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(−)-Epigallocatechin gallate causes oxidative damage to isolated and cellular DNA

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Abstract

Green tea catechins, especially (−)-epigallocatechin gallate (EGCG), are believed to mediate much of the cancer chemopreventive effects of tea. However, it was reported that green tea catechins enhanced colon carcinogenesis in rats. Experiments using ^{32}P-labeled DNA fragments obtained from human cancer-related genes showed that catechins induced DNA damage in the presence of metals such as Cu(II) and Fe(III) complexes. In the presence of Fe(III)EDTA, the order of DNA damaging ability was EGCG ≈ (−)-epigallocatechin > (−)-epicatechin gallate ≫ catechin. Catechins plus Fe(III)EDTA caused DNA damage at every nucleotide, most likely due to •OH generation from H_2O_2. In the presence of Cu(II), the order was (−)-epigallocatechin > catechin > EGCG > (−)-epicatechin gallate. Cu(II)-mediated DNA damage by EGCG occurred most frequently at T and G residues, especially of 5′-TG-3′ and GG sequences. Catalase and bathocuproine inhibited the Cu(II)-mediated DNA damage, suggesting the involvement of H_2O_2 and Cu(I). In the presence of metal ions, increased amounts of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) were found in DNA treated with EGCG. Furthermore, EGCG increased amounts of 8-oxodG in HL-60 cells, but not in the H_2O_2-resistant clone HP100. When GSH was reduced by L-buthionine-[*S*, *R*]-sulfoximine, a low concentration of EGCG increased amounts of 8-oxodG in HL-60 cells, further supporting the involvement of H_2O_2 in cellular DNA damage. It is concluded that EGCG can induce H_2O_2 generation and subsequent damage to isolated and cellular DNA, and that oxidative DNA damage may mediate the potential carcinogenicity of EGCG.

Keywords: DNA damage; Epigallocatechin gallate; 8-Oxo-7,8-dihydro-2′-deoxyguanosine; Reactive oxygen species; Metal; Double base lesion

1. Introduction

Some epidemiological studies suggest that green tea has potentially preventive effects against cancer [1–5]. The ability of tea and its components to inhibit cancer formation has been demonstrated in different animal models. Tea polyphenols, especially EGCG, are believed to mediate much of the cancer chemopreventive effects of tea [6,7]. Oral administration of EGCG inhibited chemical carcinogenesis of the gastrointestinal tract and lung in rodents [8–10]. Catechins, especially EGCG, can ameliorate free radical damage to DNA, under certain conditions [11,12]. Therefore, polyphenolic compounds present in tea have been proposed to reduce the risk of cancer.

On the other hand, green tea catechins have been reported to enhance colon carcinogenesis in rats [13]. Furthermore, several human cohort and case-control studies indicated significant positive relationships between tea consumption and cancers of various organs [14–20]. These results suggest that green tea catechins may have carcinogenic as well as anticarcinogenic effects. In fact, putative chemopreventive antioxidants also have carcinogenic potential. Previously, we demonstrated that several antioxidants, including Vitamin A and its derivative [21], Vitamin E [22], quercetin [23] and *N*-acetylcysteine [24], caused oxidative damage to cellular and isolated DNA. Therefore, these detrimental effects should be carefully studied before considering the use of green tea catechins as cancer chemopreventive agents.

In this study, we examined DNA damage induced by four catechins and the site specificity using ^{32}P-5′-end-labeled

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Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine (also known as 8-hydroxy-2′-deoxyguanosine); DTPA, diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; •OH, free hydroxyl radical; HPLC-ECD, an electrochemical detector coupled to high-pressure liquid chromatography; EGCG, (−)-epigallocatechin gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; BSO, L-buthionine-[*S*, *R*]-sulfoximine; FCS, fetal calf serum; Fpg, formamidopyrimidine-DNA glycosylase.

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DNA fragments obtained from the human *p53* and *p16* tumor suppressor genes and the c-Ha-*ras*-1 protooncogene. We investigated the formation of 8-oxodG by using an electrochemical detector coupled to high-pressure liquid chromatography (HPLC-ECD). A characteristic oxidative DNA lesion, 8-oxodG, has attracted much attention in relation to mutagenesis and carcinogenesis [25–27]. We also compared 8-oxodG formation in the human leukemia cell line HL-60 and its hydrogen peroxide (H_2O_2)-resistant clone HP100 treated with EGCG.

2. Materials and methods

2.1. Materials

Restriction enzymes were purchased from Boehringer Mannheim GmbH. T_4 polynucleotide kinase was obtained from New England Biolabs. [γ-^{32}P]ATP (222 TBq/mmol) was acquired from Amersham Biosciences. Diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA) and bathocuproine disulfonic acid were procured from Dojin Chemical Co. Acrylamide, piperidine, DMSO, $FeCl_3$, bisacrylamide and bathophenanthroline disulfonic acid disodium salt were obtained from Wako Chemical Industries, Ltd. $CuCl_2$, EDTA-Na-Fe(III) salt, ethanol, D-mannitol, sodium formate and adenosine-5′-diphosphate disodium salt were acquired from Nacalai Tesque. (+)-Catechin, (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and EGCG were obtained from Kurita Industries, Ltd. L-Buthionine-[*S*, *R*]-sulfoximine (BSO), calf thymus DNA, calf intestine phosphatase (CIP), superoxide dismutase (SOD, 3000 U/mg from bovine erythrocytes), catalase (45,000 U/mg from bovine liver), and bacterial alkaline phosphatase (BAP) were obtained from Sigma Chemical Co. Nuclease P_1 (400 U/mg) was purchased from Yamasa Shoyu Co. *Escherichia coli* formamidopyrimidine-DNA glycosylase (Fpg) was obtained from Trevigen Inc. Fetal calf serum (FCS) was obtained from GIBCO; total Se varied between 14 and 38 ng/mL (0.2–0.5 μM) in the different batches of 100% serum used.

2.2. Preparation of ^{32}P-5′-end-labeled DNA fragments

DNA fragments were obtained from the human *p53* [28] and *p16* [29] tumor suppressor genes and the c-Ha-*ras*-1 protooncogene [30]. The DNA fragment from the *p53* tumor suppressor gene was prepared from the plasmid pUC18. A singly ^{32}P-5′-end-labeled 211-bp fragment (*Hin*dIII* 13972–*Apa*I 14182) was obtained according to the previously described method [31]. Exon-containing DNA fragments were also obtained from the human *p16* tumor suppressor gene. These fragments were subcloned into the pGEM®-T Easy Vector (Promega Corporation). A 490-bp fragment was further digested with *Mro*I to obtain a singly labeled 328-bp fragment (*Eco*RI* 5841–*Mro*I 6168) and a singly labeled 158-bp fragment (*Mro*I 6173–*Eco*RI* 6330). In addition, a 460-bp fragment was further digested with *Bss*HII to obtain a singly labeled 309-bp fragment (*Eco*RI*9481–*Bss*HII 9789) and a singly labeled 147-bp fragment (*Bss*HII 9794–*Eco*RI*9940). DNA fragments from the human c-Ha-*ras*-1 protooncogene were prepared from the plasmid pbcNI, which carries a 6.6-kb *Bam*HI chromosomal DNA segment containing the c-Ha-*ras*-1 gene. A singly labeled 337-bp fragment (*Pst*I 2345–*Ava*I* 2681) and a singly labeled 261-bp fragment (*Ava*I* 1645–*Xba*I 1905) were obtained according to the previously described method [32]. Nucleotide numbering starts with the *Bam*HI site [30]. In each case, an asterisk indicates ^{32}P-labeling.

2.3. Analysis of DNA damage by EGCG in the presence of metal ions

The standard reaction mixture in a microtube (1.5 mL, Eppendorf) contained catechins, 20 μM metal ions, ^{32}P-5′-end-labeled DNA fragment and 20 μM/base of calf thymus DNA in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. After incubating at 37° for 90 min, the DNA fragments were treated with 1 M piperidine at 90° for 20 min or 10 units of Fpg protein in the reaction buffer (10 mM HEPES–KOH (pH 7.4), 100 mM KCl, 10 mM EDTA, and 0.1 mg/mL BSA) at 37° for 2 hr. Fpg protein catalyzes the excision of 8-oxodG as well as 2,6-diamino-4-hydroxy-5-formamidopyrimidine residues [33–35]. The DNA fragments were electrophoresed on 8% polyacrylamide/8 M urea gels. The autoradiograms were obtained by exposing X-ray film to the gels. The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam-Gilbert procedure [36] using a DNA-sequencing system (LKB 2010 Macrophor). The relative amounts of oligonucleotides from the treated DNA fragments were measured with a laser densitometer (LKB 2222 UltroScan XL).

2.4. Measurement of 8-oxodG formation in calf thymus DNA by EGCG plus metal ions

The amount of 8-oxodG was measured by a modified method of Kasai *et al*. [37]. Calf thymus DNA fragments (100 μM/base) were incubated with EGCG and 20 μM metal ions at 37° for 1 hr. After ethanol precipitation, DNA was digested with nuclease P_1 and CIP, and analyzed by an HPLC-ECD [38].

2.5. Analysis of 8-oxodG and GSH in cultured cells

HL-60 and HP100 cells were grown in RPMI 1640 supplemented with 6% FCS at 37° under 5% CO_2 in a humidified atmosphere. Cells (1.0×10^6 cells/mL) were incubated with EGCG for 1 hr at 37°. Where indicated,

HL-60 cells (1.0×10^6 cells/mL) were pre-incubated with 0.5 mM BSO for 5 hr at 37°. After the incubation, the medium was removed, and the cells were washed three times with cold PBS. The cells were then suspended in 0.05 mg/mL RNase and 0.5 mg/mL proteinase K in 500 μL of lysis buffer (Applied Biosystems), followed by incubation at 60° for 1 hr under the anaerobic condition. After ethanol precipitation, DNA was digested with 8 U nuclease P_1 and 1.2 U BAP, and then analyzed by an HPLC-ECD by the previously described method [38]. Furthermore, the amount of GSH was measured with an HPLC-ECD with a gold electrode (Eicom), as described previously [39].

3. Results

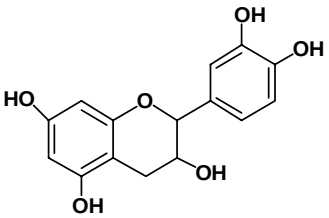
3.1. Damage to ^{32}P-labeled DNA fragments by four catechins in the presence of metal ions

We compared the intensity of DNA damage in the presence of metal ions by four catechins: catechin, EGC, ECG and EGCG. The chemical structures of these catechins are shown in Fig. 1. DNA fragments treated with various concentrations of catechins in the presence of metal ions were detected by autoradiography as shown in Fig. 2. Oligonucleotides were detected as a result of DNA damage. In the presence of Fe(III)EDTA, EGC and EGCG induced DNA damage (Fig. 2A). In addition, ECG also caused mild DNA damage (data not shown). Catechin only induced slight DNA damage (Fig. 2A). The order of DNA damaging ability was EGCG ≈ EGC > ECG ⩾ catechin. When Fe(III)ADP was added instead of Fe(III)EDTA, DNA damage was induced to a lesser extent than that observed with Fe(III)EDTA. EGCG only induced slight DNA damage in the presence of Fe(III) and Fe(III)citrate under the conditions used (data not shown).

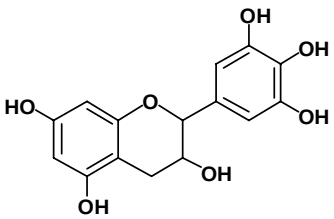
In the presence of Cu(II), all four catechins induced DNA damage (Fig. 2B). The order of DNA damaging ability was EGC > catechin > EGCG > ECG. Without piperidine treatment, direct breakage of the deoxyribose phosphate backbone by EGCG was observed in the presence of Cu(II) and Fe(III)EDTA (data not shown). Piperidine treatment enhanced DNA cleavage by EGCG, suggesting that base modification occurred. EGCG did not induce DNA damage in the presence of Co(II), Ni(II), Mn(II), or Mn(III) (data not shown).

3.2. Effects of scavengers and metal chelators on DNA damage induced by EGCG in the presence of metal ions

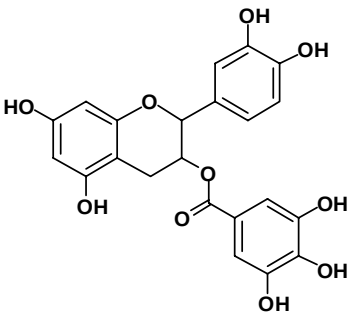
Fe(III)EDTA-mediated DNA damage caused by EGCG was inhibited by typical free hydroxy radical (•OH) scavengers, such as ethanol, mannitol, sodium formate, DMSO, and methional (Fig. 3A). The iron chelating agents, deferoxamine mesilate and bathophenanthroline, inhibited the DNA damage. Catalase significantly inhibited DNA damage, while SOD had no effect. These results indicate that •OH, hydrogen peroxide (H_2O_2), and iron are necessary for DNA damage.



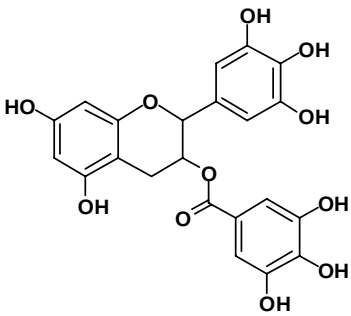
catechin



EGC: epigallocatechin



ECG: epicatechin gallate



EGCG: epigallocatechin gallate

Fig. 1. Chemical structures of catechins used in this study.

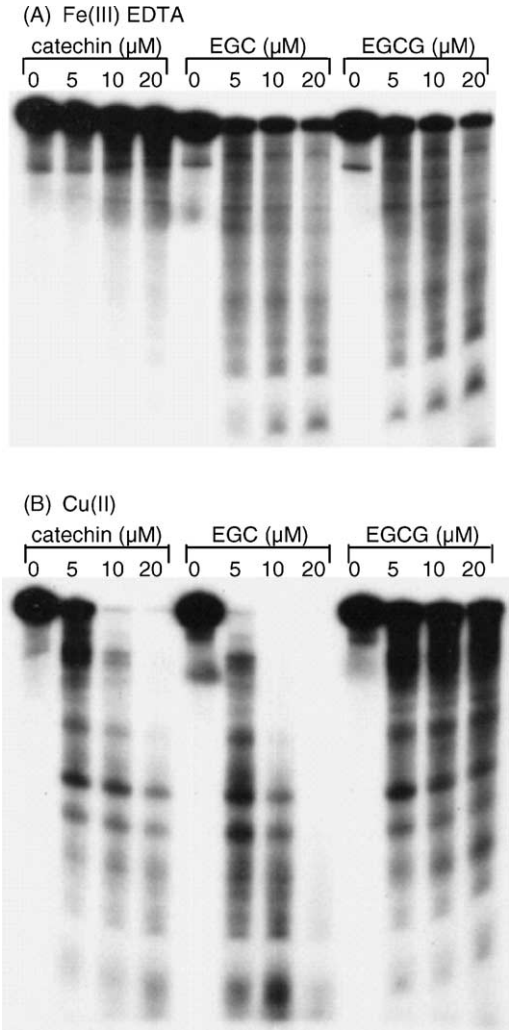


Fig. 2. Autoradiogram of ^{32}P-labeled DNA fragments incubated with catechins in the presence of metal ions. The reaction mixture contained the ^{32}P-5′-end-labeled 309-bp DNA fragment, 20 μM per base of calf thymus DNA, the indicated concentrations of catechins (catechin, EGC, and EGCG) and 20 μM Fe(III)EDTA (A) or $CuCl_2$ (B) in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. The mixture was incubated at 37° for 90 min followed by piperidine treatment. The DNA fragments were electrophoresed on an 8% polyacrylamide 8 M urea gel (12 cm × 16 cm), and autoradiograms were obtained by exposing X-ray film to the gel.

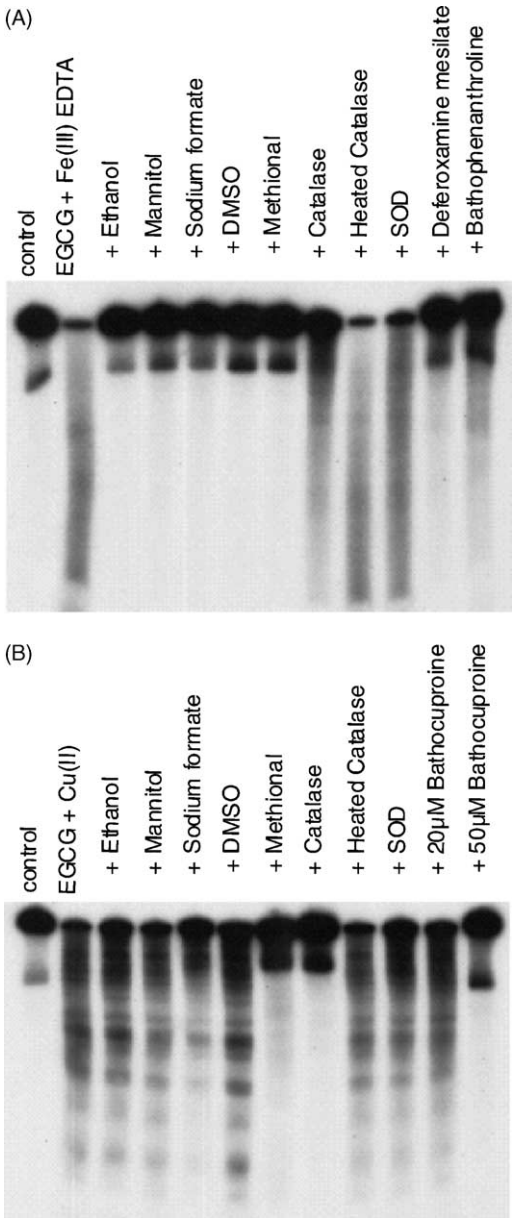


Fig. 3. Effects of scavengers and metal chelators on DNA damage induced by EGCG in the presence of metal ions. The reaction mixture contained the ^{32}P-5′-end-labeled 261-bp (A) or 309-bp DNA fragment (B), 20 μM per base of calf thymus DNA, 200 μM (A) or 100 μM (B) EGCG, 20 μM Fe(III)EDTA (A) or Cu(II) (B) in 200 μL of 10 mM sodium phosphate buffer at pH 7.8 containing 5 μM DTPA. Scavengers or metal chelators were added where indicated. After incubation at 37° for 60 min (A) or 90 min (B), followed by piperidine treatment, the DNA fragments were analyzed by the method described in Fig. 2. The concentrations of chemicals were as follows: (A) 0.8 M ethanol; 0.1 M mannitol; 0.1 M sodium formate; 0.8 M DMSO; 1.0 M methional; 30 U catalase; 30 U heated catalase; 30 U SOD; 2 mM deferoxamine mesilate; 2 mM bathophenanthroline. (B) 0.8 M ethanol; 0.1 M mannitol; 0.1 M sodium formate; 0.8 M DMSO; 1.0 M methional; 30 U catalase; 30 U heated catalase, 30 U SOD, 20 μM bathocuproine, 50 μM bathocuproine.

In the presence of Cu(II), catalase and bathocuproine, a Cu(I) chelator, inhibited the DNA damage caused by EGCG, suggesting the involvement of H_2O_2 and Cu(I) (Fig. 3B). Typical •OH scavengers did not inhibit the DNA damage, whereas methional did. Methional scavenges not only •OH, but also species with weaker reactivity than •OH [40]. These results suggest that H_2O_2 reacts with Cu(I) to produce reactive species causing DNA damage.

3.3. Site specificity of DNA damage by EGCG in the presence of metal ions

Fe(III)EDTA-mediated DNA damage induced by EGCG occurred without preference for any particular nucleotide (Fig. 4A). In contrast, the Cu(II)-mediated DNA damage caused by EGCG occurred most frequently at T and G residues (Fig. 4B). Using piperidine treatment, DNA was frequently damaged at T residues of 5′-TG-3′ and G

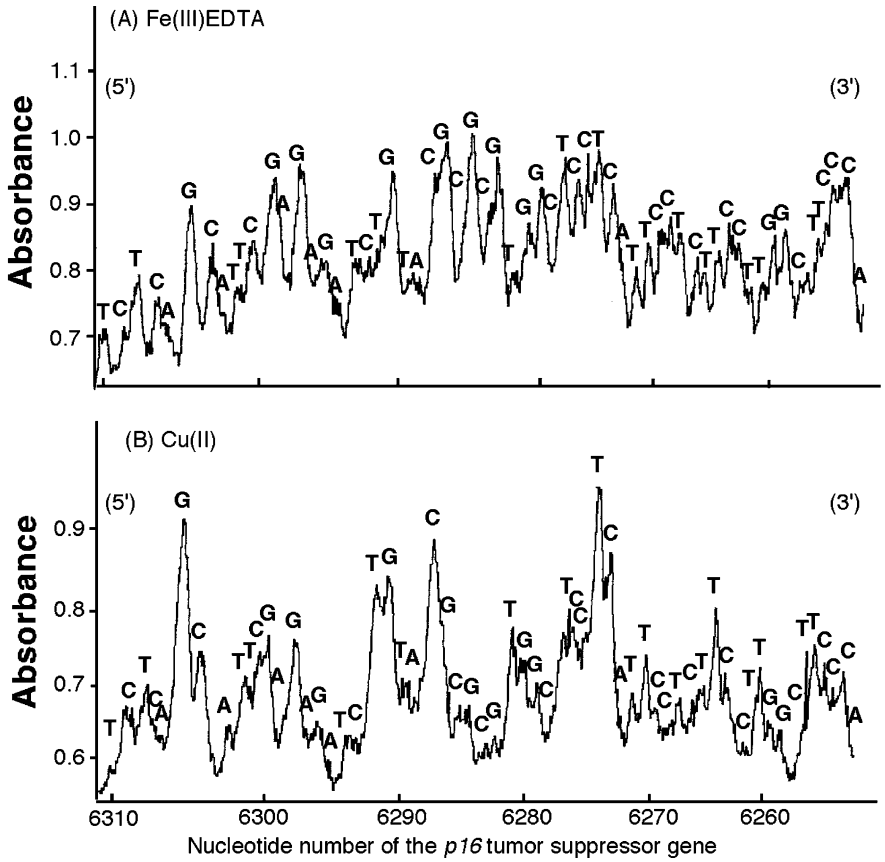


Fig. 4. Site specificity of DNA cleavage induced by EGCG in the presence of metal ions. The ^{32}P-5′-end-labeled 158-bp fragment obtained from the human *p16* tumor suppressor gene in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA and 20 μM per base of calf thymus DNA was incubated with 100 μM EGCG plus 20 μM Fe(III)EDTA (A) or $CuCl_2$ (B) at 37° for 90 min followed by piperidine treatment as described in Section 2. The DNA fragments were electrophoresed on an 8% polyacrylamide/8 M urea gel using a DNA-sequencing system and visualized by autoradiography. The relative amounts of oligonucleotide were measured by scanning the autoradiogram with a laser densitometer (LKB 2222 UltroScan XL, Pharmacia Biotech).

residues of GG (Fig. 5A). Fpg treatment frequently induced cleavage at G residues, especially of 5′-TG-3′ and GG sequences (Fig. 5B). Without piperidine or Fpg treatment, no DNA cleavage was observed under the conditions used (data not shown).

3.4. Formation of 8-oxodG in calf thymus DNA by EGCG in the presence of metal ions

We measured 8-oxodG content, a relevant indicator of oxidative base damage, in calf thymus DNA treated with EGCG. The amount of 8-oxodG increased with increasing concentrations of EGCG in the presence of metal ions (Fig. 6). At EGCG concentrations below 20 μM, the presence of Fe(III)EDTA led to more efficient formation of 8-oxodG than the presence of Cu(II). EGCG also increased formation of 8-oxodG in the presence of Fe(III)ADP (data not shown). Fe(III)ADP is a good model compound of endogenous Fe complex.

It is reported that green tea catechins, especially EGCG, partially protect DNA from $^{\bullet}$OH-induced strand breaks and base damage under certain conditions [11,12]. In order to clarify whether EGCG protects oxidative DNA damage, we examined the effect of EGCG on 8-oxodG formation by H_2O_2 in the presence of metal ions. When H_2O_2 was added to 1 μM of EGCG, the formation of 8-oxodG in the presence of Cu(II) and Fe(III)EDTA showed 60- and 10-fold increases, respectively (Fig. 6). These results showed that EGCG did not inhibit, but rather enhanced the formation of 8-oxodG by H_2O_2 in the presence of metal ions under our experimental condition.

3.5. Formation of 8-oxodG by EGCG in cultured human cells

The content of 8-oxodG in HL-60 cells treated with EGCG increased in a dose-dependent manner, and formation of 8-oxodG at 200 μM EGCG was significantly increased than that in control. However, no significant increase in 8-oxodG was observed in H_2O_2-resistant HP100 cells treated with EGCG (Fig. 7A).

Generally, cancer cell lines such as HL-60 contain higher level of GSH than normal cells [41]. In order to decrease GSH level of HL-60 cells, the cells were pretreated with BSO. The treatment with BSO at 0.5 mM for 5 hr resulted in a decrease of GSH to 60% in HL-60 cells

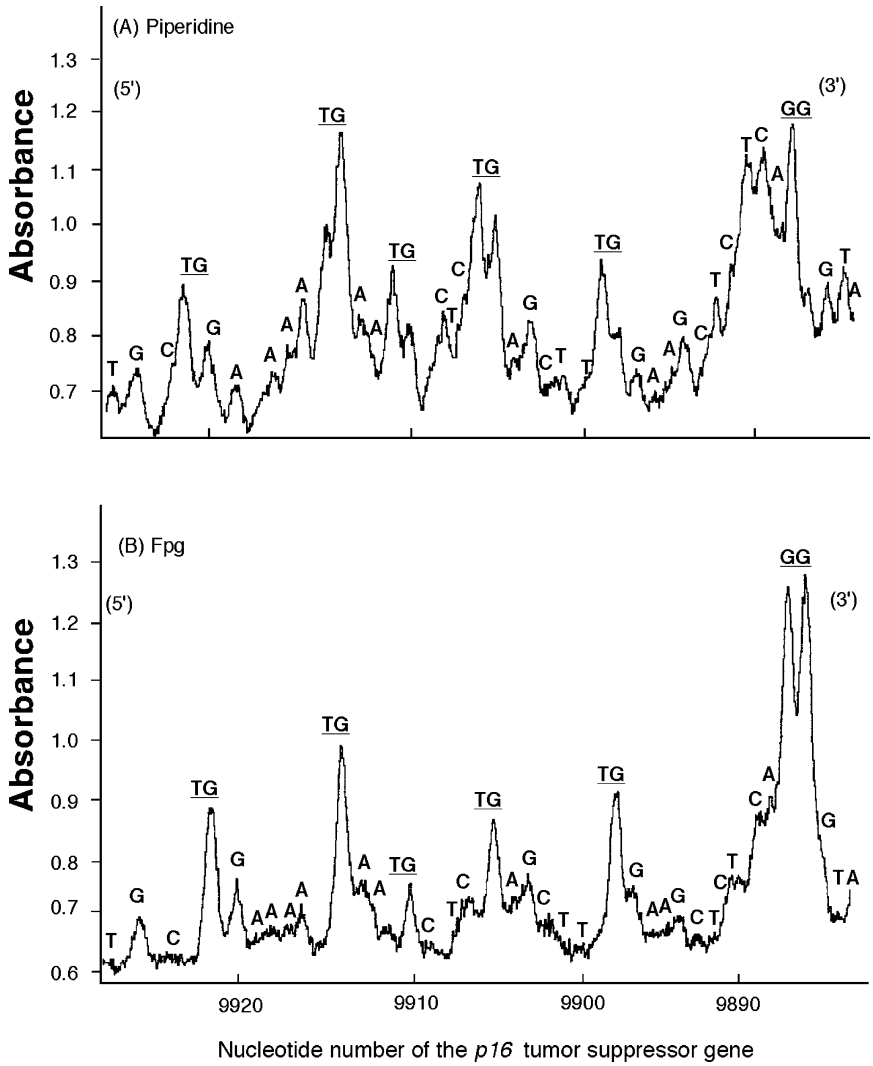


Fig. 5. The difference between piperidine-labile sites and Fpg-labile sites induced by EGCG. The ^{32}P-5′-end-labeled 147-bp fragment obtained from the human *p16* tumor suppressor gene in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA and 20 μM per base of calf thymus DNA was incubated with 100 μM EGCG plus 20 μM $CuCl_2$ at 37° for 90 min. After piperidine (A) or Fpg (B) treatment, the DNA fragments were analyzed by the method described in Fig. 4. Underlined bases represent double base lesions detected by the treatment with piperidine and Fpg protein.

(data not shown). When GSH was reduced by BSO, 20 μM EGCG significantly increased 8-oxodG formation in HL-60 cells (Fig. 7B). These results suggest that a low concentration of EGCG can cause oxidative DNA damage in normal human cells and that H_2O_2 plays a critical role in EGCG-induced DNA damage.

4. Discussion

The present study demonstrates that green tea catechins can cause DNA damage in the presence of metal ions. In the presence of Fe(III)EDTA, EGCG, EGC and ECG induced DNA damage, but catechin induced only slight DNA damage. The order of DNA damaging ability was EGCG ≈ EGC > ECG ⩾ catechin. In the presence of Cu(II), the order was EGC > catechin > EGCG > ECG. Thus, the potent DNA damaging activities of catechins are dependent on their chemical structures. The compounds having three hydroxyl groups (pyrogallol) in the B ring and/or galloyl groups in the C ring, such as EGC, ECG and EGCG, caused DNA damage in the presence of both Cu(II) and Fe(III) complexes. Catechin, which has an *o*-dihydroxy (catechol) structure in the B ring without galloyl groups in the C ring, caused stronger DNA damage in the presence of Cu(II). Interestingly, the *o*-dihydroxy structure in the B ring is an important determinant for radical scavenging and/or antioxidative potential [42,43].

EGCG is the major constituent, accounting for approximately 40% of the total polyphenolic content in green tea [7]. Much of the cancer-preventive effects of green tea are mediated by EGCG [44]. EGCG is the most expected chemopreventive agent among tea catechins. As one method to study the potential risks, we focused on the mechanism of DNA damage by EGCG. EGCG caused iron-dependent DNA damage uniformly at every nucleo-

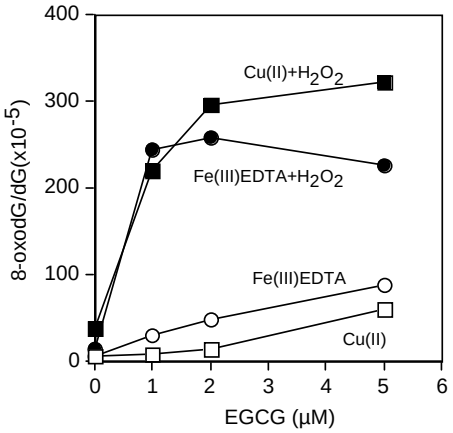


Fig. 6. Formation of 8-oxodG in calf thymus DNA induced by EGCG in the presence of metal ions. Calf thymus DNA (100 μM per base) was incubated with EGCG and 20 μM metal ions in 4 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA at 37° for 1 hr in the absence and presence of 10 μM H_2O_2. After ethanol precipitation, the DNA was subjected to enzyme digestion and analyzed by an HPLC-ECD as described in Section 2.

tide. It is known that free •OH causes DNA damage without site specificity [45–47]. The DNA damage was inhibited by •OH scavengers and catalase. On the basis of our data, we conclude that Fe(III) complex-mediated DNA cleavage by EGCG is mainly due to •OH generated *via* the Fenton reaction. Cu(II)-mediated DNA damage by EGCG occurred at T and G residues, especially at 5′-TG-3′ and GG sequences. EGCG frequently induced Cu(II)-mediated formation of piperidine-labile sites at T residues of 5′-TG-3′. Treatment of DNA with Fpg led to chain cleavage at G residues of 5′-TG-3′ and GG sequences. Fpg catalyzes the excision of piperidine-resistant 8-oxodG [34] and further oxidized piperidine-labile guanine residues [48]. We also detected 8-oxodG by means of HPLC-ECD. Collectively, EGCG efficiently induces oxidative DNA damage, including double base lesions, especially 8-oxodG formation adjacent to piperidine-labile thymine lesions. Recently, reactive oxygen species have been reported to induce double base lesions in DNA oligomers [49–51]. We consider that the initial damaging step is the oxidation of G residues followed by damage of adjacent T residues. This is supported by the report that guanine is the most easily oxidized of the DNA bases [52,53].

In this study, catalase and bathocuproine completely inhibited DNA damage induced by EGCG plus Cu(II), indicating the participation of H_2O_2 and Cu(I) in the DNA damage. Bathocuproine inhibits the activation of H_2O_2 by stabilizing Cu(I) [54,55]. Typical •OH scavengers showed little or no inhibitory effect on DNA damage induced by EGCG, suggesting that •OH does not play an important role. Methional, which scavenges not only •OH, but also species with weaker reactivity than •OH [40], inhibited the DNA damage. From these results and previous papers, we conclude that reactive species such as the Cu(I)-hydroperoxo complex participate in Cu(II)-mediated DNA damage by EGCG [56,57]. A possible mechanism shown in Fig. 8 could account for most of these observations. First, metal-mediated autooxidation of EGCG to the semiquinone radical occurs. The radicals and metal ion then react with oxygen to generate O_2^-, which is dismutated to H_2O_2. H_2O_2 can pass through the cellular membrane and induce •OH and/or metal-hydroperoxo complex formation by reaction with metal ions. Thus, H_2O_2 plays an important role in generating reactive species responsible for DNA damage. In view of the very tightly controlled physiological uptake and turnover of copper and iron, it is difficult to consider any presence of free copper and iron ions available for the postulated reactions [58]. Therefore, we envision that DNA and/or other natural

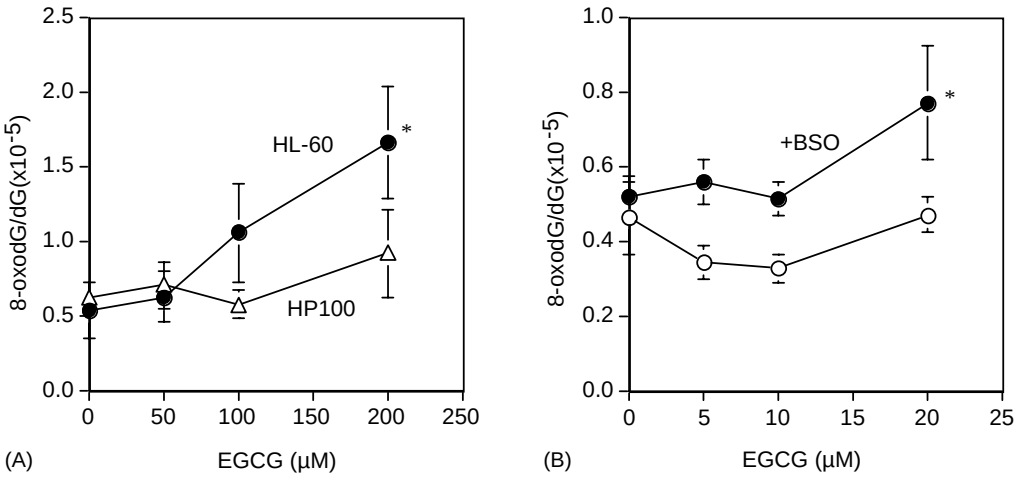


Fig. 7. Contents of 8-oxodG in cultured cells treated with EGCG. HL-60 and HP100 cells (1.0×10^6 cells/mL) were incubated with various concentrations of EGCG for 1 hr (A). HL-60 cells (1.0×10^6 cells/mL) were preincubated with 0.5 mM BSO for 5 hr at 37°, followed by incubation with various concentrations of EGCG for 1 hr at 37° (B). After the incubation, DNA was extracted immediately. The extracted DNA was subjected to enzyme digestion and analyzed by an HPLC-ECD as described in Section 2. Results are expressed as means ± SE of values obtained from four to seven independent experiments. Asterisk indicates significant differences compared with control by *t*-test ($P < 0.05$).

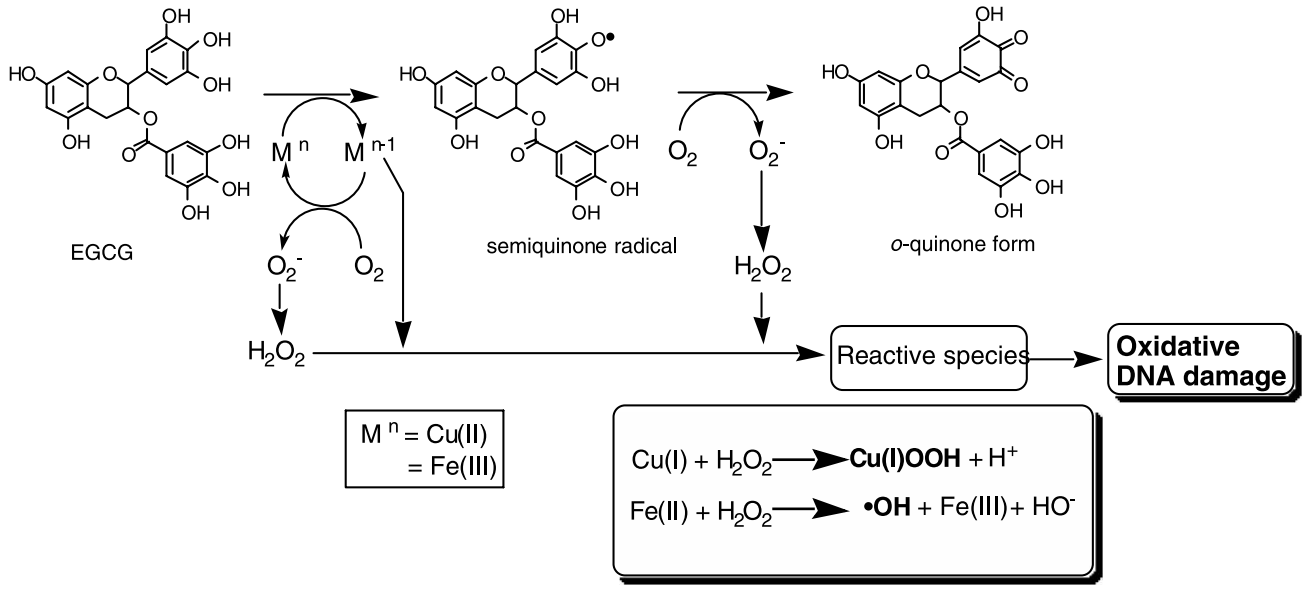


Fig. 8. A possible mechanism of oxidative DNA damage induced by EGCG in the presence of metal ions.

complexes with these metals may participate in carcinogenesis *in vivo*.

The present study demonstrated that EGCG significantly induced 8-oxodG formation in HL-60 cells, but did not significantly increase 8-oxodG in H_2O_2-resistant HP100 cells. The catalase activity of HP100 cells was 18 times higher than that of HL-60 cells [59]. Furthermore, a low concentration of EGCG significantly induced 8-oxodG formation in HL-60 cells treated with BSO. The addition of BSO resulted in decrease of GSH to 60% in HL-60 cells under the condition used. Generally, cancer cell lines, such as HL-60, contained higher level of GSH than normal cells. Therefore, the depletion of GSH by BSO may prove to be an advantageous technique for the estimate of oxidative DNA damage in normal cells.

Our result and these literatures suggest that H_2O_2 plays a critical role in EGCG-induced 8-oxodG formation in cells. 8-oxodG formation has been reported to lead to G → T transversion *via* DNA misreplication, with a resulting mutation [25–27]. In addition, Tanaka *et al*. reported that ferric nitrilotriacetate selectively damaged certain target genes such as *p15* and *p16* by homozygous or hemizygous deletion, which could be one of the major pathways responsible for carcinogenesis induced by reactive oxygen species [60]. In the presence of Cu(II), EGCG caused oxidative DNA damage, especially double base lesions. Clustered damage, which can be demonstrated in living cells, is poorly repaired [61]. Our results support the conjecture that EGCG may cause inactivation of tumor suppressor genes by clustered damage in living cells, which may account for the potential mutagenicity and carcinogenicity of EGCG.

Tea polyphenols, especially EGCG, have been believed to mediate much of the cancer chemopreventive effects of green tea. Some epidemiologic studies have shown an inverse association between drinking green tea and cancer incidence [1–5]. We evaluated whether EGCG was able to prevent damage in calf thymus DNA subjected to oxidative stress induced by H_2O_2 in the presence of metal ions. EGCG did not inhibit, but rather enhanced the formation of 8-oxodG by H_2O_2 in the presence of metal ions. These results suggested that EGCG can act not as an antioxidant, but as a pro-oxidant under the condition used. Concomitantly, several epidemiological studies indicate that tea consumption is positively associated with an increased risk of cancers in various organs [14–20]. Furthermore, green tea catechins containing EGCG as the main component enhanced colon carcinogenesis in rats [13]. Some papers reported that EGCG caused chromosomal aberrations and sister chromatid exchanges in human cultured cells, and induced DNA strand breakage [62–66]. Collectively, our data demonstrated that EGCG caused oxidative DNA damage in the presence or absence of H_2O_2. It is concluded that the oxidative DNA damage by EGCG plays an important role in the potential carcinogenicity of catechins.

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