JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 8977–8982 8977
DOI:10.1021/jf101277t

A Major Green Tea Component, (−)-Epigallocatechin-3-gallate, Ameliorates Doxorubicin-Mediated Cardiotoxicity in Cardiomyocytes of Neonatal Rats

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Cardiac injury is a major complication of the oxidative stress-generating anticancer drug doxorubicin (DOX). The green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) has been reported to play a cardioprotective role in diseases associated with oxidative stress. The objective of this study was to investigate whether EGCG can protect against DOX-induced toxicity in cardiomyocytes. The data showed that EGCG protected the cardiomyocytes from DOX-mediated cardiotoxicity, as evidenced by decreased lactate dehydrogenase (LDH) activity and increased cell viability in a dose-dependent manner. EGCG treatment also decreased malondialdehyde content and increased protein expression and activities of manganese superoxide dismutase (MnSOD), catalase, and glutathione peroxidase. Furthermore, treatment with EGCG decreased reactive oxygen species (ROS) production and apoptosis. This study suggests that EGCG could protect cardiomyocytes from DOX-induced oxidative stress by attenuating ROS production, apoptosis, and increasing activities and protein expression of endogenous antioxidant enzymes.

KEYWORDS: (−)-Epigallocatechin-3-gallate; doxorubicin; oxidative stress; cardioprotection; cardiomyocytes

INTRODUCTION

Doxorubicin (DOX, Adriamycin), an anthracycline antibiotic, is a highly effective chemotherapeutic drug used in the treatment of solid and hematopoietic tumors. However, a major limiting factor for the clinical use of DOX is its cumulative, irreversible cardiac toxicity. Although the precise cellular mechanism responsible for the chronic cardiotoxicity of DOX remains enigmatic, accumulating evidence indicates that DOX-induced cardiomyopathy is mainly caused by increased oxidative stress (*1*, *2*). Structurally, DOX is a quinone, which can generate a large amount of superoxide (O_2^-) via a redox cycling reaction catalyzed by endogenous reductases and endothelial nitric oxide synthase. O_2^- in turn gives rise to a variety of more active reactive oxygen species (ROS), including H_2O_2, OH^-, and $ONOO^-$, which trigger further oxidation of biomolecules (*3*, *4*).

One potential approach to decreasing oxidative stress associated with DOX is to use agents that mimic endogenous antioxidants (*5*). Cells are equipped with a variety of antioxidants that quench ROS produced under normal conditions. When ROS are generated in excess or when the cellular antioxidant defense system is defective, ROS may interact degeneratively with cellular components, including proteins, lipids, and nucleic acids, causing cellular dysfunction and even cell death (*6*, *7*). Appropriate antioxidant strategies could be particularly useful to limit ROS production and ROS-induced alterations and hence to protect the myocardium against DOX cardiotoxicity (*8*). Thus, it is necessary to identify naturally occurring antioxidants that may be used to mitigate oxidative stress induced by DOX.

Both epidemiological and experimental studies have demonstrated a positive correlation between the consumption of green tea (*Camellia sinensis*) and cardiovascular disease risk reduction (*9*−*11*). The potential health benefits associated with tea consumption have been partially attributed to the antioxidant properties of polyphenols, particularly catechins, among which (−)-epigallocatechin gallate (EGCG) was the most effective (*12*). EGCG, the most abundant polyphenol in green tea, has a potent antioxidant property because of the two triphenolic groups in its molecular structure. Several studies have demonstrated that EGCG enhanced antioxidant activity, which increases resistance to oxidative injury in cardiomyocytes (*13*, *14*). However, there are few investigations of the cardioprotection of EGCG against oxidative stress-related DOX cardiotoxicity in cardiomyocytes.

As DOX continues to be a mainstay in chemotherapy, there is an increasing population of cancer survivors who are at risk of developing cardiac abnormalities during and after chemotherapy. Development of cardioprotective agents, such as EGCG, is thus urgently needed. Therefore, the present study was designed to investigate the effect of EGCG against oxidative stress induced by DOX in cardiomyocytes and further examine the underlying molecular mechanisms of its action.

MATERIALS AND METHODS

Animals. Sprague−Dawley rats [1−3 days old, grade II, Certificate Number SCXK (gan) 2006-0001] were purchased from Jiangxi College of Chinese Medicine, Jiangxi Province, China. All animals used in this study

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Published on Web 07/28/2010

were cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication 85-23, 1996), and all experimental procedures were approved by the Nanchang University Animal Ethic Committee.

Chemicals and Reagents. EGCG was purchased from Shanghai Winherb Medical Science Co. Ltd. (Shanghai, China) and dissolved in phosphate-buffered saline (PBS) at a concentration of 20 g/L, and aliquots were stored at −80 °C as a stock solution (*15*). DOX was purchased from Shenzhen Wanle Pharmaceutical Co. Ltd. (Shenzhen, China). Cell culture products were obtained from Life Technologies (Paisley, Scotland). 2′,7′-Dichlorofluorescein-diacetate (DCFH-DA) was purchased from Molecular Probes Inc. (Eugene, OR). Annexin V−FITC apoptosis detection kit was obtained from BD Biosciences (San Diego, CA). Anti-MnSOD, anti-catalase, anti-glutathione peroxidase, and anti-β-actin primary antibodies, as well as the horseradish peroxidase-linked secondary antibody, were from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell Culture. Primary cultures of cardiomyocytes were prepared according to the method described by Reinecke et al. (*16*) with minor modifications. Briefly, the ventricles from neonatal rats were minced and dissociated with 0.1% trypsin five or six times at 37 °C, then filtered and centrifuged for 8 min at 120*g*, and finally resuspended in Eagle's minimum essential medium supplemented with 15% fetal calf serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). Resuspended cells were placed into a Petri dish in a humidified incubator (5% CO_2, 37 °C) for 2 h to promote early adherence of fibroblasts. Nonadherent cells were counted with a hemocytometer, and the final myocyte cultures were found to contain >90 cardiomyocytes. Approximately 1×10^6 cells in the medium were pipetted into 6-well gelatin-coated plates and incubated for 72−96 h before treatments. The cells were treated with indicated concentrations of DOX for the required time or pretreated with EGCG for 1 h before the DOX treatment. At the indicated time points, the cells were collected for different assays. Controls with vehicle were also performed in the same conditions.

Assay of Cell Viability. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide (MTT) assay. The cardiomyocytes were seeded in 96-well plates at 1×10^5/well and incubated with 0.1, 0.5, or 1 μM DOX or pretreated with different concentrations of EGCG (0, 3.13, 6.25, 12.5, 25, 50, 100, 150, and 200 μM) for 1 h. After treatment, cardiomyocytes were treated with 20 μL of MTT dye (5 g/L) for 4 h at 37 °C. The growth medium was removed, and the formazan crystals, formed by oxidation of the MTT dye, were dissolved with 150 μL of DMSO. The absorbance of the blue formazan derivative was measured at 490 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA).

Assay of Cell Death. The number of dead cells was determined with the trypan blue exclusion method. In brief, cell monolayer was rinsed with PBS once and resuspended with trypsin and EDTA. The cells were immediately stained with 0.4% trypan blue, and the number of dead cells was determined using a hemocytometer under a light microscope.

Assay of Lactate Dehydrogenase (LDH) Activity. To measure LDH activity in the culture medium of cardiomyocytes, 0.2 mL of culture medium was taken and immediately assayed for LDH activity using a commercial kit (JianCheng Bioengineering Institute, Nanjing, China) with a spectrophotometer (UV-120-02, Shanghai, China), following the manufacturer's instruction.

Measurement of Intracellular ROS Generation. The generation of ROS was assessed using the cell-permeable probe 5(6)-carboxy-2′,7′-dichlorofluorescein diacetate (cDCFH-DA). This dye is cleaved by cellular esterases to nonfluorescent 2′,7′-dichlorofluorescin (DCFH) and oxidized by ROS to a fluorescent product dichlorofluorescein (DCF). After incubation with or without EGCG, cells were harvested and washed with cold PBS. Washed cells were further incubated with 10 μM DCFH-DA at 37 °C for 20 min. Dye oxidation (increase in fluorescence) was measured using a FACStar Plus flow cytometer (Becton Dickinson, Franklin Lakes, NJ) with excitation and emission at 488 and 530 nm, respectively.

Protein Expression Analysis of MnSOD, Catalase, and Glutathione Peroxidase. Protein expression levels of MnSOD, catalase, and glutathione peroxidase in cardiomyocytes were assessed by Western blotting. Briefly, cells were lysed with lysis buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM EDTA, 1% (v/v) Triton X-100, 1 mM NaF, 1 mM Na_3VO_4, 0.2 mM phenylmethanesulfonyl fluoride, 10 μg/mL leupeptin, and 10 μg/mL aprotinin. Equivalent amounts of protein samples were resolved on 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. After blocking with 5% (w/v) nonfat milk, the membranes were blotted with polyclonal anti-MnSOD (1:100 dilution), anti-catalase (1:1000 dilution), or anti-glutathione peroxidase (1:300 dilution) antibodies and detected with a horseradish peroxidase-linked secondary antibody (1:2000 dilution). The specific bands were detected using chemiluminescence reagents on a chemiluminescence film. Densitometric analysis of the Western blot was performed with a GDS-8000 UVP photoscanner and LAB WOEK45 Image software (Bio-Rad). Normalization of results was ensured by running parallel Western blots with β-actin antibody (as an internal control).

Assays of Activities of MnSOD, Catalase, Glutathione Peroxidase, and Malondialdehyde Level. Cardiomyocytes were washed twice with PBS containing 0.05 mM ethylenediaminetetraacetate and sonicated at 4 °C. After centrifugation at 800*g*, for 5 min, the supernatants were immediately assayed for activities of MnSOD, catalase, and glutathione peroxidase and malondialdehyde contents using commercial kits (JianCheng Bioengineering Institute, Nanjing, China) with a spectrophotometer (UV-120-02, Shanghai, China), following the manufacturer's instructions.

Flow Cytometric Analysis of Apoptosis. Apoptosis was determined by annexin V and propidium iodide (PI) doubles staining. Cardiomyocytes were centrifuged to remove the medium, washed with PBS, and stained with annexin V−FITC and PI in binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$). Ten thousand events were collected for each sample. Stained cells were analyzed using a flow cytometer.

Statistical Analysis. Data are expressed as means ± SEM. Statistical significance was analyzed using one-way analysis of variance (ANOVA), followed by the Student−Newman−Keuls test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Effect of EGCG on Cell Viability in Cardiomyocytes. To examine the effect of EGCG on cardiomyocyte injury induced by DOX, we performed a series of dose- and time-response assays to determine the working concentration that led to a consistent degree of cytotoxicity. The MTT assay showed no decrease of viability of cells exposed to different concentrations of EGCG for 24 h (**Figure 1A**). Treatment with DOX at 0.1, 0.5, and 1 μM for 24 h caused a dose-dependent decrease of viability, whereas EGCG pretreatment at concentrations from 3.13 to 200 μM for 1 h prevented the decrease of viability (**Figure 1B**). The levels of 0.1, 0.5, and 1 μM fall within the concentrations of DOX found in the plasma of patients undergoing DOX therapy, among which 1 μM DOX caused the most damage to the cardiomyocytes (*17*). Thus, 1 μM DOX was used in the following experiments.

The viability of cells pretreated with EGCG at 100 μM for 1 h before exposure to 1 μM DOX was up to 80.05 ± 3.91%, whereas the higher concentrations (150 and 200 μM) did not cause any enhancement of this preventive effect. One possible reason is the cell toxicity due to the accumulation of the two drugs at high concentrations (*18*). We analyzed the effective half-maximal concentration for protection (EC_{50}) from the dose-response curves. EGCG attenuated cytotoxicity induced by DOX in cardiomyocytes with an EC_{50} of 38 μM. Therefore, we used concentrations of 38, 50, or 100 μM EGCG for our subsequent experiments. The application of 1 μM DOX induced time-dependent cytotoxicity, which was prevented by EGCG (38 μM) (**Figure 1C**). These data suggest that EGCG protected cardiomyocytes against DOX cardiotoxicity in a dose-dependent manner.

Effect of EGCG on Cell Damage in DOX-Treated Cardiomyocytes. Cell death and LDH activity are usually used as indexes of cardiomyocyte injury. To determine the effect of EGCG on cardiomyocyte injury induced by DOX, we examined the effects of EGCG on cell death and LDH activity. As shown in **Table 1**, the rate of dead cardiomyocyte in DOX-treated cells was significantly higher than that in controls. Consistent with this, LDH activity in

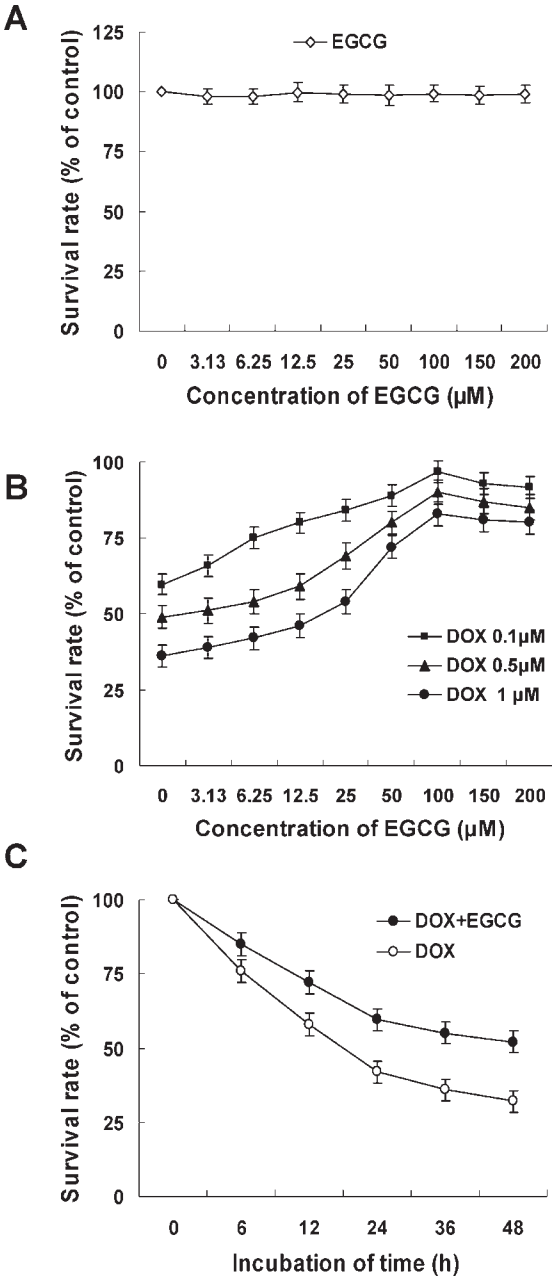

Figure 1. EGCG protects against DOX-induced cell injury in cardiomyocytes. (**A**) After the cells had been incubated with different concentrations of EGCG (0, 3.13, 12.5, 25, 50, 100, 150, 200 μM) for 24 h, cell viability was measured by MTT assay. (**B**) After the cardiomyocytes had been incubated with different concentrations of DOX (0.1, 0.5, 1 μM) for 24 h or EGCG treatment (0, 3.13, 12.5, 25, 50, 100, 150, 200 μM), cell viability was measured by MTT assay. (**C**) Cell viability after pretreatment with EGCG at 38 μM for 1 h before exposure to 1 μM DOX was estimated using the MTT assay with a time course. Values are expressed as mean ± SEM; $n = 8$.

the culture medium for the DOX-treated cells was significantly higher than for control cells. Pretreatment with EGCG attenuated DOX-induced increase of LDH activity and cardiomyocyte death. These results indicate that EGCG is a potent cardioprotective agent against DOX-induced cardiomyocyte injury.

Effect of EGCG on Malondialdehyde Content in DOX-Treated Cardiomyocytes. The concentration of malondialdehyde in the cell lysate was used as a biochemical marker for lipid peroxidation. DOX-treated cardiomyocytes had an increased malondialdehyde content compared with untreated cells. Treatment with

Table 1. Effect of EGCG on Cell Death and LDH Activity in Cardiomyocytes Subjected to DOX[a]

group	cell death (%)	LDH activity (U/L)
control	7.16 ± 0.61	4.61 ± 0.55
DOX	35.64 ± 2.96 a	29.59 ± 1.85 a
EGCG-38	28.51 ± 2.18 b	19.38 ± 1.57 b
EGCG-50	20.82 ± 2.09 b	13.62 ± 1.36 b
EGCG-100	10.26 ± 1.35 b	8.21 ± 1.25 b

[a] Data are expressed as the mean ± SEM; $n = 8$. EGCG-38, 38 μM EGCG; EGCG-50, 50 μM EGCG; EGCG-100, 100 μM EGCG. For entries followed by "a", $P < 0.01$ vs control group; for entries followed by "b", $P < 0.01$ vs DOX group.

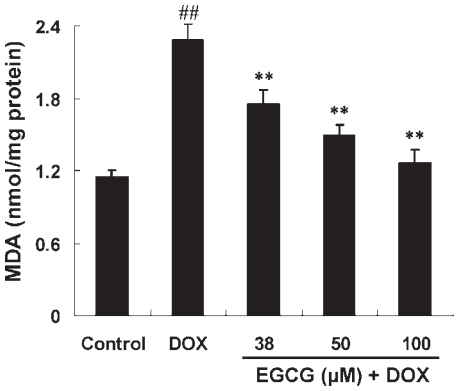

Figure 2. Effect of EGCG on DOX-induced change of malondialdehyde contents in cardiomyocytes. Values are expressed as mean ± SEM; $n = 8$. ##, $P < 0.01$ versus control group; **, $P < 0.01$ versus DOX group.

EGCG (38, 50, or 100 μM) resulted in a significant reduction in malondialdehyde contents in DOX-treated cardiomyocytes (**Figure 2**). These results suggested that EGCG attenuated lipid peroxidation induced by DOX in a dose-dependent manner.

Effect of EGCG on ROS Generation in DOX-Treated Cardiomyocytes. Intracellular ROS generation was evaluated by measuring DCF fluorescence intensity. As shown in **Figure 3**, EGCG caused a rapid and significant increase ($P < 0.01$ vs controls) in DCF fluorescence (measured in arbitrary units). However, when EGCG at 38, 50, or 100 μM was applied 1 h prior to DOX treatment, it caused a dose-dependent attenuation in DCF fluorescence (all $P < 0.01$ vs DOX). These findings indicate that EGCG has a direct ability to scavenge ROS generation.

Effects of EGCG on Proteins and Activities of MnSOD, Catalase, and Glutathione Peroxidase in DOX-Treated Cardiomyocytes. The first line of defense against oxidative stress-mediated cardiac injury comprises several antioxidant enzymes, including Mn-SOD, catalase, and glutathione peroxidase. To explore whether MnSOD, catalase, and glutathione peroxidase played a role in the observed cardioprotective effects of EGCG, we examined protein expression of MnSOD, catalase, and glutathione peroxidase in the samples after cardiomyocytes were treated with DOX or EGCG. As shown in **Figure 4**, a significant increase in the expression of MnSOD, catalase, and glutathione peroxidase was observed in the EGCG pretreatment group compared with the control and DOX groups.

Effects of EGCG on activities of MnSOD, catalase, and glutathione peroxidase were also analyzed in the present study (**Table 2**). Cardiomyocytes subjected to DOX treatments exhibited a significant decrease in activities of MnSOD, catalase, and glutathione peroxidase as compared with control cells. In EGCG (38, 50, or 100 μM) treatment groups, the activities of MnSOD, catalase, and glutathione peroxidase significantly increased as compared with those in the DOX group. These data indicate that

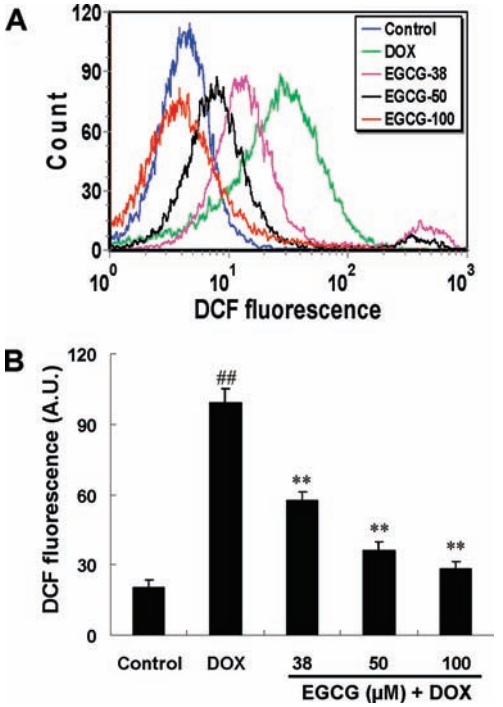

Figure 3. Effect of EGCG on ROS in cardiomyocytes exposed to DOX: (**A**) flow cytometric histograms of fluorescence of 2′,7′-dichlorofluorescein (DCF) in cardiomyocytes; (**B**) column bar graph of cell fluorescence for DCF. Values are expressed as mean ± SEM; $n = 8$. ##, $P < 0.01$ versus control group; **, $P < 0.01$ versus DOX group.

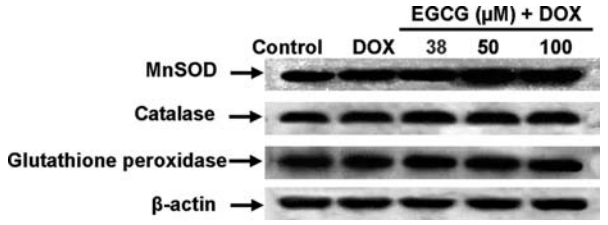

Figure 4. Effect of EGCG on expression of MnSOD, catalase, and glutathione peroxidase in cardiomyocytes subjected to DOX. Cardiomyocytes were lysed, and expression of MnSOD, catalase, glutathione peroxidase, and β-actin was analyzed by Western blot.

Table 2. Effect of EGCG on Activities of MnSOD, Catalase, and Glutathione Peroxidase in Cardiomyocytes Subjected to DOX Injury[a]

group	MnSOD (U/mg of protein)	catalase (U/mg of protein)	glutathione peroxidase (U/mg of protein)
control	108.68 ± 5.98	86.59 ± 6.01	160.04 ± 9.42
DOX	61.39 ± 4.81 a	45.26 ± 5.99 a	118.51 ± 7.05 a
EGCG-38	82.79 ± 5.92 b	59.38 ± 5.95 b	132.05 ± 9.11 b
EGCG-50	96.37 ± 6.08 b	68.98 ± 6.51 b	146.07 ± 9.81 b
EGCG-100	104.25 ± 6.27 b	71.21 ± 6.91 b	154.24 ± 11.18 b

[a] Data are expressed as the mean ± SEM; n =8. EGCG-38, 38 μM EGCG; EGCG-50, 50 μM EGCG; EGCG-100, 100 μM EGCG. For entries followed by "a", $P < 0.01$ vs control group; for entries followed by "b", $P < 0.01$ versus DOX group.

increased antioxidant enzymes protection could contribute to the cardioprotective effects of EGCG against DOX-induced cardiotoxicity in cardiomyocytes.

Effect of EGCG on Apoptosis of DOX-Treated Cardiomyocytes. To further examine whether EGCG protects against DOX-induced apoptosis in cardiomyocytes, we used flow cytometric

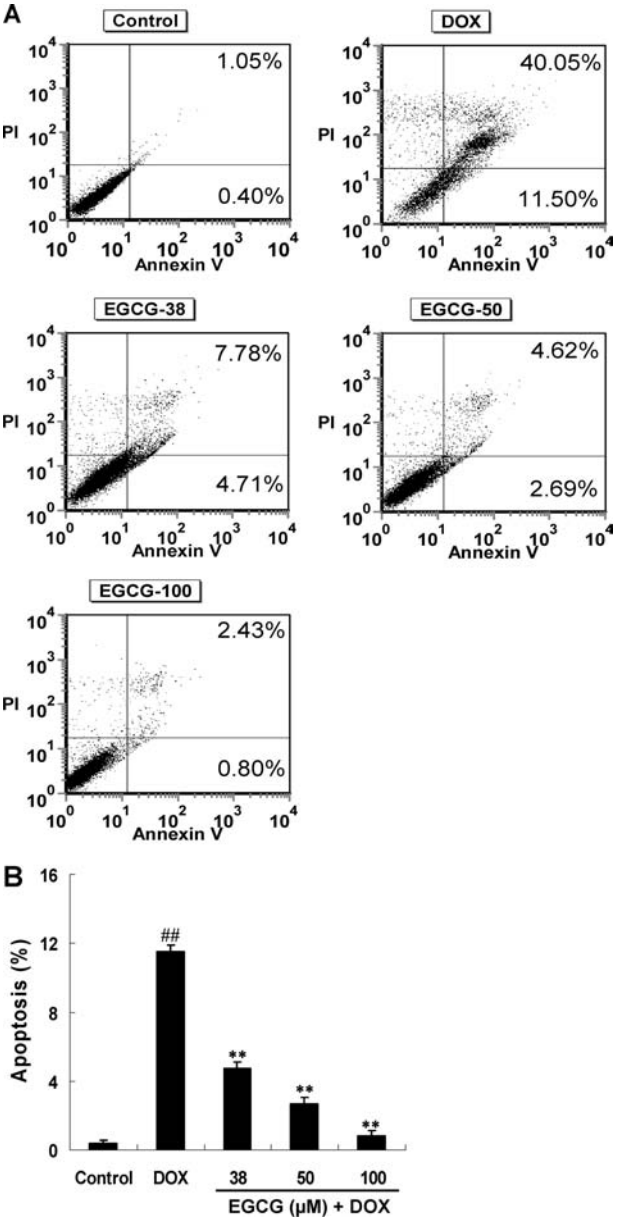

Figure 5. Effect of EGCG on apoptosis in cardiomyocytes exposed to DOX: (**A**) detection of apoptotic cells by annexin V and propidium iodide double staining (cardiomyocytes were treated with 0, 38, 50, or 100 μM EGCG, stained with annexin V−FITC and PI labeling, and analyzed by flow cytometry); (**B**) column bar graph of apoptosis. Data are expressed as the mean ± SEM; $n = 8$. ##, $P < 0.01$ versus control group; **, $P < 0.01$ versus DOX group.

analysis to quantify the rate of apoptosis using double staining of annexin V−FITC and PI. A significant increase of apoptosis was observed in DOX-treated cardiomyocytes compared with control cells. Interestingly, treatment with EGCG (38, 50, or 100 μM) showed a significant resistance in apoptosis in DOX-treated cardiomyocytes in a dose-dependent manner (**Figure 5**). These results suggested that EGCG attenuated apoptosis in DOX-treated cardiomtocytes.

DISCUSSION

Cellular defenses that protect cardiomyocytes against oxidative stress have been proposed to be an important way to reduce the progression of DOX-induced myocyte dysfunction. Our results suggest that EGCG can protect against DOX-induced oxidative stress in a neonatal rat cardiomyocyte model. The major finding in

the study was that EGCG markedly suppressed the DOX-induced decrease of cell viability in a dose-dependent manner. Moreover, EGCG protected cardiomyocytes from the release of LDH and cell death induced by DOX. These effects may be achieved by an increase in the activity and/or protein expression of MnSOD, catalase, and glutathione peroxidase, thus attenuating ROS production, malondialdehyde content, and apoptosis.

DOX, a quinone-containing anthracycline antibiotic, is used in the treatment of a broad spectrum of human cancers. However, clinical application of DOX is limited by its dose-dependent cardiotoxicity (*19*), but the mechanism of cardiotoxicity induced by DOX is not completely understood. ROS generation during intracellular metabolism of DOX and subsequent oxidative stress have been proposed to be important mechanisms underlying its cardiac toxic effects. ROS can alter the structural and functional integrity of cells by a variety of mechanisms, including lipid peroxidation, proteolysis, and shearing of the nuclear materials (*20*, *21*). In the present study, our results indicate that cardiomyocytes subjected to DOX have a rapid increase in ROS production and lipid peroxidation and a concomitant reduction of cell viability and increase of LDH activity. These results also suggest that oxidative stress plays a significant role in the myocardial cell injury induced by DOX. Appropriate antioxidant strategies may be particularly useful to limit ROS production and ROS-induced alterations and hence to protect DOX-mediated cardiac injury. Thus, it is necessary to discover new antioxidants or free radical scavengers with high potency and low toxicity.

There are two major types of tea: green tea and black tea. Green tea has gained the attention of both consumers and researchers in the past few years for its strong antioxidant properties. In fact, epidemiologic studies have shown that green tea consumption can reduce the incidence of cardiovascular diseases (*22*). Green tea has unique characteristics as an agent, with few adverse effects. In addition, it is inexpensive, can be orally consumed, and has a long history as a beverage of general tolerance among all races (*23*). Therefore, green tea seems to have the potential of becoming an ideal agent for cardioprotection. EGCG is a polyphenol that makes up ~30% of the solids in green tea. It has been found to be effective in protecting against pathological states characterized by an increase in the basal rate of ROS production (*24*, *25*). In the present study, we found that EGCG could prevent the decrease of cell viability and the increase of LDH activity resulting from DOX treatment in a dose-dependent manner. These observations suggested that EGCG could exert direct cardioprotection against the cardiotoxic effects of DOX in cardiomyocytes. Our study further showed that treatment with EGCG significantly attenuated ROS production and decreased the concentration of malondialdehyde. These data suggested that the cardioprotective effect of EGCG against DOX cardiotoxicity may be related to attenuation of oxidative stress.

Although the intracellular localization of ROS formation elicited by DOX is still under investigation, recent studies suggest that DOX accumulates over time in mitochondria, where it is enzymatically transformed to semiquinone radicals and superoxide anions (*26*). Cardiac tissue is highly susceptible to oxidative damage, because cardiomyocytes are rich in mitochondria, the site of basal ROS generation, and are also exposed to relatively high oxygen tension compared to other tissues (*27*). The first line of cell defense against oxidative stress-mediated cardiac injury comprises several endogenous antioxidant enzymes such as MnSOD, catalase, and glutathione peroxidase, which act together to scavenge ROS. MnSOD, the primary antioxidant enzyme that resides only in mitochondria, converts superoxide radicals to H_2O_2. Glutathione peroxidase and/or catalase subsequently convert H_2O_2 to water (*28*, *29*).

To explore whether endogenous antioxidants played a role in the observed cardioprotective effects of EGCG, we examined the effect of EGCG on proteins and the activities of MnSOD, catalase, and glutathione peroxidase in DOX-treated cardiomyocytes. Consistent with previous results, our current results also suggested that DOX-induced injury was associated with increased oxidative stress and depletion of the activities of antioxidatant enzymes in cardiomyocytes. Interestingly, EGCG treatment has been shown to evoke a dose-dependent increase in both protein expression and activity levels of these antioxidant enzymes. These findings suggested that EGCG might offer cardioprotection against oxidative stress-induced cell death by enhancing the activities and/or protein expression of these antioxidant enzymes in cardiomyocytes. In addition, DOX-induced apoptosis of cardiomyocytes has been proposed to play an important role in its cardiotoxicity that is linked to the formation of ROS derived from redox cycling of DOX (*30*, *31*). Apoptosis plays a critical role in a variety of biological systems and has been identified to be an important molecular basis for both the initiation and progression of DOX cardiotoxicity (*32*, *33*). In this study, we also found that cardiomyocytes subjected to DOX insults had a rapid and significant increase in apoptosis and a concomitant elevation of intracellular ROS production. This result suggests that there is a tight relationship between ROS generation and apoptosis in DOX-induced cardiomyocyte injury. EGCG treatment has been shown to evoke a dose-dependent reduction of apoptosis in cardiomyocytes subjected to DOX. This indicates that alleviating DOX-induced apoptosis may be one of the mechanisms underlying the cardioprotective effect of EGCG. Meanwhile, the change was associated with an attenuation of ROS generation and a decrease of malondialdehyde content. However, whether the alleviation of apoptosis is caused by attenuation of ROS generation remains to be determined.

In summary, the present study suggests DOX to be an oxidant that can induce cardiomyocyte death. EGCG significantly attenuates DOX-induced oxidative stress and improves cell survival in cultured cardiomyocytes. These results may be relevant to understanding of the cardiovascular health benefits of green tea consumption in humans. EGCG is a natural compound in green tea and seems to be safer than popular cardioprotective agents. Hence, EGCG may have a great potential as a novel therapeutic agent to prevent cardiotoxic effects induced by DOX.

ABBREVIATIONS USED

cDCFH-DA, (6)-carboxy-2′,7′-dichlorofluorescein diacetate; DCFH, 2′,7′-dichlorofluorescin; DOX, doxorubicin; EGCG, (−)-epigallocatechin-3-gallate; LDH, lactate dehydrogenase; MnSOD, manganese superoxide dismutase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; ROS, reactive oxygen species.

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Received for review April 5, 2010. Revised manuscript received June 13, 2010. Accepted July 15, 2010. Financial support for this study by the National High Technology Research and Development Program of China (863 Program) (2008AA10Z325) and the Objective-Oriented Project of State Key Laboratory of Food Science and Technology (SKLF-MB-200806) and Key Science and Technique Project of Jiangxi Provincial Department of Science is gratefully acknowledged.