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Activation of Antioxidant-Response Element (ARE), Mitogen-Activated Protein Kinases (MAPKs) and Caspases by Major Green Tea Polyphenol Components during Cell Survival and Death

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Green tea polyphenols (GTP) have been demonstrated to suppress tumorigenesis in several chemical-induced animal carcinogenesis models, and predicted as promising chemopreventive agents in human. Recent studies of GTP extracts showed the involvement of mitogen-activated protein kinases (MAPKs) in the regulation of Phase II enzymes gene expression and induction of apoptosis. In the current work we compared the biological actions of five green tea catechins: (1) induction of ARE reporter gene, (2) activation of MAP kinases, (3) cytotoxicity in human hepatoma HepG2-C8 cells, and (4) caspase activation in human cervical squamous carcinoma HeLa cells. For the induction of phase II gene assay, (-)-epigallocatechin-3-gallate (EGCG) and (–)-epicatechin-3-gallate (ECG) potently induced antioxidant response element (ARE)-mediated luciferase activity, with induction observed at 25 μM with EGCG. The induction of ARE reporter gene appears to be structurally related to the 3-gallate group. Comparing the activation of MAPK by the five polyphenols, only EGCG showed potent activation of all three MAPKs (ERK, JNK and p38) in a dose- and time-dependent manner, whereas EGC activated ERK and p38. In the concentration range of 25 μM to 1 mM, EGCG and ECG strongly suppressed HepG2-ARE-C8 cell-growth. To elucidate the mechanisms of green tea polyphenol-induced apoptosis, we measured the activation of an important cell death protein, caspase-3 induced by EGCG, and found that caspase-3 was activated in a dose- and time-dependent manner. Interestingly, the activation of caspase-3 was a relatively late event (peaked at 16 h), whereas activation of MAPKs was much earlier (peaked at 2 h). It is possible, that at low concentrations of EGCG, activation of MAPK leads to ARE-mediated gene expression including phase II detoxifying enzymes. Whereas at higher concentrations of EGCG, sustained activation of MAPKs such as JNK leads to apoptosis. These mechanisms are currently under investigation in our laboratory. As the most abundant catechin in GTP extract, we found that EGCG potently induced ARE-mediated gene expression, activated MAP kinase pathway, stimulated caspase-3 activity, and induced apoptosis. These mechanisms together with others, may contribute to the overall chemopreventive function of EGCG itself as well as the GTP.

Key words: Green Tea Polyphenols, EGCG, EGC, ECG, EC, Catechin, ARE-luciferase, MAP kinases, JNK, ERK, p38, Apoptosis, Caspase-3, HepG2-ARE-C8, HeLa

INTRODUCTION

Tea (*Camillia sinensis*) is one of the most popular beverages consumed daily in the world. Epidemiological survey showed drinking green tea, the unfermented form of fried tea leaves, has strong linkage to low incidence of some types of cancers in Asian countries (Weisburger, 1999). *In vivo* studies have indicated that green tea possesses chemoprotective function against tumor initiation, promotion and progression, in various carcinogenesis animal models (Conney *et al.*, 1999; Gensler *et al.*, 1996; Steele *et al.*, 1999) and human clinical trials (Li *et al.*, 1999). Because the most abundant constituents in green tea are polyphenolic compounds, including (–)-epicatechin (EC), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC) and (–)-epigallocatechin-3-gallate (EGCG) (Fig. 1), which comprise

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30-42% solid green tea extract (Mukhtar and Ahmad, 1999), numerous studies have been performed to characterize their biochemical effects and understand the molecular mechanism of chemopreventive activity in different phases of carcinogenesis. Based on chemical structure and reactivity, green tea polyphenols (GTP) are better free radical scavengers than Vitamin C and E (Zhao *et al.*, 1989), so this antioxidant property may partially contribute to their anti-tumorigenesis function. The other important chemopreventive characteristic of green tea polyphenols is the induction of Phase II detoxifying enzymes such as glutathione S-transferases (GSTs), NAD(P)H:quinone reductase, epoxide hydrolase, and UDP-glucuronosyltrans-ferases (Bu-Abbas *et al.*, 1995; Khan *et al.*, 1992; Lee *et al.*, 1995). It is known that most phase II genes generally contain *cis*-acting regulatory elements called the antioxidant response element (ARE) or electrophile-responsive element, which has been found in rat GST-Ya, rat GST-P, and rat and human quinone NAD(P)H:oxidoreductase/DT-diaphorase (Wasserman and Fahl, 1997). Recent studies from our laboratory showed that the transcriptional regulation of ARE-mediated gene expression can be regulated by the mitogen-activated protein kinase (MAP kinase) pathway, a very important cellular signal transduction pathway (Yu *et al.*, 1999; Yu *et al.*, 2000c). Responses to numerous types of extracellular signals are mediated by MAPKs, which are members of a serine/threonine kinase family. A well-defined MAPK subfamily are extracellular signal-regulated kinases (ERK1 and ERK2), which are responsible for the phosphorylation and activation of various transcription factors, including c-MYC and TCF/Elk1 (Marais and Marshall, 1996). Other members of MAPK family are c-Jun N-terminal kinases (JNK1 and JNK2) and p38, both of which belong to stress-activated protein kinases (SAPKs). Their activities can be induced by diverse physiological and environmental stimuli such as growth factors, cytokines, T-cell activators, UV irradiation, heat shock and osmotic shock. Once activated, JNK can phosphorylate various transcription factors such as AP-1, ATF2 and TCF/Elk1 leading to immediate-early gene induction, and p38 can phosphorylate MAPK-activated protein kinase 2 and 3, which subsequently phosphorylate a small heat shock protein, Hsp27 (Kyriakis and Avruch, 1996).

Besides their chemoprotective function, green tea polyphenols also possess cytotoxicity to different types of cancer cells, such as lung (Yang *et al.*, 1998), liver, breast, colon (Valcic *et al.*, 1996) and prostate (Gupta *et al.*, 2000; Paschka *et al.*, 1998). It has been shown that the inhibitory effect of green tea polyphenols on cell proliferation is mediated through inducing apoptosis and disturbing the cell cycle (Ahmad *et al.*, 1997). Apoptosis, or programmed cell death, generally can be triggered by internal signals such as mitochondria-mediated cytochrome c release (Adams and Cory, 1998), and external signals such as Fas and tumor necrosis factor, which bind to the respective

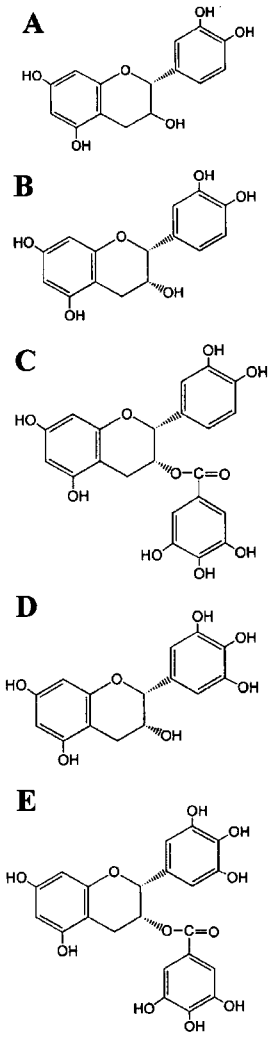

Fig. 1. Structures of catechin and catechin derivatives in green tea polyphenols extract. (A) (+)-catechin, (B) (-)-epicatechin (EC), (C) (–)-epicatechin gallate (ECG), (D) (–)-epigallocatechin (EGC) and (E) (–)-epigallocatechin gallate (EGCG).

receptors to initiate the cell death cascade (Bennett *et al.*, 1998; Laster *et al.*, 1988). One of the early cell responses to proapoptotic signals is the activation of caspase-3, a member of the protease family, which functions as initiators and effectors in the whole process of apoptosis. After proteolytic activation from procasapase-3, activated caspase-3 can recognize DEVD tetrapeptide motif in target proteins and specifically cleave the peptide chain after aspartic acid, which then leads to deregulation of protein activities and dismantlement of the cytoskeletal and cellular structure (Thornberry and Lazebnik, 1998).

Previously, we reported GTP extract can potently induce ARE-mediated CAT reporter activity in HepG2 cells transiently transfected with ARE-CAT plasmids, and the activation of ARE reporter activity was correlated with the stimulation of mitogen-activated protein kinase (MAPK) pathway (Yu *et al.*, 1997a). However, the active components that

are contained in GTP, which would activate the MAP kinase pathway and induce ARE reporter gene activity, remain unclear. In the present study, five polyphenolic catechins and GTP were used to examine ARE-mediated gene expression in HepG2-ARE-C8 cells (generated from HepG2 cells stably transfected with pARE-TI-luciferase reporter gene) (Yu *et al.*, 1999). The effects on transcriptional regulation of ARE by different polyphenols were investigated by determining the respective luciferase reporter activity, and the signal transduction mechanism was evaluated by MAPK activation. To further elucidate the role of major green tea polyphenols in the regulation of cell growth and induction of apoptosis, we compared the cytotoxicity of five polyphenolic compounds and GTP in HepG2-ARE-C8 cells using cell viability assay. The stimulation of caspase-3 activity was studied using the most potent polyphenolic compound, EGCG. In this report, our data showed that the activation of ARE coincides with the activation of JNK, ERK and p38 MAPKs in HepG2-ARE-C8 cells, and the apoptosis induced by EGCG is correlated with the activation of caspase-3 in HeLa cells.

MATERIALS AND METHODS

Cell culture

HepG2-ARE-C8 cell line was established in our laboratory. Human hepatoma cell, HepG2, which was purchased from American Type Culture Collection (ATCC), was transfected with pARE-TI-luciferase construct (provided by Dr. William Fahl, University of Wisconsin) using a FuGENE™ 6 method (Yu *et al.*, 1999). Twenty-four hours after transfection, cells were cultured in fresh medium containing 0.8 mg/ml of G418. Clonal cells were selected for growth in the presence of G418 by limiting dilution and confirmed by the inducible activity of ARE-luciferase reporter gene by *tert*-butylhydroquinone (tBHQ) and sulforaphane. One clone, named HepG2-ARE-C8, was presented in this report.

HepG2-ARE-C8 cells were maintained in modified F-12 media supplemented with 10% fetal bovine serum, 1.7 g/L sodium bicarbonate, 100 units/ml penicillin and 100 µg/ml streptomycin, essential amino acid and insulin. Cells were normally starved overnight in minimum essential medium (MEM) containing 0.5% serum before treatment.

Human cervical cancer cell line, HeLa, was purchased from ATCC. HeLa cells were maintained in MEM supplemented with 10% fetal bovine serum, 2.2 g/L sodium bicarbonate, 100 units/ml penicillin and 100 µg/ml streptomycin.

Materials

Catechin, (–)-epicatechin (EC), (–)-epicatechin gallate(ECG), (–)-epigallocatechin (EGC) and (–)-epigallocatechin gallate (EGCG) were purchased from Sigma (Sigma Chemical, St. Louis, MO). Green tea polyphenols (GTP), which is a polyphenolic fraction isolated from green tea extract containing mainly (-)-epicatechin derivatives, was purchased from LKT Laboratories (LKT Laboratories, Inc., St. Paul, MN, USA). All stock solutions, ranging from 5 mM to 1 M or from 5 mg/ml to 1 g/ml, were prepared by dissolving in dimethylsulfoxide (DMSO). Fluorogenic peptide substrate of caspase-3 (Ac-DEVD-MCA) was purchased from Peptides International (Louisville, KY). The final concentration of DMSO in the culture medium was controlled to <0.1%. The rabbit anti-phospho-ERK1/2, -JNK and -p38 polyclonal antibodies were purchased from New England Biolabs Inc. (Beverly, MA)

Assay for luciferase reporter activity

Cells were subcultured in 6-well plates at a density of 10^5 cells/well. After pre-treatment with MEM containing 0.5% serum overnight, cells were challenged with different doses of catechin, EC, ECG, EGC, EGCG and GTP for 24 h. The luciferase activity was determined according to the protocol provided by the manufacturer (Promega Corp., Madison, WI). Briefly, after treatment, cells were washed twice with ice-cold phosphate-buffered saline (PBS) and harvested in reporter lysis buffer. The homogenates were centrifuged at 13,000 × g for 15 min at 4 °C. A 20 µl supernatant was used for the assay of luciferase activity using TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). Luciferase activity was normalized against protein concentration.

Western blotting to examine phosphorylation of ERK, JNK and p38

After treatments, the cells (cultured in 10-cm petri dish) were washed with ice-cold PBS and lysed with 500 µl of lysis buffer (10 mM Tris-HCl, pH 7.4, 50 mM sodium chloride, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 100 µM sodium orthovandate, 2 mM iodoacetic acid, 5 mM $ZnCl_2$, 1 mM phenylmethylsulfonyl fluoride, and 0.5% Triton-X 100). The lysate was homogenized by passing through a 23 G needle three times or sonicating 10 sec, and kept on ice for 30 min. The homogenate was centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was transferred into a clean tube and stored at -80°C.

The protein concentration of whole cell lysates was determined by Bio-Rad protein assay kit. The equal amount of proteins was mixed with 4 × loading buffer, and preheated at 95°C for 3 min. The samples were then loaded on a 10% mini SDS-polyacrylamine gel, and run at 200 V. The proteins were transferred onto a PVDF membrane for 1.5 h using semi-dry transfer system (Fisher). The membrane was blocked in 5% bovine serum albumin (BSA) solution for 1 h at room temperature, then incubated overnight at 4°C with anti-phospho-MAPK primary antibody (1:1,000 dilution, New England Biolabs, Inc., Beverly, MA), which specifically recognized phosphorylated ERK1 (Thr202/Tyr204), JNK (Thr183/Tyr185), p38 (Thr180/Tyr182), respec-

tively. After hybridization with primary antibody, membrane was washed with TBST (Tris buffered-saline Tween-20) for three times, then incubated with HRP (Horseradish Peroxidase)-conjugated secondary antibody (1:10,000 dilution, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 45 min at room temperature and washed with TBST three times. Final detection was performed with ECL™ (Enhanced Chemiluminescence) Western blotting reagents (Amersham Pharmacia Biotech., Piscataway, NJ).

Cell viability assay (MTS Assay)

Cells were subcultured in 96-well plates at a density of 10^4 cells/well. Twenty-four hours later, cells were treated with different doses of catechin, EC, ECG, EGC, EGCG and GTP for 24 h. The MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay was performed with CellTiter 96 Aqueous nonradioactive cell proliferation assay kit (Promega Corp., Madision, WI). The absorbance was read at 490 nm on ELISA reader, and the percentage of cell survival was obtained.

Caspase activity assay

After treatments, HeLa cells were washed twice with ice-cold PBS and lysed in a hypotonic buffer containing 50 mM Tris-HCl (pH 7.4), 50 mM β-glycerophosphate, 15 mM $MgCl_2$, 15 mM EDTA, 100 μM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothrietol (DTT), and 150 μg/ml digitonin. Cell lysates were homogenized by passing through a 23-gauge needle three times. Homogenates were kept on ice for 30 min, and then centrifuged at 12,500 × g for 20 min at 4°C. The supernatants were transferred into new tubes and protein concentrations were determined by the Bradford method (Bio-Rad, CA). The enzymatic activities of caspase-3 were assayed with 200 μM Ac-DEVD-MCA fluorogenic substrates in assay buffer (100 mM HEPES, 10% sucrose, 10 mM DTT and 0.1% CHAPS) as described previously (Yu *et al.*, 1998). The fluorescence was measured on CytoFluor II fluorescence reader by setting 360 nm as excitation wavelength and 460 nm as emission wavelength.

RESULTS AND DISCUSSION

Antioxidant response element (ARE) has been shown to be a major mediator which transcriptionally induce the gene expression of many phase II detoxifying enzymes, including glutathione S-transferase and quinone reductase (Xie *et al.*, 1995). To observe cellular effect of green tea polyphenols on transcriptional regulation of ARE reporter genes, HepG2-ARE-C8 cells, which were stably transfected with pARE-TI-luciferase construct, were treated with catechin, EC, ECG, EGC, EGCG in concentrations ranging from 25 to 500 μM, and 25-500 μg/ml GTP. The luciferase

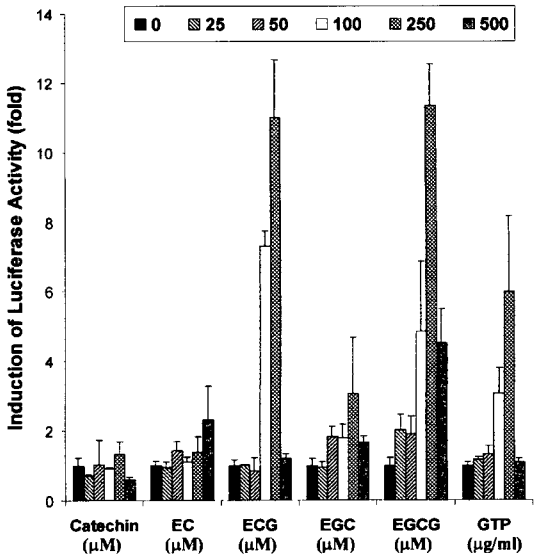

Fig. 2. Dose-dependent induction of luciferase reporter activity by green tea polyphenol compounds in HepG2-ARE-C8 cells. Stably transfected HepG2 cells with ARE-luciferase construct were selected as described under "Materials and Methods". HepG2-ARE-C8, a G418-selected positive clone, was treated with different concentrations of catechin, EC, ECG, EGC, EGCG and GTP. Luciferase activity was determined 24 h after treatment and normalized against protein concentration. The level of luciferase activity in untreated HepG2-ARE-C8 cells was set arbitrarily to 1.

activity was normalized by respective protein concentration. The fold of induction of luciferase activity was calculated by dividing treated samples over the control samples. As shown in Fig. 2, EGCG is the most potent ARE-luciferase inducer among the green tea polyphenols tested. The induction of luciferase activity by EGCG can be observed at 25 μM, then peaked at 250 μM treatment, which produced more than 11-fold induction. However, at 500 μM of EGCG, there is a decrease in ARE-luciferase activity, which may be due to the cytotoxic effect of EGCG as discussed later. Interestingly, ECG appears to be a strong inducer of ARE reporter gene, with more than 10-fold induction observed at 250 μM. EGC and EC have relatively weak inducing effect of luciferase activity, and catechin has no effect on ARE reporter activity.

Several members of the MAP kinase family have been identified to be involved in the ARE-mediated induction of Phase II enzymes by chemicals (Yu *et al.*, 1999; Yu *et al.*, 2000c). Also, in a previous study, we found that GTP activates ERK and JNK, in a dose- and time-dependent manner, and that the activation of MAP kinase is correlated with the induction of ARE reporter gene (Yu *et al.*, 1997a). To further understand the effect of the individual polyphenol on MAP kinase activation, we performed Western blotting using anti-phospho-ERK, -JNK, -p38 antibodies to detect the activated MAP kinases. First, we compared the MAP kinase activating effects of five poly-

phenols at 250 μM concentration with that of 250 μg/ml GTP in HepG2-ARE-C8 cells. The result shown in Fig. 3A indicates that treatments of EGCG and GTP lead to phosphorylation of all three MAP kinases. Whereas EGC strongly activates ERK and p38, but weakly activates JNK. The similarity between EGCG and GTP on the activation of MAP kinases further reinforces the view that EGCG may act as a major effective component in green tea. Consequently, we performed further studies using EGCG. In the kinetic time course study (Fig. 3B), the phosphorylation of JNK can be detected as early as 15 min after treatment of HepG2-ARE-C8 cells with 250 μM EGCG, whereas the activation of ERK and p38 was slightly delayed, appearing at 30 min. The maximum activation of all three MAP kinases occurred at 2 h. Next, we examined the dose response of EGCG, the activation of ERK and JNK can be detected at 25 μM concentration after 2 h of EGCG treatment, which corresponds to the induction of ARE-luciferase as described above, whereas the activation of p38 appeared at 50 μM (Fig. 3C).

Many studies have shown that tea polyphenols can elicit anti-proliferative effects in several human carcinoma cell lines (Gupta *et al.*, 2000; Paschka *et al.*, 1998; Valcic *et al.*, 1996; Yang *et al.*, 1998). To examine the growth inhibitory effect of green tea polyphenols on HepG2-ARE-C8 cells, MTS cell viability assay was performed using different concentrations of catechin, EC, ECG, EGC, EGCG and GTP after 24-h exposure. The potencies of polyphenols in the inhibition of HepG2-ARE-C8 cell growth are dose-dependent (Fig. 4). The IC50 (concentration to induce 50% growth inhibition) of EGCG and ECG occurred around 500 μM, which appears to be more potent than catechin. Because of the strong growth inhibitory effect of EGCG in many carcinoma cell lines, and since many publications have reported that apoptosis was induced by green tea polyphenols, we next studied the mechanism of EGCG-induced cell death. We chose HeLa cell line, a human cervical squamous carcinoma cell line, because it is highly sensitive to EGCG's growth inhibitory effect, and also HeLa cells are commonly used in the study of signal transduction and apoptosis mechanisms (Yu *et al.*, 1997b). We examined the stimulation of caspase-3 activity, a central protein in the execution of apoptosis and one of the indicators of apoptosis. In kinetic study, caspase-3 activity remained at the basal level during first 8 h treatment with 50 μM EGCG. However, after 12 h, caspase-3 activity increased very rapidly, and peaked at 16 h (Fig. 5A). Next, we examined the dose response of EGCG. Caspase-3 activity was stimulated by EGCG at 50 μM and peaked at 100 μM following 16 h of exposure (Fig. 5B). However, at higher concentration of 250 μM, caspase-3 activity was decreased to the basal level. This could be due to non-specific necrotic cell death as seen with extensive cell lysis under the microscopy. EC appeared to have no effect on caspase-3 activity. It is interesting to note that

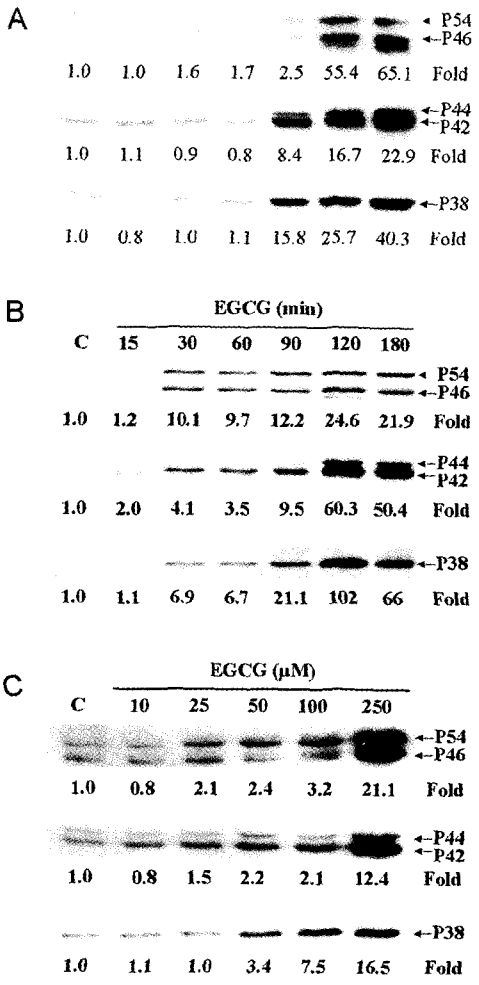

Fig. 3. Effect of green tea polyphenol compounds on the activation of mitogen-activated protein kinases (MAPKs). (A), comparison of MAP kinase activation by treating HepG2-ARE-C8 cells with 250 μM catechin, EC, ECG, EGC, EGCG and 250 μg/ml GTP for 2 h. (B), Time course of MAP kinases activities in HepG2-ARE-C8 cells treated with 250 μM EGCG. (C), Dose response of MAP kinase activities in HepG2-ARE-C8 cells after 2 h EGCG treatment. Phosphorylation of MAP kinases, JNK (p54, p46), ERK (p44, p42) and p38, was detected by *in vitro* immunoblotting with anti-phospho-MAPK antibodies (New England Bio-labs). Fold of induction of JNK, ERK and p38 activation was calcu-lated as the relative intensity of the treated cells to the control cells by densitometry.

HeLa cells were much more sensitive to EGCG growth inhibitory effects as compared to HepG2-ARE-C8 cells. This may be due to the fact that the HepG2-ARE-C8 cells were transfected with pARE-luciferase containing neomycin gene and were selected from G418-resistant single cells. Future studies would elucidate the differences in the dose-response in apoptosis between HeLa cells and HepG2-ARE-C8 cells.

Green tea has been established as a primary dietary supplement with potent chemopreventive activity as evidenced by epidemiological studies as well as animal

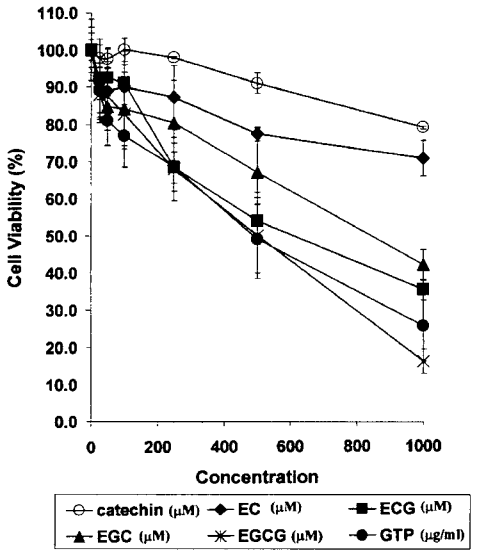

Fig. 4. Cytotoxicity of green tea polyphenols compounds to HepG2-ARE-C8 cells. HepG2-ARE-C8 cells were split in 96-well plates at a density of 10^4 cells/well. Twenty-four hours later, cells were treated with different doses of catechin, EC, ECG, EGC, EGCG and GTP for 24 h. The MTS assay was performed with CellTiter 96 Aqueous nonradioactive cell proliferation assay kit. The absorbance was read at 490 nm on ELISA plate reader.

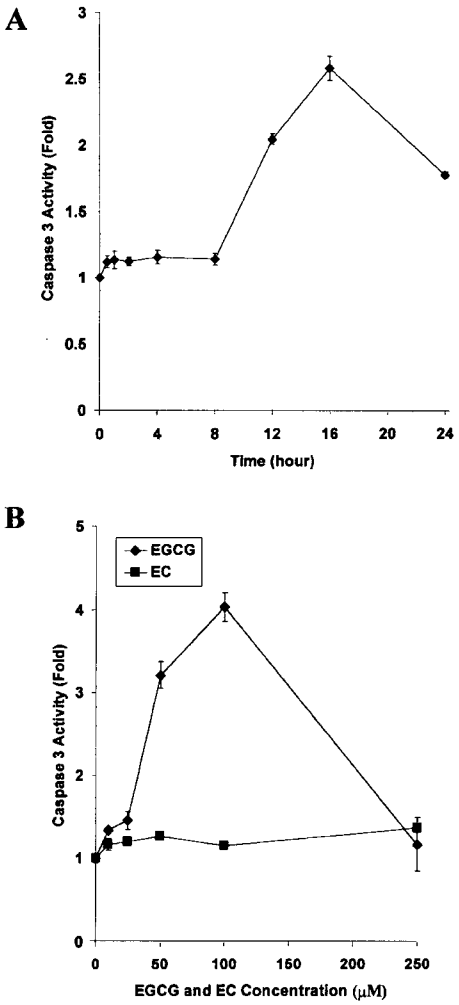

Fig. 5. Time- and dose-dependent activation of caspase 3 by EGCG in HeLa cells. HeLa cells were treated with EGCG in 6-well plates. After treatment, HeLa cells were lysed in a hypotonic lysis buffer. The caspase assay was performed in 96-well plate with 50 μM Ac-DEVD-MCA fluorogenic substrates. The absorbance was measured on CytoFluor II fluorescence reader by setting 360 nm as excitation wavelength and 460 nm as emission wavelength. Protein concentrations were determined by the Bradford method (Bio-Rad, CA).

models and *in vitro* cell models. Phase II detoxifying enzymes, one of the biomarkers of chemoprevention, are regulated by ARE, which presents in the 5'-flanking region of these genes. In the present study, our results showed that EGCG and ECG can dramatically enhance the expression of ARE reporter gene. Because of the higher content of EGCG than ECG in green tea extract, EGCG may be the major component in GTP-stimulated ARE reporter activity. In addition, examining the chemical structures of polyphenols (Fig. 1), it is possible that the 3-gallate group in EGCG and ECG may relate to the effect of GTP-stimulated ARE reporter activity and phase II gene induction. In the studies of MAP kinases activation, the similarity between EGCG and GTP further lend support to the notion that EGCG may play a major role in the biological effects of green tea. Since ERK, JNK and p38 are important nucleus-translocating kinases responsible for phosphorylating multiple transcription factors (Cobb and Goldsmith, 1995), with the subsequent regulation of gene expression related to cell proliferation and cell death, the activation of all three MAP kinases by EGCG indeed is surprising as well as intriguing. The individual role of each MAP kinase in the regulation of ARE is under investigation in our laboratory. Noticeably, our recent studies as well as studies from other laboratories have found that NF-E2-related factor 2 (Nrf2), along with small Maf protein (acting as transcriptional repressor in heterodimers), can bind to and activate the ARE (Nguyen *et al.*, 2000; Venugopal and Jaiswal, 1996; Yu *et al.*, 2000a). In future, it will be interesting to examine the role of Nrf2 in EGCG-induced ARE activation.

The cytotoxic effects of EGCG, ECG and EGC in HepG2-ARE-C8 cells display an IC50 of 500 μM following 24 h treatment, whereas the induction of ARE reporter activity and activation of MAP kinases by EGCG occurred between 25-250 μM. It suggests that, at low concentrations, EGCG may induce ARE-mediated phase II gene expression through activation of MAP kinases, whereas at high concentrations, EGCG may activate additional signalling pathways such as the caspases leading to apoptotic cell death, as seen with the HeLa cells. Interestingly, activation of caspase-3 activity (peaked at 16 h) was a relatively late event when compared

to the kinetics of activation of MAP kinases (peaked at 2 h). This delayed kinetics in caspase-3 activation is in contrast to that induced by other chemopreventive agents, such as isothiocynates (Yu *et al.*, 1998) and butylated hydroxylanisole (BHA), which peaked around 2 h (Yu *et al.*, 2000b). The molecular mechanisms of EGCG-induced caspase activation and apoptosis are under investigation.

In summary, EGCG, as the most abundant component in green tea polyphenol extract, dramatically enhanced antioxidant-response element-mediated reporter gene expression, and potently activated the MAP kinase pathway. EGCG could also stimulate caspase-3 activity and induce apoptosis. These mechanisms, together with others, may provide additional insights for our understanding of the overall chemopreventive function of EGCG itself, as well as that of the GTP.

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