Tea Catechins Protect against Lead-Induced ROS Formation, Mitochondrial Dysfunction, and Calcium Dysregulation in PC12 Cells

Liuji Chen,[†] Xianqiang Yang,[‡] Hongli Jiao,[§] and Baolu Zhao*[,§]

National Key Laboratory of Medical Neurobiology, Shanghai Medical College, Fudan University, Shanghai, China 200032, Department of Tea Science, Zhejiang University, Hangzhou, China 310029, and Laboratory of Visual Information Processing, Research Centers of Brain and Cognitive Science, Institute of Biophysics, Academia Sinica, Beijing, China 100101

Received March 25, 2003

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, which suggests that antioxidants may play an important role in the treatment of lead poisoning. The present study was designed to elucidate whether tea catechins had any protective effects on altered oxidative stress parameter in PC12 cells exposed to lead. The experimental results showed that lead decreased PC12 cell viability and induced a rapid elevation of $[Ca^{2+}]_i$, which was followed by an accumulation of ROS and a decrease of mitochondrial membrane potential (MMP). Treatment by tea catechins significantly increased cell viability, decreased intracellular Ca^{2+} levels and ROS formation, and improved MMP in PC12 cells exposed to lead. The galloylated catechins showed a greater effect on ROS formation and mitochondrial dysfunction than that of nongalloylated catechins, which was similar to the result of their scavenging ability on free radical. In view of the time course of ROS formation and mitochondrial dysfunction and their correlation, our results also suggested that the beneficial effects of tea catechins on MMP are related, at least in part, to its ability to scavenge ROS in PC12 cells exposed to 100 μM Pb^{2+}. The present results suggest that tea catechins supplementation may play a role for modulating oxidative stress in PC12 cells exposed to lead.

Introduction

Lead is one of the metals most commonly encountered in the environment (*1*). Its widespread industrial use and continued release into the environment as an exhaust emission product have made lead a serious threat to human health (*2*). Exposure to low levels of lead is associated with behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in experimental animals (*3*).

Some evidence indicates that cellular damage mediated by ROS[1] may be involved in the pathology associated with lead intoxication (*4*, *5*). The lipid peroxidation levels in blood were strongly correlated with blood lead concentration in exposed workers (*6*). The activities of the antioxidant enzymes, SOD, and glutathione peroxidase, in erythrocytes from the workers exposed occupationally to lead, were remarkably higher than in nonexposed workers (*7*). Lead increased the prooxidant/antioxidant ratio in a concentration-dependent manner in lead-treated CHO cells and rats (*4*, *5*). The results suggest that antioxidants might play an important role in the treatment of lead poisoning.

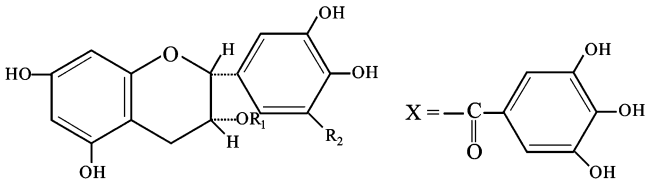

Figure 1. Chemical structures of green tea catechins. EC, (−)-epicatechin R_1=H, R_2=H; EGC, (−)-epigallocatechin R_1=H, R_2=OH; ECG, (−)-epicatechin gallate R_1=X, R_2=H; EGCG, (−)-epigallocatechin gallate R_1=X, R_2=OH.

The chemical composition of green tea contains many polyphenolic compounds, generally known as catechins. The four most abundant naturally occurring tea catechins are EC, EGC, ECG, and EGCG (Figure 1). Yang et al. reported that tea polyphenols had numerous potentially beneficial medicinal properties including inhibition of carcinogenesis, tumorigenesis, and mutagenesis, as well as the inhibition of tumor growth and metastasis (*8*). Our previous data indicated that green tea catechins had higher antioxidant activities than that of vitamin C and vitamin E (*9*). Tea catechins also exhibited powerful antioxidant activity, 20 times more potent than vitamin C, in the lipoprotein oxidation model that simulates the oxidation of low-density lipoproteins responsible for atherosclerosis (*10*, *11*). It was also found that the presence of at least an ortho-dihydroxyl group in the

* To whom correspondence should be addressed. Tel: 8610-64888569. Fax: 8610-64871293. E-mail: zhaobl@sun5.ibp.ac.cn.
† Fudan University.
‡ Zhejiang University.
§ Academia Sinica.

[1] Abbreviations: $[Ca^{2+}]_i$, intracellular free calcium; CHO, Chinese hamster ovary; DCFH, 2′,7′-dichlorofluorescin; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; DMSO, dimethyl sulfoxide; EGCG, (−)-epigallocatechin gallate; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EC, (−)-epicatechin; Fluo-3-AM, fluo-3 acetomethoxyester; KRH, Krebs−Ringer−Hepes; MMP, mitochondrial membrane potential; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliunbromide; Pb, lead; Rh123, rhodamine 123; ROS, reactive oxygen species; SOD, superoxide dismutase.

10.1021/tx0340605 CCC: $25.00 © 2003 American Chemical Society
Published on Web 08/22/2003

B-ring and a galloyl moiety at the 3-position was important in maintaining the effectiveness of their radical scavenging ability (*12*, *13*). However, catechins showed prooxidants in high concentrations or in the presence of Cu^{2+}, induced DNA cleavage, and accelerated the peroxidative property of unsaturated fatty acid (*14*, *15*). So, it is significant to elucidate the relationship between their structures and functions, concentration, and effect in different reactive or cell systems.

In view of the formation of ROS by lead exposure, tea catechins are expected to have beneficial effects on the oxidative stress-mediated pathogenesis process. Recently, we have demonstrated that exposure of HepG2 cells to Pb^{2+} decreased cell viability and stimulated lipid peroxidation of cell membranes as measured by thioburbituric acid reaction (*16*). Electron spin resonance spin labeling studies indicated that lead exposure decreased the fluidity in the polar surface of the cell membrane, but the membrane fluidity in the hydrophobic region was not affected. Tea catechins treatment significantly increased cell viability, decreased lipid peroxidation levels, and protected cell membrane fluidity in lead-exposed HepG2 cells in a concentration-dependent manner. Cotreatment with EGCG and EC and ECG and EGCG also showed a synergistically protective effect.

Lead exposure causes neuron damage, especially injury to the dopamine system, glutamate system, and *N*-methyl-α-D-Aspartate (NMDA) receptor complex, which play an important role in study and memory (*17*, *18*). PC12 cells can secrete dopamine and possess a dopamine transporter, and it has been frequently used as a neuron cell model (*19*, *20*). Using in vitro and in vivo models, recent studies suggested the protective effects of tea catechins on neuronal damage induced by free radical attack. Guo et al. reported that EGCG reduced the lipid peroxidation injury in synaptosomes (*21*). Nie et al. showed that EGCG and ECG have neuroprotective effects on 6-OHDA-induced apoptosis in PC12 cells, and the mechanism may be related to their antioxidant activities (*20*). Oral administration of EGCG alone also elevated the activity of SOD and catalase in striatum (*22*). However, to our knowledge, there is no previous study on the neuroprotective effect of tea catechins against neuronal damage following lead treatment. The aim of the present study is to further define the protective effect of tea catechins on lead-induced ROS formation, mitochondrial dysfunction, and calcium dysregulation in PC12 cells. It is clearly demonstrated that high concentrations of lead-induced cell death through a mechanism involving an increase in the levels of ROS, mitochondrial dysfunction, and dysregulation of intracellular calcium homeostasis. In the presence of tea catechins, the oxidative damage induced by lead was clearly suppressed.

Material and Methods

Reagent. RPMI 1640 medium, fetal bovine serum, and MTT were purchased from GIBCO Life Technologies. Poly-L-lysine, $DCFH_2$-DA, Fluo-3-AM, Rh123, and DMSO were purchased from Sigma. EGCG, ECG, EGC, and EC, whose purity was determinated to be >98% by HPLC, were kindly provided by Zhejiang University. All other chemicals made in China were of analytical grade.

Cell Culture. PC12 cells (a gift from the medical school of Peking University) were grown at 37 °C in RPMI 1640 medium supplemented with 10% equine serum, 5% fetal bovine serum, 100 units/mL of penicillin, 100 μg/mL of streptomycin, 100 mM Hepes, and 1.5 g/L $NaHCO_3$. The cells were plated at a density of 1×10^5 cells/mL on poly-L-lysine-coated 96 well plates and 5×10^5 cells /mL in a 40 mm tissue culture dish. Cells were exposed to lead acetate alone or pretreated with different concentrations of tea catechins 30 min before lead acetate was added. The control group received the same concentration of sodium acetate. Only cells in exponential growth were used for the experiments.

Cell Viability Assay. In the experiments, 1×10^4 cells were plated in each well of a 96 well plate and were allowed to attach to the substrate for a 24 h period. Cells were exposed for an additional 24 h to either sodium acetate (control) or lead acetate in the absence or presence of different concentrations of tea catechins. Cell viability was determined using the MTT assay (*23*). In brief, 20 μL of 5 mg/mL of MTT in PBS was added to each well and the plates were incubated at 37 °C for a further 4 h. The media were then removed, and the purple formazan crystals were 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 Intracellular ROS. ROS formation was detected with a fluorescent spectrometer using a nonfluorescent compound, $DCFH_2$-DA. Once inside the cell, the deesterified product becomes the fluorescent compound, DCFH on oxidation by ROS (*24*), and the fluorescent signal is proportional to ROS production (*25*). After exposure to sodium acetate (control) or lead acetate with or without antioxidants, the cells (10^6/mL) were incubated in 1 mL of 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5.6 mM glucose, 1.5 mM $CaCl_2$, and 20 mM Hepes-Na, pH 7.4, and allowed to take up 5 μM $DCFH_2$-DA at 37 °C for 20 min, in an atmosphere of 95% air and 5% CO_2. After loading with $DCFH_2$-DA, the cells were washed with the same buffer and fluorescence was measured at 488 nm excitation and 525 nm emission. Cell samples were then pelleted and used to normalize for protein content between samples.

Analysis of MMP. The changes in MMP were estimated using a fluorescent cationic dye Rh123, which accumulates in mitochondria as a direct function of the membrane potential and is released upon membrane depolarization (*26*). The analysis of MMP was performed as described previously with minor modifications. In brief, after exposure to sodium acetate (control) or lead acetate in the presence or absence of antioxidants, the cells (10^6/mL) were incubated for 10 min at 37 °C with Rh123 (1 μM), in an atmosphere of 95% air and 5% CO_2. The cells were then washed with 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5.6 mM glucose, 1.5 mM $CaCl_2$, and 20 mM Hepes-Na (pH 7.4) and transferred to a cuvette. The dye trapped in the cells was determined by fluorescent analysis at excitation and emission wavelengths of 490 and 515 nm, respectively (37 °C). The samples were then pelleted and used to normalize for protein content between samples.

Determination of Intracellular Calcium Concentration. The concentration of intracellular Ca^{2+} was measured with Fluo-3AM, a calcium fluorescent ester indicator (*27*). In brief, PC12 cells were detached by vigorous aspiration in condition medium, washed with Locke's buffer pH 7.4 containing 154 mM NaCl, 5.6 mM KCl, 1.0 mM $MgCl_2$, 2.3 mM $CaCl_2$, 3.6 mM $NaHCO_3$, 5.0 mM HEPES, and 5.6 mM D-glucose, and resuspended in the fresh buffer. A total 5 μL of Fluo-3 AM (5 μM) was added to the cell suspension (~10^6 cells/mL) in Locke's solution, and the mixture was incubated at 37 °C for 45 min. After incubation, the cells were then washed, resuspended in a standard medium, and transferred to thermostatically controlled cuvettes equipped with a magnetic stirrer. The fluorescence intensity of Fluo-3 was quantified with a fluorescence spectrometer at excitation and emission wavelengths of 488 and 525 nm, respectively (37 °C). $[Ca^{2+}]_i$ was calculated from the fluo-3 fluorescence intensity using the equation:

$$[Ca^{2+}]_i = K_d(F - F_{min})/(F_{max} - F)$$

F_{max} and F_{min} were the maximum and minimum fluorescence

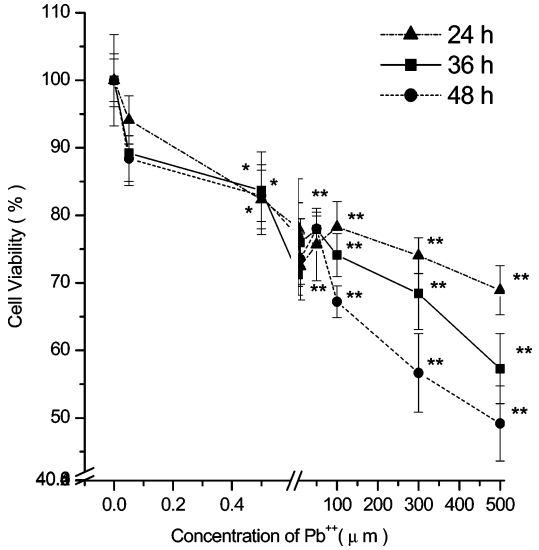

Figure 2. Dose and time dependence on lead-induced death of cultured PC12 cells. PC12 cells were cultured with various concentrations of lead acetate for different times, and cell viability was determined by MTT assay as described in Materials and Methods. Data are expressed as a percentage of the untreated control ± SE ($n = 7$). Exposure time was for 24, 36, or 48 h. *$P < 0.05$, **$P < 0.01$ vs control.

determined by adding 20 μL of 10% Triton X-100 and 20 μL of EGTA (5 mM), respectively. As K_d, 400 nm/L was used.

Data Analysis. Throughout the text, data were expressed as means ± SE of triplicate determinations, from at least three independent experiments. Statistical analysis was determined using one way ANOVA with $P < 0.05$ considered significant.

Results

Lead Decreased PC12 Cell Viability in a Concentration-Dependent Manner. After the initial 24 h attachment period, cells were exposed to sodium acetate (control) or lead acetate alone and the cytotoxicity was measured by the MTT method. It was found that the toxicity of Pb^{2+} was both time- and concentration-dependent in PC12 cells, as shown in Figure 2. When the concentration exceeded 0.5 μM, Pb^{2+} significantly decreased PC12 cell viability. It was evident that increasing exposure times showed steeper concentration−response curves, especially in high concentrations (100−500 μM, $P < 0.05$).

Time Course of MMP, Intracellular ROS, and Calcium Concentration in Lead-Induced PC12 Cells. Because the cytotoxic effect of Pb^{2+} is significant in appearance in high concentration, the following experiment was conducted in the cells exposed to 100−500 μM Pb^{2+}. Figure 3 showed the time course of MMP, intracellular ROS, and calcium concentration in PC12 cells exposed to 100 μM Pb^{2+}. When the cells were treated with 100 μM Pb^{2+}, a significant increase of intracellular calcium was found on 6 h ($P < 0.01$). When cells were treated for 12 h, intracellular ROS formation significantly increased about 30% ($P < 0.05$). While in the case of 24 h, more than about 34.5% of the cells was found to have decreased MMP as evidenced by the lower Rh123 fluorescence intensity ($P < 0.05$). The results indicated that the change of calcium preceded the increase of intracellular ROS and the decrease of MMP and the formation of ROS preceded the decrease of MMP in the cells exposed to 100 μM Pb^{2+}.

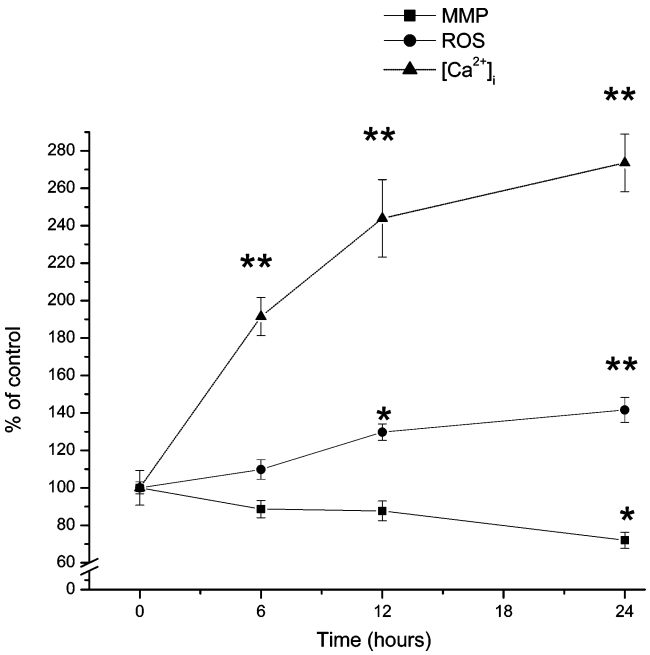

Figure 3. Time course of MMP, intracellular ROS, and calcium concentration. After the initial 24 h attachment period, PC12 cells were exposed to 100 μM Pb^{2+} for an additional 24 h and MMP, intracellular ROS, and calcium concentration were determined by fluorimetric assay at different times as described in Materials and Methods. Data are expressed as a percentage of the untreated control ± SE, $n = 5$. *$P < 0.05$, **$P < 0.01$, significant difference from control in the absence of Pb^{2+} by ANOVA.

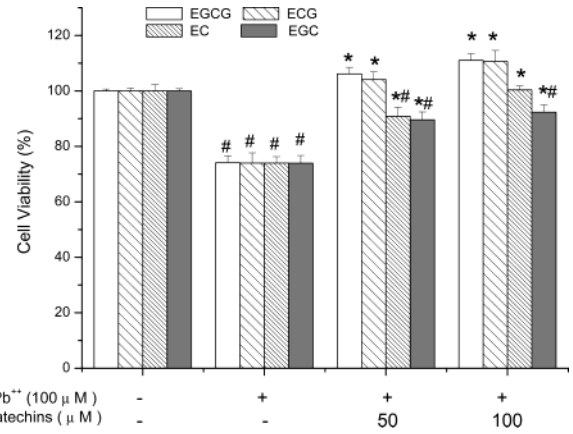

Figure 4. Effect of tea catechins on Pb^{2+}-induced cell toxicity. After the initial 24 h attachment period, PC12 cells were exposed to both Pb^{2+} (100 μM) and different concentration of tea catechins for an additional 24 h, and the cell viability was determined by MTT assay as described in Materials and Methods. Data are expressed as a percentage of the untreated control ± SE, $n = 7$. #$P < 0.05$ vs control, *$P < 0.01$ vs the group treated by only 100 μM Pb^{2+}.

Protective Effects of Tea Catechins on Lead-Exposed PC12 Cell Viability. The effects of tea catechins alone on cell viability, MMP, intracellular ROS accumulation, and Ca^{2+} levels were studied and shown to have no effect when the concentration of tea catechins was less than 100 μM (data not shown). Figure 4 shows the effects of tea catechins on lead-exposed PC12 cell viability. The results indicated that tea catechins significantly increased viability of lead-exposed PC12 cells. Comparing with that of the cells treated by Pb^{2+}, 50−100 μM ECG and EGCG, as well as 100 μM EC all restored the cell viability to a normal level ($P < 0.01$),

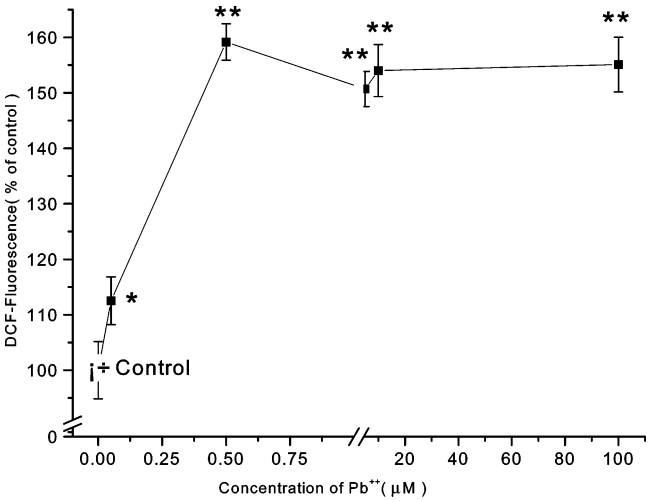

Figure 5. Lead acetate-induced intracellular accumulation of ROS in PC12 cells. Formation of ROS after 24 h of lead acetate exposure was detected using $DCFH_2$-DA oxidation and fluorescence. PC12 cells were cultured for 24 h in standard RMPI 1640 medium with different concentrations of lead acetate. The rate of DCF formation was then evaluated as described in Materials and Methods. The results, expressed as percent units of DCF fluorescence of the control, are the means ± SE of triplicate determinations of 3−5 distinct experiments. *P < 0.05, **P < 0.01, significantly different from control conditions (□), in the absence of lead acetate.

while in the cases of 50 μM EC or EGC or 100 μM EGC, the survival rate was about 90% (P < 0.05). Therefore, the decreased order of the protective effects of tea catechins was classified as ECG ≈ EGCG > EC ≥ EGC.

Inhibitory Effect of Tea Catechins on ROS Formation in PC12 Cells Exposed to Lead. To investigate the involvement of oxygen radicals in lead-induced toxicity in PC12 cells, the accumulation of ROS after lead exposure was measured utilizing a converting reaction of DCFH-DA to DCF. As compared with the control group treated by sodium acetate, ROS accumulation, determined by the measurement of DCF fluorescence, was increased 10−60% after treatment with a different concentration of lead acetate (Figure 5). Comparing with that treated by Pb^{2+}, in the presence of 100 μM EGC or 50−100 μM ECG or EGCG, the increased ROS was clearly suppressed (as shown in Figure 6, P < 0.05), whose DCF fluorescence was similar to that determined in control cells incubated in the absence of lead acetate. The inhibition effects of tea catechins on the accumulation of intracellular ROS induced by lead were in the order EGCG ≈ ECG > EGC ≥ EC.

Tea Catechins Protect PC12 Cells against Lead-Induced Impairment of Mitochondrial Function. The concentration response of MMP changes was studied when PC12 cells were treated by different concentrations of lead for 24 h. As shown in Figure 7, 50 μM Pb^{2+} failed to cause any MMP changes as compared to the control treated by sodium acetate. The higher Pb^{2+} concentrations (100−500 μM) induced a decrease of MMP. At 50 μM, nongalloylated catechins (EC and EGC) had little effect on MMP changes, while galloylated catechins (ECG and EGCG) showed stronger protective effects against mitochondrial dysfunction induced by Pb^{2+}. Four monomers of tea catechins at 100 μM all restored the MMP to the normal levels from the change caused by 100 μM Pb^{2+} (Figure 8).

Effects of Tea Catechins on Lead-Induced Calcium Dysregulation in PC12 Cells. The effect of lead

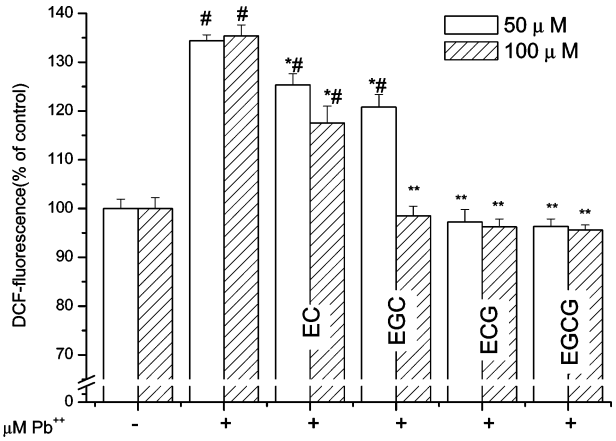

Figure 6. Effect of tea catehins on ROS accumulation induced by lead acetate in PC12 cells. Formation of ROS after 24 h of lead acetate exposure was detected using $DCFH_2$-DA oxidation and fluorescence in the presence of antioxidants. PC12 cells were cultured for 24 h in standard RMPI 1640 medium. The rate of DCF formation was then evaluated as described in Materials and Methods. The results, expressed as percent units of DCF fluorescence of the control, are the means ± SE of triplicate determinations of 3−5 distinct experiments. #P < 0.05 vs control, *P < 0.05, **P < 0.01 vs the group treated by only 100 μM Pb^{2+}.

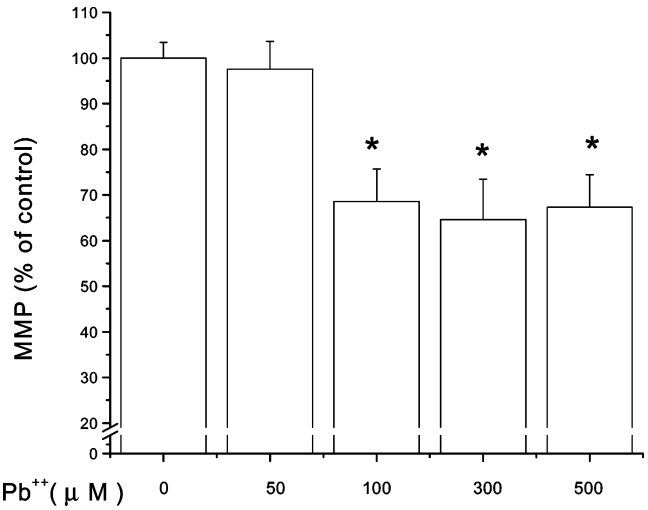

Figure 7. Effect of lead on MMP of PC12 cells. MMP was evaluated by the capacity of the cells to take up the fluorescent cationic dye Rh123 after the cells were incubated for 24 h in the absence or presence of Pb^{2+}. The results are expressed as percentage of trapped fluoresence in treated as compared to control. The data are expressed as means ± SE for 3−7 different experiments. *P < 0.05 significantly different as compared to control conditions in the absence of Pb^{2+}.

on intracellular Ca^{2+} levels in PC12 cells after exposure to various concentration of Pb^{2+} (0.05−100 μM) was examined. As shown in Figure 9, intracellular Ca^{2+} was significantly increased by Pb^{2+} from basal levels (310.5 ± 6.8 nmol/L). With 0.5 μM lead treatment, the intracellular Ca^{2+} reached a maximum (1041.2 ± 20.32 nmol/L). The intracellular Ca^{2+} remained unchanged (800 ± 65 nmol/L) over the range of 5−100 μM Pb^{2+}, which was significantly higher than that of the control. As shown in Figure 10, pretreatment of cells with 50 μM EGC or EGCG partially blocked a Pb^{2+}-induced intracellular calcium increase (P < 0.05). While at 100 μM, EC, ECG, EGC, and EGCG all significantly inhibit an intracellular calcium increase in PC12 cells induced by Pb^{2+} (P < 0.01), whose inhibitory rates were about 42.4, 33.2, and 32.3 (P < 0.01), and 22.3% (P < 0.05), respectively.

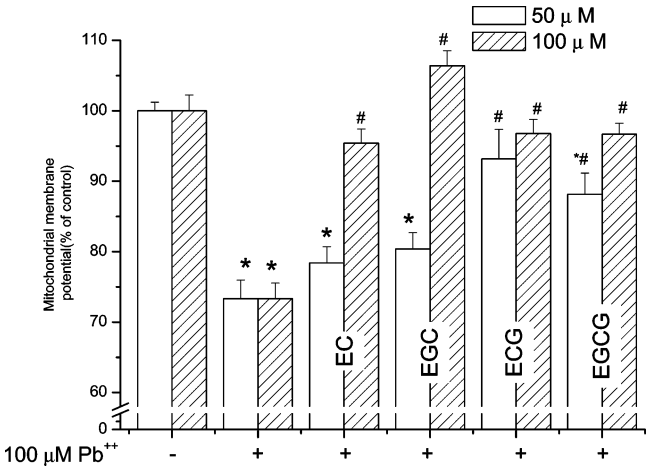

Figure 8. Effect of tea catechins on MMP in PC12 cells exposed to Pb^{2+}. MMP was evaluated by the capacity of the cells to take up the fluorescent cationic dye Rh123, and the cells were incubated for 24 h in the presence of Pb^{2+} and different antioxidants for 24 h. The results are expressed as percentage of trapped fluoresence in treated as compared to control. The data are means ± SE for 3−7 different experiments. $^*P < 0.05$ vs the control in the absence of 100 μM Pb^{2+}; $^{\#}P < 0.05$ vs the control in the presence of 100 μM Pb^{2+}.

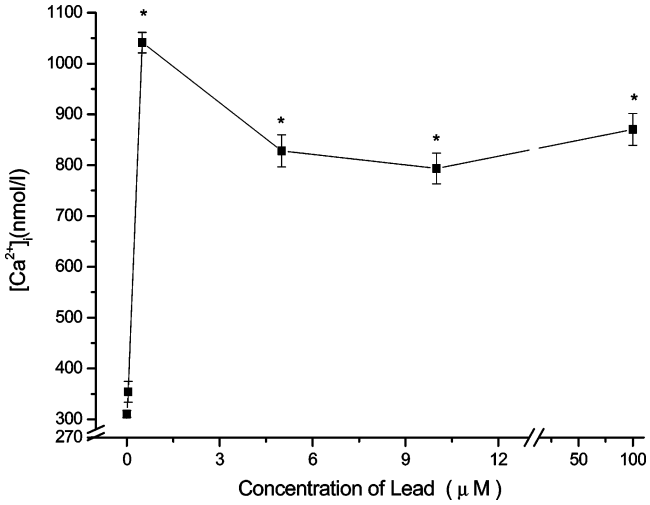

Figure 9. Lead acetate-induced intracellular increase of calcium in PC12 cells. PC12 cells were cultured for 24 h in standard RMPI 1640 medium. Intracellular Ca^{2+} levels were detected using Fluo-3AM after 24 h of lead acetate exposure. The fluorescence intensity was then evaluated as described in Materials and Methods. The results are the means ± SE of triplicate determinations for 3−5 distinct experiments. $^*P <$ 0.01, significantly different from the control conditions in the absence of lead acetate.

Discussion

Lead exposure causes neuron damage, especially injury to the dopamine system, glutamate system, and NMDA receptor complex, which play an important role in study and memory (*17*, *18*). PC12 cells can secrete dopamine and possess a dopamine transporter, and it has been frequently used as a neuron cell model (*19*, *20*). The result of this work showed that exposure to 100 μM Pb^{2+} decreased PC12 cell viability and induced a rapid elevation of $[Ca^{2+}]_i$, which was followed by accumulation of ROS and decrease of MMP. Treatment by tea catechins significantly increased cell viability, decreased intracellular Ca^{2+} levels and ROS formation, and improved MMP in PC12 cells exposed to lead in a concentration-dependent manner. Both ROS and $[Ca^{2+}]_i$ in the cell were rapidly

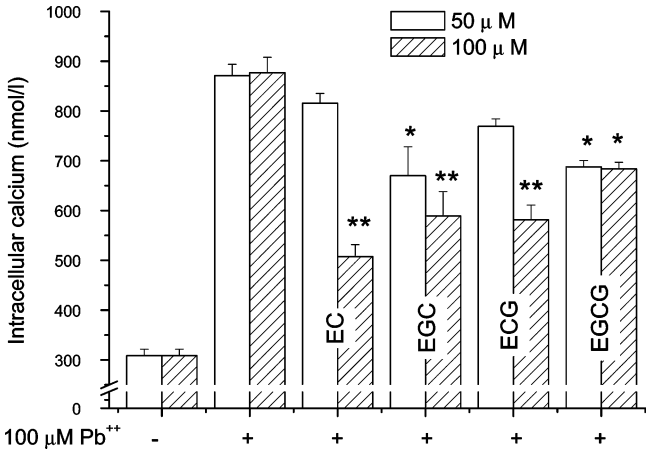

Figure 10. Effect of antioxidants on lead-induced intracellular increase of calcium in PC12 cells. PC12 cells were cultured for 24 h in standard RMPI 1640 medium. Intracellular Ca^{2+} levels were detected using Fluo-3AM after 24 h exposure to 100 μM Pb^{2+} and different tea catechins. The fluorescence intensity was then evaluated as described in Materials and Methods. The results are the means ± SE of triplicate determinations for 3−5 distinct experiments. $^*P < 0.05$, $^{**}P < 0.01$, significantly different from control conditions in the presence of 100 μM lead acetate.

elevated when the cell was exposed to low concentration of Pb^{2+} (<1 μM), but the decrease of MMP appeared in a higher concentration of Pb^{2+} (>100 μM), indicating that ROS and $[Ca^{2+}]_i$ are more sensitive to Pb^{2+} than MMP. The result of this work can be used to elucidate the toxicological mechanisms of lead on PC12 cells and protective effect of tea catechins, which may be useful for protection of neuron by antioxidant against lead toxicity.

Tea catechins are strong scavengers against superoxide, hydrogen peroxide, hydroxy radicals, and nitric oxide produced by various chemicals (*9*, *12*, *13*). They also could chelate with metals because of the catechol structure (*28*). These characteristics make tea catechins become ideal candidates for treatment of lead toxicity. The data from our studies of PC12 cells indicated that lead treatment significantly decreased cell viabilities and increased intracellular ROS formation in a concentration-dependent manner. With 0.05 μM Pb^{2+} treatment, there was not a remarkable effect on cell viability of PC12 cells, as determined by the MTT method, but the intracellular ROS formation was significantly increased with the concentration of lead treatment ($P < 0.05$), suggesting that intracellular ROS formation was more sensitive to the lead treatment for the cells. As a kind of excellent free radical scavenger, tea catechins protected PC12 cells against lead-induced inhibition of MTT reduction and reversed the effects of lead on oxidative stress parameters, further supporting the hypothesis that oxidative stress is involved in lead toxicity. As shown in Figures 4, 6, and 8, the galloylated catechins (ECG and EGCG) showed stronger protective effect against oxidative damage than that of nongalloylated catechins (EC and EGC), which is similar to the result of scavenging ability on free radical (*13*, *21*). As reported previously, tea catechins scavenged free radical in the order EGCG ≈ ECG > EGC > EC (*13*, *21*). Okabe also reported the similar order in inhibiting growth of human lung cancer cell line PC9 (*29*). EGCG is the most effective antioxidant in all of the components of green tea polyphenols. Our results are consistent with the fact that EGCG is more effective than

other catechins. Tea catechins are strong metal ion chelators because of the catechol structure, which has been shown to form stable complexes with Fe^{2+}, Ca^{2+}, Al^{3+}, Mn^{2+}, Cr^{3+}, and Pb^{2+} (*30*, *31*). The galloylated catechins containing more phenolic hydroxyl groups have a stronger chelating ability with metal ion than nongalloylated catechins (*31*). Therefore, the protective effect of tea catechins on PC12 cells exposed to lead might be related to both their scavenging ability on free radical and their chelating ability with metal ion.

Lead characteristically perturbs processes linked to calcium and the calcium messenger system because of the similarities between Ca^{2+} and Pb^{2+} (*32*). Oberto et al. provided evidence that Pb^{2+} at micromolar levels induced neuronal death through apoptosis in cerebellar granule cells, which was prevented by calcium channel antagonist, suggesting that the entry of Pb^{2+} into cells or the influx of Ca^{2+} might be important for this effect (*33*). As shown in Figure 9, the increased intracellular levels of free Ca^{2+}, observed after treatment of PC12 cells with different concentrations of lead, suggested a role for Ca^{2+} in lead-induced cell toxicity. These data are consistent with earlier papers mentioned above. There are several possible mechanisms for the Pb^{2+}-induced increase in $[Ca^{2+}]_i$; the most likely is that Pb^{2+} stimulates the opening of a Ca^{2+} channel via the activation of PKC. The ability of lead to activate PKC has been confirmed by several investigated in different cell types and under different experimental conditions (*34*, *35*). Tea catechins could partially block Pb^{2+}-induced intracellular calcium increase, which probably was associated with both their inhibitory effect on PKC (*36*) and their chelating ability with calcium ion (*31*).

In conclusion, we have shown that lead toxicity leads to the increase of $[Ca^{2+}]_i$ and ROS formation. The impairment of mitochondrial function and perturbation of calcium homeostasis may be useful to elucidate the mechanisms of lead-induced oxidative stress in PC12 cells. Furthermore, the present study also evidenced that lower concentration of tea catechins resulted in decreased intracellular Ca^{2+} level and ROS formation and improved MMP in PC12 cells treated by lead. Therefore, it can be deduced that the increased cell viability in tea catechin-treated cells along with improved ROS level and intracellular calcium homeostasis reflect the protective action of tea catechins in lead-treated PC12 cells. The results also suggest that the beneficial effects of tea catechis on MMP are related to their ability to scavenge ROS in PC12 cells exposed to lead. Further assays in vivo are currently underway to evaluate the efficacy of tea catechins as protective agents against lead toxicity.

Acknowledgment. We thank professor Zhang Jin-Zhu for her kindly help in the experiments. This work was supported by a grant from the National Natural Science Foundation of China.

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TX0340605