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The anticancer mechanism of caffeic acid phenethyl ester (CAPE): review of melanomas, lung and prostate cancers

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Abstract. – BACKGROUND: Caffeic acid phenethyl ester (CAPE), an active component of propolis extract, specifically inhibits NF-κB. It exhibits antioxidant, antiinflammatory, antiproliferative, cytostatic, and most improtantly, antineoplastic properties.

AIM: The aim of the present mini-review is to summarize and evaluate the anticancer mechanism of CAPE with examples of several cancer types.

RESULTS: In view of the mechanisms and findings in our laboratory and those of others in literature, we suggest that CAPE possess anticancer and apoptosis inducing activities.

CONCLUSIONS: Further researches are needed regarding the anticancer basis of CAPE in all disciplines of medicine. Also, clinical potential toxicities of CAPE should be revealed if it is going to be used as an anticancer agent.

Key Words:
Caffeic acid phenethyl ester (CAPE), Cancer, Effect, Treatment.

Introduction

As a natural compound bearing polyphenolic ring, CAPE (Figure 1), is originated from a biologically active bee product, propolis. It is known to have antitumoral, antiinflammatory, antineoplastic and antioxidant properties to some extend. It is a white-color powder as a commercial product with a storage temperature –20°C and soluble in ethanol, DMSO, and ethyl acetate (50 mg/ml). Its empirical formula is $C_{17}H_{16}O_4$ and has 284.31 g/mol molecular weight (Figure 1).

CAPE is a specific inhibitor of NF-κB. It has been shown to significantly suppress the lipoxygenase pathway of arachidonic acid metabolism during inflammation in μM concentrations. At a concentration of 10 μM, it completely blocks production of reactive oxygen species (ROS) in human neutrophils and the xanthine/xanthine oxidase system.

Possible Anticancer Mechanisms of CAPE

It has been suggested that CAPE potently stimulate glucose uptake in cultured skeletal muscle cells throughout the adenosine monophosphate-activated kinase-protein (AMKP) pathways showing an important anti-diabetic potential[1]. CAPE has a higher hydrophobicity and stronger inhibition potency toward xanthine oxidase (XO) and it inhibits the enzymatic activity via binding to the molybdoptrein region of its active site[2]. Because XO has an action to metabolyze both purine and pyrimidine bases, by this inhibition mechanism, CAPE can stop the nucleotide turnover salvage pathway showing anticancer activity in all cell types. It is suggested to be used in the treatment of gout and hyperuricemia because of that XO inhibitory effect[3].

Oxidative stress is also suggested to be a major cause of cellular injuries in carcinogenesis. It was tested by using erythrocyte membrane ghost lipid peroxidation, plasmid pBR322 DNA, and protein damage initiated by the water-soluble initiator 2,2'-azabis(2-amidinopropane) hydrochloride and H_2O_2 monitored by formation of hydroperoxides and by DNA nicking assay, single-cell alkaline electrophoresis, and sodium dodecyl sulphate (SDS)-polyacrylamide gel (PAGE) electrophoresis. The results showed that CAPE and its related polyphenolic acid esters elicited remarkable inhibitory effects on erythrocyte membrane lipid peroxidation, cellular DNA strand breakage, and protein fragmentation[4].

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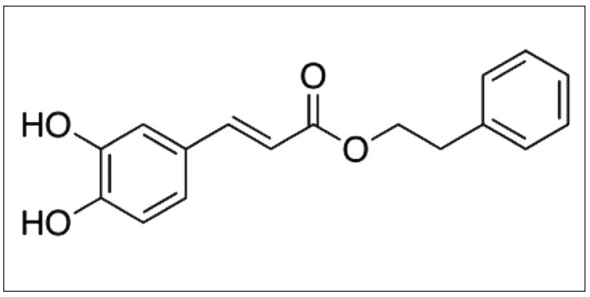

Figure 1. The chemical structure of caffeic acid phenethyl ester.

Arachidonic acid metabolites are important mediators in cancer and inflammation processes, so when we look at the lipoxygenase inhibitor effect of CAPE, we can see that CAPE has a ability to inhibit 5-lipoxygenase by a complete uncompetitive mechanism[5]. Inhibition of the tumor promoter-mediated oxidative processes by CAPE has also been reported in the culture of HeLa cells[6]. The other studies has also shown that CAPE and the other caffeic acid esters inhibit azoxymethane-induced colonic preneoplastic lesions and enzyme activities, including ornithine decarboxylase, tyrosine kinase and lipoxygenase, associated with colon carcinogenesis[7].

One of the anti-tumor promotion activities of CAPE is suggested to be the induction of apoptosis. CAPE suppressed 12-O-tetradecanylphorbol-13-acetate-induced cell transformation and induced apoptosis in mouse epidermal JB6 Cl 41 cell. No difference in induction of apoptosis was observed between nomal lymphoblasts and sphingomyelinase-deficient cell lines. CAPE treatment of two p53 mutant tumor cell lines, NCI-H358 and SK-OV-3, and p53 deficient (p53(-/-)) and Cl 41 cells caused the cleavage of caspase-3 as well as DNA fragmentation. However, caspase-3 cleavage was seen early only in cell expressing wild-type p53 (p53(+/+)) and Cl 41 cells. This shows p53 may be involved in the early stage of CAPE-induced apoptosis. In addition, CAPE may induce apoptosis through p53-dependent and -independent pathways and its anti-tumor promotion activity may have occured through the induction of apoptosis[8]. To reveal the mechanism of CAPE-induced differential cytotoxicity, nontumorigenic cloned rat embryo fibroblasts (CREF) and adenovirus-transformed CREF cells (Wt3A) were used in a study. Nucleosomal-length DNA degradation, morphological alterations by electron microscopy, *in situ* labeling of 3'OH ends, and the appearance of a hypodiploid cell population by bivariant flow cytometry, cell death induced by CAPE in the transformed Wt3A cells revealed that the mechanism is apoptosis. CAPE has this effect by modulating the redox state (as GSH level) of the cells[9]. A series of oncogene transformed, oncogene-reverted and CAPE-resistant oncogene transformed CREF cells were used to observe the mechanism underlying the increased sensitivity of transformed cells to CAPE. A direct relationship exists between the cytotoxic effects of CAPE and the induction of DNA fragmentation and apoptosis. The expression of the transformed phenotype by rodent cells evokes sensitivity to CAPE-induced toxicity through apoptosis[10]. Evidence indicated that CAPE may represent a unique compound that can specifically target progressed transformed cells for growth suppression and toxicity. An understanding of the mechanism underlying this selective effect of CAPE could result in the identification of important biochemical pathways mediating cellular transformation and progression of the transformed state[11].

Angiogenesis is a fundamental pathogenic process in cancer and it is very valuable to find some compounds that has potential inhibitory effect on angiogenesis. One of these compounds is the biomimetic dimerization product of CAPE, benzo[k,l]xanthene lignan. The lignan showed a significant, dose-related inhibitory effect on new vessel growth in the angiogenesis bioassay and it inhibited vascular endothelial growth factor (VEGF) secretion in ovarian cell culture[12]. In an *in vitro* tube formation assay, human umbilical vein endothelial cells and fibroblast cells were incubated for 14 days with VEGF for induction of proliferation and migration of cells and with potential inhibitor, CAPE. It significantly suppressed VEGF-induced *in vitro* tube formation and proliferation[13].

The effect of CAPE on tumor invasion and metastasis by determining the regulation of matrix metalloproteinases (MMPs), which are zinc-dependent proteolytic enzymes playing pivotal role in tumor metastasis by cleavage of extracellular matrix as well as nonmatrix substrates, is an attractive area. Dose dependent decreases in MMP and tissue inhibitor of MMP-2 mRNA levels were observed in CAPE-treated HT1080 human fibrosarcoma cells as detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Gelatin zymography analysis also exhibited a significant down-regulation of MMP-2 and MMP-9 expression in HT1080 cells trated with

CAPE. Moreover, CAPE inhibited the activated MMP-2 activity as well as invasion, motility, cell migration and colony formation of tumor cells[14]. One of cell culture cytotoxicity assays of CAPE in colon adenocarcinomas showed a dose-dependent decrease in cell viability, cell invasion by 47.8%, and expression of MMP-2 and -9. The other effects were inhibition of VEGF production, inhibition of pulmonary metastatic capacity accompanied with a decreased plasma VEGF (in BALB/c mice), and also prolonged survival of mice implanted by colon adenocarcinomas[15].

In view of the above-mentioned mechanisms and findings in our laboratory and those of others in literature, we suggest that CAPE possess anticancer and apoptosis inducing activities. Further researches are needed regarding the clinical potential toxicities of CAPE if it is going to be used as an anticancer agent.

The effect of CAPE on Several Cancer Types in Experimental and Cell Culture Models

Lung Cancers

Lots of chemopreventive agents have been reported to interfere with the intracellular signal that is related to carcinogenesis, the proliferation of cancer cells, apoptosis, and cell migration. The effects of the representative chemopreventive agents on the transforming growth factor-beta (TGF-β)-induced invasive phenotype using A549 lung adenocarcinoma cells as a model system was investigated by using CAPE as therapeutic agent. CAPE effectively suppressed TGF-β-enhanced cell motility and TGF-β-induced Akt (protein kinase β) activation as well as a specific inhibitor of phosphatidyl inositol 3-kinase (PI3K)/Akt pathway, LY294002[16]. By this finding, the Authors suggest that CAPE can be applied not only as chemopreventive agent but also as a anti-metastatic therapeutic agent. The useful therapeutic dose of CAPE for A549 lung cancer cell line was found to be 6 μg/ml and the tolerance dose of normal lung fibroblast for CAPE appeared to be higher than the therapeutic dose[17]. It is claimed that CAPE inhibits the growth of tumor cells using oxidative stress pathways connected to p53-independent pathways and observed to inhibit oxidative processes by decreasing the generation of intracellular hydrogen peroxide (H_2O_2) in A549 cells. The decrease in H_2O_2 production and GSH level of A549 cells was seen in a very rapid and profound manner[17]. Moreover, the depleting intracellular stores of GSH (reduced glutathione) by CAPE can render cells more susceptible to oxidative stress-induced apoptosis[18].

Prostate Cancer

Prostate cancer is a highly common malignancy in Western countries and it is resistant to apoptosis. Because of this resistance, it is essential to develop new therapeutic strategies. Because CAPE is a nuclear factor-κB (NF-κB) inhibitor and 5α reductase inhibitor, it has a potential for the treatment of prostate cancer. It was found that CAPE can inhibit NF-κB activation in prostatic cancer-3 (PC-3) cells, by blocking the ability of paclitaxel and tumor necrosis factor-alpha (TNF-α) to activate NF-κB. This effect is also associated with a reduction in the cellular levels of the inhibitors of apoptosis proteins (cIAP-1, cIAP-2 and XIAP). Note that these proteins are a family of structually homologous caspase inhibitors[19]. This is consistent with other reports showing that CAPE prevents NF-κB activation[20]. CAPE can reduce constitutive activation levels. It is suggested that it could have a role in managing prostate cancer by blocking the NF-κB survival pathway. This is because CAPE may enable lower doses of chemotherapy or radiotherapy to be used to achieve a clinical response, with possibly less overall toxicity. In a study, CAPE was used as second hit agent in association with antisense oligonucleotide to target a region within the baculovirus IAP repeat (BIR) domain of cIAP-1 and examined its ability to facilitate apoptosis in prostate cancer cells[21]. Western blotting showed a down-regulation in cIAP-1 expression and higher levels of spontaneous apoptosis in PC3 and DU145 cells with no alteration in overall cell viability.

Melanomas

CAPE is suggested to suppress reactive-oxygen speies (ROS)-induced DNA strand breakage in human melanoma A2058 cells when compared other potential protective agents[22]. It was also studied in aspect of *in vitro* biochemical mechanism of its toxicity using tyrosinase enzyme as a molecular target in human SK-MEL-28 melanoma cells. The IC(50) of CAPE towards human skin melanoma (SK-MEL-28 melanoma) cells was found to be 15 μM. Quinone formation is thought to play an important role in CAPE-induced cell toxicity. When

the role of tyrosinase was investigated in CAPE toxicity, it was noticed that CAPE led to negligible antiproliferative effect, apoptotic cell death and ROS formation in shRNA plasmid-treated cells. Moreover, CAPE selectively caused escalation in the ROS formation and intracellular GSH depletion in melanocytic human SK-MEL-28 cells which express functional tyrosinase[23]. The studies on five melanoma cell lines, B16-F0, B16F10, SK-MEL-28, SK-MEL-5, and MeWo and *in vivo* efficacy study in skin B16-F0 melanoma tumor model in C57BL/6 mice showed that CAPE (10 mg/kg/day) led to intracellular GSH depletion, 10-25 fold increase in ROS formation in B16-F0 cells, caused 5-7 fold increase in apoptosis in B16F0 cells, and led to tumor size growth inhibition[24]. Tyrosinase bioactivates CAPE to an O-quinone, which reacts with GSH to form CAPE-SH conjugate. The researchers investigated CAPE as a selective glutathione S-transferase (GST) inhibitor in the presence of tyrosinase, which is abundant in melanoma cells. 90% of CAPE was metabolized by tyrosinase after a 60 minutes incubation. It showed 70-84% GST inhibition. CAPE-SG conjugate and CAPE quinone demostrated 85% GST inhibition via reversible and irreversible mechanisms. MK-571, a selective multidrug resistance protein (MRP) inhibitor, and probenecid, a nonselective MRP inhibitor, decreased the inhibitory concentration (IC)[50] of CAPE by 13% and 21%, apoptotic cell death by 3% and 13%, and mitochondrial membrane potential in human SK-MEL-28 melanoma cells by 10% and 56%, respectively. Lastly, computational docking analyses suggest that CAPE binds to the GST catalytic active site indicating intracellularly formed quinones and GSH conjugates of CAPE may play major roles in the selective inhibition of GST in SK-MEL-28 melanoma cells[25].

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