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Antioxidant properties of (-)-epicatechin-3-gallate and its inhibition of Cr(VI)-induced DNA damage and Cr(IV)- or TPA-stimulated NF-κB activation

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Abstract

Electron spin resonance (ESR) spin trapping was utilized to investigate the scavenging effects on hydroxyl radicals ($^{\cdot}OH$) and superoxide radicals ($O_2^{\cdot-}$) by (-)-epigallocatechin-3-gallate (EGCG), one of the major anticancer compounds in tea. The spin trap used was 5,5-dimethyl-pyrroline *N*-oxide (DMPO). The Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^-$) was used as a source of $^{\cdot}OH$ radicals. EGCG efficiently scavenges $^{\cdot}OH$ radicals with reaction rate of 4.62×10^{11} $M^{-1}sec^{-1}$, which is an order of magnitude higher than several well recognized antioxidants, such as ascorbate, glutathione and cysteine. It also scavenges $O_2^{\cdot-}$ radicals as demonstrated by using xanthine and xanthine oxidase system as a source of $O_2^{\cdot-}$ radicals. Through its antioxidant properties, EGCG exhibited a protective effect against DNA damage induced by Cr(VI). EGCG also inhibited activation of nuclear transcription factor NF-κB induced by Cr(IV) and 12-*o*-tetradecanoylphorbol-13-acetate (TPA). The present studies provide a mechanistic basis for the reported anticarcinogenic properties of EGCG and related tea products. (Mol Cell Biochem **206:** 125–132, 2000)

Key words: oxygen derived free radicals, antiooxidants, cancer, tea, epigallocatechin-3-gallate

Introduction

Tea is consumed world-wide, especially in Asian countries where it is a major beverage. It is generally believed that tea exerts various biological and pharmaceutical effects, although many of them remain to be investigated [1–3]. Studies show that green tea and its major components are able to inhibit carcinogenicity induced by a variety of chemicals in a number of animal models [1, 5]. Green tea may act at the cancer initiation stage, preventing chemical-induced mutagenesis either by inhibiting the bioactivation process or facilitating deactivation of reactive intermediates [6]. Green tea may also affect the post-initiation activation phase by inhibiting cell proliferation [7]. Green tea contains relatively large amounts of polyphenols [1, 2], which are reported to have antioxidant properties [8]. They can scavenge a wide range of free radicals, including 1,1-diphenyl-2-picryl-hydrazyl (DPPH) [9], hydroxyl radicals ($^{\cdot}OH$) [10] and lipid-derived free radicals [8,10]. These radicals have been shown to play an important role in the development of certain diseases, as well as in normal physiological processes [11, 12]. The toxicity of many drugs

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and chemicals are also known to be mediated through free radical reactions.

Although a variety of polyphenols in tea exhibit antioxidant properties, (-)-epigallocatechin-3-gallate (EGCG) is especially interesting. The chemical structure of EGCG is shown in Fig. 1. This polyphenol is non-toxic and is a potential cancer chemopreventive agent [1, 2]. It has been reported that application of EGCG prior to the application of tumor promoter, teleocidin, inhibited tumor promotion on mouse skin initiated by 7,12-dimethylbenz(a)anthracene [13]. Inhibitory effects of EGCG on various chemical carcinogens have been reported [1]. Antioxidant properties of EGCG may account for some of these actions [8–10]. Although many recent studies have suggested that EGCG functions as a scavenger of oxygen free radicals, its antioxidant properties have not been studied. For example, what is the reaction rate constant of EGCG with $^{\cdot}OH$, the most reactive free radical among reactive oxygen species. Without the reaction rate constant, it is difficult, if not impossible, to compare its antioxidant properties with other well established antioxidants, such as ascorbate, glutathione and cysteine. In the present study, the reaction rate of EGCG with $^{\cdot}OH$ radical was measured using Fenton reactions as a source of $^{\cdot}OH$ radicals. The scavenging activity of EGCG on superoxide radicals ($O_2^{\cdot-}$), was examined using xanthine/xanthine oxidase system as a source of $O_2^{\cdot-}$ radicals. The Cr(VI)-induced DNA damage as well as Cr(IV) or TPA-induced nuclear transcription factor (NF-κB) activation were used as model systems to examine the possible protective effect of EGCG. The major questions to be answered in this study are as follows: (a) What is the reaction rate constant between EGCG and $^{\cdot}OH$ radical? (b) How does it compare with other well established antioxidants? (c) Does EGCG scavenge $O_2^{\cdot-}$ radical? (d) Does EGCG inhibit Cr(VI)-induced DNA damage? (e) Does EGCG inhibit Cr(IV)- or 12-*o*-tetradecanoylphorbol-13-acetate (TPA)-induced activation of nuclear transcription factor NF-κB?

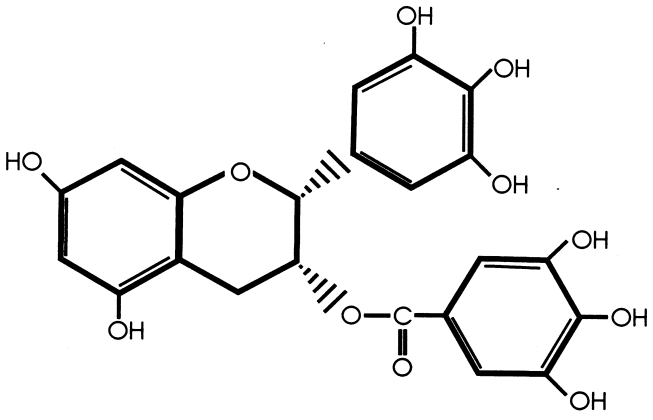

Fig. 1. The chemical structure of EGCG.

Materials and methods

Materials

Iron(II) chloride ($FeCl_2$), hydrogen peroxide (H_2O_2), potassium dichromate ($K_2Cr_2O_7$), and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were purchased from Aldrich (Milwaukee, WI, USA). NADPH, glutathione reductase, sodium formate, and TPA were purchased from Sigma (St. Louis, MO, USA). EGCG was provided by Dr. C.S. Yang of Rutgers University (Piscataway, NJ, USA). Chelex-100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA, USA). Phosphate buffer (pH 7.4) was treated with Chelex-100 to remove putative metal ion contaminants. DMPO solutions were purified using activated charcoal until free radical impurities disappeared as verified by ESR spectroscopy.

Electron spin resonance (ESR) measurements

ESR spin trapping [14] was used to detect short-lived free radical intermediates. This technique involves the addition-type reaction of a short-lived radical with a diamagnetic compound (spin trap) to form a relatively long-lived free radical product, the so-called spin adduct, which can be studied by conventional ESR. The intensity of the spin adduct signal corresponds to the amount of short-lived radicals trapped, and the hyperfine splittings of the spin adduct are generally characteristic of the original, short-lived, trapped radical. All ESR measurements were made using a Varian E-9 spectrometer and a flat cell assembly. Hyperfine splittings were measured (to 0.1 G) directly from magnetic field separations using potassium tetraperoxochromate (K_3CrO_8) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as standards. The relative radical concentration was estimated by multiplying half of the peak height by $(\Delta H_{pp})^2$ (where ΔH_{pp} represents peak-to-peak width). Reactants were mixed in test tubes in a final volume of 0.50 ml. The reaction mixture was then transferred to a flat cell for ESR measurement.

Oligonucleotide

Oligonucleotides were synthesized by the phosphoramidite method on a DNA/RNA synthesizer (Applied Biosystems, Model 392, Foster City, CA, USA). A NF-κB binding sequence in the human IL-6 gene promoter (-74 TGGGATT deprotected at 50°C overnight, dried in a speed vacuum and then resolved in the Tris-EDTA buffer. Complimentary strands were denatured at 80°C for 5 min and annealed at room temperature. The double-stranded probe was labeled with ^{32}P-dCTP (Amersham, Arlington Heights, Il, USA) using a Klenow fragment (BRL, Gaithersburg, MD, USA).

Cell culture

Jurkat cells ($CD4^+$ human lymphoblast cell line) were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 2 mM glutamine and 100 U/ml penicillin-streptomycin (complete medium). A concentration of 2×10^6/ml cells was used for the experiments.

Nuclear extraction

The nuclear extracts were prepared according to the method described earlier [16]. Briefly, 2×10^7 cells were treated with 500 µl lysis buffer (50 mM KCl, 0.5% NP-40, 25 mM HEPES pH 7.8, 1 mM PMSF, 10 µg/ml leupeptin, 20 µg/ml aprotinin, and 100 µM DTT) on ice for 4 min. After 1 min centrifugation at 14,000 rpm, the supernatant was saved as a cytoplasmic extract. The nuclei were washed once with the same volume of buffer without NP-40, then suspended in 300 µl of extraction buffer (500 mM KCl, 10% glycerol with the same concentrations of HEPES, PMSF, leupeptin, aprotinin and DTT as the lysis buffer) and pipetted several times. After centrifugation at 14,000 rpm for 5 min, the supernatant was collected as the nuclear protein extract and stored at -70°C. The protein concentration was determined by BCA (Pierce, Rockford, IL 61105, USA).

Electrophoretic mobility shift assay (EMSA)

The DNA-NF-κB binding reaction was conducted in a 24 µl reaction mixture containing 1 µg Poly dI.dC (Sigma), 3 µg nuclear protein extract, 3 µg BSA, 4×10^4 cpm of ^{32}P-labeled oligonucleotide probe and 12 µl of 2 × Y buffer (24% glycerol, 24 mM HEPES pH 7.9, 8 mM Tris-HCl pH 7.9, 2 mM EDTA, and 2 mM DTT). This mixture was incubated on ice for 10 min in the absence of the radiolabelled probe, then incubated for 20 min at room temperature in the presence of radiolabelled probe. After the incubation, the DNA-protein complexes were resolved on a 5% acrylamide gel (National Diagnostics, Atlanta, GA, USA) that had been pre-run at 110 V for 1 h with 0.5 × Tris-boric acid-EDTA buffer. The loaded gel was run at 210 V for 90 min, then dried and placed on Kodak X-OMAT film (Eastman Kodak, Rochester, NY, USA) for autoradiography. The film was developed after overnight exposure at -70°C.

DNA strand breakage assays

The DNA strand breakage assay was carried out according to the method described earlier [20]. Briefly, reactions were carried out in 10 mM phosphate buffer (pH 7.4) in 1.5 ml polypropylene tubes at 37°C. Each reaction mixture contained 10 µg DNA (λ *Hind* III digest) in a total volume of 100 µl. DNA damage was assessed for each reaction by using a 10 µl sample of this mixture. To this solution 2 µl of gel loading buffer (50 mM EDTA, 2.5% sodium dodecyl sulfate (SDS), 0.1% bromophenol blue (BPB), and 6.25% glcerol) was added and then the sample was electrophoresed in 0.7% agarose at 1-2 V/cm in 40 mM Tris-acetate buffer containing 2mM EDTA (pH 8.0). Gels were stained in ethidium bromide (5 µg/ml) for 10 min and photographed under ultraviolet transillumination.

The concentrations provided in figure legends are final concentrations. All the experiments were carried out in air at room temperature except those specifically indicated.

Results

Scavenging of $^{\cdot}OH$ by EGCG

The Fenton reaction ($Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\cdot}OH + OH^-$) was used as a source of $^{\cdot}OH$ radicals. As shown in Fig. 2a, an aqueous solution containing Fe(II), H_2O_2, and a spin trap (DMPO) in a phosphate-buffered solution (pH 7.4), generated a 1:2:2:1 quartet with hyperfine splittings of $a_N = a_H = 14.9$

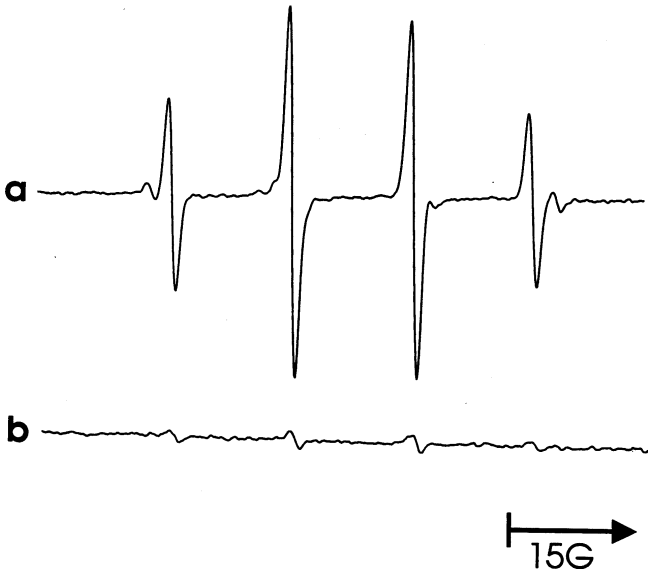

Fig. 2. ESR signals from DMPO/$^{\cdot}OH$ adducts obtained from the reaction of Fe(II) with H_2O_2. (a) ESR spectrum recorded 1 min after mixing 1.0 mM $FeCl_2$, 1.0 mM H_2O_2, and 10.0 mM DMPO in a pH 7.4 phosphate-buffered solution; (b) Same as (a) but with 0.5 mM mM EGCG added. The spectrometer settings were: receiver gain, 5.0×10^4; modulation amplitude, 0.5 G; magnetic field, 3500 ± 100 G; scan time, 4 min.

G. Based on these splitting constants, the 1:2:2:1 quartet was assigned to a DMPO/·OH adduct as indirect evidence for ·OH generation [16, 17]. Addition of 1 mM EGCG effectively reduced the intensity of the DMPO/·OH spin adduct signal (Fig. 2b), demonstrating that EGCG is an ·OH radical scavenger. The control, which has an equivalent volume of EGCG vehicle (without EGCG), did not alter the spectral intensity (data not shown), demonstrating that the effect of EGCG was not due to its vehicle.

Calculation of reaction rate constant of EGCG with ·OH

To determine the reaction rate constant of EGCG with ·OH radicals, we carried out kinetic studies according to methods reported earlier for the reaction of ethanol with ·OH [18, 19]. The reaction steps may be written as:

$$\cdot OH + DMPO \xrightarrow{k_d} DMPO/\cdot OH \quad [1]$$

$$k_d = 2.1 \times 10^9\ M^{-1}sec^{-1}$$

$$\cdot OH + EGCG \xrightarrow{k_E} product \quad [2]$$

$$-d[\cdot OH]/dt = k_d[DMPO][\cdot OH] + k_E[\cdot OH][EGCG] \quad [3]$$

$$d[DMPO/\cdot OH]/dt = k_d[DMPO][\cdot OH] \quad [4]$$

Dividing equation [3] by equation [4], one obtains equation [5].

$$\frac{-d[\cdot OH]/dt}{d[DMPO/\cdot OH]/dt} = 1 + \frac{k_E[EGCG]}{k_d[DMPO]} \quad [5]$$

At a saturating level of DMPO and in the absence of EGCG, the rate of ·OH spin trapping is equal to the rate of ·OH generation, d[·OH]/dt. If V and v represent the rate of ·OH spin trapping in the absence and in the presence of EGCG, respectively, one obtains equation [6].

$$V/v = 1 + k_E[EGCG]/k_d[DMPO] \quad [6]$$

or

$$V/v - 1 = \frac{k_E[EGCG]}{k_d[DMPO]} \quad [7]$$

Figure 3 shows the dose-dependent inhibition of ·OH by EGCG. The data were plotted according to equation [7]. A straight line is obtained with a slope of 22, which is the ratio of k_E/k_d (Fig. 3). Using the value of $k_d = 2.1 \times 10^9\ M^{-1}sec^{-1}$ for

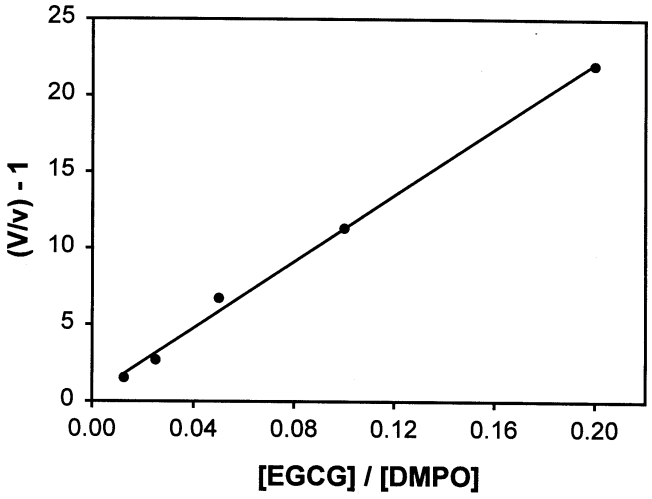

Fig. 3. Scavenging of ·OH radical by EGCG. The ·OH radicals were produced by the reaction of 1.0 mM $FeCl_2$, 1.0 mM H_2O_2 in the presence of 10.0 mM DMPO. The data were plotted according to V/v - 1 = $k_E[EGCG]/k_d[DMPO]$, as explained in the text.

the ·OH trapping by DMPO [20], the value of k_E is calculated as follows:

$$k_E = 22\ k_d$$

$$= 22 \times 2.1 \times 10^9\ M^{-1}sec^{-1}$$

$$= 4.62 \times 10^{11}\ M^{-1}sec^{-1}$$

It should be noted that the rate constant k_E as calculated above may not be very accurate. For example, this method did not include the decay of the spin adduct after its formation [21]. However, it does provide a convenient method for obtaining a relative value.

Scavenging of $O_2^{\cdot-}$ by EGCG

Xanthine and xanthine oxidase system was used as a source of $O_2^{\cdot-}$ radicals. As shown in Fig. 4a, a mixture of xanthine and xanthine oxidase in the presence of DMPO generated a spin adduct spectrum. Analysis of this spectrum shows hyperfine splittings of $a_N = 14.2$ G, $a_H = 11.5$ G, and $a_H^{\lambda} = 1.2$ G. These splittings are typical of the DMPO/$O_2^{\cdot-}$ [16]. Addition of EGCG decreased the intensity of DMPO/$O_2^{\cdot-}$ (Fig. 4b). The control, which has an equivalent volume of PDTC vehicle (without EGCG), did not alter the spectral intensity (data not shown), demonstrating that the effect of EGCG was not due to its vehicle. Thus EGCG appears to have $O_2^{\cdot-}$ scavenging activity.

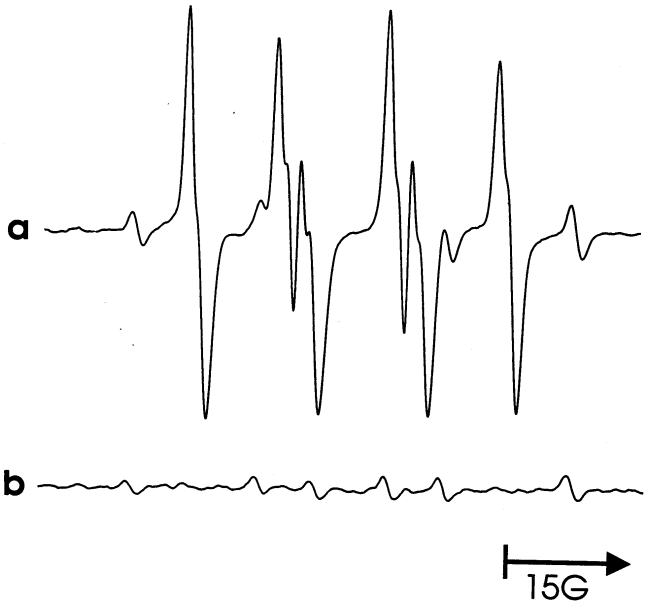

Fig. 4. ESR signals from DMPO/$O_2^{\cdot-}$ adducts obtained from a mixture of 0.05 U/ml xanthine oxidase, 0.3 mM xanthine and 50 mM DMPO in pH 7.4 phosphate-buffered solution. (a) ESR spectrum recorded 4 min after reaction initiation; (b) Same as (a) but with 1.25 mM EGCG added. The spectrometer settings were: receiver gain, 5.0×10^4; modulation amplitude, 0.5 G; magnetic field, 3500 ± 100 G; scan time, 4 min.

Inhibition of Cr(IV)- or TPA- induced NF-κB activation

A previous study has shown that Cr(IV) is able to induce NF-κB activation via ·OH radical-mediated reactions [22]. The present study investigates the possible protective effect of EGCG. Jurkat cells were used to detect Cr(IV)-induced NF-κB activation. The cell were incubated in the presence of Cr(IV) for 3 h and then were harvested for extraction of nuclear proteins. These proteins were analyzed by EMSA for the DNA binding activity of NF-κB. As shown in Fig. 5, the untreated Jurkat cells did not exhibit NF-κB activation (Fig. 5, lane 1). Cells treated with 2 μM C(IV) show enhanced DNA binding activity (Fig.5, lane 2). Addition of EGCG caused a dose dependent decrease in Cr(IV)-induced NF-κB activation (Fig. 5, lanes 3–6).

Cr(IV)-induced NF-κB activation was verified using competition and supershift assays. NF-κB complexes usually generate two bands in the gel. The upper band is formed by the p50/p65 heterodimer, and the lower band by the p50/50 homodimer. Since the upper band and lower band were both induced, the p65 and p50 subunits of NF-κB were both activated. The Jurkat cell nuclear protein formed a typical pattern of NF-κB bands with the radiolabeled probe. This was confirmed with oligonucleotide competition and antibody supershift studies although only p50 antibody was used (Fig. 6). Fig. 6, lane 1 shows NF-κB complexes stimulated by TPA. Unlabeled NF-κB probe effectively competed with the

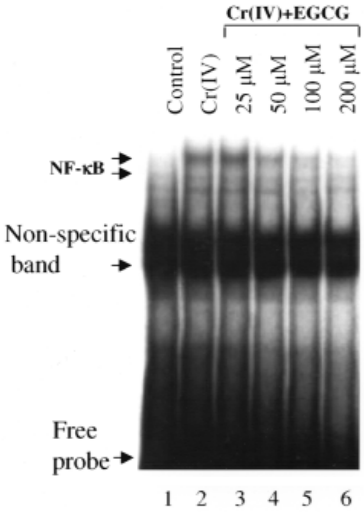

Fig. 5. Induction of DNA binding activity of NF-κB protein by Cr(IV) and its inhibition by EGCG. Jurkat cells were adjusted to a density of 2×10^6/ml and treated for 3 h with Cr(IV) or Cr(IV) and different concentrations of EGCG. Cells were then subjected to extraction of the nuclear protein as stated in the Materials and methods. DNA binding activity of the NF-κB protein was detected with a probe of ^{32}P-labeled double-strand NF-κB binding oligonucleotide by an EMSA assay. Lane 1, untreated cells; lane 2, 2 μM Cr(IV); lane 3, 2 μM Cr(IV) + 25 μM EGCG; lane 4, 2 μM Cr(IV) + 50 μM EGCG; lane 5, 2 μM Cr(IV) + 100 μM EGCG; lane 6, 2 μM Cr(IV) + 200 μM EGCG.

radiolabeled probe in NF-κB protein binding (Fig. 6, lane 2), while unlabeled AP-1 probe had no effect (Fig. 6, lane 3). The antibody against p50 subunit of NF-κB shifted the p50/p50 band and reduced the p50/p65 band (Fig. 6, lane 4), while

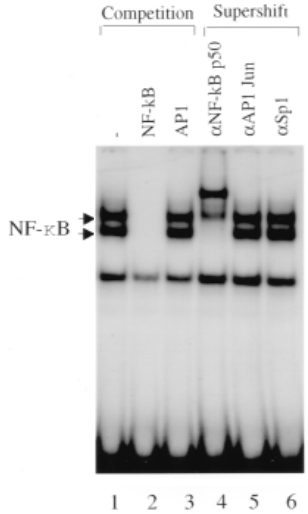

Fig. 6. Confirmation of NF-κB complexes. Lane 1 shows NF-κB complexes stimulated by TPA. The unlabeled NF-κB probe (100 μg) was used in lane 2 as a specific competitor. The same amount of unlabeled AP-1 probe was used in lane 3 for a nonspecific competition. The antibody (200 μg) against the p50 subunit of NF-κB protein was added in lane 4 to confirm the protein nature of DNA protein complexes. Lanes 5 and 6 contained 200 μg antibody against Sp-1 and c-Jun subunit of AP-1 protein, respectively, to serve as nonspecific antibodies.

unrelated antibodies to Sp-1 or AP-1 had no effect (Fig. 6, lanes 5 and 6).

TPA is a potent NF-κB inducer. Antioxidants, such as N-acetyl-L-cysteine, inhibit TPA-induced NF-κB activation [23]. In the present study, TPA-induced NF-κB activation in Jurkat cells was used as another model system to examine the possible protective effect of EGCG. Fig. 7 shows the activation of NF-κB by TPA and the effect of EGCG. Fig. 7, lane 1 shows the untreated cells as a control. TPA induced NF-κB activation (Fig. 7, lane 2). Addition of EGCG caused a dose dependent decrease in TPA-stimulated NF-κB activation (Fig. 7, lanes 3–7).

Inhibition of Cr(VI)-induced DNA damage

Cr(VI)-induced DNA damage was used as a model system to examine the protective effect of EGCG. Earlier studies have shown that glutathione reductase is able to reduce Cr(VI) to Cr(V) using NADPH as a cofactor [17, 24]. During the reduction process, molecular oxygen is reduced to H_2O_2 via $O_2^{\cdot-}$ as an intermediate. Cr(VI) reacts with H_2O_2 to generate ^{C}OH radicals, which cause DNA damage. Fig. 8, lane 1 shows the DNA alone as a control. Cr(VI), NADPH glutathione reductase (generating $^{\cdot}OH$ radicals via Cr(V), H_2O_2 as intermediates) caused DNA damage (Fig. 8, lane 2). Sodium formate, an $^{\cdot}OH$ radical scavenger, inhibited this DNA damage (Fig. 8, lane 3). EGCG at a concentration of 0.2 mM exhibited no protection (Fig. 8, lane 4). An increase in EGCG concentration enhanced the protective effect (Fig. 8, lanes 4–6).

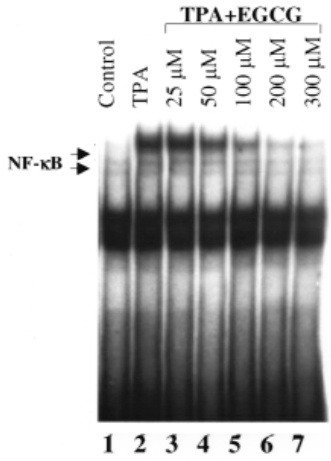

Fig. 7. Induction of DNA binding activity of NF-κB protein by TPA and its inhibition by EGCG. Jurkat cells were adjusted to a density of 2×10^6/ml and treated for 3 h with TPA or TPA plus different concentrations of EGCG. Cells were then subjected to extraction of the nuclear protein as stated in the Materials and methods. DNA binding activity of the NF-κB protein was detected with a probe of ^{32}P-labeled double-strand NF-κB binding oligonucleotide by an EMSA assay. Lane 1, untreated cells; lane 2, 10 ng/ml TPA; lane 3, 10 ng/ml TPA + 25 μM EGCG; lane 4, 10 ng/ml TPA + 50 μM EGCG; lane 5, 10 ng/ml TPA + 100 μM EGCG;lane 6, 10 ng/ml TPA + 200 μM EGCG; lane 7, 10 ng/ml TPA + 300 μM EGCG.

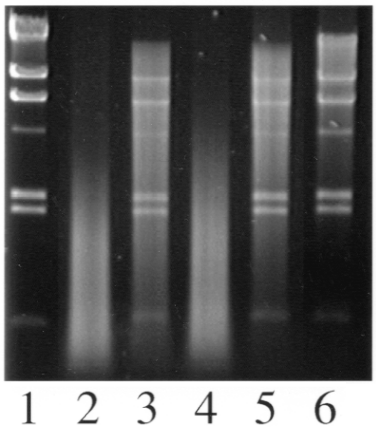

Fig. 8. DNA strand breaks by Cr(VI) reactions. (a) Lane 1, untreated control λ *Hin*d III-digested DNA in pH 7.4 phosphate buffer; lane 2, same as lane 1 but with 2 mM Cr(VI), 1 mM NADPH and 0.5 mg/ml glutathione reductase added; lane 3, same as lane 2 but with 25 mM sodium formate added; lane 4, same as lane 2 but with 0.2 mM ECGC added; lane 5, same as lane 2 but with 2 mM ECGC added; lane 6, same as lane 2 but with 4 mM ECGC added.

Discussion

Free radical reactions have been found to be involved in the pathogenesis of a variety of diseases. Use of antioxidants is an important strategy against diseases caused by free radical reactions. In recent years, there has been a growing interest in identifying potentially important antioxidants against free radicals, specially those from naturally occurring substances. These substances include fruits and vegetables. Epidemiological studies have shown an association between those with diets rich in fresh fruits and vegetables and a decreased risk of cancer and other diseases [25]. While further studies remain to be done, it is generally believed that the antioxidant constituents contribute to these protective effects. The polyphenolic components of certain plants may function as antioxidants providing an anticarcinogenic action. Tea contains a relatively high concentration of these compounds. EGCG is one of them. Using ESR spin trapping with DMSO as a spin trap, the present study shows that EGCG is an efficient $^{\cdot}OH$ radical scavenger. The reaction rate constant is 4.62×10^{11} $M^{-1}sec^{-1}$, which is an order of magnitude higher than other well established antioxidants, such as ascorbate ($1.2 \times 10^{10} M^{-1}sec^{-1}$), GSH ($1.5 \times 10^{10} M^{-1}sec^{-1}$) and cysteine ($1.3 \times 10^{10} M^{-1}sec^{-1}$) (Table 1). This large rate constant may be due to the existance of 8 hydroxyl groups in the structure of EGCG molecule (Fig. 1). Using xanthine and xanthine oxidase as a source of $O_2^{\cdot-}$ radicals, the present

Table 1. Reaction rate constants of some antioxidants with ·OH radical

Antioxidant	k ($M^{-1}sec^{-1}$)	References
ascorbate	1.3×10^{10}	(26)
glutathione	1.5×10^{10}	(26)
cysteine	1.5×10^{10}	(26)
azide	1.1×10^{10}	(26)
EGCG	4.62×10^{11}	Present study

study demonstrates that EGCG is also able to scavenge $O_2^{\cdot-}$ radicals.

It may be noted that the concentration of EGCG used spin trapping is very high. It is the rate constant and not the concentrations used to calculate this constant that determine whether EGCG has antioxidant properties. For increasing the ·OH radical trapping efficiency and determining a relatively more accurate reaction rate of k_E, a relatively high concentration, of DMPO is used. Because spin trapping agent itself is an excellent ·OH scavenger, DMPO will react with ·OH radical in competition with EGCG. In the absence of DMPO, EGCG will efficiently react with ·OH radical and other oxygen free radicals.

In the present study, EGCG was examined for its possible inhibitory effect on NF-κB activation. NF-κB is considered a primary oxidative stress response transcription factor that functions to enhance the transcription of a variety of genes, including certain oncogenes, such as c-*myc*. Previous studies have shown that ·OH radicals generated by chromium-mediated free radical reactions play a prominent role in the chromium-induced NF-κB activation [22, 27]. Since this transcription factor is considered to play a key role in the mechanism of Cr(VI)-induced carcinogenesis, EGCG may exhibit its protection against carcinogenesis induced by Cr(VI). Similarly, EGCG may also inhibit NF-κB activation induced by other carcinogens, such as nickel, silica and asbestos, via the scavenging of ·OH radicals.

Another important result is that EGCG is able to inhibit TPA-induced NF-κB activation. TPA is a potent tumor promoter. It induces a wide range of other biological effects in cultured cells. It has been reported that topical application of a polyphenolic fraction isolated from green tea protects against TPA-induced tumor promotion in 7,12-dimethylbenz[a]anthracene-initiated SENCAR mouse skin in a dose-dependent manner [28]. One might speculate that EGCG may inhibit TPA-induction tumor promotion via inhibition of NF-κB activation.

In the present study, EGCG has been examined for its ability to inhibit DNA damage caused by free radical reactions. Previous studies have shown that glutathione reductase in the presence of NADPH is able to reduce Cr(VI) to generate Cr(V) [17, 24]. In the reduction processes, molecular oxygen is reduced to $O_2^{\cdot-}$, which generates H_2O_2 via dismutation. Cr(V) will react with H_2O_2 to generate ·OH radical [17, 24, 29]. The ·OH radicals generated by this reaction caused DNA strand breaks. Although Cr(VI) can be reduced by various reductants, such as ascorbate and glutathione, recent in vivo ESR studies have shown that glutathione reductase is likely to be the major reductant responsible for Cr(VI) reduction in vivo [30]. It is generally believed that free radical reactions mediated by chromium intermediates, such as Cr(V), play a key role in the mechanism of Cr(VI)-induced carcinogenesis [31]. The results obtained from the present study demonstrate that EGCG can inhibit Cr(VI)-induced DNA damage by scavenging ·OH radicals. It may be noted that many carcinogens, such as nickel, silica and asbestos, exert their carcinogenic effects by producing free radicals. EGCG has the potential to act as a therapeutic agent against carcinogenesis induced by these carcinogens.

In conclusion, the results presented here demonstrate that (a) EGCG is an efficient ·OH radical scavenger with reaction rate constant of $k = 4.62 \times 10^{11} M^{-1}sec^{-1}$, which is comparable to several well recognized antioxidants, such as ascorbate, glutathione and cysteine. (b) EGCG is also a scavenger $O_2^{\cdot-}$ radical. (c) EGCG inhibits Cr(VI)-induced DNA damage. (d) EGCG inhibits Cr(VI)- and TPA-induced NF-κB activation.

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