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Inhibitory Effect of Curcumin on TPA (12-O-Tetradecanoyl Phorbol-13-acetate)-Induced Activation of Protein Kinase C Isoenzyme-Epsilon and c-fos Protein Level in Human Keratinocytes

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ABSTRACT: Protein Kinase C (PKC) is a family of isoenzymes that are normally expressed in human keratinocytes. The expression pattern of PKC under induction of 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a potent tumor promoter, is herein reported. Curcumin was evaluated for its effect on TPA-activated PKC-ε because PKC-ε has been found to have unique oncogenic potential and substrate specificity. Curcumin was shown previously to inhibit TPA-induced AP-1 activity. c-fos protein is inducible by TPA and thus is associated with c-jun to result in an increased AP-1 activity. In the present study the effect of curcumin on TPA-induced c-fos protein level was determined. Human keratinocytes were treated with 160 nM TPA for 1, 2 and 18 h. Upon stimulation by TPA for 1 or 2 h, PKC-α and -ε isoenzymes were translocated from cytosol to membrane by 70 or 85 % and they were completely down regulated after treatment for 18 h. However, effects were not found in PKC-δ isoenzyme. Treatment with 20, 40 and 50 μM curcumin inhibited TPA-induced translocation of PKC-ε isoenzyme from the cytosol to the membrane in a dose-dependent manner. Curcumin alone did not affect the translocation of PKC-ε isoenzyme. The expression level of c-fos protein in human keratinocytes was induced by 160 nM TPA with the maximum result at 2 h, as measured by enhanced chemiluminesence Western blotting detection system. Curcumin pretreated cells decreased TPA-induced c-fos expression in a dose-dependent manner. In conclusion, the induction of c-fos expression and PKC-ε activation by TPA occurred in human keratinocytes and this signalling was inhibited by curcumin.

KEYWORDS: Curcumin, protein kinase C (PKC), human keratinocytes, TPA, c-fos.

INTRODUCTION

Turmeric is a perennial herb widely cultivated in tropical regions of Asia. It has been widely used in many parts of the world as spice, colorant, and cosmetic [1]. It contains about 1-5 % curcumin (diferuloylmethane), a phenolic compound that has been identified as the major pigment in turmeric, and it possesses both anti-inflammatory and antioxidant properties[2]. It has been shown to inhibit mutagenesis, carcinogenesis and DNA-carcinogen adduct formation[3]. With its perceived human safety, following centuries of use, curcumin becomes a potent phytochemical candidate for cancer prevention. However, how curcumin elicits this chemopreventive efficacy on carcinogenesis is an ongoing research project in many laboratories. A large body of data has demonstrated that curcumin is engaged in multiple anti-tumor promoting pathways. It has been reported that curcumin inhibited a variety of biological activities of 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a potent skin tumor promoter. TPA induces several biosynthetic processes, namely enhanced expression of cellular oncogenes such as c-jun, c-fos and c-myc[4] and elevation or translocation of protein kinase C (PKC)[5]. PKC is a multigene family consisting of at least ten distinct serine/threonine kinases which play a key role in signal transduction and growth control. PKC isoenzymes are classified into three groups based on activation by Ca^{2+} and phospholipid: Ca^{2+} dependence or conventional isoenzymes (PKC-α, β, γ) , Ca^{2+} independence or novel isoenzymes (PKC-δ, ε, θ, η) and not requiring Ca^{2+} or lipid (PKC-ζ, λ)[6]. Conventional and novel PKC isoenzymes are the major cellular receptors for TPA. In addition, PKC isoenzymes display selective organ and cellular expression. It was found that PKC-α, -δ and -ε isoenzymes are normally expressed in human keratinocytes[7]. Little is known concerning the role of individual PKC isoenzymes in human keratinocytes. Among the PKC isoenzymes it has been reported that PKC-ε isoenzyme exhibited full oncogenic potential[8,9] and is implicated in the regulation of other biological

processes, including antiviral resistance, hormonal secretion, transporter regulation and golgi function modulation[10,11]. Thus, this study focused on PKC-ε isoenzyme and determined the effect of curcumin on TPA-induced translocation or downregulation of PKC-ε isoenzyme in human keratinocytes. In most studies, the inhibitory effect of curcumin on TPA action has been studied using mouse skin model. The effect of curcumin on TPA actions in human skin model has not been established. In this paper we demonstrated for the first time the protective effect of curcumin on TPA induced PKC-ε activation. The inhibition of TPA-induced PKC-ε by curcumin might explain the decrease in c-fos protein level in human keratinocytes.

Materials and Methods

Human Keratinocytes Culture

Primary keratinocytes were isolated from keratome biopsies of juvenile foreskins (2-3 years old), which were obtained from Dr. Aram Limtrakul (Chiang Mai Public Health Promotion Center). The skin was placed in transport medium (DMEM free serum) and moved to the laboratory, where it was processed as soon as possible. Using a pair of scissors, all excess dermis and connective tissue were removed, the skin sections were sterilized by immersing in absolute ethanol for 1 min and soaked in antibiotic solution (20% penicillin-streptomycin and 10% penicillin – streptomycin) at 4 °C for 30 min. The tissue was transferred to dispase solution and incubated at 4 °C for 48 h. Then the epidermis layer was removed and treated with 2 ml trypsin-EDTA at 37 °C for 30-45 min, with gentle mixing every 5 min. Addition of 4 ml soybean trypsin-inhibitor stopped the action of trypsin. Then cells were centrifuged at low speed centrifugation (500 rpm) for 5 min at room temperature. Supernatant was removed and cell pellet resuspended in keratinocyte-SFM 5 ml. Using syringe with needle size 22G1/2, the suspension was mixed very gently to form a single cell suspension. The primary keratinocytes were cultured at a density of $3x10^6$ cells/culture in T-25 flasks in keratinocyte-SFM medium supplemented with epidermal growth factor (EGF, 5 ng/ml) and bovine pituitary extract (BPE, 50 µg/ml). The Ca^{2+} concentration of the medium was kept at 0.05 mM to maintain undifferentiated keratinocytes. The cells were maintained in a CO_2-incubator (37 °C, 5% CO_2) and the medium was changed every 2-3 days. Passages 3-5 were used in our study.

Preparation of Cytosolic and Membrane Fractions of PKC

For detection of PKC, cytosolic and membrane fractions were prepared from subconfluent cell culture as described before[12]. Briefly, cells were washed twice with ice cold Ca^{2+} and Mg^{2+} - free phosphate-buffer saline (PBS) and detached by adding 0.05% of trypsin-EDTA. The reaction was stopped with trypsin-inhibitor. Cells were collected by low speed centrifugation and then washed once with PBS. The cell pellets were resuspended in buffer A (25 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM EGTA, 0.05 mM DTT, 0.02% triton X-100, 10 µg/ml leupeptin, 25 µg/ml aprotinin, 2 mM PMSF) and homogenized using a Dounce homogenizer (pestle A). Cell homogenates were centrifuged at 100,000*g* for 1 h. The supernatant was collected as a cytosolic PKC fraction. The pellet was washed once in buffer B (25 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM EGTA, 2 mM PMSF), then treated on ice for 1 h with 0.2% triton X-100 in buffer A and centrifuged at 100,000*g* for 1 h. The supernatant (membrane PKC fraction) was collected and concentrated using Centricon 30 concentrators (Amicon Corp.) and membrane protein concentrations were determined by Lowry's method [13]. The PKC levels in cytosolic and membrane fractions were measured by Western-blot analysis.

Preparation of Nuclear Extracts of c-fos Protein

The nuclear extract was prepared by the method of Dignam et al.[14] as follows. The pellet obtained from the low speed centrifugation of the homogenate was resuspended in buffer C (20 mM HEPES, pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT) and was stirred gently with a magnetic stirring bar for 30 min at 4 °C and then centrifuged for 30 min at 25,000*g*. The supernatant was dialysed against 50 volumes of buffer D (20 mM HEPES, pH 7.9, 20% glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT) for 5 h. The dialysate was centrifuged for 20 min at 25,000*g* and the resulting precipitate discarded. The supernatant, designated as the nuclear extract, was frozen as aliquots in liquid nitrogen and stored at -80 °C.

Western Blotting for PKC and c-fos Proteins

Cells were placed in growth factor-free medium (keratinocyte-SFM without hydrocortisone, insulin, BPE and EGF) for 48 h. The cells were then treated with test compounds as described in the text. For detection of PKC protein, proteins (30 µg) from cytosolic and membrane fractions were electrophoresed in discontinuous 7.5% SDS-polyacrylamide gels. The proteins were electroblotted onto Hybond ECL membrane in a Trans-blot electrophoretic transfer cell (Bio-Rad Laboratories) as described before[15]. The blots were blocked with 5% nonfat dry milk dissolved in tris buffered saline containing 0.2% Tween-20 (TBST). To

detect PKC-ε (93 kD), the blot was incubated for 2 h with anti-PKC-ε antibody at a dilution of 1:2500 in TBST. Whereas for PKC-α (82 kD) and -δ (76 kD), the blots were incubated with the anti- PKC-α and -δ antibodies for 1 h at a dilution of 1:2500. Following washing, immunoreactive proteins were visualized using the enhanced chemiluminnescence Western blotting detection system (Amersham). The autoradiograms were scanned with an Image Master sharp laser densitometric scanner, and the peak areas representing PKC bands were determined. Western blot analysis of the fos protein was performed by the same protocol as described above except in the detection system, the specific antibody to c-fos protein (p62, 62 kD) was used instead of specific antibody to PKC proteins.

Data Manipulation and Statistical Analysis

The levels of PKC were presented as percentages of the cytosolic PKC level of DMSO-treated cells (CO, control). The levels of c-fos were presented as percentages of the c-fos level after 2 hr TPA treatment. Results are the means ± SD from three independent experiments. Differences between the means were analyzed by one-way analysis of variance. Results were considered to be statistically significant when $p<0.05$.

Results

Translocation and Downregulation of PKC-Alpha (α), -Delta (δ), and -Epsilon (ε) Isoenzymes in Response to TPA

PKC isoenzyme activation is associated with translocation from cytosol to membrane. The translocations of PKC-α, -δ and -ε isoenzymes, which are TPA responsive isotypes in human keratinocytes, were evaluated in this study. The 80 % confluent human keratinocytes at passages 3-5 were placed in growth factor-free medium for 48 h. The cells were treated with 160 nM TPA for 1, 2 and 18 h. Control cells received 0.4% DMSO. Treatment of keratinocytes with TPA for 1 or 2 h induced translocation of PKC-α and -ε isoenzymes from the cytosol that was associated with increasing amounts in the membrane by about 70 or 85 % (Figs.1 A and B). Long-term treatment with TPA (18 h) induced complete downregulation (i.e., loss of >99%) of PKC-α and -ε. In contrast, densitometric analysis of Western blots for PKC-δ revealed no significant reproducible changes in either cytosolic or membrane fractions in response to TPA (Fig.1C). Our data indicated that in human keratinocytes there were two PKC isoenzymes, α and ε, which were activated by 160 nM TPA and demonstrated the specific activation of PKC isoenzymes in response to TPA.

Effect of TPA on the Total Level of the PKC-Epsilon (ε) in Whole Cell Lysates

The activation by TPA may increase the total level of PKC-ε isoenzyme in whole cell lysates. To examine this possibility, whole cell lysates of keratinocytes treated with 160 nM TPA for 0, 1, 2 and 18 h were assayed by Western blot (Fig. 2). No significant difference between the band intensities of the control and 1 and 2 h TPA-treated keratinocytes were observed. This finding suggests that when human keratinocytes were treated with TPA, PKC-ε was activated by translocation from cytosol to the membrane but TPA did not affect the total level of PKC protein in the cells. Long-term treatment with TPA (18 h) induced complete downregulation of PKC level.

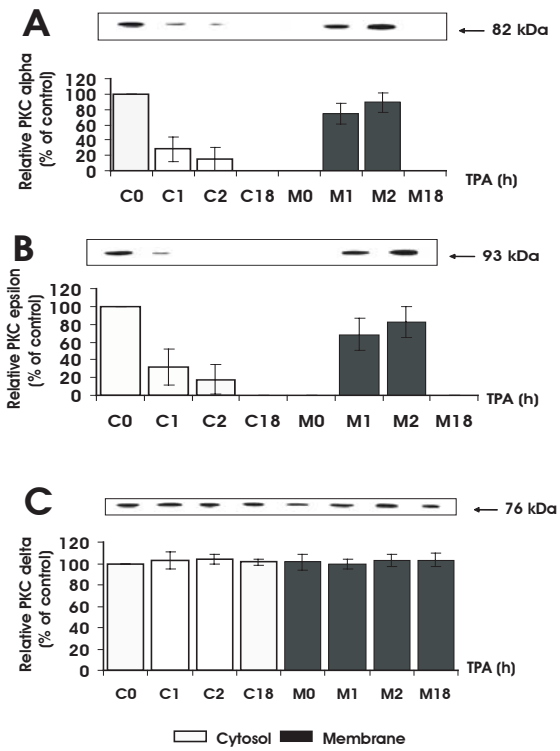

Fig 1. Translocation and downregulation of PKC-α,ε, δ isoenzymes in response to TPA. Cytosolic (C) and membrane (M) fractions (30 μg protein) were prepared from keratinocytes treated with TPA, separated by SDS-PAGE, and immunoblotted as described. *Upper*: Western blot analysis of PKC-α (A),ε (B) and δ(C) protein expression using anti PKC-α, ε and δ antibodies, respectively. C0 and M0 are cytosolic and membrane fractions of DMSO-treated cells (control). C1 and M1, C2 and M2, C18 and M18 are cytosolic and membrane fractions of TPA-treated cells for 1, 2 and 18 h respectively. *Lower* : Relative intensity of the immunoblots calculated as means ±S.E. Each histogram obtained from three independent experiments performed in triplicate.

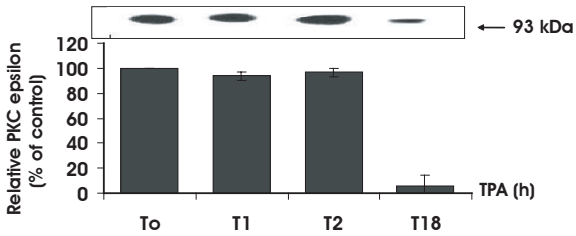

Fig 2. Effect of TPA on the total level of PKC-ε isoenzyme in human keratinocytes. Whole cell lysates (30 µg protein) were prepared from keratinocytes treated with 160 nM TPA for 1 (T1), 2 (T2) and 18 (T18) h respectively, separated by SDS-PAGE, and immunoblotted as described. *Upper*: Western blot analysis of PKC-ε protein expression using anti PKC-ε antibodies. *Lower*: Relative intensity of the immunoblots calculated as means ±S.E.. Each histogram was obtained from three independent experiments performed in triplicate. The arrow indicates molecular weight of PKC-ε isoenzyme.

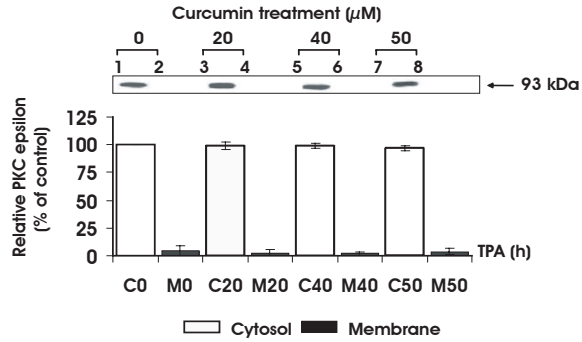

Fig 3. Effect of curcumin on translocation of PKC-ε isoenzyme. Cytosolic (C) and membrane (M) fractions (30 µg protein) were prepared from keratinocytes treated with curcumin (0, 20, 40 and 50 µM) for 1 h, separated by SDS-PAGE, and analyzed for PKC-ε isoenzymes. Lanes 1, 3, 5, 7 and 2, 4, 6, 8 are cytosolic and membrane fractions, respectively. Lanes 1 and 2, control (C0 and M0); lanes 3 and 4, 20 µM curcumin (C20 and M20); lanes 5 and 6, 40 µM curcumin (C40 and M40) and lanes 7 and 8, 50 µM curcumin (C50 and M50). Upper: Western blot analysis of PKC-ε expression using anti-PKC-ε antibody. Lower : Relative intensity of the immunoblots calculated as means ±S.E. Each histogram was obtained from three independent experiments performed in triplicate. The arrow indicates molecular weight of PKC-ε isoenzyme.

Effect of Curcumin on Translocation of PKC-Epsilon (ε)

To explore whether curcumin itself affects translocation of PKC-ε in human keratinocytes, the cells were incubated at 37 °C for 1 h at different concentrations of curcumin (0, 20, 40, 50 µM). It was found that the PKC bands were only detected in cytosolic fractions, and there were no significant differences among control and curcumin treated cells (Fig. 3). Thus, it can be suggested that curcumin itself did not induce translocation of PKC-ε enzyme from the cytosol to the membrane in human keratinocytes.

Effect of Curcumin on TPA-Induced PKC-Epsilon (ε) Activation

We previously reported that curcumin inhibited TPA-induced tumor promotion on mouse skin[19, 20]. However the molecular mechanism of this inhibition remained to be explored in human skin model. Therefore this study examined the effect of curcumin on translocation of TPA-induced PKC-ε which has not yet been reported in other studies. The cells were preincubated for 1 h with different concentrations of curcumin (20, 40, 50 µM) followed by treatment with 160 nM TPA for 1 h at 37 °C. The control cells received 0.4% DMSO. Cytosolic and membrane fractions were prepared and analyzed for the level of PKC-ε isoenzyme by Western blotting. The result indicated that curcumin inhibit the TPA induced PKC-ε in a dose dependent manner (Fig. 4A).

Effect of Time on Curcumin Addition on TPA-Induced PKC-Epsilon (ε)

It appears that the molecular action of curcumin is quite complicated because the targets of its action vary from DNA to RNA and protein level (enzyme level). Accordingly, this study examined kinetics of TPA-dependent PKC-ε activation by curcumin. Primary culture of human keratinocytes were treated with 50 µM curcumin for 1 h prior to addition of TPA (pre-treatment), at the same time as the addition of TPA (co-treatment), or 1 h after addition of TPA (post-treatment). After these treatments cytosolic and membrane fractions were prepared and then assayed for PKC-ε as described earlier. It was found that TPA-dependent PKC-ε activation was inhibited only when cells were pretreated with curcumin. The co-treatment and post-treatment were not effective (Fig. 4B). Therefore, this study showed that curcumin inhibited the effect of TPA in the signal transduction cascade before TPA induced the translocation or downregulation of PKC-epsilon.

Effect of Curcumin on TPA-Induced c-fos Gene Expression

One of the important biological activities of TPA required for tumor promotion is enhanced expression of cellular oncogenes such as c-jun, c-fos and c-myc. In this study, the effect of curcumin on TPA-induced c-fos gene expression in human keratinocytes was evaluated. The 80% confluent human keratinocytes were incubated with 160 nM TPA for 0, 0.5, 1, 2, 4 and

18 h. After these treatments, nuclear extracts were prepared and then assayed for c-fos protein as described. TPA appeared to increase the c-fos protein level after 0.5 h incubation (Fig. 5). This increase in c-fos protein level induced by TPA appeared to be maximum at 2 h. After 2 h of TPA incubation, the level of c-fos protein declined. We observed complete disappearance of c-fos protein level after long-term treatment with TPA (18 h). The data in Fig. 5 indicated that TPA activation of c-fos protein peaked at 2 h; therefore it was chosen for study of the effect of curcumin. Human keratinocytes were preincubated for 1 h with different concentrations of curcumin (20, 40 and 50 μM) followed by treatment with 160 nM TPA for 2 h. After these treatments nuclear extracts were prepared and then assayed for c-fos protein as described in Materials and Methods. The result showed that curcumin inhibited TPA-induced expression of c-fos in human keatinocytes in a dose dependent manner (Fig. 6). This finding showed for the first time that TPA-induced c-fos protein level was suppressed by curcumin in a human skin model.

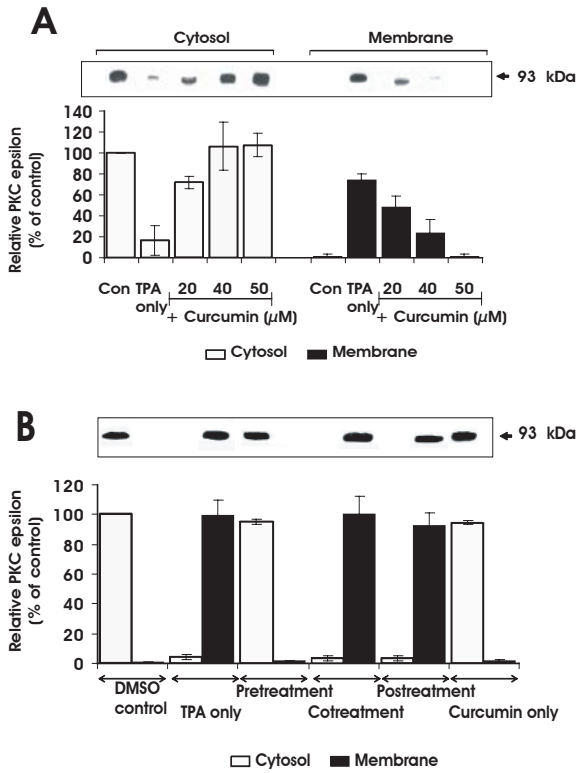

Fig 4.A: Dose response inhibition of TPA-induced PKC-ε activation by curcumin. Keratinocytes were preincubated for 1 h with different concentrations of curcumin followed by treatment with 160 nM TPA for 1 h as described in the text. After these treatments cytosolic (C) and membrane (M) fractions (30 μg protein) were prepared and then analyzed for PKC-ε isoenzymes. **B:** Effect of time on curcumin addition on TPA-induced PKC-ε isoenzyme. Human keratinocytes were treated with 50 μM curcumin for 1 h prior to addition of TPA (pre-treatment), at the same time as the addition of TPA (co-treatment) or 1 h after addition of TPA (post- treatment). After these treatments cytosolic and membrane fractions were prepared and then assayed for PKC-ε as described. Upper: Western blot analysis of PKC-ε expression using anti-PKC-ε antibody from three independent experiments. Lower : Relative intensity of the immunoblots calculated as means ±S.E. Each histogram obtained from three independent experiments performed in triplicate. The arrow indicates molecular weight of PKC-ε isoenzyme.

Discussion

The previous study from our laboratory indicated that topical application of curcumin[16] and dietary curcumin[15,17] had marked inhibitory effect on TPA induced tumor promotion in mouse skin , which was consistent with the findings of many previous reports[18,19]. In order to further investigate the mechanism by which curcumin causes inhibition, our study examined the effect of curcumin on the level and distribution of TPA induced PKC-ε isoenzyme in primary human keratinocytes. The findings of our study demonstrated the chemopreventive potential of curcumin on TPA-induced PKC-ε isoenzyme, i.e., the isoenzyme that could be a molecular target for tumor promoting phorbol esters in skin chemical carcinogenesis models.

Human keratinocytes are the main cell type (>95%)

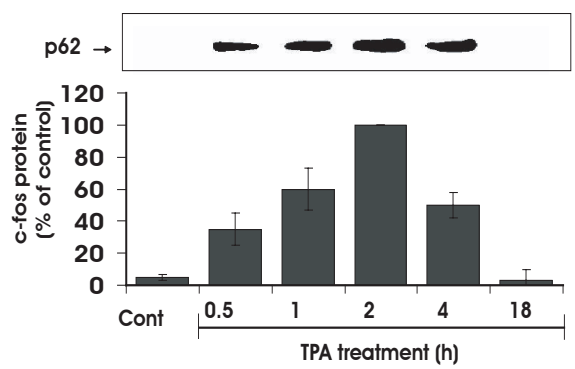

Fig 5. Effect of TPA on c-fos gene expression in human keratinocytes. Human keratinocytes were incubated with 160 nM TPA for 0, 0.5, 1, 2, 4 and 18 h. After these treatments nuclear extracts were prepared and assayed for c-fos protein. Upper: Western blot analysis of c-fos protein using anti-c-fos antibody. Lower : Relative intensity of the immunoblots calculated as means ±S.E. Each histogram was obtained from three independent experiments performed in triplicate.

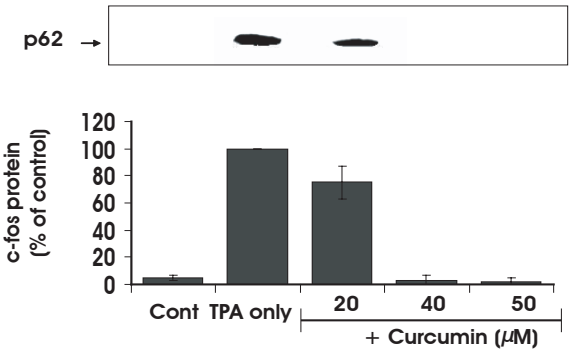

Fig 6. Dose response of inhibition of TPA-induced c-fos expression by curcumin. Human keratinocytes were preincubated for 1 h with different concentrations of curcumin (20, 40 and 50 μM) followed by treatment with 160 nM. TPA for 2 h. After these treatments nuclear extracts were prepared and then assayed for c-fos protein by Western blotting. Upper: Western blot analysis of c-fos protein using anti-c-fos antibody. Lower : Scanning densitometry of the relative intensity of the immunoblots calculated as means ±S.E. Each histogram was obtained from three independent experiments performed in triplicate.

of the epidermis which express both mRNA and protein of PKC-α, -δ and -ε isoenzymes. Among the first signaling proteins, PKC isoenzymes have been shown to change sub-cellular localization upon activation[20]. Our study found that 160 nM TPA-treatment for 1 or 2 h caused PKC-α and -ε isoenzymes to translocate from cytosol to membrane. The levels of cytosolic PKC-α and -ε isoenzymes were significantly reduced with a concomitant increase in the membrane fraction, indicating specific activation of both isoenzymes (Fig. 1, A and B). For a long time, it has been proven that the binding site of TPA on PKC isoenzymes is C1 domain whose membrane affinity is increased dramatically upon binding to TPA[21]. This could explain the reason why the level of PKC-α and - ε isoenzyme on the cell membrane were higher after 2 h TPA exposure than 1 h. In addition, this study found that PKC-α and -ε isoenzymes underwent downregulation after treatment with 160 nM TPA for 18 h. (Fig. 1, A and B). Translocation of PKC from cytosol to the membrane by TPA seems to activate the enzyme in intact cells and down regulation occurs after the translocation. Little is known about the molecular mechanism(s) and in vivo determinants of the process of the downregulation through proteolysis of PKC. However, one protease cleavage site has been mapped to the border of the hinge region and catalytic domain of the PKC isoenzymes. There is evidence that the proteolytic process caused by neutral Ca^{2+}-dependent proteinase, which was found in the membrane, resulted in two fragments of PKC: a 50 KD protein which was released into the cytoplasm and displayed a phospholipid- and Ca^{2+}-independent protein kinase C activity (Protein kinase M, PKM), and a 35 KD protein which retained TPA binding activity. The role of 35 KD protein in gene regulation has been considered [22,23,24]. Therefore this study combined with preliminary information can suggest that activation of PKCs is usually associated with their translocation from the cytosol to the membrane. Concomitantly, there is a cleavage of the catalytic domain from the regulatory domain by proteinase, which may occur at the border of the hinge region and catalytic domain. Then activated PKC (catalytic domain) functions at the membrane or may translocate to specific targets such as the nucleus[24]. However, exactly how PKC translocates to specific targets has not yet been clearly understood. It has been reported that eventually, the activated PKC undergoes further breakdown. Thus, continuous exposure to activators lead to activation and down-regulation of PKC, and it is possible that either activation or down-regulation is responsible for the biological effects.

The roles of PKC-α and -ε isoenzymes in human keratinocytes were linked to epidermal differentiation program and carcinogenesis respectively [25]. However, the other crucial role of PKC isoenzyme in human keratinocytes is apoptosis. A recent study revealed that in normal human keratinocytes, PKC-δ isoenzyme is correlated with the induction of apoptosis by UV exposure [26], which is a distinct pathway from TPA activation. Those findings were consistent with this study because this study found that with either short-term TPA treatment (160 nM TPA for 1 and 2 h) or long-term treatment (160 nM TPA for 18 h) the PKC-δ level remained relatively unchanged (Fig. 1C).

Previous studies have shown that PKC-ε isoenzyme in human keratinocytes was involved with oncogenic potential. We, therefore, investigate the effect of curcumin on TPA-induced PKC-ε activation. We found that TPA did not affect the total level of PKC-ε isoenzymes (Fig. 2), suggesting that TPA had no effect on new PKC synthesis, but was directly involved with the translocation of preexisting PKC-ε from the cytosol to the membrane. Our study showed that curcumin completely inhibited the translocation of PKC-ε isoenzyme induced by TPA (Fig. 3) in a dose-dependent manner. In addition, curcumin itself at any concentration tested (20, 40, 50 μM), did not affect the translocation or down-regulation of PKC-ε isoenzyme (Fig. 4A). Therefore, curcumin had no mitogenic activity on human keratinocytes, unlike the TPA actions. Moreover TPA-induced translocation of PKC-ε isoenzyme was inhibited only in case of pretreatment with curcumin (Fig. 4B). Co- or post-treatment with curcumin was not effective. This result was consistent with the known effect of curcumin on NF-kB

activation[27], which was also found that the inhibitory effect of curcumin was accomplished only in case of pre-treatment, whereas co- or post-treatment did not. This result, combined with the characteristic structure of curcumin, which is exceedingly nonpolar, suggests that curcumin may be inserted into membrane, bind to cytosolic PKC at C1 domain, and then inhibit the molecule. However, this study proposed that the affinity of TPA is much higher than curcumin, and therefore the inhibition effect was observed only in pretreatment with curcumin.

Curcumin has been demonstrated to be a potent inhibitor of NF-kB activation in human myeloid cells[27], AP-1 activation in mouse fibroblast cells and the expression of c-jun, c-fos and c-myc in JB6 cells and in mouse epidermis[28]. In the present study, we report the suppression of c-fos protein level by curcumin in primary human keratinocytes. The c-fos protein is inducible by TPA and thus is associated with c-jun to result in an increased AP-1 activity. Curcumin may also block TPA-induced AP-1 activation by inhibiting a protein kinase such as PKC-ε isoenzyme in human keratinocytes. Functional activation of the transcriptional factor AP-1 is believed to play an important role in signal transduction of TPA-induced tumor promotion. In conclusion, the data described here demonstrated for the first time that the suppression of TPA-induced c-fos protein level and PKC-ε activation in human keratinocytes by curcumin may suppress tumor promotion by blocking signal transduction pathways in the target cells.

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