

Green Tea Polyphenol Epigallocatechin-3-Gallate Attenuates Behavioral Abnormality in Hemi-Parkinsonian Rat

Tourandokht Baluchnejadmojarad^{*1} and Mehrdad Roghani²

¹Dept. of Physiology, School of Medicine, Iran University of Medical Sciences; ²Dept. of Physiology, School of Medicine, Shahed University, Tehran, Iran.

Received 11 November 2005; revised 18 February 2006; accepted 12 March 2006

ABSTRACT

Background: Epigallocatechin gallate (EGCG), a major constituent of green tea, has been introduced as a potent free radical scavenger and can effectively reduce free radical-induced lipid peroxidation. Since free radical injury plays an important role in neuronal damage in Parkinson's disease (PD), this study examined whether EGCG administration would reduce functional asymmetry in an experimental model of PD in male Wistar rats. **Methods:** For this purpose, unilateral intrastratial 6-hydroxydopamine-lesioned rats were intraperitoneally pretreated with EGCG (40 mg/Kg) 2 hours before surgery and daily (20 mg/Kg) for a period of 2 weeks post-surgery. Apomorphine- and amphetamine-induced rotations were measured pre- and post-surgery after 2 weeks. **Results:** The results showed that there are 35.1% ($P<0.05$) and 33.2% ($P<0.05$) reductions in controversies apomorphine- and ipsiversive amphetamine-induced rotations in EGCG-treated-lesioned group respectively as compared to the untreated lesioned group at 2nd week post-surgery. **Conclusion:** Taken together, these results showed that two-week administration of EGCG could attenuate the drug-induced behavioral abnormalities in this model of PD. *Iran. Biomed. J. 10 (4): 203-207, 2006*

Keywords: Epigallocatechin gallate (EGCG), Parkinson's disease (PD), 6-hydroxydopamine (6-OHDA)

INTRODUCTION

Parkinson's disease (PD) is a neuropathological disorder involving the degeneration of dopaminergic neurons in the substantia nigra, with the subsequent loss of their terminals in the striatum. The ensuing loss of dopamine causes most of the debilitating motor disturbances associated with PD [1]. Oxidative stress and increased lipid peroxidation, low glutathione levels, DNA damage and iron deposition has been reported as the main causes of dopaminergic neurons degeneration in PD [2]. Oxidative stress not only destroys the dopaminergic neurons, but also it compromises mitochondrial oxidative phosphorylation leading to decreased energy output by these organelle and eventually to secondary death of the cells [3].

On the other hand, unilateral damage of the nigrostriatal dopaminergic system through intrastratial injection of 6-hydroxydopamine (6-OHDA) is followed by a reduction in the striatal

dopamine level and an upregulation of dopaminergic postsynaptic receptors at the same side [4]. These changes produce a prominent functional and motor asymmetry that can be evaluated by direct acting (apomorphine) and indirect-acting (amphetamine) dopaminergic agonists [5]. These rotations, especially those induced by apomorphine, are considered as reliable indicators of nigrostriatal dopamine depletion [6].

Current PD medications treat symptoms without halting or retarding degeneration of dopaminergic neurons [7]. Tea polyphenols have been reported to be potent antioxidants and beneficial in oxidative stress-related diseases [8, 9]. In addition, there are multiple lines of compelling evidence from epidemiologic and laboratory studies supporting that frequent consumption of green tea is inversely associated with the risk of chronic human diseases including neurodegenerative disorders such as Alzheimer's disease. The chemopreventive and chemoprotective effects of green tea have been

*Corresponding Author; Tel. (+98-21) 880 58709; Fax: (+98-21) 880 58719; E-mail: tmojarad@yahoo.com

largely attributed to antioxidative and anti-inflammatory activities of its polyphenolic constituents, such as epigallocatechin gallate (EGCG) [10, 11]. On the other hand, it has been shown that the green tea polyphenol (-)-epigallocatechin-3-gallate can effectively prevent neuronal cell death caused by several neurotoxins and may have a neuroprotective effect when it is administered even after the induction of cell damage [13-15]. Therefore, this study was conducted to investigate the beneficial effect of two-week EGCG administration in an early model of PD in rat.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (the Pasteur's Institute of Iran, Tehran), weighing 230-270 g, at the start of the experiment were housed three to four per cage in a temperature-controlled colony room under light/dark cycle with food and water available *ad libitum*. They were held in the colony room for at least 10 days before being tested. Only rats not showing any biased rotational behavior (net rotations less than 30/hour) following intraperitoneal injection of apomorphine hydrochloride (0.5 mg/kg) were selected for the present study. The animals ($n = 50$) were randomly divided into four groups: sham-operated group (SH, $n = 10$, which stereotactically received an identical volume of ascorbate-saline solution into the left striatum), EGCG-treated SH operated group (SH + EGCG, $n = 10$, which also intraperitoneally pretreated with EGCG (40 mg/Kg) two hours before surgery and daily (20 mg/Kg) for a period of 2 weeks post-surgery), lesion group (6-OHDA, $n = 14$, which received a single injection of 5 μ l of 0.9% saline containing 2.5 μ g/ μ l of 6-OHDA-HCl (6-OHDA, Sigma) and 0.2% ascorbic acid (weight/volume)), and EGCG-treated lesion group (6-OHDA + EGCG, $n = 16$, which also received EGCG as before). Unilateral intrastratial 6-OHDA injection was performed through a 10- μ l Hamilton syringe on anesthetized rats (ketamine 100 mg/kg and xylazine 5 mg/kg, *i.p.*) using stereotaxic apparatus (Stoelting, USA) at the coordinates: L-3 mm, AP +9.2 mm, V + 4.5 mm from the center of the interaural line, according to the atlas of Paxinos and Watson [16]. At the end of injection, the needle was left in place for an additional 5 min and then withdrawn at a rate of one mm/min.

Behavioral testing. The animals were tested for rotational behavior by apomorphine hydrochloride

(0.5 mg/kg, *i.p.*) and amphetamine sulphate (5 mg/kg, *i.p.*) one week before (baseline) and at the end of second week after the surgery. Testing with apomorphine and amphetamine were conducted at least two days apart. The rotations were measured according to a method as described previously by Fujita *et al.* [17]. Briefly, the animals were allowed to habituate for 10 min and then 1 min after the injection of drugs, full rotations were counted in a cylindrical container (a diameter of 33 cm and a height of 35 cm) at 10-min intervals for the first 60 min in a quiet isolated room. The number of ipsilateral rotations (toward the lesioned side) was counted as positive scores for amphetamine and as negative scores for apomorphine and inversely for that of contralateral rotations. Net number of rotations was defined as the positive scores minus the negative scores.

Statistical analysis. All data were expressed as mean \pm S.E.M. For the drug-induced rotational behavior, one-way ANOVA followed by post hoc test was used. Meanwhile, the relationship between apomorphine- and amphetamine-induced rotations was determined using Pearson's (linear) regression analysis. In addition, for comparing data in the same group before surgery and at the end of study, student's paired *t*-test was used. In all analyses, the null hypothesis was rejected at 0.05 level.

RESULTS

There was no mortality in rats due to surgical operation and the injected materials. In addition, the injection site was histologically verified in the center of neostriatum of all samples.

To evaluate the beneficial effect of EGCG, the turning behavior following dopaminergic agonists (*i.e.* apomorphine and amphetamine) was quantitatively measured. Regarding apomorphine-induced rotations, there were no significant differences among the groups one week before surgery (one-way ANOVA) (Fig. 1A). Statistical analysis of the total net number of rotations made over a 60-min period in the second week after surgery (Fig. 1B) showed that apomorphine causes a very significant contralateral turning in the rats of the 6-OHDA group as compared to data one week before surgery (paired student's *t*-test) ($P < 0.001$). Meanwhile, EGCG pretreatment for two weeks caused a significant reduction (by 35.1%) in EGCG + 6-OHDA group in comparison with untreated 6-

OHDA group (unpaired student's *t*-test) ($P < 0.05$). On the other hand, EGCG treatment did not produce any significant change in apomorphine-induced rotational behavior in SH group (paired student's *t*-test).

Following amphetamine, there was a higher rate of rotations in rats of all groups as compared to apomorphine. In addition, there were no significant differences among the groups one week before surgery (one-way ANOVA) (Fig. 2A). Statistical analysis of the total net number of rotations made over a 60-min period in the 2nd week after surgery (Fig. 2B) showed that amphetamine causes a very significant contralateral turning in the rats of the 6-OHDA group as compared to data one week before surgery (paired student's *t*-test) ($P < 0.001$).

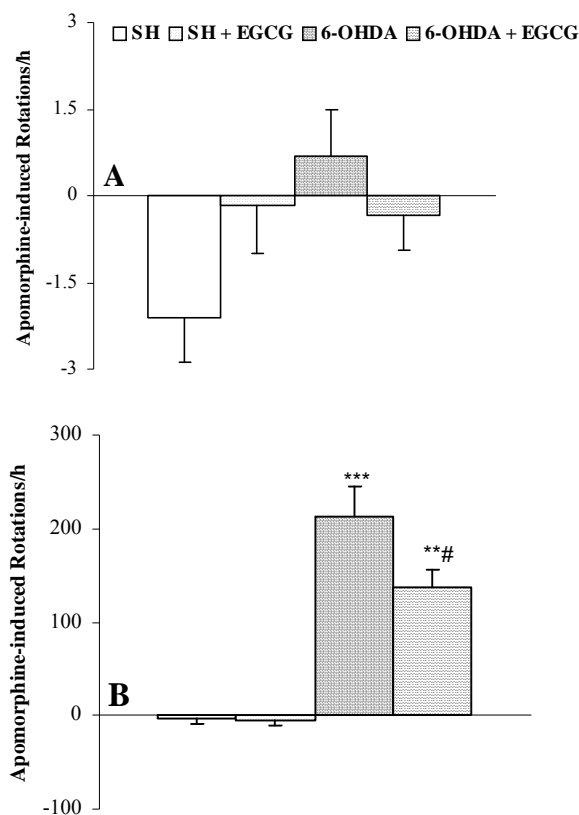


Fig. 1. Total net number of rotations (Mean \pm S.E.M.) induced by apomorphine (0.5 mg/kg, i.p.) over a period of 60 min pre-surgery (A) and at the end of second week post-surgery (B). Note that the positive values indicate turns contralateral to the side of lesion. Meanwhile, SH, SH + EGCG, 6-OHDA, and 6-OHDA + EGCG represent sham-operated, epigallocatechin-3-gallate-treated sham, lesion, and EGCG-treated lesion groups, respectively. ** $P < 0.005$, *** $P < 0.001$ (versus SH); # $P < 0.05$ (versus 6-OHDA group).

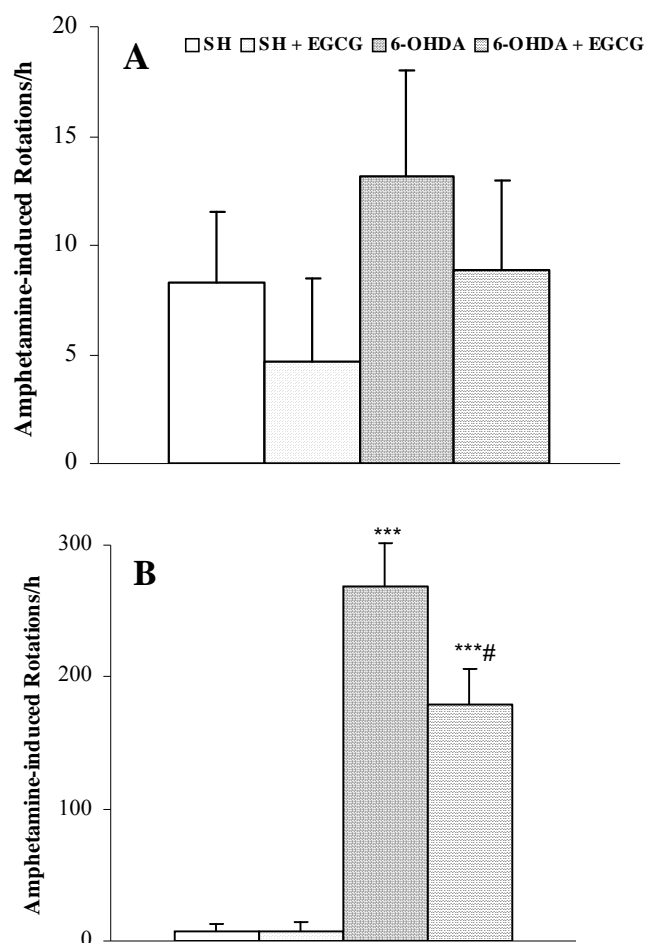


Fig. 2. Total net number of rotations (mean \pm S.E.M.) induced by and amphetamine (5 mg/kg, i.p.) over a period of 60 min pre-surgery (A) and at the end of second week post-surgery (B). Note that the positive values indicate turns ipsilateral to the side of the lesion. Meanwhile, SH, SH + EGCG, 6-OHDA, and 6-OHDA + EGCG represent sham-operated, epigallocatechin-3-gallate-treated sham, lesion, and EGCG-treated lesion groups, respectively. *** $P < 0.001$ (versus SH) 1, # $P < 0.05$ (versus 6-OHDA).

Furthermore, EGCG pretreatment for two weeks caused a significant reduction (by 33.2%) in EGCG-treated 6-OHDA group in comparison with untreated 6-OHDA group (unpaired student's *t*-test) ($P < 0.05$). On the other hand, EGCG treatment did not produce any significant change in apomorphine-induced rotational behavior in SH group.

Further analyses of rotational response for apomorphine and amphetamine showed that there is a significant correlation (Pearson's linear regression analysis) ($r = 0.76$, $P < 0.05$) in EGCG-treated lesion group (Fig. 3).

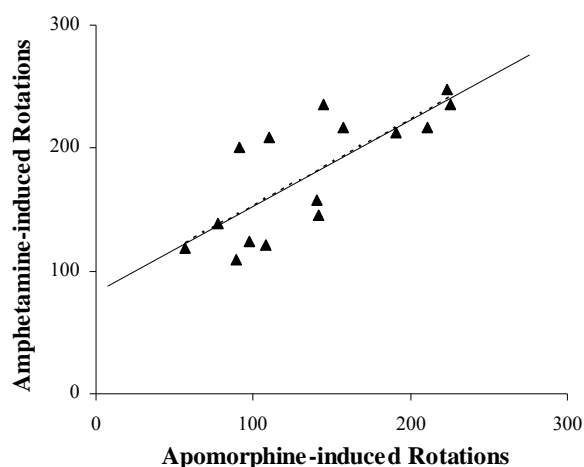


Fig. 3. Relationship between net number of amphetamine- and apomorphine-induced rotations over a period of 60 min at the end of second week post-lesion in EGCG-treated lesion group. In this respect, a Pearson regression coefficient of 0.76 ($P = 0.027$) was obtained. Meanwhile, SH, SH + EGCG, 6-OHDA, and 6-OHDA + EGCG represent sham-operated, epigallocatechin-3-gallate-treated sham, lesion, and EGCG-treated lesion groups, respectively.

DISCUSSION

These results demonstrated that two-week intraperitoneal administration of EGCG, a major constituent of green tea, could significantly attenuate the drug-induced behavioral abnormalities (apomorphine- and amphetamine-induced rotational tests), as observed in 6-OHDA-EGCG rats.

In the present study, a significant attenuation of the drug-induced rotational behavior was observed in the EGCG-treated 6-OHDA-lesioned group in the second week after the study for both apomorphine and amphetamine. The presented results clearly demonstrate that the nigrostriatal dopaminergic neurons are preserved and maintained against degenerative effects induced by the neurotoxin 6-OHDA. Consequently, a reduction was observed in apomorphine- and amphetamine-induced rotational behavior. It is a well-established fact that reactive oxygen species are implicated as the leading biochemical cause of neuronal death in various neurological disorders, including PD [3]. Tea polyphenols have been reported to be potent antioxidants and beneficial in oxidative stress-related diseases [11]. According to previous reports, the protective effect of EGCG in this study might be attributed to its alteration of oxidative stress, glutathione pools, and/or cytochrome P450 activity

in different cellular compartments [13]. In addition, the correlative neurite outgrowth activity of EGCG on PC12 cell line may also contribute to its neurorescue and neuroprotective effect [10]. On the other hand, neuroprotective capacity of EGCG may well be accounted for by their biological actions. In this regard, EGCG could protect against oxidative stress-induced cell death through stimulation of PKC and modulation of cell survival/cell cycle [18]. Further research studies are required to clarify the involved mechanisms.

In summary, these results may have implications for therapeutic use of tea polyphenols *in vivo*. In this respect, EGCG may have a beneficial effect in reversing behavioral abnormalities in an experimental model of PD in rat.

REFERENCES

1. Sauer, H. and Oertel, W.H. (1994) Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience* 59: 401-415.
2. Schwarting, R.K. and Huston, J.P. (1997) Behavioral and neurochemical dynamics of neurotoxic meso-striatal dopamine lesions. *Neurotoxicology* 18: 689-708.
3. Dauer, W. and Przedborski, S. (2003) Parkinson's disease: mechanisms and models. *Neuron* 39: 889-909.
4. Baluchnejadmojarad, T. and Roghani, M. (2004) Evaluation of functional asymmetry in rats with dose-dependent lesions of dopaminergic nigrostriatal system using elevated body swing test. *Physiol. Behav.* 82: 369-373.
5. Schwarting, R.K., Bonatz, A.E., Carey, R.J. and Huston, J.P. (1991) Relationships between indices of behavioral asymmetries and neurochemical changes following mesencephalic 6-hydroxydopamine injections. *Brain Res.* 554: 46-55.
6. Shapiro, R.M., Glick, S.D. and Camarota, N.A. (1987) A two-population model of rat rotational behavior: effects of unilateral nigrostriatal 6-hydroxydopamine on striatal neurochemistry and amphetamine-induced rotation. *Brain Res.* 426: 323-331.
7. Ross, G.W. and Petrovitch, H. (2001) Current evidence for neuroprotective effects of nicotine and caffeine against Parkinson's disease. *Drugs Aging* 18: 797-806.
8. Raza, H. and John, A. (2005) Green tea polyphenol epigallocatechin-3-gallate differentially modulates oxidative stress in PC12 cell compartments. *Toxicol.*

- Appl. Pharmacol.* 207: 212-220.
9. Lee, J.S., Oh, T.Y., Kim, Y.K., Baik, J.H., So, S., Hahm, K.B. and Surh, Y.J. (2005) Protective effects of green tea polyphenol extracts against ethanol-induced gastric mucosal damages in rats: stress-responsive transcription factors and MAP kinases as potential targets. *Mutat. Res.* 579: 214-224.
 10. Reznichenko, L., Amit, T., Youdim, M.B. and Mandel, S. (2005) Green tea polyphenol (-)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite outgrowth. *J. Neurochem.* 93: 1157-1167.
 11. Mandel, S., Maor, G. and Youdim, M.B. (2004) Iron and alpha-synuclein in the substantia nigra of MPTP-treated mice: effect of neuroprotective drugs R-apomorphine and green tea polyphenol (-)-epigallocatechin-3-gallate. *J. Mol. Neurosci.* 24: 401-416.
 12. Lin, J., Della-Fera, M.A. and Baile, C.A. (2005) Green tea polyphenol epigallocatechin gallate inhibits adipogenesis and induces apoptosis in 3T3-L1 adipocytes. *Obes. Res.* 13: 982-990.
 13. Aneja, R., Hake, P.W., Burroughs, T.J., Denenberg, A.G., Wong, H.R. and Zingarelli, B. (2004) Epigallocatechin, a green tea polyphenol, attenuates myocardial ischemia reperfusion injury in rats. *Mol. Med.* 10: 55-62.
 14. Weinreb, O., Mandel, S., Amit, T. and Youdim, M.B. (2004) Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J. Nutr. Biochem.* 15: 506-516.
 15. Lee, H., Bae, J.H. and Lee, S.R. (2004) Protective effect of green tea polyphenol EGCG against neuronal damage and brain edema after unilateral cerebral ischemia in gerbils. *J. Neurosci. Res.* 77: 892-900.
 16. Paxinos, G. and Watson, C. (1986) The rat brain in stereotaxic coordinates, 2nd ed., Academic Press, San Diego.
 17. Fujita, M., Nishino, H., Kumazaki, M., Shimada, S., Tohyama, M. and Nishimura, T. (1996) Expression of dopamine transporter mRNA and its binding site in fetal nigral cells transplanted into the striatum of 6-OHDA lesioned rat. *Mol. Brain. Res.* 39: 127-136.
 18. Levites, Y., Amit, T., Youdim, M.B. and Mandel, S. (2002) Involvement of protein kinase C activation and cell survival/cell cycle genes in green tea polyphenol (-)-epigallocatechin 3-gallate neuroprotective action. *J. Biol. Chem.* 277: 30574-30580.