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Black Tea Polyphenols, Theaflavins, Prevent Cellular DNA Damage by Inhibiting Oxidative Stress and Suppressing Cytochrome P450 1A1 in Cell Cultures

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Tea polyphenols have been demonstrated as chemopreventive agents in a number of experimental models. However, less is known about the mechanism of chemoprevention by black tea compared with that of green tea. Some beneficial properties of theaflavins, the black tea polyphenols, were investigated in the present study. Theaflavins showed inhibitory effects on H_2O_2- and *tert*-butyl hydroperoxide (tBuOOH)-induced cytotoxicity (evaluated by tetrazolium bromide reduction), cellular oxidative stress (detected by oxidation of 2′, 7′-dichlorofluorescin), and DNA damage (measured by amount of 8-OHdG and comet assay) in rat normal liver epithelium cell RL-34 cell lines. In addition, theaflavins also exhibited suppression of cytochrome P450 1A1 induced by omeprazole in the human hepatoma HepG2 cell line. Furthermore, when HepG2 cells were pretreated with omeprazole to induce CYP1A1, then exposed to benzo[*a*]pyrene (B[a]P), DNA damage was observed using the comet assay. However, theaflavins could inhibit this DNA damage. These results indicated that theaflavins could prevent cellular DNA damage by inhibiting oxidative stress and suppressing cytochrome P450 1A1 in cell cultures.

KEYWORDS: Theaflavins; intracellular oxidative stress; cytoprotection; DNA damage; CYP1A1

INTRODUCTION

Experimental and epidemiological data have revealed that tea prevents various types of cancer including skin, lung, stomach, colon, esophagus, and mammary gland (reviewed in refs *1* and *2*). This is highly attributed to the polyphenolic compounds in the tea. The most abundant polyphenol in green tea is epigallocatechin gallate (EGCG). It has been demonstrated that EGCG has a wide range of chemopreventive properties (reviewed in refs *3* and *4*). The typical polyphenols in black tea are theaflavins which have TF1, TF2, and TF3 chemical structures (Figure 1). They are formed by fermentation from green tea and account for 2−6% of the dry weight of solids in brewed black tea (*5*). Theaflavins are the color and flavor components of black tea. Theaflavins have antioxidative properties against lipids oxidation detected in the rabbit erythrocyte ghost system (*6*) and rat liver homogenates (*7*), and against LDL oxidation in mouse macrophage cells (*8*). They have antimutagenicity in the rat liver S9 fraction and are suspected to inhibit cytochrome P450 enzymes (*9*). Theaflavins were also found to block the signal transduction of cell proliferation (*10*, reviewed in ref *11*). The protective effect of theaflavins on DNA oxidative damage was detected in the cell-free systems (*6*). Even so, studies conducted with black tea are limited in comparison with those of green tea. Therefore, the potential chemopreventive activities of black tea polyphenols need to be determined.

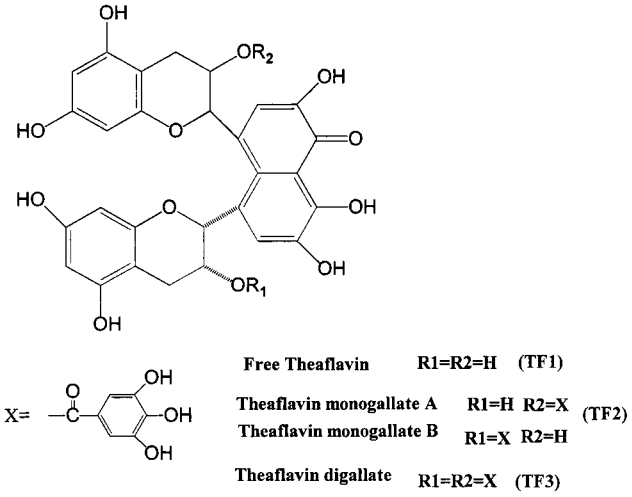

Figure 1. Chemical structures of theaflavins.

In this study, we investigated the effects of theaflavins on oxidative stress-induced cytotoxicity, DNA damage in normal rat liver epithelial cell RL-34 cells, and the carcinogen-induced

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DNA damage by elevated cytochrome P450 1A1 (CYP1A1) in human hepatoma HepG2 cells.

Although tea polyphenols are known antioxidants, it was also recently reported that tea polyphenols could produce H_2O_2 in cell culture medium (*12*). Theaflavins were also reported to produce H_2O_2 and induce apoptosis in several cell lines (*13*). This could be due to the prooxidative activity of the polyphenols. Some antioxidants such as phenolics do have prooxidative activity (*14*). Antioxidant/prooxidant activity of polyphenols is dependent on many factors such as metal-reducing potential, chelating behavior, pH, solubility characteristics, the bioavailability, and stability in tissues and cells (*14*). In the present study, we confirmed that theaflavins are still powerful antioxidants that can protect the normal rat liver cells against oxidative stress induced by H_2O_2 and *tert*-butyl hydroperoxide (tBuOOH) and prevent cellular oxidative DNA damage.

Many chemical carcinogens require metabolic activation before exerting their carcinogenic effects. A lot of cytochrome P450 enzymes take part in such an activation. CYP1A1 can activate the procarcinogens such as benzo[*a*]pyrene (B[a]P) and dioxin, etc. (*15*). CYP1A1 can be induced by the aromatic hydrocarbon receptor (AhR) ligand and non-AhR ligand (reviewed in ref *16*). In the current study, we found that theaflavins could suppress CYP1A1 induced by omeprazole, a non-AhR ligand, and inhibit the DNA damage caused by activated B[a]P in HepG2 cells. HepG2 cells were used because of their good properties by maintaining cytochrome P450 monooxygenase activities in vitro (*17*).

MATERIALS AND METHODS

Reagents. The purified tea polyphenols, EGCG, theaflavin (TF1), theaflavin monogallate (TF2) and theaflavin digallate (TF3) were kindly provided by Mitsui Norin Co., Ltd. (Japan). The purity of the tea polyphenols was more than 95%. TF2 contained 35% theaflavin monogallate A and 60% monogallate B. Glucose oxidase was purchased from Sigma. Glucose, tBuOOH, B[a]P, and omeprazole were from Wako Chemical Co. (Osaka, Japan). Tea polyphenols were dissolved in dimethyl sulfoxide (DMSO, the final concentration of DMSO to cells was no more than 0.1%). The treatment with DMSO was always taken as a control.

Cell Culture. RL-34 and $HepG_2$ cells were from the Japanese Cancer Research Resources Bank. Both cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 100 U/mL penicillin, 100 μg/mL streptomycin, 588 μg/mL L-glutamine, 0.16% $NaHCO_3$, and a specific concentration of heat-inactivated fetal bovine serum (FBS, 5% for RL-34 cells, 10% for $HepG_2$ cells). Cells were grown at 37 °C in an atmosphere of 95% air and 5% CO_2.

Cell Cytotoxicity. RL-34 cells suspended in the medium with FBS were plated at 10^4 cells/well in 96-well plates. After a 24 h incubation at 37 °C, the cells were exposed to the indicated compounds for a specific time, then treated with 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) for 4 h, followed by adding 100 μL of 0.04 N HCl−2-propanol solution. Cell viability was determined by formazan production from a diphenyltetrazolium salt using a multiplate reader at 570 nm (630 nm reference filter).

Intercellular Reactive Oxygen Species (ROS). RL-34 cells were treated with the indicated compounds for 30 min at 37 °C and then incubated with 5 μM 2′,7′-dichlorofluorescin diacetate (DCFH-DA) for another 30 min. After chilling on ice, the cells were washed with ice-cold phosphate-buffered saline (PBS, pH 7.0), scraped from the plate, and resuspended at 10^6 cells/mL in PBS containing 10 μM EDTA. The fluorescence was measured using a flow cytometer (Epics XL, Beckmam Coulter).

8-Hydroxy-2′-deoxyguanosine (8-OHdG) Measurement. RL-34 cells were plated in 90-mm dishes. After treatment with different agents, the cell DNA was isolated using the G NOME DNA isolation kit (BIO 101, Vistar, CA). Ten μg DNA was single-stranded by incubation with 180 units of Exonuclease III (Takara Biotech. Co., Ltd., Japan) at 37 °C for 1 h. After being heated at 95 °C for 5 min and rapidly chilled on ice, the DNA was digested to nucleosides by incubation with 0.6 unit nulease P1 (Takara) at 37 °C for 1 h and then with 0.6 unit E. colic 75 alkaline phosphatase (Takara) for another 1 h. The supernatant from centrifugation was used for the 8-OHdG assay. The amount of 8-OHdG was measured according to the protocol of the competitive ELISA kit (8-OHdG check, Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan).

Comet Assay. The comet assay was performed according to Singh et al. (*18*) with slight modification. Briefly, a frosted micro slide glass (Matsunami Glass Ind., Ltd, Japan) was covered, in order, with 0.65% agarose (Takara), 0.5% low-melting-point (LMP) agarose (Seaplaque, USA) mixed with freshly prepared cell suspensions and mere 0.5% LMP agarose when each agarose was dried. The slide was then coded and carefully immersed in a freshly prepared ice-cold lysing solution (2.5 M NaCl, 100 mM EDTA-2Na, 10 mM Tris, 34 mM sodium lauroyl sarcosinate, adjusting pH to 10 with NaOH, and adding 1% Triton and 10% DMSO) for 1 h. After lysis, the slide was placed in the electrophoresis buffer (1 mM EDTA-2Na and 300 mM NaOH) to unwind the DNA for 15 min, and electrophored for 30 min using a field strength of 0.7 V/cm (25 V, 300 mA). The operations till the end of the electrophoresis were performed at 4 °C. After the electrophoresis, the slide was neutralized with 0.4 mM Tris−HCl (pH 7.5) and stained with 20 μg/mL ethidium bromide (EtBr). The "comet tail" was observed using a fluorescence microscope (Olympus BX60F-3, Olympus Optical, Tokyo, Japan) equipped with a 515−560-nm excitation filter and a 590-nm barrier filter.

Cells with DNA damage displayed migration of the DNA from the nucleus toward the anode. The damaged nucleoid appears in the form of a comet and the undamaged one appears as a halo. The comet tail-length was measured using NIH Image 1.62 software. Quantification of the DNA damage was estimated as the comet tail length.

Ethoxyresorufin-*O*-deethylase (EROD) Activity Assay. The EROD activity assay method was modified according to Kennedy et al. (*19*). Briefly, $HepG_2$ cells were evenly seeded in 96-well plates and cultured till 90% confluent. After treatment with omeprazole (OMZ) and theaflavins for 24 h, the cells were washed with PBS. The multiplate was treated to several freeze and thaw cycles to make the cells permeable and then used for the activity assay. A 100-μL final volume of the reaction liquid containing 50 mM Tris buffer (pH 7.2), 1.3 mg/mL BSA, 5 μM ethoxyresorufin, and 1.7 μM NADPH was added to each well. The plate was kept at 37 °C for 15 min then the reaction was stopped by adding 150 μL of ice-cold methanol. After the reaction, the liquids were transferred to a fluorescence plate for 15 min, then the plate was scanned by a Fluorescence Scan II plate reader (Dainippon Pharmacology. Co., Japan) for fomed resorufin with a 530-nm excitation filter and a 590-nm emission filter. Each sample occupied 4 wells (1 for control, 3 for assay detection). The control wells contained Tris buffer instead of NADPH.

Immunoblotting. The treated cells were washed with PBS and lysed with lysis buffer (50 mM Tris−HCl, pH 7.0, 150 mM NaCl, 1% TriotonX-100, 0.5% sodium deoxycholate, 0.1% SDS, and 100 μg/mL phenylmethylsulfonyl fluoride). A 30-μg sample of the cell protein was subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE). For the immunoblots, proteins were transferred to a nitrocellulose membrane using a semidry blotting technique. After blocking with 5% skim milk, the membrane was probed with antiserum for human CYP1A1 (Daiich Nippon Pharmacology Co., Japan), then followed by the addition of horseradish peroxidase conjugated to IgG and ECL reagent. The signals were visualized and quantified using the Lightcapture system (ATTO, Japan).

RT-Polymerase Chain Reaction (RT-PCR). The treated cells were washed twice with PBS, and the total RNA was isolated using ISOGEN reagent (Wako, Japan). The RT reaction was performed with 10 μg of total RNA and an oligo (dT) primer using the First-Strand cDNA synthesis kit (Life Technology Inc., Rockville, MD). The Biologica Co. synthesized the polymerase chain reaction (PCR) primers. The primer sequences of CYP1A1 and the PCR reaction were according to Guigal et al. (*20*). As the internal control, the amount of GAPDH mRNA was also measured. The primers of GAPDH were (F):

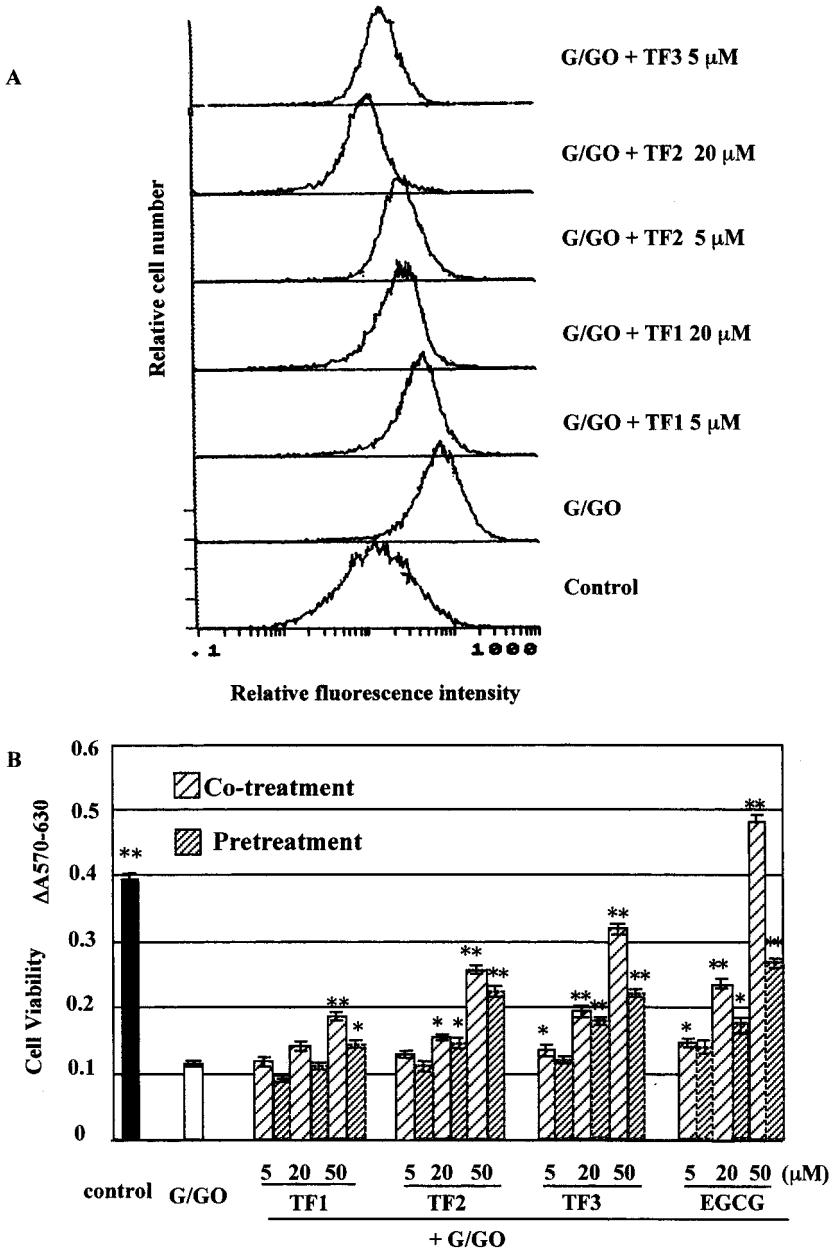

Figure 2. (A) Effect of theaflavins on glucose/glucose oxidase (G/GO)-induced intracellular oxidative stress. Cells were treated with 1.4 U/mL GO and 5 mM glucose in the presence of TF1, TF2, or TF3 (5 or 20 μM) for 2 h, and then intracellular ROS was measured as described in Materials and Methods. (B) Effect of theaflavins and EGCG on G/GO-induced cytotoxicity. Cells were treated with 1.4 U/mL GO and 5 mM glucose and 5–50 μM of teapolyphenols for 2 h (cotreatment), or cells were pretreated with 5–50 μM of tea polyphenols for 1 h, then washed by PBS, and incubated with medium containing G/GO for another 2 h (pretreatment). Cell viability was measured by MTT assay. Results are mean ± SD, $n = 4$. *$P < 0.05$, **$P < 0.01$, compared with G/GO treated cells.

5′-ATCCCATCACCATCTTCCAGGAGC-3′; (R): 5′-CACAGTCT-TCTGGGTGCAGTGAT-3′ according to Tso et al (*21*). The PCR cycles were 25 for CYP1A1, and 36 for GAPDH. The PCR products were analyzed by electropheresis on 1.5% agarose gel and visualized by EtBr staining. The amount of PCR product was also measured using the light capture system.

Statistics. Data represent means ± SD. ANOVA was used to evaluate differences between multiple groups. If significance was observed between two groups, a Student's *t*-test was used to compare the means of the two specific groups. A value of $P < 0.05$ was accepted as statistically significant.

RESULTS

Theaflavins Dispelled Intracellular Oxidative Stress and Protected Cells. Glucose/glucose oxidase (G/GO) is a system which continuously produces H_2O_2. When RL-34 cells were treated with G/GO, exogenous H_2O_2, the intracellular oxidative stress was increased; it was detected by using DCFH-DA as an intracellular fluorescence probe. TF1, TF2, and TF3 inhibited this intracellular ROS in the dose-dependent manner, and TF3 was the most effective one: namely, TF3 inhibited almost all the intracellular ROS even at 5 μM (Figure 2A).

At the same time, theaflavins protected cells against oxidative stress-induced cytotoxicity (Figure 2B). Co-incubation of RL-34 cells with TF1, TF2, TF3, and G/GO alleviated the G/GO cytotoxicity in the dose-dependent manner; especially 50 μM TF3 could recover more than 90% of the cell viability. Pretreatment of RL-34 cells with TF1 (50 μM), TF2, and TF3 (>20 μM) for 1 h also abated the cytotoxicity. Pretreatment

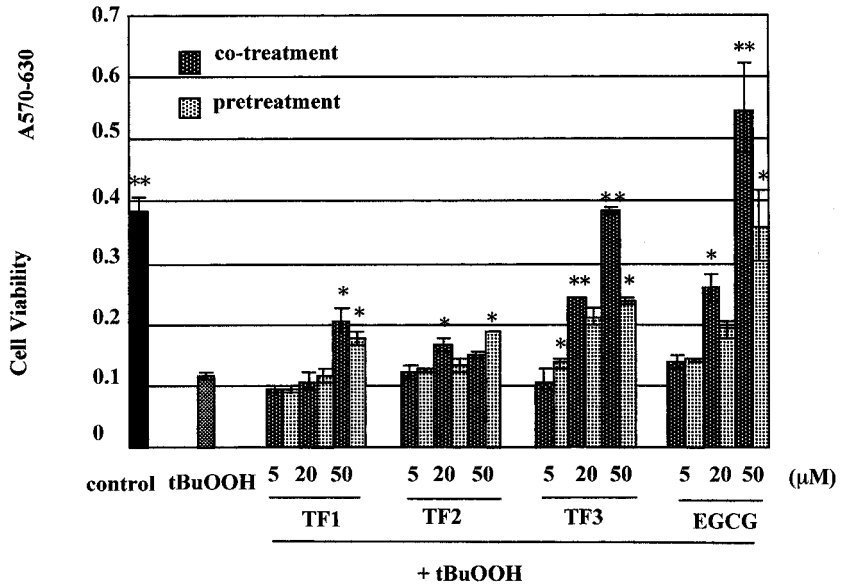

Figure 3. Effect of theaflavins and EGCG on tBuOOH-induced cytotoxicity in RL-34 cells. Cells were treated with 1 mM tBuOOH and 5–50 μM of tea polyphenols for 2 h (co-treatment), or cells were treated with 5–50 μM of tea polyphenols for 1 h, then washed by PBS, and incubated with medium containing 1 mM tBuOOH for another 2 h (pretreatment). Cell viability was measured by MTT assay. Results are mean ± SD, $n = 4$. *$P < 0.05$, **$P < 0.01$, compared with tBuOOH treated cells.

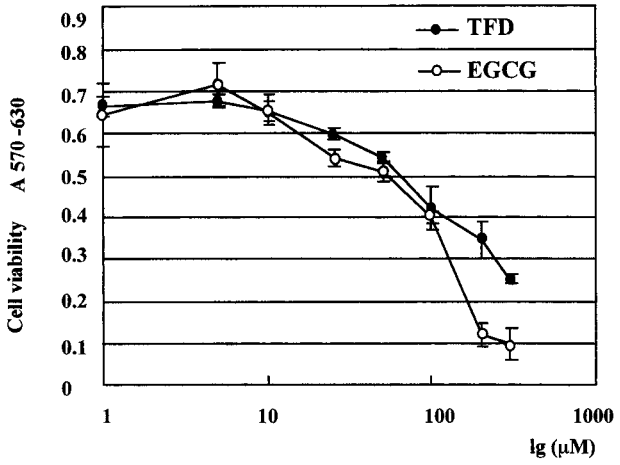

Figure 4. Cytotoxicity of TF3 and EGCG themselves in RL-34 cells. Cells were treated with 1–300 μM TF3 or EGCG for 24 h, and cell viability was measured by MTT assay. Results are mean ± SD, $n = 4$.

with 50 μM TF2 and TF3 had the same effect on cytoprotection. A similar result was obtained when the cells were treated with tBuOOH, another producer of reactive oxygen free radicals (Figure 3). The cytoprotection of TF3 was nearly the same as that of EGCG (Figures 2B and 3).

It is known that all polyphenols themselves will produce cytotoxicity at high concentrations. However, TF3 had less cytotoxicity than EGCG at high concentrations (Figure 4).

TF3 Prevented Oxidative Stress-Induced DNA Damage. Because TF3 showed the most effective cytoprotection and inhibition of intracellular ROS, we then investigated its effect on DNA protection. The amount of 8-OHdG, which is a DNA oxidative marker, was measured in DNA isolated from RL-34 cells treated with G/GO. As shown in Figure 5A, the cellular 8-OHdG level was increased about 30% in the G/GO treated cells, compared with that in control cells. But TF3 inhibited this increase in the dose-dependent way regardless of whether co-treatment or pretreatment was used. In addition, TF3 also inhibited tBuOOH-induced DNA damage detected by the comet assay in RL-34 cells (Figure 5B). When DNA damage occurs, the comet tail can be seen under fluorescence microscopy (*18*). In this study, comet assay was so sensitive that it could detect DNA damage caused by even 100 nM tBuOOH. tBuOOH at 100 nM made the comet tail 2.5 times longer, but co-incubation of TF3 and tBuOOH could significantly shorten the comet tail, as near to the control level. For pretreatment, the comet tail was also significantly shortened by the addition of 50 μM TF3.

Theaflavins Suppressed Omeprazole (OMZ)-Induced CYP1A1. OMZ, a non-AhR ligand, induces CYP1A1 in vivo (*22*, *23*) and in several cell lines (*24*, *25*). In our study, 200 μM OMZ induced EROD activity at about 2.5-fold in HepG2 cells, but TF1, TF2, and TF3 effectively inhibited the increased EROD activity. The inhibitory effect of >20 μM TF2 and TF3 was significant (Figure 6A). Moreover, 50 μM TF2 and TF3 could strongly suppress 200 μM OMZ-induced CYP1A1 protein and the mRNA level measured by immunoblotting and RT-PCR (Figure 6B, 6C).

Genistein is known as an inhibitor of OMZ-induced CYP1A1 (*24*−*26*). The treatment of cells with 50 μM genistein and 200 μM OMZ decreased by 50% the CYP1A1 protein level, and 50 μM TF2 and TF3 decreased the CYP1A1 protein level by 20−35% (Figure 7).

Theaflavins Inhibited DNA Damage Caused by Activated B[a]P. B[a]P is almost completely metabolically activated by CYP1A1 before it exerts carcinogenesis. Extensive DNA damage may be one of the first steps leading to carcinogenesis. Whether B[a]P could cause DNA damage in HepG2 cells pretreated with OMZ to induce CYP1A1 was examined by comet assay. As Figure 8 shows, when cells were treated with 200 μM OMZ for 22 h to induce CYP1A1, then 10 μM B[a]P was added, the comet tail became 2 times longer than those of cells treated only with vehicle, OMZ, and B[a]P. This indicated OMZ-induced CYP1A1 could activate B[a]P, thus causing DNA damage to occur. However, when cells were treated with OMZ in the presence of 50 μM TF2 or TF3, the DNA damage caused by B[a]P was reduced. This was mostly because TF2 and TF3 exhibited a strong suppressive effect on the OMZ-induced CYP1A1.

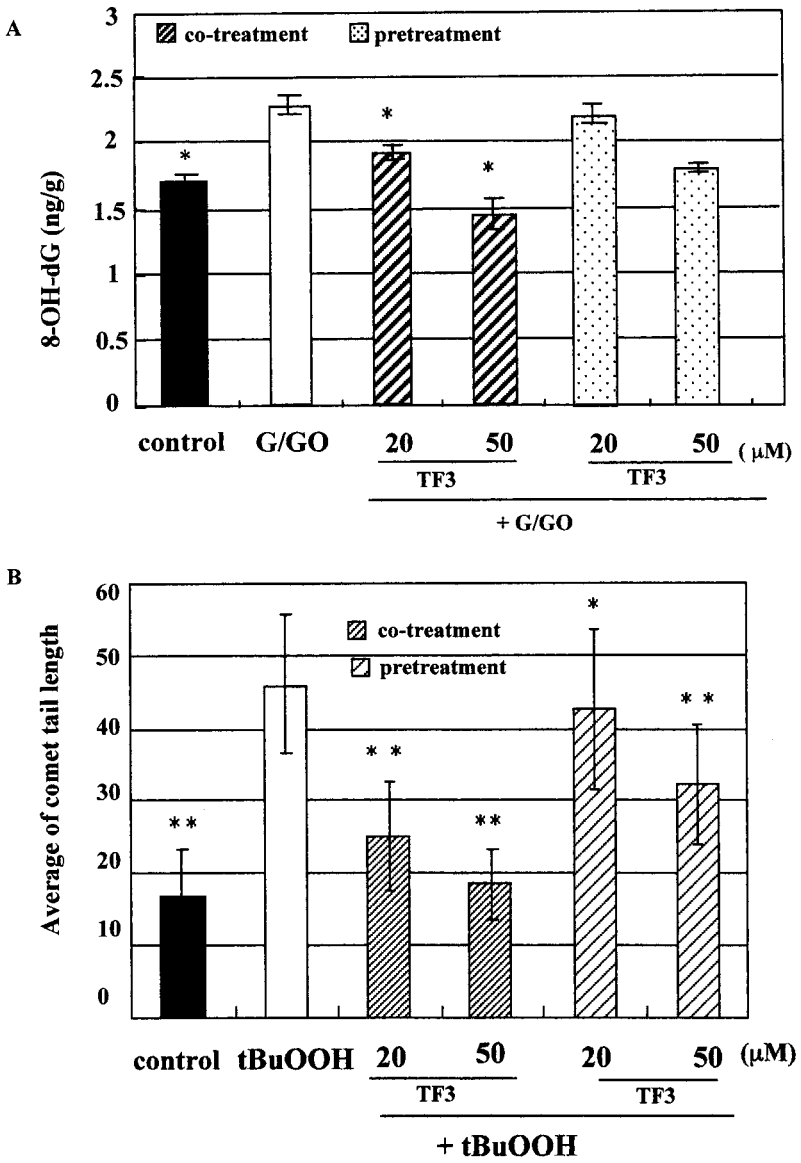

Figure 5. Effect of TF3 on oxidative DNA damage in RL-34 cells. (A) Cells were treated with 1.4 U/mL GO, 5 mM glucose, and 20 or 50 μM TF3 for 2 h (co-treatment), or cells were treated with 20 or 50 μM TF3 for 1 h, then washed by PBS, and incubated with medium containing 1.4 U/mL GO and 5 mM glucose for another 2 h (pretreatment). The amount of 8-OHdG of the cells was measured. Results are mean ± SD, $n = 3$. *$P < 0.05$, compared with G/GO treatment. (B). Cells were co-treated with 100 nM tBuOOH and TF3 for 2 h, or pretreated with TF3 for 1 h, then TF3 was washed out, and the cells were incubated with 100 nM tBuOOH for another 2 h. Comet assay was carried out as described in Materials and Methods. Results represent mean ± SD, $n = 50$ (cells). *$P < 0.05$, **$P < 0.01$, compared with tBuOOH treatment.

DISCUSSION

Many studies have demonstrated that DNA damage is related to cancer risk. DNA can be attacked by endogenous and exogenous reactive oxygen species (ROS) and xenobiotics resulting in DNA damage. The present study demonstrated that the black tea polyphenols, theaflavins, not only have a protective effect on oxidative stress-induced cytotoxicity and cellular DNA damage, but they also inhibit activated carcinogen-related DNA damage by suppressing the elevated CYP1A1 in cells.

The results of the co-treatment of RL-34 cells with theaflavins and oxidants shows that the protective properties of the theaflavins against oxidative-stress are TF3 > TF2 > TF1. This is in accordance with other reports; that is, the number of hydroxy groups and their positioning and arrangement influence the antioxidant activity of the theaflavins (*6*). However, in almost all the previous reports, theaflavins were co-incubated with oxidants. In this study, pretreatment of the cells with theaflavins was also carried out. Oxidants were added to the cells after removing the theaflavins-supplemented medium and washing the cells with PBS. In the case of pretreatment, theaflavins also effectively protected cells and prevented oxidative DNA damage. This suggests that theaflavins can be absorbed by the cells, and that theaflavins remaining on the cell surface or within the cells can also work as well. This agrees with Yoshida et al.'s data in which the pretreatment of cells by the theaflavins inhibited the cell-mediated oxidization of LDL (*8*).

Why theaflaivns provide cytoprotection and DNA protection against oxidative stress maybe involve the following suggested mechanisms. The hydroxyl groups in the aromatic B ring are considered important in scavenging free radicals (*27*). The additional hydroxyl groups in TF3 make it the most effective in ROS scavenging. Moreover, theaflavins can significantly suppress the intracellular ROS (Figure 2A), which favors their cytoprotection and DNA protection. Furthermore, a recent study

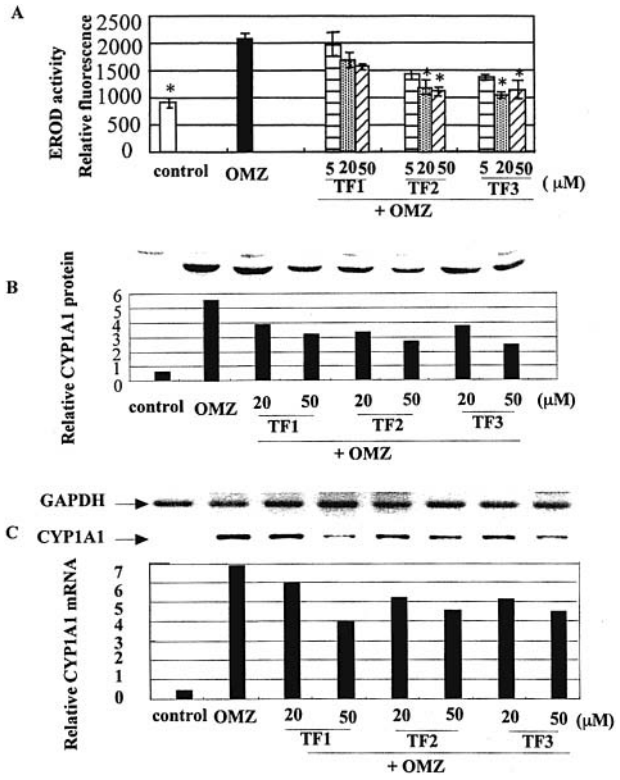

Figure 6. Effect of theaflavins on omeprazole (OMZ) induced CYP1A1 in HepG2 cells. Cells were treated with 200 μM OMZ and various concentration of TF1, TF2, or TF3 for 24 h. Then, EROD activity assay (A), immunoblot (B), and RT-PCR (C) were performed as described in Materials and Methods. In (A), each value represents mean ± SD, for $n = 3$. *P < 0.05, compared with OMZ treatment. In (B), bar graph indicates the corresponded bands of CYP1A1 protein level. In (C), the amount of CYP1A1 mRNA was normalized to the amount of GAPDH mRNA as the bar graph shows.

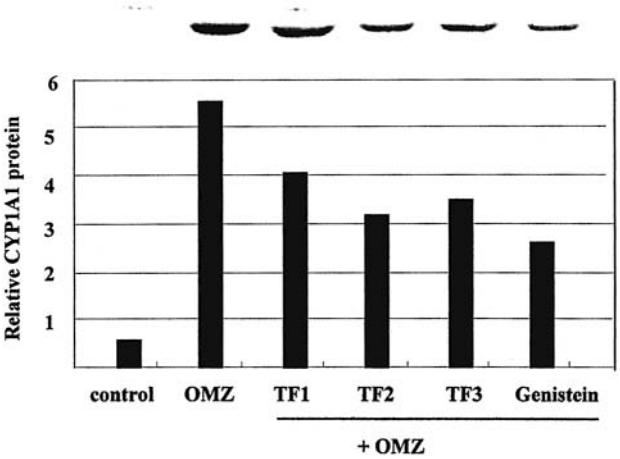

Figure 7. Comparison of inhibitory effect of theaflavins and genestein on omeprazole (OMZ)-induced CYP1A1 protein level in HepG2 cells. Cells were treated with 200 μM OMZ and 50 μM theaflavins or genistein. Then, cells were harvested and used for western blot analysis.

demonstrated that TF3 could strongly block the xanthine oxidase activity in HL-60 cells, which inhibits superoxide formation resulting from xanthine oxidase catalytic reaction (*28*). This implied that theaflavins might reduce ROS formation by blocking the ROS-generating enzymes and related oxidative signal transducers.

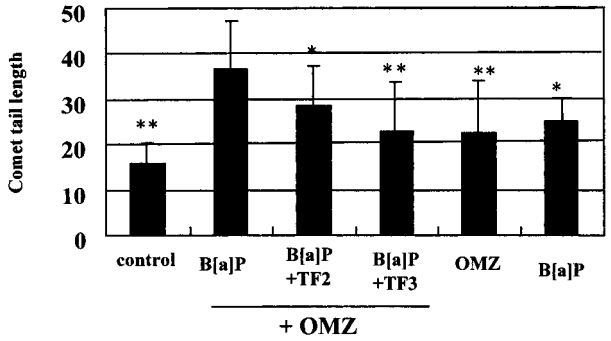

Figure 8. Effect of theaflavins on activated B[a]P-related DNA damages in HepG2 cells. Cells were treated with 200 μM OMZ in the absence or precence of TF2 or TF3 for 22 h, then incubated with 10 μM B[a]P for another 2 h. After that, comet assay was carried out. Results are means ± SD, $n = 50$ (cells). *P < 0.05, **P < 0.01, compared with B[a]P + OMZ treatment.

There are several methods to measure DNA damage (*29*). 8-OHdG is recognized as a useful marker for the estimation of DNA damage produced by oxygen radicals. Oxidation at the C8 position of guanine is one of the more popular types and is also a major mutagenic lesion producing predominately G−T transversion mutation. Comet assay measures the DNA strand breaks in single cells by electrophoresis under alkaline conditions. It can detect DNA lesions not only caused by oxidative damage, but also by carcinogens attack (reviewed in ref *30*). In this study, G/GO (H_2O_2)-induced oxidative DNA damage, but not that induced by tBuOOH, was successfully detected by 8-OHdG measurement utilizing ELISA. Whereas, by the comet assay, we were able to detect the tBuOOH-induced oxidative DNA damage, but not that triggered by G/GO. This may be due to the different oxidative effect of G/GO (produces H_2O_2) and tBuOOH (produces *tert*-butoxyl radicals) on the cellular DNA, thus resulting in the detected difference of the two methods, which may be due to their unique specificity.

On the other hand, theaflavins inhibited the activated B[a]P-induced DNA damage in HepG2 cells in our experiment. B[a]P is a well-known carcinogen and has been shown to induce gene mutation, chromosomal aberrations, and other types of genotoxic effects in various experimental systems, both in vitro and in vivo (*31*, *32*). It is bioactivated almost completely by CYP1A1 and results in different toxic compounds (reviewed in ref *33*). The activated B[a]P-induced DNA adduct and DNA damage were identified in vivo (*34*, *35*) and in vitro (*36*, *37*). Recently, theaflavins has been reported to inhibit B[a]P binding to human epithelial cell DNA (*38*). However, there is no direct evidence that the inhibition of mutation induced by activated carcinogens is due to blocking the P450. In our study, we demonstrated that theaflavins could significantly suppress omeprazole-induced CYP1A1, and prevent DNA damage induced by activated B[a]P. In the present study, CYP1A1 is again shown to take part in activation of procarcinogens, and another kind of CYP1A1 inducer, omeprazle, may also have a potential risk in the activation of procarcinogenesis. Of course, omeprazole's role in carcinogenesis remains to be elucidated.

Omeprazole, an inhibitor of H^+/K^+-ATPase, is a drug for the treatment of gastric ulcers. It is known that the induction of CYP1A1 by omeprazole is initiated from a protein tyrosine kinase-mediated signal transduction pathway, a different pathway from the classical activation cascade of the AhR (*24*−*26*). Our data showed that TF2 and TF3 were active but weaker than genistein, an inhibitor of tyrosine kinase. Liang et al. pointed out that TF3 is a better inhibitor of the tyrosine receptor kinase

(*10*). Therefore, the blocking of omeprazole-induced CYP1A1 by theaflavins is possibly due to its tyrosine receptor kinase property.

The benefit of drinking tea is highly dependent on its metabolism in vivo. The metabolisms of the green tea polyphenols in vivo and their metabolites' function have been recently studied. For example, catechins can be metabolized to glucuronides or sulfate conjugates and methylated conjugates in vivo (*39*), and the metabolites of catechin play an important role in scavenging ROS in the biosystems (*40*, *41*). However, there is little known about the metabolism of theaflavins in vivo. How many theaflavins can be effectively absorbed in vivo still needs to be investigated.

Black tea accounts for 80% of the tea consumption in the world, but its health advantages are less studied than those of green tea. To our knowledge, this is the first paper to report the protective effect of theaflavins on cellular oxidative DNA damage, and also the first to provide direct evidence for theaflavins that inhibition of activated carcinogen-induced DNA damage is due to suppression of elevated cytochrome P450 1A1. This report provides supplementary information on the chemopreventive properties of the black tea polyphenols.

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