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Zinc Ion Enhances GABA Tea-Mediated Oxidative DNA Damage

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ABSTRACT: GABA tea is a tea product that contains a high level of γ-aminobutyric acid (GABA). Previous study has demonstrated a synergistic effect of GABA tea and copper ions on DNA breakage. This study further explored whether zinc (Zn), a nonredox metal, modulated DNA cleavage induced by GABA tea extract. In a cell-free system, Zn^{2+} significantly enhanced GABA tea extract and (−)-epigallocatechin-3-gallate (EGCG)- or H_2O_2-induced DNA damage at 24 h of incubation. Additionally, low dosages of GABA tea extract (1−10 μg/mL) possessed pro-oxidant activity to increase H_2O_2/Zn^{2+}-induced DNA cleavage in a dose-dependent profile. By use of various reactive oxygen scavengers, it was observed that glutathione, catalase, and potassium iodide effectively inhibited DNA degradation caused by the GABA tea extract/H_2O_2/Zn^{2+} system. Moreover, the data showed that the GABA tea extract itself (0.5−5 mg/mL) could induce DNA cleavage in a long-term exposure (48 h). EGCG, but not the GABA tea extract, enhanced H_2O_2-induced DNA cleavage. In contrast, GABA decreased H_2O_2- and EGCG-induced DNA cleavage, suggesting that GABA might contribute the major effect on the antioxidant activity of GABA tea extract. Furthermore, a comet assay revealed that GABA tea extract (0.25 mg/mL) and GABA had antioxidant activity on H_2O_2-induced DNA breakage in human peripheral lymphocytes. Taken together, these findings indicate that GABA tea has the potential of both pro-oxidant and antioxidant. It is proposed that a balance between EGCG-induced pro-oxidation and GABA-mediated antioxidation may occur in a complex mixture of GABA tea extract.

KEYWORDS: *GABA tea, zinc, EGCG, DNA cleavage, pro-oxidant, antioxidant*

INTRODUCTION

Tea made from the leaves of the plant *Camellia sinensis* is one of the most commonly consumed beverages worldwide. GABA tea contains a higher level of γ-aminobutyric acid (GABA) than green tea. The basic steps of manufacturing GABA tea are similar to green tea, except in anaerobic condition.[1,2] The different production methods alter the chemical composition of the dried tea leaves. However, Wang et al.[2] have reported that GABA tea contains many polyphenolic compounds, which are similar to those in green tea.

Tea constituents have been demonstrated to possess cancer preventive activity in many animal models.[3,4] Although little is known about the anticancer potential of GABA tea, a possible mechanism is suggested by its polyphenolic antioxidant constituents.[4] For instance, catechins are known as the characteristic polyphenolic compounds in tea. (−)-Epigallocatechin-3-gallate (EGCG) is the most abundant and active catechin in green tea.[3,5] Additionally, GABA tea extract exhibits antioxidant ability by chelating ferrous ion and scavenging 2-diphenyl-1-picrylhydrazyl radical, hydroxyl radical, and superoxide anion.[1] In contrast to the anticancer activity demonstrated in animal models, the results from epidemiological studies assessing associations between tea consumption and human cancer risk have not yielded a clear conclusion.[4] So far, it has not been clearly elucidated whether EGCG plays a critical role in the process of anticancer. On the other hand, a serious concern exists about possible toxicities in individuals with high EGCG supplement intakes. For example, EGCG-induced liver damage has been reported in studies from France, Spain, and Canada.[6,7] Kanadzu et al.[8] have reported that EGCG at concentrations of 1−100 μM increases DNA strand breakage in purified blood lymphocytes, but suppresses the DNA breakage induced by bleomycin at lower concentrations from 10 to 100 nM, indicating that EGCG is both antioxidant and pro-oxidant.[8]

Zinc (Zn^{2+}) is the second most abundant trace element in the human body. In most mammalian cells, the normal intracellular concentration of Zn^{2+} is in the range of 100−500 μM. Most (∼90%) of the total Zn^{2+} is bound to proteins. Although the concentration of free Zn^{2+} ions is extremely low (∼200 nM) in plasma/interstitial fluid,[9] Zn^{2+} is released into the synaptic cleft of glutamatergic neurons at concentrations of 100−300 μM during synaptic transmission.[10] Zn^{2+} is necessary for human health. For example, Zn^{2+} is required for cell proliferation and growth. However, the role of Zn^{2+} in tumorigenesis is still controversial and mystery. Recent evidence and information have evolved regarding the involvement and importance of Zn^{2+} in the development of several cancers.[11] Cui et al.[12] have revealed a correlation between high Zn^{2+} levels and the onset of carcinogenesis in breast tissue.[12] Indeed, numerous studies have shown an increase in Zn^{2+} in breast cancer.[13−16] Furthermore, Zn^{2+} has been demonstrated to accumulate in *N*-methyl-*N*-nitrosourea (MNU)-induced mammary tumors in rats, and low Zn^{2+} intake can suppress MNU-induced mammary tumorigenesis in rats.[17,18] These results support the idea that increased cell proliferation during tumorigenesis generates a need for a

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continuous and sufficient supply of Zn^{2+} to sustain tumor growth. A highly significant role of Zn^{2+} in tumorigenesis is also observed in the development and progression of pancreatic cancer. Moreover, both in vitro and in vivo evidence pointed to an increase in ZIP4 expression being associated with increased Zn^{2+} intake, increased cell proliferation, and increased tumor growth.[19] These findings may provide a biochemical target for selectively enhancing cytotoxicity in human breast and pancreatic cancer cells. Jayaraman and Jayaraman[20] have indicated that malignant cells are more sensitive to Zn^{2+} exposure than nonmalignant cells. Thus, the extensive role of Zn in cancer cells may provide interesting avenues for novel therapeutic interventions.

Several previous studies have reported that the cytotoxicity of catechins is relatively specific to tumor cells when compared with normal cells.[21−24] Hadi et al.[25] suggest that cancer cells may be more subject to electron transfer between coppers and polyphenols to generate reactive oxygen species (ROS) responsible for DNA damage.[25] It is likely that the preferential cytotoxicity of metal ion−polyphenol interaction accounts for the anticancer effect of polyphenol. Our previous data have demonstrated that GABA tea exhibits pro-oxidant properties, leading to oxidative strand breakage in DNA in the presence of low doses of Cu^{2+} ions.[26] These findings implicate the potential anticancer activity of GABA tea extract. Although Cu^{2+} is one of the most redox-active of metal ions in living cells, the major ions in the nucleus are Cu^{2+} and Zn^{2+} (redox-inert). However, the effect of Zn^{2+} on GABA tea bioactivities has not been thoroughly investigated. To understand the role of Zn^{2+} in DNA cleavage upon GABA tea extract exposure, we tried to determine whether GABA tea extract was capable of damaging DNA in the presence of Zn^{2+}. Furthermore, we also elucidated the mechanism of GABA tea extract/Zn^{2+} system-induced DNA damage.

MATERIALS AND METHODS

Chemicals and Reagents. Ammonium ferrous sulfate, EGCG, glutathione, *N*-sodium lauroyl sarcosinate, sodium azide, Triton X-100, xylenol orange, and zinc chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Lymphocyte separation medium (Ficoll-Paque PLUS) was acquired from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Supercoiled ΦX174 phage DNA was purchased from Promega Corp. (Madison, WI). Hydrogen peroxide (H_2O_2) was purchased from Wako Co. (Osaka, Japan). Low-melting-point agarose (LMPA) and normal-melting-point agarose were obtained from Cambrex Bio Science Rockland, Inc. (Rockland, ME). RPMI 1640 medium, fetal bovine serum, trypsin, penicillin, streptomycin, sodium pyruvate, and nonessential amino acid were from HyClone Laboratories Inc. (Logan, UT). All other chemicals used in this study were of analytical grade.

Preparation of GABA Tea Extract. The experimental procedures were described previously.[26] GABA tea leaves were purchased from a local tea market (I-Min Corp., Ltd.) in Nantou, Taiwan. Six grams of GABA tea leaves was steeped twice in 120 mL of boiling water for 5 min. After quickly cooling to room temperature, the infusion was filtered twice through a filter paper (Whatman no. 1) and freeze-dried under vacuum condition (Kingmech Corp., Ltd., Taiwan). GABA tea extract was dissolved in deionized water before using as a stock of 100 mg/mL solution. The level of zinc was below the detection limit. Wang et al.[2] have reported the content of bioactive components in GABA tea.

DNA Cleavage Assay. The experimental procedures were based on a previously described method.[26] Briefly, supercoiled ΦX174 phage DNA was incubated with various concentrations of GABA tea extract, GABA, or EGCG in the absence or presence of H_2O_2 or $ZnCl_2$ at 37 °C for the indicated periods, respectively. The total volume was 10 μL in 1× phosphate-buffered saline (PBS) (Ca^{2+}- and Mg^{2+}-free). After incubation, each sample was mixed with 5 μL of the staining dye solution (bromophenol blue and xylene cyanole in 50% glycerol), and then the mixture was loaded onto a preprepared 1% agarose gel. The gel was run at 100 V for 30 min. Due to single-strand DNA cleavage, the supercoiled Form-I DNA was converted into a relaxed open circular Form-II DNA. The two DNA forms were separated by agarose gel electrophoresis and quantified to obtain the percent of DNA cleavage.

Lymphocyte Isolation. Human peripheral blood was obtained from healthy subjects in a 10 mL tube containing heparin. Lymphocytes were isolated by a previously described method.[26] Briefly, 3 mL of whole blood was diluted 1:1 with RPMI 1640 and carefully layered on the top of lymphocyte separation medium in a centrifugation tube in a ratio of 1:1. After centrifugation for 15 min at 400*g*, the white layer of lymphocytes at the interface between blood plasma and the medium was carefully transferred into a tube containing 5 mL of culture medium, RPMI 1640. The lymphocytes were then washed twice with RPMI 1640 and centrifuged at 250*g* for 10 min. The cell pellet was resuspended in 6 mL of RPMI 1640 medium containing 10% fetal bovine serum and 2 mM glutamine. The cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell Culture and Chemical Treatment. One milliliter of lymphocytes in RPMI 1640 medium was incubated with various concentrations of GABA tea extract or GABA in the absence or presence of H_2O_2 for 2 h. The reaction mixture was centrifuged at 2000*g*. The cell pellet was washed in 1× PBS (Ca^{2+}- and Mg^{2+}-free) and used for the comet assay. Cell viability was determined before the start and end of the reaction.

Comet Assay (Single-Cell Gel Electrophoresis). The comet assay was adapted from the method of Wang et al.[26] One milliliter of lymphocyte pellet was mixed with 500 μL of 1% LMPA in PBS at 37 °C, and 140 μL of this mixture was applied onto a frosted glass microscope slide precoated with a layer of 1% normal-melting-point agarose (150 μL). After application of a third layer of 1% normal-melting-point agarose (150 μL), the slides were immersed in cold-lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% *N*-sodium lauroyl sarcosinate, 1% Triton X-100, 10% DMSO) for 1 h at 4 °C. The slides were then placed in an electrophoresis tank, allowing the DNA to unwind for 15 min in the alkaline solution (300 mM NaOH and 1 mM Na_2EDTA). Electrophoresis was then performed at 300 mA for 20 min in the same alkaline solution at room temperature. The slides were then neutralized with 0.4 M Tris-HCl buffer (pH 7.4) and stained with ethidium bromide. The image was then read with an Olympus (BX51) fluorescence microscope equipped with integrated CC camera (Moticam 2000). To assess lymphocyte DNA breakage, comets were scored at 400× magnification. Images from 60 cells (20 from each triplet slide) were analyzed by computer using Image Pro Plus 6.0 software (Media Cybernetics). The percent of DNA fragmentation is used as DNA damage parameter for this study and defined as the percent of DNA in the comet tail versus total DNA from both comet tail (DNA fragments) and comet head (cell nucleus).

Statistical Analysis. The statistical analysis was performed as described previously[26] and is expressed as the median ± SD of three independent experiments. A Student's *t* test was used to examine statistically significant differences. Analysis of variance was performed by using ANOVA. p values of <0.05 were considered to be statistically significant.

RESULTS

DNA Cleavage Ability of GABA Tea Extract in the Presence of Zn^{2+}. In previous study, we have demonstrated that low dosages of GABA tea extract (1−10 μg/mL) are capable of degrading cellular DNA in the presence of redox metals (Cu^{2+}).[26] To explore whether nonredox metal (Zn^{2+}) modulated DNA cleavage in the presence of GABA tea extract, ΦX174 phage DNA was exposed to different concentrations of GABA tea extract in the presence of Zn^{2+} for 24 h. The results

given in Figure 1A show that the levels of DNA cleavage were slightly induced by GABA tea extract from 100 to 200 μg/mL

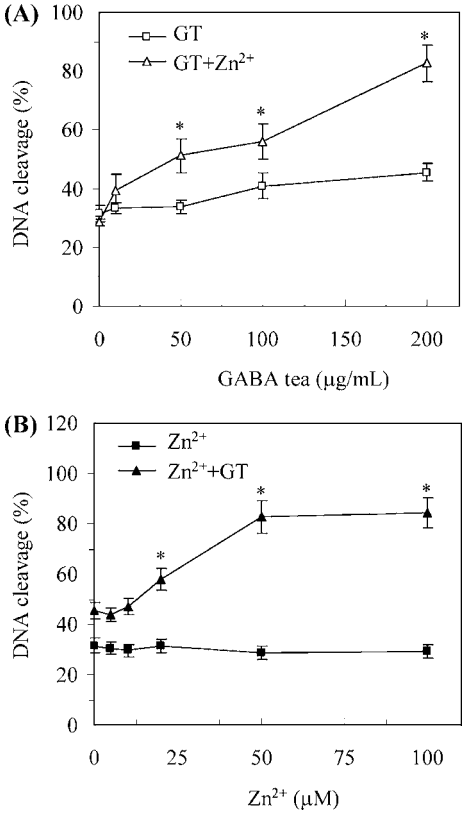

Figure 1. GABA tea extract (GT)/Zn^{2+} coexposure was capable of inducing DNA strand breakage: (A) effect of DNA cleavage induced by GT in the absence or presence of Zn^{2+} (ΦX174 phage DNA was cotreated with various concentrations of GT in the presence or absence of Zn^{2+} (50 μM) for 24 h; ∗ indicates $p < 0.05$ as compared with GT alone); (B) effect of increasing concentrations of Zn^{2+} (5−100 μM) on GT (0.2 mg/mL)-induced DNA cleavage (∗ indicates $p < 0.05$ as compared with Zn^{2+} alone).

(Figure 1A). Zn^{2+} (50 μM) further enhanced the levels of DNA cleavage in the presence of increasing concentrations of GABA tea extract (50−200 μg/mL) (Figure 1A), implying that GABA tea extract and Zn^{2+} coexposure could lead to DNA damage. To further investigate the effect of GABA tea extract and Zn^{2+} in DNA cleavage, ΦX174 phage DNA was treated with increasing concentrations of Zn^{2+} at 200 μg/mL GABA tea extract. As shown in Figure 1B, although Zn^{2+} alone did not render any effect on DNA cleavage, Zn^{2+} significantly enhanced GABA tea extract-induced DNA cleavage in a dose-dependent manner (20−100 μM). These results indicated that GABA tea extract/Zn^{2+} coexposure is capable of inducing DNA strand breakage.

Role of EGCG on GABA Tea Extract-Induced DNA Cleavage in the Presence of Zn^{2+}. EGCG is one of the major tea catechins in GABA tea extract.[2] It is conceivable that EGCG might contribute the major effect on GABA tea extract-induced DNA cleavage. To address this idea, ΦX174 phage DNA was exposed to different concentrations of EGCG in the absence or presence of 50 μM Zn^{2+} for 24 h, and the DNA cleavage was assessed as mentioned in Figure 1. As shown in Figure 2A, EGCG alone significantly caused DNA cleavage in a dose-dependent manner (50−200 μM), implying that EGCG did not exhibit the antioxidant ability by scavenging ROS in our

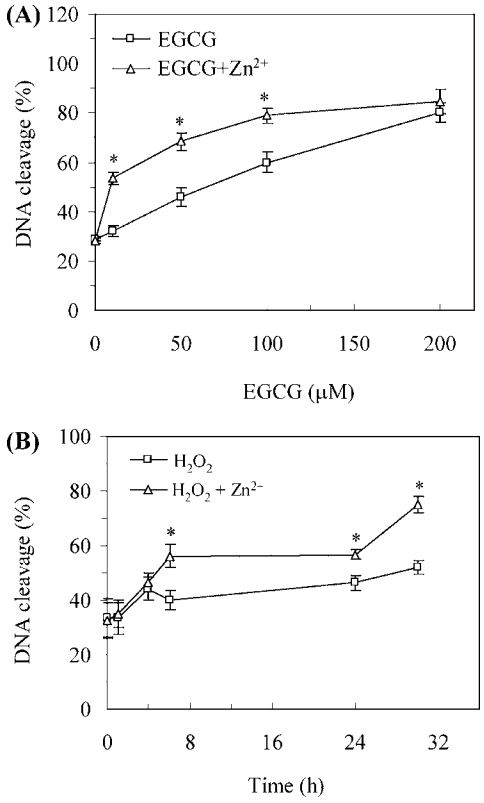

Figure 2. DNA cleavage ability of EGCG in the absence or presence of Zn^{2+}: (A) effect of DNA cleavage induced by EGCG in the absence or presence of Zn^{2+} (ΦX174 phage DNA was cotreated with various concentrations of EGCG in the presence or absence of Zn^{2+} (50 μM) for 24 h; ∗ indicates $p < 0.05$ as compared with GT alone); (B) effect of Zn^{2+} on H_2O_2-induced DNA cleavage (ΦX174 phage DNA was cotreated with Zn^{2+} (50 μM) and H_2O_2 (100 μM) for the indicated time; ∗ indicates $p < 0.05$ as compared with H_2O_2 alone).

experiment condition. Zn^{2+} (50 μM) remarkably enhanced EGCG-mediated DNA cleavage. In contrast, GABA, the other major constituent in GABA tea extract, did not induce DNA cleavage in the presence of 50 μM Zn^{2+}, even though the GABA concentration was as high as 50 mM (data not shown). It is likely that EGCG might play a crucial role in GABA tea extract/Zn^{2+}-mediated DNA cleavage.

Previous studies have demonstrated that EGCG can cause oxidative DNA breakage through H_2O_2 generation.[27−29] Thus, we further investigated whether Zn^{2+} increased EGCG-induced DNA cleavage mainly through the interaction of H_2O_2 and Zn^{2+}. ΦX174 phage DNA was exposed to H_2O_2 in the presence or absence of Zn^{2+}, and the levels of DNA cleavage were assayed. The results given in Figure 2B show that 100 μM H_2O_2 alone slightly induced DNA cleavage. Zn^{2+} (50 μM) cotreatment significantly enhanced the level of DNA cleavage in a time-dependent profile. As compared with H_2O_2 alone, the level of cleaved DNA was slightly elevated by 10.3 and 23.1% in H_2O_2/Zn^{2+}cotreatment for 24 and 30 h, respectively (Figure 2B). From these results, we suggest that the increase of EGCG-mediated DNA cleavage by Zn^{2+} might be partially ascribed to the interaction of H_2O_2 and Zn^{2+}.

Pro-oxidant Activity of GABA Tea Extract on H_2O_2-Induced DNA Cleavage in the Presence of Zn^{2+}. Previous studies have found that H_2O_2 production and Zn level are significantly higher in breast cancer patients, as compared to the respective controls,[30−33] suggesting persistent oxidative stress

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within carcinoma cells. Thus, we investigated the effect of GABA tea extract on H_2O_2-induced DNA cleavage in the presence of Zn^{2+}. Figure 3A shows that 50 μM Zn^{2+} slightly but

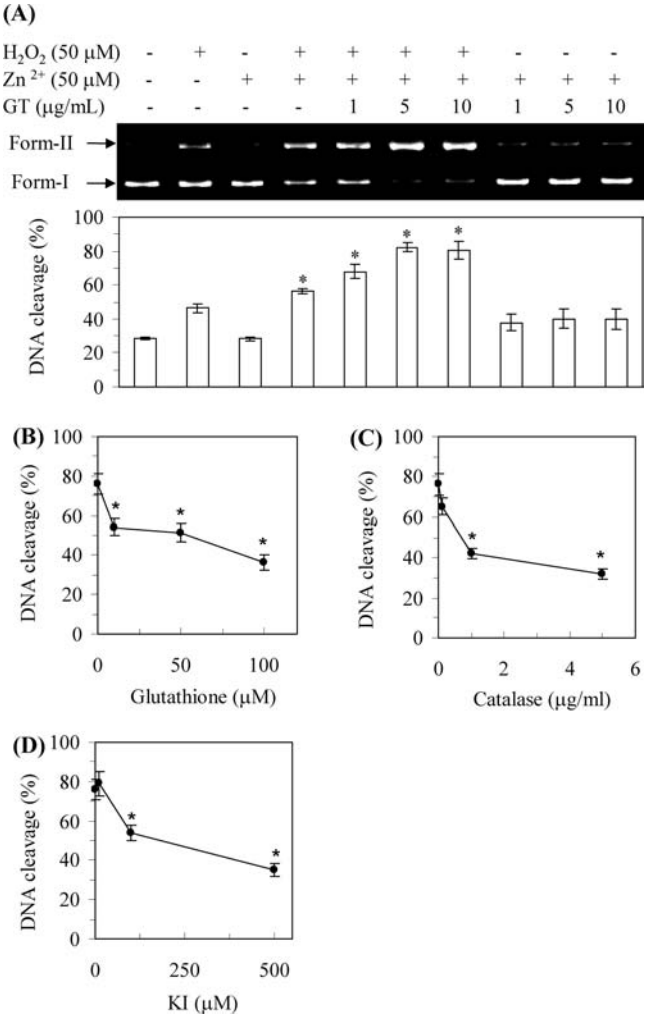

Figure 3. Low dosages of GABA tea extract (GT) promoted H_2O_2-induced DNA cleavage in the presence of Zn^{2+}: (A) ΦX174 phage DNA was cotreated with GT and Zn^{2+} in the absence or presence of H_2O_2 for 24 h (∗ indicates $p < 0.05$ as compared with H_2O_2 alone); (B) effect of glutathione on GT/H_2O_2/Zn^{2+} system-induced DNA cleavage; (C) effect of catalase on GT/H_2O_2/Zn^{2+} system-induced DNA cleavage; (D) effect of potassium iodide (KI) on GT/H_2O_2/Zn^{2+} system-induced DNA cleavage (ΦX174 phage DNA was cotreated with 10 μg/mL GT, 50 μM H_2O_2, 50 μM Zn^{2+}, and various concentrations of free radical scavengers for 24 h; ∗ indicates $p < 0.05$ as compared with DNA cleavage induced by GT/H_2O_2/Zn^{2+} system).

significantly enhanced 50 μM H_2O_2-induced DNA cleavage (lane 4), consistent with the results of Figure 2B. GABA tea extract (1−10 μg/mL) and Zn^{2+} cotreatment did not cause DNA breakage (lanes 8−10). Interestingly, low dosages of GABA tea extract remarkably augmented H_2O_2/Zn^{2+}-induced DNA cleavage in a concentration-dependent profile (Figure 3A, lanes 5−7), implying that GABA tea extract might possess a pro-oxidant activity to mediate the DNA damage effect of H_2O_2/Zn^{2+} coexposure. To further explore whether ROS participated in the molecular event of DNA cleavage induced by the GABA tea extract/H_2O_2/Zn^{2+} system, various active oxygen scavengers were used in reaction mixture. As shown in Figure 3B−D, DNA cleavage was substantially inhibited by the addition of free radical scavengers. GABA tea extract/H_2O_2/Zn^{2+}-induced DNA cleavage was significantly inhibited in the presence of glutathione in a dose-dependent manner (Figure 3B). Furthermore, catalase (1 μg/mL) completely abolished DNA cleavage induced by the GABA tea extract/H_2O_2/Zn^{2+} system, suggesting the essential role of H_2O_2 in the reaction (Figure 3C). The similar effectiveness of potassium iodide (a singlet excited-state quencher) was also observed (Figure 3D). However, superoxide dismutase (superoxide anion scavenger), sodium azide (singlet oxygen scavenger), and mannitol and sodium benzoate (hydroxyl radical scavengers) were ineffective in the prevention of DNA cleavage in our experimental conditions (data not shown), indicating that these ROS may be not the main factors to cause DNA cleavage in the reaction. These data suggest that DNA cleavage caused by the GABA tea extract/H_2O_2/Zn^{2+} system might be not mainly through the generation of ROS, but additional pathways such as polyphenolic radicals might also be involved.

Pro-oxidant Activity of GABA Tea Extract in a Cell-free System. We further determined whether GABA tea extract alone could cause cleavage of supercoiled ΦX174 phage DNA. As shown in Figure 4A, during 4−24 h of incubation, 0.1 mg/mL GABA tea extract did not significantly induce DNA cleavage. However, 24.6% increase of DNA cleavage was observed when ΦX174 phage DNA was exposed to GABA tea extract for 48 h. In addition, the level of DNA cleavage (62.2 ± 3.3%) was reached maximally at 0.5 mg/mL GABA tea extract for 48 h of incubation and not further increased at 1−5 mg/mL GABA tea extract (Figure 4B). Moreover, 100 μg/mL GABA tea extract did not alter 200 μM H_2O_2-induced DNA cleavage in vitro (Figure 4C). Most importantly, 50 μM H_2O_2 enhanced EGCG-induced DNA cleavage (Figure 4D), further suggesting that EGCG could act as a pro-oxidant in GABA tea extract. On the basis of our data, we suggest that the effect of GABA tea extract on DNA cleavage may be mediated through intricate pathways, depending on individual constituents. For instance, each constituent could interact with each other to display either antioxidant or pro-oxidant potentials involved in the complex mixtures.

Role of GABA in Antioxidant Activity of GABA Tea Extract. GABA, the other major constituent in GABA tea extract, has antioxidant activity.[34−36] Thus, we speculated that GABA might contribute the major effect on the antioxidant activity of GABA tea extract. To address this idea, ΦX174 phage DNA was treated with various concentrations of GABA in the presence of H_2O_2 for 48 h. As shown in Figure 5A, GABA significantly inhibited H_2O_2-induced DNA cleavage in a dose-dependent manner. The percentages of inhibition were 10.9, 57.5, and 72.0% at 1, 5, and 10 mM GABA, respectively, confirming that GABA exhibited antioxidant activity. Interestingly, the aforementioned evidence indicated that GABA tea extract alone did not prevent DNA cleavage from H_2O_2 in a cell-free system (Figure 4C). It is anticipated that the interaction of antioxidant (GABA) and pro-oxidant (EGCG) may occur in GABA tea extract. Thus, the effect of GABA on EGCG-induced DNA cleavage was further tested. As expected, GABA repressed EGCG-induced DNA cleavage. The percentages of inhibition were 2.1, 21.9, 38.3, and 72.9% at 1, 5, 10, and 50 mM GABA, respectively (Figure 5B). These results demonstrated that GABA possessed the antioxidant ability to inhibit H_2O_2- and EGCG-induced DNA cleavage.

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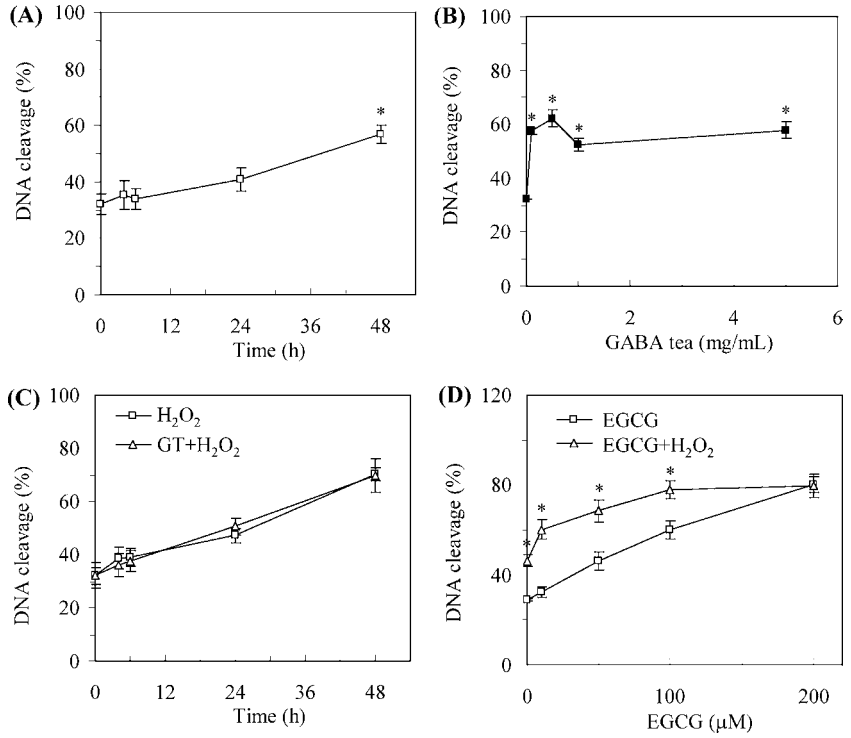

Figure 4. GABA tea extract (GT)-induced DNA damage in a cell-free system: (A) ΦX174 phage DNA was treated with 100 μg/mL GT for the indicated times; (B) ΦX174 phage DNA was treated with various concentrations of GT for 48 h (∗ indicates $p < 0.05$ as compared with control untreated); (C) ΦX174 phage DNA was cotreated with 100 μg/mL GT in the presence of H_2O_2 (200 μM) for the indicated periods; (D) ΦX174 phage DNA was treated with various concentrations of EGCG in the absence or presence of H_2O_2 (50 μM) for 24 h (∗ indicates $p < 0.05$ as compared with EGCG alone).

Antioxidant Activity of GABA Tea Extract in Lymphocyte. The aforementioned evidence shows that GABA significantly inhibited H_2O_2-induced DNA cleavage. To investigate whether GABA tea extract had antioxidant activity on H_2O_2-induced DNA breakage in lymphocytes, the comet assay was performed under alkaline conditions. Photographs of comets seen on treatment of lymphocytes with GABA or GABA tea extract either alone or in the presence of H_2O_2 are shown in Figure 6A. Cells were intact without a tail after exposure to 10 mM GABA alone (Figure 6Ab), indicating GABA alone does not render any effect on DNA breakage in this condition. When cells were treated with 0.25 mg/mL GABA tea extract, a comet with a slight tail was induced (Figure 6Ac). A comet with a significant tail indicative of DNA breakage could be seen when lymphocytes were treated with 100 μM H_2O_2 (Figure 6Ad). Interestingly, 46.0 and 70.3% decreases of H_2O_2-induced DNA breakage were obtained at 1 and 10 mM GABA cotreatment, respectively (Figure 6Aef,B). DNA breakage was also inhibited by the addition of 0.25 mg/mL GABA tea extract (36.3% decrease) (Figure 6Ah,B). These results demonstrated that GABA tea extract exhibited antioxidative activity at lower dosages (0.25 mg/mL) to suppress the production of DNA breakage induced by H_2O_2. In conjunction with a previous finding that GABA tea extract alone induced DNA breakage in a concentration-dependent manner (0.5−2 mg/mL) in lymphocytes,[26] we suggest that GABA tea extract indeed has a dual function of pro-oxidant and antioxidant potentials in lymphocytes. Collectively, our results have a great impact on GABA tea bioactivity, depending on the constituents and trace metal ions.

DISCUSSION

In this study, we have elucidated that GABA tea extract exhibited pro-oxidant properties, leading to oxidative strand breakage in DNA in the presence of Zn^{2+}. Our previous study has shown that a low dosage of GABA tea is capable of degrading cellular DNA in the presence of Cu^{2+} ion.[26] These results imply that GABA tea consumption could be associated with genotoxicity, when metal ions (Zn^{2+} or Cu^{2+}) coexist. In addition, this study indicate that GABA tea exhibited a dual function of pro-oxidant and antioxidant. EGCG, one of the major tea catechins in GABA tea extract, might behave as a pro-oxidant that promotes free radicals to damage DNA during a quinone redox cycle. However, GABA, the other main constituent, might act as a radical scavenger to prevent DNA cleavage. In conjunction with previous findings and ours, we propose that a balance between EGCG-induced pro-oxidation and GABA-mediated antioxidation may simultaneously occur in a complex mixture of GABA tea extract.

Our data show that GABA tea extract/Zn^{2+} induced DNA cleavage after 24 h of coexposure (Figure 1). In addition, we observed that Zn^{2+} could significantly enhance DNA cleavage caused by EGCG (Figure 2A), suggesting that Zn^{2+} has a pro-oxidant activity. Our previous study has demonstrated that during 1 h of incubation, GABA tea extract/Cu^{2+} cotreatment caused DNA degradation, mediated through redox cycle (between Cu^{+} and Cu^{2+}) and singlet oxygen generation.[26] Cu^{2+} is a redox-active biometal that can participate in electron transfer reactions with the consequent production of oxidant species. Although Zn^{2+} is redox-inert, our data further show that Zn^{2+} slightly increased H_2O_2-induced DNA cleavage for 24 h of incubation (Figure 2B). These results are consistent with a

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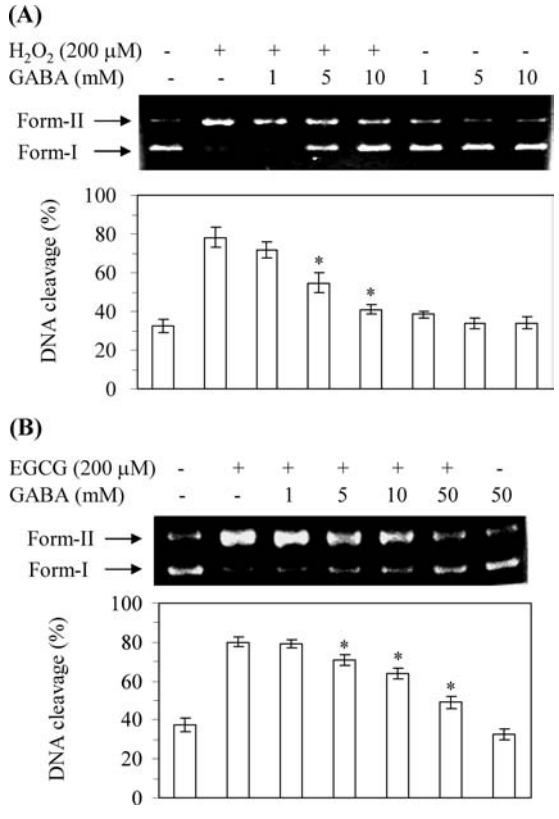

Figure 5. Inhibitory effects of GABA on DNA cleavage induced by H_2O_2 (A) or EGCG (B): (A) ΦX174 phage DNA was cotreated with GABA and H_2O_2 for 48 h; (B) ΦX174 phage DNA was cotreated with GABA and EGCG for 24 h (∗ indicates $p < 0.05$ as compared with H_2O_2 alone).

previous report that the yield of acyclic pBluescript K+ plasmid DNA increased with increasing concentrations of Zn^{2+} up to 50 μM, reaching maxima at 69% in the presence of H_2O_2 (1 mM) for 15 min of incubation.[37] Several studies have shown that EGCG caused oxidative DNA breakage through H_2O_2 generation.[27−29] We suggest that H_2O_2 may be generated in the reaction of EGCG and Zn^{2+} interaction. H_2O_2 then may react instantly with Zn^{2+} to further promote ROS generation by Fenton reaction (data not shown). As shown in Figure 7, we propose that the interaction of H_2O_2 and Zn^{2+} may involve the enhancement of GABA tea-induced DNA cleavage.

Cancer cells have been demonstrated to increase steady-state level of ROS, when compared to the normal cells, suggesting that carcinoma cells in vitro and in vivo are frequently under persistent oxidative stress.[30,38] Szatrowski and Nathan[39] have reported that a substantial amount of H_2O_2 is produced by human tumor cells. In breast cancer patients, H_2O_2 production was significantly higher, as compared to the respective controls.[32,33] On the other hand, numerous studies have implicated Zn^{2+} hyperaccumulation in the etiology of breast cancer.[13−16] These biochemical changes may provide a new paradigm useful to the design of cancer therapies that selectively enhance cell killing of human cancer cells.[11,20,30,31,38] Our data show that low dosages of GABA tea extract promoted H_2O_2/Zn^{2+}-induced DNA damage (Figure 3A). We further found that that the generation of ROS is not the main pathway to cause DNA cleavage in the GABA tea extract/H_2O_2/Zn^{2+} system (Figure 3B−D). An alternative possibility is that EGCG−Zn^{2+} interaction might play a critical role in DNA damage mediated by the GABA tea extract/H_2O_2/Zn^{2+} system. EGCG has been observed to possess two strong bidentate metal binding sites (two adjacent hydroxyl groups) in the B and D rings, respectively.[40,41] Hagerman et al.[41] have demonstrated that EGCG can react via comproportionation to yield catechol semiquinone radicals, which spin stabilized with Zn^{2+}.[41] Additionally, Zn^{2+}-stabilized EGCG radicals can cause irreversible damage to biological molecules such as proteins.[41] Quesada et al.[42] have also reported that EGCG displays a high affinity for Zn^{2+} in solution at very low concentrations (0.1 μM), suggesting that EGCG may be able to displace zinc loosely bound to protein within the cell cytoplasm. Furthermore, Tan et al.[43] have reported that quercetin−Zn^{2+} complexes significantly promote DNA strand breaks via a hydrolytic pathway, not an oxidative pathway. In this regard, we suggest that the generation of EGCG semiquinone radicals might be involved in DNA degradation. Through the formation of EGCG−Zn^{2+} complexes, Zn^{2+} may enhance EGCG-induced DNA cleavage (Figure 2A). Thus, we propose that the DNA cleavage caused by the GABA tea extract/H_2O_2/Zn^{2+} system might also be mediated through Zn^{2+}-stabilized polyphenolic radicals. Our working model is presented in Figure 7.

Several lines of evidence have demonstrated that EGCG can chelate Zn^{2+} to form a metal complex that has a higher antitumor activity than EGCG alone.[44] Frezza et al.[31] have reported that metal complexs containing Zn^{2+} have received considerable attention as potential anticancer agents.[31] Quercetin−Zn^{2+} complexes significantly induce apoptosis of tumor cells via their intercalation into the stacked DNA base pairs to cause DNA strand breaks,[45] suggesting that quercetin−metal complexes, as antitumor drugs, might be used in target-based cancer therapy.[46] Collectively, these findings provide evidence that the use of Zn-binding ligands to target tumor zinc could provide a novel strategy for cancer selective treatment. On the basis of our data, we propose that GABA tea may exhibit anticancer and apoptosis properties through promoting DNA cleavage via its interactions with metal ions. Further evidence will be needed to perform to support our hypothesis.

In this study, we found the inhibitory effect of GABA tea extract on H_2O_2-induced DNA breakage in lymphocyte (Figure 6), although this effect was not obtained in a cell-free system (Figure 4C). Thus, we suggest that the effect of GABA tea extract on DNA cleavage may be mediated through intricate pathways, depending on individual constituents. GABA tea (0.25 mg/mL) contains less EGCG (17.75 μM) and more GABA (4.39 mM) than green tea.[2] Kanadzu et al.[8] have reported that EGCG at concentrations of 10−100 nM suppressed H_2O_2-induced DNA strand breakage, whereas it induced DNA strand breakage at concentrations of 1−100 μM.[8] Our data demonstrated that long-term exposure (24 h) of DNA to EGCG could induce DNA cleavage (Figures 2A and 4D), although our previous study did not obtain the DNA cleavage ability caused by EGCG alone after 1 h of incubation.[26] Additionally, we observed that EGCG did not suppress, but increased, H_2O_2-induced DNA cleavage (Figure 4D), indicating that EGCG may be mainly involved in the pro-oxidant activity of GABA tea. On the other hand, Wang et al.[2] have found that GABA tea has an antioxidant activity similar to that of green tea. The level of EGCG is lower in GABA tea (3.26 mg/100 mg) than in green tea (4.69 mg/100 mg), whereas the content of GABA is significantly higher in GABA tea (180.97 mg/100 g) than in green tea (16.94 mg/100 g).[2] As

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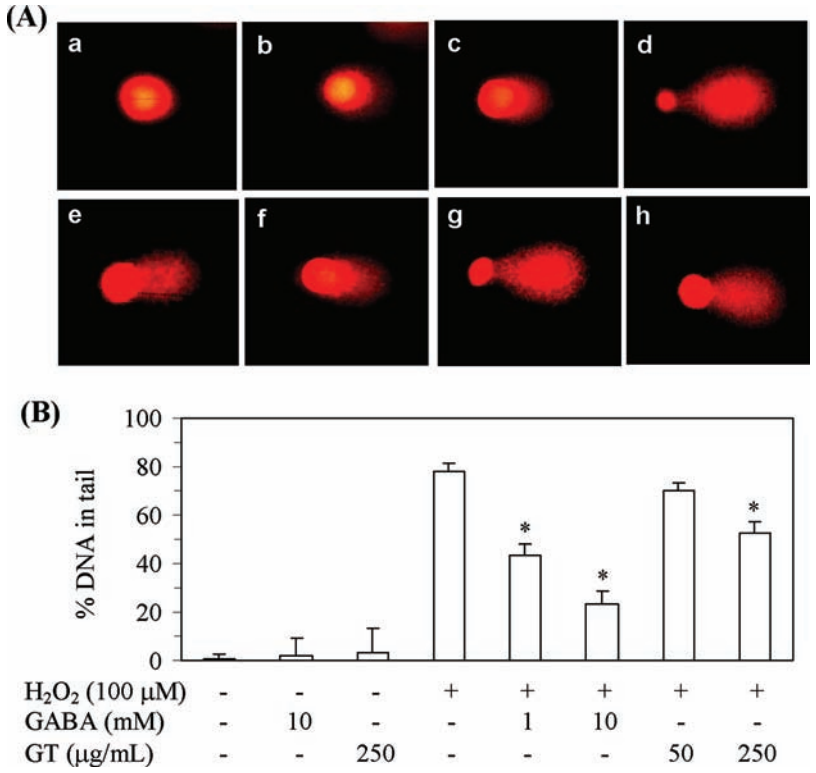

Figure 6. Inhibitory effects of GABA or GABA tea extract (GT) on H_2O_2-induced DNA breakage in human peripheral lymphocytes: (A) photographs of comets (400×) after treatment with solvent (a), 10 mM GABA (b), 250 μg/mL GT (c), 100 μM H_2O_2 alone (d), 1 mM GABA and 100 μM H_2O_2 (e), 10 mM GABA and 100 μM H_2O_2 (f), 50 μg/mL GT and 100 μM H_2O_2 (g), or 250 μg/mL GT and 100 μM H_2O_2 (h) for 2 h; (B) DNA breakage induced by 100 μM H_2O_2 in the absence or presence of GABA or GT (∗ indicates $p < 0.05$ as compared with cells treated H_2O_2 alone).

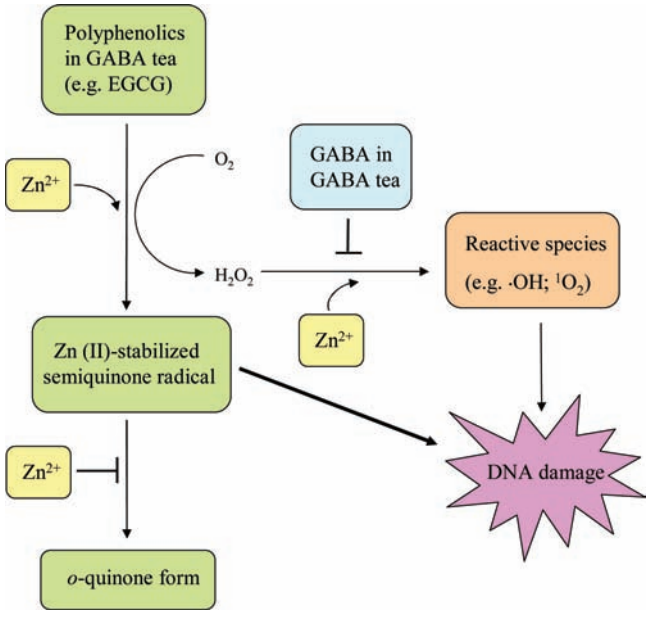

Figure 7. Proposed mechanism of DNA damage induced by GABA tea extract in the presence of Zn^{2+}.

anticipated, several studies have demonstrated that GABA has antioxidant activity.[34−36] Our present data show that GABA possessed antioxidant ability to inhibit H_2O_2-induced DNA damage in a cell-free system and in lymphocyte (Figures 5A and 6). We further demonstrated that GABA decreased EGCG-induced DNA cleavage (Figure 5B), supporting the idea that each constituent can interact with each other in the complex mixture of GABA tea. Although the inhibitory effect was less than that on H_2O_2-induced DNA damage (Figure 5A), this difference further supports our assumption that the generation of EGCG semiquinone radicals may be involved in EGCG-induced DNA degradation. Taken together, these results suggest that GABA-mediated antioxidation might be involved in decreasing the ability of DNA degradation induced by GABA tea, as shown in Figure 7.

In summary, our data demonstrate that Zn^{2+} could act as a pro-oxidant to enhance GABA tea extract-mediated DNA cleavage. Additionally, we observed that GABA tea has a dual function of pro-oxidant and antioxidant activities. These findings will provide implications for the potential of GABA tea in anticancer therapy.

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Notes

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ABBREVIATIONS USED

EGCG, (−)-epigallocatechin-3-gallate; GABA, γ-aminobutyric acid; GT, GABA tea extract; KI, potassium iodide; LMPA, low-melting-point agarose; MNU, *N*-methyl-*N*-nitrosourea; PBS, phosphate-buffered saline; ROS, reactive oxygen species.

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