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Modulation of response to estrogens in cultured human female bone cells by a non-calcemic Vitamin D analog: changes in nuclear and membranal binding☆

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Abstract

Estradiol17β (E_2) and the phytoestrogens genistein (G), and daidzein (D) increase creatine kinase (CK) specific activity in primary cell cultures of human female to a greater extent in cells from pre-menopausal than post-menopausal women. Pretreatment with the non-calcemic analog of Vitamin D, JK 1624 F2-2 (JKF), upregulated this estrogenic response at all ages. In contrast, biochainin A (BA) and quercertin (Qu) increased CK with no age dependence or modulation by JKF pretreatment. Both ERα and ERβ present in the cells were upregulated by pretreatment with JKF, as measured by Western blot analysis. Real time PCR showed no significant change in ERα mRNA but a marked decrease in ERβ mRNA in both age groups after JKF treatment. Cells from both age groups had surface binding sites for E_2, shown by assays using cell impermeable Europium labeled ovalbumin-E_2 conjugate (Eu-Ov-E_2). Binding of [^{3}H]-E_2 to intracellular E_2 receptors (ERs) was similar in both age groups with differences in phytoestrogenic competition. JKF pretreatment increased nuclear but decreased membranal binding in both age groups. These results provide evidence for membranal, in addition to nuclear estrogen receptors which are differentially modulated by a Vitamin D analog.

Keywords: Estrogen receptors α and β; Membranal binding; "Non-hypercalcemic" Vitamin D analogs; Phytoestrogens

1. Introduction

The secosteroid 1,25 dihydroxyvitamin D_3 (1,25) generates a spectrum of biological responses via genomic [1] and nongenomic mechanisms [2]. For optimal bone growth and prevention of osteoporosis in post-menopausal women, adequate concentrations of both 17-β estradiol (E_2) and Vitamin D_3 are required [3].

We have studied the interaction of Vitamin D analogs and estrogens in a rat model, [4], using the increase in the specific activity of creatine kinase (CK) as a response marker. This marker can be used to measure the response to of E_2 in cells containing low concentrations of E_2 receptors (ERs) [5,6]. We found that pretreatment with the demonstrably non hypercalcemic Vitamin D analog JKF 1624 F2-2 (JKF) [7] upregulated responsiveness and sensitivity to E_2 in human bone derived cell cultures as measured by the stimulation of CK [8]. This increase was accompanied by an increase in estrogen receptors [9].

There is growing evidence that several estrogen-dependent effects are induced via cell membrane associated signaling rather than by the classical nuclear receptor route of steroid hormone action [10]. The present study was undertaken to determine whether estrogenic modulation of human primary bone cell cultures involves putative membranal estrogen receptors, as well as changes in the classical nuclear ERα and ERβ.

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2. Materials and methods

2.1. Cell cultures

Human bones were obtained from biopsies of patients, and cells were isolated and cultured in DMEM medium without Ca^{2+} as described previously [11].

2.2. Association of $^{3}[H]$-estradiol with bone cells

Cells were incubated with ^{3}[H]-E_2 [12] at 37 °C, and binding was performed and analyzed by radioactivity determination by scintillation counting as described elsewhere [12,13].

2.3. Association of the membrane impermeant, Europium labeled estradiol–Ov conjugate with bone cells

Cells were incubated with the steroid protein conjugates (E_2–Ov) [14] labeled with Eu at 4 °C, and binding was performed and analyzed by time-resolved fluorescence using an Arcus time-resolved fluorometer (Wallac, Turku, Finland) as described before [15].

3. Results

Human female bone cells treated with 30 nM estradiol 17β (E_2) or 3 μM genistein (G), daidzein (D), biochainin A (BA) or quercetin (Qu), showed a significant increase in CK activity with greater response to E_2, G or D in cells derived from pre-menopausal women compared to cells derived from post-menopausal women and an equal response to BA or Qu . In cell cultures derived from pre-menopausal women, 3 days pretreatment with JKF upregulated the CK specific activity response to E_2 to G or to D by 50 ± 8, 85 ± 5, and 120 ± 12% respectively, with no effect on the response to BA or to Qu. In cells derived from bones of post-menopausal women, JKF upregulated CK response only to E_2, G, and D by 110 ± 8, 110 ± 5, and 105 ± 10% respectively, as in the pre-menopausal cells.

Western immunobloting of cell extracts, detected two forms of both ERα (32 and 67k) and ERβ (37 and 63k). In pre-menopausal bone cells, JKF increased the expression of both ER forms: α by 3- and 4.5-fold and β by 3- and 1.5-fold. In post-menopausal bone cells, the increase of 32k ERα was 2.7-fold, with no change in 67k ERα. Both 37 and 63k ERβ forms, were increased by 11- and 4-fold, respectively [8].

Real time PCR products of mRNA, extracted from cultured bone cells, show that JKF increased mRNA for ERα by 10% in cells derived from pre-menopausal bones and by 30% in cells from post-menopausal bones. In both age groups mRNA for ERβ was decreased by 60 and 50%, respectively.

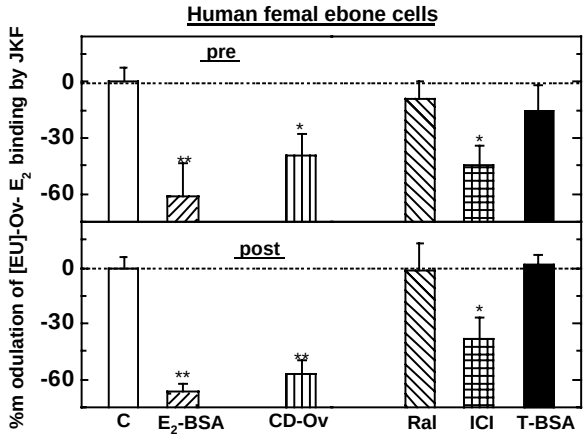

Fig. 1. Modulation by JKF of membranal binding, in bone cells, from pre-menopausal (pre) and post-menopausal (post) women. Cells were incubated in the absence or presence of 500 fold excess of estradiol–BSA (E_2–BSA), carboxy daidzein–ovalbumin (CD–Ov), raloxifene (Ral), ICI 16480 (ICI) or testosterone–BSA (T–BSA). Results are expressed as Eu-Ov-E_2 binding in the presence of a defined competitor as a percentage of binding in the absence of competitor and are means ± S.E.M. of three experiments, each performed in triplicate, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with total binding (C) by ANOVA.

3.1. Modulation by JKF of total Eu-Ov-E_2 binding to putative membrane binding sites in human bone cells

Incubation of cells with Eu-Ov-E_2, showed specific binding in both age groups, which showed competition by E_2–BSA, carboxy genistein–ovalbumin (CG–Ov), carboxy daidzein–ovalbumin (CD–Ov) or ICI 16480 (ICI) but not with raloxifene or testosterone–BSA (T–BSA). JKF (1 nM

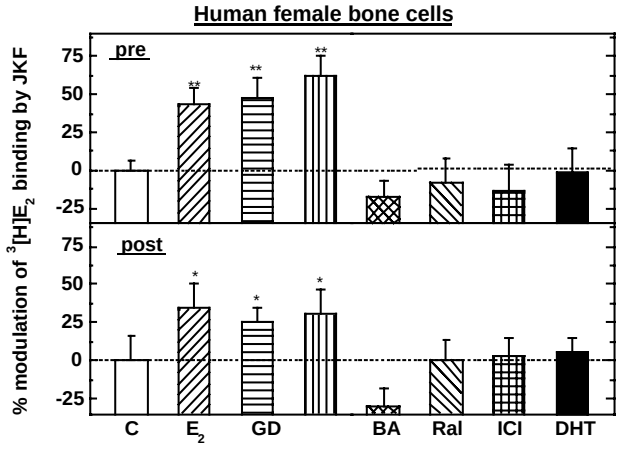

Fig. 2. Modulation by JKF of specific nuclear binding of ^{3}[H]-E_2 in human bone cells from pre-menopausal (pre) and post-menopausal (post) women. Cells were incubated in the absence or presence of 500-fold excess of E_2, daidzein (D), raloxifene (Ral), ICI 16480 (ICI) or dihydrotestosterone (DHT). Results are expressed as ^{3}[H]-E_2 binding in the presence of a defined competitor as a percentage of binding in the absence of any competitor, and are the means ± S.E.M. of three experiments, each performed in triplicate, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with total binding (C) by ANOVA.

for 3 days) decreased specific membranal binding in bone cells from both age groups (Fig. 1).

3.2. Modulation by JKF of total ^{3}[*H*]-E_2 binding to intracellular binding sites in human bone cells

Incubation of cells with ^{3}[H]-E_2 showed specific binding in both age groups which showed competition with E_2, genistein, daidzein, biochainin A, quercetin, ICI 16480 or raloxifene, but not by dihydrotestosterone (DHT). JKF (1 nM for 3 days) increased specific binding of ^{3}[H]-E_2 in bone cells from both age groups (Fig. 2).

4. Discussion

The increased responsiveness to estrogens after pretreatment with Vitamin D, previously demonstrated in skeletal-derived cells in vitro [9] was demonstrated as well in primary human bone cells, apparently due to modulation of ERs [8]. All forms of ERs were modulated significantly by JKF treatment except the 67k ERα in post-menopausal women. Real time PCR analysis demonstrates that JKF slightly increased ERα mRNA, but markedly decreased ERβ mRNA, in cells from both age groups. JKF upregulated the responses to E_2 and to phytoestrogens that show higher activity in pre-menopausal human bone, but not to phytoestrogens, which show no age related, decline in their response.

Binding of ^{3}[H]-E_2 to intracellular receptors is similar in both ages with JKF increasing this specific intracellular binding as found for both vascular and bone cells [9,13]. Binding of Eu-Ov-E_2 to putative membranal receptors is similar in both age groups with JKF pretreatment decreasing this specific binding, raising the question of a possible reciprocal relationship between nuclear and membranal receptors.

JKF upregulated response of bone cells to estrogenic compounds is presumed to be due to increased expression of ERα, as well as a decrease of ERβ, since ERβ, ERα coexpression was shown to decrease transcriptional capacity of an estrogen reporter gene in osteoblasts [16]. In addition, since the cells in this study contain putative E_2 membranal receptors, mediating some of the estrogenic effects [10] and JKF down regulates these receptors, in cells from both pre- and post-menopausal women, the role of each of the various types of receptor has still to be determined in the biology of bone cells.

References

[1] K. Ozono, T. Sone, W. Pike, The genomic mechanism of action of 1,25-dihydroxyvitamin D_3, J. Bone Miner. Res. 6 (1991) 1021–1027.

[2] A. Revelli, M. Massobrino, J. Tesarik, Nongenomic effects of 1α25-dihydroxyvitamin D_3, Trends Endocrinol. Metab. 9 (1998) 419–427.

[3] J.C. Gallagher, Drug therapy of osteoporosis: calcium, estrogen and Vitamin D, in: B.L. Riggs, L.J. Melton III (Eds.), Osteoporosis: Etiology, Diagnosis and Management, vol. 1, Raven Press, New York, 1988, pp. 389–401.

[4] D. Somjen, A. Waisman, J.-K. Lee, G.H. Posner, A.M. Kaye, A non-calcemic analog of 1α, 25 dihydroxy Vitamin D_3 (JKF) upregulates the induction of creatine kinase B by 17β estradiol in osteoblast-like ROS 17/2.8 cells and in rat diaphysis, J. Steroid Biochem. Mol. Biol. 77 (2001) 205–212.

[5] N. Reiss, A.M. Kaye, Identification of the major component of the estrogen induced protein of rat uterus as the BB isozyme of creatine kinase, J. Biol. Chem. 256 (1981) 5741–5749.

[6] S.D.H. Malnick, A. Shaer, H. Soreq, A.M. Kaye, Estrogen induced creatine kinase in the reproductive system of the immature female rat, Endocrinology 19 (1983) 1907–1909.

[7] G.H. Posner, J.K. Lee, Q. Wang, S. Peleg, M. Burke, H. Brom, P. Dolan, T.W. Kensler, Noncalcemic, antiproliferative, transcriptionally active, 24-fluorinated hybrid analogues of the hormone 1α25-dihydroxyvitamin D_3. Synthesis and preliminary biological evaluation, J. Med. Chem. 41 (1998) 3008–3014.

[8] S. Katzburg, D. Hendel, A. Waisman, G.H. Posner, A.M. Kaye, D. Somjen, Treatment with "non-hypercalcemic" analogs of 1,25 dihydroxyvitamin D_3 increases responsiveness to 17-β estradiol, dihydrotestosterone or raloxifene in primary human osteoblasts, J. Steroid Biochem. Mol. Biol. 88 (2004) 213–219.

[9] B. Fournier, S. Haring, A.M. Kaye, D. Somjen, Stimulation of creatine kinase specific activity in human osteoblast and endometrial cells by estrogens and anti-estrogens and its modulation by calciotropic hormones, J. Endocrinology 150 (1996) 275–285.

[10] A. Nadal, A.B. Ropero, O. Laribi, M. Maillet, E. Fuentes, B. Soria, Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor alpha and estrogen receptor beta, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11603–11608.

[11] S. Katzburg, A. Ornoy, D. Hendel, M. Lieberherr, A.M. Kaye, D. Somjen, Age and gender specific stimulation of creatine kinase specific activity by gonadal steroids in human bone-derived cells in culture, J. Endocrinol. Invest. 24 (2001) 166–172.

[12] S. Katzburg, A.M. Kaye, B. Gayer, F. Kohen, G.H. Posner, D. Somjen, Age dependent responsiveness to estrogen and to phytoestrogens in cultured human female bone cells: the role of nuclear and membrane estrogen receptors, Annual Meeting of the Israel Endocrine Society, Tel Aviv, Israel, December 2002, p. A25, (abstract).

[13] D. Somjen, F. Kohen, B. Gayer, O. Sharon, R. Limor, T. Kulik, E. Knoll, N. Stern, Role of putative membrane receptors in the effects of estradiol on human vascular cell growth, Am. J. Hypertens. (2004) in press.

[14] F. Kohen, S. Bauminger, H. Lindner, Preparation of antigenic steroid-protein conjugates, in: E.D.H. Cameron, S.G. Hillier, K. Griffiths, Steroid Immunoassay, Alpha Omega Publishing, Cardiff, 1975, pp 11–31.

[15] O. Mazor, M. Hillairet de Boisferon, A. Lombet, A. Gruaz-Guyon, B. Gayer, D. Skrzydelsky, F. Kohen, P. Forgez, A. Scherz, W. Rostene, Y. Salomon, Europium-labeled epidermal growth factor and enurotensin: novel probes for receptor-binding studies, Anal. Biochem. 301 (2002) 75–81.

[16] D.G. Monroe, S.A. Johnsen, M. Subramaniam, B.J. Getz, S. Kosla, B.L. Riggs, T.C. Spelsberg, Mutual antagonism of estrogen receptors alpha and beta and their preferred interactions with steroid receptor coactivators in human osteoblastic cell lines, J. Endocrinol. 176 (2003) 349–357.