Enhanced neuroprotective effect by combination of bromocriptine and *Hypericum perforatum* extract against MPTP-induced neurotoxicity in mice

M. Mohanasundari a, M.S. Srinivasan a, S. Sethupathy b, M. Sabesan a,*

a Department of Zoology, Annamalai University, Annamalainagar-608002, Tamilnadu, India
b Division of Biochemistry, Rajah Muthiah Medical College and Hospital Annamalai University, Annamalainagar, Tamilnadu, India

Received 15 August 2005; received in revised form 5 June 2006; accepted 8 June 2006
Available online 31 July 2006

Abstract

The present study has been designed to evaluate the combined effect of bromocriptine (BRC) and *Hypericum perforatum* extract (HPE) on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson’s disease in male Swiss Albino mice, which were randomly divided into seven groups of six animals each. Group I served as control. Groups II and III were given 300 mg/kg HPE (po) and 10 mg/kg BRC (i.p.) respectively, once daily for 7 days. The four doses of MPTP (20 mg/kg) were administered intraperitoneally with an interval of 2 h to the groups IV, V, VI and VII. The drug treatment was given to fifth group (10 mg/kg BRC; i.p), sixth group (300 mg/kg HPE; po) and seventh group (300 mg/kg HPE; po and 10 mg/kg BRC; i.p.) once in a day for 7 days and the dose on the first day was given 30 min prior to first MPTP injection. The rotarod test, hang test and forepaw stride length revealed significant improvement by the combined treatment. Dopamine and DOPAC levels were significantly improved (p<0.05). There was a significant reduction in lipid peroxidation after the combined treatment (p<0.05) and the antioxidant status was improved. These findings suggest that the combined effect of BRC and HPE was more pronounced than BRC or HPE alone. So it is concluded that the combined treatment might be preferable to either BRC or HPE alone in the effective clinical management of Parkinson’s disease.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Parkinson’s disease; Dopamine; Antioxidants; Lipid peroxidation

1. Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin, selectively destroys dopaminergic neurons in the striatal area of rodents and primates [1]. The neurotoxicity of MPTP requires its oxidation to MPP⁺ (1-methyl-4-phenylpyridinium) by monoamine oxidase B an event that most likely occurs in glial cells [2]. The MPP⁺ is taken up by the high affinity dopamine uptake systems and subsequently accumulates within the mitochondria of nigrostriatal dopaminergic cells [3]. Mitochondrial dysfunction and free radical generations were implicated as major mechanism of neuronal death in neurodegenerative diseases [4]. BRC has recently been shown to possess strong free radical scavenging action in vivo. Thus, BRC could afford protection against methamphetamine-induced dopamine (DA) depletion [5] and 6-hydroxy dopamine-induced DA-ergic toxicity [6]. BRC has also been shown to block MPTP-induced behavioral dysfunction indicating its potent neuroprotective action [7]. However, patients who receive BRC monotherapy may also experience adverse effects, including confusion, visual hallucinations, and paranoia [8,9].

There is an increasing interest in natural antioxidants, namely phenols, present in medicinal and dietary plants, that might help prevent oxidative damage [10]. The reinforcement of endogenous antioxidants via intake of dietary antioxidant may be of particular importance in attenuating
the cumulative effect of oxidatively damaged molecules [11].

Ethanolic extract of HPE contains many phenolic compounds, namely flavonoids and phenolic acids, suggesting that they could have important antioxidant properties [12] and could possibly reduce the side effects of BRC. So the present study has been done to evaluate the combined effect of HPE and bromocriptine against MPTP-induced Parkinson’s disease in mice.

2. Materials and methods

2.1. Chemicals

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), bromocriptine mesylate, thiobarbituric acid (TBA), reduced glutathione, and 3,5-dithio-bis-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. USA. All other reagents were of analytical grade.

2.2. Animals

Albino male mice (20–25 g) were obtained from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, Annamalai University, India. All animals were housed in 1:1 h light and dark cycle. Institute’s animal ethical committee clearance has been obtained for this study.

2.3. Preparation of extract

Hypericum perforatum plants (Gluceace) were collected from high altitude of Western Ghats of the Nilgiris, Tamil Nadu, South India. It was dried in shade, powdered and extracted with methanol. Extracts were evaporated under low pressure using Buchi type rotary evaporator. The concentrated extract was kept in vacuum desiccator until the constant weight of solvent free extract was obtained. The extract was dissolved in Tween 80 solution with distilled water 1:9 ratio (Tween 80:D.W.; 1:9) [13].

2.4. Experimental design

The mice were divided into seven groups and each group had six animals. The first group of mice was kept as a control without the following treatment. The second group was treated with HPE 300 mg kg$^{-1}$ (po) [14] for 7 days. The third group of mice was treated with BRC (10 mg kg$^{-1}$ body weight) i.p. [7] for 7 days of the experimental period.

The four doses of MPTP (20 mg/kg) were administered intraperitoneally with an interval of 2 h [15] to the groups IV, V, VI and VII. The drug treatment was given to fifth group (10 mg/kg BRC; i.p), sixth group (300 mg/kg HPE; po) and seventh group (300 mg/kg HPE; po and 10 mg/kg BRC; i.p) once a day for 7 days and the dose on the first day was given 30 min prior to first MPTP injection. All groups were sacrificed on the eighth day as per animal ethics.

2.5. Behavioral study

2.5.1. Rotarod test

Motor co-ordination was measured on the seventh day using an automated rotarod (Amni, Rotar Instrumentation, Columbus, OH, USA). Animals were exposed to 10 trials on rotating rod at various rpm such as 25, 20, 15, 10 and 5 with 5 min intervals and the cut-off time was kept as 180 s by the method of Rozas et al. [16]. The rotor was divided into two compartments, which could allow two mice at a time. The average of the retention time on the rod was calculated.

2.5.2. Hang test

Neuromuscular strength was determined on the seventh day in the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid (grid 12 cm$^2$ opening 0.5 cm$^2$) and supported until they grabbed the grid with both their fore and hind paws. The grid was then inverted so that the mice were allowed to hang upside down. The grid was mounted 20 cm above a hard surface, to discourage falling or injury in case of falling. The apparatus was equipped with a 3-inch wall to prevent animals from transversing to the upper side of the grid. Animals were allowed to stay on the grid on seventh day for 30 s and 10 chances were given with 1 min interval and the best fall values were recorded. The percentage of success was recorded as maximum time hanging/30 s × 100 [17].

2.5.3. Forepaw stride length during walking

Animals had their forepaws placed in black ink and the length of forepaw steps during normal walking (the animals were trained to walk along a straight line only prior to injection) across a clean sheet of paper was measured. Stride length was determined by measuring the distance between each step of the same side of the body of the animal. Measurement of the stride length was calculated from the mid digit toe of the first step to the heel of the second step [17].

2.6. Estimation of catecholamines

The animals were killed by cervical decapitation after 24 h of the last dose and their striatum was immediately homogenized in 0.1 mol/L perchloric acid for HPLC (high performance liquid chromatography) analysis of DA, and 3,4-dihydroxyphenyl acetic acid (DOPAC) [18].

2.7. Estimation of antioxidants and lipid peroxidation

Reduced glutathione (GSH) by the method of Beutler [19], the activity of superoxide dismutase (SOD) by the method of Kakkar et al. [20], catalase activity by the method of Sinha [21], and the activity of glutathione peroxidase (GPx) by the method of Rotruck et al. [22] were assayed. Lipid peroxidation (TBARS) in tissue was estimated by the method of Ohkawa et al. [23] and protein by the method of Lowry et al. [24].
2.8. Statistical analysis

Data have been presented as mean values±SEM, the statistical significance between individual data was assessed using ANOVA (Scheffe’s F test), and \( p<0.05 \) was accepted as significant.

3. Results

3.1. Rotarod test

The retention time on the rod significantly decreased in MPTP group when compared with the control group. The retention time significantly increased \( (p<0.05) \) in pretreated groups 5, 6 and 7 respectively when compared with group 4. Combined treatment group 7 markedly showed an improvement \( (p<0.05) \) when compared with group 4 and either alone group respectively (Fig. 1).

3.2. Hang test

The hang time significantly decreased \( (p<0.05) \) in group 4 when compared with the control group. In groups 5, 6 and 7, it significantly increased when compared with group 4. Group 7 showed a marked improvement in hang time when compared with group 5 and 6 \( (p<0.05) \) (Fig. 2).

3.3. Forepaw stride length

The forepaw step distance significantly decreased \( (p<0.05) \) in MPTP-treated group when compared with the control group. Combined treatment group significantly improved \( (p<0.05) \) when compared with the MPTP treated group (Fig. 3).

3.4. Dopamine and DOPAC contents

Striatal dopamine, DOPAC and HVA levels in the MPTP-treated group decreased significantly \( (p<0.05) \) when compared with the control group. Combined treatment group significantly improved \( (p<0.05) \) when compared with the MPTP treated group (Fig. 3).
0.02; HVA 0.73±0.01 vs. 1.66±0.02). Treatment with BRC, HPE groups significantly (p<0.05) improved striatal dopamine, DOPAC and HVA levels when compared with the MPTP-treated group (BRC+MPTP; dopamine: 6.13±0.17; DOPAC: 1.49±0.02; HVA: 0.91±0.01 and HPE+MPTP: 6.53±0.18; 1.61±0.02; 0.96±0.05) whereas in the combined treatment (HPE+BRC+MPTP) the levels were improved markedly (p<0.05) when compared with BRC+MPTP, HPE+MPTP-treated groups (HPE+BRC +MPTP; dopamine: 7.88±0.19; DOPAC: 1.75±0.01; HVA: 1.25±0.01) (Fig. 4).

3.5. Antioxidants and lipid peroxidation

Striatal antioxidant level of SOD and catalase decreased significantly by 48% and 67%, respectively; GPx and GSH level were also significantly decreased (p<0.05) in the MPTP-treated group when compared with control. Catalase and SOD activities restored to near normal by BRC and HPE combined treatment (Table 1). Lipid peroxidation increased in the MPTP-treated group when compared with control (91%). HPE and BRC combined treatment lowered lipid peroxidation to a significant level (p<0.05) (Fig. 5).

4. Discussion

The rotarod test, which requires animal to balance and walk on a rotating cylinder, is widely used to test and measure the co-ordinated motor skills [25]. In the present study, the rotarod, hang test and forepaw stride length performances significantly reduced in the MPTP group when compared with control. Treatment with BRC (i.p) combination of HPE (po) showed marked improvement in behavioral performances in comparison with MPTP group. BRC reversed MPTP-induced akinesia, catalepsy hind limb abduction and long lasting motor impairment as reported earlier [26]. Earlier study has shown that HPE inhibited the scopolamine-induced deficit of passive avoidance retention in rats and mice [12].

The dopamine level was significantly reduced in MPTP treated animals. MPTP destroys DA containing neurons in the substantia nigra resulting in severe depletion of DA in the nerve terminals region, nucleus caudate putamen (NCP) following systemic administration in mice [27]. In the BRC-treated group, striatal dopamine status significantly improved.

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase (μmol of H2O₂ utilized/min/mg of protein)</th>
<th>SOD (U/ mg of protein)</th>
<th>GPx (μmol of GSH consumed/min/mg of protein)</th>
<th>GSH (μmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.58±0.01</td>
<td>3.83±0.22</td>
<td>12.21±0.17</td>
<td>0.62±0.01</td>
</tr>
<tr>
<td>HPE</td>
<td>0.63±0.01</td>
<td>3.75±0.23</td>
<td>12.85±0.03</td>
<td>0.57±0.01</td>
</tr>
<tr>
<td>BRC</td>
<td>0.61±0.01</td>
<td>3.81±0.02</td>
<td>12.65±0.07</td>
<td>0.59±0.01</td>
</tr>
<tr>
<td>MPTP</td>
<td>0.19±0.01*</td>
<td>1.97±0.12*</td>
<td>8.6±0.02*</td>
<td>0.28±0.02*</td>
</tr>
<tr>
<td>HPE+MPTP</td>
<td>0.44±0.01*</td>
<td>3.38±0.26*</td>
<td>10.57±0.04*</td>
<td>0.49±0.01*</td>
</tr>
<tr>
<td>BRC+MPTP</td>
<td>0.41±0.02*</td>
<td>3.0±0.05*</td>
<td>10.26±0.03*</td>
<td>0.45±0.01*</td>
</tr>
<tr>
<td>HPE+BRC+MPTP</td>
<td>0.57±0.02*</td>
<td>3.51±0.08*</td>
<td>12.0±0.07*</td>
<td>0.53±0.02*</td>
</tr>
</tbody>
</table>

Values expressed as mean±SEM (n=6).
U*, enzyme concentration required to inhibit the NBT 50%.
*, significantly different from control group (p<0.05).
†, significantly different from MPTP group (p<0.05).
ANOVA followed by Scheffe’s ‘F’ test.
Chronic dietary supplementation of BRC has also been reported to protect nigral neuronal integrity in aged rats by supporting the turn over of DA through a presynaptic D2 receptor mechanism [28]. HPE also improved the dopamine level. HPE as well as hyperforin treatment has been shown to modulate neurotransmitter levels in the brain of rodents [29].

Antioxidant enzymes SOD and catalase were significantly decreased in MPTP treated animals, which is an indirect evidence of excessive oxidative stress beyond its capacity and due to the depletion of GSH resulting in damage to enzymes [27]. Depletion of GSH in the MPTP-treated groups is reversed by BRC and HPE. HPE has been shown to have antioxidant and antidepressant with memory enhancing properties [12]. It is evident that BRC and HPE might render significant antioxidant protection against MPTP-induced neurotoxicity when compared with either alone.

5. Conclusion

In the present study, behavioral results clearly show that mice treated with MPTP could develop a variety of behavioral deficits such as loss of motor co-ordination, neuromuscular strength and inability (or) lack of initiative of the animals to return to preinjection behavior. The prior administration of both Hypericum perforatum alcoholic extract and BRC given alone clearly prevented the behavioral deficits and biochemical alterations. The combined treatment has shown better effects when compared with either alone. So it is concluded that the combination of BRC and HPE has enhanced neuroprotective effect and may be useful in effective clinical management of Parkinson’s disease.

References
