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The Potential of Soy Isoflavones (*Glycine max*) and Magnetic Hydroxyapatite Nanoparticles as Osteoporosis Therapy for Menopausal Women

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ABSTRACT

Background: Menopausal women experience amenorrhea for 12 consecutive months. In Indonesia, one of four women aged 50 – 80 years has a risk of osteoporosis. This age range is related to the menopause phase. Low estrogen levels in menopausal women cause decreased bone mineral density resulting in osteoporosis. Osteoporosis is a degenerative disease characterized by decreased bone density and bone strength that causes fractures. This literature review aims to determine the potential of soy isoflavones (*Glycine max*) and magnetic hydroxyapatite nanoparticles as alternative therapies for osteoporosis in menopausal women. **Reviews:** Soy isoflavones are phytoestrogens because they have estrogen-like structures and functions. Some studies explain that phytoestrogens have benefits in osteoporosis therapy by maintaining bone density through decreasing osteoclast resorption and stimulating osteoblasts. Consuming enough soy supplements everyday has been shown to increase bone mineral density. This effect is strengthened by coating the soy isoflavones with magnetic hydroxyapatite nanoparticles through two mechanisms, as a therapeutic agent and drug carrier. The magnetic field of the magnetic nanoparticles produces force and torque to increase the temperature until 42°C and trigger organelles movement, causing apoptosis of osteoclasts. Hydroxyapatite is a major component of bone mineral that replaces bone tissue deformity and has an osteoblastic effect in bone regeneration. Nano-sized hydroxyapatite will increase material properties and stability in high temperatures. However, hydroxyapatite has less magnetic activity so this function is completed by magnetic nanoparticles. **Summary:** Therefore, the combination of soy isoflavones and magnetic hydroxyapatite nanoparticles works synergistically as an alternative therapy for osteoporosis in menopausal women.

Keywords: Soy Isoflavones, Magnetic Hydroxyapatite Nanoaparticles, Osteoporosis, Menopausal women

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INTRODUCTION

Menopausal women are women who do not experience menstruation (amenorrhea) for 12 consecutive months, determined retrospectively and are the permanent end of menstruation.[1] In menopausal women, there is a decrease in the hormone estrogen which should have a protective role against bones so that they are more susceptible to osteoporosis. Osteoporosis is a degenerative disease characterized by low bone mass density, disruption of bone microarchitecture or mineralization and decreased bone strength.[2] This process is influenced by the balance of osteoblast and osteoclast activity. If there is interference, the bones can become porous and easily fracture.[3]

The prevalence of age-related bone loss is still high. According to data from the Indonesian Ministry of Health, 1 of 4 women in the age range of 50–80 years has a risk of osteoporosis. Besides, the incidence of osteoporosis is 4 times greater in women than men.[4] Every year, 200 million people with osteoporosis experience fracture.

The composition of osteoporosis cases consists of women of childbearing age by 30%, menopausal women 40%, and men 30%.[2] Osteoporosis caused by menopausal period is referred to as primary osteoporosis. Some risk factors for osteoporosis are age, white or Asian race, low body mass index (BMI), family history, low calcium intake, smoking, alcohol, and low physical activity.

Current guidelines still suggest bisphosphonate as the first-line therapy, but gastrointestinal adverse effects such as nausea, vomiting, abdominal pain, dyspepsia, esophagitis, and reflux, may be found after this drug administration. A less common but surprising adverse effect that may emerge is the fracture of the jaw, in contrast with the benefit of reducing vertebral and nonvertebral fracture.[5] Seeing that there is still a lack of osteoporosis therapy, an alternative strategy is needed to reduce mortality and morbidity in menopausal women. A combination of natural ingredients and the latest technology is expected to overcome this problem.

METHOD

The method used in the preparation of this paper by searching literature through searching using online searches with instruments Pubmed, ScienceDirect, and Google Scholar. The keywords used are soy isoflavones, hydroxyapatite magnetic nanoparticles, osteoporosis, and menopausal women with English and Indonesian publications in the last 10 years, obtained 40 literatures that are considered relevant. Level of evidence 1A2C determined based on the classification issued by Oxford Centre for Evidence-based Medicine Level of Evidence.[6]

REVIEW

Soy Isoflavones (*Glycine max*) as Osteoporosis Therapy

Antioxidants are substances needed to neutralize free radicals in the body. These free radicals can cause oxidative stress so can accelerate the occurrence of degenerative diseases.[7] Actually, the body can produce antioxidants through several antioxidant mechanisms, but the amount can be less than needed because humans tend to be often exposed to pollutants caused by depletion of the ozone layer, cigarette smoke, and other air pollution agents. In addition, physical stress such as over-exercises can also increase the number of reactive oxygen species (ROS) so the body needs exogenous antioxidants.[8]

Soybeans have antioxidant effects due to flavonoid content. Isoflavones, flavonoid group, has the most optimum absorption and bioavailability.[9] Isoflavones are found in nuts, especially soybeans. Isoflavones have 4 forms, there are aglycone (non-sugar), glycosides, acetal glycosides, and malonyl glycosides. Among these forms, aglycone isoflavones have the highest antioxidant activity to neutralize free radicals and are easily absorbed by the small intestine.[10] The isoflavone content in soybeans is dominated by genistein and daidzein from the aglycone group.[11]

Isoflavones are bioactive compounds that have functional structures and activities such as estrogen that are referred to as phytoestrogens.[10,12] Phytoestrogens have

health benefits due to contain high antioxidant, which can prevent several diseases such as breast cancer, colon cancer, osteoporosis, and other degenerative diseases.[10,12–14] In postmenopausal women, the hormone estrogen in the body decreases which results in an increased risk of bone remodeling imbalances that are dominated by osteoclast activity, thereby reducing mineral density in the bone which will cause osteoporosis or even fracture.[15] Phytoestrogens are proposed as an effort to solve the problem of decreasing the hormone estrogen in postmenopausal women which has an impact on health, especially osteoporosis.[12]

The estrogen has a direct effect on the activity of osteoblasts, osteocytes, and osteoclasts through estrogen receptors.[16] Genistein and Daidzein of phytoestrogens have a higher estrogenic ability to inhibit bone mass reduction.[13] The basis of the protective role of isoflavones in maintaining postmenopausal women bone density. This is related to the affinity of phytoestrogens for estrogen receptors β (ERβ) which determine the ability to inhibit osteoclast resorption activity and stimulate osteoblasts.[14]

Phytoestrogens stimulate osteoblasts by producing c-fos gene, a proto-oncogen as a activator transcription, and core binding factor (c-bfa) genes that work to differentiate mesenchymal cells and osteoblast progenitor cells develop into mature and functional osteoblast cells.[17] This maturation is also assisted by the Indian Hedgehog (IHH) which is a pathway to regulate cartilage growth. IHH will help the differentiation process of various stage of osteoblasts, including regulating the transition from osteoprogenitors to functional osteoblasts.[18] In addition, osteoblasts that have been formed will produce collagen and glycosaminoglycans (GAG) which help the osteoblasts themselves in the formation of new bones (bone formation). Estrogen has the ability to inhibit bone resorption by inhibiting the maturation of osteoclasts. Osteoblast progenitor cells secrete receptor activator of nuclear factor (RANK) and osteoclast differentiation factor (ODF) that form active bonds with RANK in osteoclast progenitor cells. This bond will cause the maturation of osteoclasts to be functional. At the same time, estrogen produces osteoclastogenesis or osteoprotegerin (OPG) inhibitors which will bind to RANK ligands so osteoclast formation does not occur. Estrogen will increase osteoprotegerin secretion which will be related to the RANK ligand in inhibiting bone resorption.[17]

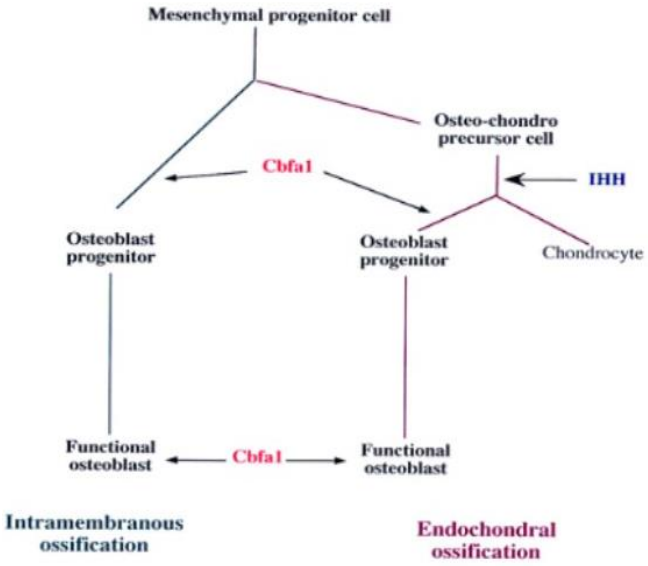

Figure 1. The Role of the Cbfa-1 Gene in Maturation of Osteoblasts and Osteoclasts[17]

Bone mineral density (BMD) is a marker to determine the activity of bone remodeling. Lower BMD indicates higher bone remodeling activity and increases the risk of fracture. Urine deoxypyridinoline (DPD), urinary calcium and N-telopeptide (NTX), and type I collagen cross-linked C-telopeptide (CTX) are bone resorption markers. If the bone resorption activity so high, it will increase the amount of urine DPD, NTX, and CTX.[19]

In a meta-analysis study by Pan Wei, daily consumption soy isoflavones for 2 months - 2 years increased BMD by 54% and decreased bone resorption marker urinary DPD by 23% compared to baseline.[20] This is also supported by Taku's meta-analysis study which stated that daily consumption 56mg of isoflavones for 12 months decreased bone resorption marker urinary DPD 14.1% compared to placebo. This meta-analysis study is devoted to menopausal women. Overall, the effect of isoflavone consumption on urinary DPD

decreased significantly by 18.0% compared to placebo.[19] Another meta-analysis study conducted by Taku showed that daily consumption an average of 82 (47–150)mg of soy isoflavone for 6-12 months increased spine BMD by 22.25mg/cm^2 compared to the control group.[21]

Isoflavones that enter the body will be reabsorbed in the small intestine to form Equol. Equol is produced from the metabolism of daidzein by hydrolysis of β-glucosidase. This hydrolysis begins by the action of brush border membrane. Until now, there is no proven data show about the bacteria that produces equol. Equol has high estrogenic and antioxidant activity. In plasma, equol will maintain its form for a longer time, more stable, and more easily absorbed than genistein dan daidzein form.[22] Therefore, the presence of equol will increase the effectiveness of isoflavones and prevent osteoporosis.

A prospective observational study conducted by Yoshikata in Japan involved 74 middle-aged (44–74 years old) Japanese women to investigate the effect of equol on bone. In the study, participants received a 10mg/day equol supplement for 12 months. In the study showed that urinary NTX decreases after equol intervention.[23] Another study showed that a daily consumption of 10mg equol for 12 months showed a reduction in urinary DPD compared with the placebo group.[24] This suggests that equol as a metabolite of daidzein can inhibit osteoclast formation.

A study from Tousen describes the effect of equol on bone mineral density. The study involved 98 postmenopausal Japanese women who were divided into 4 groups, the placebo group, 2 mg/day equol supplementation group, 6 mg/day equol supplementation group, and 10mg/day equol supplementation group. The study showed that there was a significant difference in BMD after consuming 10mg/day equol supplementation for 12 months, which was 1.040 g/cm^2 compared to the placebo group of 0.994mg/cm^2.[24] The effect of soy isoflvone and its metabolite (equol) in BMD and bone resorption markers was robust, including in a menopausal women.

Magnetic Nanoparticle as Osteoporosis Therapy

To potentiate the isoflavone role in preventing osteoporosis progression in menopausal women, nanoparticles might be added for a more effective drug delivery system. This addition is due to degradation reduction in order to deliver the right amount of the substance to the target area. There are many types of beneficial nanoparticles evidenced in hormone replacement therapy, such as ceramics, metals, polymers, and composites which promote osteoblasts and osteointegration. The two types that have been proposed as novel osteoporosis treatment are the magnetic and hydroxyapatite nanoparticles. Magnetic nanoparticles are ever used in nanostructured bisphosphonate (Bis)-conjugated iron (II, III) oxide (Fe_3O_4), while hydroxyapatite nanoparticles are used in nanostructured risedronate/ zinc-hydroxyapatite.[25]

A nanoparticle is a term for a particle that has a size of less than 100 nm.[26] The bone structure is composed of nano-sized components that using a nanoparticle to target this site is no longer an impossible mission. Nanoparticle might protect the substance from the degradation process and establish a better pharmacokinetic and pharmacodynamic. Nanoparticle also preserves the delivery agent side effects, such as inflammation or uncontrolled bone formation.[27]

The most important molecule used in a magnetic nanoparticle is the ferum oxide (Fe_3O_4). Thermotherapy is the mechanism used in this process. High temperature is applied to demolish the osteoclast because when the molecule is ingested by the osteoclast, the Fe_3O_4 will induce a thermolysis process. Moreover, this molecule is relatively stable, non-toxic, and efficiently affordable.[26]

Thermotherapy utilizes a magnetic field to produce molecule movement and thus produce energy. This process is called magnetic hysteresis. To reach an effect to destroy a cell, the temperature must reach 42°C. Moreover, mechanical force or torque is also produced from a change of magnetic field, usually in a form of superparamagnetic and isotropy. Changes in the magnetic field

make organelles movement and induce apoptosis. Associated with the function to induce nanoparticle magnetic movement, the right amount of external magnetic stimulation is needed to transform motion energy into heat energy. Electron Spin Resonance (ESR) is used to overcome this problem. The ESR signal intensity tested in human blood for 15 minutes is 3.00×10^8 a.u. (arbitrary unit). The magnetic nanoparticle is also able to induce the permeabilization of eight lysosome membranes. Reactive oxygen species are resulted to make the apoptosis of osteoclast.[28] With a reduction of osteoclast cell number, this nanoparticle gives authority to osteocytes to make new bone tissue. This makes the magnetic nanoparticle has a therapeutic role.

Each magnetic element maintains its temperature around the Curie point. Curie temperature is the temperature when a magnet loses its magnetic properties. After reaching that temperature, the molecules stop to move and the magnetic temperature will drop. However, after the temperature drops, the molecules regain their magnetic property and the cycle will go on. The temperature that can induce apoptosis of the target cell without harming other cells is 42°C. To regulate the Curie temperature of Fe_3O_4 at this temperature, it is necessary to add metallic elements such as Mn and Zn. By adding the correct proportion of Mn and Zn, the form of Mn_xZn_1-xFe_2O_4 (Mn Zn ferrite) can convert the motion energy produced from magnets into thermal energy when it has not reached the Curie point of 42°C. Mn Zn ferrite nanoparticles can control temperature under the stimulation of external magnetic fields. In addition, Mn Zn ferrite has an advantage in magnetic thermotherapy, which has good biocompatibility and can effectively kill target cells without injuring other cells.[29]

Hydroxyapatite Nanoparticles as Osteoporosis Therapy

Low esterogen levels in menopausal women cause a decrease of mineral bone density. Consequently, fracture occurs easily either due to minor or major trauma. Calcium supplementation is the therapy that usually used. Hydroxyapatite is a type of calcium that is used.[30] Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is a major component of bone mineral.[31] Hydroxyapatite is included as calcium phosphate which contains hydroxide with calcium and phosphate ratio of 1.67. The hydroxyapatite crystal phase is the most stable phase, with a hexagonal (closed-packed) structure.[32] Hydroxyapatite is an important material in the medical field needed for bone regeneration, bone and dental implants, also orthopedics. The material is biocompatible which can be applied directly to the body and it is non-toxic, so it does not cause an inflammatory and irritating response.[3] Hydroxyapatite also has good biocompatibility and bioactivity related to cells in bone and tissue. That is because the components are the same as hard tissue and can be absorbed by bone tissue in the body so that the material can be used as fillers to replace thinned or broken bone tissue.[32]

Another function of hydroxyapatite is as a drug delivery agent and anticancer treatment. However, hydroxyapatite has a poor magnetic property that is enhanced by magnetic nanoparticles.[27,33] Then, to improve the performance possessed by hydroxyapatite, is created nanoparticle and monolithic structures. This system plays a significant role in a variety of biomedical applications because of its unique functional properties which have a high surface area of volume and an ultrafine structure similar to biological apatite.[34]

This type of hydroxyapatite has been considered primarily in the form of particulates for bone care because of its easy manufacturing, handling, and surface contact with the surrounding tissue. So that this material can encourage bone growth and can accelerate the healing process in the bones. In addition, the structure of hydroxyapatite nanoparticles will improve material properties such as traction, stiffness, hardness, resistance to fatigue, and stability at high temperatures.[32] The advantages described above justify a further review of the synthesis, characterization, and behavior of nanocrystalline hydroxyapatite in contact with cells. Hydroxyapatite particles like needles are suitable for preparing filters for ion exchange between cations or the absorption of proteins.[34]

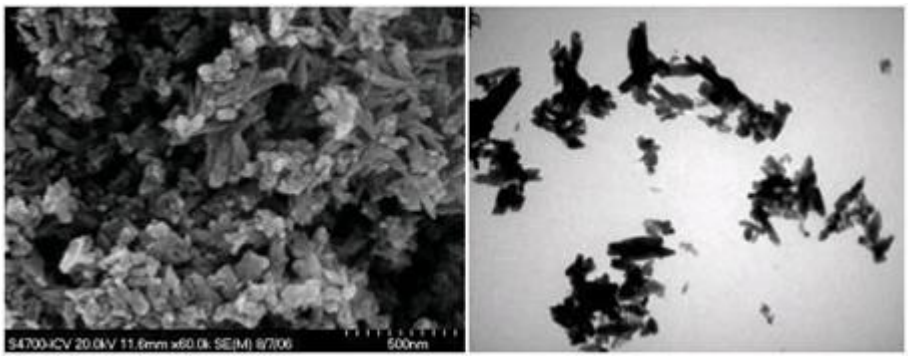

Figure 2. SEM (Left) and TEM (Right) *Micrographs*. Nanocrystal Morphology Characteristics in Hydroxyapatite Nanoparticles. Nanoparticle's size 80x20(SEM, *scale bar*=500nm; TEM, 12000x)[34]

In general, the synthesis of hydroxyapatite nanoparticles uses some methods including mechanochemistry, sol-gel, biomineralization, chemical precipitation, emulsion techniques, and batch hydrothermal processes. Some of these methods have some disadvantages such as requiring very precise control of reaction conditions, expensive starting materials, large amounts of organic solvents, or time-consuming. From these methods, chemical precipitation is the most prominent process because it is simple and produces good results without the need for many instruments, equipment, or sophisticated reagents. Hydroxyapatite nanoparticle that can be useful as nanocrystals, aggregates, powders, or also suitable discs and microspheres, has become versatile materials in bone techniques with many fields of action.[34]

Several studies have also been carried out to improve the performance of hydroxyapatite nanoparticles including calcium hydroxyapatite substitution with magnetite, hydroxyapatite substitution with Ce, load changes during bone deformation processes from hydroxyapatite and collagen.[35–37]

The Combination of Soy Isoflavones and Hydroxyapatite Magnetic Nanoparticles as Osteoporosis Therapy in Menopausal Women

To overcome porous tissue, we need substances that can fill and stimulate bone tissue regeneration in menopausal women because of estrogen insufficiency. Substances that are considered to have the ability to fill the empty spaces in bone are hydrogels. Hydrogels can be implemented as a skeleton at porous bone sites with an administration route in the form of injection.[38] Hydrogels that already contain these substances will carry out their respective duties. These tasks can be summarized in the explanation below:

1. Soy isoflavones that have estrogen-like effects will occupy the estrogen receptors in the bone, which is the ERβ receptor. The stimulation of these receptors will increase the expression of the cbfa gene so that the number of osteoblasts will increase from the differentiation of osteoblast progenitors. With an increase in the number of osteoblasts, the process of osteogenesis will increase. In addition, the resorption process is also inhibited due to an increase in the expression of osteoprotegerin genes so that the RANK ligand cannot bind to its receptors in carrying out the osteoclast maturation process.
2. The magnetic nanoparticles (Fe_3O_4) which have been added to Mn and Zn will reach the target cell because it has a ligand that can bind to specific receptors on osteoclasts, namely calcitonin receptor (CTR). After attaching to the target cell, Mn Zn ferrite nanoparticles will induce changes in motion energy to heat energy so that osteoclasts will undergo apoptosis.
3. Hydroxyapatite is a major component of bone. In the presence of hydroxyapatite, material to start osteogenesis is available so that it can fill areas that have become porous.

In the body, isoflavones are known to have high bioavailability, whereas magnetic

nanoparticles and hydroxyapatite are reported to be stable and have low toxic effects. The process of degradation of isoflavone glycosides is converted to the form of aglycones in the free form by the release of glucose from glycosides. Then the aglycone compound will be catalyzed by the enzyme glucosidase in the small intestine. In the bloodstream, some isoflavones are fat-soluble and some bind weakly to protein. Isoflavones will be distributed to the liver for recycling as part of bile and enterohepatic circulation. Final excretion through feces and urine.[3]

Therapy using a combination of isoflavones with hydroxyapatite nanoparticles aims to increase bone mineral density (BMD) and reduce the factors of fracture caused by osteoporosis. This therapy is given to osteoporosis women with fracture risk factors, such as women over 65 years and menopausal women under 65 years with a family history of osteoporosis, alcohol consumption and smoking, malabsorption, long-term drug use (steroids), and chronic body inflammation (Rheumatoid arthritis, Chronic Obstructive Pulmonary Disease, and Crohn's disease).[39] Measurement of bone mineral density that is precise and used almost all over the world is using dual X-ray absorptiometry (DXA) in the form of T-score. WHO determines osteoporosis if BMD is below -2.5 SD, osteopenia if BMD is -1 to -2.5 SD, and normal if the bone mineral density is above -1 SD.[40]

SUMMARY

Menopausal women experience amenorrhea for 12 consecutive months that cause low-level of estrogen. Soy isoflavones (*Glycine max*) as phytoestrogens are able to maintain bone density through decreased stimulation of osteoblast formation and decreased osteoclast activation to resorb bone. The magnetic nanoparticles in the form of Fe_3O_4 with Mn and Zn will bind to the calcitonin receptor (CTR) and induce changes in magnetic energy into heat energy by magnetic hysteresis mechanism that causes osteoclast apoptosis. Hydroxyapatite nanoparticles which resemble the main component of the bone composition may replace thinning bone tissue and induce an osteoblastic effect. Hydrogels containing a combination of soy isoflavones and hydroxyapatite magnetic nanoparticles can fill the empty spaces in bones and release their components to perform synergistically.

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