CHAPTER I

INTRODUCTION

It is known that estrogen depletion leads to various problems in different systems especially cardiovascular system and bone remodeling, producing cardiovascular diseases and osteoporosis. Endothelial dysfunction and inflammation-induced imbalance of bone formation and bone resorption are mainly focused for the underlining causes of both pathogenesis.

Estrogen replacement therapy (ERT) has significant potential benefits for postmenopausal women, such as improvement of menopausal symptoms, cardiovascular disease and protection from osteoporosis (Chow *et al.*, 1992). Epidemiological studies have demonstrated the protective effects of ERT on the cardiovascular system and the maintenance of bone health. Both indirect mediation through lipoprotein metabolism and direct effect on vascular system have been suggested for such cardiovascular protection (Farhat *et al.*, 1996). Several underlying mechanisms of these effects of estrogen have been postulated, including the favorable lipid profile, serving as antioxidant, inhibiting the synthesis of thrombotic proteins, and stimulating nitric oxide (NO) generation (Gonzales *et al.*, 2001 and Farhat *et al.*, 1996). Several studies indicated that estrogen could inhibit bone remodeling, bone resorption and bone formation and activity of the endothelial nitric oxide synthase (eNOS) (Hayashi *et al.*, 1995), whereas the possibility of nitric oxide derived from the eNOS pathway plays role in mediating the effects of sex hormones on bone formation(Armour and Ralston, 1998).

Recently, Makraldi *et al.* (2003) reported that estrogen administration fully prevented not only the ovariectomy (OVX)-induced changes in bone remodeling but also the morphological alterations observed in bone vessels. 17β-Estradiol, in addition to its efficacy in preventing the bone OVX-induced changes, is totally effective in

counteracting OVX-induced vascular changes in both the intramedullary vascular number and vascular area. After 14 days of treatment, estrogen replacement in OVX rats stimulated vascular endothelial growth factor (VEGF) gene expression and restored normal expression of constitutive NOS. In addition, these changes occurred in concomitant with stimulation of bone cell activity and before detectable bone loss. Barou *et al.* (2002) has developed a technique allowing simultaneous visualization of blood vessels and bone cellular activities and has found evidence for a relationship between vessel number and histodynamic bone formation parameters within the tibial metaphysis of male rats. Dulax *et al.*, (2000) reported that endogenous nitric oxide enhanced VEGF expression by vascular smooth muscle cells. The induction of VEGF synthesis by NO may be of great importance in the maintenance of vascular homeostasis.

However, ERT may raise a risk of breast cancer, endometrial hyperplasia, hypercoagulable stage, hypertriglyceridemia and angiogenesis (Baker *et al.*, 2000; Lissin and cooke, 2000). Therefore, many investigators have much interest in development for other modalities. The idea behind potential health promoting effects of phytoestrogens arose mainly from epidemiological studies on Asian populations (Adlercreutz, 1998), which benefit from a high soy isoflavone intake. The beneficial effects of phytoestrogens are thought to occur in four main domains, in relation with a possible alternative to ERT: menopausal symptoms, osteoporosis, cardiovascular disease and hormone dependent cancers. The hypothesis that phytoestrogens may have fovorable effects on menopausal symptoms came from the observation that Asian women suffer less from hot flashes than population from western countries.

A group of compounds which has been used by many investigators is phytoestrogens, particularly genistein. Phytoestrogens represent a family of plant compounds that have both estrogenic and antiestrogenic properties (Lorraine and Fitzpatrick, 1999; Yoon-Bok *et al.*, 2004 and Squadrito *et al.*, 2000). Most of the research on phytoestrogens has concentrated on soy and its isoflavones, mainly genistein. It has a wide variety of pharmacological effects in animal cells and provides

the benefit for ERT but does not lead to estrogen –dependent risk and side effects.

In previous study, it has been shown that genistein can enhance the vascular response to acetylcholine, the same way as estrogen does (Honore *et al.*, 1997). Khemapech *et al.* (2003) and Squadrito *et al.* (2000) reported that genistein could exert the same protection as estrogen against the reduction of vascular function in ovariectomized rats. Khemapech *et al.*, has showed that genistein supplementation could prevent endothelial dysfunction in mesenteric microcirculation of ovariectomized rat at 3-6 weeks. Moreover, treatment with genistein could prevent trabecular and compact bone loss in rodent ovariectomy without hypertrophic effects on the uterus (Ishimi *et al.*, 2000 and Fanti *et al.*, 1998).

Several studies have shown that bone loss may be attributed to osteoclast recruitment induced by mediators of inflammation. The studies of estrogen for its role in bone metabolism focused on its action on proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), granulocyte colony-stimulating factor (G-CSF). All of these proinflammatory cytokines, which increase bone resorption by increasing the pool of preosteoclasts in bone marrow, are down-regulated by estrogen (Manolagas and Jilka, 1995 and Miyaura *et al.*, 1995). Administration of genistein was associated with higher bone formation rate per tissue volume and with a trend toward a higher number of osteoblasts per bone perimeter. In contrast, Binbin and Shifeng (2003), reported that genistein could prevent bone resorption by the promotion of bone formation. Moreover, several studies reported that genistein could prevent the decrease in bone density and strength caused by ovariectomy.

Estrogen is able to prevent alterations in vessel number related to bone loss. However, the effect of genistein on preventing changes of bone vascularization related to bone loss has not been clarified yet. **Therefore, the present study aims at : 1) studying the effects of genistein on preventing the changes of bone vascularization and bone remodeling, and 2) studying whether the mechanism(s) of genistein action is mediated by VEGF and proinflammatory-cytokines inhibition or not.**

Research questions

Whether genistein (in the dose that could prevent endothelial dysfunction: 0.25mg/kg/day) could prevent bone loss in ovariectomized rat or not? If it could, what is its possible mechanism(s)?

Objectives

1. To study the effects of genistein on bone mineral content and levels of osteocalcin and alkaline phosphatase in ovariectomized rats.
2. To study the effect of genistein on increased proinflammatory cytokines TNF-α and IL-6 in ovariectomized rats.
3. To study the effect of genistein on reduction of VEGF and bone capillary density in ovariectomized rats.

Hypothesis

Genistein (0.25 mg/kg/day) can prevent the alterations of bone vascularization and bone remodeling in ovariectomized rats by inhibiting increased proinflammatory cytokines and decreased VEGF.