Original Communication

Epigallocatechin Gallate Protects against TNFα- or H_2O_2-Induced Apoptosis by Modulating Iron Related Proteins in a Cell Culture Model

Qi Xu[1,2], Anumantha G. Kanthasamy[3], and Manju B. Reddy[1]

1 Department of Food Science and Human Nutrition, Iowa State University, Ames, USA
2 School of Public Health, Shanghai University of Traditional Chinese Medicine, China
3 Department of Biomedical Sciences, Iowa State University, Ames, USA

Received: February 23, 2017; Accepted: July 20, 2017

Abstract: Oxidative stress, iron dysregulation, and inflammation have been implicated in the pathogenesis of Parkinson's disease (PD). Considering the entwined relationship among these factors, epigallocatechin gallate (EGCG) may be a good candidate for PD treatment due to its protective effects against those factors. The objective of this study is to determine whether EGCG protects N27 dopaminergic neuronal cells from H_2O_2 – and TNFα- induced neurotoxicity. Seven treatments were included: control, H_2O_2, TNFα , $FeSO_4$, H_2O_2+EGCG, TNFα+EGCG, $FeSO_4$+EGCG. Cells were pretreated with 10 μM EGCG, followed by 50 μM H_2O_2, 30 ng/ml TNFα or 50 μM $FeSO_4$. Neuroprotective effects of EGCG were assessed by cell viability assay, caspase-3 activity, intracellular reactive oxygen species (ROS) generation, and iron related protein expressions. Caspase-3 activity was increased to 2.8 fold ($P < 0.001$) and 1.5 fold ($P < 0.01$) with H_2O_2 and TNFα treatment; However, EGCG pretreatment significantly decreased the caspase activity by 50.2 % ($P < 0.001$) and 30.1 % ($P < 0.05$). Similarly, cell viability was reduced to 69.2 % ($P < 0.01$) and 89 % ($P < 0.01$) by H_2O_2 and TNFα, which was partially blocked by EGCG pretreatment. Also, EGCG significantly ($P < 0.001$) protected against H_2O_2- induced ROS in a time dependent manner. In addition, both H_2O_2 and TNFα significantly ($P < 0.05$) upregulated hepcidin expression and marginally reduced ferroportin (Fpn) expression unlike iron treatment alone. Collectively, our results show that EGCG protects against both TNFα- and H_2O_2- induced neuronal apoptosis. The observed neuroprotection may be through the inhibition of oxidative stress and inflammation which is possibly mediated mainly by hepcidin and partially by Fpn.

Keywords: Parkinson's disease, EGCG, iron MPTP

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting about 1.5 % global population over 65 years old [1]. It is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which manifests as motor dysfunction including tremor, bradykinesia, postural instability and rigidity [2]. Current therapies of PD include the precursor of dopamine levodopa, the monoamine oxidase B inhibitor, dopamine receptor antagonist, catechol-o-methyl transferase inhibitor. Although these available therapies can relieve the symptoms and improve the functional capacity of the patients, but can't stop or reverse the progression of the neurodegenerative process [3]. Therefore, there is a great need to understand the pathogenesis and develop the new neuroprotective agents for the treatment or prevention of PD.

Although the etiology of PD has to be established, it is widely accepted that many factors including oxidative stress, inflammation, iron overloading may lead to neurodegeneration and development of PD [4]. Oxidative stress is a condition caused by the imbalance in the production of reactive oxygen species (ROS) and the biological system's antioxidant capacity to detoxify those species and repair the resulting damage [5]. The major consequence of oxidative stress includes damage to nuclei, lipids and proteins, which severely affects cellular function and may induce cell death [6]. Oxidative stress has been thought to be involved in both idiopathic and genetic cases of PD, and oxidative damage such as increased levels of oxidized lipids, proteins, and DNA, and decreased levels of reduced glutathione have

https://doi.org/10.1024/0300-9831/a000493

been observed in substantia nigra (SN) of PD patients [7]. A number of sources and mechanisms responsible for the generation of ROS including the metabolism of dopamine, mitochondrion dysfunction and aging have also been implicated in PD pathogenesis [8]. Neuroinflammation is considered as another major component in the pathogenesis of PD, which is demonstrated by the presence of activated microglia in the SN of PD patients or neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tertrahydropyridine (MPTP)- induced animal models [9–10]. Microglia are the resident macrophages of the central nervous system playing an essential role in the immune response [11]. However, over-activated or chronically activated microglia are a significant source of oxidative stress and damage the neighboring neurons through the secretion of cytotoxic substances such as nitric oxide or superoxide radicals [12]. In addition, microglia produced pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), can mediate direct apoptosis in neurons through the activation of caspase 8 [13].

The role of iron has gained increasing attention in PD due to its complicated interplay with other pathological factors including oxidative stress and neuroinflammation [14]. Although iron possesses essential physiological roles in all organisms, excess iron can participate in Fenton reaction to generate highly reactive hydroxyl radicals leading to lipid peroxidation, DNA and protein damage [14]. Moreover, iron accumulation might stimulate the activation of glial cells leading to the release of neurotoxic substances such as TNFα, IL-6, nitric oxide contributing to the progression of PD [15]. Accumulated evidence has demonstrated that iron accumulation is a hallmark of several neurodegenerative disorders including PD [15]. It is demonstrated that iron concentration is significantly elevated in SN in PD patients as well as neurotoxins, such as 6-hydroxydopamine (6-OHDA)-, 1-methyl-4-phenyl-1,2,3,6- tertahydropyridine (MPTP)-, and rotenone- induced PD model in animals [16, 17]. The iron accumulation in PD may be due to altered expression of iron related proteins, such as increased expression of cellular iron import proteins including divalent metal transporter-1 (DMT-1) and transferrin receptor (TFR), or decreased expression of cellular iron export proteins ferroportin (Fpn) and ceruloplasmin, or altered expression of iron storage proteins ferritin or neuromelanin [18]. Hepcidin is a small peptide that controls intracellular iron balance by binding to the sole cellular iron exporter Fpn and inducing its degradation [19]. Recent studies have shown that hepcidin and Fpn are widely expressed in the central nervous system and dysregulated hepcidin-Fpn axis might account for iron accumulation in neurodegenerative disorders [20, 21].

Based on the multifactorial pathogenesis of PD, natural compounds targeted to affect multiple functions are ideal candidates for the prevention or treatment of the disease. Epigallocatechin gallate (EGCG) is the major polyphenol in green tea and it gained attention due to its antioxidant, iron chelating and anti-inflammatory properties [22]. Both in vitro and in vivo studies have shown that EGCG prevented neurotoxin 1-methyl-4-phenylpyridinium (MPP^+)- induced neuronal cell death and MPTP- induced striatal dopamine depletion and loss of TH positive neurons, respectively [23–25]. In agreement with these findings, our recent in vitro study also showed that EGCG protected against 6-OHDA-induced neurotoxicity by regulating gene and protein expression of DMT-1, hepcidin, and ferroportin, which are involved in brain iron homeostasis [26]. The objective of this study is to further investigate whether EGCG could protect dopaminergic neurons from oxidative stress or inflammation induced apoptosis. Our hypothesis is that EGCG exerts neuroprotection against hydrogen peroxide (H_2O_2)- and TNFα- induced neurotoxicity through regulating iron related proteins, hepcidin and Fpn.

Materials and Methods

Chemicals

The immortalized rat mesencephalic dopaminergic neuronal cell line (1RB3AN27, generally referred to as N27) was a gift from Dr. Kedar N. Prasad, University of Colorado Health Sciences Center (Denver, CO). RPMI-1640 medium, fetal bovine serum, L-glutamine, penicillin, and streptomycin,5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) were purchased from Invitrogen (Carlsbad, CA). EGCG, $FeSO_4$, ascorbic acid, mouse β-actin antibody, and H_2O_2 were purchased from Sigma Aldrich (St. Louis, MO). Substrate for caspase-3, Acetyl-Asp-Glu-Val-Asp-AFC was obtained from Calbiochem (San Diego, CA). Rat TNFα recombinant was purchased from peprotech (Rocky Hill, NJ). The Cell Titer 96® AQueous Non-Radioactive Cell Proliferation assay kit was bought from Promega (Madison, WI). The rabbit polyclonal antibody for Fpn or hepcidin was purchased from Abcam (Cambridge, MA). Alexa Fluor 680 conjugated anti-mouse IgG and IRdye 800 conjugated anti-rabbit IgG were purchased from Invitrogen (Carlsbad, CA) and Rockland Inc (Gilbertsville, PA), respectively. All solutions were prepared fresh prior to each assay.

Cell culture and treatment

N27 cells were grown in RPMI-1640 medium supplemented with 10 % fetal bovine serum, 2 mmol/l L-glutamine, 50 units penicillin, and 50 μg/ml streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO_2 as described in our previous studies [27]. We used N27 dopaminergic neuronal cell model since this cell line has the potential to differentiate and produce dopamine and widely used as a cell culture model for PD [28]. Cells from passage numbers 5–8 were treated with different concentrations of EGCG, H_2O_2, TNFα to determine the optimal doses for the experiments. To investigate the protective effect of EGCG against H_2O_2- or TNFα- induced cytotoxicity, cells were pretreated with 10 μM EGCG for 3 h, followed by 50 μM H_2O_2 30 ng/ml TNFα or 50 μM $FeSO_4$ for another 15 or 24 h. Cells were collected at the end of each treatment for the future assay.

Cell viability assay

Cell viability was measured using the Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation kit as described earlier [28]. Briefly, cells were incubated with 10 μL tetrazolium compound MTS solution reagent mix at 37 °C for 45 min, followed by adding 25 μL DMSO to dissolve the formazan crystals. The absorbance was read at 490 nm with a reference wavelength of 670 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

Caspase-3 activity assay

Caspase-3 activity was measured as described previously [29]. The cell pellet was lysed with Tris buffer (50mM Tris-HCL, 1 mM EDTA, and 10 mM EGTA at pH = 7.4) containing 10 μmol/L digitonin for 20 min at 37°C. Lysates were quickly centrifuged and cell free supernatants were incubated with 50 μM Ac-DEVD-AFC as the fluorometric caspase-3 substrate for 1 h at 37°C. The caspase-3 activity was measured using a fluorescence microplate reader with the excitation at 400 nm and emission at 505 nm. The caspase-3 activity was expressed as fluorescent units (FU)/mg protein.

Intracellular ROS measurement

The formation of intracellular ROS was measured using the CM-H_2DCFDA fluorescent probe as described in our early publication with minor modifications [28]. In brief, cells were incubated with 10 μM CM-H_2DCFDA along with the treatment. Fluorescence intensity was continuously measured using a fluorescence microplate reader with the excitation 488 nm and emission 515 nm with 30 min interval for 2 h.

Western blot

Cell pellets were lysed using a modified radioimmunoprecipitation assay (RIPA) buffer as described previously [30]. Cell lysates containing equal amount of protein were loaded and separated on 12% SDS-PAGE (for Fpn) gels or 15% Tricine-SDS-PAGE gels (for hepcidin). After separation, the proteins were transferred onto a nitrocellulose membrane (12% SDS-PAGE gel) or polyvinylidene difluoride (PVDF) membrane (15% Tricine-SDS-PAGE gel) and probed with proper antibody directed against hepcidin rabbit polyclonal (1:500) or Fpn rabbit polyclonal (1:1000), followed by IR-dye 800 anti-rabbit secondary antibody (1:5000). Membranes were visualized on Odyssey Infrared Imaging system (LICOR, Lincoln, NE) and β-actin was used as an internal control.

Statistics

Data were analyzed with Prism 5.0 software (Graph Software, San Diego, CA). The measurements were normalized to the respective controls in each experiment. The differences among the treatments were compared with ANOVA with Dunnett's or Tukey's Multiple Comparison and considered significant $P < 0.05$.

Results

Cytotoxic effect of EGCG, H_2O_2 and TNFα

To determine the optimal dose of EGCG, H_2O_2 and TNFα, we first measured the dose response of EGCG, H_2O_2 and TNFα on cytotoxicity using MTS assay. As shown in Table 1, no cytotoxic effect was found when cells were treated with 5 or 10 μM EGCG for 24 h. However, N27 cell viability was reduced to 61.6% ($P < 0.001$), 31.2% ($P < 0.001$) and 31.6% ($P < 0.001$) after 24 h incubation of 25 μM, 50 μM, and 100 μM EGCG, respectively. The cytotoxic effects of different concentration of H_2O_2 and TNFα were also shown in Table 1. Cytotoxicity was not found with 10 μM H_2O_2 after 24 h incubation, but a reduction of 12% ($P < 0.01$) and 48.3% ($P < 0.001$) of cell viability was found with 30 μM and 100 μM. Similarly, 24 h treatment of TNFα 10 ng/ml didn't affect cell viability but 30 ng/ml, 60 ng/ml, 100 ng/ml significantly decreased cell viability by 24.4% ($P < 0.05$), 38.6% ($P < 0.001$), 29.9% ($P < 0.01$), respectively. Therefore, 50 μM H_2O_2 and 30 ng/ml TNFα were chosen to induce cytotoxicity, and 10 μM EGCG was selected as safe dose for the evaluation of its neuroprotective effect in the subsequent experiments.

EGCG protects N27 cells from both H_2O_2- and TNFα- induced cytotoxicity

Protective effects of EGCG against H_2O_2- and TNFα- induced cytotoxicity was evaluated by MTS (Figure 1A and Figure 1B), caspase-3 activity (Figure 1C and Figure 1D), and intracellular ROS measurement (Figure 1E). Cell viability was decreased to 69.2% ($P<0.01$) and 89% ($P<0.01$) after treating with H_2O_2 or TNFα. However, EGCG shows marginal protection and increased cell viability to 88.5% and 94.8% respectively. Similarly, caspase-3 activity was increased to 2.8 fold ($P<0.001$) and 1.5 fold ($P<0.01$) after treatment with H_2O_2 or TNFα, but EGCG significantly protected against H_2O_2- or TNFα- induced apoptosis by reducing caspase-3 activity by 50.2% ($P<0.001$) and 30.1% ($P<0.05$), respectively. In addition, intracellular ROS was increased by 4.5% ($P<0.001$), 5.5% ($P<0.001$), 6.6% ($P<0.001$) after incubating with H_2O_2 for 60 min, 90 min, 120 min, and EGCG pretreatment significantly counteracted the effect ($P<0.001$) and protected against H_2O_2- induced ROS in a time dependent manner.

EGCG protects N27 cells from both H_2O_2- and TNFα- induced cytotoxicity through downregulation of hepcidin and upregulation of Fpn

To further explore the mechanisms involved in the protective effect of EGCG against H_2O_2- and TNFα- induced cytotoxicity, we assessed the hepcidin and Fpn protein expressions, as shown in (Figure 2A and Figure 2B). As expected, 24 h of EGCG alone treatment didn't affect either hepcidin or Fpn expressions. However, H_2O_2 and TNFα significantly upregulated hepcidin expression by 66.3% ($P<0.05$) and 64.1% ($P<0.05$), and 3h pretreatment of EGCG down regulated hepcidin expression induced by H_2O_2 by 34.1% ($P<0.05$) and induced by TNFα by 32.1% ($P>0.05$), respectively (Figure 2A and Figure 2C). Although not significant, Fpn expression was reduced modestly and EGCG pretreatment marginally counteracted this effect by increasing Fpn expression by 42.5% ($P>0.05$) and 44.5% ($P>0.05$), respectively (Figure 2B and Figure 2D). As a positive control, we also tested the effect of 50 μM iron on hepcidin and Fpn expressions (Figure 2E and Figure 2F). Iron partially elevated hepcidin expression by 79.9% and significantly reduced Fpn by 47.6% ($P<0.05$), and EGCG pretreatment reversed these effects and decreased hepcidin expression by 19.7% and increased Fpn to the control level ($P<0.01$).

Table 1. Dose response effects of 24 hours exposure of EGCG (5–100μM), H_2O_2 (10–100μM) and TNFα (10–100μM) on Dopaminergic neuronal N27 cell viability.

EGCG (μM)		H_2O_2 (μM)		TNFα (ng/ml)	
0	100.0	0	100.0	0	100.0
5	92.0	10	93.1	10	81.4
10	99.9	30	88.0**	30	75.6*
25	61.6***	100	51.7***	60	61.4***
50	31.2***			100	70.1**
100	31.6***				

The values (mean ± SEM) represent percentage of the respective controls (no treatment); ANOVA with Dunnett's multiple Comparisons test was used to detect the differences between the treatments and controls of each treatment; *$P<0.05$; **$P<0.01$; ***$P<0.001$. EGCG: epigallocatechin gallate; H_2O_2: hydrogen peroxide; TNFα: tumor necrosis factor alpha.

Discussion

The goal of this current study was to investigate the antioxidant and anti-inflammatory effects of EGCG in a cell culture model of PD. We used H_2O_2 and TNFα to induce oxidative stress or inflammation mediated damage in dopaminergic neurons. Hydrogen peroxide is produced from the enzymatic or spontaneous dismutation of superoxide and further converted to highly toxic hydroxyl radicals via Fenton reaction [31]. Hydrogen peroxide, generated during dopamine turnover and auto-oxidation of dopamine, can produce free radicals, which are implicated in neurotoxins MPTP or rotenone mediated neuronal death [32]. Therefore, H_2O_2 is extensively used in vitro studies to elicit the mechanisms of which oxidative damage induced neuronal apoptosis, as well as to screen neuroprotective agents in neurodegenerative diseases [33–35]. A previous study has shown that 250 μM H_2O_2 increased intracellular ROS by 50% after 24 h treatment and caspase 3 activity by 210% after 8 h treatment [36]. Similarly, our current study showed a lower dose of 50 μM H_2O_2 started to increase intracellular ROS after 60 min, and significantly elevate caspase 3 activity after 15 h and induce cell death after 24 h treatment. Our study also found that TNFα had similar effects on dopaminergic N27 cells as H_2O_2. TNFα is a pro-inflammatory cytokine that is secreted by microglia in response to various stimuli, and has been considered to play a key role in the neuroinflammation mediated cell death in neurodegenerative disorders including PD [37]. TNFα not only activates and recruits immune cells to propagate inflammation, but also directly induces oxidative stress by the activation of ROS generation [13]. This might explain why TNFα and H_2O_2 have similar toxic effects and induce caspase activity and apoptosis in dopaminergic neurons in our current study. The protection of EGCG against both TNFα- and H_2O_2-induced apoptosis suggests both antioxidant and anti-inflammatory properties of EGCG. Since ROS and inflammation can have synergistic effect and eventually result in a feed-forward loop of neurodegeneration in PD [14], EGCG might be a promising candidate for prevention or halting the progression of the disease.

Hepcidin-Fpn axis is a master regulation of cellular iron metabolism and controls cellular iron export in response to iron stores, oxidative stress, inflammation [38]. Although the role of hepcidin in neurodegenerative disorders is very limited, recent research has shown that hepcidin and Fpn were widely expressed in the central nervous system and might be involved in neuroinflammation and brain iron dysregulation [20,21,39]. One recent in vivo study shows intracerebroventricular injection of lipopolysaccharides (LPS) in the rat brain upregulated hepcidin and downregulated Fpn in cortex and SN [39]. Another in vitro study showed that inflammatory cytokines such as TNFα upregulated the expressions of iron importer divalent metal transporter 1 (DMT-1), suppressed Fpn expression, which is mediated in part by hepcidin, resulting in iron accumulation in neurons or astrocytes [20]. Our previous study found that neurotox-

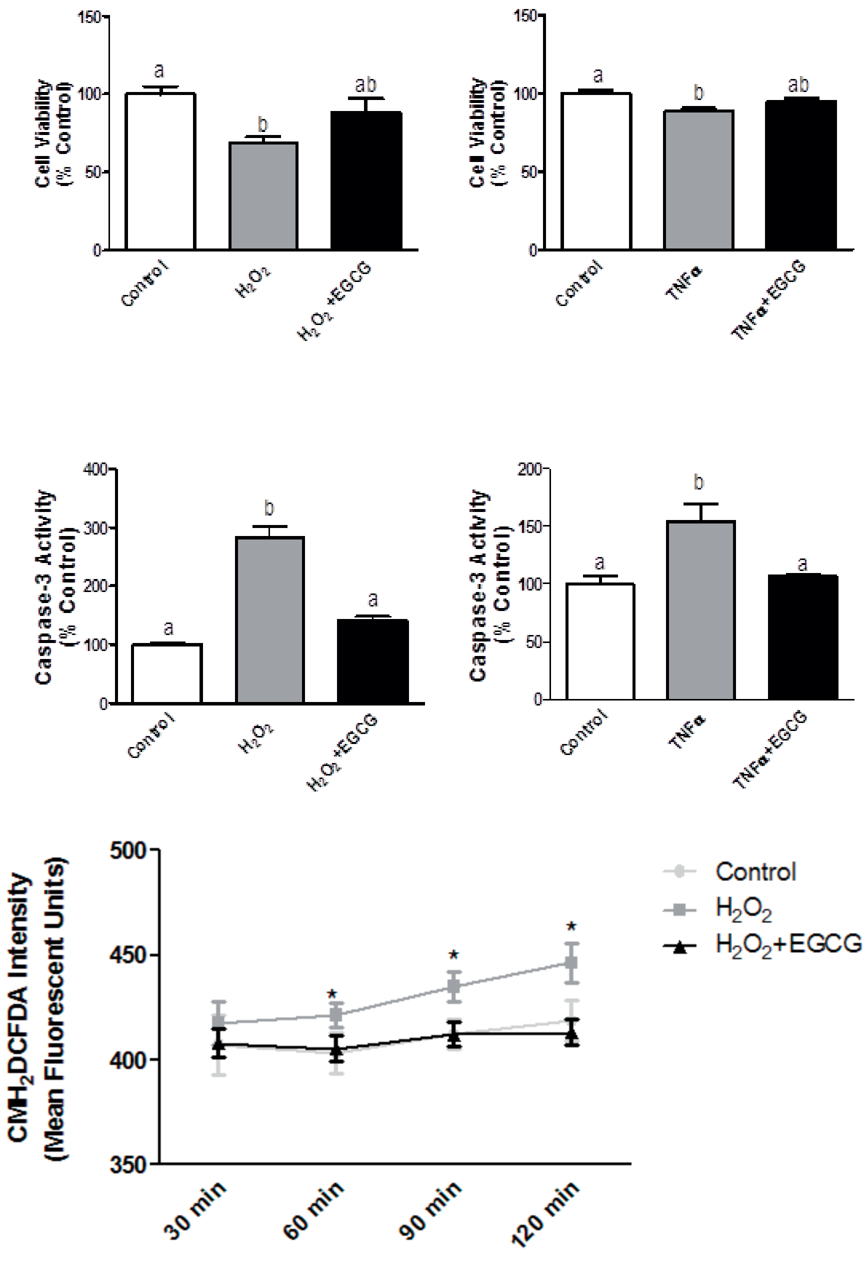

Figure 1. The protective effect of EGCG against H_2O_2 or TNFα induced neurotoxicity measured by MTS (A, n = 8; B, n = 8), caspase-3 activity (C, n = 4; D, n = 5) and intracellular ROS (E, n = 8) in N27 cells; Cells were treated with 10 μM EGCG, followed by the treatment of 50 μM H_2O_2 or 30 ng/ml TNFα for another 15 or 24 h. The values (mean + SEM) are normalized to their respective controls and ANOVA with Tukey's Multiple Comparison was used to detect the differences among the treatments and controls; $^*P < 0.001$. Bars sharing same letters are not significantly different.

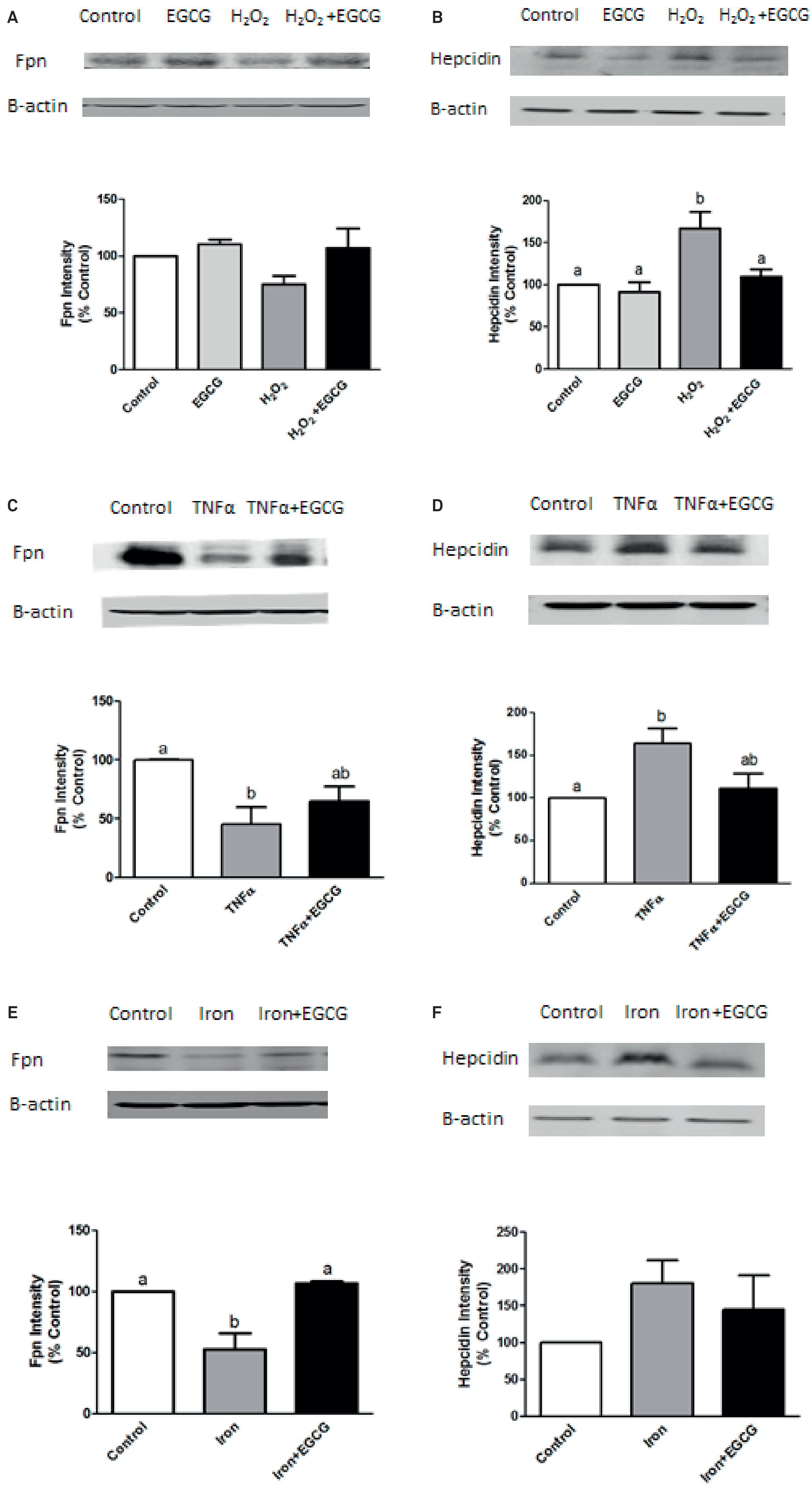

Figure 2. The protective effect of EGCG against H_2O_2 (A and B), TNFα (C and D), or ferrous sulfate (E and D) induced altered expressions of hepcidin or ferroportin in N27 cells (n = 3); Cells were treated with 10 μM EGCG with 3 h, followed by the treatment of 50 μM H_2O_2 or 30 ng/ml TNFα or 50 μM ferrous sulfate for another 15 or 24 h. The values (mean+SEM) are normalized to their respective controls and ANOVA with Tukey's Multiple Comparison was used to detect the differences among the treatments and controls; Bars sharing same letters are not significantly different.

in 6-OHDA increased the expression hepcidin and decreased the expression of Fpn, leading to iron accumulation in dopaminergic neurons [26]. In agreement with the above studies, this study shows that H_2O_2 and TNFα can significantly upregulate hepcidin expression and marginally reduce Fpn expression. These results further implicate the role of iron related proteins in both oxidative stress and inflammation mediated cell damage and demonstrated the link among iron dysregulation, oxidative stress and neuroinflammation. We also found that iron alone treatment on dopaminergic neurons had more significant effects on Fpn expression than hepcidin. Since Fpn is regulated not only by hepcidin at the post-translation level, but also by iron regulatory protein/iron responsive element at the posttranscriptional level [40] or degraded at the posttranslational level due to the absence of ceruloplasmin [41], iron alone treatment might have reduced Fpn expression independent of hepcidin, at least in neuronal cells. These results are consistent with study showing that in vivo iron overload mediated hepcidin upregulation is not reproduced in cell culture model [42], since hepcidin production in response to iron may be mediated via humoral factors such as cytokines that is secreted by cells like reticuloendothelial monocytes [43].

Epigallocatechin gallate is the most abundant polyphenol in green tea and has been shown to prevent neurotoxin MPTP- and 6-OHDA- induced neurodegeneration in both in vitro and in vivo studies [24, 44–46]. In addition, EGCG might be a good candidate for neurodegenerative disorder since in vivo study shows it could be easily absorbed from the digestive tract and widely distributed in various organs, including the brain, which had a similar concentration to the level found in the liver, kidney, lung, heart, spleen and pancreas [47]. The underlying protective mechanisms may be due to its antioxidant, anti-inflammatory, iron chelating properties, its ability to interfere with protein aggregation and intracellular signaling pathways such as Nrf2 [48, 49]. Our current study demonstrates that EGCG can prevent both oxidative stress or inflammation mediated neurodegeneration may be mainly through hepcidin and partially by Fpn.

Caution should be taken in extrapolating the results from in vitro studies because it is difficult to mimic the in vivo metabolism of EGCG and interpret the response at physiological doses applicable to humans. Thus further study is needed to confirm the role of hepcidin-Fpn axis in EGCG mediated protection in an in vivo model of PD. Moreover, research has reported that the concentration of EGCG in human plasma can be reached around 1μM when the subjects drank an excess of catechin liquid [50]. Although our dose (10μM) is higher than the normal physiological dose, it can be achieved through dietary supplements.

Overall, our study suggests EGCG can protect against H_2O_2- and TNFα- induced neurotoxicity may be through the mediation of iron regulated proteins such as hepcidin and Fpn. Our study sheds light on some of the mechanisms by which EGCG provides protection in PD.

References

1. Blesa, J. and Przedborski, S. (2014) Parkinson's disease: animal models and dopaminergic cell vulnerability. Front Neuroanat. 8, 155.
2. Magalingam, K.B., Radhakrishnan, A.K. and Haleagrahara, N. (2015) Protective mechanisms of flavonoids in Parkinson's disease. Oxid Med Cell Longev. 314560.
3. Singh, N., Pillay, V. and Choonara, Y.E. (2007) Advances in the treatment of Parkinson's disease. Prog Neurobiol. 81, 29–44.
4. Dexter, D.T.and Jenner, P. (2013) Parkinson disease: from pathology to molecular disease mechanisms. Free Radic Biol Med. 62, 132–144.
5. Shukla, V., Mishra, S.K. and Pant, H.C. (2011) Oxidative stress in neurodegeneration. Adv Pharmacol Sci.572634.
6. Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D. and Milzani, A. (2006) Biomarkers of oxidative damage in human disease. Clin Chem. 52, 601–623.
7. Hwang, O. (2013) Role of oxidative stress in Parkinson's disease. Exp Neurobiol. 22, 11–17.
8. Dias, V., Junn, E. and Mouradian, M.M. (2013) The role of oxidative stress in Parkinson's disease. J Parkinsons Dis. 3, 461–491.
9. Hirsch, E.C., Vyas, S. and Hunot, S. (2012) Neuroinflammation in Parkinson's disease. Parkinsonism Relat Disord. 18 Suppl 1, S210–212.
10. Yasuda, Y., Shinagawa, R., Yamada, M., Mori, T., Tateishi, N. and Fujita, S. (2007) Long-lasting reactive changes observed in microglia in the striatal and substantia nigral of mice after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Brain Res. 1138, 196–202.
11. More, S.V., Kumar, H., Kim, I.S., Song, S.Y. and Choi, D.K. (2013) Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson's disease. Mediators Inflamm. 952375.
12. Guadagno, J., Xu, X., Karajgikar, M., Brown, A. and Cregan, S.P. (2013) Microglia-derived TNFalpha induces apoptosis in neural precursor cells via transcriptional activation of the Bcl-2 family member Puma. Cell Death Dis. 4, e538.
13. Fischer, R. and Maier, O. (2015) Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. Oxid Med Cell Longev. 610813.
14. Urrutia, P.J., Mena, N.P.and Nunez, M.T. (2014) The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. Front Pharmacol. 5,38.
15. Zecca, L., Youdim, M.B., Riederer, P., Connor, J.R. and Crichton, R.R. (2004) Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci. 5, 863–873.
16. Weinreb, O., Mandel, S., Youdim, M.B. and Amit, T. (2013) Targeting dysregulation of brain iron homeostasis in Parkinson's disease by iron chelators. Free Radic Biol Med. 62, 52–64.
17. Gotz, M.E., Double, K., Gerlach, M., Youdim, M.B. and Riederer, P. (2004) The relevance of iron in the pathogenesis of Parkinson's disease. Ann N Y Acad Sci. 1012, 193–208.
18. Le, W. (2014) Role of iron in UPS impairment model of Parkinson's disease. Parkinsonism Relat Disord. 20 Suppl 1, S158–161.
19. Myhre, O., Utkilen, H., Duale, N., Brunborg, G. and Hofer, T. (2013) Metal dyshomeostasis and inflammation in Alzheimer's and Parkinson's diseases: possible impact of environmental exposures. Oxid Med Cell Longev. 726954.

20. Urrutia, P., Aguirre, P., Esparza, A., Tapia, V., Mena, N.P., Arredondo, M., Gonzalez-Billault, C. and Nunez, M.T. (2013) Inflammation alters the expression of DMT1, FPN1 and hepcidin, and it causes iron accumulation in central nervous system cells. J Neurochem. 126, 541–549.
21. Wang, S.M., Fu, L.J., Duan, X.L., Crooks, D.R., Yu, P., Qian, Z.M., Di, X.J., Li, J., Rouault, T.A. and Chang, Y.Z. (2010) Role of hepcidin in murine brain iron metabolism. Cell Mol Life Sci. 67, 123–133.
22. Higdon, J.V. and Frei, B. (2003) Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr. 43, 89–143.
23. Levites, Y., Weinreb, O., Maor, G., Youdim, M.B. and Mandel, S. (2001) Green tea polyphenol (-)-epigallocatechin-3-gallate preventsN-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induceddopaminergic neurodegeneration. J Neurochem. 78, 1073–1082.
24. Choi, J.Y., Park, C.S., Kim, D.J., Cho, M.H., Jin, B.K., Pie, J.E. and Chung, W.G. (2002) Prevention of nitric oxide-mediated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease in mice by tea phenolic epigallocatechin 3-gallate. Neurotoxicology, 23, 367–374.
25. Ye, Q., Ye, L., Xu, X., Huang, B., Zhang, X., Zhu, Y. and Chen, X. (2012) Epigallocatechin-3-gallate suppresses 1-methyl-4-phenyl-pyridine-induced oxidative stress in PC12 cells via the SIRT1/PGC-1alpha signaling pathway. BMC Complement Altern Med. 12, 82.
26. Chen, D., Kanthasamy, A. and Reddy, M.B. (2015) EGCG protects against 6-OHDA-induced neurotoxicity in a cell culture model. Parkinsons Dis. 843906.
27. Xu, Q., Kanthasamy, A.G. and Reddy, M.B. (2008) Neuroprotective effect of the natural iron chelator, phytic acid in a cell culture model of Parkinson's disease. Toxicology. 245, 101–108.
28. Charli, A., Jin, H., Anantharam, V., Kanthasamy, A. and Kanthasamy, A.G. (2015) Alterations in mitochondrial dynamics induced by tebufenpyrad and pyridaben in a dopaminergic neuronal cell culture model. Neurotoxicology. 53, 302–313
29. Sun, F., Kanthasamy, A., Song, C., Yang, Y., Anantharam, V. and Kanthasamy, A.G. (2008) Proteasome inhibitor-induced apoptosis is mediated by positive feedback amplification of PKCdelta proteolytic activation and mitochondrial translocation. J Cell Mol Me. 12, 2467–2481.
30. Gordon, R., Anantharam, V., Kanthasamy, A.G. and Kanthasamy, A. (2012) Proteolytic activation of proapoptotic kinase protein kinase Cdelta by tumor necrosis factor alpha death receptor signaling in dopaminergic neurons during neuroinflammation. J Neuroinflammation. 9, 82.
31. Konyalioglu, S., Armagan, G., Yalcin, A., Atalayin, C. and Dagci, T. (2013) Effects of resveratrol on hydrogen peroxide-induced oxidative stress in embryonic neural stem cells. Neural Regen Res. 8, 485–495.
32. Liddell, J.R., Obando, D., Liu, J., Ganio, G., Volitakis, I., Mok, S.S., Crouch, P.J., White, A.R. and Codd, R. (2013) Lipophilic adamantyl- or deferasirox-based conjugates of desferrioxamine B have enhanced neuroprotective capacity: implications for Parkinson disease. Free Radic Biol Med. 60, 147–156.
33. Whittemore, E.R., Loo, D.T., Watt, J.A. and Cotman, C.W. (1995) A detailed analysis of hydrogen peroxide-induced cell death in primary neuronal culture. Neuroscience. 67, 921–932.
34. Chetsawang, B., Putthaprasart, C., Phansuwan-Pujito, P. and Govitrapong, P. (2006) Melatonin protects against hydrogen peroxide-induced cell death signaling in SH-SY5Y cultured cells: involvement of nuclear factor kappa B, Bax and Bcl-2. J Pineal Res. 41, 116–123.
35. Ricart, K.C. and Fiszman, M.L. (2001) Hydrogen peroxide-induced neurotoxicity in cultured cortical cells grown in serum-free and serum-containing media. Neurochem Res. 26, 801–808.
36. Jiang, D., Jha, N., Boonplueang, R. and Andersen, J.K. (2001) Caspase 3 inhibition attenuates hydrogen peroxide-induced DNA fragmentation but not cell death in neuronal PC12 cells. J Neurochem. 76, 1745–1755.
37. Tweedie, D., Sambamurti, K. and Greig, N.H. (2007) TNF-alpha inhibition as a treatment strategy for neurodegenerative disorders: new drug candidates and targets. Curr Alzheimer Res. 4, 378–385.
38. Fleming, M.D. (2008) The regulation of hepcidin and its effects on systemic and cellular iron metabolism. Hematology Am Soc Hematol Educ Program. 151–158.
39. Qian, Z.M., He, X., Liang, T., Wu, K.C., Yan, Y.C., Lu, L.N., Yang, G., Luo, Q.Q., Yung, W.H. and Ke, Y. (2014) Lipopolysaccharides upregulate hepcidin in neuron via microglia and the IL-6/STAT3 signaling pathway. Mol Neurobio. 50, 811–820.
40. Zhang, D.L., Senecal, T., Ghosh, M.C., Ollivierre-Wilson, H., Tu, T. and Rouault, T.A. (2011) Hepcidin regulates ferroportin expression and intracellular iron homeostasis of erythroblasts. Blood. 118, 2868–2877.
41. Ward, D.M. and Kaplan, J. (2012) Ferroportin-mediated iron transport: expression and regulation. Biochim Biophys Acta. 1823, 1426–1433.
42. Jacolot, S., Ferec, C. and Mura, C. (2008) Iron responses in hepatic, intestinal and macrophage/monocyte cell lines under different culture conditions. Blood Cells Mol Dis. 41, 100–108.
43. Sasaki, Y., Shimonaka, Y., Ikuta, K., Hosoki, T., Sasaki, K., Torimoto, Y., Kanada, H., Moriguchi, Y. and Kohgo, Y. (2014) Hepcidin production in response to iron is controlled by monocyte-derived humoral factors. Int J Hematol. 99, 12–20.
44. Bitu Pinto, N., da Silva Alexandre, B., Neves, K.R., Silva, A.H., Leal, L.K. and Viana, G.S. (2015) Neuroprotective properties of the standardized extract from Camellia sinensis (Green Tea) and its main bioactive components, Epicatechin and Epigallocatechin Gallate, in the 6-OHDA model of Parkinson's disease. Evid Based Complement Alternat Med. 161092.
45. Li, R., Peng, N., Du, F., Li, X.P. and Le, W.D. (2006) Epigallocatechin gallate protects dopaminergic neurons against 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity by inhibiting microglial cell activation. Nan Fang Yi Ke Da Xue Xue Bao. 26, 376–380.
46. Wang, L., Xu, S., Xu, X. and Chan, P. (2009) (-)-Epigallocatechin-3-Gallate protects SH-SY5Y cells against 6-OHDA-induced cell death through STAT3 activation. J Alzheimers Dis. 17, 295–304.
47. Levites, Y., Weinreb, O., Maor, G., Youdim, M.B. and Mandel, S. (2001) Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. J Neurochem. 78, 1073–1082.
48. Caruana, M. and Vassallo, N. (2015) Tea polyphenols in Parkinson's disease. Adv Exp Med Biol. 863, 117–137.
49. Bai, Q., Lyu, Z., Yang, X., Pan, Z., Lou, J. and Dong, T5.(2017) Epigallocatechin-3-gallate promotes angiogenesis via up-regulation of Nfr2 signaling pathway in a mouse model of ischemic stroke. Behav Brain Res. 321, 79–86.
50. Sugisawa, A.and Umegaki, K.(2002) Physiological concentrations of (-)-epigallocatechin-3-O-gallate (EGCg) prevent chromosomal damage induced by reactive oxygen species in WIL2-NS cells. J Nutr, 132(7), 1836–1839.

Manju B. Reddy, PhD
Food Sci and Human Nutr
Iowa State University
220 Mackay Hall
Ames, Iowa, 50011
USA

mbreddy@iastate.edu

