Research Article

Cancer Prevention Research

Green Tea Polyphenols and Metabolites in Prostatectomy Tissue: Implications for Cancer Prevention

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Abstract

Epidemiologic, preclinical, and clinical trials suggest that green tea consumption may prevent prostate cancer through the action of green tea polyphenols including (−)-epigallocatechin-3-gallate (EGCG). To study the metabolism and bioactivity of green tea polyphenols in human prostate tissue, men with clinically localized prostate cancer consumed six cups of green tea (n = 8) daily or water (n = 9) for 3 to 6 weeks before undergoing radical prostatectomy. Using high-performance liquid chromatography, 4″-*O*-methyl EGCG (4″-MeEGCG) and EGCG were identified in comparable amounts, and (−)-epicatechin-3-gallate was identified in lower amounts in prostatectomy tissue from men consuming green tea (38.9 ± 19.5, 42.1 ± 32.4, and 17.8 ± 10.1 pmol/g tissue, respectively). The majority of EGCG and other green tea polyphenols were not conjugated. Green tea polyphenols were not detected in prostate tissue or urine from men consuming water preoperatively. In the urine of men consuming green tea, 50% to 60% of both (−)-epigallocatechin and (−)-epicatechin were present in methylated form with 4′-*O*-MeEGC being the major methylated form of (−)-epigallocatechin. When incubated with EGCG, LNCaP prostate cancer cells were able to methylate EGCG to 4″-MeEGCG. The capacity of 4″-MeEGCG to inhibit proliferation and NF-κB activation and induce apoptosis in LNCaP cells was decreased significantly compared with EGCG. In summary, methylated and nonmethylated forms of EGCG are detectable in prostate tissue following a short-term green tea intervention, and the methylation status of EGCG may potentially modulate its preventive effect on prostate cancer, possibly based on genetic polymorphisms of catechol *O*-methyltransferase. *Cancer Prev Res; 3(8); 985–93. ©2010 AACR.*

Introduction

The consumption of green tea, brewed from the leaves of *Camellia sinensis*, may be protective for several chronic diseases including cardiovascular disease, neurodegenerative disease, osteoporosis, and cancer (1–4). The active phytochemicals in green tea are polyphenols, also known as flavan-3-ols, including (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin (EC), and (−)-epicatechin-3-gallate (ECG; Fig. 1), with EGCG being the most abundant and possibly the most bioactive of the green tea polyphenols (GTP; ref. 5).

In addition to their antioxidant activity, GTPs selectively induce apoptosis and cell cycle arrest in cancer cells without affecting normal cells (6–8). In LNCaP prostate cancer cells, the induction of apoptosis by GTPs is regulated through the activation of specific caspases, particularly caspase-3, and modulation of apoptosis-related genes, through the downregulation of Bcl-2 and upregulation of Bax (9). Additionally, GTPs exert chemopreventive and anti-inflammatory activity by modulating cell signaling pathways such as the NF-κB pathway, mitogen-activated protein kinase pathway, epidermal growth factor receptor–mediated pathway, and the insulin-like growth factor–mediated pathway (10, 11).

The chemopreventive and anticancer effects of green tea and GTPs have been shown in cell culture studies and in various animal models (12–14). The majority of epidemiologic studies support an association between green tea consumption and a reduced risk of prostate cancer (15–17). In a clinical case-control trial, Bettuzzi et al. (18) showed a reduced incidence of prostate cancer in men with prostatic intraepithelial neoplasia after a 1-year green tea supplement intervention compared with a group of men receiving placebo. Likewise, in a single-arm preprostatectomy trial of a green tea supplement, McLarty et al. (19) showed a decrease in serum prostate–specific antigen levels, and decreased prostate tissue vascular endothelial growth factor and hepatocyte growth factor concentrations.

It has been proposed that the limited bioavailability of GTPs and their conversion *in vivo* into less-active

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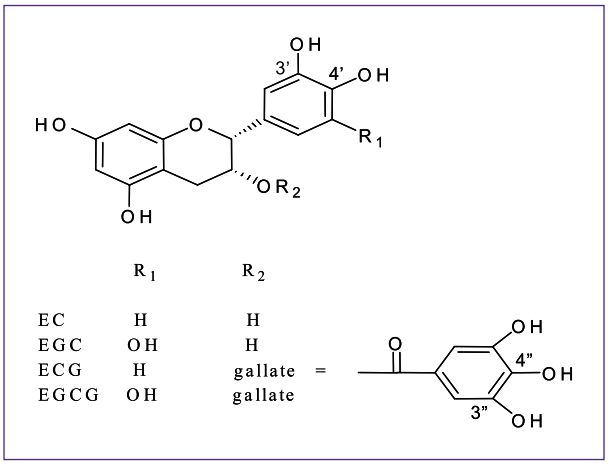

Fig. 1. Chemical structure of GTPs.

metabolites could affect their chemopreventive potential (20). The majority of EGCG in the human blood circulates in the free form, and only a small amount occurs in the glucuronidated or sulfated form (21, 22). Methylation of EGCG by catechol-*O*-methyltransferase (COMT) has been described *in vitro* and in animal studies (22, 23). However, to date, the biotransformation in human tissue *in vivo* has not been studied. The present study was carried out both to determine the degree of methylation of EGCG in human prostate glands obtained in men consuming green tea for up to six weeks before prostatectomy, and to determine the potential effect of the methylation of EGCG on its preventive actions on prostate cancer cells.

Materials and Methods

Green tea intervention in men diagnosed with prostate cancer

The following protocol was approved by the University of California at Los Angeles and Veterans Administration human subjects' committees, and informed consent was signed by all study subjects. In 2008, a randomized intervention trial was initiated in which men with clinically localized prostate cancer were randomized to six cups daily of green tea, black tea, or water for 3 to 6 weeks before undergoing radical prostatectomy. We report here on results from the first 17 subjects randomized to the green tea and water groups. Subjects randomized to the green tea group received detailed instructions to prepare the tea using one tea bag in 240 mL of boiling water to be brewed for 5 minutes. Green tea bags (authentic green tea) were generously provided by Celestial Seasonings. All tea bags were from the same production lot number. The control group was instructed to consume six cups of water daily. A tea consumption diary was kept to record compliance. A first voided urine was collected at baseline, one to two times during the intervention, and at the end of the intervention. Fasting blood was collected before the intervention and within 5 days of the surgery to measure liver function tests and prostate-specific antigen concentration. Liver function tests (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) were measured by the University of California at Los Angeles clinical laboratory. Serum prostate-specific antigen concentration was analyzed by competitive chemiluminescent immunoassay using the DPC Immulite analyzer (Siemens Healthcare Diagnostics) available in our laboratory according to the manufacturer's instruction. Fresh prostate tissue obtained from the prostatectomy was wrapped in foil, frozen in liquid nitrogen, and stored at −70°C.

Tissue and urine sample extraction and high-performance liquid chromatography detection

For tissue GTP analysis, 300 mg of prostate tissue in 200 μL of 2% ascorbic acid solution were homogenized with (−)-catechingallate as an internal standard. The homogenates were buffered with 300 μL of 0.4 mol/L of phosphate solution (pH 6.5) incubated without enzyme, or with 1,000 units of β-glucuronidase (G7896, Sigma) alone, or 1,000 units of β-glucuronidase and 40 units of sulfatase (S-9754, Sigma) at 37°C for 45 minutes for the detection of free, glucuronidated, or total form of GTPs. The sulfated GTPs were calculated by subtraction of free and glucuronidated forms from the total form. After incubation, the mixture was adjusted to pH 3 with trifluoroacetic acid and centrifuged at 3,000 rpm for 5 minutes. The pellets were washed twice with 300 μL of 0.05 mol/L of phosphate buffer (pH 3.0); the supernatants were combined; and solid phase extraction was done using preconditioned HLB cartridges (Waters Corp.). The cartridges were washed (1 mL of water, 1 mL of 5% methanol), and analytes were eluted (70% dimethylformamide in methanol), dried in vacuum, and reconstituted for detection by high-performance liquid chromatography-CoulArray electrochemical detection system (ESA) as previously described (24). The detection limit was 0.2 pmol/g tissue.

The method for urinary GTP detection was previously described (21) with minor modifications. Briefly, an aliquot of 250 μL urine sample was incubated without enzyme, or with 50 units of β-glucuronidase alone, or 50 units of β-glucuronidase and 20 units of sulfatase buffered with 100 μL of ascorbic acid-EDTA solution at 37°C for 2 hours for the detection of free, glucuronidated, or total form. The analytes were separated and detected using high-performance liquid chromatography after solid phase extraction with the conditions as described above. The detection limit was 1 μg/L GTPs in urine. The identities of the tea polyphenols and their metabolites found in the prostate tissues and urine samples were confirmed using mass spectrometry (liquid chromatography tandem mass spectrometry). The GTP concentrations were adjusted by urinary creatinine concentration.

Cell line and cell culture

The human prostate adenocarcinoma LNCaP cell line was obtained from the American Type Culture Collection

and cultured in RPMI 1640 with glutamine supplemented with 10% (v:v) of fetal bovine serum (USA Scientific), 100 IU/mL of penicillin, and 100 μg/mL of streptomycin (Invitrogen, Inc.) at 37°C in a 5% CO_2 incubator. At pH above 6.5, EGCG undergoes autoxidation and dimerization, and forms hydrogen peroxide (25). To minimize the hydrogen peroxide effect, 50 U/mL of catalase were added to the medium before EGCG (Sigma) and 4″-*O*-methyl EGCG (4″-MeEGCG; Nacalai USA, Inc.).

Cellular absorption of EGCG and 4″-MeEGCG

LNCaP cells were allowed to grow to 60% to 70% confluency in 100-mm Petri dishes. In one experiment, cells were incubated with fresh serum-complete medium containing 40 or 80 μmol/L EGCG for 2, 24, 48, or 72 hours, respectively. In the other experiment, cells were incubated with 80 μmol/L of EGCG or 4″-MeEGCG for 2 or 24 hours. The procedures for cell harvest was described by Lambert et al. (26) with some modifications. Briefly, the medium was removed and the dishes were washed with 10 mL of PBS for three times. The dishes were placed on ice, and 100 μL of 2% ascorbic acid in water was added. Cells were scraped and collected for homogenization. The homogenate was centrifuged at 10,000 rpm for 15 minutes, and the supernatant was transferred with a little left for detection of cytosolic protein concentration. Equal volume of cold methanol was added to the transferred supernatant to precipitate proteins. The mixture was centrifuged at 14,000 rpm for 15 minutes, and the supernatant was transferred, dried under nitrogen flow, and reconstituted for high-performance liquid chromatography-CoulArray detection. Cytosolic EGCG and 4″-MeEGCG concentrations were normalized by cytosolic protein determined by the Bio-Rad protein assay according to the manufacturer's protocol (Bio-Rad Laboratories). All the experiments were repeated thrice.

Inhibition of cell proliferation by GTPs

LNCaP cells were seeded into 96-well plates at a density of 1×10^4 per well with RPMI 1640 serum-complete medium and treated with the following: vehicle control (NT), EGCG, or 4″-MeEGCG at 40 and 80 μmol/L for 24, 48, or 72 hours. Catalase (50 U/mL) was added to the medium before EGCG and 4″-MeEGCG. Cell viability was determined by trypan blue dye exclusion assay. Triplicate wells of viable cells for each concentration were counted on a hemacytometer after trypsinization. Each well had three repeats of counting. The experiment was repeated thrice.

Inhibition of NF-κB activation by GTPs

LNCaP cells were allowed to grow to 70% to 80% confluency in six-well plates and incubated with 40 or 80 μmol/L of EGCG or 4″-MeEGCG in fresh complete RPMI 1640 for 24 hours. Tumor necrosis factor (TNF)-α (10 ng/mL; R&D Systems) was added to the medium to incubate with the cells for 15 minutes. After that, the medium was aspirated and the cells were washed twice with cold PBS. The cells were lysed in cold lysis buffer [25 mmol/L HEPES, 150 mmol/L sodium chloride, 1% (v:v) Triton 100, 1% (v:v) protease inhibitor (Sigma), 5 mmol/L EDTA] for 5 minutes on ice and collected in a microfuge tube. The crude lysate was passed through 26½ (1 of 2) G needle and cleared by centrifugation at 14,000 *g* for 10 minutes at 4°C. The protein concentration was measured by the Bio-Rad protein assay according to the manufacturer's protocol (Bio-Rad Laboratories).

For the Western blot analysis, 50 μg of protein were loaded onto a 15% Tris-HCl gel. Separated proteins were electrotransferred to polyvinylidene difluoride membranes and blocked in TBS with 0.1% Tween 20 and 5% nonfat milk for 1 hour at room temperature. Membranes were incubated with rabbit anti-human NF-κB inhibitor protein κB (IκB)-α (sc-203; Santa Cruz) at a dilution of 1:500 overnight at 4°C. Goat anti-rabbit IgG-horseradish peroxidase (sc 2030; Santa Cruz) was used as the second antibody. Protein was visualized and analyzed using a ChemiDoc XRS (Bio-Rad Laboratories) chemiluminescent detection and imaging system. After striping the membrane, monoclonal antibody to β-actin (ab 6276; Abcam, Inc.) was applied as loading control.

Induction of apoptosis by GTPs

To compare the capacity of 4″-MeEGCG to EGCG in inducing apoptosis, LNCaP cells were allowed to grow to 50% to 60% confluency in six-well plates and treated with 40 or 80 μmol/L of EGCG or 4″-MeEGCG in fresh complete RPMI 1640 for 24, 48, or 72 hours. Catalase (50 U/mL) was added to the medium before EGCG and 4″-MeEGCG. Following treatment, the cells were harvested, and Western blot was done as described for NF-κB using the rabbit anti-human cleaved caspase-3 (Cell Signaling), which targets the activated caspase-3 fragments at a dilution of 1: 200.

Statistical analysis

SPSS (version 17.0) was used for statistical analyses. Mean value, median, and SD were calculated using descriptive statistics. Comparison of means was done by two independent samples *t* test, or one-way ANOVA with Tukey's posttest when data were normally distributed. Otherwise, the Kruskal-Wallis test or Wilcoxon rank-sum test were used to compare the differences if data were not normally distributed. Differences were considered significant if $P < 0.05$.

Results

Clinical characteristics and compliance

There were no significant differences between the green tea and control groups with regard to mean Gleason score (6.6 ± 0.5 and 6.9 ± 0.3, respectively) and serum baseline prostate-specific antigen concentration (7.2 ± 5.1 versus 8.8 ± 5.7 μg/L, respectively). The mean duration of the intervention in the green tea and the control group was 25 and 27 days, respectively, with an average compliance of 94.5 ± 3.0% and 93.4% for the tea and water control groups. No adverse events were reported in the green tea or control group, and there was no liver toxicity as measured by preintervention and postintervention serum

Table 1. Tea polyphenol content of the green tea used for the clinical intervention trial

	EGC*	EGCG	EC	ECG	Catechin
Tea water (mg/L)	202.3 ± 10.9	396.8 ± 28.3	52.1 ± 4.7	62.1 ± 4.6	7.9 ± 1.7
Tea leaf (mg/g)	24.1 ± 0.2	47.2 ± 1.4	6.2 ± 0.3	7.4 ± 0.3	0.9 ± 0.2

*The tea was brewed using one tea bag in 240 mL of boiling water for 5 min.

levels of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase (data not shown).

GTPs and metabolites in human prostate tissue and urine

EGCG was the most abundant polyphenol in the green tea leaves and brewed green tea preparations used in the clinical trial (Table 1). The recovery rates for tissue analysis using solid phase extraction for EGC, EC, EGCG, 4″-MeEGCG, and ECG were 65%, 69%, 56%, 56%, and 52%, respectively, and 82%, 86%, 74%, 71%, and 75%, respectively, in urine. GTPs were found in prostate tissue of all the subjects in the green tea group but with considerable individual variation in the concentration (Table 2). An average of 48% of total EGCG was found in methylated form (4″-MeEGCG) in the prostate of subjects in the green tea group (Table 2). No GTPs were detected in the control group either in prostate tissue or in urine (data not shown). In the prostate tissue, EGCG was mainly found in the free form (>60%) along with a small portion being glucuronidated and/or sulfated (Fig. 2). EGC and methyl EGC were found in urine throughout the study in all of the eight participants in the green tea group (Table 3). An average of 61% of EGC was found in methylated form in the urines collected at the end of the study (Table 3). Sulfated and a small amount of glucuronidated methyl EC was found in the urine by liquid chromatography/tandem mass spectrometry analysis with peak areas similar to that of EC. The identities of the methylated EC will be addressed when methyl EC standards are available. In addition, 100% of urinary EGC was found in conjugated form (data not shown).

Methylation of EGCG in LNCaP prostate cancer cells

EGCG degraded quickly (>25%) in cell culture medium during the first 2 hours (Fig. 3A). 4″-MeEGCG showed the same degradation pattern in cell culture medium as EGCG (data not shown). When LNCaP cells were treated with EGCG alone, both EGCG and its metabolite 4″-MeEGCG were detected in the cells in equal concentrations (Fig. 3B and C), and trace amounts of 4′, 4″-DiMeEGCG was detected by liquid chromatography/tandem mass spectrometry. The cellular content of GTPs decreased by 90% between 2 and 24 hours, but remained stable between 24 and 72 hours (Fig. 3B and C). A dose-dependent increase of cellular EGCG and 4″-MeEGCG concentration was observed when the cells were treated with 40 and 80 μmol/L of EGCG (Fig. 3B and C). When LNCaP cells were treated with 4″-MeEGCG alone, there was no EGCG detectable in the cells at 2 and 24 hours (Fig. 3D), and an equivalent amount of 4″-MeEGCG was absorbed compared with the amount of intracellular EGCG following the EGCG treatment (Fig. 3C and D).

Inhibition of cell growth by EGCG or 4″-MeEGCG treatment

EGCG and 4″-MeEGCG treatment of LNCaP cells was associated with a dose- and time-dependent decrease of cell viability compared with the vehicle-treated control group ($P < 0.05$). Relative to the vehicle treated control group, at 72 hours, the cell proliferation was inhibited by 16.9 ± 7.9% (in mean ± SD) after treatment with 40 μmol/L of 4″-MeEGCG compared with 28.2 ± 3.7% after treatment with the same concentration of EGCG, and an inhibition of 33.4 ± 6.3% compared with 53.6 ± 3.7% was observed after treatment with 80 μmol/L of 4″-MeEGCG and EGCG, respectively (Fig. 4).

Inhibition of NF-κB activation

The activation of NF-κB, represented by decreased levels of IκB-α protein, was significantly inhibited by both EGCG and 4″-MeEGCG; however, the inhibition was significantly lower when using 4″-MeEGCG ($P < 0.05$). Relative to the vehicle-treated control group, NF-κB activation was inhibited by 14.1 ± 4.5% (in mean ± SD) after treatment with 40 μmol/L of 4″-MeEGCG compared with 41.3 ± 4.0% after treatment with the same concentration of EGCG, and an inhibition of 55.5 ± 5.3% compared

Table 2. GTP and methyl-metabolite concentration in the prostate tissues of men in the green tea intervention group ($n = 8$)

	GTPs (pmol/g tissue)		
	Mean ± SD	Median	Range
EGCG	42.1 ± 32.4*	26.4	9.6-104.8
4″-MeEGCG	38.9 ± 19.5*	35.8	5.9-69.7
ECG	17.8 ± 10.1*	17.2	0.2†-31.9

NOTE: In the control group, GTP and methyl-metabolite concentrations were below detection limit.
*Compared with the control group, $P < 0.01$.
†Nondetectable values are represented by detection limit.

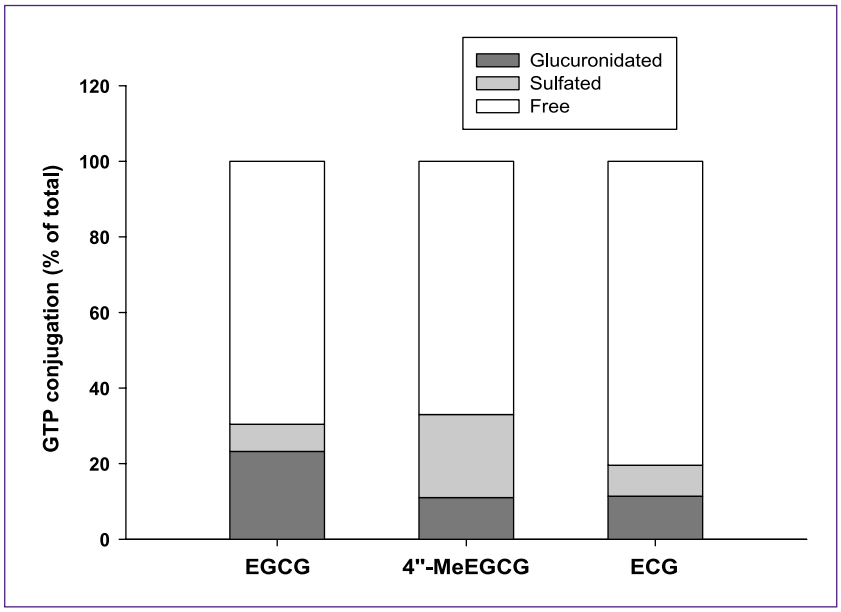

Fig. 2. Glucuronide and sulfate conjugation rate of EGCG, 4″-MeEGCG, and ECG in prostate tissue of men in the tea intervention group expressed as percent of total of each GTP (means of eight participants).

with 121.1 ± 8.9% was observed after treatment with 80 μmol/L of 4″-MeEGCG and EGCG, respectively (Fig. 5).

Induction of apoptosis

Both EGCG and 4″-MeEGCG induced apoptosis in LNCaP cells in a dose- and time-dependent manner as determined by caspase-3 protein expression. Relative to the control group, at 72 hours, caspase-3 protein expression was induced by 95.6 ± 4.7% (in mean ± SD) after treatment with 40 μmol/L of 4″-MeEGCG compared with 203.2 ± 12.1% by the same concentration of EGCG, and an induction of 171.0 ± 8.5% compared with 353.9 ± 7.9% was observed after treatment with 80 μmol/L of 4″-MeEGCG and EGCG, respectively (Fig. 6).

Discussion

The bioavailability and biotransformation of green tea seems to play a key role in the biological activity of GTPs in target tissues (22, 27). The major biotransformation affecting EGCG seems to be *O*-methylation by COMT (23). Prior studies from our laboratory showed that GTPs are present in the human prostate after the consumption of six cups of tea daily (28). However, our current study is the first to show that in prostate tissue collected from participants consuming six cups of green tea daily, 48% of EGCG was present in the methylated form as 4″-MeEGCG with a majority of both EGCG and 4″-MeEGCG in the free form (nonglucuronidated/sulfated). A different methylated EGCG metabolite (4′, 4″-DiMeEGCG) was found in rat and mouse tissue by Meng et al. (22) after green tea or EGCG treatment, which may reflect a species variation. In addition, the presence of 4′, 4″-DiMeEGCG may depend on the concentration of EGCG because we only found it in trace amounts in cell culture extracts but not in human prostate tissue extracts. In the current study, none of the nongallated GTPs including EGC and EC or their metabolites were found in the prostate tissue. This may be due to the extensive glucuronidation and sulfation of the nongallated GTPs during intestinal absorption and liver metabolism leading to enhanced urinary excretion (21, 29). Our findings confirmed that the majority of GTPs excreted in urine were present in conjugated form. In addition, about equal amounts of urinary GTPs were found in methylated form in the present study supporting other publications (30), which may affect the total methylation status of the body.

The findings of a considerable amount of methyl EGCG in the human prostate led us to investigate whether EGCG methylation occurs in the prostate or whether EGCG is absorbed in methylated form. Studies of other investigators using liver homogenates showed *in vitro* that EGCG was methylated by liver cytosolic COMT to 4″-MeEGCG and 4′,4″-diMeEGCG, and EGC was methylated to 4'-MeEGC (23). However, only trace

Table 3. Concentration of tea polyphenols and methyl-metabolites in urine of participants in the green tea group (n = 8)

Time	Concentration (μmol/g creatinine)*		
	EGC	EC	4'-MeEGC
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 1	8.2 ± 3.9†	7.6 ± 3.8†	9.3 ± 4.3†
Week 2	5.2 ± 4.2†	3.4 ± 2.4†	7.4 ± 3.7†
Week 3	7.8 ± 3.9†	6.9 ± 3.4†	12.4 ± 5.9†

*Data are presented as mean ± SEM.
†Compared with either the control group or the baseline levels, $P < 0.05$.

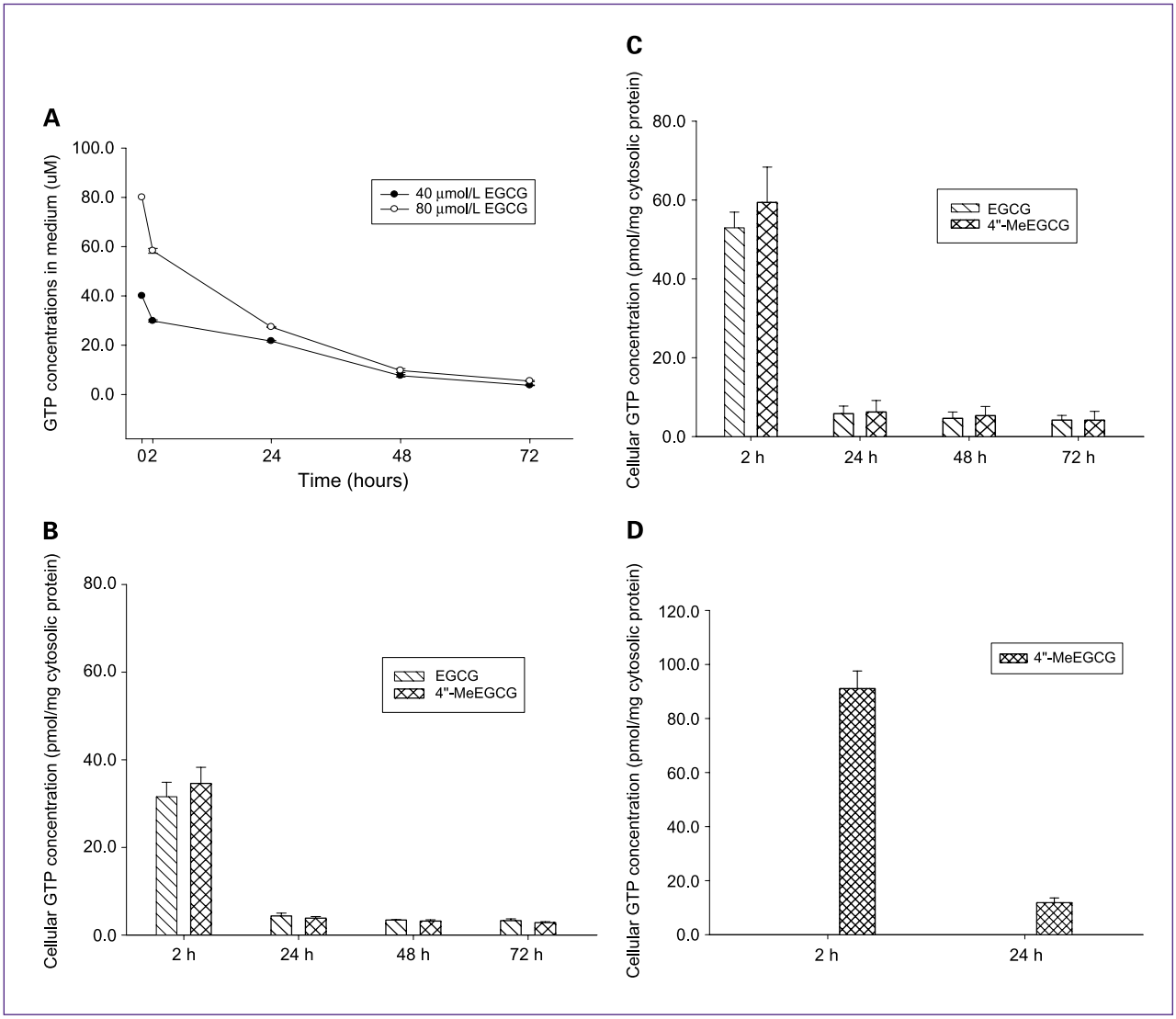

Fig. 3. Cellular uptake and metabolism of EGCG in LNCaP cells. EGCG concentration in medium after treatment of LNCaP cells with 40 or 80 μmol/L of EGCG (A). Intracellular concentration of EGCG and 4″-MeEGCG after treatment with 40 μmol/L of EGCG (B), 80 μmol/L of EGCG (C), or 80 μmol/L of 4″-MeEGCG (D).

amounts of 4″-MeEGCG has been reported in the plasma after green tea consumption (22, 31), which may not constitute a major source for 4″-MeEGCG in tissues. In the present study, we found that treatment of cultured prostate cancer cells with EGCG resulted in methylation of 50% of EGCG within cells after 2 hours. However, no demethylation of 4″-MeEGCG was observed in cultured LNCaP cells. These results indicate that methylation of EGCG may occur in prostate tissue by cytosolic COMT.

The catechol structure of polyphenols plays a critical role in their antioxidant activity (20). Therefore, it is of concern that conjugation of the catechol hydroxyl groups of tea polyphenols may result in altered chemopreventive activities. In the current study, we examined whether the anticarcinogenic activity of EGCG was influenced by methylation at the 4″-position of EGCG. The results revealed a significantly decreased activity of 4″-MeEGCG in inhibiting LNCaP cell viability. In addition, methylation of EGCG reduced the ability of EGCG to induce apoptosis significantly. EGCG is an effective inhibitor of NF-κB (32, 33). Extracellular stimuli, such as reactive oxygen species and proinflammatory cytokine TNF-α, can trigger the activation of NF-κB by dissociation and degradation of the NF-κB inhibitor protein IκB (34). Once released NF-κB migrates to the nucleus to initiate the transcription of many genes promoting inflammation and suppressing apoptosis (35–37). In the present study, the methylated form of EGCG decreased TNF-α–induced NF-κB inhibitory activity significantly compared with unmethylated EGCG. Similarly, other investigators showed that methylation of EGCG and ECG

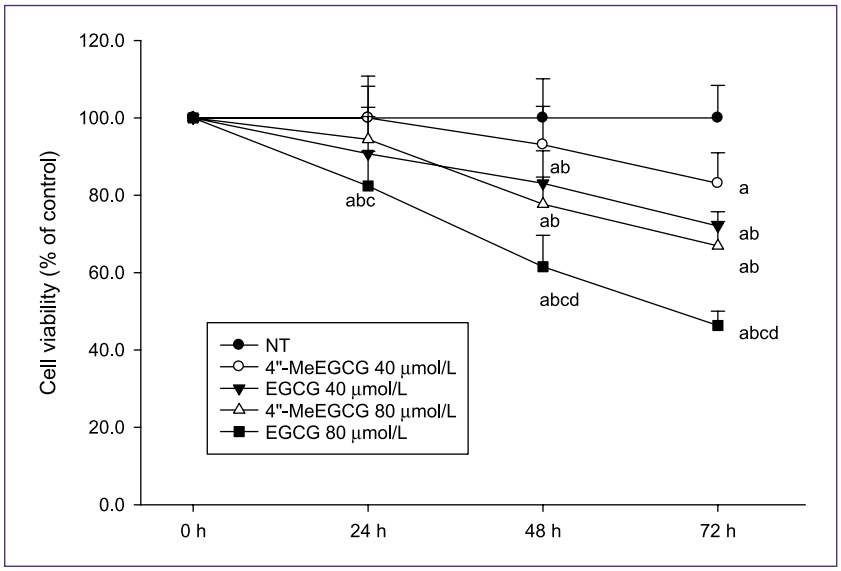

Fig. 4. Cell viability was inhibited by EGCG or 4″-MeEGCG. LNCaP cells were incubated with EGCG or 4″-MeEGCG (40 or 80 μmol/L) for different time points. Cell viability was detected by trypan blue exclusion assay. Superscript letters, significant difference between groups ($P < 0.05$): a, compared with vehicle control; b, compared with 40 μmol/L of 4″-MeEGCG treatment; c, compared with 40 μmol/L EGCG treatment; d, compared with 80 μmol/L 4″-MeEGCG treatment. Points, mean; bars, SD.

decreased the proteasome-inhibitory activity in human leukemic Jurkat T cells, which may reduce their chemopreventive effect (38).

Our observation that methylation decreased the anticarcinogenic activity of EGCG provides a potential explanation for the outcome of an epidemiologic study in Asian-American women that showed a significantly decrease in breast cancer risk only among those tea drinkers possessing at least one low-activity COMT allele (39). Common polymorphisms result in slow methylation and increased bioactivity of EGCG from green tea. Future green tea intervention studies will benefit from the evaluation of interindividual variations in methylation and conjugation processes, which may impact on the chemopreventive effects of tea polyphenols in prostate cancer and possibly other malignancies.

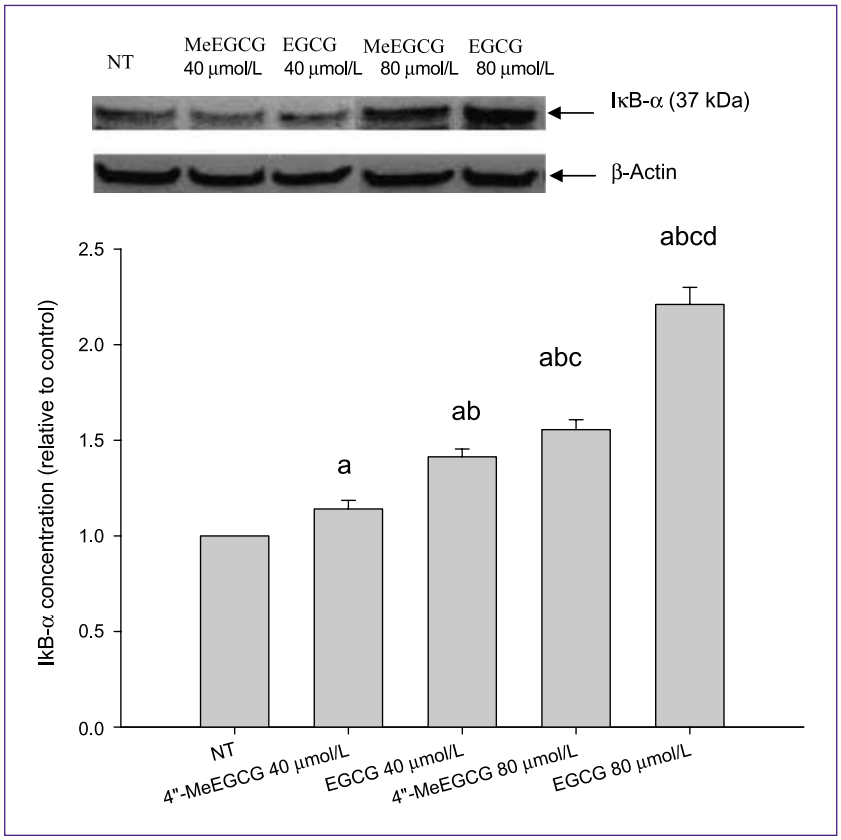

Fig. 5. Inhibition of NF-κB activation by EGCG or 4″-MeEGCG. LNCaP cells were incubated with 40 or 80 μmol/L of EGCG or 4″-MeEGCG for 24 h. NF-κB was stimulated by treatment with 10 ng/mL of TNF-α for 15 min. The IκB-α protein was determined by Western blotting. Superscript letters, significant difference between groups ($P < 0.05$): a, compared with vehicle control; b, compared with 40 μmol/L of 4″-MeEGCG treatment; c, compared with 40 μmol/L of EGCG treatment; d, compared with 80 μmol/L of 4″-MeEGCG treatment. Columns, mean; bars, SD.

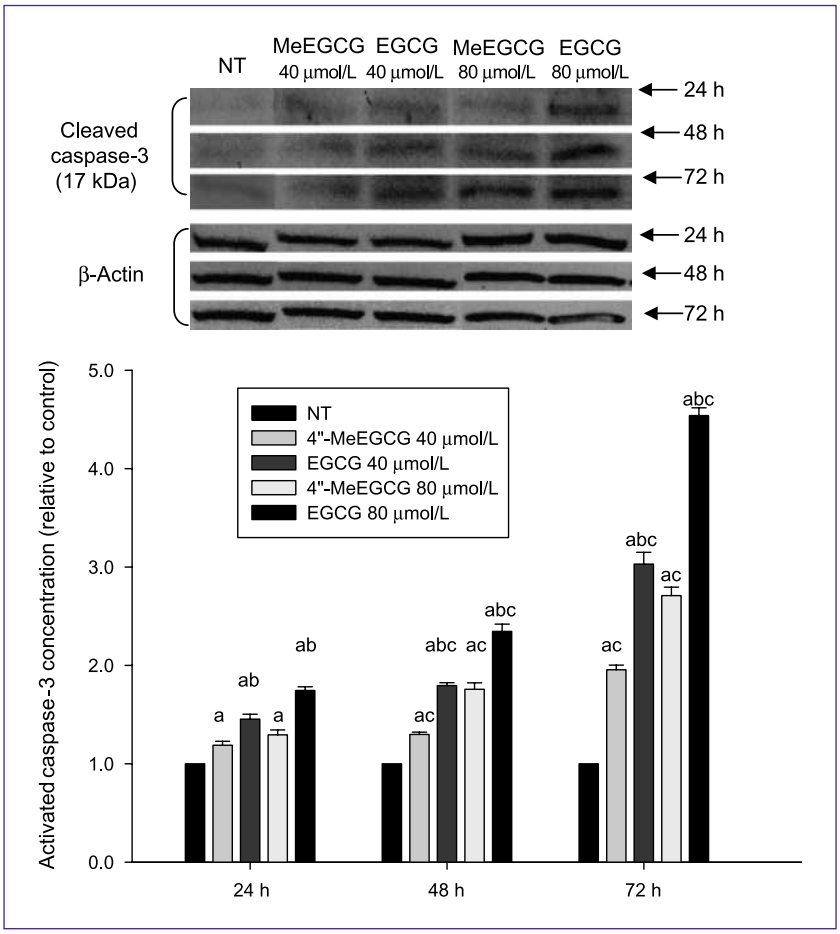

Fig. 6. Induction of apoptosis by EGCG or 4″-MeEGCG. LNCaP cells were incubated with 40 and 80 μmol/L of EGCG or 4″-MeEGCG for different time points. The protein concentration of activated caspase-3 was determined by Western blotting. Superscript letters, significant difference between groups ($P < 0.05$): a, compared with vehicle control; b, compared with 4″-MeEGCG treatment at the same dose level; c, compared with the previous time point. Columns, mean; bars, SD.

In summary, we have shown that the predominant GTPs present in human prostate tissue following a green tea intervention are EGCG and methylated EGCG and, to a lesser extent, EGC. Due to their similarity to human prostate tissue in performing methylation processes, LNCaP prostate cancer cells provide an appropriate *in vitro* model to study GTP chemopreventive activity. Future tea intervention studies will need to address interindividual variation in methylation and conjugation processes, which may potentially modulate the chemopreventive effects of tea polyphenols in prostate cancer and possibly in other cancers based on common polymorphisms of the gene coding for COMT.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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