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

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ABSTRACT

Vitamin D metabolites or its less-calcemic analogs (JKF or QW) are beneficial for bone biology. We analyzed whether or not 25(OH)D_3 (25), 1,25$(OH)_2D_3$ (1,25), JKF or QW regulate lipooxygenase (LO) enzymes expression and their products hydroxyeicosatetraenoic acid (12 and 15 HETE) formation as well as reactive oxygen species (ROS) production in human bone cell lines (SaOS2 and hFOB) and primary cultured human bone cells (Obs) from males or females. All compounds except 25 increased LOs mRNA expression and HETE production in female or male Obs. ROS formation was induced by JKF and QW in both cell lines, and was inhibited by different inhibitors. Baicalein (baic) an inhibitor of 12 and 15 LO activity, inhibited partially ROS formation by JKF or QW in SaSO2 and hFOB. JKF-stimulated DNA synthesis in female Obs was inhibited by baic but unchanged by addition of HETE or HETE with baic. These results indicate that vitamin D increased oxidative stress in bone cells is in part via induction of LO expression and activity. This new feature of vitamin D is probably mediated by intracellular and/or membranal receptors and its potential hazard could lead to potential damage due to increased lipid oxidation.

1. Introduction

Vitamin D affects skeletal cells via genomic [1,2] and non-genomic mechanisms [3]. Adequate availability of vitamin D_3 and its active metabolite, 1,25 is essential for skeletal health and modulation of cell growth and differentiation of both Obs and osteoclasts [4], but it is hypercalcemic [4,5] and therefore optimal bone growth and prevention of osteoporosis which requires its adequate concentrations [6,7] needs the use of its less-calcemic analogs having no adverse calcemic activity. We tested structurally modified less-calcemic analogs for their activity and found that JKF1624F_2-2(JKF) and QW1624F_2-2(QW) [8,9] stimulated Obs [8,9] and pre-treatment with them up-regulated both responsiveness and sensitivity to estrogens [8–10]. In the present study we analyzed the expression and regulation of LOs as well as ROS formation by these analogs. We focused on these enzymes since LO products were shown to induce ROS formation [11], proliferation or survival, playing a role in promoting cell growth [12]. We hypothesized that the growth modulating effects of vitamin D in human Obs 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 biological activities.

2. Materials and methods

Reagents: JKF and QW were synthesized by us [13]. 12 and 15 HETE were from Biomol (Biomol International, Plymouth Meeting, PA). Nitro blue tetrazolium (NBT), N-acetylcysteine (Nac) and diphenyleneiodonium chloride (DPI) were from Sigma Chemicals Co. (St. Louis, MO).

Cell cultures: (*a*) Primary human bone cells (Obs) from females or males were prepared by us [9]. (*b*) Human bone cell lines: SaOS2- and hFOB-human bone cell lines were obtained from ATCC (Manassas, VA, USA).

Hormonal treatment: Sub-confluent cells were treated daily with vehicle, JKF or QW at 1 nM [9] (*a*) for 3 days and mRNA for 12 and 15 LO type 1 were determined [11] or (*b*) for 1 h with serum-free medium, followed by the addition of vehicle or vitamin D compounds at 1 nM for 10 min and HETE were extracted and assayed [11] or (*c*) for 24 h with JKF or QW at 1 nM and DNA synthesis was assayed [11].

Determination of mRNA for 12 and 15 LO by RT-PCR: RNA was extracted and expression of 12 and 15 LO enzymes was carried out by RT-PCR [11].

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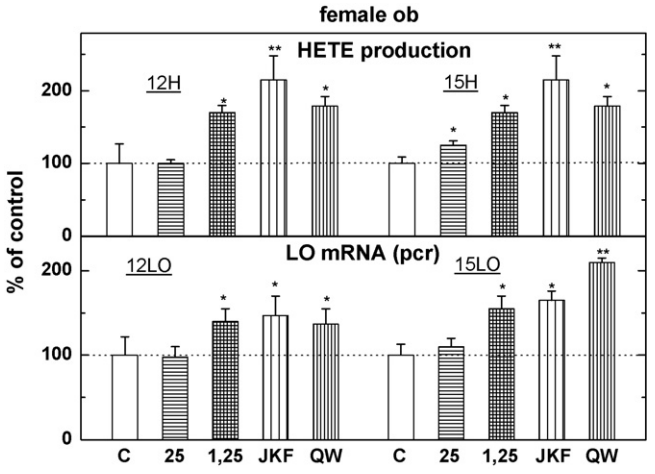

Fig. 1. The effect of vitamin D metabolites and analogs on LO mRNAs expression and the production of HETE in female Obs. Details are given in Section 2.

Determination of levels of 12 and 15 HETE by HPLC: Cells and medium were extracted for HETE and analyzed by HPLC [11].

Determination of ROS formation: Cells were treated for 1 h with vehicle or vitamin D analogs at 1 nM [9] for ROS formation using NBT colorimetric method [14].

Assessment of DNA synthesis: Twenty-two hours after hormonal treatment, [^{3}H] thymidine was added for 2 h and its incorporation into DNA was determined [11].

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

Stimulation of LO mRNA expression and HETE formation in female Obs by vitamin D: Three daily additions of 25, 1,25, JKF or QW at 1 nM to female Obs, modulated the expression of mRNA for 12 and 15 LO (Fig. 1, upper panel) and single treatment for 1 h modulated the formation of 12 and 15 HETE (Fig. 1, lower panel). All hormones stimulated all parameters; 25 stimulated only 15 H formation (Fig. 1, lower panel).

The effect of JKF, 12 and 15 HETE with/without baicalein on DNA synthesis in female Obs: Treatment of female Obs with 1 nM JKF for 24 h or 1 μM 12 or 15 HETE increased DNA synthesis (Fig. 2). HETE did not affect the cellular response to JKF, but the LO activity inhibitor baicalein (baic) inhibited JKF stimulated DNA synthesis, but not the response to HETE (Fig. 2).

The effect of JKF or QW with/without baicalein, DPI or Nac on ROS formation in SaSO2 and hFOB: Human bone cell lines SaSO2 (Fig. 3, upper panel) and hFOB (Fig. 3, lower panel) treated for 1 h with 1 nM JKF or QW, increased ROS formation which was inhibited by DPI and Nac (Fig. 3).

The effect of JKF or QW with/without baicalein or DPI on DNA synthesis in SaSO2: Human bone cell line SaSO2 was treated for 24 h with 1 nM JKF or QW, there was an increased DNA synthesis (Fig. 4), blocked completely by DPI (Fig. 4, lower panel) or baic (Fig. 4, upper panel).

4. Discussion

The effects of vitamin D on ROS formation complements our findings in human skeletal cells *in vitro*, where the hybrid fluorinated analogs JKF and QW stimulate cell proliferation and membranal responses [9]. Although reports suggest that 12 and 15 HETE interact with multiple signals promoting cell replication,

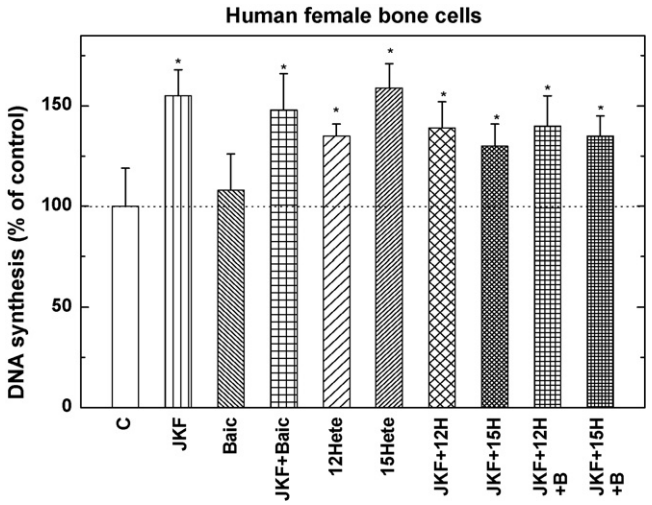

Fig. 2. The effect of vitamin D analog JKF with and without baicalein or 12 or 15 H or HETE with baicalein on DNA synthesis in female Obs. Details are given in Section 2.

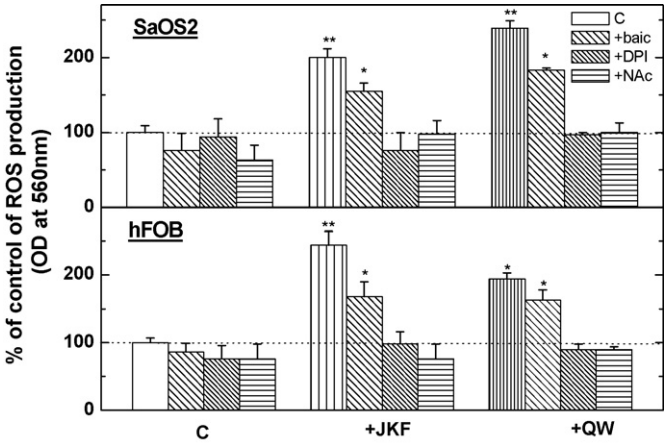

Fig. 3. The effect of vitamin D analogs JKF and QW on ROS formation with baicalein or DPI or N-Acetyl cysteine (Nac) in human bone cell lines SaOS2 and hFOB. Details are given in Section 2.

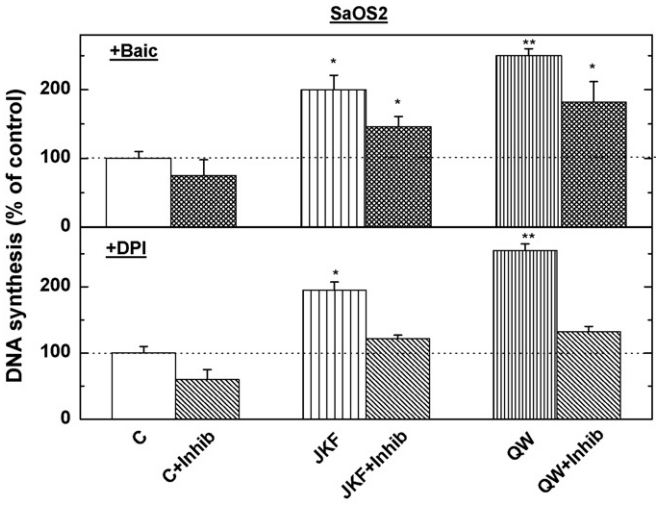

Fig. 4. The effect of vitamin D analogs JKF and QW with baicalein or DPI on DNA synthesis in human bone cell line SaOS2. Details are given in Section 2.

their effects are probably not redundant, since inhibition of their production hinders normal cell growth in a variety of cell types [15,16]. Recent publications linked also 12/15 LO or 15 and 12 LO (platelet type) to bone density [17,18]. In this study we provide the first direct evidence for the expression and biological role of LOs in bone cell biology. We found mRNA expression of three types of LOs in cultured human bone cells and lines, i.e., the platelet type 12 LO, 15 LO type 1 and 15 LO type 2. Here we show that the expression of the LOs in these cells is modulated by vitamin D metabolites and analogs. The expression of these enzymes results in the ability of Obs to secrete 12 and 15 HETE, the products of LOs. Moreover, the generation of HETE which is driven by vitamin D metabolites and analogs is linked to vitamin D-dependent Obs growth. The HETE raise local oxidative stress as was shown here by measurements of ROS formation induced by vitamin D analogs. Because oxidative stress, in turn, may lead to inhibition of differentiation of bone osteoblasts-like cells [17] and acceleration of osteoclast differentiation [18], its induction may also result in the release of oxidizing fatty acids which unfavourably affect overall bone osteoblast/osteoclast homeostasis through enhanced oxidative stress. Presently, however, these potential secondary sequels of vitamin D-stimulated LO activities in bone remain entirely conjectural and are the subject of future investigation.

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