cells: ↑ PKD/p38 phosphorylation
- Bone marrow stromal cells: ↑ Osteoestrogenesis by blocking RANKL activity
- Bone marrow stromal cells: ↑ Bone formation
- Bone marrow stromal cells: ↑ Bone loss
- Bone marrow stromal cells: ↑ Secretion of PPARγ2
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- Bone marrow stromal cells: ↑ mRNA expression ALP, OCN, Oex & Col-I
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BMP, bone morphogenetic protein; RANKL, receptor activator of nuclear factor kappa-B ligand; NF-κB, nuclear factor kappa-B; JNK, c-Jun N-terminal kinases; AMPK, AMP-activated protein kinase; Nfatc1, nuclear factor of activated T-cells, cytoplasmic 1; BMP-2, bone morphogenetic protein 2; PNS, Panax notoginseng saponins; OPG, osteoprotegerin; cbfa1, core binding factor alpha-1; PPARγ2, peroxisome proliferator-activated receptors γ2; ERK, extracellular signal-regulated kinases; BMD, bone mineral density; cAMP, cyclic adenosine monophosphate; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; TRAP, tartrate-resistant acid phosphatase; NK, natural killer cells; iNOS, isoform nitric oxide synthases; NO, nitric oxide; OVC, ovariectomised rats; DPD/Cr, urinary deoxypyridinoline/creatinine; BV/TV, trabecular bone volume over total bone volume; Conn.D, connectivity density; Tb.N, trabecular number; Tb.Th, trabecular thickness; BMSCs, bone marrow stromal cells; Runx 2, runt-related transcription factor 2; BSP, bone sialoprotein.

The most pivotal role of the BMPs is to the transforming growth factor (TGF)-β superfamily and BMP-2 plays an especially significant role in osteoblastogenesis. When BMP-2 binds to a transmembrane protein such as a bone morphogenetic protein receptor II, it phosphorylates type I receptor, and hence activates the Smad complex (Smad 1, 4, 5, and 8) signaling pathways [26–29], which can aid in the activation of osteoblast-specific transcriptional regulation genes involved in bone formation, such as osteocalcin (OCN), collagen type I, osteonectin, osteopontin, osterix, and bone sialoprotein (BSP) (Fig. 2). Recently it has been suggested that retinoblastoma binding protein 1 may be the co-activator of runt-related transcription factor 2 (Runx2) [30,31].

Different therapeutic agents have been used for the treatment, management, and prevention of osteoporosis, including denosumab, bisphosphonate, raloxifene, calcitriol, teriparatide, strontium ranelate, and hormone replacement therapy, but these drugs may have side effects. Recently, it has been found that bisphosphonate causes esophagitis, esophageal cancer, and atypical femur fractures when it is used for more than five years. Similarly, it has been suggested that hormone replacement therapy may cause breast cancer, coronary heart disease, stroke, and venous thromboembolism [32–35].

Modern pharmacological therapy is costly and produces many side effects, resulting in significant patient non-compliance. Thus, there is a strong need to explore...
alternative therapies, particularly from herbal sources as these are cost-effective and have minimal side effects. Over the past decades, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play a central role in the healthcare of much of the world population.

Panax ginseng Meyer commonly known as Korean ginseng belongs to the family Araliaceae. The name, ginseng, is derived from the Chinese word “rénshēn,” which means “man root.” The plant is native to Asia and North America. As much as 2000 years ago the roots of American ginseng (P. quinquefolius) and Korean ginseng (P. ginseng Meyer) were traditionally used for the treatment of many diseases [53,54]. Among the 15 Panax species, 4 different species include P. ginseng, P. notoginseng, P. japonicus, P. quinquefolius were used commercially. The Korean ginseng can be divided into nine cultivars and three lines.

The ginseng plant contains many active ingredients, including steroidal saponins with a dammarane skeleton as well as protopanaxadiol and protopanaxatriol, commonly known as ginsenosides, which is the energetic part of glycoside plus an aglycone. Recently 128 ginsenosides have been identified from P. ginseng [55].

Although ginsenosides have been reported to possess antioxidant, anti-cancer, anti-diabetic, anti-adipocyte, and sexual enhancement properties, very few studies have been conducted on their anti-osteoporotic activity [56-60]. Cell and animal studies of the anti-osteoporotic activities of different ginsenosides have been conducted. Protopanaxadiol-type saponins (Rb1, Rg3, Rd, and Rh2), protopanaxatriol-type saponins (Re and Rg1), P. notoginseng saponins (PNS: Rg1, Rb1, and R1), and ginseng mixtures have been reported to show anti-osteoporotic activity in these studies (Table 1).

Even though the exact mechanisms of ginseng’s anti-osteoporotic effects are not fully understood, in vitro and in vivo data suggest three possibilities: 1) modulation of osteoblastogenesis and bone formation, 2) modulation of osteoclastogenesis and bone resorption, and 3) modulation of osteoporosis. It is obvious from the Table 1 that Rh2, Rd, Rh2(S), and (TR) ginsenosides stimulate the secretion and phosphorylation of alkaline phosphatase (ALP), OCN, osteopontin, osterix, Col-I, BMP-2, protein kinase D (PKD)/AMP-activated protein kinase, PKD/p38, and cAMP and increase bone mineral density [36,39,40,44]. Similarly, He et al. [42] and Li et al. [43] demonstrated that P. notoginseng has the ability to affect the mRNA level of ALP, BSP, core-binding factor subunit alpha-1, and OPG, increases phosphorylation of extracellular-signal-regulated kinases and p38, and inhibits the secretion of peroxisome proliferator-activated receptors γ (PPARγ2) and RANKL. On the other hand, ginsenosides Rh2(R), Rb1, Rh2, and Rg1 may have the ability to suppress the secretion and activity of osteoclasts, RANKL, NF-κB, JNK, c-Fos, NFAc1, and TNF-α, interleukin (IL)-6, and lipopolysaccharide [37,38,41,45]. Monroe et al. [46], Kim et al. [47], and Yovel et al. [48] suggest that red ginseng acidic polysaccharide can increase the tumoricidal activity of isofom nitric oxide synthases of natural killer cells in ovariectomised rats (OVX), while it may increase TNF-α, nitric oxide, and IL-1 expression by peritoneal macrophages. However, calcium and hydroxyproline secretion in the urine of male Wistar rats was significantly suppressed by treatment with a liquid extract from Siberian ginseng (Eleutherococcus senticosus), while also increasing the strength of femoral diaphyses and vertebrae [49]. According to Kim et al. [50], the ginseng mixture (HER-S) has the ability to induce the deposition of BMD, increase femoral trabecular width and estrogen levels, and decrease TRAP activity in OVX. Similarly Shen et al. [51] and Li et al. [52] reported that PNS decreased the loss of BMD, PPARγ2, microstructure corrosion in trabecular bone, and urinary deoxypyridinoline/creatinine, while increasing trabecular bone volume over total bone volume, connectivity density, trabecular number, trabecular thickness, alkaline phosphatase, Runx2, OC, and BSP levels in bone marrow stromal cells. Kim et al. [61] found clinically that treatment of postmenopausal osteoporosis patients with Korean red ginseng together with maltose capsules had no significant consequences.

CONCLUSION

Ginseng that is used as a folk and conventional medicine for the treatment of different diseases for decades has been recognized as one of the valuable source in drug discovery and development. The fundamental of ginseng’s ability is its pharmacological active ingredients called ginsenosides. Ginseng has widespread pharmacological beneficial activities and mechanism of action. Many studies demonstrate that ginsenosides can decrease osteoporosis by inhibiting production of NF-κB, stimulate ALP, Col-I, Runx2, increase blood circulation, and enhance memory [42]. Furthermore many studies indicate that ginsenosides have a huge number of activities in both pathological and physiological circumstances concerning with bone disease. However the effects and...
mechanisms of action of ginsenosides are still not yet entirely understood.

Data from in vitro and in vivo studies have revealed that ginseng saponins (ginsenosides) have beneficial effects in the treatment of osteoporosis and may increase the osteogenesis of bone marrow stromal cells and pre-osteoblast cells. Even though some ginsenosides have been studied for the treatment of osteoporosis, their functions at the pharmacokinetic and pharmacodynamic levels are not well understood, and are important for understanding the inhibition of bone resorption and osteoclastogenesis. Future studies about osteoporosis with ginsenosides should include detailed mode of action and mechanisms both in vitro and in vivo.

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