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Toxicology and Applied Pharmacology 207 (2005) 212–220

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

Green tea polyphenol epigallocatechin-3-gallate differentially modulates oxidative stress in PC12 cell compartments

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Received 27 October 2004; accepted 5 January 2005
Available online 23 February 2005

Abstract

Tea polyphenols have been reported to be potent antioxidants and beneficial in oxidative stress related diseases. Prooxidant effects of tea polyphenols have also been reported in cell culture systems. In the present study, we have studied oxidative stress in the subcellular compartments of PC12 cells after treatment with different concentrations of the green tea polyphenol, epigallocatechin-3-gallate (EGCG). We have demonstrated that EGCG has differentially affected the production of reactive oxygen species (ROS), glutathione (GSH) metabolism and cytochrome P450 2E1 activity in the different subcellular compartments in PC12 cells. Our results have shown that although the cell survival was not inhibited by EGCG, there was, however, an increased DNA breakdown and activation of apoptotic markers, caspase 3 and poly- (ADP-ribose) polymerase (PARP) at higher concentrations of EGCG treatment. Our results suggest that the differential effects of EGCG might be related to the alterations in oxidative stress, GSH pools and CYP2E1 activity in different cellular compartments. These results may have implications in determining the chemopreventive therapeutic use of tea polyphenols in vivo.

Keywords: Tea polyphenol -EGCG; PC12 cells; GSH metabolism; Oxidative stress; Apoptosis

Introduction

Tea (*Camellia sinesis*) is one of the most common beverages worldwide. Green tea polyphenols have strong antioxidant activity (Durfresne and Farnworth, 2001; Higdon and Frei, 2003; Rice-Evans, 1999). The major green tea polyphenols are (−) epicatechin (EC), (−) epigallocatechin (EGC), (−) epicatechin gallate (ECG) and (−) epigallocatechin-3-gallate (EGCG). These catechins are potent scavengers of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide produced by various chemicals (Guo et al., 1996; Schroeder et al., 2003; Vinson et al., 1995). The antioxidant effects of tea polyphenols are also considered to be associated with their ability to stimulate antioxidant defense metabolism through the redox-regulated transcription factors and mitogen-activated protein kinases (MAPK)-dependent cell cycle regulation (Jiao et al., 2003; Katiyar et al., 2001; Williams et al., 2004). Tea polyphenols, especially EGCG, a major constituent of green tea, has been associated with reduced risk of cancer, diabetes and cardiovascular disease (Lambert and Yang, 2003; Waltner-Law et al., 2002). Tea catechins differ significantly in their pharmacokinetic properties and bioavailability and may attain very high concentrations in vivo within a short time of consumption (Lee et al., 2002; Warden et al., 2001).

Epidemiological cell culture and in vivo studies, including in humans, have shown that tea polyphenols, particularly EGCG, are potent chemopreventive agents protecting against many types of cancer. These effects are mediated through the inhibition of growth, modulation of intracellular

Abbreviations: CDNB, 1-chloro 2, 4-dinitrobenzene; CYP2E1, cytochrome P450 2E1; DMNA, *N*, *N*-dimethylnitrosamine; EGCG, epigallocatechin-3-gallate; GSH, reduced glutathione; GST, glutathione S-transferase; LPO, lipid peroxidation; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; PARP, poly- (ADP- ribose) polymerase; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2005.01.004

calcium, induction of apoptosis, inhibition of nuclear factor kappa B, and cell cycle dysregulation through IGF-I, phosphoinositol-3-kinase/Akt pathways (Adhami et al., 2004; Ahmad et al., 2000; Gupta et al., 2004; Koh et al., 2003; Lee et al., 2004; Yang et al., 1998). Furthermore, it has also been reported that EGCG has pronounced cell growth inhibitory effects and induce apoptosis selectively in cancer cells but not in normal cells (Chung et al., 2003; Gupta et al., 2004). The exact mechanism of the differential effects of EGCG and other tea polyphenols on tumor cells and normal cells is not clearly understood. However, it is suggested that these compounds may exert beneficial and/or cytotoxic actions through their modulation of cell cycle regulation and signal cascades, which may be different in cancer cells as compared to normal cells. It has also been demonstrated that the higher concentrations of tea polyphenols in cell culture systems produce H_2O_2, which may be an important factor responsible for cellular toxicity (Johnson and Loo, 2000; Long et al., 2000; Sakagami et al., 2001; Yamamoto et al., 2003; Yang et al., 2000). On the other hand, H_2O_2-induced erythrocyte membrane damage has been reported to be inhibited by EGCG treatment (Saffari and Sadrzadeh, 2004). In tumor cells, a differential oxidative stress environment and induction of apoptosis by tea polyphenols compared to the normal cells have been reported (Yamamoto et al., 2003; Yang et al., 2000). The differences in the action of EGCG in the tumor and normal cells may be related to the differences in oxidative stress and antioxidative defense mechanisms in these cells. The majority of studies, using cell culture systems, have focused to elucidate the antioxidant effects of EGCG after the treatment of cells with pro-oxidants. The aim of our present study, however, was to evaluate the alteration in oxidative stress in different subcellular compartments of the PC12 cells after treatment with EGCG. We have demonstrated that EGCG exert differential effects in altering the oxidative stress, glutathione (GSH) metabolism, reactive oxygen species production and lipid peroxidation (LPO) in different subcellular compartments.

Materials and methods

Reagents. Fetal bovine serum and RPMI 1640 were purchased from Gibco BRL (Grand Island, NY, USA). Horse serum, NADPH, NADH, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide, (MTT), lucigenin, reduced glutathione (GSH), 1-chloro 2,4-dinitrobenzene (CDNB), malonedialdehyde (MDA), *N*,*N*-dimethylnitrosamine (DMNA), and (−)-epigallocatechin gallate (EGCG), were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). PC12 cells were purchased from American Type Culture Collection (Manassas, VA, USA). A kit for lipid peroxidation assay was purchased from Oxis International Inc. (Portland, OR, USA). Polyclonal antibodies for cytochrome *c*, caspase 3, and poly (ADP-ribose) polymerase (PARP) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibody for cytochrome P450 2E1 (CYP2E1) was purchased from Amersham International, Plc. (Amersham, UK). Reagents for SDS-PAGE and Western blot analysis were purchased from Bio Rad Laboratories (Richmond, CA, USA). All other chemicals and reagents were of highest purity grade either from Sigma-Aldrich, Bio Rad Laboratories or from Gibco BRL.

PC12 cell culture and treatment with EGCG. Cells were grown in poly-L-lysine coated 75 cm^2 flasks (~2.0–2.5 × 10^6 cells/ml) in RPMI1640 medium supplemented with 2 mM glutamine, 5% heat inactivated horse serum, 10% heat inactivated FBS and 1× antibiotic–antimycotic mixture (Sigma), in the presence of 5% CO_2–95% air with relative 100% humidified atmosphere at 37 °C. Cells were grown to 60% confluency before splitting. Media was changed every 2–3 days. Cells were treated with different concentrations of EGCG diluted in sterile phosphate buffered saline (PBS), pH 7.4 to attain the desired concentrations of 10 μM, 50 μM, 100 μM, 200 μM, 400 μM, 600 μM and 800 μM. No addition was made in the control flasks. For every set, at least 4–6 flasks were prepared. Cells were allowed to grow for 16 h after treatment with the EGCG, which was found to be an appropriate time based on our previous study (Ahmed et al., 2002). The cells were then harvested, washed with PBS and homogenized in 10 mM phosphate buffer pH 7.4 containing 0.15 M KCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF at 4 °C. Mitochondria and postmitochondrial fractions were prepared by centrifugation as described (Raza et al., 2004).

Effect of EGCG on oxidative stress in PC12 cells. PC12 cells were treated with different concentrations of EGCG for 16 h as described above. Total cell lysate, mitochondria and post-mitochondrial supernatant (PMS) were used to measure the various oxidative stress-related parameters. ROS was measured by lucigenin-enhanced chemiluminescence method as described before (Raza et al., 2004). LPO was measured as the total MDA and 4-HNE formed using the kit. Subcellular concentration of GSH and GST (CDNB substrate) and CYP2E1 enzyme activities (DMNA substrate) were measured as described before (Raza et al., 2002, 2004).

MTT cell viability assay. Cell viability (0.5 × 10^5 cells/well on 96-well plates), based on mitochondrial dehydrogenase activity, was determined by MTT-formazan assay after treatment with different concentrations of EGCG after 16 h. The assay is based on the ability of living cells to reduce MTT to insoluble formazan; therefore, the amount of formazan produced is proportional to the number of living cells. Formazan production was measured at 570 nm using an ELISA microplate reader (Anthos Laboratories, Salzburg, Germany) as described previously (Ahmed et al., 2002).