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Effect of propolis and caffeic acid phenethyl ester (CAPE) on NFκB activation by HTLV-1 Tax

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ABSTRACT

HTLV-1 is the etiological agent of aggressive malignancy of the $CD4^+$ T-cells, adult T-cell leukemia (ATL), and other severe clinical disorders. The viral Tax protein is a key factor in HTLV-1 pathogenicity. A major part of Tax oncogenic potential is accounted for by its capacity of inducing the transcriptional activity of the NFκB factors, which regulate the expression of numerous cellular genes. Propolis (PE), a natural product produced by honeybees, has been used for a long time in folk medicine. One of PE active components, caffeic acid phenylethyl ester (CAPE), was well characterized and found to be a potent inhibitor of NFκB activation. Therefore, the aim of this study was to pursue the possibility of blocking Tax oncogenic effects by treatment with these natural products. Human T-cell lines were used in this study since these cells are the main targets of HTLV-1 infections. We tried to determine which step of Tax-induced NFκB activation is blocked by these products. Our results showed that both tested products substantially inhibited the activation of NFκB-dependent promoter by Tax. However, only PE could efficiently inhibit also the Tax-induced activation of SRF- and CREB-dependent promoters. Our results showed also that PE and CAPE strongly prevented both Tax binding to IκBα and its induced degradation by Tax. However, both products did not interfere in the nuclear transport of Tax or NFκB proteins.

1. Introduction

HTLV-1 is the etiological agent of aggressive lethal malignancy of CD4 T-lymphocytes called adult T-cell leukemia (ATL) and certain other clinical disorders (Norris et al., 2010; Yoshida, 2005). Although the mechanism of HTLV-1 pathogenicity is not completely clear yet, the Tax oncoprotein is regarded as a central player in the initiation of the leukemogenic process leading to ATL (Schavinsky-Khrapunsky et al., 2003; Watters et al., 2010). A major part of Tax oncogenic potential is accounted for its capacity of activating the transcriptional activity of the NFκB factors (Higuchi and Fujii, 2009; Peloponese and Jeang, 2006), which regulate the expression of numerous cellular genes (Pahl, 1999) associated with diverse biological processes, such as embryonic development, immune and inflammatory responses, cell growth, apoptosis, stress responses and oncogenesis (Tergaonkar, 2006; Wang et al., 2010). Unlike the transiently acting external signals, Tax renders the NFκB factors constitutively active in HTLV-1 infected cells (Tabakin-Fix et al., 2006). Persistent activity of NFκB factors has been proved to play a central role in the pathophysiology of ATL and other clinical disorders (Peloponese and Jeang, 2006). Intense effort is, currently, focused on developing means for blocking the persistent NFκB activity as a therapeutic strategy for these clinical disorders (Horie et al., 2006; Peloponese and Jeang, 2006). The NFκB family consists of five transcription factors, p50(NFκB1), p52(NFκB2), p65(RelA), RelB and c-Rel, acting in various combinations of homo- and heterodimers that display distinct specificities (Campbell and Perkins, 2006; Tergaonkar, 2006). The most prominent dimers involved in NFκB-dependent transcriptional gene activation are p65:p65 and p65:p50, whereas p50:p50 dimer is rather inhibitory (Campbell and Perkins, 2006). In the non-activated state NFκB factors are trapped in the cytoplasm, tightly associated with inhibitory proteins called IκBs, which includes IκBα, IκBβ, IκBε, p105, p100 and Bcl-3, but most of the signal-induced activity is regulated via IκBα and IκBβ (Campbell and Perkins, 2006). NFκB factors are activated in response to a wide variety of external signals which induce proteasomal degradation of the sequestering IκBs and enable, thereby, the released NFκBs to translocate to the nucleus where they exert their regulatory effect on target genes expression (Campbell and Perkins, 2006; Tergaonkar, 2006). The external signals activate a large multi-subunit IκB kinase (IKK) complex by certain protein kinases (Yamamoto and Gaynor, 2004). IKK complex consists of two catalytic subunits, IKKα and IKKβ (Zandi et al., 1997) and a regulatory subunit called IKKγ or NFκB essential modulator (NEMO). The catalytic function of IKKα and IKKβ is activated in response to their phosphorylation at specific serine residues by these signaling kinases. Then the activated IKKα and IKKβ phosphorylate specific

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serine residues of the sequestering IκBs and render them accessible for ubiquitination and subsequent proteasomal degradation (Karin and Ben-Neriah, 2000). However, for inducing NFκB-dependent gene expression, nuclear translocation needs to be complemented by a second phase, in which the nuclear p65 factor is linked to the CBP/p300 and P/CAF co-activators that are essential for the transcriptional competence of p65 containing dimmers (Sheppard et al., 1999). Linking to these co-activators requires phosphorylation of p65 at specific serine residues, which is carried out by PKA (Zhong et al., 2002) or certain other signal-activated serine kinases (Catley et al., 2004) (Fig. 1). Previous studies have suggested that Tax may release NFκB from IκB by different ways such as: (a) Tax can induce constitutive phosphorylation of IKKα and IKKβ independently of external signals (Azran et al., 2004). (b) Tax can physically interact with IKKχ which promotes its binding to IKKα and IKKβ thereby leading to activation of their kinase activity (Chu et al., 1998, 1999; Harhaj and Sun, 1999; Higuchi and Fujii, 2009; Jin et al., 1999) and (c) Tax can bind directly to IκBs and induce their proteasomal degradation independently of their phosphorylation by IKK (Suzuki et al., 1995). Also, Tax can link the free p65 to CBP/p300 by bridging them and enhance, in this manner, the transcriptional competence of the p65-containing nuclear NFκB dimers independently of p65-phosphorylation (Bex et al., 1997) (Fig. 1).

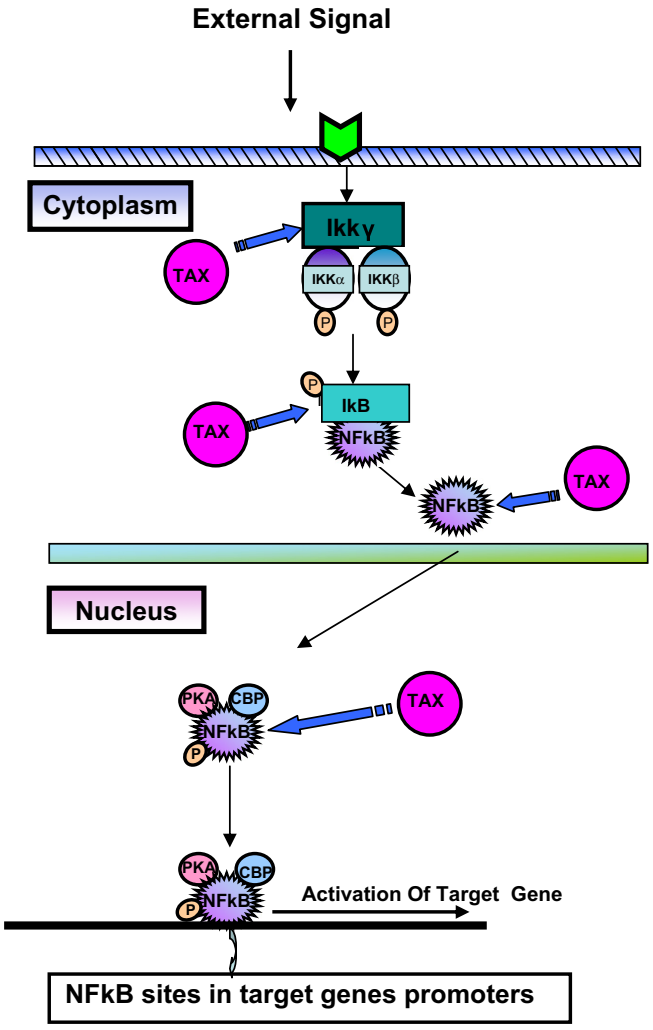

Fig. 1. A schematic presentation on how NFκB activation is regulated by IKB degradation as follows: IKB degradation is dependent on its phosphorylation mediated by activated complex of IKKα and IKKβ which is regulated by IKKχ. Activation of IKK complex is induced by their phosphorylation by a specific kinase which is activated by external stimuli. The activated IKKs phosphorylate serine residues located in the carboxyl terminus of IKBs, which leads to their ubiquitinylation and proteasomal degradation. The degradation of IKBs releases NFκB complexes for nuclear translocation. In the nucleus NFκB is linked to the CBP/p300 co-activators. Linking to these co-activators requires phosphorylation of NFκB at specific serine residues, which is carried out by activated serine kinases. Tax may release NFκB from IκB by different ways such as: (a) Tax can physically interact with IKKχ which promotes its binding to IKKα and IKKβ resulting thereby in activation of their kinase and (b) Tax can bind directly to IκBs and induce their proteasomal degradation independently of their phosphorylation by IKK. Then, it seems that Tax can enhance the nuclear transport of the released NFκB. Also, in the nucleus Tax can link the free NFκB to CBP/p300 by bridging them and enhance, in this manner, the transcriptional competence of the nuclear NFκB independently of its phosphorylation.

Propolis (PE), a natural product produced by honeybees and which is based on resins collected by bees from certain trees and plants, has been used for thousands of years in folk medicine. The chemical composition of PE is complex and has not been completely established. It is known that the most important group of compounds in terms of amount and biochemical activity are the flavonoids, which are considered to play a significant role in its bioactivities (Pratsinis et al., 2010; Russo et al., 2006; Umthong et al., 2009). Over the past two decades, this material has been the subject of intensive experimental and clinical studies, in which its antioxidant, antimicrobial, antifungal, anti-inflammatory and antitumor properties have been investigated (Huleihel and Eshanu, 2001; Libério et al., 2009; Russo et al., 2006; Szliszka et al., 2009; Yang et al., 2005). The most active and studied component of PE is caffeic acid phenethyl ester (CAPE) which is a potent inhibitor of NFκB activation (Natarajan et al., 1996). A major part of CAPE's biological activities is related to its inhibitory effect on NFκB activation (Ang et al., 2009; Lee et al., 2010; Márquez et al., 2004; Onori et al., 2009; Yang et al., 2005). Hence, it is of high interest to examine the ability of PE and CAPE to abrogate the various Tax oncogenic functions through blocking its NFκB activation. Our results showed that PE and CAPE strongly inhibited the activation of NFκB-dependent promoter by Tax probably due to preventing the Tax-induced degradation of IκBα.

2. Materials and methods

2.1. Cells

In this study we used the following cells: Jurkat, uninfected human T-cell lines, and MT2 which are HTLV-1 infected humanT-cells. The cells were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum, glutamine and antibiotics.

2.2. Propolis and CAPE

Propolis was obtained from the beehives of Kibbutz Yad Mordecai, Israel. An aqueous extract of propolis (20% of row propolis) (PE) was prepared in 95% ethyl alcohol and kept at room temperature and darkness for 2 days by shaking. The solution was filtered and then its pH was adjusted to 7.1–7.2 with sodium borate. The required concentrations were prepared by dilution of the concentrated extract with RPMI culture medium. As a control, we used phosphate-buffered saline (PBS) (pH 7.2) containing the same amount of sodium borate, which was necessary to adjust the pH of PE.

CAPE was purchased as a powder from Sigma–Aldrich Corporation. A stock solution of this product was prepared by dissolving it in dimethyl sulfoxide (DMSO) and then making the appropriate concentrations for examining its activity by dilution with RPMI growth medium (RPMI).

2.3. Plasmids and transient transfection assay

Reporter plasmids expressing luciferase through a minimal promoter linked to three copies of the consensus NFκB responsive element (pNF-κB-Luc, Clontech Labs, Palo Alto, CA), through SRF

responsive element and through HTLV-1-LTR were used to measure the NFκB and HTLV-1-LTR transcriptional competence. The plasmid expressing the HTLV-1 wild type (w.t.) *Tax* through the hCMV promoter, was provided by Francoise Bex (Laboratorie de Microbiologie, Université Libre de Bruxelles, B-1070, Brussels, Belgium). The indicated quantities of the various plasmids were transfected by jetPEI™ Transfection reagent kit (Polyplus-transfection company, France) into 5×10^5 cells/well of six well plate and the total DNA was completed in each transfection mixture to 2 μg with an empty plasmid (dLTR). The pRL-null vector expressing *Renilla* (pRL-renilla, Promega Corporation, Madison, WI), was included (0.1 μg) in each transfection assay for assessing the relative transfection efficiency. The enzymatic activities were assayed in the cell extracts 24 or 48 h after transfection and the luciferase activity was normalized according to that of *Renilla* and expressed as fold of the relevant control of each experiment.

2.4. Western blot analysis of the expression and subcellular distribution of the examined proteins

To follow the expression and subcellular distribution of the examined proteins, cytoplasmic and nuclear fractions were prepared from the transfected cells (by Nucbuster protein extraction kit, Novagen, Madison, WI) and aliquots of 50 μg protein were analyzed for the level of these proteins by Western blot analysis as described elsewhere (Mor-Vaknin et al., 1997) with the corresponding monoclonal antibodies (Tax, NFκB p65 and IKBα specific monoclonal antibodies, ENCO Scientific Services Ltd., Israel). Equal loading of the different samples of the cytoplasmic fractions was assessed by stripping the blot from the first detecting antibodies and re-analyzing it with anti actin antibody. The loading of the nuclear extract samples was assessed with anti-lamin B antibody. Variation in transfection efficiency was assessed by including 0.1 μg of pRL-Renilla plasmid in each transfection and measuring the enzymatic activity in extracts of the transfected cells.

2.5. Immuno co-precipitation analysis

Aliquots of 150 μg protein of the indicated sub-cellular fractions were immuno-precipitated with the specified antibody and the immunoprecipitates were assayed by Western blotting for the indicated co-precipitating proteins with the respective antibody as previously described (Torgeman et al., 2001).

3. Results

3.1. Toxicity of PE and CAPE

The cytotoxic effect of *PE* and *CAPE* on Jurkat and MT2 cell lines was evaluated by:

1. Measuring the levels of [^{3}H]Thymidine incorporation. Cells were grown in the presence or absence of increasing concentrations of *PE* or *CAPE* for 72 h and [^{3}H]Thymidine incorporation was measured.
2. Cell counting. Cells were treated with increasing doses of *PE* or *CAPE* for 72 h. Samples of cells were stained and viable cells were counted.

The obtained results (Fig. 2A and B) show that CC_{50} (concentration caused 50% inhibition of cell growth) of PE is about 5% and of CAPE is about 90 μM in all examined cells.

3.2. Effect of PE and CAPE on HTLV-1 Tax activation of various promoters

The effect of PE and CAPE on Tax activation of different promoters was examined in cell culture. Plasmids expressing reporter genes via NFκB-, CREB- or SRF-responsive promoters were transfected into Jurkat and MT2 cells; Jurkat cells were transfected also with 0.5 μg of *Tax* plasmid. The cells were treated with increasing

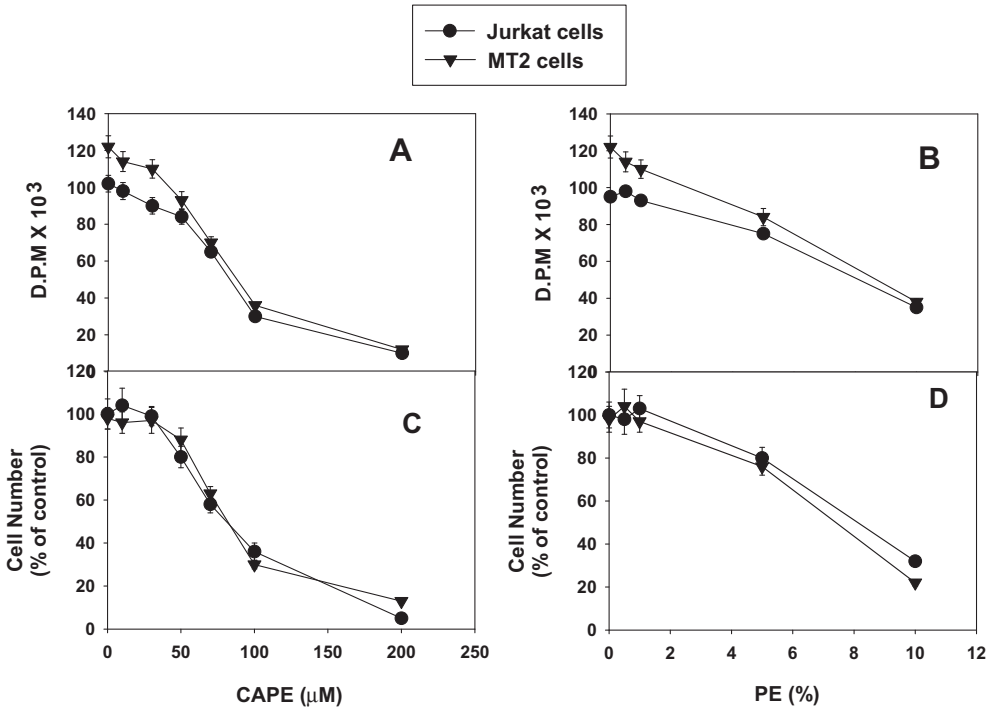

Fig. 2. Effect of PE and CAPE on cell proliferation. Cells were grown in increasing doses of PE (A and C) or CAPE (B and D) for 72 h. Cell proliferation was estimated either by [^{3}H]Thymidine incorporation, which was measured by liquid scintillation counting (A and B) or by direct cell counting with a Neubauer hemacytometer (C and D). Results are represented as mean ± SE of three different experiments.

doses of PE or CAPE at 2 h post-transfection and the treatment was continued up to the end of the experiment. The luciferase activity was measured in the cell lysates at 24 h post-transfection.

The results presented in Fig. 2 demonstrate that when transfected separately, the w.t. *Tax* strongly activates all the tested promoters (NFκB, SRF and CREB/ATF dependent HTLV-1-LTR). As can be seen from Fig. 3 both CAPE (Fig. 3A) and PE (Fig. 3B) inhibited Tax activation of NFκB dependent gene expression in a concentration-dependent manner; at concentrations of 5 μM and 0.1%, respectively, they completely abolished this Tax activation. However, only PE was able to inhibit also Tax activation of the CREB-dependent (Fig. 3D) and SRF-dependent(Fig. 3F) gene expression, while CAPE had no effect on Tax activation of these promoters (Fig. 3C and E). In addition, it is worthwhile to note that treatment of the above cells with either PE or CAPE had no effect on the expression of Tax as confirmed by Western blot assay (data not shown).

In order to confirm CAPE and PE inhibition of Tax-induced NFκB pathway, we examined whether CAPE or PE can interfere with the expression of an NFκB endogenous target gene product, inducible nitric oxide synthetase (iNOS), in MT2 cells. Cells were treated with 0.1 % PE or 5 μM and at 24 h later, 150 μg aliquots of the total cellular extracts of the tested cells were examined for Tax and iNOS protein levels by Western blot analysis with Tax and iNOS specific monoclonal antibodies. The results presented in Fig. 3G showed that both PE and CAPE significantly inhibited the Tax-induced expression of iNOS protein.

3.3. Effect of PE and CAPE on NFκB-induced activation by TNFα and Tax

NFκB-dependent gene expression is known to be induced by various pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α (Lee et al., 2010), which initiates an intracellular signaling cascade, resulting in the phosphorylation and subsequent degradation of IκBα by the 26S-proteasome (Tanaka et al., 2001). The potential of natural antioxidants, like CAPE, to prevent this induced activation of NFκB had been previously demonstrated (Schubert et al., 2002; Song et al., 2008). In the present study we examined the effect PE and CAPE on possible synergistic or additive activity between Tax and TNF-α (as a representative of NFκB external stimuli) against NFκB-mediated activation. Jurkat cells were transfected with 0.5 μg Plasmid expressing a reporter gene via the NFκB responsive promoter and 0.5 μg of *Tax* plasmid. The cells were treated with 0.1% PE or 5 μM CAPE at 2 h post-transfection and the treatment was continued up to the end of the experiment. At 24 h post-transfection the appropriate cells were treated also with 5 ng/ml TNFα and 6 h later the luciferase activity was measured in the cell lysates. The results presented in Fig. 4 show a significant synergistic activity between Tax and TNFα on NFκB activation. It can be seen also from these results that both PE and CAPE significantly prevented NFκB-induced activation by Tax, TNFα and both.

3.4. Effect of PE and CAPE on Tax-induced NFκB activation and IκBα degradation

Jurkat cells, which were transfected with 0.5 μg of Tax plasmid, and MT2 cells were treated with 0.1% PE or 5 μM CAPE. At 24 h later, 150 μg aliquots of the cytoplasmic fractions of the tested cells were examined for Tax, NFκB and IκBα protein levels by Western blot analysis with Tax, NFκB and IκBα specific monoclonal antibodies. The results presented in Fig. 5 showed that both PE and CAPE significantly prevented both cytoplasmic NFκB release and IκBα degradation induced by Tax in both cells examined. These products had no effect on Tax nuclear transport.

3.5. Effect of PE and CAPE on binding of Tax to IκBα

One of the mechanisms proposed for Tax activation of NFκB involves direct physical Tax binding to the IκBs. The ability of PE and CAPE to block IκBα binding to Tax was examined in MT2 and Jurkat cells transfected with 0.5 μg Tax plasmid. In order to prevent the rapid proteasomal degradation of IκB, all cells examined were treated with 10 nM PS-341 (an inhibitor of the 26S proteasome) in addition to their treatment with 1% PE or 5 μM CAPE at 2 h post-transfection. At 24 h later 150 μg aliquots of the cytoplasmic fractions of the tested cells were immunoprecipitated with anti-Tax monoclonal antibody. The immunoprecipitates were examined for the presence of IκBα by Western blot analysis with anti-IκBα monoclonal antibody. Reciprocal co-precipitation was performed for confirmation. Fig. 6A and B shows a significant amount of immuno co-precipitating Tax and IκBα proteins in infected and Tax-transfected cells. Both PE and CAPE almost completely prevented the formation of this Tax-IκBα complex in infected and transfected cells. Most of the Tax and IκBα proteins present in the input (Fig. 6C1 and D1) were successfully immunoprecipitated with the Tax specific monoclonal antibody, while there was no significant immunoprecipitation of Tax or IκBα proteins with non-specific IgG (Fig. 6C2 and D2).

3.6. Effect of PE and CAPE on Tax and NFκB nuclear translocation

Tax protein is known for its ability to induce NFκB transport from the cytoplasm to the nucleus. In this study we examined whether PE or CAPE interferes with this mediated NFκB transport. In order to get free NFκB in the cytoplasm, tested cells were transfected with a plasmid expressing p65(RelA), since PE and CAPE prevent the endogenous NFκB release in the cytoplasm as shown above (Fig. 5). Jurkat cells were transfected with 0.5 μg plasmid expressing p65(RelA) and 0.5 μg of Tax-Flag plasmid while MT2 cells were transfected only with 0.5 μg plasmid expressing p65(RelA). At 2 h post-transfection, all examined cells were treated with 0.1% PE or 5 μM CAPE. Western blot analysis of p65(RelA) and Tax in the nuclear and cytoplasmic cell extracts were done at 24 h post-treatment. The results presented in Fig. 7 show that Tax enhances the nuclear transport of p65(RelA), and that both PE and CAPE have no effect on this transport in all tested cells.

4. Discussion

Transcription factors of the NFκB family regulate the expression of many cellular and viral genes (Bassères et al., 2010; de Jong et al., 2010; Puvvada et al., 2010; Santoro et al., 2003; Tabakin-Fix et al., 2004) and, consequently, affect diverse biological processes, including oncogenesis (Chalet and Thomas, 2002; Li and Verma, 2002; Wang et al., 2010). Therefore, a substantial part of the oncogenic potential of HTLV-1 Tax protein is ascribed to its capacity of inducing a constitutive activation of the transcriptional competence of these factors (Peloponese and Jeang, 2006). The current view is that Tax affects NFκB through cytoplasmic and nuclear functions. In the cytoplasm, Tax acts so as to release NFκBs from their IκB proteins and facilitate their transport to the nucleus. Then, in the nucleus *Tax* interacts with the p65 member of the NFκB family and links it with the CBP/p300 and P/CAF coactivators, which are essential for the transcriptional activity of p65-containing NFκB dimers. This and other *Tax* nuclear functions activate a wide range of cellular genes involved in central biological pathways contributes to *Tax* oncogenic potential (Azran et al., 2004). Abrogating the Tax activation of NFκB can prevent a major part of Tax pathogenicity. Several natural and synthetic compounds have been found to inhibit NFκB activation, and to exert anti-

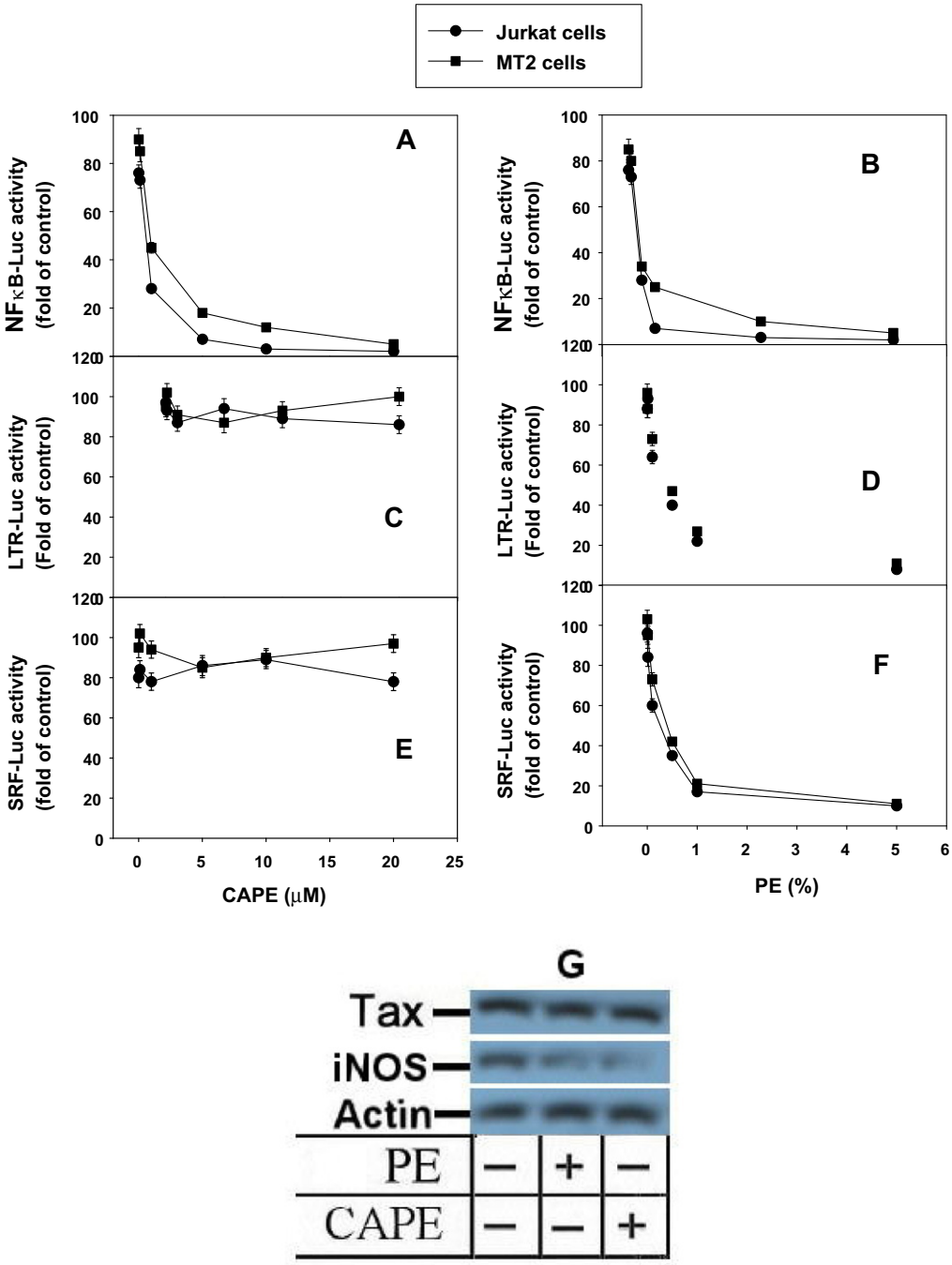

Fig. 3. Effect of CAPE and PE on NFκB-, CREB- or SRF-responsive promoters activation by Tax. 0.5 μg of Plasmids expressing reporter genes via NFκB- (A and B), CREB- (C and D) or SRF- (E and F) responsive promoters were transfected into Jurkat and MT2 cells; Jurkat cells were transfected also with 0.5 μg of *Tax* plasmid. The cells were treated with increasing doses of CAPE (A, C and E) or PE (B, D and F) at 2 h post-transfection and the treatment was continued up to the end of the experiment. Luciferase activity was measured in the cell lysates at 24 h post-transfection. The results presented are an average of three repeated experiments ± SE. In order to confirm CAPE and PE inhibition of Tax-induced NFκB pathway, we examined the effect of CAPE and PE on the expression of an NFκB target gene product, iNOS (D). MT2 cells were treated with 0.1% PE or 5 μM and at 24 h later, 150 μg aliquots of the total cellular extracts of the tested cells were examined for Tax and iNOS protein levels by Western blot analysis with Tax and iNOS specific monoclonal antibodies.

inflammatory effects in vitro and in vivo (Olivier et al., 2006; Umezawa et al., 2000). CAPE, one of the active components of PE derived from honeybee hives, has been reported to be a selective inhibitor of NFκB (Grunberger et al., 1988; Natarajan et al., 1996). In the present study, for the first time as far we know, we examined the effect of CAPE and PE on Tax-induced activation of NFκB. Our results showed that PE and CAPE strongly inhibited NFκB transcriptional activation induced by Tax in HTLV-1-uninfected and infected T-cells in culture (Fig. 3). Similar effect of PE and CAPE on NFκB transcriptional activation induced by Tax was obtained also in primary T-lymphocytes obtained from normal healthy individuals (data not shown). Our results showed also that CAPE has no effect on Tax activation of other promoters which are not NFκB responsive promoters such as HTLV-1 LTR and SRF, whereas, PE strongly inhibited Tax activation of these promoters too (Fig. 3C–F). It seems that additional component/s of PE other

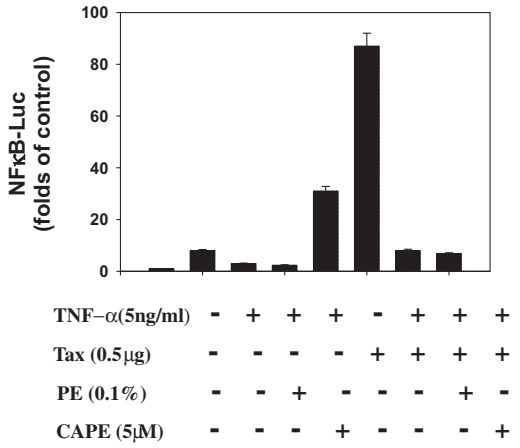

Fig. 4. Effect of PE and CAPE on NFκB-induced activation by TNFα and Tax. Jurkat and MT2 cells were transfected with 0.5 μg plasmid expressing a reporter gene via NFκB responsive promoter; Jurkat cells were transfected also with 0.5 μg of *Tax* plasmid. The cells were treated with 0.1% PE or 5 μM CAPE at 2 h post-transfection and the treatment was continued up to the end of the experiment. At 24 h post-transfection the appropriate cells were treated also with 5 ng/ml TNFα and 6 h later the luciferase activity was measured in the cell lysates.

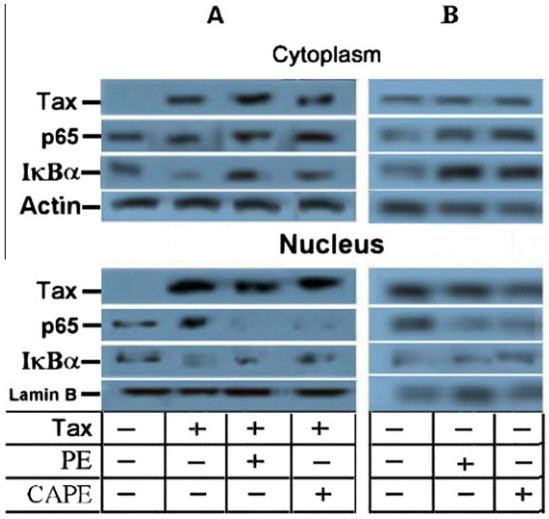

Fig. 5. Effect of PE and CAPE on Tax induced IκBα degradation. Jurkat cells (A), which were transfected with 0.5 μg of Tax plasmid, and MT2 cells (B) were treated with 0.1% PE or 5 μM CAPE at 2 h post-transfection for 24 h. Then 150 μg aliquots of the cytoplasmic fractions of the tested cells were examined for Tax, p65 and IκBα protein levels by Western blot analysis with Tax, p65 and IκBα specific monoclonal antibodies.

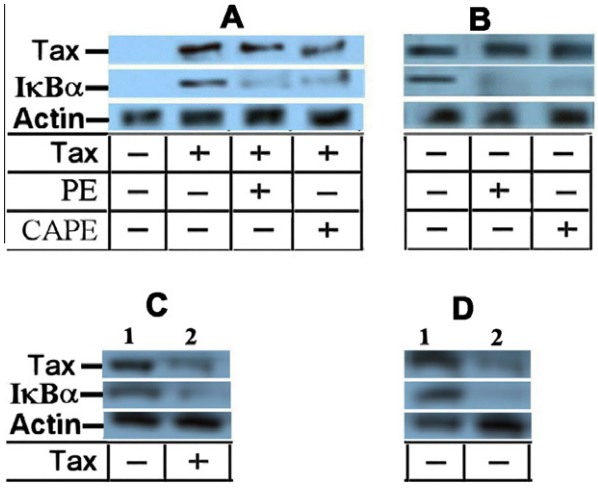

Fig. 6. Effect of PE and CAPE on binding of Tax to IκBα. Jurkat cells (transfected with 0.5 μg Tax plasmid) (A and C) and MT2 (B and D) cells were treated with 10 nM PS-341 (an inhibitor of the 26S proteasome) and 0.1% PE or 5 μM CAPE at 2 h post-transfection. At 24 h later 150 μg aliquots of the cytoplasmic fractions of these cells were immunoprecipitated with anti-Tax monoclonal antibody. The immunoprecipitates were examined for the presence of IκBα and Tax by Western blot analysis with IκBα and Tax monoclonal antibodies. Reciprocal co-precipitation was performed for confirmation. As a control, 150 μg aliquots of the cytoplasmic fractions of Tax transfected Jurkat cells (C) or MT2 cells (D) were examined for Tax and IκBα total protein levels by Western blot analysis with Tax and IκBα specific goat monoclonal antibodies (lanes C1 and D1) or with non-specific goat IgG (lanes C2 and D2).

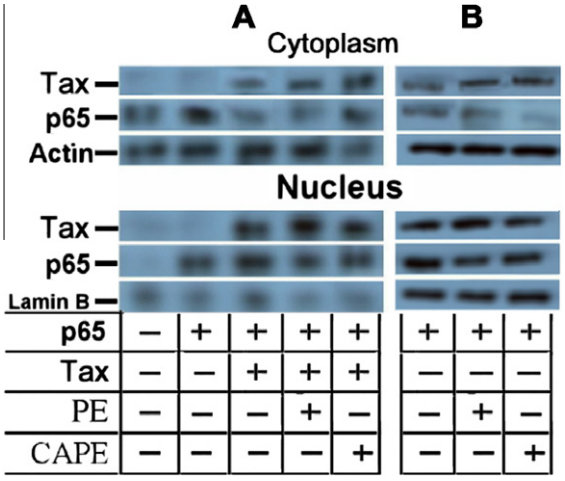

Fig. 7. Effect of PE and CAPE on Tax and NFκB nuclear translocation. Jurkat cells (A) were transfected with 0.5 μg plasmid expressing p65(RelA) and 0.5 μg of Tax plasmid while MT2 cells (B) were transfected only with 0.5 μg plasmid expressing p65(RelA). At 2 h post-transfection, all examined cells were treated with 0.1% PE or 5 μM CAPE. Western blot analysis of p65(RelA) and Tax determination in the cytoplasmic and nuclear cell extracts were done at 24 h post-treatment.

than CAPE is/are responsible for this activity. In agreement therewith, polyphenols from different plants such as green tea (Li et al., 2000; Sonoda et al., 2004) and *Scutellaria baicalensis georgi* – baicalin (Baylor et al., 1992) were found previously to have an inhibitory effect on HTLV-1 replication as well as on ATL growth.

Trying to elucidate the inhibitory mechanism of PE and CAPE on Tax activation of NF-κB, we examined the effect of PE and CAPE on Tax and NFκB nuclear translocation. Although from the results presented in Fig. 5 it seems that both products are able to prevent the Tax-induced nuclear translocation of cellular NF-κB, still this inhibition might be a consequence of blocking NFκB release from IκBs by these products. In order to examine this point, the tested cells were transfected with a plasmid expressing p65(RelA), providing a free p65(RelA) in the cytoplasm, and treated with PE or CAPE. The obtained results showed that both products have no effect on the nuclear translocation of either NFκB or Tax proteins (Fig. 7).

Previous studies have suggested that Tax may release NFκB from IκB by different ways such as direct binding to IκBs and induction of their proteasomal degradation independently of their phosphorylation by IKK (Suzuki et al., 1995). Based on these studies we examined the effect of PE and CAPE on Tax binding to IκBα and its induced degradation. Our results show that these products strongly prevented both Tax binding to IκBα (Fig. 6) and its induced degradation by Tax (Fig. 5). It seems that at least one possible way of inhibiting Tax activation of NFκB is by blocking binding to IκBs and probably preventing, by this way, Tax-induced degradation.

In fact, intervention of CAPE in IκBα-p65 pathway resulting in NFκB inhibition seems to be dependent on cells type and experimental conditions. Natarajan et al. (1996) reported that CAPE interferes with DNA binding of p65 without prevention of IκBα degradation (Ang et al., 2009). Ang et al. (2009) found that it delays IκBα degradation, preventing p65 translocation into nucleus and Lee et al. (2010) showed that CAPE prevents IκBα degradation concomitantly with attenuation of p65 nuclear accumulation. Although our results are in agreement with part of these previous findings and in contrast with others, but it is worthwhile to mention that we examined, in this study, the effect of CAPE and PE on Tax-induced NFκB activations, while in all previous studies the effect of CAPE on NFκB activation was examined by other stimuli such as TNF-α. The partial disagreement with some of the previous findings could be due to different mechanism of Tax-induced NFκB activation.

In addition, the synergistic activity of Tax and TNF-α on NFκB activation proved by our results (Fig. 4) may indicate complementary pathways of NFκB activation by Tax and other stimuli such as TNF-α CAPE and PE, in agreement with previous studies (Lee et al., 2010; Song et al., 2008), strongly prevented NFκB activation by TNFα and they also conferred complete inhibition of the synergistic activation of NFκB by Tax and TNF-α.

These natural products may interfere in the additional steps during the Tax-induced NFκB activation process. Therefore, it seems worthy to continue elucidating the mechanism of action of these natural products against Tax–NFκB activation. Exploring the inhibitory potential of these natural products against Tax induced NFκB activation which is considered as a major factor of Tax oncogenic activity (Chalet and Thomas, 2002; Peloponese and Jeang, 2006) and elucidating their mechanism of action will pave the way for developing such products as preventive/measures or drugs for ALT and perhaps other HTLV-1 related diseases.

Acknowledgments

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