Anticancer Effect of Caffeic Acid Phenethyl Ester

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Abstract: Background: Caffeic Acid Phenethyl Ester (CAPE), a bioactive component of honeybee hive propolis, is known to exhibit anti-mitogenic, anti-carcinogenic, anti-inflammatory, anti-viral and immuno-modulatory properties. Result: In this review study, we summarize the known effect and possible mechanism of CAPE on suppressing proliferation of different cancer cells as well as inhibiting growth and metastasis of different tumors. Conclusion: Recent observations suggest CAPE administration as a potential adjuvant therapy for several types of cancer.

Key words: Necrosis, hepatocarcinogenesis, CAPE, anti-mitogenic

INTRODUCTION

Caffeic Acid Phenethyl Ester (CAPE) (Fig. 1), a lipophilic derivatives of caffeic acid and a phenolic antioxidant structurally related to 3, 4-dihydroxyphenylacetic acid, is one of the active components extracted from honeybee hive product propolis. CAPE has been used in folk medicine as a potent anti-inflammatory agent and is known to exhibit anti-mitogenic, anti-carcinogenic, anti-inflammatory, anti-viral and immuno-modulatory properties.

CAPE is well known as a NF-κB inhibitor. CAPE (50-80 μM) specifically inhibits the activation of nuclear transcription factor NF-κB induced by Tumor Necrosis Factor (TNF) and inflammatory agents as well as prevented the translocation of p65 unit of NF-κB. CAPE inhibits the binding between NF-κB and DNA but had no effect on other transcription factors (Natarajan et al., 1996). Reducing agent such as DTT, 2, 3-dimercaptopropanol and 2-mercaptoethanol reverses the effect of CAPE-induced inhibition of NF-κB activation (Natarajan et al., 1996). CAPE is also a strong antioxidant (Bhimani et al., 1993; Jaiswal et al., 1997; Sudina et al., 1993). CAPE dosage-dependently inhibits the oxidative stress induced by H2O2, oxidized species 8-Hydroxy-2'-Deoxyguanosine (8-OHdG), 5-Hydroxymethyl-2'-Deoxyuridine (HMDU) and 12-O-Tetradecanoylphorbol-13-acetate (TPA) in HeLa cells (Bhimani et al., 1993).

CAPE also inhibits activity of Xanthine Oxidase (XO), which is the major source of Reactive Oxygen Species (ROS). CAPE treatment in human hepatoma HepG2 cells dramatically stimulates gene expression antioxidant response element-mediated NAD(P)H quinone oxidoreductase (NQO1) gene expression (Jaiswal et al., 1997). Several studies indicate that CAPE may be an alternative adjuvant therapy for several types of cancer with little or no side effect. We therefore summarized the important researches that have been done in the past 20 years and discussed the effect and anti-cancer mechanism of CAPE.

Anti-cancer effect of CAPE on different cancer cells: CAPE has been reported to inhibit transformation of normal cells to cancer cells. CAPE (10 μM) selectively inhibited cloned rat embryo fibroblast cells transformed to neoplastic cells. CAPE also inhibited cell proliferation in other cancer cells lines such as MCF-7, A549, and U87-MG.

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CAPE induces cell cycle arrest in CREF cells but induces apoptosis in type 5 adenovirus-transformed W3A cells (Chiao et al., 1995). CAPE (5 μM) treatment also suppresses TPA-induced cell transformation and induces apoptosis in mouse epidermal JB6 Cl 41 cells (Nomura et al., 2001).

Different cancer cell lines showed different sensitivity to CAPE treatment (Table 1). CAPE treatment suppresses proliferation of several human cancer cell lines, including MCF-7 breast cancer cells (Nomura et al., 2001), PC-3 prostate cancer cells (Watabe et al., 2004), HL-60 leukemia cells (Chen et al., 2001b; McElney et al., 2004), A549 and H1299 non-small cell lung cancer cells (Chen et al., 2004; Chen et al., 2001a; Lin et al., 2011; Usia et al., 2002), HT-1080 fibrosarcoma cells (Chen et al., 2004), HeLa cervical cancer cells (Chen et al., 2004), CT26, HCT116, SW480 colon cancer cells (Liao et al., 2003), Shigeoka et al., 2004; Wang et al., 2005), GNM neck metastasis of Gingiva carcinoma (Xiang et al., 2006), TSCC tongue squamous carcinoma cells (Xiang et al., 2006), SAS oral squamous carcinoma cells (Lee et al., 2000), Meng 1 oral epidermal carcinoma cells (Lee et al., 2000), Daoy medulloblastoma cells (Lee et al., 2005), Nalm6, Farage, Pfeiffer, Ramos, HDMAR lymphoma cells (Lin et al., 2006) and U973 myeloid leukemia cells (Berger et al., 2007). Among the different type of cancer cell lines being tested, cervical cancer HeLa cells (Usia et al., 2002) and multiple lymphoma cell lines (Berger et al., 2007) are relative sensitive to CAPE treatment, suggesting the possibility that cervical cancer and lymphoma cancer patients may benefit from CAPE treatment. Non-cancer cells, such as human immortal lung fibroblast WI-38 cells (Chen et al., 2004), Human Normal Umbilical Vein Epithelial Cells (HUVEC) (Usia et al., 2002), or Human Normal Oral Fibroblast (NHO) cells (Lee et al., 2005) are much more resistant to CAPE treatment, indicating potential selective cytotoxic effect against cancer cells of CAPE treatment.

CAPE treatment causes apoptosis and cell cycle arrest. CAPE induces apoptosis in many cancer types through stimulation of Bel-2-associated X protein (Bax) (Chen et al., 2001a; Jin et al., 2008; Lee et al., 2003; Watabe et al., 2004), Bak (Lee et al., 2003), p53 (Hung et al., 2003, Lee et al., 2003; Nomura et al., 2001), p21 (Hing et al., 2003), extracellular signal-regulated kinase (ERKs) (Lee et al., 2003), c-Jun (Hung et al., 2003), c-Jun N-terminal kinase (JNK) (Watabe et al., 2004), p38 mitogen-activated protein kinase (p38 MAPK) (Lee et al., 2003; Watabe et al., 2004), Fas ligand (Watabe et al., 2004), caspase activity (Chen et al., 2001a; Hung et al., 2003; Jin et al., 2008; Lee et al., 2003), down-regulation of Bel-2 (Jin et al., 2008; Lee et al., 1994), the cellular inhibitor of apoptosis proteins 1 and 2 (cIAP-1 and cIAP-2, respectively) and X-linked Inhibitor of Apoptosis Protein (XIAP) (Chen et al., 2001a; McElney et al., 2004), release of cytochrome C (Jin et al., 2008; Lee et al., 2003), loss of mitochondrial transmembrane potential (Hung et al., 2003), decrease in McI-1 (Hung et al., 2003), as well as by inhibiting the functions of NF-kB (McElney et al., 2004; Watabe et al., 2004). Treatment of p38 MAPK inhibitor SB203580 partially suppresses CAPE-induced p53 activation, Bax expression and apoptosis (Watabe et al., 2004). Expression of dominant negative e-Jun which inhibits the JNK signal, also suppresses CAPE-induced apoptosis, suggesting MAPKs are involved in CAPE-induced apoptosis.

Table 1: Effect of CAPE on proliferation of different cancer cell lines. Either IC50 of the dosages being used to show suppressive effect of CAPE on cancer are shown in table.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Cancer type</th>
<th>IC50 (μM)</th>
<th>Dosage (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>Breast cancer</td>
<td>-</td>
<td>10-100</td>
</tr>
<tr>
<td>PC-3</td>
<td>Prostate cancer</td>
<td>-</td>
<td>88</td>
</tr>
<tr>
<td>HL-60</td>
<td>Leukemia</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>A549</td>
<td>Lung cancer</td>
<td>20.9</td>
<td>-</td>
</tr>
<tr>
<td>H1299</td>
<td>Lung cancer</td>
<td>21.2</td>
<td>-</td>
</tr>
<tr>
<td>HT-1080</td>
<td>Fibrosarcoma</td>
<td>9.5</td>
<td>-</td>
</tr>
<tr>
<td>HeLa</td>
<td>Cervical cancer</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>CT26</td>
<td>Colon cancer</td>
<td>35.0</td>
<td>3.5-24</td>
</tr>
<tr>
<td>HCT116</td>
<td>Colon cancer</td>
<td>-</td>
<td>9-182</td>
</tr>
<tr>
<td>SW480</td>
<td>Colon cancer neck metastasis</td>
<td>-</td>
<td>9-182</td>
</tr>
<tr>
<td>GNM</td>
<td>Gingiva carcinoma tongue squamous cell</td>
<td>-</td>
<td>25-200</td>
</tr>
<tr>
<td>TSCCA</td>
<td>Carcinoma oral squamous cell</td>
<td>-</td>
<td>25-200</td>
</tr>
<tr>
<td>SAS</td>
<td>Carcinoma oral cell epidermoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Meng 1</td>
<td>Carcinoma</td>
<td>-</td>
<td>50-200</td>
</tr>
<tr>
<td>Daoy</td>
<td>Medulloblastoma acute lymphoblastic</td>
<td>-</td>
<td>1-100</td>
</tr>
<tr>
<td>Nalm6</td>
<td>Lymphoma diffuse large cell</td>
<td>3.1</td>
<td>-</td>
</tr>
<tr>
<td>Farage</td>
<td>Lymphoma diffuse large cell</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Pfeiffer</td>
<td>Lymphoma</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>Ramosi</td>
<td>Burkitt’s lymphoma</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>HDMAR</td>
<td>T-cell lymphoma</td>
<td>2.1</td>
<td>-</td>
</tr>
<tr>
<td>U937</td>
<td>Myeloid leukemia</td>
<td>-</td>
<td>0.4-53</td>
</tr>
</tbody>
</table>
apoptosis (Watabe et al., 2004). Overexpression of Bel-2 rescues apoptosis induced by CAPE (Su et al., 1994).

In addition, CAPE treatment induces G1 or G2 cell cycle arrest in several cancer cells through suppression of cyclin B1 (Chen et al., 2004; Lin et al., 2006), cyclin D1 (He et al., 2006; Kuo et al., 2006), cyclin E (Kuo et al., 2006), e-cadherin expression (He et al., 2006), phosphorylation of Rb (Kuo et al., 2006), cytoplasmic and total and nuclear β-catenin (Wang et al., 2005; Xiang et al., 2006) and increased expression of the cyclin dependent kinase inhibitors p21WAF1 (Kuo et al., 2006), p27Kip1 (Kuo et al., 2006) and p16INK4A (Kuo et al., 2006). CAPE may also induce necrosis (Berger et al., 2007).

CAPE treatment also suppresses cancer cell motility and invasiveness via suppression of Akt phosphorylation (Shigekawa et al., 2004), phosphorylation of Focal Adhesion Kinase (FAK) (Weyant et al., 2000), expression of matrix metalloproteinase MMP-2 and MMP-9 (Usia et al., 2002), Vascular Endothelial Growth Factor (VEGF) (Usia et al., 2002) and disrupts the arrangement of actin cytoskeleton (Weyant et al., 2000).

Inhibition of tumor growth and metastasis by CAPE:

Several animal studies suggest that CAPE can prevent carcinogenesis in vivo. C57BL/6J-Min+/− mice bear a germ line mutation in the Apc gene and spontaneously develop several intestinal adenomas by 15 weeks of age. A dietary level of 0.15% CAPE decreases 63% of tumor formation in these mice. Examination of intestinal tissue from the treated animals reveals that CAPE treatment increases apoptosis and cell proliferation of enteroxyte and decreases expression of the oncoprotein β-catenin in the enterocytes (Mahmoud et al., 2000). Rats treated with 50 mg kg−1 CAPE i.p., reduced the formation of aberrant crypt foci and colon-rectal carcinoma induced by azoxymethane (Borrelli et al., 2002). Male Wistar rats are medium-term rat hepatocarcinogenesis model. These rats will generate Altered Hepatic Foci (AHF), an early sign of hepatocarcinoma, when subjected to a carcinogenic treatment (diethylnitrosamine (DEN), 2-AAF) to. A single

20 mg kg−1 dosage treatment of CAPE given 12 h before initiation of the carcinogenic treatment reduces γ-glutamyl transpeptidase (GGT) positive AHF by 84%, possibly through an anti-oxidative and free-radical scavenging mechanism (Carrasco-Legleu et al., 2006). Gavage of CAPE (20 mg kg−1) decreases the number and area of GGT-positive AHF in Male Wistar rats exposed to diethylnitrosamine by 91 and 97%, respectively (Carrasco-Legleu et al., 2004). Nuclear localization of the p65 subunit of NF-κB was decreased by 85% by CAPE treatment as well (Carrasco-Legleu et al., 2004).

CAPE treatment also suppresses tumor growth and metastasis in animal models. Growth of rat C6 glioma xenografts in nude mice are dose-dependently inhibited by CAPE treatment (1-10 mg/kg/day, i.p.). CAPE treatment significantly reduces the number of mitotic cells and Proliferating Cell Nuclear Antigen (PCNA)-positive cells (Lee et al., 2005). Oral administration of CAPE (100-250 mg/kg/day) for 7 days after murine colon 26-L5 cancer cells inoculation decreases the tumor weight and the number of tumor nodules in the lung by around 50 and 50%, respectively, compared to the control (Nagoaka et al., 2003). CAPE treatment (10 mg/kg/day, i.p.) in Balb/c mice reduces 80% of the pulmonary metastatic foci of CT26 murine colon tumors, decreased 60% plasma VEGF level and prolonged the survival of mice (Liao et al., 2003). Gauge of single dosage (50 or 150 mg kg−1) of CAPE 15, 10 and 5 days before metastases in the lung generated by intravenous (i.v.) injection of transplantable mammary carcinoma in CBA mice reduces 50-67% of lung metastases (Orcoli et al., 2004). Subcutaneous or oral administration of CAPE (5 mg kg−1) three times a week suppresses 45-55% of the tumor volume and 70-85% of number of liver metastasis of HepG2 xenografts in nude mice (Chung et al., 2004).

**DISCUSSION**

The possible mechanism involved in the anti-cancer effect of CAPE is summarized in Fig. 2. As we discuss

![Fig. 2: Potential mechanism of the anti-cancer activity of CAPE. The signaling molecules being activated by CAPE are shown in red and those being suppressed by CAPE are shown in blue](image-url)
above, CAPE can induce apoptosis, G1 or G2 cell cycle arrest and necrosis while it can reduce motility and invasiveness in cancer cells depends on the concentration of CAPE being used and the types of cancer cells being treated. CAPE also suppresses development, growth and metastasis of tumors in animal models. These observations suggest that CAPE might be a potential therapeutic agent for cancers. The achievable concentration of CAPE in human serum is around 5.0 μg mL⁻¹ (17 μM) (Celli et al., 2007). This concentration (17 μM) is not enough to eradicate all types of cancer cells (Table 1). However, CAPE can be used in combination with current standard treatments. We believe that further clinical trials should be performed to determine if caffeic acid phenethyl ester can be used as a safe and effective adjuvant therapy for variable types of cancers.

Disclosure of Potential Conflicts of Interest: No potential conflicts of interest were disclosed.

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