Original Research

The Major Green Tea Polyphenol, (-)-Epigallocatechin-3-Gallate, Induces Heme Oxygenase in Rat Neurons and Acts as an Effective Neuroprotective Agent against Oxidative Stress

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Key words: (-)-epigallocatechin-3-gallate, green tea, polyphenols, oxidative stress, heme oxygenase

Background: Oxidative stress induced by hyperglycemia is a key factor in the pathogenesis of diabetic complications, such as neuropathy. Recently, green tea catechins have received much attention, as they can facilitate a number of antioxidative mechanisms and improve glycemic control.

Objective: The aim of this study was to investigate the cytoprotective effects of (-)-epigallocatechin-3-gallate (EGCG) against oxidative stress damage in a cell line of rat neurons. The role of heme oxygenase 1 (HO-1) induction by EGCG and the transcriptional mechanisms involved were also evaluated.

Methods: Immortalized rat neurons (H 19-7) were exposed to various concentrations of EGCG (10–200 μM). After treatments (6 or 24 hours), cells were harvested for the determination of heme oxygenase activity, mRNA levels, and protein expression. Nuclear levels of Nrf2, a transcriptional factor involved in HO-1 activation, were also measured. Neurons were pretreated for 12 hours with EGCG 50 μM or EGCG 50 μM + zinc protoporphyrin IX 10 μM and then exposed for 2 hours to 50 mμ/mL glucose-oxidase before cell viability was determined.

Results: In cultured neurons, elevated expression of HO-1 mRNA and protein were detected after 6 hours of incubation with 25–100 μM EGCG, and its induction relates with the activation of Nrf2. Interestingly, pre-incubation (12 hours) with EGCG 50 μM resulted in an enhanced cellular resistance to glucose oxidase–mediated oxidative damage; this cytoprotective effect was considerably attenuated by zinc protoporphyrin IX, an inhibitor of heme oxygenase activity.

Conclusions: In this study, we demonstrated that EGCG, the major green tea catechin, induced HO-1 expression in cultured neurons, possibly by activation of the transcription factor Nrf2, and by this mechanism was able to protect against oxidative stress–induced cell death.

INTRODUCTION

Green tea, one of the most widely consumed beverages, has recently attracted scientific attention as a potential nutritional strategy to prevent a broad range of age-related chronic disorders, including cardiovascular diseases [1,2], cancer [3], obesity [4], diabetes [5], and neurodegenerative pathologies [6]. Moreover, a number of epidemiological studies have suggested that consuming green tea on a daily basis, as part of a healthy lifestyle, may reduce the onset of all causes of mortality and improve longevity [7]. The health-promoting effects of green tea consumption are mainly attributed to its

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Abbreviations: EGCG = (-)-epigallocatechin-3-gallate, HO-1 = heme oxygenase 1, GOX = glucose-oxidase, ZnPP IX = zinc protoporphyrin IX, Nrf2 = transcription factor NF-E2-related factor-2

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polyphenol content, which represents 35% of its dry weight [8]. Compared to black tea, green tea is particularly rich in catechins, which include (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate, (-)-epigallocatechin, and epicatechin. EGCG is the most active and abundant compound in green tea, representing approximately 43% of the total phenols. Many of the aforementioned beneficial effects of green tea on cancer, obesity, type 2 diabetes, and cardiovascular risk factors have been related to its EGCG content. In fact, this compound appears to have a number of different molecular targets, impinging on several signaling pathways and showing pleiotropic activity on cells and tissues. EGCG possesses antioxidant and anti-inflammatory properties, which include the capacity to inhibit overexpression of cyclooxygenase-2 [9] and nitric oxide synthase [10]. It also induces apoptosis in several types of cancer cells by inactivating some transcription factors, such as nuclear factor kappa B [11], activator protein 1 [12], and signal transducer and activator of transcription 1 (STAT-1) [13]. EGCG prevents cancer cell invasion, angiogenesis, and metastasis by down-regulating the expression of matrix metalloproteinases and by inhibiting the cell adhesion function [14–16]. Several reports have also shown that EGCG is able to induce a general xenobiotic response in the target cells, activating multiple defense genes [14]. EGCG could be considered a representative member of a more general group of bioactive phytochemicals, which also includes curcumin, resveratrol, caffeic acid phenethyl ester, sulforaphane, and ethyl ferulate [17–19], that exhibit Michael acceptor function and possess the ability to activate the transcription factor Nrf2, and by this to induce expression of phase II detoxifying/antioxidant enzymes and heme oxygenase-1 (HO-1). HO-1 is a ubiquitous and redox-sensitive inducible stress protein that catabolizes heme to produce carbon monoxide and bilirubin [20]. A substantial body of evidence demonstrates that both bilirubin and carbon monoxide effectively contribute to modulate important physiological processes within the cardiovascular, immune, and nervous systems [21]. These include the regulation of vessel tone, inhibition of platelet aggregation, and prevention of cell death and tissue injury. Furthermore, it is now widely accepted that induction of HO-1 expression represents an adaptive response that critically increases cell resistance to oxidative injury [22,23]. In this study, we analyzed the potency of EGCG as an inducer of HO-1 expression in cultured neurons and explored whether this induction contributed to the protective effects of this polyphenolic compound against oxidative damage.

MATERIALS AND METHODS

Chemicals and Reagents

EGCG, glucose oxidase (GOX), which generates hydrogen peroxide in the culture medium, and all other reagents were from Sigma unless otherwise specified. Zinc protoporphyrin IX (ZnPP-IX), a specific inhibitor of HO activity, was from Porphyrin Products Ltd (Logan, UT). Rabbit polyclonal antibodies directed against HO-1 and HO-2 were obtained from Stressgen (Victoria, BC, Canada).

Cell Culture

Rat hippocampal neurons (H 19-7) [24] were purchased from the American Type Culture Collection (Manassas, VA) and cultured according to the manufacturer's instructions. Cells were grown in 75-cm^2 flasks and maintained at 37°C in a humid atmosphere of air and 5% carbon dioxide. Confluent cells were exposed to various concentrations of EGCG. After each treatment (6 or 24 hours), cells were harvested for the determination of HO activity; HO-1, HO-2 and heat shock protein 72 (HsP72) mRNAs; and HO-1 and HO-2 protein levels. Neurons, growing in 24 wells, were also exposed to different concentrations of EGCG, and cell viability was determined at 24 hours. A group of cultured neurons was pretreated for 12 hours with EGCG 50 μM or EGCG 50 μM + ZnPP-IX 10 μM and then exposed for 2 hours to 50 mU/ml GOX before cell viability was determined.

Cell Viability Assay

Neurons were exposed to EGCG for 24 hours, and cell viability was assessed with the use of an Alamar Blue assay according to the manufacturer's instructions (Serotec, Oxford, United Kingdom), as reported previously [17]. At the end of each treatment, cells were washed twice and incubated for an additional 5 hours in complete medium containing 1% Alamar Blue solution. The optical density of each sample was measured using a plate reader (Molecular Devices, Crawley, United Kingdom). The intensity of the color developed in the medium is proportional to the viability of cells, which was calculated as the difference in absorbance between 570 and 600 nm and expressed as percentage of control.

Reverse-Transcriptase Quantitative Polymerase Chain Reaction

Total RNA from cell cultures was extracted using Trizol (Sigma, St. Louis, MO) and treated with RNase-free DNase to remove any residual genomic DNA. Single-stranded cDNAs were synthesized incubating total RNA (1 μg) with SuperScript II RNase H-reverse transcriptase (200 U), oligo-$(dT)_{12–18}$ primer (100 nM), deoxynucleotide triphosphates (1 mM), and RNase inhibitor (40 U) at 42°C for 1 hour in a final volume of 20 μl. Reaction was terminated by incubating at 70°C for 10 minutes.

Forward (FP) and reverse (RP) primers used to amplify HO isoforms and Hsp72, and expected amplification products, are listed in Table 1. To control the integrity of RNA and to control for differences attributable to errors in experimental

Table 1. Sequences of Oligonucleotide Primers for HO and Hsp70 Isoforms mRNAs

Name	GenBank Accession No.	Sequence	PCR product
HO-1-F	NM_012580.1	TGCTCGCATGAACACTCTG	331 bp
HO-1-R	NM_012580.1	TCCTCTGTCAGCAGTGCCT	
HO-2-F	J05405.1	CACCACTGCACTTTACTTCA	224 bp
HO-2-R	J05405.1	AGTGCTGGGGAGTTTTAGTG	
Hsp72-F	NM_031971	CACCACTGCACTTTACTTCA	302 bp
Hsp72-R	NM_031971	TCCTCTGTCAGCAGTGCCT	
PGK1-F	M31788	AGGTGCTCAACAACATGGAG	183 bp
PGK1-R	M31788	TACCAGAGGCCACAGTAGCT	

manipulation from tube to tube, primers for rat phosphoglycerate kinase 1 (PGK1), a housekeeping gene that is consistently expressed in brain tissues, were used in separate polymerase chain reactions (PCR).

Aliquots of cDNA (0.1 and 0.2 μg) and known amounts of external standard (purified PCR product, 10^2 to 10^8 copies) were amplified in parallel reactions using the FP and RP. Each PCR reaction (final volume 20 μl) contained 0.5 μM of primers, 2.5 mM Mg^{2+}, and 1× Light cycler DNA master SYBR Green (Roche Diagnostics, Indianapolis, IN). PCR amplifications were performed with a Light-Cycler (Roche Molecular Biochemicals) using the following four cycle programs: (1) denaturation of cDNA (1 cycle: 95°C for 10 minutes); (2) amplification (40 cycles: 95°C for 0 seconds, 58°C for 5 seconds, 72°C for 10 seconds); (3) melting curve analysis (1 cycle: 95°C for 0 seconds, 70°C for 10 seconds, 95°C for 0 seconds); (4) cooling (1 cycle: 40°C for 3 minutes). The temperature transition rate was 20°C/s except for the third segment of the melting curve analysis, where it was 0.2°C/s. Fluorimeter gain value was 6. Real-time detection of fluorimetric intensity of SYBR Green I, indicating the amount of PCR product formed, was measured at the end of each elongation phase. Quantification was performed by comparing the fluorescence of PCR products of unknown concentration with the fluorescence of the external standards. For this analysis, fluorescence values measured in the log-linear phase of amplification were considered using the second derivative maximum method of the Light-Cycler Data Analysis software (Roche Molecular Biochemicals). Specificity of PCR products obtained was characterized by melting curve analysis, followed by electrophoretic gel visualized by ethidium bromide staining and by DNA sequencing.

Western Blot Analysis for HO-1 and HO-2

After treatment with EGCG, samples of neurons were analyzed for HO-1 and HO-2 protein expression using a Western immunoblot technique, as described previously [18]. Briefly, an equal amount of proteins (30 μg) for each sample was separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes; the nonspecific binding of antibodies was blocked with 3% nonfat dried milk in Tris-buffered saline (TBS-T). Membranes were then probed with a polyclonal rabbit anti–HO-1 and anti–HO-2 antibodies (Stressgen) (1:1000 dilution in TBS-T, pH 7.4) for 2 hours at room temperature. After three washes with TBS-T, blots were visualized using an amplified alkaline phosphatase kit from Sigma (Extra-3A), and the relative density of bands was analyzed by the use of an imaging densitometer (model GS-700; Bio-Rad, Hertfordshire, United Kingdom). Blots shown are representative of three independent experiments.

HO Activity Assay

HO activity was determined at the end of each treatment as described previously [17]. Briefly, microsomes from harvested cells were added to a reaction mixture containing nicotinamide adenine dinucleotide phosphate, glucose-6-phosphate dehydrogenase, rat liver cytosol as a source of biliverdin reductase, and the substrate hemin. The mixture was incubated in the dark at 37°C for 1 hour and the reaction was stopped by the addition of 1 ml of chloroform. After vigorous vortex and centrifugation, the extracted bilirubin in the chloroform layer was measured by the difference in absorbance between 464 and 530 nm ($\varepsilon = 40\ mM^{-1}cm^{-1}$).

Preparation of Nuclear Extract and Western Blot for Nrf2

Neurons were washed twice with phosphate-buffered saline (1×). Cells were then harvested in 1 ml phosphate-buffered saline 1× and centrifuged at 3000 rpm for 3 minutes at 4°C. The cell pellet was carefully resuspended in 200 μl of cold buffer containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES) (pH = 7.9), 10 mM potassium chloride, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM ethyleneglycoltetraacetic acid (EGTA), 1 μM dithiothreitol (DTT), and complete protease inhibitor cocktail (Roche, Mannheim, Germany). The pellet was then incubated on ice for 15 minutes to allow cells to swell. After this time, 15 μl of 10% nonyl phenoxylpolyethoxylethanol-40 (NP-40) was added and the tube was vortexed for 10 seconds. The homogenate was then centrifuged at 3000 rpm for 3 minutes at 4°C and the nuclear pellet was resuspended in 30 μl of cold buffer consisting of 20 mM HEPES (pH = 7.9), 0.4 M sodium

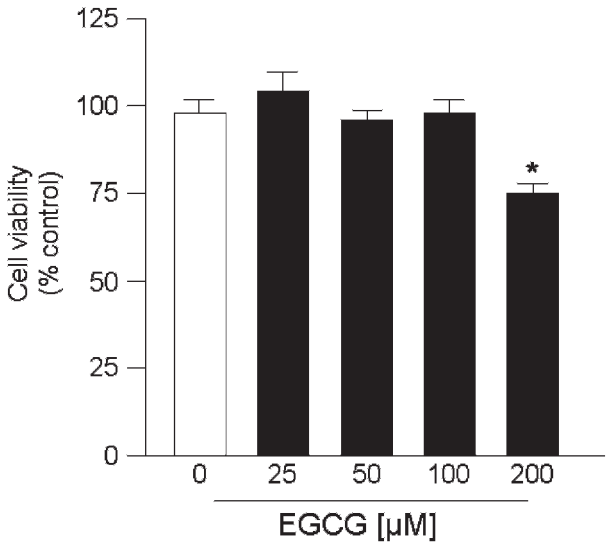

Fig. 1. Effect of EGCG on cell viability. Cells were exposed for 24 hours to various concentrations (0–200 μM) of EGCG in complete medium. Cell viability was measured spectrophotometrically using an Alamar Blue assay. Data are expressed as the mean ± SEM of six independent experiments. * $p < 0.05$ versus 0 μM of EGCG.

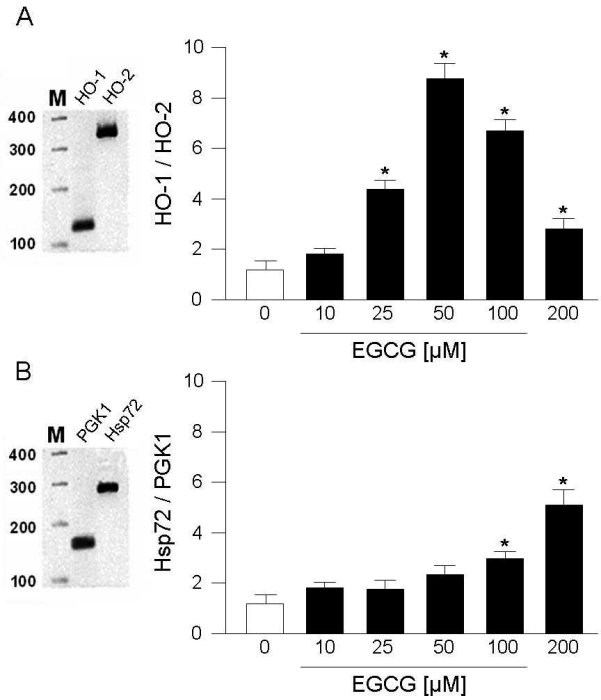

Fig. 2. Real-time quantification of HO-1, HO-2, and Hsp72 mRNA levels by PCR in neurons treated with EGCG. Specific primers for HO-1, HO-2, and Hsp72 were used to amplify rat RNA. Total RNA from different samples and known amounts of external standards (purified PCR product, 102 to 108 copies) was amplified in parallel reactions. Fluorimetric intensity of SYBR Green I, indicating the amount of PCR product formed, was measured at the end of each elongation phase. Quantification was performed by comparing the fluorescence of PCR products of unknown concentration with the fluorescence of the external standards. The specificity of the products amplified was evaluated by melting curve analysis. Cellular expression of HO-1 transcript relative to the expression of HO-2 and of Hsp72 transcript relative to the expression of PGK1 (mean ± SEM) is shown for the different EGCG concentration after 6 hours of treatment (A,B). * $p < 0.05$ versus 0 μM of EGCG.

chloride, 1 mM EDTA, 1 mM EGTA, 1 μM DTT, and protease inhibitors. The pellet was then incubated on ice for 15 minutes and vortexed for 10 to 15 seconds every 2 minutes. The nuclear extract was finally centrifuged at 13,000 rpm for 5 minutes at 4°C. The supernatant containing the nuclear proteins was resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel and submitted to immunoblot analysis using anti-Nrf2 (1:500 dilution) and anti-Sp1 (1:500 dilution) antibodies.

Statistical Analysis

Differences in the data among the groups were analyzed by using one-way analysis of variance combined with the Bonferroni test. Values were expressed as the mean ± standard error of the mean (SEM), and differences between groups were considered to be significant at $p < 0.05$.

RESULTS

HO Activity, HO-1 mRNA, and Protein Expression in Cultured Hippocampal Neurons

Cell viability (determined using the Alamar Blue assay) was fully preserved, when the concentration of EGCG did not exceed 100 μM (Fig. 1). The exposure of neurons for 6 h to different concentrations of EGCG (10, 25, 50, 100, and 200 μM) resulted in a gradual and significant ($p < 0.05$) increase in HO-1 mRNA, as shown by quantitative real-time PCR (Fig. 2A), with a maximal value at 50 μM, followed by a decrease with higher concentration, in a bell-shaped way. This gene activation was strongly associated with a marked up-regulation of HO-1 protein. HO activity and protein expression were maximally up-regulated after 6 hours of treatment with 50 μM EGCG, although to a lesser extent, an increase in HO activity was also found in cells after 24 hours of EGCG treatment (Fig. 3A,B). In contrast, no increase in HO-1 expression and activity was found when higher concentrations (200 μM) of this drug were used. However, at higher concentrations, the induction of Hsp72 mRNA, an inducible heat shock protein, was observed (Fig. 2B). Hsp72 up-regulation and the reduced ability of EGCG to increase HO expression and activity at high concentrations (200 μM), correlated with a cytotoxic effect exerted by this compound (Fig. 1). To determine the potential toxic effect of EGCG on neurons, cells grown to confluence in 24 wells were incubated in increasing concentrations for 24 hours. When the concentration of these drugs did not exceed 100 μM, cell viability

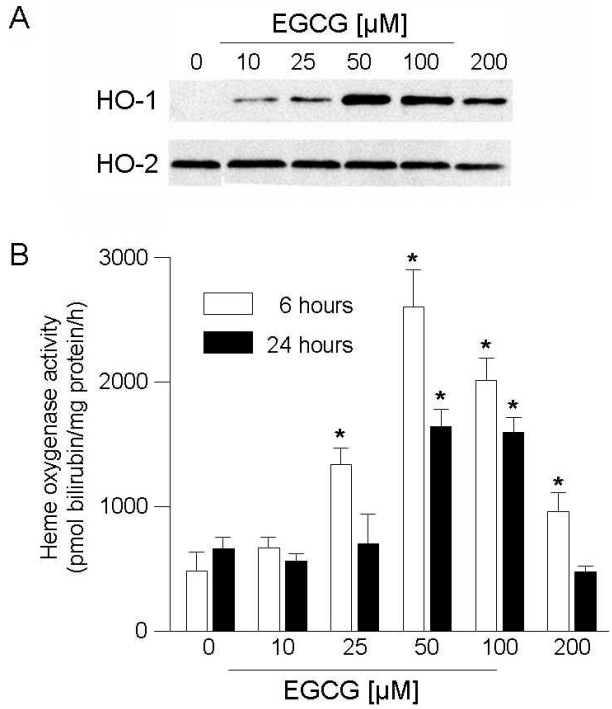

Fig. 3. Effect of EGCG on HO-1 and HO-2 protein expression and HO activity in neurons. Western blots showing HO-1 and HO-2 protein levels in cultured neurons after treatment with EGCG (0–200 μM) for 6 hours. These panels are representative of four different experiments with similar results. HO activity was measured in cells after short (6 hours) or prolonged (24 hours) exposure to various concentrations of EGCG (0–200 μM). Each bar represents the mean ± SEM of five independent experiments. * $p < 0.05$ versus 0 μM of EGCG.

(determined using the Alamar Blue assay) and cell morphology (observed under the microscope) were fully preserved throughout the incubation period. Treatment of neurons with 200 μM of EGCG was significantly cytotoxic, resulting in a 25% reduction in cell viability.

Nrf2 Expression in Neurons

Cells were exposed to EGCG at the final concentration of 50 μM to evaluate the expression of Nrf2 protein over time. As shown in Fig. 4, treatment with EGCG caused a significant time-dependent increase in Nrf2 protein expression in the nuclear extracts. Quantification of three independent Western blots showed that after 1 hour of exposure to 50 μM of EGCG, Nrf2 expression significantly increased and remained up-regulated for 12 hours, whereas the levels of the housekeeping transcription factor Sp1 were stable.

HO Expression by EGCG Protects Neuronal Cells from GOX-Induced Cell Death

We examined the effects of EGCG pretreatment on GOX-induced cell death in H 19-7 rat hippocampal neurons. The

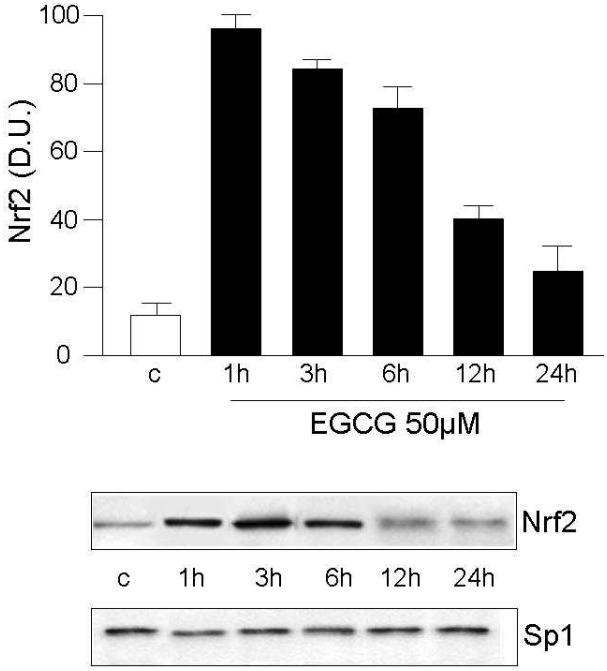

Fig. 4. EGCG increases the levels of the Nrf2 transcription factor in neurons nuclear extracts. Western blots showing increases in Nrf2 protein levels in nuclear extracts of cells treated with 50 μM EGCG, whereas the levels of the housekeeping transcription factor Sp1 were stable.

neuronal cells were pretreated for 12 hours with EGCG at a concentration of 25 μM and then exposed to 50 mU/ml GOX for 2 hours. Exposure of the cells for 2 hours to 50 mU/ml GOX caused a 74% decrease in cell viability ($p < 0.05$; Fig. 5). However, pretreatment of the cells with EGCG helped to maintain the viability of the cells to 72% of control levels ($p < 0.05$; Fig. 5). The involvement of HO-1 in the cytoprotective effect of EGCG was confirmed using an inhibitor of HO activity, ZnPP-IX, which at a concentration of 10 μM significantly blocked EGCG-mediated suppression of GOX-induced cell death (Fig. 5). These data indicate that the cytoprotective effect of EGCG might be a result, in part, of the induction of HO-1.

DISCUSSION

Oxidative stress refers to a disrupted redox equilibrium between the production of free radicals and the ability of cells to protect against damage caused by these molecules. A high concentration of glucose has been implicated as a causal factor in the initiation and progression of diabetic complications, and hyperglycemia has been shown to directly increase the production of free radicals and oxidative stress [25,26]. Prevention of synthesis and tissue accumulation of advanced glycation end products or oxidative-derived end products could constitute a major advance in the treatment of diabetic

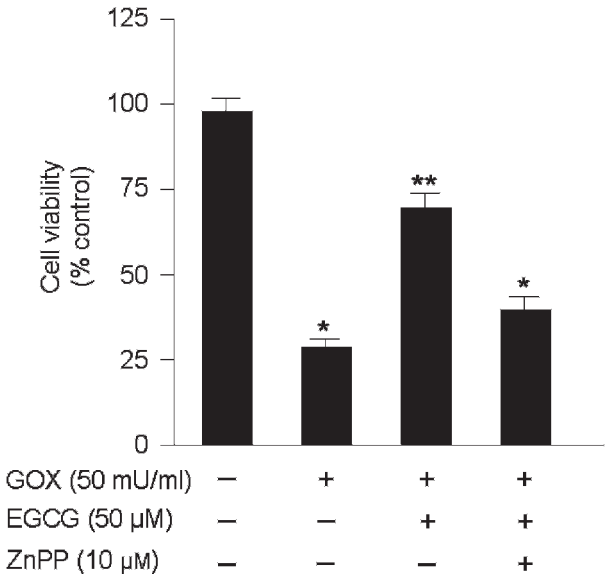

Fig. 5. Neuroprotective effect of EGCG against GOX-induced cell death. Rat hippocampal neurons were treated with 50 μM of EGCG for 12 hours in the presence or absence of 10 μM of ZnPP-IX. After this pretreatment, cells were incubated for 2 hours with GOX 50 mU/ml. After these treatments, cells were washed and viability was assessed by Alamar Blue assay. *$p < 0.05$ versus GOX; ** $p < 0.05$ versus GOX plus EGCG.

complications, such as diabetic neuropathy [27]. Therefore, compounds that scavenge reactive oxygen species may confer regulatory effects on high glucose-induced cell damage. There is currently an intensive search for natural ingredients for the prevention and treatment of diabetes [28]. Among others, green tea catechins have received a great deal of attention recently. Epidemiological evidence indicates that populations with high intakes of green tea catechins benefit in terms of body weight and body fat, glucose homeostasis, and cardiovascular health. A large epidemiological study conducted in Japan showed that subjects with an average habitual consumption of >6 cups of green tea per day had a decreased risk of developing diabetes [29]. Overall, the potential beneficial effects of green tea catechins on glucose metabolism may be mediated by various mechanisms, including decreased carbohydrate absorption, decreased hepatic glucose production, increased insulin secretion and insulin sensitivity, and increased uptake of glucose into skeletal muscle [30–32]. EGCG may also protect against the development of long-term complications of diabetes as a result of its strong antioxidant effects [33]. In particular, some studies have recently demonstrated that the neuroprotective mechanisms of EGCG are partly a result of increasing activities of antioxidant enzymes and decreasing advanced glycation end product–induced damage in rat brain or neuronal cells [34,35]. Protecting cells from oxidative stress may be achieved either by directly scavenging reactive oxygen species or by fortifying the body's antioxidant defenses through induction of antioxidant gene expression.

In this study, we attempted to determine the molecular mechanisms underlying the antioxidant effects of EGCG in neurons exposed to oxidative stress by focusing on the ability of this compound to up-regulate HO-1 expression, a critical adaptive enzyme involved in cellular stress response. We demonstrated that cells treated with different concentrations of EGCG (25 to 100 μM) did not show significant cytotoxicity but did show increased HO activity with a bell-shaped dose-response curve; the latter finding was accompanied by a parallel bell-shaped increase in HO-1 mRNA and protein expression (Figs. 2A,3). Higher concentrations of EGCG (200 μM) induced cellular toxicity and caused different stress adaptive system activation, such as Hsp72 expression (Fig. 2B), but did not induce HO-1 expression. Thus, we suggest that the ability of low-dose EGCG to stimulate HO-1 expression is mediated by the activation of a specific signal transduction protein. Previous papers have identified that Nrf2 is a key transcriptional factor that activates the antioxidant-reactive element (ARE) and in turn regulates the expression of antioxidant phase II detoxifying enzymes and other antioxidant proteins containing the ARE sequence in the gene promoter region, such as HO-1 [36]. It has been shown that some polyphenols, including EGCG, up-regulate HO-1 through the Nrf2 signaling pathway [37]. In the present research, Nrf2 was found to be markedly up-regulated in neurons exposed to nontoxic concentrations of EGCG. Our data therefore suggest that in neurons, EGCG may induce HO-1 and other ARE-dependent genes via the activation of Nrf2. In this study, we also demonstrated that the cytoprotective effects of EGCG against oxidative damage in neuronal cells is strictly linked to this pathway. Pretreatment of cultured neurons with 50 μM of EGCG up-regulated HO-1 expression, which conferred neuroprotection against GOX-induced oxidative injury and significantly reduced cell death. ZnPP-IX, a specific inhibitor of HO activity, abrogated the protective effect of EGCG, demonstrating a direct involvement of HO-1 induction in the antioxidant mechanisms promoted by this catechin. HO-1 is central to the regulation of oxidative injury, but the advantages to pharmacologically target this enzyme appear to be more extensive. With regard to glycemic homeostasis and diabetes, HO-1 induction improved insulin sensitivity and glucose tolerance and decreased insulin levels [38]. Thus it has been suggested that increasing HO-1 expression and HO activity by pharmacological or nutritional compounds might represent a promising strategy to prevent and mitigate detrimental side effects of diabetes [39].

CONCLUSIONS

Recently, much attention has been focused on polyphenolic compounds, as they can facilitate a number of antioxidative

mechanisms and activate defensive enzymes, thus protecting against both oxidative and electrophilic cell damage. A corollary of our study is that pharmacological activation of HO-1 by the food-related compound EGCG, probably via the activation of Nrf2 nuclear translocation, efficiently protects neurons from oxidative stress and should be evaluated as a new therapeutic approach for treatment and prevention of diseases that correlate with oxidative damage, such as diabetic neuropathy.

REFERENCES

1. Sano J, Inami S, Seimiya K, Ohba T, Sakai S, Takano T, Mizuno K: Effects of green tea intake on the development of coronary artery disease. Circ J 68:665–670, 2004.
2. Wolfram S: Effects of green tea and EGCG on cardiovascular and metabolic health. J Am Coll Nutr 26:373S–388S, 2007.
3. Moyers SB, Kumar NB: Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials. Nutr Rev 62:204–211, 2004.
4. Boschmann M, Thielecke F: The effects of epigallocatechin-3-gallate on thermogenesis and fat oxidation in obese men: a pilot study. J Am Coll Nutr 26:389S–395S, 2007.
5. Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, Kim JA, Quon MJ, Montagnani M: Epigallocatechin gallate, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial ischemia/reperfusion injury in spontaneously hypertensive rats (SHR). Am J Physiol Endocrinol Metab 292:E1378–E1387, 2007.
6. Mandel S, Weinreb O, Amit T, Youdim MB: Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. J Neurochem 88:1555–1569, 2004.
7. Khan N, Mukhtar H: Tea polyphenols for health promotion. Life Sci 81:519–533, 2007.
8. Yang CS, Landau JM: Effects of tea consumption on nutrition and health. J Nutr 130:2409–2412, 2000.
9. Hussain T, Gupta S, Adhami VM, Mukhtar H: Green tea constituent epigallocatechin-3-gallate selectively inhibits COX-2 without affecting COX-1 expression in human prostate carcinoma cells. Int J Cancer 113:660–669, 2005.
10. Ahmed S, Rahman A, Hasnain A, Lalonde M, Goldberg VM, Haqqi TM: Green tea polyphenol epigallocatechin-3-gallate inhibits the IL-1 beta-induced activity and expression of cyclooxygenase-2 and nitric oxide synthase-2 in human chondrocytes. Free Radic Biol Med 33:1097–1095, 2002.
11. Kim SJ, Jeong HJ, Lee KM, Myung NY, An NH, Yang WM, Park SK, Lee HJ, Hong SH, Kim HM, Um JY: Epigallocatechin-3-gallate suppresses NF-kappaB activation and phosphorylation of p38 MAPK and JNK in human astrocytoma U373MG cells. J Nutr Biochem 18:587–596, 2007.
12. Dong Z, Ma W, Huang C, Yang CS: Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (-)-epigallocatechin gallate, and theaflavins. Cancer Res 57:4414–4419, 1997.
13. Townsend PA, Scarabelli TM, Pasini E, Gitti G, Menegazzi M, Suzuki H, Knight RA, Latchman DS, Stephanou A: Epigallocatechin-3-gallate inhibits STAT-1 activation and protects cardiac myocytes from ischemia/reperfusion-induced apoptosis. FASEB J 18:1621–1623, 2004.
14. Khan N, Mukhtar H: Multitargeted therapy of cancer by green tea polyphenols. Cancer Lett 269:269–280, 2008.
15. Sartippour MR, Shao ZM, Heber D, Beatty P, Zhang L, Liu C, Ellis L, Liu W, Go VL, Brooks MN: Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. J Nutr 132:2307–2311, 2002.
16. Ahmed S, Wang N, Lalonde M, Goldberg VM, Haqqi TM: Green tea polyphenol epigallocatechin-3-gallate (EGCG) differentially inhibits interleukin-1 beta-induced expression of matrix metalloproteinase-1 and -13 in human chondrocytes. J Pharmacol Exp Ther 308:767–773, 2004.
17. Scapagnini G, Foresti R, Calabrese V, Giuffrida Stella AM, Green CJ, Motterlini R: Caffeic acid phenethyl ester and curcumin: a novel class of heme oxygenase-1 inducers. Mol Pharmacol 61: 554–561, 2002.
18. Scapagnini G, Butterfield DA, Colombrita C, Sultana R, Pascale A, Calabrese V: Ethyl ferulate, a lipophilic polyphenol, induces HO-1 and protects rat neurons against oxidative stress. Antioxid Redox Signal 6:811–818, 2004.
19. Talalay P, Fahey JW, Healy ZR, Wehage SL, Benedict AL, Min C, Dinkova-Kostova AT: Sulforaphane mobilizes cellular defenses that protect skin against damage by UV radiation. Proc Natl Acad Sci USA 104:17500–17505, 2007.
20. Maines MD, Trakshel GM, Kutty RK: Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible. J Biol Chem 261:411–419, 1986.
21. Abraham NG, Kappas A: Pharmacological and clinical aspects of heme oxygenase. Pharmacol Rev 60:79–127, 2008.
22. Clark JE, Foresti R, Green CJ, Motterlini R: Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress. Biochem J 348 Pt 3:615–619, 2000.
23. Chen K, Gunter K and Maines MD: Neurons overexpressing heme oxygenase-1 resist oxidative stress-mediated cell death. J Neurochem 75:304–313, 2000.
24. Eves EM, Tucker MS, Roback JD, Downen M, Rosner MR, Wainer BH: Immortal rat hippocampal cell lines exhibit neuronal and glial lineages and neurotrophin gene expression. Proc Natl Acad Sci USA 89:4373–4377, 1992.
25. Rolo AP, Palmeira CM: Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicol Appl Pharmacol 212:167–178, 2006.
26. Shi H, Liu KJ: Effects of glucose concentration on redox status in rat primary cortical neurons under hypoxia. Neurosci Lett 410:57–61, 2006.
27. Pop-Busui R, Sima A, Stevens M: Diabetic neuropathy and oxidative stress. Diabetes Metab Res Rev 22:257–273, 2006.
28. Dembinska-Kiec A, Mykkänen O, Kiec-Wilk B, Mykkänen H: Antioxidant phytochemicals against type 2 diabetes. Br J Nutr 99(E Suppl 1):ES109–ES117, 2008.

29. Iso H, Date C, Wakai K, Fukui M, Tamakoshi A, JACC Study Group: The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med 144:554–562, 2006.
30. Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Riegger C, Weber P: Epigallocatechin gallate supplementation alleviates diabetes in rodents. J Nutr 136:2512–2518, 2006.
31. Thielecke F, Boschmann M: The potential role of green tea catechins in the prevention of the metabolic syndrome—a review. Phytochemistry 70(1):11–24, 2009.
32. Fukino Y, Ikeda A, Maruyama K, Aoki N, Okubo T, Iso H: Randomized controlled trial for an effect of green tea-extract powder supplementation on glucose abnormalities. Eur J Clin Nutr 62:953–960, 2008.
33. Rizvi SI, Zaid MA, Anis R, Mishra N: Protective role of tea catechins against oxidation-induced damage of type 2 diabetic erythrocytes. Clin Exp Pharmacol Physiol 32:70–75, 2005.
34. Srividhya R, Jyothilakshmi V, Arulmathi K, Senthilkumaran V, Kalaiselvi P: Attenuation of senescence-induced oxidative exacerbations in aged rat brain by (-)-epigallocatechin-3-gallate. Int J Dev Neurosci 26:217–223, 2008.
35. Lee SJ, Lee KW: Protective effect of (-)-epigallocatechin gallate against advanced glycation endproducts-induced injury in neuronal cells. Biol Pharm Bull 30:1369–1373, 2007.
36. Jaiswal AK: Nrf2 signaling in coordinated activation of antioxidant gene expression. Free Radic Biol Med 36:1199–1207, 2004.
37. Kweon MH, Adhami VM, Lee JS, Mukhtar H: Constitutive overexpression of Nrf2-dependent heme oxygenase-1 in A549 cells contributes to resistance to apoptosis induced by epigallocatechin 3-gallate. J Biol Chem 281:33761–33772, 2006.
38. Li M, Kim DH, Tsenovoy PL, Peterson SJ, Rezzani R, Rodella LF, Aronow WS, Ikehara S, Abraham NG: Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. Diabetes 57:1526–1535, 2008.
39. Abraham NG, Tsenovoy PL, McClung J, Drummond GS: Heme oxygenase: a target gene for anti-diabetic and obesity. Curr Pharm Des 14:412–421, 2008.

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