8-2014

Neuroprotective Effects of (-)-Epigallocatechin 3-O-gallate (EGCG) on l-methyl-4- phenyl-l,2,3,6-tetrahydropyridine (MPTP) and Hydrogen Peroxide (H2O2) Stressed PC 12 Cells

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by
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A Master's Thesis Submitted to the Faculty of
Montclair State University
In Partial Fulfillment of the Requirements
For the Degree of
Master of Science
August 2014

College of Science & Mathematics

Biology Department

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ABSTRACT

Despite the fact that Parkinson's disease (PD) is the second most common neurodegenerative disease, much of its etiology and pathogenesis remains to be discovered. The main pathological feature of PD is the progressive death of specific dopaminergic (DAergic) neurons in the brain. Oxidative stress induced cell death has been hypothesized as one mechanism responsible for PD pathogenesis.

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) that has been implicated in PD as a by-product of dopamine (DA) degradation. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to produce a Parkinsonian syndrome via an oxidative stress induced mechanism. For these reasons, $H2O_2$ and MPTP were used in this study to treat DAergic PC12 cells to produce a model of PD.

We hypothesized that (-)-epigallocatechin 3-O-gallate (EGCG), a principal chemical component of green tea known for a plethora of health promoting bioactive properties, protects PC12 cells from H_2O_2- and MPTP-induced stress.

PC12 cells treated with EGCG showed increased cell count when compared to untreated cells or cells only treated with H_2O_2 or MPTP. Cell count increased as EGCG concentration increased.

The results of this study demonstrate that EGCG has neuroprotective effects on H_2O_2 and MPTP stressed PC12 cells.

NEUROPROTECTIVE EFFECTS OF (-)-EPIGALLOCATECHIN 3-O-GALLATE (EGCG) ON 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) AND HYDROGEN PEROXIDE (H_2O_2) STRESSED PC12 CELLS

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Master of Science

By

Elizabeth M. Appert

Montclair State University

Montclair, New Jersey

2014

ACKNOWLEDGEMENTS

Dr. Vladislav Snitsarev – Dr. Snitsarev, thank you for providing me with the opportunity to work with you this past year. You have taught me so many different things that will prove invaluable to me as I continue my scientific pursuits. Thank you.

Dr. Elena Petroff – Dr. Petroff, I would like to thank you for all of the advice that you have given to me over the past two years. I am extremely grateful for all of your direction and help. Talking to you has not only cemented my decision to pursue a career in research, but I now also feel more ready for the next steps after Montclair. Thank you.

Dr. David Rotella – Dr. David Rotella, thank you for taking the time to be a part of my thesis committee.

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List of Abbreviations

DA	Dopamine
DAergic	Dopaminergic
EGCG	(-)-Epigallocatechin-3-O-gallate
H_2O_2	Hydrogen peroxide
L-DOPA	Levodopa
MOA	Monoamine oxidase
MPP^+	1-methyl-4-phenylpyridine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
6-OHDA	6-Hydroxydopamine
PD	Parkinson's disease
ROS	Reactive oxygen species
SEM	Standard Error of the Mean

Introduction

Parkinson's Disease (PD)

PD is the second most common neurodegenerative disorder after Alzheimer's disease (reviewed in (Bove et al. 2005, Danielson and Andersen 2008, Dauer and Przedborski 2003). In the United States alone, there are over one million cases of PD (reviewed in (Drechsel and Patel 2008)), with approximately 50,000 new diagnoses each year (reviewed in (Bove et al. 2005)). While genetic mutations in several proteins, including α-synuclein, LRRK1 and others, have been found to cause PD, the majority of PD cases occur sporadically (reviewed in (Cali et al. 2011, Lotharius and Brundin 2002)). The etiology of sporadic PD remains unknown (reviewed in (Deumens et al. 2002, Hald and Lotharius 2005)). The hallmark clinical symptoms of PD include bradykinesia, resting tremor, rigidity and postural instability, and in some cases, dementia (reviewed in (Drechsel and Patel 2008, Exner et al. 2012, Mosley et al. 2006)). These symptoms are the result of the progressive loss of DAergic neurons, particularly those found in the substantia nigra pars compacta (reviewed in (Danielson and Andersen 2008, Drechsel and Patel 2008, Hastings 2009)). For yet unknown reasons, the DAergic neuron population in the substantia nigra pars compacta show particular susceptibility in comparison to those in the ventral tegmental region of the brain (reviewed in (Przedborski et al. 2001)). The loss of these neurons leads to decreased levels of DA in the striatum, which is responsible for movement control (reviewed in (Dauer and Przedborski 2003, Drechsel and Patel 2008)). Symptoms are not typically observed in a PD patient until 60 to 70% of the DAergic neurons have already been lost and over 80% of DA levels have been depleted (reviewed in (Deumens et al. 2002, Drechsel and Patel 2008, Hald and Lotharius 2005,

Schulz and Falkenburger 2004)). For these reasons, further research is required to establish the cause of PD and discover ways to prevent the initiation and progression of the disease.

PD Treatment

Currently, treatment is limited to treating the symptoms of the disease, not the underlying cause of the disease; the most common treatment for PD is levodopa (L-DOPA) (reviewed in (Bargiotas and Konitsiotis 2013)). L-DOPA, a precursor to DA, is able to cross the blood brain barrier into the brain, where it is converted to DA in neurons (Gey and Pletscher 1964). The purpose of treatment with L-DOPA is to elevate DA levels in the brain; however, as the population of neurons responsible for converting L-DOPA is declining in PD, and as DA itself could contribute to the pathology of PD, L-DOPA is not a very effective treatment in the long term (reviewed in (Miyazaki and Asanuma 2008)). Furthermore, chronic treatment with L-DOPA leads to adverse effects including a number of deficits in motor response such as L-DOPA induced dyskinesia, hallucinations and other symptoms, in addition to the PD symptoms already present (reviewed in (Bargiotas and Konitsiotis 2013, Miyazaki and Asanuma 2008)). While L-DOPA is the most effective treatment currently for relieving PD symptoms, it cannot halt the progression of the disease (reviewed in (Bargiotas and Konitsiotis 2013)). The limitations in current treatment advocate the importance of further elucidating the mechanisms underlying the progression of PD; therefore, more successful ways to treat PD and to stop its progression must still be discovered.

Reactive Oxygen Species (ROS) in PD

While the initial cause of the disease remains unknown, several hypotheses point to a number of mechanisms of specific oxidative stress in which elevated production of ROS results in cellular damage of extracellular and intracellular components and ultimately leads to cell death of DAergic neurons in the nigrostriatal pathway (reviewed in (Drechsel and Patel 2008, Hald and Lotharius 2005, Mosley et al. 2006, Sherer et al. 2002)). The production of ROS is a normal physiological occurrence and ROS are usually enzymatically converted to harmless molecules such as water (reviewed in (Mosley et al. 2006)). However, in diseases such as PD, increased levels of ROS overwhelm normal degradation mechanisms and cause damage of cellular components, which ultimately leads to cell death (reviewed in (Mosley et al. 2006)). In PD, the DAergic neurons are particularly susceptible to oxidative stress as DA metabolism results in various toxic species including hydrogen peroxide (H_2O_2) (reviewed in (Drechsel and Patel 2008, Lotharius and Brundin 2002)). The degradation of DA by monoamine oxidase A (MAO-A) and the auto-oxidation of dopamine were shown to be coupled with the production of endogenous H_2O_2 (reviewed in (Hald and Lotharius 2005)). As DA accumulates in the cytosol, it is prone to undergo auto-degradation and enzymatic degradation (reviewed in (Lotharius and Brundin 2002)). A product of DA degradation, dopamine-quinone (reviewed in (Asanuma et al. 2004) (Hald and Lotharius 2005)), also inhibits complex I in the mitochondrial electron transport chain (reviewed in (Hald and Lotharius 2005, Lotharius and Brundin 2002)). Complex I is responsible for pumping protons across the mitochondrial membrane to create a concentration gradient, and when this process is inhibited, there are more electrons available for the production of superoxide and other

ROS (reviewed in (Cali et al. 2011)). Mitochondrial damage is also found in glial cells in the PD brain(Schmidt et al. 2011). Thus, DA itself may contribute to oxidative stress induced cell death implicated in PD by two mechanisms, DA degradation and inhibition of the mitochondrial electron transport chain (reviewed in (Drechsel and Patel 2008, Hald and Lotharius 2005)). Studies have also shown an increase in exogenous ROS resulting from an inflammatory response by proximal microglia (Wu et al. 2003).

PC12 cells as a PD model

PC12 cell line was developed from a rat adrenal pheochromocytoma as a way to study the nervous system at a single cell and multi-cell level; norepinephrine and DA are predominant catecholamines found in these cells (Greene and Tischler 1976). MAO is also found in PC12 cells and is responsible for the enzymatic degradation of catecholamines (Greene and Tischler 1976). For these reasons, PC12 cells are widely used as a model for PD when treated with toxins that reproduce PD symptoms such as 6-OHDA, MPTP, H_2O_2, and rotenone (Fonck and Baudry 2001, Grau and Greene 2012, Lu et al. 2010, Snitsarev et al. 2013, Thakur and Nehru 2014).

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

In the 1980s, drug users developed a Parkinsonian syndrome after an intravenous injection of synthetic opioid-like drugs traced with MPTP, a by-product of illicit drug synthesis (Langston et al. 1983, Langston et al. 1999). Since MPTP was found to induce many of the clinical symptoms associated with PD in humans, it has been widely used as a toxin to create PD models (Chiba et al. 1984). MPTP is highly lipophilic and as such, is able to readily cross the blood brain barrier to the brain where it is converted into the active metabolite 1-methyl-4-phenylpyridine (MPP^+) by the enzyme MAO-B in non-

DAergic glial cells (reviewed in (Dauer and Przedborski 2003)). MPP^+ is taken up via the DA transporter into DAergic neurons where it acts as a potent neurotoxin (reviewed in (Cali et al. 2011, Dauer and Przedborski 2003)). In DAergic neurons, MPP^+ inhibits complex I of the electron transport chain, resulting in the increased production of ROS (reviewed in (Bove et al. 2005, Dauer and Przedborski 2003)). When MPP^+ utilizes the DA transporter, it causes displacement of DA into the cytosol, where it is more readily degraded and produces ROS (reviewed in (Lotharius and Brundin 2002)). MPTP specifically damages DAergic neurons in the nigrastriatal pathway (Langston et al. 1999), thus reproducing clinical and pathological aspects of PD (reviewed in (Bove et al. 2005, Dauer and Przedborski 2003)).

(-)-Epigallocatechin 3-O-gallate (EGCG)

After water, tea is the second most consumed beverage around the world (reviewed in (Kanwar et al. 2012, Steinmann et al. 2013)). Catechins are the main components of green tea with EGCG constituting approximately 59% of the total catechins content (reviewed in (Steinmann et al. 2013)). In addition to its established antiviral, antibacterial and antifungal effects, EGCG has also been shown to have various beneficial effects at the cellular level (reviewed (Steinmann et al. 2013)). Studies have established the anticancerous effects of EGCG (reviewed in (Yang C. S. et al. 2002)) including its ability to inhibit growth by reducing oncogene expression, increasing expression of tumor suppressor genes and inhibiting telomerase (reviewed in (Chen et al. 2011, Kanwar et al. 2012)). In the 6-hydroxydopamine (6-OHDA) treated rat model of PD, EGCG was found to protect DAergic neurons through inhibition of the ROS-NO pathway, which is implicated in 6-OHDA induced cell death and in PD neuronal death (Guo et al. 2007).

Hypothesis

EGCG protects PC12 cells from H_2O_2- and MPTP-induced stress.

Materials and Methods

PC12 cells

PC12 cells were cultured similar to (Yermolaieva et al. 2000) (Yermolaieva et al. 2004). Briefly, PC12 cells were plated at 10^4 cells/cm^2 in 60 (35 mm) Petri dishes in 2.5 mL of the DMEM culture medium supplemented with 10% horse serum, 5% fetal bovine serum, 1% L-Glutamine, and 0.5% Pen/Strep (Life Technologies, Carlsbad, CA). PC12 cells were used at a passage less than thirty. Five wells were used as a control with no treatment. Five wells were treated with varying EGCG concentrations, 10^{-8} M, 10^{-7} M, and 10^{-6} M. Five wells were treated only with 10^{-4} M H_2O_2. 15 wells were treated with 10^{-4} M H_2O_2 and different concentrations of EGCG: five with 10^{-8} M, five with 10^{-7} M and five with 10^{-6} M. Five wells were treated only with 10^{-5} M MPTP. 15 wells were treated with 10^{-5} M MPTP and different concentrations of EGCG, five with 10^{-8} M, five with 10^{-7} M and five with 10^{-6} M. EGCG was applied 5 min after the toxin treatment. The PC12 cells with respective treatments were incubated for 24 hours in 5% CO_2/95% air at 37°C.

PC12 cells images

Four bright field pictures were taken of each 35 mm Petri dish using a Nikon Eclipse TI-S Microscope (Nikon Instruments Inc., Melville, NY) and Photometrics CoolSnap EZ camera (Photometrics, Tucson, AZ) at an objective of 20x at random locations on the plate. Each picture encompassed a rectangular space of 670 μm by 900 μm for a total

area of 603 mm^2 (Fig. 1, upper panels). The number of cells in each image was counted manually.

Statistical analysis

The mean, standard error of the mean (SEM) and p-values were calculated in Excel (Microsoft Office, Microsoft, Redmond, Washington, USA). The p-values were calculated with a student t-test for a two-tail distribution and two-sample unequal variance. P-values less than 0.05 are considered significant.

Chemicals

EGCG, MPTP and H_2O_2 were purchased from Sigma-Aldrich (St. Louis, MO).

PC12 were purchased from the American Type Culture Collection (Manassas, VA).

Results

Control (no H_2O_2 or MPTP treatment)

Fig. 1 shows representative images of the cells for each treatment. A visual observation of the images indicates that cells treated with EGCG show increased cell count. Petri dishes treated with higher concentrations of EGCG show higher cell counts. In cells treated with EGCG in concentrations of 10^{-8} M, 10^{-7} M and 10^{-6} M, average cell count significantly increased by 1.6 fold, 1.6 fold and 2.6 fold, respectively, from untreated cells (Fig. 2A, Table 1)

H_2O_2 treatment

Treatment of PC12 cells with H_2O_2 (10^{-4} M) resulted in 68% ± 3% (SEM) cell death during 24 hours. Fig. 1B shows the decrease in cell count compared to the cell count seen in Fig. 1A, Control. Visual observation of the images indicates that cells treated with H_2O_2 (10^{-4} M) and increasing concentrations of EGCG showed increased cell count compared to cells treated only with H_2O_2. For cells treated with H_2O_2 and 10^{-8} M EGCG, 10^{-7} M EGCG and 10^{-6} M EGCG, average cell count significantly increased in a dose-dependent manner by 1.4 fold, 4.0 fold and 6.7 fold, respectively (Fig. 2A, Table 2). Cells treated with H_2O_2 and 10^{-7} M EGCG showed no significant difference in average cell number from untreated cells. Cells treated with H_2O_2 and 10^{-6} M EGCG showed a significant increase in cell count compared to untreated cells (Table 1).

MPTP treatment

Treatment of PC12 cells with MPTP (10^{-5} M) resulted in 44% ± 4% (SEM) cell death during 24 hours. The difference in cell count between control and MPTP treated cells can

be seen in a comparison of Fig. 1A and Fig. 1C. Visual observation of the images in Fig. 1C indicates that increasing the concentration of EGCG results in increasing cell counts. In cells treated with MPTP and 10^{-8} M EGCG, 10^{-7} M EGCG and 10^{-6} M EGCG, average cell count significantly increased by 1.5 fold, 2.2 fold and 4.1 fold, respectively (Fig. 2A, Table 2). Cells treated with MPTP and 10^{-7} M EGCG showed no significant difference in average cell number from untreated cells. Cells treated with MPTP and 10^{-6} M EGCG showed a significant increase in cell count compared to untreated cells (Table 1).

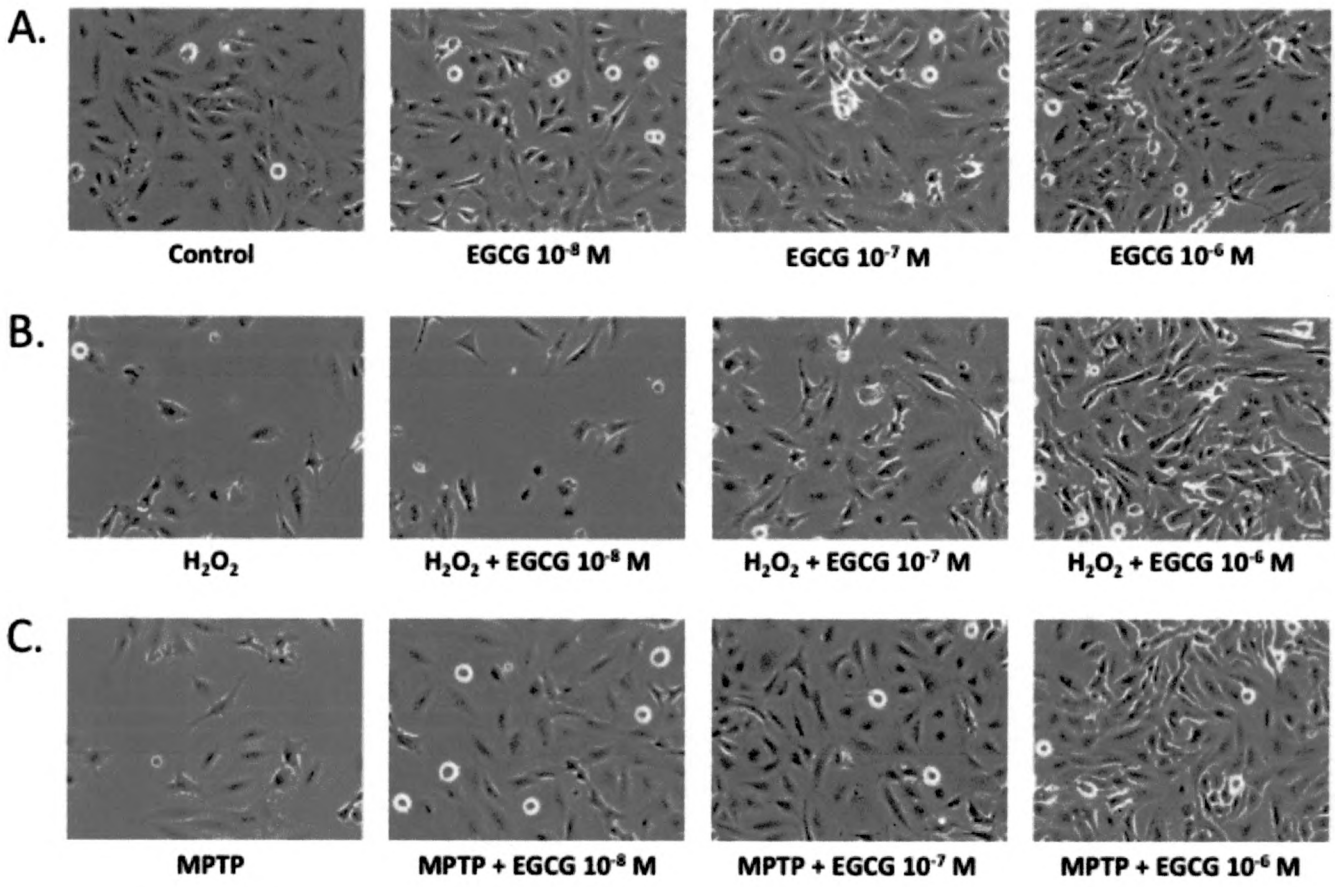

Figure 1. Representative images of each cell treatment.

A. Control and treatment with EGGC in varying concentrations.

B. Cells treated with H_2O_2 (10^{-4} M) and varying concentrations of EGCG.

C. Cells treated with MPTP (10^{-5} M) and varying concentrations of EGCG.

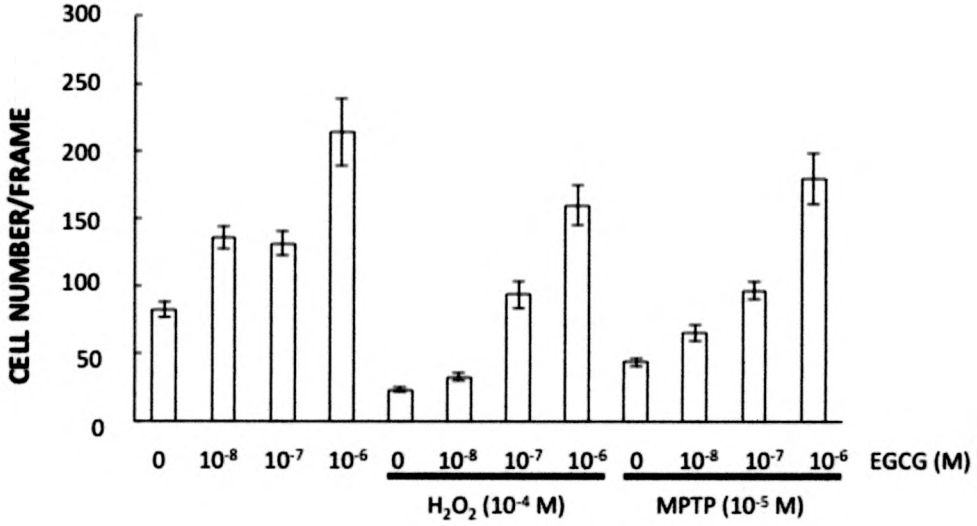

Figure 2. Comparisons between different cell treatments. The number of cells per image frame for each cell treatment. Average values ± SEM are shown.

Table 1. Cell number for each cell treatment and the p-value when compared to the non-treated cells. Average values are shown ± SEM.

	Cell Number	P-Value
Control	82.6 ± 5.6	----
EGCG 10^{-8} M	135.7 ± 8.4	< 0.001
EGCG 10^{-7} M	131.3 ± 9.0	< 0.001
EGCG 10^{-6} M	214.2 ± 25.2	< 0.001
H_2O_2	23.8 ± 1.6	< 0.001
H_2O_2 + EGCG 10^{-7} M	94.1 ± 10.2	0.327
H_2O_2 + EGCG 10^{-6} M	159.9 ± 14.6	< 0.001
MPTP	44.05 ± 2.9	< 0.001
MPTP + EGCG 10^{-8} M	65.7 ± 5.9	0.044
MPTP + EGCG 10^{-7} M	97.2 ± 6.8	0.106
MPTP + EGCG 10^{-6} M	180.0 ± 19.1	< 0.001

Table 2. Cell number for each treatment with H_2O_2 and MPTP and the p-value of each treatment with EGCG compared to treatment only with the toxins. Average values are shown ± SEM.

	H_2O_2 (23.8 ± 1.6)		MPTP (44.05 ± 2.9)	
	Mean	P-Value	Mean	P-Value
EGCG 10^{-8} M	33.3 ± 2.7	0.005	65.7 ± 5.9	0.002
EGCG 10^{-7} M	94.1 ± 10.2	< 0.001	97.2 ± 6.8	< 0.001
EGCG 10^{-6} M	159.9 ± 14.6	< 0.001	180.0 ± 19.1	< 0.001

Discussion

Effects of EGCG on PC12 cells growth

For these experiments, 10^{-8} M, 10^{-7} M and 10^{-6} M EGCG were used because peak plasma levels of EGCG in humans after typical green tea consumption were found to be between approximately 10^{-7} M and 10^{-6} M (Chow et al. 2003, Lee et al. 2002). When compared to untreated cells, increasing the concentration of EGCG resulted in an increasing number of cells in culture (Fig.1A and 2). This observation indicates that EGCG is not cytotoxic at these concentrations. Cells in culture can only grow to a certain extent due to restrictions on the size of the plate and the availability of nutrients. EGCG allowed for more cells to survive in a limited space, possibly by reducing the harmful effects of endogenously produced ROS that could otherwise inhibit cell proliferation in culture. In this study, the observed beneficial effect of EGCG in micromolar and submicromolar concentrations on the growth of PC12 cells, a cancerous cell line, contradicts the widely reviewed anticancerous effects of green tea consumption in humans (Chen et al. 2011, Kanwar et al. 2012). In cancer cell lines, the cytotoxic effects of EGCG are observed for concentrations of EGCG at 10 μM and higher (Noguchi et al. 2006, Qiao et al. 2009, Spinella et al. 2006), significantly exceeding the concentrations used in this study (10^{-8} M, 10^{-7} M and 10^{-6} M) as well as the levels of EGCG found in human plasma after typical consumption of green tea, between approximately 10^{-7} M and 10^{-6} M (Chow et al. 2003, Lee et al. 2002). It is also possible that EGCG has cell specific effects, and whether it will promote cell survival or induce apoptosis is dependent on the particular cell type or status (Yamamoto et al. 2003). Further research into this apparent inconsistency is necessary.

Previous Studies with PC12 cells and EGCG

Previous studies using PC12 cells and EGCG have produced inconsistent results. While some have indicated that EGCG has no protective effects in PC12 cells and may worsen the effects stressors have on cells or induce apoptosis (Crispo et al. 2010a, Crispo et al. 2010b, Mazzio et al. 2001, Raza and John 2005, 2008), others suggest that EGCG does protect against oxidative stress (Flueraru et al. 2005, Koh et al. 2003, Srividhya and Kalaiselvi 2013, Ye et al. 2012).

Not all the investigators found that EGCG protected PC12 cells against the effects of MPTP toxicity, as is evident in Mazzio et al.'s study (Mazzio et al. 2001). This study used a population of PC12 cells that had evolved from the original cell line. In this evolution process, they may have also mutated to resist the effects of EGCG. In our study, PC12 cells were used and not a line that had evolved from PC 12 cells.

In the Crispo et al. study, *Protective effects of methyl gallate on H_2O_2-induced apoptosis in PC12 cells*, it was determined that EGCG exacerbates the stress caused by H_2O_2. However, a higher concentration (10 μM) of EGCG was used than that which was used in our study (Crispo et al. 2010b). Higher concentrations of EGCG (greater than 10^{-5} M) are potentially cytotoxic (Raza and John 2005). Crispo et al.'s study, *Protective effects of polyphenolic compounds on oxidative stress-induced cytotoxicity in PC12 cells*, EGCG showed no rescue effect on PC12 cells treated with H_2O_2. 10^{-4} M EGCG was also used, which is again, a higher concentration of EGCG than used in this study and higher than that which is found in the body after green tea consumption (Crispo et al. 2010a).

A range of concentrations of EGCG were used for the Flueraru et al. study. When cells were treated solely with EGCG, increasing concentrations of EGCG to greater than 10^{-4}

M resulted in higher cytotoxicity. However, when cells were treated with 2,2'-azobis(2-amidinopropane) hydrochloride (AAPH) to induce oxidative stress, lower concentrations of EGCG were used (approximately 10^{-5} M) and the cells were protected against oxidative stress induced cell death. This concentration of EGCG is higher than the one which was used in our study, but it showed similar results. One thing to consider in this study is that the cells were pretreated with EGCG. Thus, the results may not be accurate as EGCG may have neutralized AAPH before it reached the cell (Flueraru et al. 2005, Kondo et al. 1999).

Surprising, the Koh et al. study shows that increasing EGCG concentrations up to 200 μM results in increased cell viability and decreased apoptosis. This is a much greater concentration of EGCG than what was used in our study. However, the cells were pretreated with EGCG. While the paper notes that the wells were washed before addition of H_2O_2, the possibility remains that EGCG neutralized H_2O_2 within the cells due to the scavenging effects of EGCG. Thus, while these results appear inconsistent with the rest of the studies in that such high concentrations of EGCG result in increased cell viability; this could be result of EGCG scavenging before H_2O_2 has damaged the cell (Koh et al. 2003, Kondo et al. 1999).

Raza and John's 2005 study shows that increasing the concentration of EGCG in PC12 cells causes the increased production of ROS. However at 10^{-5} M, no significant increase was seen. It is concluded that at lower concentrations, such as the ones we used in our study, EGCG acts as an antioxidant. They also determined that EGCG has differential effects in various cellular compartments (Raza and John 2005).

Raza and John's 2008 study showed that using 10^{-4} M EGCG, a higher concentration that which was used in our study, resulted in the decreased production of ROS in PC 12 cells and an increase in apoptosis. While this appears to contradict their previous study, the PC 12 cells in this study were stressed using 4-HNE whereas the PC 12 cells used in the previous study were unstressed. Even though a decrease in the production of ROS is observed, apoptosis is still increased which supports the idea that higher concentrations of EGCG are cytotoxic (Raza and John 2008).

In the study performed by Srividhya et al., cells were not pretreated with EGCG thus eliminating some of the potential scavenging effects. 20 μM of EGCG were used, which is higher than the concentrations used in our study but less than the studies where apoptosis was induced by EGCG (Srividhya and Kalaiselvi 2013). Similarly, Ye et al.'s study did not treat the cells with EGCG before treating with MPP+. The highest concentration of EGCG used was 10^{-5} M and the cells were protected against stress (Ye et al. 2012). These results are similar to the ones observed in our study.

In summary, from a review of these studies, it appears that concentrations greater than 10^{-5} M EGCG result in apoptosis while concentrations less than 10^{-5} M EGCG have antioxidant and protective effects in cells. For our study, we used concentrations of EGCG between 10^{-8} M and 10^{-6} M, as these are the concentrations found in blood plasma levels after typical green tea consumption. These concentrations of EGCG are not toxic and have protective effects against stressors. Also, in our study, cells were treated with EGCG after being treated with the toxins. Our results suggest that EGCG can repair damage previously inflicted by MPTP and H_2O_2.

EGCG protects PC12 cells from H_2O_2 and MPTP-induced damage

The other groups of cells were treated with 10^{-4} M of H_2O_2 or 10^{-5} M MPTP (Fig. 1B, 1C and 2). H_2O_2 is implicated in the pathogenesis of PD as a byproduct of dopamine degradation and a ROS (reviewed in (Danielson and Andersen 2008)). We applied 10^{-4} M of H_2O_2 because this concentration of H_2O_2 caused about 50% or greater cell death in PC12 cells (Jiang et al. 2007, Lu et al. 2010). MPTP has been developed as a stressor to replicate PD symptoms, as it follows a similar pathway in causing oxidative stress induced cell death by inhibiting complex I of the mitochondrial electron transport chain (reviewed in (Chung et al. 2005, Danielson and Andersen 2008, Drechsel and Patel 2008)). We applied 10^{-5} M of MPTP because this concentration of MPTP caused about 40% death or greater in PC12 cells (Hracsko et al. 2011, Yang S. F. et al. 2001). Compared to untreated colonies, cell count was significantly decreased in cells treated with H_2O_2 (Fig. 1 and 2A, Table 1) or MPTP (Fig. 1 and 2A, Table 1), indicating that H_2O_2 and MPTP can cause cell death. When these groups of cells were also treated with different concentrations of EGCG, there was a significant increase in the number of cells in each sample (Fig. 1B, 1C and 2A). Cell count increased in a dose-dependent manner. Cells treated with H_2O_2 or MPTP and 10^{-7} M EGCG showed no significant difference in average cell number compared to untreated cells. This would appear to indicate that this treatment of EGCG was able to increase cell count to a similar cell count observed in non-PD. Cells treated with H_2O_2 or MPTP and 10^{-6} M EGCG showed significant increase in cell count compared to untreated cells. Therefore, the results demonstrate the neuroprotective effects of EGCG on PC12 cells that are undergoing oxidative stress induced cell damage and death.

Limitations

PC12 cells as a PD model

While PC12 cells provide a suitable model for studying PD, future studies should use human DA producing neuronal cells to determine if these results can be duplicated in the cells actually affected in PD. PC 12 cells are tumor cells, which could impact how EGCG affects cellular processes. Also, while the results show that cell count is increased with application of EGCG, nothing can be determined about how the transport or storage of DA is affected. Any treatment will only be effective if it can increase cell count and ensure that DA is being transported effectively.

PC12 cell images

Taking pictures at four random locations of each well could potentially provide for an inaccurate portrayal of the effects of different treatments. Future studies should use smaller plate sizes or multiwall clusters that could be totally captured in one image.

PC 12 cells counting

Due to the nature of PC12 cell growth, cell counting needed to be done manually. As previously stated, this method is not only time consuming be could introduce human error into the study. The need for this will have to be eliminated for future studies.

EGCG

Finally, EGCG is unstable and has low bioavailability. EGCG derivatives may prove more effective to determine their effectiveness in functioning as a neuroprotective agent (Landis-Piwowar et al. 2013).

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Proceedings of the National Academy of Sciences of the United States of America 101: 1159-1164.