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Aromatase in bone: roles of Vitamin D_3 and androgens☆

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Abstract

We have mainly focused on the regulatory mechanism of cytochrome P450 aromatize in bone cells. Our previous study demonstrated a strong positive correlation of serum dehydroepiandrosterone sulfate (DHEA-S) and estrone (E1) with BMD in postmenopausal women but no correlation between serum estradiol (E2) and BMD in the same group. In addition, administration of DHEA to ovariectomized rat significantly increased BMD. These in vivo findings strongly suggested that circulating adrenal androgen may be converted to estrogen in osteoblast and may contribute to BMD maintenance. Actually, in cultured human osteoblast cells, DHEA was found to convert to androstenedione by 3β-hydroxysteroid dehydrogenase (3β-HSD) activity and then androstenedione to estrone through the apparent aromatase activity. The aromatase activity in cultured human osteoblast cells was significantly increased by dexamethasone (DEX). Interestingly, DEX and 1α,25-dihydroxyvitamin D_3 (VD_3) synergistically enhanced aromatase activity as well as P450arom mRNA expression. A little stronger induction of aromatase activity by DEX and VD_3 was observed in cultured human fibroblasts. The increase of the aromatase activity by DEX and VD_3 was accompanied with the increase of luciferase activity of fibroblast cells transfected with Exon 1b-promoter-luciferase construct, but not of osteoblasts transfected with the same construct, suggesting a different regulatory mechanism of aromatase by DEX and 1α,25-dihydroxyvitamin D_3 (VD_3) between these two cells despite the same promotor usuage. In human bone cells, intracrine mechanism through aromatase activity, together with a positive regulation of aromatase activity by glucocorticoid and VD_3, may contribute to the local production of estrogens, thus leading to protective effect against osteoporosis especially after menopause. The effect of sex steroids (estrogen versus testosterone) in bone remodeling was also briefly reviewed based on several recent findings in this field.
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1. Introduction

The biosynthesis of estrogens from C19 steroids is catalyzed by aromatase cytochrome P450 (P450arom) which is the *CYP19* gene. The importance of the aromatase in bone has recently been well characterized by two groups using aromatase deficient mice and both have reported osteoporotic phenotype in these mice although sexual dimorphic response is somewhat inconsistent with each other [1–3]. And human aromatase mutant is also osteoporotic, showing tall height because of unfused epiphysis [4–6]. These results clearly indicate the importance of estrogens in bone remodeling. However, it is still difficult to conclude the major source of estrogen responsible for the maintenance of bone mineralization. The local production of estrogens at extragonadal sites is thought to be dependent on a supply of circulating C19 androgenic substrate like dehydroepiandrosterone (DHEA). The first focus of this paper is the intracrine regulatory mechanism of arornatase in osteoblast, especially in respect of a significance of DHEA as a possible source for estrogen formation in bone cells and also the importance of Vitamin D_3 in aromatase activity in osteoblasts. Secondary, the significance of testostosterone in comparison to estrogen in bone remodeling was also discussed by reviewing several recent findings in this field.

2. Aromatase in bone

It is well established that estradiol (E2) sharply declines after menopause while DHEA is gradually decreased with aging after puberty. In our previous study, an age-associated significant decrease in BMD was observed in postmenopausal women over 50 years old. Significant positive correlation between BMD and serum DHEA-S was

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found in 127 postmenopausal women, but no correlation was seen between BMD and serum estradiol [7]. In subset analysis, serum DHEA-S and serum estrone (E1) showed positive correlation with BMD in postmenopausal women from 50 to 70 years old, suggesting that the conversion of adrenal androgens to estrogens in peripheral tissues including osteoblasts may greatly contribute to the maintenance of BMD in postmenopausal women [7]. This was also evidenced by the fact that administration of DHEA to ovariectomized rat for 12 weeks significantly increased BMD and decreased relative osteoid volume in femur of rat [7].

Aromatase activity is surely present in human osteoblasts [8]. Namely, when primary cultured human osteoblasts were treated with 10^{-3} M dexamethasone (DEX) and incubated with ^{3}H androstendione, ^{3}H water was released during from the conversion of androstenedione to estrone. The reaction showed typical Micheles–Menten kinetics with K_m for 5 nM and V_{max} around 200–300 fmol/mg per protein. The K_m value in the osteoblast was lower than those reported in adipose tissue and skin by about one order of magnitude, suggesting that circulating androstenedione may be easily converted to estrone in osteoblasts. The K_m or V_{max} showed no correlation with the age, or sex of the individuals.

The treatment with 10^{-7} M of sex steroids such as estradiol, DHEA or androgen analogue, R1881 had no effect on osteoblast aromatase activity. Retinoic acids, cyclic AMP and TPA had no effects either. Preincubation with 1,25-$(OH)_2$ Vitamin D_3 or 24*R*,25-dihydroxy Vitamin D_3 alone had no effect on aromatase activity, but very interestingly, when these Vitamin D were added together with dexamethasone, the magnitude of aromatase activity was 1.5–2.0 times higher than that observed with dexamethasone alone. This synergistic enhancing effect by Vitamin D was much potent with 1,25-$(OH)_2$ D_3 than with 24*R*,25-dihydroxy Vitamin D_3. (Fig. 1) [8]. The time course in the effect of dexamethasone and/or 1,25-$(OH)_2$ D_3 on aromatase activity in human osteoblasts and fibroblasts were clearly different. Osteoblast aromatase activity was increased by preincubation with 10^{-7} M dexamethasone reaching a maximum at 12 h preincubation. The addition of Vitamin D_3 strongly enhanced the effect of dexamethasone and it was maintained for 24 h incubation or further increased at 48 h incubation. This is a striking contrast to the marked decrease by the treatment with dexamethasone alone after 24 h. On the other hand, in fibroblasts, Vitamin D_3 similarly enhanced the dexamathsone-induced aromatase activity but its enhancement was a little more potent and very continuous compared with the case in osteoblasts (unpublished observation). The time course in the change of aromatase mRNA level was almost the same as observed as activities in these cells. The 1,25-$(OH)_2$ Vitamin D_3-induced enhancement of osteoblast aromatase activity varied from 1–2.4-fold on each of the samples from 15 cases. Interestingly, the magnitude of Vitamin D_3-induced enhancement of aromatase activity was significantly correlated with the level of Vitamin D receptor mRNA [8].

P450arom gene has multiple promoters and the activation of these promoters is tissue-specific. We previously reported that human osteoblasts predominantly express exon 1b (I-4) [8]. Glucocorticoid responsive element (GRE) exists at 319-upstream from a transcription start site of exon 1b, but typical Vitamin D receptor responsive element (VDRE) was not found within 1.1 kb of the 5′-upstream region of exon 1b. In order to do luciferase assay, a DNA fragment containing 1.1 kb of the 5′-upstream region of exon 1b and some deleted sequence were constructed in luciferase reporter gene and transfected into human osteoblasts. The addition of 10^{-7} M dexamethasone caused 7–10-fold increase of the luciferase activity of the 1.1 kb construct in fibroblasts. Deletion of the 5′-promoter region from −1101 to −500 showed little effect, but the deletion to −267, which deleted the GRE, completely abolished the effect of dexamethasone. The simultaneous addition of Vitamin D_3 further enhanced 2–3 times the luciferase activity compared with that by the treatment of dexamethasone alone. This synergistic effect of Vitamin D_3 and dexamethasone was completely abolished

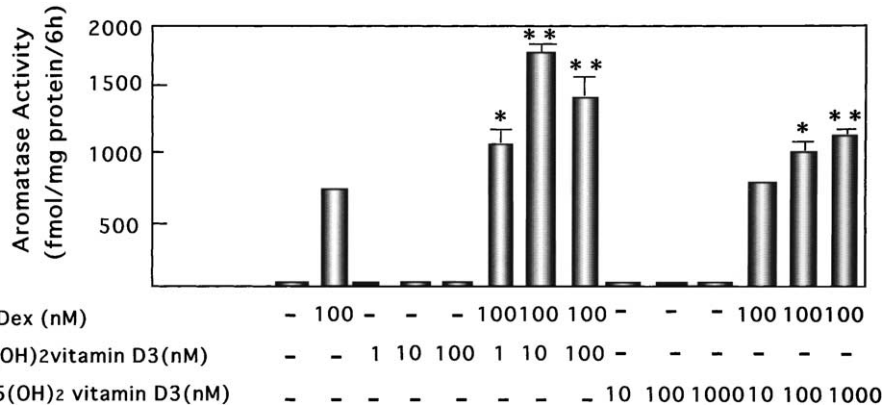

Fig. 1. Effect of 1,25-$(OH)_2$ Vitamin D_3 and 24*R*,25-$(OH)_2$ Vitamin D_3 on aromatase activity in human osteoblasts [8]. Cells were preincubated in the presence and absence of 10^{-7} M dexamethasone with or without 10^{-9} to 10^{-7} M, 1,25-$(OH)_2$ Vitamin D_3 and 10^{-8} to 10^{-6} M 24*R*,25-$(OH)_2$ D_3 for 12 h. $^{*}P < 0.005$; $^{**}P < 0.001$ vs. cells preincubated with dexamethasone alone.

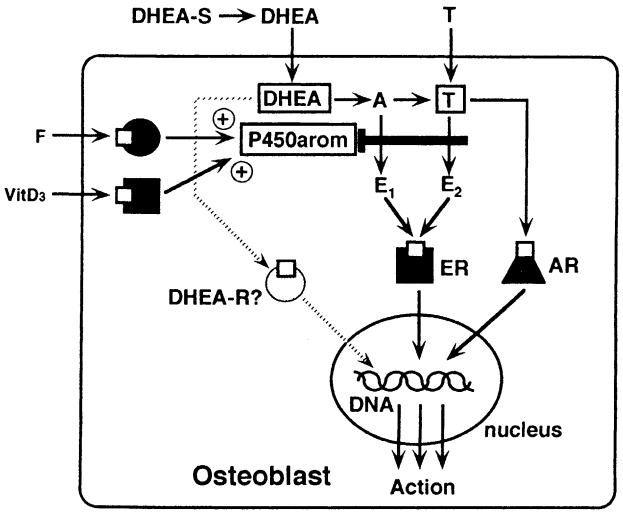

Fig. 2. Schematic representation of intracrine formation of estrogen from DHEA-S in osteoblast [7]. F: cortisol; A: androstenedione; E1: estrone; E2: estradiol; ER: estrogen receptor; AR: androgen receptor.

when the 5′-promoter region was deleted to −500 bp. Similarly, co-expression of Vitamin D_3 resulted in much more induction of dexamethasone induced-luciferase activity in −888 1b-promoter but not in −500 1b-promoter in fibroblasts. These results indicate that synergistic effect of dexamethsone and Vitamin D_3 in human fibroblasts is, if not all, regulated at the transcripional level. On the other hand, enhancement by Vitamin D_3 on dexamethasone-induced aromatase activity observed in osteoblasts was not accompanied by the increase of luciferase activity in any construct, suggesting a post transcriptional modulation of aromatase gene by Vitamin D_3 (unpublished observation).

Fig. 2 summarizes schematically the osteoblastic aromatase proposed in this paper. It has been reported that osteoblast has DHEA-S sulfatase, indicating that osteoblast can uptake DHEA-S and convert it to DHEA. DHEA is possibly converted to δ4-androstenedione by 3β-HSD and, then estrone by aromatase. Estorone can be converted estradiol by 17β-HSD. These estrogens bind to estrogen receptor in osteoblasts. Aromatase is a key enzyme for this pathway and is uniquely regulated by glucocorticoid and Vitamin D_3. Namely, a circulating level of cortisol (0.1–0.4 μM) binds to glucocorticoid receptor in osteoblast and induces the expression of aromatase, transiently. However, Vitamin D_3 can maintain its expression, dependent on Vitamin D receptor density of osteoblasts. The physiological concentration of cortisol and Vitamin D_3 is thus very important for continuous maintenance of aromatase activity in osteoblasts. In addition, circulating levels of androgens including testosterone and DHEA-S are important source for estrogen formation in bone. The importance of DHEA in bone remodeling has been shown in several trials of DHEA supplementation to men [9–11]. And if not all, two reports namely by Dr. Labrie [9] and by Dr. Baulieu [10] demonstrated that 10% DHEA cream or 50 mg DHEA was very effective in increasing BMD and elicit changes biochemical markers of bone remodeling, respectively. Thus, the intracrine mechanism for estrogen formation in bone cells, demonstrated in the present study, may propose a modified therapy for osteoporosis in aged people, namely a combined therapy with a supplementive dose of DHEA to compensate for the decline in aging and a low dose of Vitamin D_3. This combined therapy may have more effect on the maintenance of aromatase activity in bone than that with DHEA alone (Fig. 3).

3. Estrogens versus androgens in bone

The importance of estrogen synthesis or action in maintenance of bone mineral density especially in male has been

	ER α KO mouse (male)	Aromtase KO mouse (male)	AR KO mouse (male)
BMD	↓	↓	↓
Bone Resorption	↑↑	↑↑	↑↑
Bone Formation	↑	↑	↑
Androgen	↑	↑	↓ (Hypoplastic Testis)
Estrogen	↑	↓	~

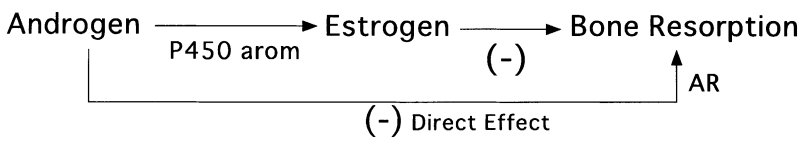

Fig. 3. Comparison of bone phenotypes observed in ERα KO mouse, aromatase KO mouse, AR (androgen receptor) KO mouse: (↑) increase; (↓) decrease; (−) suppresive.

well established especially from the bone phenotype in ERα knockout mice [12] and aromatase knockout mice [1–3]. Mutations of these two genes have been actually found in male young patients, leading to osteoporosis and tall height [4–6,13]. Since these young patients have high level of testosterone production, predominant importance of estrogens in bone metabolism over testosterone especially in male adolescence has been suggested. As to the androgen and bone, clinical studies have suggested that combined therapy of estrogens plus androgens may enhance bone mineral density and bone mass to a more significant degree than estrogen therapy alone in postmenopausal women [14]. However, the mechanism of androgen action on bone metabolism remains controversial. The effect is partly through the effects of aromatase to transform the androgen to estrogen as demonstrated in this paper. Alternatively, androgen may directly acts on bone. This is because AR is expressed in chondrocytes, osteoblasts, osteocytes [15] and osteoclasts [16]. And also administration of antiandrogens to female mice resulted in osteopenia, suggesting the direct role of AR in bone metabolism [17].

Most recently, two groups [18,19] independently published the phenotypes of AR knock out mouse. Interestingly, AR knock out mouse showed osteopenia with rather enhanced bone resorption [18]. This is basically very similar to the bone phenotype observed in ERα or aromatase knockout mouse (AR KO) (Fig. 3). Since it has been thought that testosterone affects bone formation rather than bone resorption [20], the mechanism seems to be somewhat unexpected in that bone resorption was more dominant in AR knock out mouse mouse. Since estrogen level in AR KO mouse was shown to be normal, the direct effect of androgen on bone is thought to be indeed important for BMD maintenance. However, patients with androgen insensitivity syndrome, a good model for the AR effect on human bone, does not show so dramatic decrease in BMD, and results in a moderate deficit in spinal or femoral neck BMD [21]. Considering the moderate deficits in BMD of AIS patients compared with the severe BMD loss in patients with ERα or aromatase deficiency, we may safely say that estrogen likely plays more dominant role than testosterone in total effects of sex steroids on bone metabolism.

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