Neuro-rescue role of epicatechin-3-gallate on 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine induced parkinson mice.

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ABSTRACT

Green tea polyphenols are known to possess various neuropharmacological properties. In the recent past, mice models using 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) have been widely used to study underlying mechanism of Parkinson’s disease. In this study, we examined the neurorescue properties of Epicatechin-3-gallate (ECG) against MPTP induced oxidative damage, neurotoxicity and behavioural deficits. Mice were orally treated with ECG (6mg/kg body weight) for 7 days had significant increase in dopamine, DOPAC and HVA levels, diminutive in the TBARS and increased in the level of GSH and activities of GPx compared to the PD mice with significantly decreased dopamine, DOPAC and HVA levels, high TBARS level and reduced GSH level and GPx activity. To conclude, our results suggest that ECG offered protection against MPTP induced oxidative stress and markedly reduced dopamine depletion. The ECG treated mice exhibited better results in behavioral analysis (Rotarod, Hang test and Stride length measurements) which clearly shows the neurorescue potential of ECG in PD.

Keywords: Epicatechin-3-gallate, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, Oxidative stress, Dopamine, behaviour.

INTRODUCTION

Parkinson’s disease (PD) is a progressive neurodegenerative movement disorder results primarily from the death of dopaminergic neurons in the substantia nigra and depletion of striatal dopamine [3]. The diminution of the dopamine concentration in the main projection area of the striatum induces the characteristic neurological motor symptoms of the disease, which include akinesia, resting tremor and rigidity. Experimental PD are generated through the administration of neurotoxins; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent neurotoxin, known to destroy central dopaminergic neurons and induces Parkinson’s disease in various experimental animals including monkey, mice, cats, dogs, rats and even gold fishes [3].

The pathogenesis of Parkinson’s disease is multifactorial with toxic reactions including mitochondrial dysfunction, oxidative stress, inflammation, the glutamatergic toxicity, the ubiquitin/ proteasome system activation of apoptosis pathways and elevation of iron and nitric oxide. The multifactorial etiology of this diseases suggests that drugs with multiple targets could have therapeutical potential for this pathology.

Tea mostly in the form of green tea or black tea or oolong tea is one of the most frequently consumed beverages in the world next to water for almost 50 centuries [24]. Green tea is considered as an important dietary source of polyphenols, particularly flavonoids including catechins (flavan-3-ols). The four major catechins of green tea are (–) - epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG) and (–)-epicatechin (EC) [23]. Levites et al [11] have reported the neuroprotective effect of green tea extract and the green tea polyphenol EGCG in experimental PD.

The prevalence of Parkinson’s disease is likely to increase in the coming decades as the number of elderly people will increase. Therefore, it is of utmost importance to develop new treatments that slow or halt the rate of progression of Parkinson’s disease. This present study is designed to investigate the neuroprotective role of epicatechin-3-gallate (ECG) by analyzing the levels of striatal dopamine and its metabolites, oxidative stress and behaviour patterns in MPTP induced Parkinsonism mice model.

MATERIALS AND METHODS

All the experiments were carried out in inbred adult male C57BL/6 mice (30-35g) from the National Institute of Nutrition, Hyderabad. They were housed in polypropylene cages bedded with husk, renewed every 24 h. Animals were under 12/12h : light /dark cycle at around 22°C and had free access to pellet diet (Pranav Agro Industries Ltd., Maharatra, India) and pure water. The experimental protocols met with the National Guidelines on the Proper Care and Use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, 2000) and were approved by the Animal Ethics Committee of the Institute (Approval No. 602/12-2008).

The mice were divided into four groups each group having six animals. The first group of mice was kept as a control. The second group had intra peritoneal administration of MPTP (20 mg/kg body weight) for 4 days [16]. The third group was orally treated with ECG (6 mg/kg body weight) [19] for seven days and received MPTP for 4 consecutive days (from 4th day to 7th day). The fourth group was treated with ECG alone.

At the end of the experiment (8th day), behavioral analysis were carried out by performing rotarod test, hang test and stride length measurement [15,16]. Then animals were sacrificed by cervical dislocation and decapitation and the brain was dissected to procure striatum and midbrain by the method described by Glowinski and Iversen [9]. Analysis of thiobarbituric acid reactive substances (TBARS) [23], reduced glutathione (GSH) [10], and glutathione...
peroxidase (GPx) was carried out in midbrain. The dopamine and its metabolites DOPAC and HVA are estimated by HPLC using electrochemical detector in striatum.

RESULT

(Table 1) ECG treatment enhanced the levels of dopamine, DOPAC and HVA in striatum of PD mice (group III) compared to the untreated PD mice group II that showed lowered neurochemical levels. The ECG alone treated group III showed no significant changes compared to the control group I.

Table 1. Changes in the levels of Dopamine and its metabolites DOPAC and HVA in control and experimental mice striata.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dopamine µg tissue</th>
<th>DOPAC µg tissue</th>
<th>HVA µg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.18 ± 0.86^a</td>
<td>2.45 ± 0.19^a</td>
<td>1.86 ± 0.14^a</td>
</tr>
<tr>
<td>MPTP</td>
<td>4.11 ± 0.32^b</td>
<td>1.74 ± 0.14^b</td>
<td>0.72 ± 0.04^b</td>
</tr>
<tr>
<td>MPTP+ ECG</td>
<td>7.55 ± 0.4^c</td>
<td>2.08 ± 0.11^c</td>
<td>1.13 ± 0.09^c</td>
</tr>
<tr>
<td>ECG</td>
<td>14.16±0.84^a</td>
<td>2.48±0.17^a</td>
<td>1.88±0.13^a</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

(Table 2) ECG treatment lowered the levels of TBARS in PD mice (group III) compared to the untreated PD mice group II that showed enhanced TBARS level. The levels of GSH and activities of GPx were significantly enhanced in the mice group III fed with ECG (Table 2) compared to the group II PD mice showed diminished levels of GSH and GPx. The ECG alone treated group III showed no significant changes compared to the control group I.

Table 2. Changes in the levels of TBARS and activities of GSH and GPx in control and experimental mice midbrain.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (µmol/g)</th>
<th>GSH (µg/gram tissue)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.46± 0.09^a</td>
<td>0.38 ±0.04^a</td>
<td>5.03 ±0.16^a</td>
</tr>
<tr>
<td>MPTP</td>
<td>3.11 ± 0.23^b</td>
<td>0.13 ±0.02^b</td>
<td>4.06 ±0.17^b</td>
</tr>
<tr>
<td>MPTP+ ECG</td>
<td>2.42 ± 0.16^c</td>
<td>0.27 ±0.02^c</td>
<td>4.43 ±0.13^c</td>
</tr>
<tr>
<td>ECG</td>
<td>1.45±0.10^a</td>
<td>0.38±0.02^a</td>
<td>5.01±0.34^a</td>
</tr>
</tbody>
</table>

^a= amount of glutathione utilized/minute .Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Fig.1) and 2) ECG treatment enhanced retention time on rotarod, neuromuscular strength (Hang test) and (Table. 3) stride length in PD mice group (III) compared to the untreated PD mice group II that showed very low score. ECG alone treated group III showed no significant changes in behavior compared to the control group I.

DISCUSSION

In the present study, the levels of dopamine and its metabolites were decreased in striatum of PD animals are due to the impairment of dopaminergic neurons. Systemic administration of MPTP to mice causes marked destruction of dopaminergic neurons in the pars compacta of the substantia nigra (SNpc) and depletion of striatal dopamine and considered as a standard model of idiopathic Parkinson’s disease. Both green tea extract and EGCG at low concentration (one of the related catechin of ECG) exerts neuroprotective role in MPTP mouse model of PD by protecting brain DA neurons and 6-hydroxydopamine induced rat PC12 and human blastoma SH-SY5Y cell death in culture. Various behavioral tests such as rotarod test (to detect the ability to balance and walk), Hang test (to study the neuro-muscular strength and coordination), Stride length measurement (to study the PD gait), open field test (to study the motor and exploratory activity) etc., are used as indices to measure the movement (motor) impairments in MPTP-induced animal models.

The cells of the substantia nigra use dopamine (a neurotransmitter-chemical messenger between brain and nerve cells) to communicate with the cells in another region of the brain called the stratum. Thus, a reduction in nigral dopamine levels results in a decrease in striatal dopamine that is believed to cause PD symptoms such as tremor, muscular rigidity, akinesia, and bradykinesia slowness in initiating and executing movements. Restoration of depleted striatal dopamine by tea polyphenol ECG may help in the restoration of behavior patterns.

Oxidation plays an important role in the degeneration of dopaminergic neurons, particularly those in the substantia nigra result in Parkinsonism. Generation of free radicals is considered to be one of the major factors in the genesis and progression of PD. The result of present study reveals that MPTP treatment increased lipid peroxidation in the brain and decreased

Fig. 2 depicts the hang test in control, PD and ECG treated mice groups and the significantly improved neuro muscular coordination and strength in ECG treated compared to PD mice group (< 0.05).

Table 3. Changes in the Forelimbs and hind limbs stride length in control and experimental mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fore limb paw stride length (cm)</th>
<th>Hind limb paw stride length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.20± 0.55^a</td>
<td>6.10± 0.46^a</td>
</tr>
<tr>
<td>MPTP</td>
<td>4.70 ± 0.36^b</td>
<td>4.71± 0.42^b</td>
</tr>
<tr>
<td>MPTP+ ECG</td>
<td>5.91 ± 0.45^c</td>
<td>5.44± 0.41^c</td>
</tr>
<tr>
<td>ECG</td>
<td>7.23±0.50^a</td>
<td>6.13±0.42^a</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).
GSH levels and its dependent GPx activities. A number of preclinical and clinical studies reported the increased lipid peroxidation in the brain of Parkinsonian patients or in MPTP-induced parkinsonian monkeys.

Pretreatment of PD mouse with ECG reduces the TBARS level and this shows that ECG reduces the lipid peroxidation. Glutathione and GPx protect cells from oxidative stress and the depletion of GSH is the earliest biochemical change in the brains of PD patients. A depletion of GSH has been found both in PD patients and in animal models. The enzyme GPx is involved in reducing hydrogen peroxide to water, in the presence of reduced glutathione as a powerful free radical scavenger. Thus, a decreased GSH and GPx levels serves as an indicator of oxidative stress. The decreased levels of GSH, and GPx in MPTP intoxicated mice in our study also, confirms that MPTP induce a state of oxidative stress in the brain. Interestingly treatment of PD mouse with ECG makes a notable improvement in the levels of GSH and GPx in the PD mouse. This shows that ECG interferes with oxidative damage seen in the PD model mouse.

Green tea polyphenols are having neuroprotective role, since they are with multipotent activities like radical scavenging, iron chelating and anti-inflammatory actions and so having promising role in preventive effect in PD with multifactorial etiology. Green tea catechins lower the incidence of cancers, arthritis and UV damage in skin which are reported in different previous findings. ECG is reported to have greatest antioxidant activity than other tea polyphenols. It has been shown to be effective in treating disorders with the influence of oxidative stress through cell line studies. The pretreatment of animals with ECG has significantly reversed these toxic effects of MPTP by increasing the levels of dopamine, its metabolites, GSH and GPx and reducing the levels of TBARS at significant level shows the antioxidant activity of ECG which is supported by the previous findings with other green tea polyphenols. Chao-fang et al have reported the neuroprotective effect of ECG on the 6-hydroxy dopamine induced apoptosis in PC-12 cells.

The present study supports the notion that ECG, a potent antioxidant could be used as a neuroprotective agent for treatment of Parkinson’s disease. However further study is needed to find out the anti-inflammatory, anti-apoptotic, monoamine oxidase inhibitory and mitochondrial function modulatory activities of ECG to prove their neuroprotective action to elucidate the proper mechanisms and appropriate dosage profiles.

REFERENCES