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Tea Catechins Protect against Lead-Induced Cytotoxicity, Lipid Peroxidation, and Membrane Fluidity in HepG2 Cells

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Recent studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and reducing the antioxidant defense system of cells. This suggests that antioxidants may play an important role in the treatment of lead poisoning as a kind of excellent scavenger of free radicals and chelator of heavy metal. Whether tea catechins have protective effects against oxidative stress after lead treatment in cell systems remains unclear. The present study was designed to elucidate if tea catechins have any protective effects on lipid peroxidation damage in lead-exposed HepG2 cells. Exposure of HepG2 cells to Pb^{++} decreased cell viability and stimulated lipid peroxidation of cell membranes as measured by the thioburbituric acid reaction. Electron spin resonance (ESR) spin-labeling studies indicated that lead exposure could decrease the fluidity in the polar surface of cell membranes. Tea catechin treatment significantly increased cell viability, decreased lipid peroxidation levels, and protected cell membrane fluidity in lead-exposed HepG2 cells in a concentration-dependent manner. The galloylated catechins showed a stronger effect than nongalloylated catechins. Cotreatment with (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG) showed a synergistically protective effect. The results suggest that tea catechin supplementation may have a role to play in modulating oxidative stress in lead-exposed HepG2 cells.

Key Words: **tea; catechins; lead toxicity; lipid peroxidation; antioxidants; oxidative stress; ESR spin labeling; membrane fluidity.**

Lead (Pb) is one of the first discovered and most widely used metals in human history and is, therefore, one of the metals most commonly encountered in the environment (Shotyk *et al.*, 1998). Its continued release into the environment as an exhaust emission product, as well as its widespread industrial use, has made lead a serious threat to human health (Juberg *et al.*, 1997). Pregnant women, children, and inhabitants of large cities are at risk of lead intoxication, and lead poisoning is a serious occupational disease in some industries. Exposure to low levels of lead has been associated with behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in experimental animals (Cory-Slechta *et al.*, 1995).

A growing amount of evidence indicates that cellular damage mediated by reactive oxygen species (ROS) may be involved in the pathology associated with lead intoxication (Bechara *et al.*, 1993; Hermes-Lima *et al.*, 1991). The malondialdehyde levels in blood were strongly correlated with lead concentration in the blood of exposed workers (Jiun and Hsien, 1994). In erythrocytes from the workers exposed occupationally to lead, the activities of the antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase, were remarkably higher than that in non-exposed workers (Monteiro *et al.*, 1985). Gurer *et al.* demonstrated that lead increased the prooxidant/antioxidant ratio in a concentration-dependent manner in lead-treated CHO cells and in rats (Gurer *et al.*, 1999). The results suggest that antioxidants might play an important role in the treatment of lead poisoning.

Tea, including black, green, and oolong tea, is one of the most widely consumed beverages in the world. During the last decade, numerous *in vitro* and *in vivo* studies had suggested that tea and tea polyphenols had strong antioxidant activity (Guo *et al.*, 1996, 1999; Shen *et al.*, 1993), and had numerous potentially beneficial medicinal properties including inhibition of carcinogenesis, tumorigenesis, and mutagenesis, as well as the inhibition of tumor growth and metastasis (Yang *et al.*, 1993). The major polyphenolic compounds in tea are catechins. The four most abundant naturally occurring tea catechins, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG) are shown in Figure 1.

The antioxidant activities of tea catechins have been examined by various methods *in vitro* and *in vivo*. Using the chemiluminescence method and the ESR spin-trapping technique, our previous data indicated that green tea polyphenols had higher antioxidant activity than that of vitamins C and E (Zhao *et al.*, 1989). In the lipoprotein oxidation model, which simulates the oxidation of low-density lipoproteins responsible for atherosclerosis, tea catechins also exhibited powerful antioxidant activity: 20 times more potent than vitamin C (Craig, 1999; Vinson *et al.*, 1995). It also has been found that the presence of at least an ortho-dihydroxyl group in the B ring and a galloyl moiety at the 3 position were important in maintain-

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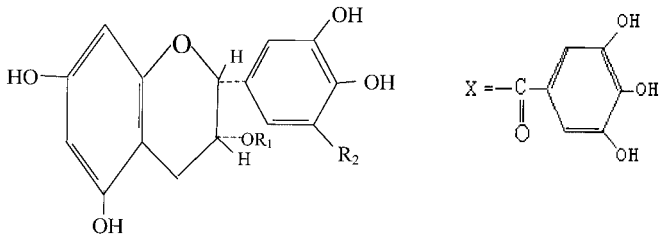

FIG. 1. Chemical structures of green tea catechins. (EC):(-)-Epicatichin R_1 = H, R_2 = H; (EGC): (-)-Epigallocatichin R_1 = H, R_2 = OH; (ECG): (-)-Epicatichin gallate R_1 = X, R_2 = H; (EGCG): (-)-Epigallocatichin gallate R_1 = X, R_2 = OH.

ing the effectiveness of their radical scavenging ability (Hayakawa *et al.*, 1999; Guo *et al.*, 1999). However, catechins in high concentration, or in the presence of Cu^{2+}, induced DNA cleavage and accelerated the peroxidative property of unsaturated fatty acid, showing prooxidant (Hayakawa *et al.*, 1997; Shen *et al.*, 1992). Therefore, it is important to elucidate the relationship between their structures and functions, concentration, and effect in a cell system.

Tea is a kind of excellent scavenger of free radicals and chelator of heavy metal (Guo *et al.*, 1991; Kumamoto *et al.*, 2001), but whether tea catechins have protective effects on oxidative stress after lead treatment remains unclear. The present study showed tea catechins could reduce the toxicity of lead in HepG2 cells by examination of the effect of lead on cell viability, malondialdehyde (MDA) levels, and cell-membrane fluidity in the presence or absence of different kinds of tea catechins.

MATERIALS AND METHODS

Reagents. Dulbecco's modified Eagle's medium (DMEM), newborn calf serum and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliunbromide) were purchased from GIBCO Life Technologies. 5-Doxylstearic acid (5-doxyl), 16-doxylstearic acid (16-doxyl), and dimethyl sulphoxide (DMSO) were purchased from Sigma. EGCG, ECG, EGC, and EC were kindly provided by Zhejiang University, and their purity was determined to be >98% by HPLC. All other chemicals made in China were analytical grade.

Cell culture. The human hepatocellular carcinoma cell line HepG2 retains many parenchymal cell functions. It has been shown that it is useful for evaluations of the mechanism of toxicity (Borenfreund *et al.*, 1990; Marinovitch *et al.*, 1988). HepG2 cells were grown as monolayer cultures in DMEM, supplemented with 10% heat-inactivated newborn calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 1% non-essential amino acid, and 2 mM glutamine. Only cells in exponential growth were used for the experiments.

Cells were grown at 37°C in disposable plastic bottles (Nunc, USA) in a humidified atmosphere of 5% CO_2, 95% air. The medium was replaced twice a week, and cells were trypsinized and diluted every 7 days at a ratio of 1:3.

Cell viability assay. In the experiments, 1×10^4 cells were plated in each well of 96-well plates, and were allowed to attach to the substrate for a 24-h period. Cells were exposed to lead for an additional 24 h in the absence or presence of different concentrations of tea catechins. Cell viability was determined using the MTT assay (Mosmann *et al.*, 1983). In brief, 20 μl of 5 mg/ml MTT in PBS was added to each well and the plates incubated at 37° for a further 4 h. The media were then removed and the purple formazan crystals dissolved in 150 μl DMSO. The absorbency of each well was then measured at 570 nm with a Bio-RAD 3350 microplate reader, and the percentage viability was calculated.

Measurement of TBARS. Lipid peroxidation was assayed by determining the production rate of thiobarbituric acid reactive substances (TBARS) and was expressed as malondialdehyde (MDA) equivalents. In brief, cells grown on 6-well plates were washed with 0.01 M PBS, scraped, and resuspended in 1 ml PBS. An aliquot was taken out for a protein assay, and 0.5 ml TBA reagent (100 mg trichloroacetic acid, 3.35 mg thiobarbituric acid) was added to each tube and vortexed. The reaction mixture was incubated at 90°C for 20 min and stopped on ice. After cooling to room temperature, TBARS were extracted with 1.0 ml n-butanol and separated at $3000 \times g$ centrifugation for 5 min. The absorbency of the total TBARS was measured at 532 nm. Tetraethoxypropane in absolute ethanol was used to prepare MDA standards. The measurements were performed in triplicate and the results were expressed as nmol equivalent of MDA/mg protein.

Spin labeling the cells with 5-doxyl or 16-doxyl. Fatty acid spin-labels of 5-doxyl and 16-doxyl, which have a stable nitroxide radical ring at the C-5 and C-16 positions, respectively, were used as a lipid probe in the cell membrane. They are well dissolved in lipids and their ordering and dynamics reflect the motion of the surrounding phospholipid hydrocarbon chains. In brief, 100 μl HepG2 cell suspension (10^7 cells/ml) was mixed with 5 μl of 5-doxyl or 16-doxyl (1.0 mM) spin label, incubated at 37° for 60 min, then the free labels washed out by 0.01 M PBS from the cell system until there were no ESR signals in the supernatant. ESR measurement condition: microwave power 20 mW, modulation amplitude 0.2 mT, X-band, modulation frequency 100 KHz, sweep width 10 mT, and temperature 298K.

Membrane fluidity calculation. The membrane fluidity characteristics were estimated from the line width and shape of the ESR spectra. Lower order and faster motion means higher membrane fluidity. The order of membrane hydrocarbon chains is described by the order parameter (S) and their motion is described by the rotational correlation time (τ_c). They are defined as follows (Juntao *et al.*, 2001):

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - 0.5(A_{xx} + A_{yy})}$$

$$\tau_c = 6.51 \times 10^{-10} \times \Delta H(0)[\sqrt{h(0)/h(-1)} + \sqrt{h(0)/h(1)} - 2]$$

where h(0), h(1), and h(−1) are the peak height of the center, low, and high field lines, respectively; $\Delta H(0)$ is the width of the central line; and $A_{\parallel}$ and $A_{\perp}$ are parallel and perpendicular hyperfine splitting parameters of the spectrum, respectively, as shown in Figure 2.

Data analysis. Throughout the text, data were expressed as mean ± SE of triplicate determinations, from at least three independent experiments. Statistical analysis was determined using a 1-way analysis of variance (ANOVA) with $p < 0.05$ considered significant.

RESULTS

The maximum non-cytotoxic concentrations of tea catechins. To select appropriate concentrations of tea catechins to be used in this study, the maximum non-cytotoxic concentrations were determined. After the initial 24-h attachment period, cells were exposed to different concentrations of catechins for an additional 24 h, and the cytotoxicity was measured by the MTT method. As shown in Figure 3, the maximum non-cytotoxic concentrations of EGCG, ECG, EGC and EC were about 15, 5, 80, and 20 μM, respectively.

Protective effects of tea catechins on lead-exposed HepG2 cell viability. Using the MTT method, it was found that the toxicity of Pb^{++} was both time- and concentration-dependent

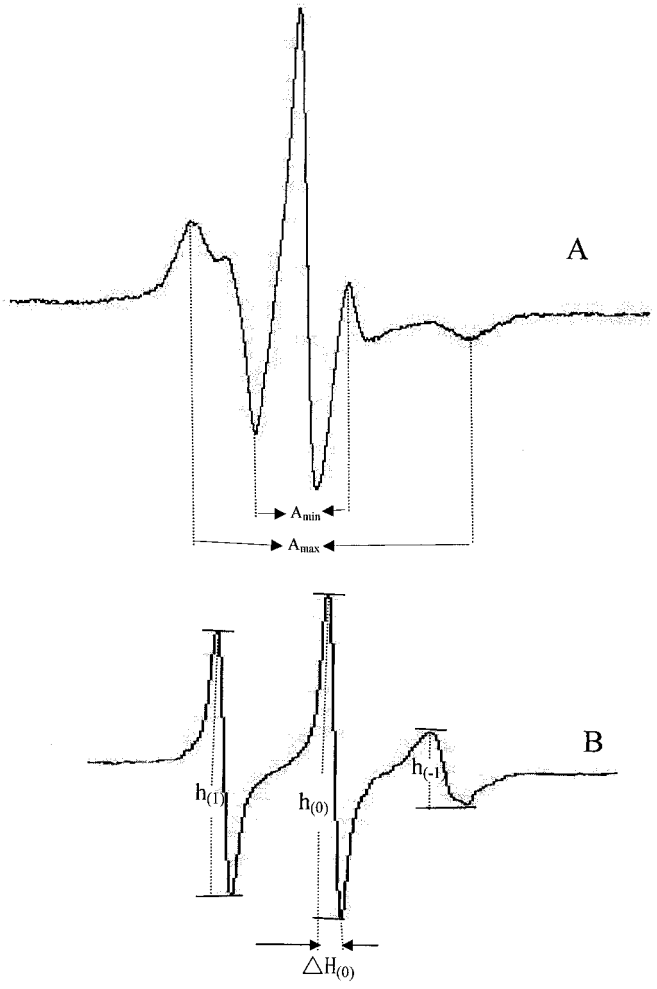

FIG. 2. Typical ESR spectra of the 5-doxyl (A) and 16-doxy l (B) labeled HepG2 cell. The detailed experimental conditions were described in Materials and Methods.

in HepG2 cells, as shown in Figure 4 when the concentration exceeded to 1.0 μM, Pb^{++} significantly decreased HepG2 cell viability. It was evident that increasing exposure times showed steeper concentration-response curves.

In order to test the protective effects of tea catechins, HepG2 cells were simultaneously exposed to 100 μM Pb^{++} and tea catechins. As shown in Figure 5, the results indicated that tea catechins significantly increased viability of lead-exposed HepG2 cells in the used concentration. The effective protective concentration for EC EGC, ECG, and EGCG were about 15–20 μM, 40–50 μM, 3–5 μM, and 8–15 μM, respectively. As far as EC and EGCG were concerned, in the range of nontoxic concentration, the higher the concentration was, the stronger the protective effect became. It was surprising to find that 80 μM EGC synergistically promoted lead-induced cell toxicity.

Furthermore, the synergistic protective effect of tea catechins against Pb^{++}-induced cell toxicity was studied. As shown in Figure 6, simultaneous treatment by two kinds of

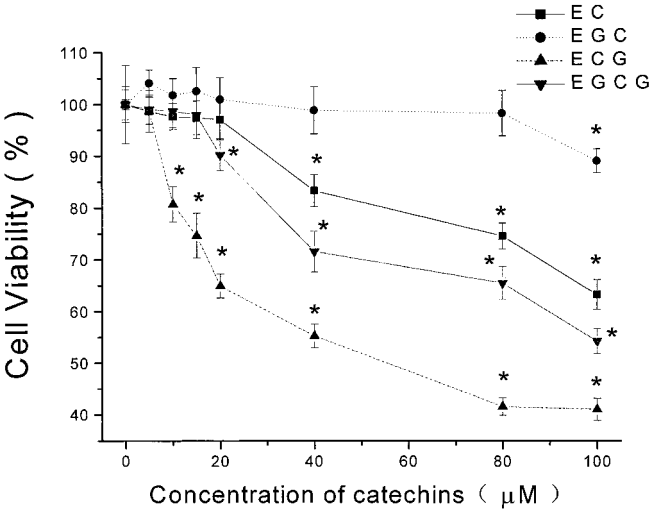

FIG. 3. Effect of different concentrations of tea catechins on HepG2 cell viability. HepG2 cells were cultured with different catechins for 24 h and cell viability was determined by the MTT method. Data are expressed as a percentage of the untreated control ± SE, $n = 7$. *Significant difference from control by ANOVA, $p < 0.05$.

catechins showed better protective effect on cell viability than one catechin, among which EC + EGCG was the best one.

Inhibitory effect of tea catechins on TBARS formation in HepG2 cell exposed to lead. In recent studies, the toxic effects of lead have been attributed to lead-induced oxidative stress and -stimulated lipid peroxidation of membrane lipids.

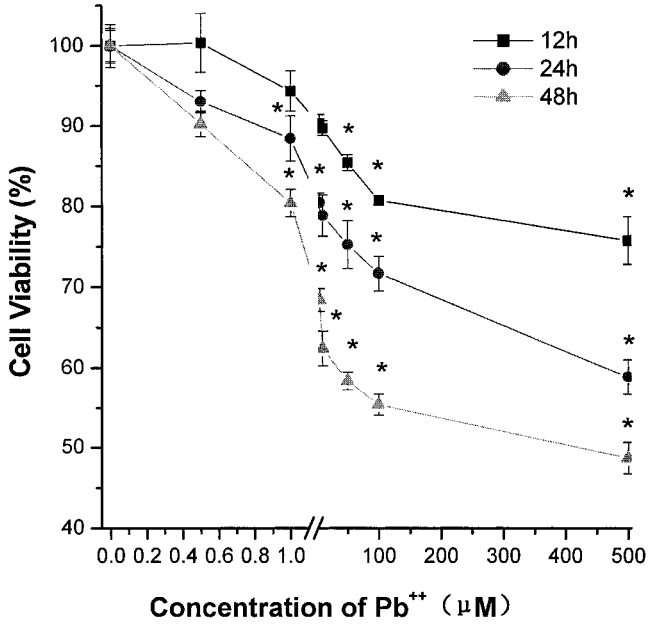

FIG. 4. Effect of Pb^{++} on HepG2 cell viability. HepG2 cells were cultured with various concentrations of Pb^{++} for different times, and cell viability was determined by MTT assay. Data are expressed as a percentage of the untreated control ± SE, $n = 7$. *Significant difference from control by ANOVA; $p < 0.05$.

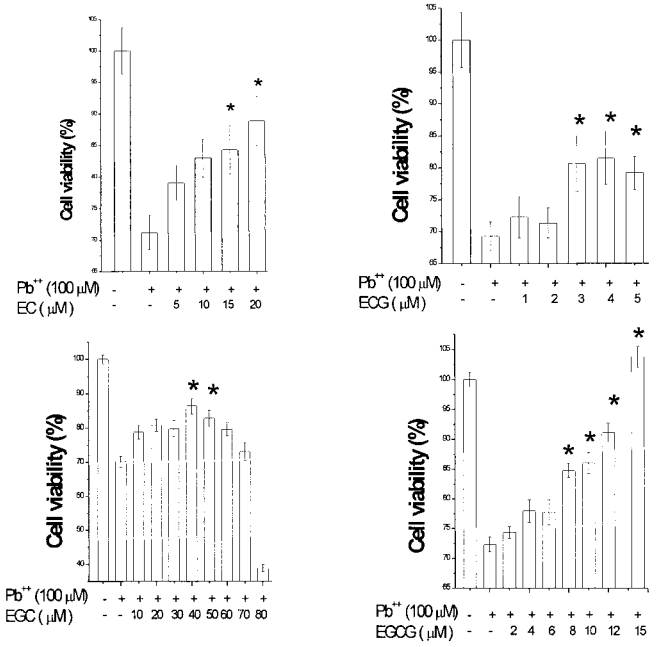

FIG. 5. Effect of tea catechins on Pb^{++}-induced cell toxicity. After the initial 24-h attachment period, HepG2 cells were exposed to both Pb^{++} (100 μM) and different concentrations of tea catechins for an additional 24 h, and cell viability was determined by MTT assay. Data are expressed as a percentage of the untreated control ± SE, $n = 7$. *Significant difference from control in the presence of 100 μM Pb^{++} by ANOVA; $p < 0.05$.

This process results in the production of lipid radicals and in the formation of a complex mixture of lipid degradation products (MDA and other aldehydes), which are extremely toxic for the cells. As shown in Figure 7, 0.01 μM Pb^{++} had no effect on TBARS formation, but when the concentration of Pb^{++} exceeded 0.1 μM, Pb^{++} significantly promoted TBARS formation in hepG2 cells in a concentration-dependent manner

In order to determine the structure-activity relation of different catechins, the effects of tea catechins on TBARS formation in HepG2 cells treated by Pb^{++} were studied. Compared with the control, only treated by 100 μM Pb^{++}, all catechins significantly decreased TBARS formation in the range of the maximum non-cytotoxic concentrations, except that 80 μM EGC showed notably prooxidant (Fig. 8). The synergistic inhibitory effect of tea catechins on TBARS formation in HepG2 cells exposed to lead is shown in Figure 9. It was found that co-treatment with EC and EGC, EGCG and EC, ECG, and EGCG had a synergistic inhibitory effect against TBARS formation.

Protective effect of tea catechins on membrane fluidity in HepG2 cells exposed to lead. The order parameter (S) calculated from the spectra is shown in Figure 10. From the data labeled by 5-doxyl, it was found that Pb^{++} increased the order parameter (S) in a concentration-dependent manner, and the difference was significant when its concentration reached 100 μM ($p < 0.05$). In addition, from the data labeled with 16-doxyl, it was found that 100 μM Pb^{++} also lightly increased the rotational correlation time (τ_c), but the difference was not significant, even if the concentration of Pb^{++} reached 500 μM (data not shown). The results suggested that 100 μM Pb^{++} exposure decreased the fluidity near the polar surface of the cell membrane, but the membrane fluidity in the hydrophobic region was not affected significantly.

Tea catechins decreased the order parameter (S) of Pb^{++}-exposed HepG2 cell membrane in a concentration-dependent manner, as shown in Figure 11. The order parameter (S) treated by both 5 μM ECG and 15 μM EGCG showed little difference from the control. As shown in Figure 12, both EGCG + ECG and EC + EGCG showed synergistic effects on order parameter (S), which is similar to those of both cell viability and TBARS formation.

DISCUSSION

Many pieces of evidence suggest that cellular damage mediated by oxidative stress may be involved in some of the pathologies associated with lead toxicity (Adonaylo *et al.*, 1999; Sandhir *et al.*, 1995). Lead stimulated oxidative hemolysis of erythrocytes, decreased erythrocyte SOD activity and accelerated conversion of oxyhemoglobin to methemoglobin (Gurer *et al.*, 2000). An inverse relationship was observed between blood-lead concentration and serum levels of α-tocopherol and ascorbic acid in pregnant women (West *et al.*, 1994). Therefore, it is reasonable to believe that antioxidants should be considered as a component of an effective treatment for lead poisoning.

Tea catechins are strong scavengers against superoxide, hydrogen peroxide, hydroxy radicals, and nitric oxide produced by various chemicals. They also could chelate with metals because of the catechol structure (Rice-Evans *et al.*, 1997). These characteristics make tea catechins ideal candidates for treatment of lead toxicity. The data from our studies of HepG2 cells indicated that the higher concentration of lead treatment decreased cell viabilities and increased lipid peroxidation levels. Treatment by tea catechins increased cell viability and reversed the effects of lead on oxidative stress parameters in a concentration-dependent manner. The galloylated catechins showed stronger protective effect against oxidative damage than that of nongalloylated catechins, which is similar to the result of scavenging ability on free radicals (Guo *et al.*, 1996, 1999). Galloylated catechins containing more phenolic hydroxyl groups had stronger chelating ability with metal ions than nongalloylated catechins (Guo *et al.*, 1991). Therefore, the protective effect of tea catechins on oxidative damage in HepG2 cells exposed to lead might be related to both their ability to scavenge free radicals and to chelate metal ions.

ESR spin labeling technique is a sensitive and reliable method to study the physical state of cell membranes. Order parameter (S) and rotational correlation time (τ_c) represent the degree of hydrocarbon chains′ long-range alignments along the membrane and the motion state of these chains. As shown in Figure 7, the increase of lipid peroxidation levels indicated that

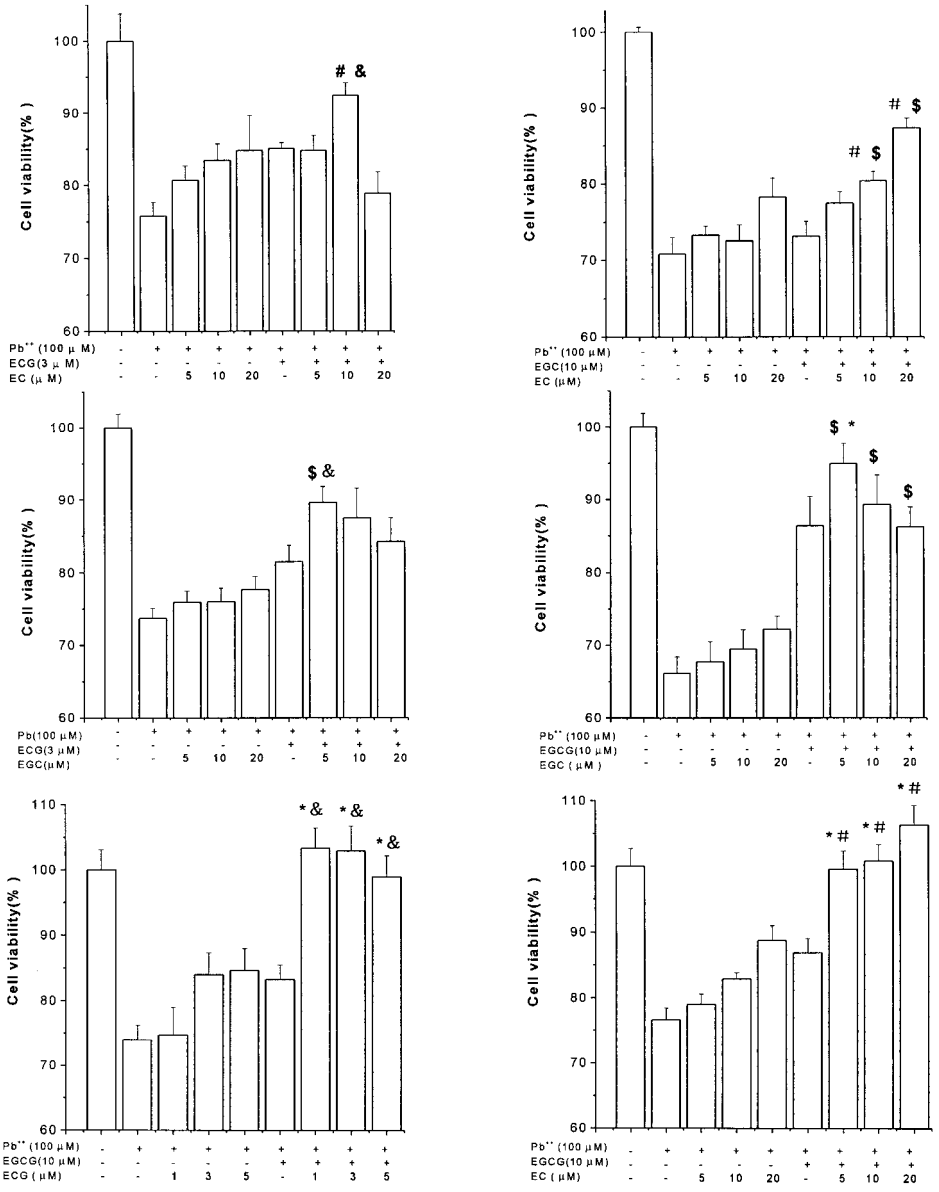

FIG. 6. Synergistic effect of tea catechins on Pb^{++}-induced cell toxicity. After the initial 24-h attachment period, HepG2 cells were exposed to both Pb^{++} (100 μM) and different tea catechins for an additional 24 h, and cell viability was determined by MTT assay. Data are expressed as a percentage of the untreated control ± SE, $n = 7$. #, &, $, *: Significant difference from the corresponding concentration of EC, ECG, EGC, and EGCG, respectively; $p < 0.05$.

lead caused oxidative damage to hepatic cell membranes. The peroxidation of hepatic cell membrane phospholipids and accumulation of lipid peroxides are expected to modulate the membrane fluidity and consequently the membrane function. The observed changes in the rotational correlation time (τ_c) and order parameter (S) (Fig. 10) indicated that the fluidity near the surface of the membrane was decreased after 100 μM-lead treatment, but the fluidity in the hydrophobic core of the membrane was not affected after the treatment. Lead induced arachidonic acid augmentation (Lawton *et al.*, 1991) and bound strongly to phosphatidylcholine membranes *in vitro* (Shafiq-ur-Rehman *et al.*, 1993), which could result in altered membrane integrity, permeability, and fluidity. These might be connected with the enhanced lipid peroxidation in HepG2 cells.

Tea catechins are mainly composed of 50–60% EGCG, 8–12% EGC, 15–20% ECG, and 4–7% EC. As reported previously, tea catechins scavenged free radicals in the order: EGCG ≈ ECG > EGC > EC (Guo *et al.*, 1996, 1999). Okabe also reported the similar order in inhibiting growth of human lung cancer cell line PC-9 (Okabe *et al.*, 1997). Because of its high activity and content, EGCG seems to be the most effective antioxidant in all the components of green tea catechins. However, several researches showed that the tea catechin complex had a stronger effect than EGCG in the scavenging capacity of free-radical and anticarcinogenic activities (Shen *et al.*, 1993). This allows us to think that the constituents of tea catechin complex together have synergistic or additive effects on scavenging free-radical and cancer-preventive activity. Support for this activity was obtained from Suganuma's study that (^{3}H) EGCG incorporation into PC-9 cells was significantly enhanced by EC. Also, co-treatment with EGCG, EC, ECG, EC,

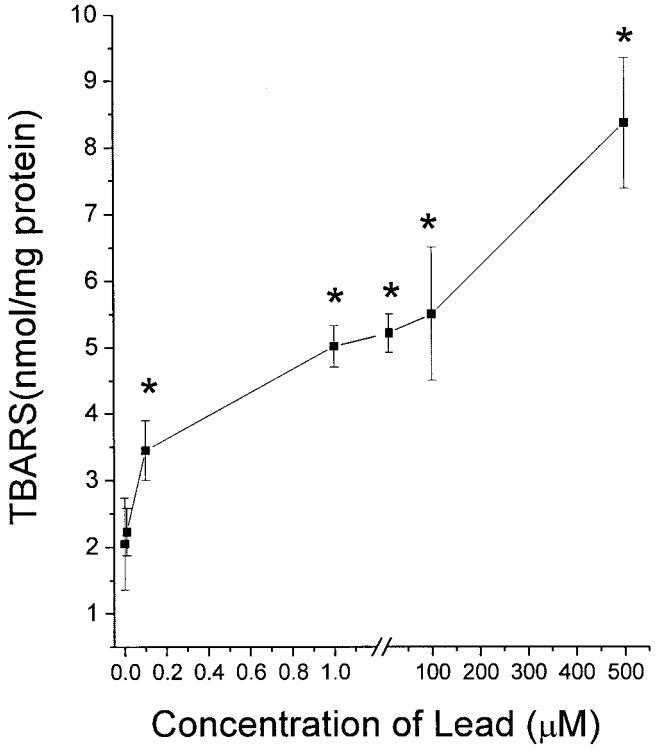

FIG. 7. Effects of different concentrations of Pb^{++} on TBARS formation in HepG2 cells. HepG2 cells were cultured with various concentrations of Pb^{++} for 24 h and the lipid peroxidation level was determined as described in Materials and Methods. *Significant difference from control by ANOVA; $p < 0.05$.

EGC, and EC synergistically induced apoptosis of PC-9 cells and inhibited tumor necrosis factor-α release from BALB/c-3T3 cells (Okabe *et al.*, 1997; Suganuma *et al.*, 1999).

Our previous research also demonstrated that various catechins in tea polyphenols constituted an antioxidant cycle, in accordance with the decreasing order of their first reductive potentials, and produced a coordinating, strengthening effect (Shen *et al.*, 1993). As shown in Figures 6, 9, and 12, the current data indicated that both EC and ECG significantly promoted the protective effect of EGCG. The mechanisms of action of ECG and EC are thought to be different because ECG did not stimulate EGCG incorporation into cells, whereas EC did (Suganuma *et al.*, 1999). Although the co-treatment with ECG and EGCG produced interesting results, the mechanisms of the action have not been well identified. Hashimoto *et al.* (1999) found that ECG had the highest affinity for the lipid bilayer in membrane, followed by EGCG, EC, and EGC, with the partition coefficients of ECG in *n*-octanol/PBS being highest. Our former research suggested that the closer the first reductive potentials were, the more significant the coordinating and strengthening effects became (Shen *et al.*, 1993). Therefore, it is reasonable to deduce that the closer first reductive potential of EGCG and ECG, as well as their stronger affinity for lipid bilayer, allows them to easily enter the cell membrane and to show synergic effect. But the mechanism should be further investigated.

Recently, much attention has been paid to the prooxidant quality of natural products. It has been reported that, in the presence of the copper (II) ion under aerobic conditions, tea catechins induced DNA cleavage, accelerated the peroxidation of unsaturated fatty acid (Hayakawa *et al.*, 1997), and killed *Escherichia coli*. (Kimura *et al.*, 1998). These effects were apparently due to the prooxidant property of catechins. Our previous research also showed that both tea catechins complex and EGCG produced superoxide anion radical and semiquinone anion radicals in alkaline solution *in vitro* (Shen *et al.*, 1992). The results in this paper showed that even in the range of maximum nontoxic concentration, EGC demonstrated significant prooxidant signs, as shown in Figures 5 and 8. These might be correlated with the toxicological effect of tea catechins. The investigation also placed catechins, under certain conditions, into radical-generating toxicological agents. Therefore, much consideration for safety should be required when tea catechins are used as therapeutic reagents or nutrition supplements.

Tea catechins are strong metal ion chelators because of the catechol structure (Guo *et al.*, 1991; Kumamoto *et al.*, 2001; Rice-Evans *et al.*, 1997). Though they have been shown to form stable complexes with Fe^{2+}, Ca^{2+}, Al^{3+}, Mn^{2+}, Cr^{3+}, and

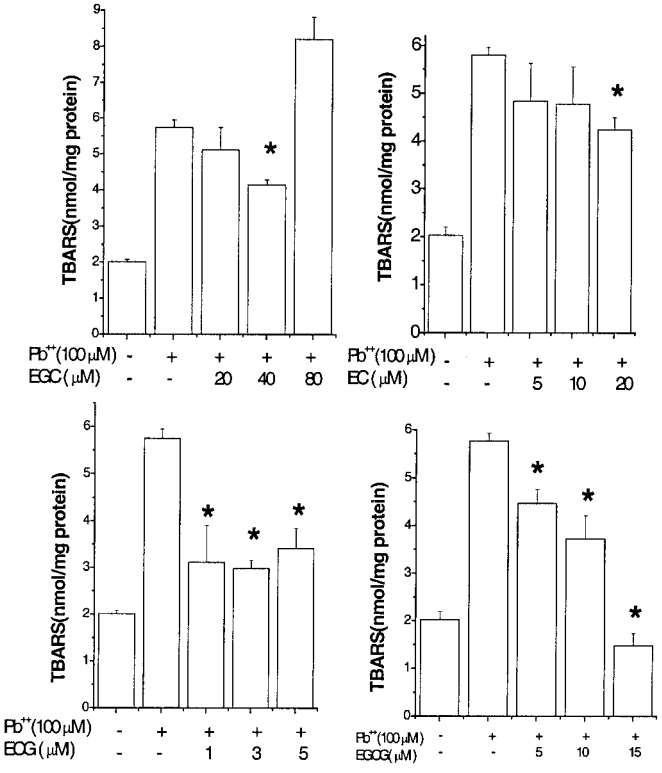

FIG. 8. Effect of tea catechins on TBARS formation in HepG2 cells treated by Pb^{++}. HepG2 cells were cultured with various concentrations of tea catechins and 100 μM Pb^{++} for 24 h and the lipid peroxidation level was determined as described in Materials and Methods. *Significant difference from control in the presence of 100 μM Pb^{++} by ANOVA; $p < 0.05$.

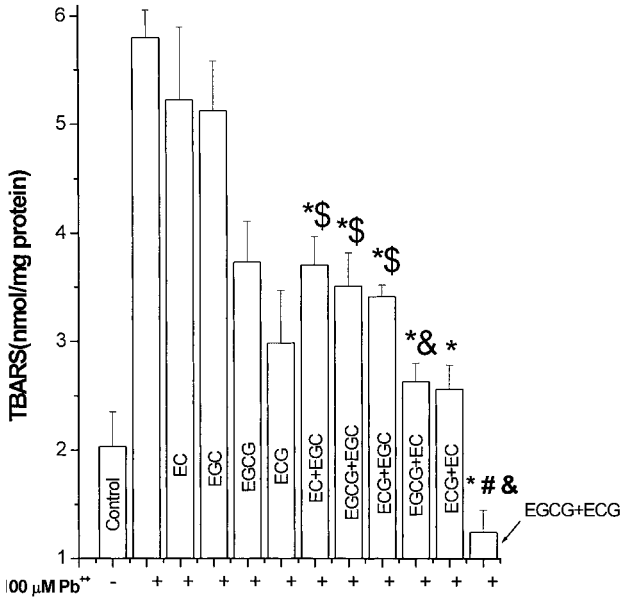

FIG. 9. Synergistic effect of tea catechins on TBARS formation in HepG2 cells exposed to Pb^{++}. After the initial 24-h attachment period, HepG2 cells were exposed to both 100 μM Pb^{++} and different tea catechins for an additional 24 h, and the lipid peroxidation level was determined as described in Materials and Methods. The concentrations of EC, ECG, EGC, and EGCG were 10 μM, 3 μM, 10 μM, and 10 μM, respectively. *, #, &, and $: Significant differences from EC; ECG; EGCG, and EGC, respectively; $p < 0.05$.

Pb^{2+} (Guo *et al.*, 1991; Kumamoto *et al.*, 2001), further investigation is needed as to whether tea catechins are capable of removing lead from the blood stream and target organs.

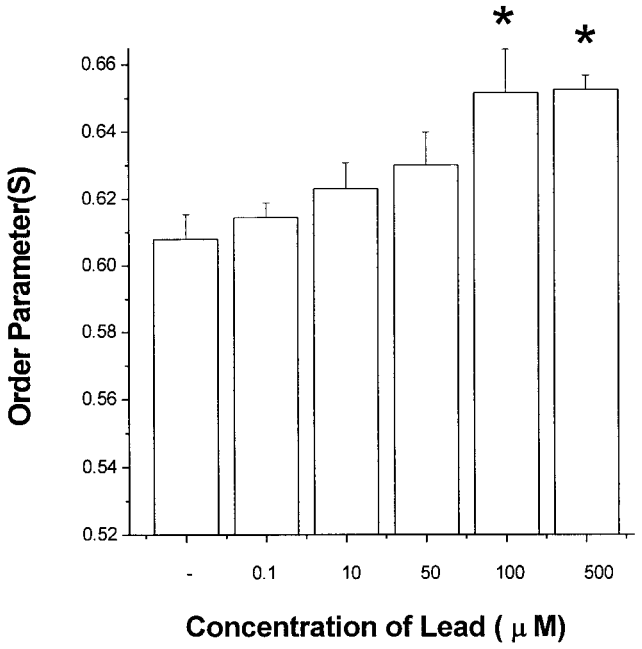

FIG. 10. Effect of different concentrations of Pb^{++} on order parameters (S) of HepG2 cells. HepG2 cells were cultured with various concentrations of Pb^{++} and order parameter was determined by ESR spin-trapping technique. *Significant difference from control by ANOVA, $p < 0.05$.

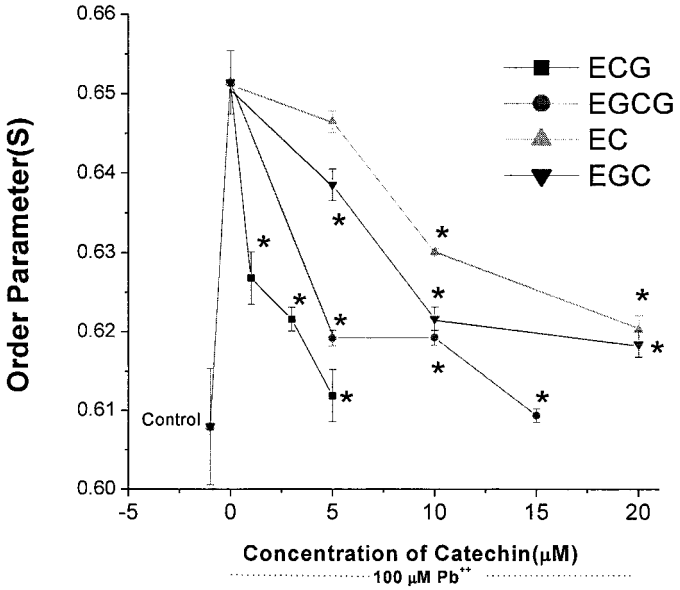

FIG. 11. Effects of tea catechins on order parameters (S) of HepG2 cells exposed to Pb^{++}. HepG2 cells were cultured with various concentrations of tea catechins and 100 μM Pb^{++} for 24 h, and the order parameter (S) was determined by ESR spin-labeling technique. *Significant difference from control in the presence of 100 μM Pb^{++} by ANOVA, $p < 0.05$.

The present study was designed to elucidate whether tea catechins resulted in decreased lipid peroxidation in HepG2 cells treated by lead. The hypothesis was evidenced in tea catechin-treated HepG2 cells exposed to lead. Therefore it can

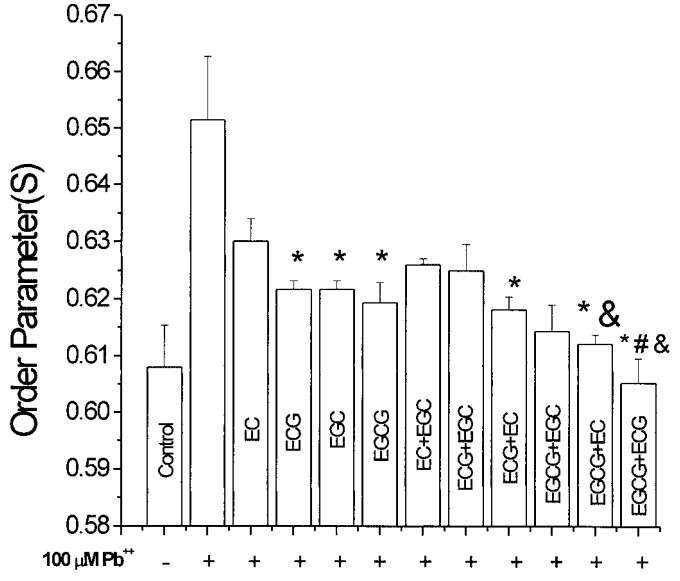

FIG. 12. Synergistic effects of tea catechins on order parameter of HepG2 cells exposed to Pb^{++}. After the initial 24-h attachment period, HepG2 cells were exposed to both Pb^{++} (100 μM) and different tea catechins for an additional 24 h, and order parameter was determined by ESR spin-labeling technique. The concentrations of EC, ECG, EGC, and EGCG were 10 μM, 3 μM, 10 μM, and 10 μM, respectively. *, #, and &: Significant difference from EC, ECG, and EGCG, respectively; $p < 0.05$.

be deduced that the increased cell viability in tea catechin-treated cells, along with improved lipid peroxidation levels, reflects the antioxidant action of tea catechins in lead-treated cells. Results from the study of cell membrane fluidity suggest that the beneficial effects of tea catechins on lipid peroxidation are related to its ability to protect cell membrane against damage by lead.

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