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Vitamin D metabolites and analogs induce lipoxygenase mRNA expression and activity as well as reactive oxygen species (ROS) production in human bone cell line

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ABSTRACT

Vitamin D metabolites and its less-calcemic analogs (vitamin D compounds) are beneficial for bone and modulate cell growth and energy metabolism. We now analyze whether 25(OH)D_3 (25D), 1,25$(OH)_2D_3$ (1,25D), 24,25$(OH)_2D_3$ (24,25D), JKF1624F_2-2 (JKF) or QW1624F_2-2 (QW) regulate lipooxygenase (LO) mRNA expression and its products; hydroxyl-eicosatetraenoic acid (12 and 15HETE) formation, as well as reactive oxygen species (ROS) production in human bone cell line (SaOS2) and their interplay with modulation of cell proliferation and energy metabolism. All compounds except 25D increased 12LO mRNA expression and modulated 12 and 15HETE production whereas ROS production was increased by all compounds, and inhibited by NADPH oxidase inhibitors diphenyleneiodonium (DPI) and N-acetylcysteine (NAc). Baicaleine (baic) the inhibitor of 12 and 15LO activity blocked only slightly the stimulation of DNA synthesis by all compounds, whereas DPI inhibited almost completely the stimulation of DNA and CK by all compounds. Treatments of cells with 12 or 15HETE increased DNA synthesis and CK that were only slightly inhibited by DPI. These results indicate that vitamin D compounds increased oxidative stress in osteoblasts in part via induction of LO expression and activity. The increased ROS production mediates partially elevated cell proliferation and energy metabolism, whereas the LO mediation is not essential. This new feature of vitamin D compounds is mediated by intracellular and/or membranal binding sites and its potential hazard could lead to damage due to increased lipid oxidation, although the transient mediation of ROS in cell proliferation is beneficial to bone growth in a yet unknown mechanism.

1. Introduction

Vitamin D metabolites and its less-calcemic analogs (vitamin D compounds) affect the biology of skeletal cells via genomic [1,2] and non-genomic mechanisms [3]. Adequate availability of vitamin D_3 and its main active metabolite 1,25$(OH)_2D_3$ (1,25D) is essential for skeletal health and modulation of cell growth and differentiation of both osteoblasts and osteoclasts [4]. On the other hand 1,25D causes hypercalcemia [4,5] and therefore optimal bone growth and prevention of osteoporosis which requires its adequate concentrations [6,7], needs the use of the less-calcemic analogs having no adverse calcemic activity [8]. There are also other native metabolites of vitamin D such as the 24,25$(OH)_2D_3$ (24,25D) and 25(OH)D_3 (25D) which are biologically active in the skeletal cells in addition to 1,25D, inducing changes in specific markers of osteoblasts in cultured osteoblasts [9,10] with no effect on calcium metabolism. The metabolite 24,25D binds to specific receptors different from those of 1,25D in skeletal cells such as chondroblasts and "young" less differentiated Obs and regulates different biological functions in a variety of skeletal cells [11,12]. Whether 25D has an independent biological role or it functions only after its conversion to the more hydroxylated metabolites the 1,25D and/or 24,25D is not clear.

We have tested previously the activity of structurally modified less-calcemic analogs of vitamin D and found that JKF1624F_2-2 (JKF) and QW1624F_2-2 (QW) stimulated different parameters in primary cultured human female derived osteoblasts and in human bone cell lines [13,14]. In addition, pre-treatment of the osteoblast-like cells with these analogs up-regulated both their responsiveness and sensitivity to estrogenic compounds and modulated the different estrogen nuclear and membranal receptors [13–15].

In the present report we studied the regulation of LO mRNA expression and activity through the formation of 12- and 15-hydroxyeicosatetraenoic acid (HETE), the arachidonic acid derived metabolites of these enzymes as well as ROS production by the vitamin D compounds. We also studied the involvement of LO, HETE and ROS in the stimulation of cell proliferation (DNA) and energy

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metabolism (CK). We focused on these enzymes since LO products [16] were shown to induce ROS formation [9], proliferation or survival, playing a role in promoting cell growth [13]. We hypothesized that the growth modulating effects of vitamin D compounds in human osteoblasts might be associated with accelerated production of LO metabolites, whose putative action including effects on ROS production may explain some of the new links between the LO system and other biological activities [17]. This is a complementation of our data presented in the last 14th vitamin D workshop [9], which was on the effects of the vitamin D compounds on primary human female bone cells cultures, and using mainly the less calcemic analogs and not the natural metabolites.

2. Materials and methods

2.1. Materials

12 and 15HETE were obtained from Biomol (Biomol International, Plymouth Meeting, PA). Nitro-blue tetrazolium (NBT), N-acetylcysteine (NAc) and diphenyleneiodonium chloride (DPI) were obtained from Sigma Chemicals Co. (St. Louis, MO). 2′,7′-dichloro-fluorescein diacetate (DCF) was obtained from Molecular Probes (Eugene, OR). Vitamin D metabolites were obtained from Enzo life science (Lausanne, Switzerland) whereas the less-calcemic analogs of vitamin D were synthesized by us [8]. All other chemical used were of analytical grade.

2.2. Cell cultures

SaOS2 human bone cell line was obtained from ATCC (Manassas, VA, USA) and grown according to the instructions.

2.3. Hormonal treatment

Sub-confluent cultured cells were treated with vehicle, 25D at 50 nM; 1,25D at 25 nM; 24,25D at 125 nM; JKF or QW at 1 nM [14];

a. for 1 h with serum-free medium, followed by the addition of vehicle or vitamin D compounds at the concentrations mentioned above for 10 min and HETE were extracted and assayed as previously described [16] or;
b. for 1 h by the addition of vehicle or vitamin D compounds, at the concentrations mentioned above for ROS assay as previously described [9] or;
c. for 24 h with the addition of vehicle or vitamin D compounds, at the concentrations mentioned above for DNA synthesis (DNA) and for creatine kinase (CK) specific activity as previously described [13].

2.4. Determination of mRNA for 12 and 15LO by RT-PCR

RNA was extracted and the expression of mRNA of 12 and 15LO or ERα and ERβ was carried out by RT-PCR as previously described [16].

2.5. Determination of the levels of 12 and 15HETE by HPLC

Cells and medium were extracted for HETE formation and analyzed by HPLC as previously described [16].

2.6. Determination of ROS formation

After hormonal treatment for 1 h, and ROS formation [9] using NBT colorimetric method as previously described [18] or for fluorescent microscopy by using 2′,7′-dichloro-fluorescein diacetate (DCF) [19] was determined.

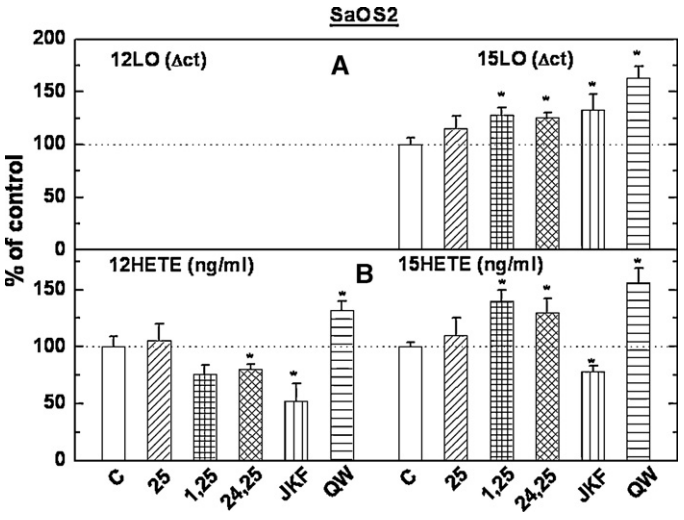

Fig. 1. The effect of vitamin D compounds 25D, 1,25D, 24,25D, JKF and QW on LO mRNAs expression and the production of HETE.
Vitamin D compounds were added daily at their optimal conditions for 3 days to determine LO mRNAs expression (A, Δct) and added for 1 h for the production of HETEs (B, ng/ml) in SaOS2 bone cell line. Details are given in the materials and methods, $^{*}p < 0.05$.

2.7. Assessment of DNA synthesis

After hormonal treatment for 22 h, ^{3}[H] thymidine was added for 2 h and its incorporation into DNA was determined as previously described [14].

2.8. Assessment of creatine kinase specific activity

After hormonal treatment for 24 h, CK was extracted and assayed as previously described [14].

2.9. Statistical analysis

The significance of differences between experimental and control means was evaluated using Student's *t*-test or ANOVA, in which $n = 5–8$ number of cultures.

3. Results

3.1. Modulation of LO mRNA expression and HETE formation in SaOS2 cell line by vitamin D compounds

Three daily additions of 25D (50 nM); 1,25D (25 nM); 24, 25D (125 nM); JKF or QW (1 nM) to SaOS2 cells modulated the expression of mRNA for 15LO but not mRNA for 12LO (Fig. 1, upper panel), which was below detectability in this study. Single treatment for 1 h with the different vitamin D compounds modulated the formation of 12 and 15HETE (Fig. 1, lower panel). All hormones with the exception of 25D stimulated 15LO mRNA expression (Fig. 1, upper panel) and 15HETE formation, whereas JKF inhibited it (Fig. 1, lower panel). On the other hand all hormones with the exception of 25D inhibited 12HETE formation and only QW stimulating it (Fig. 1, lower panel).

3.2. The effect of vitamin D compounds and 12 or 15HETE with/without DPI on ROS formation in SaOS2 cell line

Human bone cell line SaOS2 (Fig. 2) treated for 1 h with 25D (50 nM); 1,25D (25 nM); 24, 25D (125 nM); JKF or QW (1 nM), or 12 and 15HETE at 1 μM showed increased ROS formation as measured