Effect of estrogen on osteoclasts and osteoblasts

Osteoporosis is a disease that afflicts many people, especially women. This disease is very debilitating and is characterized by excessive bone loss which results in severe fractures. There are two types of osteoporosis: Type I and Type II. Type I is the more severe type and is prevalent in post-menopausal women. There have been numerous hypothesis and studies as to the cause of osteoporosis and it’s relation to menopause (Riggs, 2002). The findings suggest that estrogen plays a major role. Estrogen receptors have been identified in bone and are involved in the production and maintenance of both osteoclasts and osteoblasts (Eriksen, 1988; Girasole, 1992). Osteoclasts function in bone resorption, and osteoblasts function in synthesizing new bone, hence these two cell types have opposite effects on bone (Saladin, 2010). Studies have shown that a decrease in estrogen levels in post-menopausal women is the primary cause of this reduction in bone density (Girasole 1992; Menolagas, 2002).

Estrogen is a steroid hormone that has many different functions. It is primarily involved in sexual differentiation and maturation, but also has some less obvious effects including thermoregulation and the maintenance of bone mineral deposition. Estrogen is a lipophilic hormone and therefore is capable of diffusing through the cell membrane and binding its two intracellular receptors, ERα and ERβ There are three female sex hormones: estrone (E1), estradiol (E2) and estriol (E3), however, estrogen is the common name used to refer to all three, though estradiol is the main form of estrogen (Carlsten, 2005).

Estrogen is mainly synthesized in the ovaries, though its synthesis is not limited to the ovaries. Some peripheral tissues, such as adipose tissue, are

capable of producing estrogen by way of steroid precursors (Nelson, 2001; Simpson 1981). Synthesis of estrogen involves many different precursors the first of which is cholesterol. Cholesterol is converted in the ovarian follicle to pregnenolone which can be converted to 17α-hydroxypregnenolone. 17 α-hydroxypregnenolone is then converted to dehydroepiandrosterone which is converted to androstenedione which undergoes a conversion to the androgen, testosterone. Aromatase then converts testosterone to esradiol (E2). Estradiol is then secreted from the follicle and can either act on its target tissue or undergo another conversion to estrone (E1) and estriol (E3) which takes place in the liver.

Estrogen has two main receptors (ERα and ERβ) that mediate its primary effects. These receptors belong to the nuclear receptor family and are transcription factors that are regulated by ligands (Carleson, 2005). Estrogen receptors require numerous coregulatory proteins that have cell-specific expressions. These cell specific expressions delineate some of the specific actions of estrogen in its various target tissues (Heldring, 2007). The two estrogen receptors maintain some highly conserved regions such as their DNA binding domains; both ERα and ERβ bind the same DNA response elements. Other domains are not at all conserved, such as the amino-terminal which exhibits significant variability in sequence as well as in length. The ligand-binding domain is located at the C-terminal and is a multifunctional domain. Both the N-terminal and the C-terminal contain activation functions, AF-1 and Af-2, respectively. These activation functions, work to activate transcription by recruiting coregulatory proteins to the DNA-binding domain. Though ERα and ERβ are fairly homologous, they are

actually derived from separate genes which are located on separate chromosomes. ERα and ERβ also give very different splice variants (Heldring, 2007). ERα and ERβ regularaly act as antagonists of each other when expressed in the same cells; hence, estrogen signaling functions as a balance between these two contradictory receptors (Carleson, 2005). It appears as if ERβ works to inhibit the effect of ERα by not only altering the recruitment of transcription factors essential for of ERα-dependent transcription, but also by increasing the degredation of ERα by way of ERβ2, a splice variant of ERβ (Heldring, 2007). Estrogen receptors have been found in many non-reproductive tissues including bone. This fact lends credence to the theory of estrogen's involvement in the maintenance of bone.

Estrogen Signaling

There are a few distinct pathways that are involved in estrogen signaling. Three of these signaling pathways are ligand-dependent, the fourth is ligand-independent. The first ligand-dependent pathway is the classical or direct pathway (Fig. 1a.) in which the ligand (usually estradiol) binds the receptor and this ligand receptor complex then dimerizes with another ligand-receptor complex in order to bind estrogen response elements in the promoters of target genes (Carleston, 2005).

The second ligand-dependent pathway is referred to as the tethered pathway (Fig. 1b.). The tethered pathway involves protein-protein interactions with transcription factors. These interactions occur after the receptor has been activated by the ligand. Hence, the receptor activates transcription by an indirect DNA binding mechanism (Carleston, 2005).

The non-genomic pathway (Fig 1c.) is also ligand-dependent but is not as well understood as the previous two. It is known that the receptor is activated by the ligand, which then initiates a signaling cascade resulting in the activation of second messenger systems (Heldring, 2007). Studies have shown that the activation of these second messenger systems display some common effects including an increase in the production of cAMP levels as well as the activation of the MAPK pathway (Lim, 2006). This activation of second messenger systems ultimately leads to a rapid physiological response without involving gene regulation (Heldring, 2007).

The fourth signaling pathway is ligand-independent (Fig. 1d.) and involves activation by way of other signaling pathways such as that of Growth Hormone which ultimately leads to the activation of gene regulation. This activation of gene regulation occurs due to the activation of protein kinases that work to phosphorylate the estrogen receptor. This phosphorylation causes receptor dimerization which allows the receptors to then bind the DNA and activate gene transcription (Carleson, 2005; Heldring, 2007).

a.) The direct ligand dependent pathway in which the ligand directly binds the receptor which dimerizes and binds the DNA promoter region.

b.) The tethered pathway is indirect and involves protein interactions with transcription factors that allow for the binding of the transcription factor to the promoter region

c.) The non-genomic pathway involvs activation by the ligand which then can cause a signal transduction pathway resulting in activation of second-messenger systems.

d.) The ligand-independent pathwayin which activation occurs by other signalling pathways (i. e. GH) and eventually leads to activation of gene transcription.

Source: Heldring, Pike, Andersson et al. Estrogen Receptors: How do they signal and What are Their Targets. Physiol. Rev. 87: 905-931. 2007.

Estrogen and Osteoporosis

Osteoporosis is a disease that is characterized by a decrease in bone mineral density and hence an increase in the frequency of bone fractures (Though osteoporosis is often associated with post-menopausal women, the disease is not necessarily limited by gender. Osteoporosis was separated into two classes in 1983 by Riggs and Melton. The two types of osteoporosis (Type I and Type II) differ in regions of bone mineral density, patterns of fracture, causal mechanisms and hormonal changes. Type I osteoporosis is the more severe form of osteoporosis, however, type II is more common especially in the elderly (70+) (Riggs, 2003).

Type I Osteoporosis is prevalent in post-menopausal women. It usually arises within 20 years after menopause and is associated with excessive cancellous bone loss. Fractures occur at sites that are rich in cancellous bone. Type I osteoporosis is associated with an increase in osteoclast function and a decrease in osteoblast function. This is thought to be due to a decrease in the levels of estrogen present in post-menopausal women (Girasole, 1992; Ribot, 1997). Osteoporosis has been found to occur in men also; primarily elderly men. The underlying explanation for osteoporosis is that the sex steroids play a role in the remodeling process of bones. Hence, when ovarian

function ceases due to menopause in women, estrogen levels decrease and bone remodeling is therefore disrupted in a deleterious fashion. Osteoporosis in men is associated with a loss of androgens which is generally due to either castration or aging (Manolagas, 2002).

Type II osteoporosis is prevalent in both men and women and can occur at any age, though it is more often associated with the elderly (above age 70). Type II osteoporosis is characterized by the loss of trabecular bone. It is generally due to aging effects such as hyperparathyroidism and impaired bone formation, and also a decrease in vitamin D and PTH levels. There is some speculation as to whether or not Type II osteoporosis may also be due to late effects of decreased estrogen levels (Riggs, 2003).

Evidence for estrogen’s involvement in osteoporosis and its actions on maintaining bone mass can be seen in the study conducted by Riggs et al. which involved 36 women with vertebral fractures due to type I osteoporosis. These women all displayed a high bone turnover rate. The women with type I osteoporosis were compared with 36 normal women (women who did not exhibit Type I osteoporosis) after they were given small amounts of the sex steroids: estradiol, estrone, and testosterone. Blood and urine samples were continuously taken (about every 24 hours) and analyzed to reveal that the levels of the sex steroids were equally apparent in both groups of women. However, the amount of all bone biochemical markers (involved in bone turnover) appeared higher in the osteoporotic women. Though the experiment did not detect a difference in the sex steroid concentrations between the two groups of women, post-experimental power calculations were done to show that there are differences between the two groups when

α = . 05 and 1-β = 0. 8. The differences for serum estrone, estradiol and testosterone were as follows: 6. 3%, 9. 9%, and 4. 4%, respectively. Some of the women with osteoporosis then underwent another study in which they were split into two groups. One group of osteoporotic women received one-year's worth of treatment with transdermal estrogen while the other group of osteoporotic women received a year's worth of treatment with a placebo. The women who underwent estrogen treatment displayed a remarkably larger decrease in bone turnover markers than the women treated with the placebo (Riggs, 2002).

Osteoclasts, Osteoblasts and the Estrogen Connection

Osteoclasts are cells involved in maintaing bone homeostasis and are located on the bone surface in the anterior portion where they work to digest old bone (Manolagas, 2002). Osteoclasts are derived from macrophages, and are very large multinuclear cells formed from the fusion of multiple stem cells (Saladin, 2010). Osteoclasts function in digesting bone; hence they are involved in bone resorption. Bone resorption is a process that releases calcium back into the circulatory system by digesting bone tissue (Saladin, 2010). The function of osteoclasts is essential to the broader process of bone remodeling.

Osteoblasts are also involved in maintaining bone homeostasis and are active in the process of bone remodeling. Osteoblasts are located in the posterior portion of the bone surface and work to produce new bone in the areas that underwent excavation by osteoclasts (Manolagas, 2002). Osteoblasts are derived from mesenchymal stem cells (Zallone, 2006). The mesenchymal stem cells give rise to osteogenic cells which give rise to most

other bone cell types including osteoblasts. Osteoblasts are immature bone cells that are located beneath the endosteum and periosteum of the bone. Osteoblasts synthesize the components of the bone matrix. The matrix undergoes mineral deposition which causes it to harden, and hence the osteoblasts become trapped within the matrix. When osteoblasts mature, they become osteocytes which function in maintaining bone (Saladin, 2010).

Osteoblasts function in making new bone. They deposit calcium salts into the bone matrix in order to make hydroxyapate which is the calcium reserve in bone. Osteoblasts fill in the cavities that were excavated by osteoclasts with new bone. Osteoblasts may be stimulated by various signals and hormones such as calcitonin and estrogen which both function in decreasing blood calcium levels and maintaining calcium levels in bone (Saladin, 2010).

Estrogen receptors (ERα and ERβ) as well as androgen receptors (AR) have been identified in both osteoblasts and osteoclasts and their parental cells. The presence of these receptors indicates that estrogen has a direct effect in mediating the process of bone remodeling (Eriksen, 1988; Girasole, 1992; Manolagas, 2002). Estrogen and androgens also have an indirect effect on the process of bone remodeling via the cytokine, interleukin-6 (IL-6) which is an important factor in the process osteoclastogenesis in bone marrow stromal cells (Carleston, 2005; Manolagas, 2002). The Study conducted by Girasole et al. has shown that estrogen has an inhibitory effect on IL-6, which results in a decrease in the production of osteoclasts. This decrease in osteoclastogenesis ultimately causes a decrease in bone resorption (Girasole, 1992).

Estrogen and androgens inhibit the production of IL-6 in vitro by inhibiting Interleukin-1 (IL-1) and tumor-necrosis factor (TNF)-α. IL-1 and TNF-α are involved in the synthesis of IL-6 (Ribot, 1997). Some studies suggest that estrogen has more of an effect on TNF-mediated production of IL-6, than on IL-1 mediated production (Girasole et al. 1992). The expression of the IL-6 receptor subunits, gp130 and IL-6-Rα, are also suppressed in bone marrow stromal cells and in osteoblast progenitor cells (Manolagas, 2002). Studies done in mice have shown that when the IL-6 gene is knocked out or neutralized (via antibodies) the upregulation of colony-forming unit-granulocyte/macrophage (CFU-GM) (which osteoclasts are derived from) in bone marrow is prevented. Hence, there is not an increase in osteoclast production. The results showed that the antigen caused the estradiol-induced inhibition of bone resorption to itself be inhibited (Ribot, 1997).

A decrease in estrogen (as well as androgens) also has an effect on osteoblasts. One mechanism by which osteoblasts are regulated by estrogen can be observed in bipotential stromal cells; these cells express the estrogen receptors. The bipotential stromal cells are parents to both adipocytes and osteoblasts. A study conducted by Okazaki et al. in mouse bone marrow stromal cell lines, found that estrogen works to mediate the differentiation of the parental bipolar stromal cells towards the production of osteoblasts (Okazaki, 2002). Post-menopausal women who exhibit bone loss have been observed to have an increased amount of lipid concentration in their bone marrow. Hence, a decrease in estrogen would cause an increase in adipogenesis and a decrease in osteoblastogenesis (Okazaki, 2002). This decrease in osteoblastogenesis would result in a decrease in bone formation.

Estrogen Replacement Therapy

A study that was conducted on post-menopausal Chinese women by Sun et al. was also useful in determining the effects of estrogen treatment for osteoporosis. These women all ranged from 41-58 years of age and had undergone menopause for more than a year. The women were split into 4 different groups. Each group received different levels of 17β-estradiol (E2) gel along with a form of progesterone, either micronized progesterone (MP) or medroxyprogesterone (MPA). The progesterone was added along with the estrogen in order to prevent endometrial hyperplasia which may occur as a result of estrogen replacement therapy. The dosages differed in both progesterone type and concentration as well as in estrogen concentration. The exact dosages for each group were as follows: group 1 received 1. 5 mg E2 and 100 mg MP, group 2 received 1. 5 mg E2 and 2 mg MPA, group 3 received 0. 75 mg E2 and 100 mg MP, and group 4 received 0. 75 mg of E2 and 2 mg MPA. These dosages were administered once a day, 25 days a month for at least a year (some of the subjects were studied for two or three years). Blood and urine samples were taken from these women and monitored for bone mineral density (BMD). The results showed that after about a year of this treatment, the average increase in BMD in cancellous bone of the 4 groups ranged from 4. 6%–6. 4%. After 36 months an increase in the BMD of the bones in the neck and the lumbar vertebrae were observed; the averages ranged from 4. 3%-7. 5% and 4. 2%-6. 2%, respectively. This study also found that the BMD in the hip (an area that is prone to fractures), had significantly higher levels than the baseline levels. The main purpose of the study was to determine an appropriate dosage of estrogen and progestin for post-menopausal Chinese women; the results of

the study indicated that either 0. 75 mg or 1. 5 mg of E2 daily is sufficient for prevention of bone loss in Chinese women. The study suggests that hormone replacement therapy has a significant effect on bone, especially during the first two years of treatment (Sun, 2002).

The knowledge of estrogen’s actions on bone and the inhibitory effect it has on osteoclastogenesis, has led to the utilization of estrogen as a treatment for osteoporosis. Estrogen’s ability to maintain bone mass in post-menopausal women has made estrogen replacement therapy a valuable form of treatment. This treatment utilizes the protective properties of estrogen on bone and functions in increasing bone mineral density (BMD) in post-menopausal women (Sun, 2002). Estrogen’s positive effects on bone are amplified during the treatment; hence, estrogen induces stimulation of osteoblastogenesis and inhibits osteoclastogenesis via IL-6 (Girasole, 1992; Okazaki, 2002). The type of estrogen administered (other than estriol) seems to have no difference in effectiveness; hence, synthetic estrogen, 17β-estridiol, and equine estrogen all have equal effects on the maintenance of bone mass and all work to decrease the amount of bone turnover (Ribot, 1997). The method by which estrogen is administered (i. e. transdermally, percutaneously, etc.) also does not appear to make a difference in its effectiveness. The dosages of estrogen as well as the duration of the treatment seem to be the only variables involved in determining the effectiveness of estrogen replacement therapy (Ribot., 1997). In order for estrogen replacement therapy to have a long term effect, estrogen must be administered for about 5-7 years (Cauley 1995; Ribot, 1997). Estrogen replacement therapy is also most effective if it is initiated early after

menopause. A study conducted by Cauley et al. found that women who started estrogen treatment within 5 years of menopause and/or underwent treatment for 10 years or more, had the most effective and long-lasting results (Cauley 1995).

Though Estrogen Replacement Therapy is a promising and effective mechanism for treatment of osteoporosis, it is associated with some serious physiological risks. Long-term usage of estrogen has been known to cause endometrial cancers. However, when estrogen is administered in conjunction with progestins, the risk of endometrial hyperplasia is significantly reduced. (Ribot, 1997; Sun, 2002). Many studies have been conducted to determine whether or not there is a relationship between estrogen and breast cancer. Some studies suggest that there is a correlation between the risk of breast cancer and use of estrogen (Lim, 2006). Other studies suggest that there is no correlation (Ribot, 1997). Though estrogen replacement therapy is associated with a few serious risks, it also has other positive physiological effects on other areas of the body, not just bone. For example, estrogen is thought to have a preventive effect on Alzheimer’s disease and also on Coronary Heart Disease (CHD) in post-menopausal women (Ribot, 1997; Tang, 1996).

The onset of type I osteoporosis is generally characterized by a loss of ovarian function which therefore results in a loss of estrogen in postmenopausal women. (Manolagas 2002). Estrogen can be used as a treatment for osteoporosis due to the presence of estrogen receptors in osteoclasts, osteoblasts and their precursors, as well as in bone marrow stromal cells (Zallone, 2006). The positive actions of estrogen on bone are

mainly due to the suppressive actions of estrogen on bone resorption by osteoclasts (Okazaki, 2002). When Estrogen levels are decreased, the normal regeneration process, which involves bone resorption followed by an appropriate amount of bone formation, is disturbed (Zallone, 2006). Various in vitro studies have been conducted that demonstrate that the presence of estrogen (as well as androgens) increases the action of factors that work to inhibit the process of osteoclastogenesis (Bellido, 1995). The decrease in the production of osteoclasts due to estrogen would therefore cause a decrease in the process of bone resorption (Okazaki 2002). Estrogen also has an effect on the production and differentiation of osteoblastic cells. However, it has the opposite effect on osteoblasts and thereby stimulates their production by shifting the mechanism of bipolar stromal cells towards the production of osteoblastic cells rather than that of adipocytes (Okazaki, 2006). Hence, estrogen works to decrease bone resorption and increase bone formation, thereby creating a protective effect on bone which can be utilized to treat such debilitating diseases as osteoporosis.