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Vitamin E activates CRABP-II gene expression in cultured human fibroblasts, role of protein kinase C

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Abstract The treatment of human fibroblasts with different tocopherols in the presence of retinol caused an increase in cytoplasmic retinoic acid binding protein II (CRABP-II) mRNA and protein. The possibility of an involvement of protein kinase C (PKC) in the response to tocopherols was supported by the results obtained with the PKC-specific inhibitors, calphostin C and bisindolylmaleimide I. The effect of α-tocopherol was prevented by okadaic acid, suggesting that a protein phosphatase is responsible for PKC dephosphorylation produced by the presence of tocopherols. The results shown support the hypothesis that phosphorylation/dephosphorylation of RXRα via PKC may be involved in the regulation of CRABP-II gene expression.

Keywords: Cytoplasmic retinoic acid binding protein II; Fibroblast; Protein kinase C; Protein phosphatase; Retinoid X receptor α; Vitamin E

1. Introduction

Retinoids (vitamin A and its derivatives) are essential regulators of epithelial cell growth and cellular differentiation in both fetal and adult tissues. Skin is a major target of retinoids both in normal and pathological states [1].

Different intracellular retinoid-binding proteins have been identified. Retinol and retinoic acid are bound to specific cellular binding proteins. These proteins are involved in the regulation of the intracellular concentration of these retinoids by acting as shuttle proteins, facilitating their transport, or as storage proteins, maintaining constant levels of free retinol and retinoic acid [2].

Two cytoplasmic retinoic acid binding proteins, CRABP-I and CRABP-II, have been characterized. CRABP-I is expressed almost ubiquitously, whereas CRABP-II expression is localized almost exclusively in skin, basically in keratinocytes and fibroblasts [3]. Furthermore, the expression of CRABP-II is upregulated by retinol and retinoic acid in human skin as well as in fibroblasts and keratinocytes; however, CRABP-I mRNA expression was not induced by retinoic acid and was undetectable in normal human skin by Northern blot analysis [4]. The human CRABP-II gene promoter contains two *cis*-acting DNA retinoic acid response elements (RAREs), which mediate the induction of CRABP-II by the ligand-inducible *trans* regulators retinoic acid receptors (RARs α, β and γ) and retinoid X receptors (RXRs α, β and γ), which bind much more efficiently to RAREs as RAR–RXR heterodimers than homodimers [5]. In human skin, the predominant RAR transcript is RARγ and the predominant RXR transcript is RXRα [6] and CRABP-II is known to be controlled by RARγ/RXRα heterodimer [7].

Vitamin E is considered as an important antioxidant molecule which prevents diseases associated with oxidative stress like cardiovascular disease, chronic inflammation, cancer and neurologic disorders. In addition, novel functions of vitamin E have been discovered such as inhibition of cell proliferation [8,9] and gene expression [10–13], and upregulation of α-tropomyosin [14] and α-tocopherol transfer protein [15]. The link between some of these functions of vitamin E appears to be protein kinase C (PKC) [8,9,13,16]. PKC belongs to a family of related Ser/Thr kinases with a key role in the transduction of signals mediated by cytokines, growth factors, and hormones. PKC is a family of several isoenzymes and it was found that only PKCα was inhibited by α-tocopherol [17]. Inhibition of PKCα occurs because α-tocopherol induces the activation of protein phosphatase 2A which causes dephosphorylation and inactivation of PKCα [17].

It has been also demonstrated that PKC is able to phosphorylate RAR in vitro and in vivo [18,19]. This phosphorylation decreases the ability of human RARα to dimerize with RXRα, affecting the transcriptional activity modulated by the heterodimer RAR–RXR [18]. Protein phosphatases 1 and 2A are also involved in the transcriptional and DNA binding activities of RAR–RXR heterodimers [20,21]. Phosphorylation of RXRα by mitogen-activated protein kinase (MAPK) also impairs the transcriptional activity of RAR/RXR [22,23] and VDR/RXR [24] heterodimers.

In this study, we show that tocopherol (α, γ and δ) treatment of human cultured fibroblasts in the presence of retinol leads to an increase in CRABP-II mRNA and protein expression. This expression appears to be regulated by PKC because the treatment of fibroblasts with specific PKC or protein phosphatase inhibitors affects CRABP-II expression. Western blot analysis with anti-phosphoserine antibody showed that RXRα

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Abbreviations: CRABP, cytoplasmic retinoic acid binding protein; RARE, retinoic acid response element; RAR, retinoic acid receptor; RXR, retinoid X receptor; PKC, protein kinase C; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

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is phosphorylated in dermal fibroblast and the treatment with α-tocopherol or PKC inhibitors decreased their phosphorylation levels. The results are consistent with a model in which tocopherols cause inactivation of PKC with a concomitant dephosphorylation of RXRα and an increase in the amounts of mRNA and protein for CRABP-II.

2. Materials and methods

2.1. Materials

(+)-α-Tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol, all *trans*-retinol, calphostin C and okadaic acid were all purchased from Sigma (St. Louis, MO). Bisindolylmaleimide I hydrochloride and monoclonal antibody against phosphoserine (clone16B4) were purchased from Calbiochem–Novabiochem GmbH (Darmstadt, Germany). Dynabeads® M-280 (sheep anti-rabbit IgG) were purchased from Dynal Biotech. (Oslo, Norway). CRABP-II monoclonal antibody was a generous gift of Dr. Cecile Rochette-Egly, β-actin goat polyclonal, RARγ rabbit polyclonal and RXRα rabbit polyclonal antibodies were purchased from St. Cruz Biotechnology (St. Cruz, CA). All other chemicals used were of the purest grade commercially available.

(+)-α-Tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol and all *trans*-retinol were prepared in ethanol with 0.01% butylated hidroxytoluene, 1000 times more concentrated than the final concentration in the culture medium. Calphostin C, bisindolylmaleimide I and okadaic acid were prepared in DMSO, 1000 times more concentrated than the final concentration in the culture medium. The final concentration of ethanol and DMSO in the culture medium was 0.1% (v/v), this concentration had no detectable effect by itself. All experiments were performed using freshly dissolved material. All the experiments presented in this study were repeated at least three separate times with similar results.

2.2. Cell culture

Human dermal fibroblasts were obtained from juvenile foreskins and grown as described [25]. Fibroblasts between passage number 4 and 12 were used throughout all the experiments. After confluence was reached, the serum content of the medium was reduced to 0.5% and the fibroblasts were treated with the test compounds plus 1 μM retinol.

2.3. Isolation of total RNA and analysis of mRNA expression by RT-PCR

Total RNA isolation and reverse transcription reactions were done as described [25]. The PCR analysis for CRABP-II and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed using Biotools DNA polymerase gel-form from B & M Labs, S.A. (Madrid, Spain) following the instructions of the manufacturer. The conditions for each PCR were determined in preliminary experiments and optimized for each set of primers. The specific primers (5′ to 3′) were for CRABP-II: ATGCCCAACTTCTCTGGCAA and CGTCATGGTCAGGATCAGTT and for GAPDH: TCACCACCATGGAGAAGGCT and AAGGCCATGCCAGTGAGCTT. The amplification conditions were 1 cycle at 85 °C for 2 min and 1 cycle at 94 °C for 2 min. Then, for CRABP-II 25 cycles of denaturation (94 °C for 30 s), annealing (59 °C for 30 s) and extension (70 °C for 1 min) with a final extension of 70 °C for 1 min and for GAPDH 25 cycles of denaturation (94 °C for 30 s), annealing (53 °C for 30 s) and extension (70 °C for 1 min) with a final extension of 70 °C for 1 min. The resulting PCR products were separated by electrophoresis in a 1.5% agarose gel in TBE and stained with ethidium bromide. The expected sizes for the PCR products were as follows: CRABP-II, 375 bp and GAPDH, 390 bp. The intensity of the bands was measured by densitometry using the GeneGenius system and the GeneTools analysis software (Syngene, Cambridge, UK).

2.4. Immunoprecipitation and Western blot analysis

Serine phosphorylation of RXRα or RARγ from human dermal fibroblasts was determined by immunoprecipitating RXRα or RARγ, respectively, from 175 μg of total protein from whole cell extracts through overnight incubation at 4 °C with 5 μg of a polyclonal anti-RXRα or anti-RARγ antibody bound to Dynabeads M-280 following the instructions of the manufacturer. Immunoprecipitates were then washed three times with PBS containing 0.25 mM phenylmethyl sulfonyl fluoride and finally boiled for 5 min in electrophoresis sample buffer. Western blot analysis was done as described [25] except that the membranes were blocked in 5% bovine serum albumin instead of 3% non-fat milk in TBST to avoid milk phosphoproteins.

2.5. Transfection and luciferase assays

Fibroblast cells were cultured in 6-well plates and transfected with the luciferase reporter plasmids (2 μg/plate), pGL3-RARP-Luc, containing RARE from position −55 to −35 of the human RAR-β promoter or pDR5-TK-Luc, containing direct repeats of AGGTC separated by five nucleotides in the context of heterologous TK promoter [23], both kindly provided by Dr. J.M. Kurie. Transfections were performed using lipofectamine reagent (Invitrogen, Life Technologies, Inc) as described [26]. Transfected cells were washed twice with Opti-MEM® (Gibco, Life Technologies, Inc), incubated in 10% FCS containing medium for 12 h and then cultured overnight in medium containing 0.5% serum and treated with 1 μM retinol alone or with 1 μM retinol plus α-tocopherol, calphostin C, bisindolylmaleimide I or okadaic acid plus α-tocopherol. Luciferase activity of each cell lysate was measured using the luciferase assay system, as described by the manufacturer (Promega; Madison, WI).

3. Results and discussion

We reported [25] that the treatment of human dermal fibroblasts with retinoic acid or retinol produced an increase of CRABP-II mRNA compared to controls as expected from previous results [27]. Interestingly, in our work [25], the treatment of fibroblasts with retinol together with vitamin E produced a major increase of CRABP-II mRNA compared to the treatment of fibroblasts with retinoic acid or retinol alone. In light of these results, we decided to study the effect of

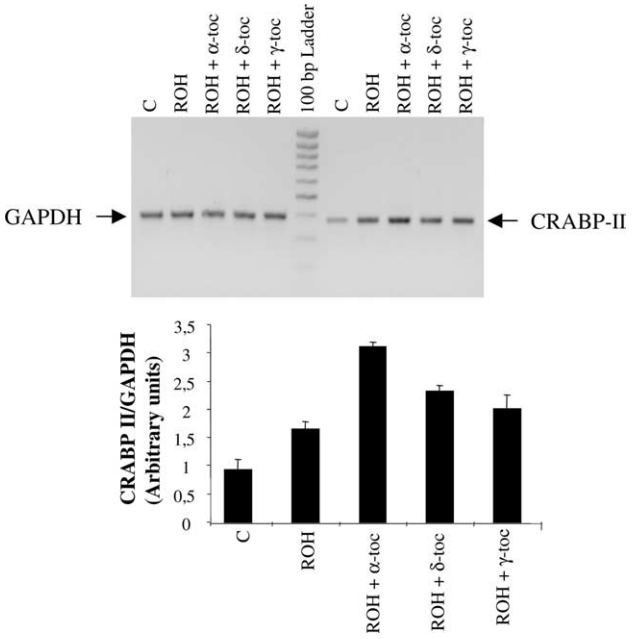

Fig. 1. Effect of different tocopherols on CRABP-II mRNA. Total RNA was isolated from untreated fibroblasts (C) or fibroblasts treated with retinol (ROH), retinol plus 100 μM of α-tocopherol (ROH + α-toc), retinol plus 100 μM γ-tocopherol (ROH + γ-toc) or retinol plus 100 μM δ-tocopherol (ROH + δ-toc) for 14 h and amplified by RT-PCR using specific primers for CRABP-II and GAPDH as described in Section 2. One fifth of the total amplified product was then analyzed on 1.5% agarose gel stained with ethidium bromide. The graphs show the ratios of CRABP-II to GAPDH for each experimental condition and represent the means (± S.D.) of three independent experiments.

different commercially available tocopherols (mono- (δ-), di- (γ-) and trimethyl- (α) tocols) on CRABP-II mRNA and protein expression.

Human dermal fibroblasts were incubated with 100 μM α-, γ- or δ-tocopherol during 14 h, in the presence of retinol. Total RNA was isolated as described in Section 2 and reverse transcription-polymerase chain reaction (RT-PCR) analysis of equal amounts of RNA was done. Reverse transcription and amplification conditions were established previously to permit the comparison of the mRNA levels in the different experimental conditions. The results of representative RT-PCR experiments involving treatment of fibroblasts with the different tocopherols (α, γ or δ) are shown in Fig. 1. As observed, the three tocopherol isomers used induced CRABP-II mRNA. α-Tocopherol produced the greatest response (3.4-fold), followed by δ-tocopherol (2.5-fold) and γ-tocopherol (2.2-fold).

Western blot analysis with anti-CRABP-II antibody showed that treatment of human dermal fibroblasts for 24 h with 100 μM α-tocopherol plus retinol (Fig. 2A, lane 2), 100 μM γ-tocopherol plus retinol (Fig. 2A, lane 3) and 100 μM δ-tocopherol plus retinol (Fig. 2A, lane 4) resulted in an increase of CRABP-II protein levels compared with the control (Fig. 2A, lane 1), as was observed at the mRNA level (Fig. 1).

Because PKC inhibition by different tocopherol isomers has been reported [16], it was interesting to establish whether the observed increase in CRABP-II expression was due to a tocopherol-mediated PKC inhibition. Calphostin C, a selective PKC enzyme inhibitor, provoked an increase in CRABP-II protein levels (Fig. 2A, lane 5), similar to that observed with the different tocopherol isomers (compare lane 5 with lanes 2–4). It has also been demonstrated that α-tocopherol induces the activation of protein phosphatase 2A resulting in the dephosphorylation and consequent inactivation of PKCα [17]. In our experimental model, the possible inhibitory effect of α-tocopherol on PKC was reversed by okadaic acid, a potent inhibitor of protein phosphatases 1 and 2A [28]. In Fig. 2A (lane 6), we observed that fibroblast treatment with α-tocopherol plus retinol and 2 nM okadaic acid reduced CRABP-II protein almost to control levels (lane 1). Similar results were obtained by RT-PCR analysis (Fig. 2B). Because the observed effect was produced with 2 nM okadaic acid and the IC_{50} for this inhibitor is 10–15 nM for protein phosphatase 1 and 0.1 nM for protein phosphatase 2A, we can assume that the protein phosphatase 2A is the phosphatase responsible for the observed PKC inactivation.

Under the same culture conditions, luciferase expression increased after treatment of fibroblasts with 1 μM retinol as expected. Moreover, the treatment of fibroblasts with α-tocopherol, calphostin C or bisindolylmaleimide I together with 1 μM retinol provoked the maximum effect (Fig. 3). Thus, our results overall consistently indicate that CRABP-II expression was increased through RARE by α-tocopherol or PKC inhibitors in the presence of retinol.

Phosphorylation events can inactivate nuclear receptors (for a review, see [29,30]). In the case of RARα, inhibition of the transcriptional activity occurs by phosphorylation of residues located within the DNA-binding domain by PKC [18]. Since CRABP II gene expression is regulated by the binding of RARγ–RXRα heterodimer to their RAREs [7], we have investigated if the effect observed in CRABP II expression by

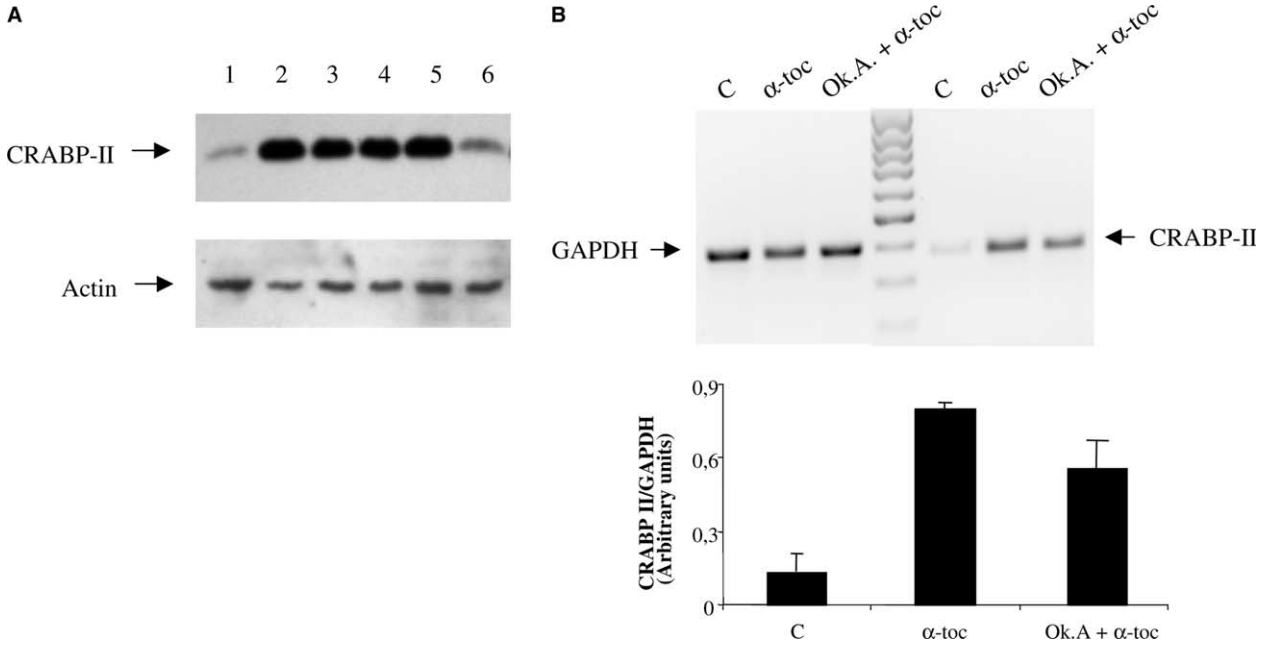

Fig. 2. Effect of tocopherols, calphostin C and okadaic acid plus α-tocopherol on CRABP-II expression. (A) Western blot analysis of equal amounts of protein (5 μg) derived from fibroblasts treated for 24 h. with retinol alone (lane 1) or fibroblasts treated with 100 μM α-tocopherol plus retinol (lane 2), 100 μM γ-tocopherol plus retinol (lane 3), 100 μM δ-tocopherol plus retinol (lane 4), 100 nM calphostin C plus retinol (lane 5) or 100 μM α-tocopherol plus retinol and 2 nM okadaic acid (lane 6) were probed with anti-CRABP-II as described in Section 2. The same blot was reprobed with an antibody specific for β-actin. (B) Total RNA was isolated from untreated fibroblasts (C) or fibroblasts treated with 100 μM of α-tocopherol plus retinol (α-toc) or 100 μM α-tocopherol plus retinol and 2 nM okadaic acid (Ok.A. + α-toc) for 14 h and amplified by RT-PCR using specific primers for CRABP-II and GAPDH as described in Section 2. One fifth of the total amplified product was then analyzed on 1.5% agarose gel stained with ethidium bromide. The graphs show the ratios of CRABP-II to GAPDH for each experimental condition and represent the means (±S.D.) of three independent experiments.

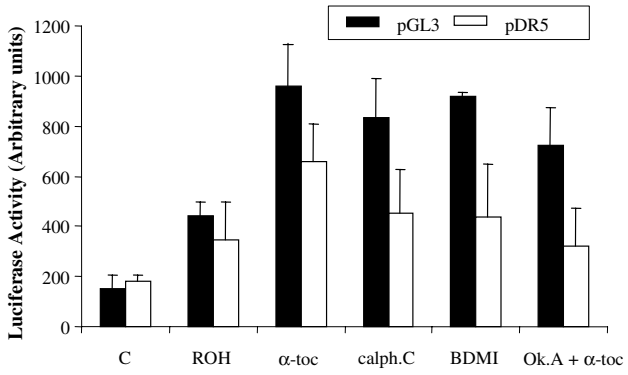

Fig. 3. Luciferase activity in human dermal fibroblasts transiently transfected with reporter plasmids containing RAREs. Luciferase assays were performed on fibroblast cells transiently transfected with pGL3-RARP-Luc or pDR5-TK-Luc as described in Section 2. Transfected cells were treated with 1 μM retinol (ROH), 100 μM α-tocopherol plus 1 μM retinol (α-toc), 100 nM calphostin C plus 1 μM retinol (calph. C), 50 nM bisindolylmaleimide I plus 1 μM retinol (BDM I), 100 μM α-tocopherol plus 1 μM retinol and 2 nM okadaic acid (Ok.A. + α-toc) or without any treatment (C). Data presented in the figure represent the means (±S.D.) of three independent experiments using 20 μg of cell extract.

tocopherol was due to changes in the phosphorylation status of RARγ and/or RXRα.

Western blot analysis of whole cell extracts immunoprecipitated with an antibody against RXRα showed that the amount of RXRα protein did not change in any of the different incubation conditions of the fibroblasts (Fig. 4A, upper panel). However, when the same blot was probed with a monoclonal antiphosphoserine antibody, we observed that the treatment of dermal fibroblast with α-tocopherol plus retinol (Fig. 4A, lower panel, lane 2), calphostin C plus retinol (lane 3) and bisindolylmaleimide I plus retinol (lane 4) decreased the phosphorylation levels of RXRα compared with fibroblasts treated with retinol alone (lane 1). The treatment of dermal fibroblasts with α-tocopherol plus retinol and okadaic acid (lane 5) increased the phosphorylation of RXRα almost to half of control level. Curiously, the phosphorylation level in serine residues of RXRα in untreated fibroblast (lane 6) is less than that observed in the fibroblast treated with retinol alone (lane 1), suggesting that ligand binding to RXRα provokes structural changes in RXRα which facilitate the action of PKC. This observation is in agreement with a previous report [18]. These results suggest that the effect of PKC on RXRα involved the modulation of the phosphorylation state rather than affecting the levels of RXRα protein. Western blot analysis using the same antiphosphoserine antibody did not indicate any differences in RARγ phosphorylation in human dermal fibroblasts (see Fig. 4B). Our results are in agreement with those of Delmotte et al. [18] where they showed that PKCα and γ were able to phosphorylate RARα in vitro and this phosphorylation, which occurs on a single serine residue, inhibits the formation of RAR–RXR heterodimers and consequently their transcriptional activity. Furthermore, our results are consistent with a model in which tocopherol induces the activation of protein phosphatase 2A, which dephosphorylates and inactivates PKC, which in turn leads to the dephosphorylation of RXRα, increasing the ability of this factor to heterodimerize with RARγ and, in the presence of the ligand, RARγ–RXRα heterodimers activate the expression of CRABP-II.

Other nuclear receptors in addition to RAR and RXR have been described as substrates of PKC: thyroid hormone receptor (T_3R) [31] and vitamin D_3 receptor (VDR) [32]. In the case of VDR, PKC activation inhibited VDR-mediated transcription by a mechanism involving phosphorylation of serine 51 located in the DNA binding domain of VDR.

Besides PKC, other kinases have been implicated in the phosphorylation of nuclear receptors including RXR [29,30].

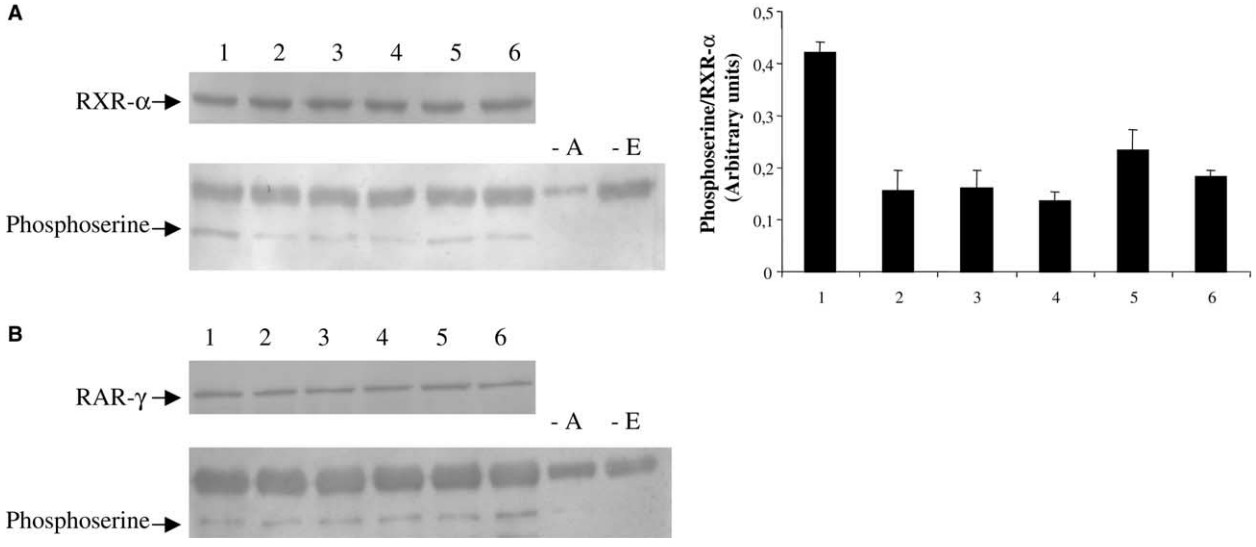

Fig. 4. Phosphorylation of RXR and RAR on serine residues in human dermal fibroblasts. Whole cell extracts were prepared from human dermal fibroblasts treated for 24 h. with retinol (lane 1), 100 μM α-tocopherol plus retinol (lane 2), 100 nM calphostin C plus retinol (lane 3), 50 nM bisindolylmaleimide I plus retinol (lane 4), 100 μM α-tocopherol plus retinol and 2 nM okadaic acid (lane 5) or without any treatment (lane 6); immunoprecipitated with an anti-RXR or anti-RAR antibody as described in Section 2, followed by SDS–PAGE and Western blotting analysis using specific antibody to RXR, RAR or phosphoserine. The lanes indicated as (–A) and (–E) correspond to immunoprecipitate without antibody and without whole cell extract, respectively. The graphs show the ratios of RXRα phosphorylated on serine residues to total RXRα immunoprecipitated for each experimental condition and represent the means (±S.D.) of three independent experiments.

Indeed, phosphorylation of RXRα mediated by MAPK impairs the transcripitional activity of RAR/RXR [22,23] and VDR/RXR [24] heterodimers, suggestting that PKC, as an activator of the MAPK pathway [33,34], might participate, indirectly, in the control of the transcriptional activity of RXR and other nuclear receptors.

Recently, it has been shown that α-, γ- and δ-tocopherol are able to activate gene expression via the pregnane X receptor (PXR). This nuclear receptor acts by binding to the direct repeat response element DR3 as a PXR–RXR heterodimer, regulating the expression of different drug metabolizing enzymes [35].

In summary, we have shown a direct transcriptional gene activation by tocopherols via PKC inhibition and mediated by RXRα.

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References

[1] Gudas, L.J., Sporn, M.B. and Roberts, A.B. (1994) Cellular biology and biochemistry of the retinoids. in: The Retinoids: Biology, Chemistry and Medicine (Sporn, M.B., Roberts, A.B. and Goodman, D.S., Eds.), pp. 443–520, Raven Press, New York.
[2] Roos, T.C., Jugert, F.K., Merk, H.F. and Bickers, D.R. (1998) Pharmacol. Rev. 50, 315–333.
[3] Li, E. and Norris, A.W. (1996) Annu. Rev. Nutr. 16, 205–234.
[4] Aström, A., Tavakkol, A., Pettersson, U., Cromie, M., Elder, J.T. and Voorhees, J.J. (1991) J. Biol. Chem. 266, 17662–17666.
[5] Durand, B., Saunders, M., Leroy, P., Leid, M. and Chambon, P. (1992) Cell 71, 73–85.
[6] Fisher, G.J., Talwar, H.S., Xiao, J.H., Datta, S.C., Reddy, A.P., Gaub, M.P., Rochette-Egly, C., Chambon, P. and Voorhees, J.J. (1994) J. Biol. Chem. 269, 20629–20635.
[7] Bastien, J., Adam-Stitah, S., Plassat, J.L., Chambon, P. and Rochette-Egly, C. (2000) J. Biol. Chem. 277, 28683–28689.
[8] Boscoboinik, D., Szewczyk, A., Hensey, C. and Azzi, A. (1991) J. Biol. Chem 266, 6188–6194.
[9] Azzi, A., Aratri, E., Boscoboinik, D., Clement, S., Ozer, N.K., Ricciarelli, R. and Spycher, S. (1998) Biofactors 7, 3–14.
[10] Ricciarelli, R., Zingg, J.M. and Azzi, A. (2000) Circulation 102, 82–87.
[11] Houglum, K., Brenner, D.A. and Chojkier, M. (1991) J. Clin. Invest. 87, 2230–2235.
[12] Chojkier, M., Houglum, K., Lee, K.S. and Buck, M. (1998) J. Physiol. 275, G1480–G1485.
[13] Ricciarelli, R., Maroni, P., Özer, N., Zingg, J.M. and Azzi, A. (1999) Free Radic. Biol. Med. 27, 729–737.
[14] Aratri, E., Spycher, S.E., Breyer, I. and Azzi, A. (1999) FEBS Lett. 447, 91–94.
[15] Fechner, H., Schlame, M., Guthmann, F., Stevens, P.A. and Rustow, B. (1998) Biochem. J. 331, 577–581.
[16] Brigelius-Flohé, R., Kelly, F.J., Salonen, J.T., Neuzil, J., Zingg, J.M. and Azzi, A. (2002) Am. J. Clin. Nutr. 76, 703–716.
[17] Ricciarelli, R., Tasinato, A., Clement, S., Özer, N.K., Boscoboinik, D. and Azzi, A. (1998) Biochem. J. 334, 243–249.
[18] Delmotte, M.H., Tahagato, A., Formstecher, P. and Lefebvre, P. (1999) J. Biol. Chem. 53, 38225–38231.
[19] Boskovic, G., Desai, D. and Niles, R.M. (2002) J. Biol. Chem. 277, 26113–26119.
[20] Lefebvre, P., Gaub, M.P., Tahayato, A., Rochette-Egly, C. and Formstecher, P. (1995) J. Biol. Chem. 270, 10806–10816.
[21] Matkovits, T. and Christakos, S. (1995) Mol. Endocrinol. 9, 232–242.
[22] Matsushima-Nishiwaki, R., Okuno, M., Adachi, S., Sano, T., Akita, K., Moriwaki, H., Friedman, S.L. and Kojima, S. (2001) Cancer Res. 61, 7675–7682.
[23] Lee, H.Y., Suh, Y.A., Robinson, M.J., Clifford, J.L., Hong, W.K., Woodgett, J.R., Cobb, M.H., Mangelsdorf, D.J. and Kurie, J.M. (2000) J. Biol. Chem. 275, 32193–32199.
[24] Solomon, C., White, J.H. and Kremer, R. (1999) J. Clin. Invest. 103, 1729–1735.
[25] Gimeno, A., Zaragozá, R., Vivó-Sesé, I., Viña, J.R. and Miralles, V.J. (2004) Exp. Dermatol. 13, 45–54.
[26] Xu, G., Bochaton-Piallat, M.L., Andreutti, D., Low, R.B., Gabbiani, G. and Neuville, P. (2001) J. Cell. Physiol. 187, 315–325.
[27] Elder, J.T., Kaplan, A., Matthew, A.C., Kang, S. and Voorhees, J.J. (1996) J. Invest. Dermatol. 106, 517–521.
[28] Cohen, P. (1989) Annu. Rev. Biochem. 58, 453–508.
[29] Shao, D. and Lazar, M.A. (1999) J. Clin. Invest. 103, 1617–1618.
[30] Rochette-Egly, C. (2003) Cell Signal. 15, 355–366.
[31] Goldberg, Y., Glineur, C., Gesquiere, J.C., Ricouart, A., Sap, J., Vennstrom, B. and Ghysdael, J. (1988) EMBO J. 7, 2425–2433.
[32] Hsieh, J.C., Jurutka, P.W., Galligan, M.A., Terpening, C.M., Haussler, C.A., Samuels, D.S., Shimizu, Y., Shimizu, N. and Haussler, M.R. (1991) Proc. Natl. Acad. Sci. USA 88, 9315–9319.
[33] Sozeri, O., Vollmer, K., Liyanage, M., Frith, D., Kour, G., Mark, G.E. and Stabel, S. (1992) Oncogene 7, 2259–2262.
[34] Marquardt, B., Frith, D. and Stabel, S. (1994) Oncogene 9, 3213–3218.
[35] Landes, N., Pfluger, P., Kluth, D., Birringer, M., Rúhl, R., Ból, G.F., Glatt, H. and Brigelius-Flohé, R. (2003) Biochem. Pharmacol. 65, 269–273.