Involvement of Protein Kinase C Activation and Cell Survival/Cell Cycle Genes in Green Tea Polyphenol (−)-Epigallocatechin 3-Gallate Neuroprotective Action*

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Studies from our laboratory have demonstrated that the major green tea polyphenol, (−)-epigallocatechin 3-gallate (EGCG), exerts potent neuroprotective actions in the mice model of Parkinson’s disease. These studies were extended to neuronal cell culture employing the parkinsonism-inducing neurotoxin, 6-hydroxydopamine (6-OHDA). Pretreatment with EGCG (0.1–10 μM) attenuated human neuroblastoma (NB) SH-SY5Y cell death, induced by a 24-h exposure to 6-OHDA (50 μM). Potential cell signaling candidates involved in this neuroprotective effect were further examined. EGCG restored the reduced protein kinase C (PKC) and extracellular signal-regulated kinases (ERK1/2) activities caused by 6-OHDA toxicity. However, the neuroprotective effect of EGCG on cell survival was abolished by pretreatment with PKC inhibitor GF 109203X (1 μM). Because EGCG increased phosphorylated PKC, we suggest that PKC isoforms are involved in the neuroprotective action of EGCG against 6-OHDA. In addition, gene expression analysis revealed that EGCG prevented both the 6-OHDA-induced expression of several mRNAs, such as Bax, Bad, and Mdm2, and the decrease in Bcl-2, Bcl-w, and Bcl-xL. These results suggest that the neuroprotective mechanism of EGCG against oxidative stress-induced cell death includes stimulation of PKC and modulation of cell survival/cell cycle genes.

Biochemical evidence points to central roles for oxidative stress (OS)1 and inflammation in neuronal death in idiopathic Parkinson’s disease (1, 2). Consistent with these findings is the fact that antioxidants and iron chelator-based neuroprotective strategies are at the focus of attention in determining how to neutralize OS-induced damage. Dietary antioxidants, especially tea and tea polyphenols, have attracted increasing interest because of their well reported biological effects including radical scavenging (3, 4) iron chelating (5, anti-carcinogenic (6, 7), and anti-inflammatory actions (8, 9). In line with these results we recently reported (10) that green tea extract inhibited iron-induced brain lipid peroxidation and protected rat pheochromocytoma (PC12) and human neuroblastoma (NB) SH-SY5Y cells against 6-hydroxydopamine (6-OHDA), a neurotoxin known to act via production of reactive oxygen species (11). This effect was achieved by preventing the translocation to the nucleus of the inflammatory and OS-responsive transcription factor, nuclear factor-κB (NF-κB) (10). On the other hand, tea or more specifically its polyphenolic fraction has been reported to decrease the incidence of carcigen-inducing malignancies in animal models (12) because of its pro-oxidant and pro-apoptotic activities.

We have recently demonstrated potent neuroprotective properties of both green tea extract and the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) against striatal dopamine depletion and substantia nigra dopaminergic neuron loss, caused by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice (13). Similarly, administration of tea polyphenols immediately after ischemia improved the memory impairment and reduced hippocampal neuronal damage in mice (14, 15), whereas the consumption of flavonoid-rich blueberries or strawberries reversed the age-related cognitive and motor behavioral deficits in rats (16). Clinical trials with Alzheimer’s disease patients have also demonstrated potential benefits from treatment with the antioxidant extract of Ginkgo biloba, known to be enriched with flavonoids (for review see Ref. 17). These findings are supported by in vitro studies, where EGCG or the Ginkgo biloba extract were shown to prevent amyloid β peptide (Aβ)-induced neurotoxicity in cultured hippocampal neurons (18, 19) and in PC12 cells2 Moreover, the flavonoid epicatechin was shown to attenuate neurotoxicity induced by oxidized low density lipoprotein in mouse-derived striatal neurons (20).

Neuronal damage, emerging from OS, has been reported to involve regulation of cell signaling molecules, as well as activation of apoptotic pathways (21–23). Several studies have suggested a fundamental role for protein kinase C (PKC) in the regulation of cell survival and programmed cell death (24–27). In this line, it was shown that brain-derived neurotrophic factor prevented the decline in PKC activity as a consequence of N-methyl-D-aspartate (NMDA)-induced excitotoxicity in pri-

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mary rat cortical neurons (28). Consistent with that, activation of PKC by phorbol 12-myristate 13-acetate (PMA) protected the hippocampal cell line from glutamate toxicity, and this effect was mediated through activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and c-Jun N-terminal kinase (JNK) (26). Moreover, the regulation of ERK1/2 and JNK activities in neurodegeneration induced by reactive oxygen species has been reported previously (21). The involvement of mitogen-activated protein kinases (MAPKs) is supported by the fact that specific pathway inhibitors conferred protection against different cell death-promoting agents (22, 29). It was shown that flavonoids can activate MAPKs signaling cascades in both neuronal and extraneuronal tissues; the flavonoid catechin was shown to counteract the induction of both ERK1/2 and JNK, induced by oxidized low density lipoprotein in primary cultures of mice striatal neurons (30). Furthermore, green tea polyphenol fraction and EGCG were demonstrated to modulate ERK, JNK, and p38 pathways in human hepatoma HepG2 (31) and cervical squamous carcinoma HeLa cells (32) and/or inhibit Ras-MAPK pathway in human prostate cancer DU145 cells (33).

The aim of the present study was to investigate the different aspects involved in neuronal damage induced by 6-OHDA, a neurotoxin that induces cell death via OS (34, 35), and in neuroprotection afforded by EGCG. We have shown that EGCG prevents neuronal cell damage at low concentrations, whereas high doses promote cell death. Our results suggest the involvement of PKC and ERK1/2 as well as modulation of cell survival/cell cycle genes in the neuroprotective action of EGCG.

EXPERIMENTAL PROCEDURES

Materials—EGCG, 6-OHDA, phosphatase inhibitor, TriReagent™ isolation reagent, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo- lium bromide (MTT) were purchased from Sigma. 1-Methyl-4-phenylpyridinium was purchased from RBI (Natick, MA). Dulbecco’s modified Eagle’s Medium (DMEM) was purchased from Invitrogen, fetal calf serum (FCS) was purchased from Biological Industries (Bet Haemek, Israel). ERK1/2 and JNK assay kits were purchased from Cell Signaling Technology, Inc. Protein inhibitor mixture Complete™ was purchased from Roche Molecular Biochemicals GmbH (Mannheim, Germany), and dNTPs, DNase, and Moloney murine leukemia virus reverse transcriptase were purchased from Promega (Madison, WI). The Nonrad-GEArray™ pathway-specific expression arrays were purchased from SuperArray Inc. (Bethesda, MD). PMA and GF 109203X were from Calbiochem (La Jolla, CA). Other chemicals and reagents were of the highest analytical grade and were purchased from local commercial sources.

Cell Culture and MTT Assay—NB SH-SY5Y cells were grown at 37 °C, in a humid 5% CO₂, 95% air environment, in growth medium DMEM supplemented with glucose (4.5 mg/ml) streptomycin/penicillin (100 units/ml), sodium pyruvate (110 mg/liter), and 10% FCS.

Cells were detached by vigorous washing, centrifuged at 200 × g for 5 min, and resuspended in DMEM with low serum content (2% FCS). The cells were plated into wells of microtiter plates (96 wells) at a density of 1.5–2 × 10⁴ cells/well and were allowed to attach for 24 h before treatment. One row contained medium only for background subtraction. EGCG was added 15 min before insult with 6-OHDA for a subsequent 24 or 48 h, respectively. The inhibitor of PKC, GF 109203X, was added 30 min before treatment with EGCG.

Neuronal cell injury was evaluated by a colorimetric assay for mitochondrial function using the MTT test, as described previously (36). The absorption was determined in a Tecan Sunrise Eliza-Reader (Switzerland) at λ = 570/650 nm after automatic subtraction of background readings. The results are expressed as percentage of the untreated control.

Preparation of Cell Lysates and Western Immunoblotting—NB SH-SY5Y cells were grown as described above, plated in 6-well culture dishes, and allowed to attach for 48 h. Afterward, the medium was removed and replaced with DMEM containing 0.5% FCS for an additional 24 h. The medium was replaced again, and cells were treated with 6-OHDA and/or different concentrations of EGCG for different time periods as specified. The reactions were stopped by placing cells on ice and aspirating the medium. Then, 500 µl of cold lysis buffer consisting of 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 1 mM sodium orthovanadate, 1% Triton X-100, provided with protease and phosphatase inhibitor mixtures were added, and the cells were left on ice for 30 min until solubilization. After centrifugation (14,000 × g for 10 min at 4 °C), protein concentration in the supernatant was determined by the Bradford reagent. Cell lysates containing 25 µg of protein (pre-denatured in boiling buffer) were separated on NuPAGE 4–12% bis-tris electrophoresis gel (Invitrogen, Groningen, The Netherlands), immunoblotted, and identified using the following antibodies: rabbit polyclonal antibodies against phosphorylated pan-PKC (detects PKC-α, -β, -δ, -ε, -γ, -η, and -λ, phosphorylated at a carboxyl-terminal residue homologous to Ser-660 of PKC-βII), total or phosphorylated ERK1/2 or JNK/SAPK (all diluted 1:1000 in 5% non-fat milk). Membranes were incubated overnight at 4 °C, followed by a 1-h exposure to polyclonal anti-rabbit IgG conjugated with horseradish peroxidase (1:2000) (primary and secondary antibodies are from Cell Signaling, Beverly, MA). The immunoblots were developed with the Western blotting detection ECL reagents (Amersham Biosciences). Quantification of results was accomplished by measuring the optical density of the phosphorylated and non-phosphorylated kinase-derived autoradiogram bands, using the computerized imaging program Quantity One (Bio-Rad). The values were normalized to the total MAP kinase intensity levels.

Total RNA Extraction—Cells were cultured on 100-mm dishes in DMEM containing 10% FCS until confluence. At the day of the experiment the medium was replaced, and EGCG (1 and 50 µM) was administered for 6 h. At the end of the incubation, the medium was removed and isolation of total RNA was performed, using TriReagent™ isolation reagent. Total RNA was treated with DNase/RNase free (Roche Diagnostics, Mannheim, Germany) for 30 min at 37 °C and subsequently extracted by a round of phenol/chloroform/isooamy alcohol (25:24:1) followed by one of chloroform. After precipitation with NaOAc (0.3 M)...
and ethyl alcohol the RNA pellet was washed with 80% ethyl alcohol (14,000 rpm for 10 min) and resuspended in 50–100 µl of diethylpyrocarbonate-treated water and incubated for 10–15 min at 56 °C to facilitate resuspension.

**GEArray™ Membrane Analysis**—Nonrad-GEArray™ pathway-specific expression arrays were used for determining apoptosis-related gene profiling induced by EGCG (1 and 50 µM). Total RNA from NB SH-SY5Y cells, treated with EGCG as described above, was reverse-transcribed with biotinylated dUTP (Roche Diagnostics) and a gene-specific primer mixture, as supplied by the manufacturer. The probes were hybridized to a cDNA expression array membrane consisting of 25 genes related to apoptosis and cell survival. The hybridization pattern was assessed by ImageMaster VDS-CL (Amersham Biosciences). The relative expression level of a given mRNA was assessed by normalizing to the housekeeping gene β-actin provided on the membrane and comparing to control values.

**Statistics**—One-way analysis of variance followed by the Tukey test, or Student’s t test, was performed using the scientific statistic software GraphPad Instat™ version 2.04. p values of less than 0.05 were considered significant.

**RESULTS**

**Neuroprotective Effects of EGCG**—The possible protective effect of EGCG against OS-induced cell death was evaluated in NB SH-SY5Y cells. NB SH-SY5Y viability was markedly reduced by a 24-h treatment with 6-OHDA (50 µM, 38 ± 2.7% of control), whereas pretreatment for 15 min with EGCG (0.1–10 µM) conferred significant protection against 6-OHDA neurotoxicity, the maximal effect being observed with 1 µM EGCG (93 ± 4.5% control, Fig. 1A). As shown in Fig. 1B, EGCG did not affect NB SH-SY5Y cell viability up to 10 µM, whereas higher concentrations induced a gradual decrease in cell survival.

**Involvement of PKC in the Neuroprotective Effect of EGCG**—PKC activity has been previously coupled with the preservation of cell survival. Its loss of function, especially upon OS exposure, correlates with the severity of the damage (26, 37). Therefore, we investigated the possible involvement of PKC in the neuroprotective activity of EGCG against 6-OHDA-induced neurotoxicity. Accordingly, we first examined the effect of a general PKC inhibitor, GF 109203X, on the ability of EGCG to protect NB SH-SY5Y cells against 6-OHDA-oxidative toxicity. Similar to results shown in Fig. 1, pretreatment of NB SH-SY5Y cells with two protective concentrations of EGCG (1 or 10 µM) doubled the percentage of cell survival versus treatment.
with the toxin. However, prior exposure to GF 109203X (1 μM) completely abolished this effect, indicating the involvement of PKC in the neuroprotective action of EGCG (Fig. 2). GF 109203X alone did not affect 6-OHDA-induced cell damage. In addition, pretreatment for 15 min with PMA (100 nM), a direct activator of PKC, significantly attenuated protection against 6-OHDA-induced cell toxicity (84 ± 9.3% of control) (Fig. 2), further suggesting the mediation of PKC activation in the neuroprotection effect. Consistent with these data, PKC phosphorylation, as assessed by detection with anti-phospho-PKC (pan) antibody, was decreased as soon as 1 h after 6-OHDA administration; maximal effect was observed at 2 h, although (pan) antibody, was decreased as soon as 1 h after 6-OHDA administration (50 μM), 6-OHDA in combination with EGCG (1 μM), or EGCG alone for 6 h, were hybridized to the arrays. In addition a high toxic concentration (50 μM) was also examined. The intensity of each gene was normalized to β-actin, and these values were compared with control-unreated cells. A shown in Fig. 6, 6-OHDA induced an increase in the expression of mRNAs coding for the pro-apoptotic proteins Bax, Bad, Mdm2, NF-κB p105 subunit, and caspase 1, 6, 7, and 10 (150–300% of control) and a decrease in Bcl-2, Bcl-w, and Bcl-xL (50–60% of control). However, pre-treatment with the low (1 μM) EGCG protective concentration abolished all these gene alterations, possibly contributing as well to its neuroprotective action. In addition, this concentration of EGCG decreased the expression of pro-apoptotic Bcl-2 family members Bax (47% of control), caspase 1 (69% of control), cyclin-dependent kinase (cdk) inhibitor p21 (Waf1, 58% of control), Mdm2 (49% of control), growth arrest and DNA damage-inducible protein Gadd45β (Gadd45β, 63% of control), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, 49% of control) mRNAs. These results indicate a positive protective effect of EGCG on a variety of survival factors, which may contribute in the counteraction of 6-OHDA toxicity. On the contrary, the high 50 μM toxic concentration of EGCG exerted a decrease in the expression of Bcl-xL and Bcl-w mRNAs (55 and 47% of control, respectively), whereas Bad, Bax, Fas, Gadd45β, p21Waf1, and caspase 6 mRNAs were increased (155–240% of control, Table 1).

**DISCUSSION**

This study demonstrates the neuroprotective effect of EGCG against 6-OHDA-induced oxidative damage. Detailed investigation on the mechanism mediating this neuroprotective effect shows, for the first time, that restoration of the reduced PKC and ERK1/2 phosphorylation levels induced by 6-OHDA, as well as modulation of antiapoptotic-related genes, mediate EGCG action. Given that the neuroprotective action of EGCG was blocked by inhibition of PKC activity, we suggest that activation of PKC is part of the neuroprotective mechanisms that are activated by this polyphenol to counteract death signals in neuronal cells. However, the possible contribution of the established radical scavenging action of EGCG cannot be ruled out.

These results are in accordance with our recent studies showing that green tea extract or its individual EGCG polyphenol can prevent mice striatal dopamine depletion and nigral dopaminergic neuronal loss in the MPTP mice model of Parkinson’s disease (13). Further support comes from in vitro studies, where green tea and black tea extracts were shown to exhibit potent neuroprotection against 6-OHDA-induced damage in NB SH-SY5Y and PC12 cells, via inhibition of nuclear translocation of the OS and inflammatory-responsive transcription factor NF-κB (10). Moreover, EGCG or Ginkgo biloba...
extract, known to be enriched with flavonoids, protects hippocampal neurons from β-amyloid-derived peptide (Aβ) or nitric oxide-induced neurotoxicity (18, 19, 39). Furthermore, the polyphenol epicatechin was shown to attenuate neurotoxicity induced by oxidized low density lipoprotein in mouse-derived cortical neurons (18, 19, 39). Moreover, the polyphenol epicatechin is known to be enriched with flavonoids, protects hippocampal neurons from β-amyloid-derived peptide (Aβ) or nitric oxide-induced neurotoxicity (18, 19, 39).

There is evidence from neuronal and non-neuronal models that PKC may be necessary for the regulation of cell proliferation and differentiation, and it has also been implicated in the control of cell survival and programmed cell death (24–27, 42).

The rapid loss of neuronal PKC activity is a common consequence of several brain damages, such as ischemia (43, 44) and glucose deprivation (45). Therefore, PKC activation has been suggested to represent a pivotal therapeutic target. Only a small number of compounds have been reported to prevent the decline in PKC activity, observed in response to a variety of insults. These include brain-derived neurotrophic factor or vasoactive intestinal peptide, which were shown to protect primary rat cortical neurons (28) and mice white matter tissue (46), respectively, against excitotoxic insults, through activation of PKC. We found that 6-OHDA toxicity is accompanied by a decline in phosphorylated PKC levels, suggesting that promotion of cell death by 6-OHDA involves inactivation of PKC. This effect was completely abolished by pretreatment with EGCG. In addition, EGCG increased phosphorylated PKC, suggesting that EGCG-mediated protection of neuronal cells from 6-OHDA damage is dependent on PKC activation. This is supported by the findings that the general PKC inhibitor GF 109203X completely abolished the protection induced by EGCG in response to 6-OHDA toxicity. PMA, a direct activator of PKC, mimicked the protection afforded by EGCG against the toxin, further indicating the crucial role of PKC in cell survival.

EGCG significantly increases the levels of PKC-α and -ε isoforms in mouse hippocampus (2). These isoforms play a crucial role in cell survival pathways (24, 26, 47). The mechanism by which EGCG affects the expression of PKC isoforms is being investigated.

In an attempt to characterize the signaling pathways involved in 6-OHDA-induced cell death and in neuroprotection by EGCG, we examined the activity of two major MAPKs, which have been implicated in neuronal survival, ERK1/2, and JNK (21, 22). Activation of ERK1/2 is associated with pro-survival signaling (48), whereas JNK activation has been strongly linked to apoptotic signaling (49) through phosphorylation of c-Jun (50). The observation that ERK1/2 signaling is involved in neuronal survival has also been demonstrated in primary cortical neurons intoxicated with glutamate (29) and in PC12 cells, in which apoptosis was induced by nerve growth factor withdrawal (21).

Accordingly, we found that 6-OHDA decreased ERK1/2 activity, whereas treatment with EGCG prevented this effect. Further support for the role of this pathway in cell survival is provided by studies showing that activation of ERK1/2 plays a major role in the neuroprotective effect of EGCG.

**Neuroprotective Action of EGCG via PKC Activation**

**Fig. 6. Modulation of cell survival/cell cycle genes by EGCG and 6-OHDA.** NB SH-SY5Y cells were treated with EGCG (1 μM), 6-OHDA (50 μM), or the combination of the two for 6 h. cDNA probes were hybridized to a custom array containing 25 genes, related to cell survival and apoptotic pathways. The amount of each product was normalized to β-actin and expressed as fold stimulation of untreated control, arbitrarily set as 1. The results are the mean ± S.E. of two separate experiments, performed in duplicate. t test: a, p < 0.05; b, p < 0.01 versus control; †, p < 0.05; ††, p < 0.01 versus 6-OHDA.

**Table 1**

| Unigene accession number | Gene name | EGCG (50 μM) vs. control |
|--------------------------|-----------|--------------------------|
| L22474                   | Bax       | 1.693 ± 0.114*           |
| U65879                   | Bad       | 1.594 ± 0.119*           |
| U59747                   | Bcl-2     | 0.547 ± 0.096*           |
| Z23115                   | Caspase 6 | 1.663 ± 0.072*           |
| U20557                   | Caspase 1 | 0.927 ± 0.080           |
| X63717                   | Fas       | 1.779 ± 0.123*           |
| F078077                  | Gadd45p   | 1.694 ± 0.173*           |
| L47233                   | p21(WAF1) | 1.644 ± 0.197           |
| U37518                   | TRAIL     | 0.921 ± 0.028           |
| Z13290                   | Mdm2      | 2.402 ± 0.107*           |
| M59803                   | NF-κB(p105)| 0.862 ± 0.03           |

* p < 0.001 versus control, t test.

† p < 0.01 versus control, t test.

‡ p < 0.05 versus control, t test.
brain-derived neurotrophic factor (51) or activation of glial metabolotropic glutamate receptors (52) against drug-induced apoptosis or N-methyl-D-aspartate-induced toxicity in primary neuronal cultures, respectively. In addition, 6-OHDA caused an increase in JNK phosphorylation, but EGCG failed to prevent the activation induced by the toxin, suggesting that activation of this MAPK superfamily pathway might be necessary for full explanation see “Discussion.”

Previous studies have shown that the neurotoxicity of 6-OHDA involves nuclear translocation of NF-κB (10), increase of reactive oxygen species, activation of JNK, inhibition of complex I of the mitochondrial respiratory chain (34), and apoptotic cell death (23, 35, 53). The increased expression of the apoptotic-related genes and the decrease in Bcl-2 or Bcl-xL may determine the susceptibility of a cell to a death signal. Decreased Bax mRNA levels, as a result of EGCG treatment, may contribute to an increase in the ratio of Bcl-2 or Bcl-xL to Bax (56), thus suggesting potential neuroprotective/antiapoptotic features of EGCG. There is no clear evidence whether the observed inhibition of p21Waf1 and Gadd45β cell cycle regulators by EGCG may have any protective effects against oxidative stress-induced damage, because most studies have investigated their effects as tumor suppressors.

Mdm2 is the major negative regulator of p53 and is itself a positive transcriptional target of p53. Its expression is often elevated subsequent to induction of p53 activity. Mdm2 is a ubiquitin ligase that targets p53 to proteasomal degradation, thus defining a negative feedback loop to regulate p53 levels (for review see Ref. 58). Thus, down-regulation of Mdm2 transcript by EGCG might consequently preserve intact p53 protein and also contribute to the protective action against 6-OHDA, where high Mdm2 mRNA levels were observed.

In contrast to the neuroprotective properties of EGCG at low concentrations, EGCG is more often known for its antitumorigenic/pro-apoptotic properties. The cell death promotion and increased expression of pro-apoptotic and cell cycle regulator-linked genes, induced by a high EGCG concentration (50 μM), supports and extends previous reports: increased consumption of relatively high concentrations of tea polyphenols has been found to correlate with reduced incidence of certain cancers (6) and to induce apoptosis and cell cycle arrest (59). Recently, EGCG was shown to induce apoptosis of neck squamous cell carcinoma cells and to cause a decrease in phosphorylated ERK1/2, Bcl-2, and Bcl-xL proteins, an increase in Bax, and an activation of caspase 9 (60). In the present work we show that high EGCG concentration also decreased ERK1/2 activity and increased that of JNK, further supporting its pro-apoptotic/antiproliferative effect. The comparative actions of EGCG, as presented in our study, at its protective and toxic concentrations is depicted in Table II.

The mechanism underlying the effectiveness of EGCG against 6-OHDA neurotoxicity is not fully known but may involve several events. A hypothetical model diagramming the multiple potential targets of EGCG effects is shown in Fig. 7. (a) Direct scavenging of oxidized 6-OHDA or of oxygen species derived from 6-OHDA, because green tea polyphenols are known to be radical scavengers and iron chelators. Nevertheless, the concentrations of EGCG required for neuroprotection are far lower than those of 6-OHDA used in the assay, arguing

| Comparision of the effects of a protective (1 μM) versus a toxic (50 μM) concentration of EGCG |  |
|----|----|
| | EGCG (1 μM) | EGCG (50 μM) |
| Cell viability | NC | Neurotoxic |
| Neuroprotective properties against 6-OHDA (50 μM) | Neuroprotective | Increased neurotoxicity |
| MPP+ (400 μM) | Neuroprotective | Increased neurotoxicity |
| Aβ(25–35, 1–40, 1–42) (10 μM)* | Neuroprotective/Neurorescuing | Increased neurotoxicity |
| | | |
| Gene signaling activation | | |
| PKC | Activation | NC |
| ERK1/2 | NC | Reduced |
| JNK | | Increased |
| | Decreased | Increased |
| Expression of apoptotic/antiproliferative genes | | |
| | | |
| EGCG (50 μM) | Neuroprotective/Neurorescuing | |
| 6-OHDA | | |
| GT/EGCG | | |
| 6-OHDA | | |
| | | |
| ROS | | |
| | | |
| PKC | | |
| GT | | |
| EGCG | | |
| BAD, BAX, Caspase 10, Caspase 7, Caspase 1, Mdm2 | | |
| BCL-2, BCL-xL | | |
| | | |
| APOPTOSIS | | |
| | | |

* Data not shown; NC, not changed; Aβ, β-amyloid peptide.
against a simple stoichiometric reaction. This assumption is also supported by the fact that EGCG concentrations higher than 10 μM could not prevent the toxic effects of 6-OHDA and even exacerbated it (data not shown). (b) Counteracting the negative effect of 6-OHDA on both PKC and ERK1/2 pathways because alternatively ERK1/2 might be subjected to direct regulation of PKC; thus, activation of this kinase by EGCG may lead to restoration of the reduced phosphorylated ERK1/2 levels. (c) Inhibiting the translocation of NF-κB to the nucleus, as previously shown for green tea extract (10). (d) Preventing the expression of cell death and cell cycle regulators genes. (e) Other mechanism(s) not yet defined.

In conclusion, this study provides the first evidence of a neuroprotective mechanistic action of EGCG against 6-OHDA-induced toxicity by stimulating PKC and modulating the expression of cell survival factors.

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