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Estrogen Enhances Vitamin D_3-mediated Expression of Osteocalcin mRNA by Increasing Vitamin D_3 Receptor Expression in Osteoblasts

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Abstract: Regulation of osteocalcin (OC) gene expression in osteoblasts by estrogen has been controversial. We thus investigated the effect of 17β-estradiol (E_2) on 1α,25-dihydroxyvitamin D_3 (VD_3)-mediated increase in the transcription of OC gene in rat osteoblastic ROS 17/2.8 cells. Treatment of the cells with E_2 or VD_3 alone did not increase OC mRNA. However, combined treatment with E_2 and VD_3 significantly increased the OC mRNA levels, suggesting that VD_3-dependent up-regulation of OC expression requires E_2 action. Since it was demonstrated that treatment with E_2 alone increased the expression of VD_3 receptor mRNA, it was suggested that E_2-mediated increase in the receptor number is contributing to VD_3-mediated increase in OC gene transcription.

Key words: estrogen, 1α,25-dihydroxyvitamin D_3, osteocalcin, 1α,25-dihydroxyvitamin D_3 receptor

OC is the most abundant noncollagenous protein of bone matrix produced by osteoblasts.[1)] It is well established that VD_3 increases the transcription of OC gene in osteoblasts.[2,3)] In the promoter region of OC gene, VD_3-responsive element has been defined.[4)]

On the other hand, regulation of OC gene expression by estrogen has been controversial. E_2 has been reported to stimulate, inhibit, and have no effect on OC gene expression.[5)] Since it has been shown that estrogen increases the number of VD_3 receptor in ROS17/2.8 cells,[6)] we investigated the effect of 17β-estradiol (E_2) on 1α,25-dihydroxyvitamin D_3 (VD_3)-mediated increase in the transcription of OC gene in rat osteoblastic ROS 17/2.8 cells.

Materials and Methods

Cell culture

Rat osteoblast-like cells (ROS 17/2.8)[7)] were cultured to 80% confluence in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere of 5% CO_2- 95% air. The cells were then cultured for 2 days in the phenol red-free DMEM supplemented with 10% serum of male rat and epidermal growth factor (Sigma Co., USA, final concentration 100 ng/ml). Then the cells were treated with 1 nM 17β-estradiol (E_2) and/or 1 nM 1α,25-dihydroxyvitamin D_3 (VD_3) for 24 hours.

Northern blot analysis

The total RNA was extracted from the cells by the acid guanidine phenol/chloroform method.[8)] After 10 μg of total RNA was electrophoresed on 0.8% agarose gel, the RNA was blotted onto a nitrocellulose membrane (Gene Screen Plus, New England Nuclear, USA). The membrane was hybridized with rat OC, VD_3 receptor (VDR) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) cDNAs which were labeled with ^{32}P-dCTP (New England Nuclear, Boston, MA, USA) using Random Primed Labeling kit (Boehringer Mannheim, Germany). After washing the membrane, radioactivities of bands were estimated by an image analyzer (BAS 2000, Fuji Film Co, Tokyo, Japan). The amounts of OC and VDR mRNA were normalized by that of GAPDH mRNA, and expressed by mean ± standard error.

Results

Before treatment with E_2 and/or VD_3, ROS 17/2.8 cells were cultured for 2 days in a phenol red-free medium supplemented with 10% serum of male rat. This preincubation aimed to deplete estrogen in the medium because phenol red and fetal bovine serum include substantial estrogenic activity. As shown in Fig. 1, treatment of the cells with VD_3 or E_2 alone did not increase OC mRNA levels. However, combined treatment with VD_3 and E_2 significantly increased the OC mRNA levels. Furthermore, E_2 alone resulted in a significant increase

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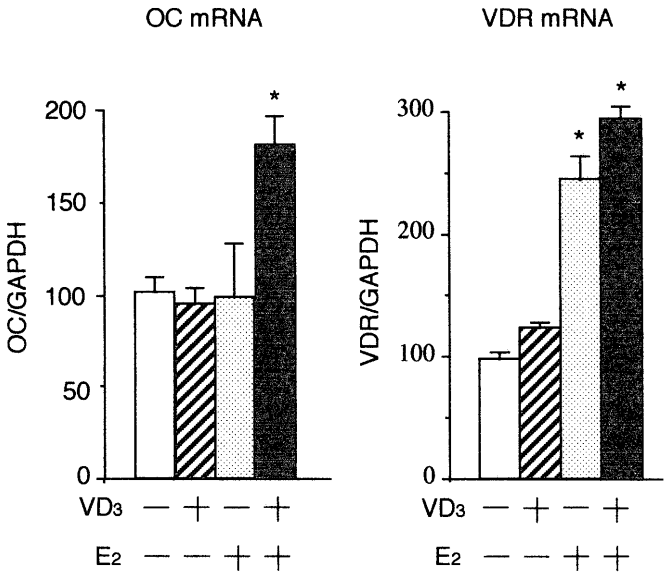

Fig. 1 Effects of E_2 and VD_3 on OC and VDR mRNA levels in ROS17/2.8 cells.
ROS 17/2.8 cells cultured in the phenol red-free DMEM supplemented with 10% serum of male rat were treated with 1 nM 17β-estradiol (E_2) and/or 1 nM 1,25-dihydroxyvitamin D_3 (VD_3) for 24 hours. Then total RNA was extracted, and Northern blot analysis was performed using rat OC, VD_3 receptor (VDR) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) cDNAs as probes. The radioactivities of bands were measured by BAS 2000. The amounts of OC and VDR mRNA were normalized by that of GAPDH mRNA, and expressed by mean ± standard error (n=3, *$p<0.05$)

in the expression of VDR mRNA in agreement with a previous report.[6] VD_3 alone did not increase the mRNA levels and had no influence on E_2-induced increase in VDR mRNA levels.

Discussion

It has been shown that VD_3 increases the transcription of OC gene.[2-4] To our surprise, VD_3 did not increase OC mRNA expression in ROS 17/2.8 cell. However, the combined treatment with VD_3 and E_2 resulted in the increase in OC mRNA, suggesting the requirement of E_2 action for transcriptional activation of its gene by VD_3.

It is not clear why VD_3 alone did not increase OC mRNA since VD_3 receptor mRNA could be detected in the cells not treated E_2. The finding that E_2-mediated increase in the expression of VD_3 receptor mRNA is associated with VD_3-dependent increase in OC expression may indicate that there could be a threshold of VD_3 receptor number to evoke VD_3 action. Further study is required whether translation of VD_3 receptor mRNA or stability of the receptor is affected by E_2.

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