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Pre-treatment with silymarin reduces brain myeloperoxidase activity and inflammatory cytokines in 6-OHDA hemi-parkinsonian rats

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HIGHLIGHTS

- Intra-SNc injection of 6-OHDA induce catalepsy in rats.
- Silymarin prevented from 6-OHDA induce catalepsy.
- Brain MPO activity and CSF levels of TNF-α and IL-6 increased in hemi-parkinsonian rats.
- Silymarin pre-treatment suppressed brain MPO activity and reduced CSF concentration of inflammatory cytokines in hemi-parkinsonian rats.

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ABSTRACT

Most chronic neurodegenerative diseases such as Parkinson's disease (PD) are accompanied by neuroinflammation which is associated with glial cells activation and production of different inflammatory cytokines. In the present study we evaluated the anti-cataleptic effect of silymarin pre-treatment in 6-hydroxydopamine (6-OHDA)-lesioned rats, striatum myeloperoxidase (MPO) activity and cerebrospinal fluid (CSF) levels of inflammatory cytokines. Male Wistar rats were pre-treated with intraperitoneal (i.p.) injections of silymarin (100, 200 and 300 mg/kg) for 5 consecutive days. Then, catalepsy was induced by unilateral infusion of 6-OHDA (8 μg/2 μl/rat) into the central region of the SNc. The anti-cataleptic effect of silymarin was assessed by the bar test 3-weeks after neurotoxin injection. Striatal myeloperoxidase activity and CSF levels of TNF-α and IL-6 were assessed at the end of behavioral experiments. Our data demonstrated that silymarin pre-treatment decreased catalepsy. The most anti-cataleptic effect was observed at the dose of 300 mg/kg of silymarin ($p < 0.001$). There was a significant ($p < 0.001$) increase in MPO activity of 6-OHDA-lesioned rats whereas; in silymarin (in all 3 doses, i.p. for 5 days) pre-treated hemi-parkinsonian rats' MPO activity was decreased markedly ($p < 0.001$). Furthermore the CSF levels of TNF-α and IL-6 were decreased ($p < 0.001$) in silymarin (100, 200 and 300 mg/kg) pre-treated rats up to the range of normal non-parkinsonian animals. From these results, it may be concluded that pre-treatment with silymarin attenuates 6-OHDA-induced catalepsy by decreasing striatal MPO activity and restores CSF concentration of inflammatory cytokines, TNF-α and IL-6 to the levels of normal non-parkinsonian rats.

1. Introduction

Parkinson's disease (PD) is the second most common and progressive neurodegenerative disease characterized by progressive deficits in motor function secondary to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). Its cardinal features include resting tremor, bradykinesia, akinesia, rigidity, and postural instability [38]. The primary cause of PD is still unknown although aging appears to be a major risk factor. Epidemiological findings suggest that inflammation may has part in the pathogenesis of PD, so that, chronic use of some anti-inflammatory drugs may reduce the risk of developing PD [31]. McGeer et al., in 1988 were the first to report microglia activation in the SNc of PD patients brain, which was the first document for involving of neuroinflammation in PD [17]. It appears that cytokines act as messengers between the immune system and the brain. They not only play a role in the neurodegeneration but also their signaling mechanisms seem to involve a balance between advancing cell survival, apoptosis, and pro-inflammatory responses [32].

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Microglia are macrophage-like cells in the CNS which have important roles in the development and preservation of the neural environment [15]. It has been found that activated microglia increases in the striatum and the substantia nigra of patients suffering from PD [17]. Several studies have demonstrated that microglial activation cause to the loss of dopaminergic neurons (DA-neurons) in PD patients [3,7,13]. A main origin of cytokines and oxidizing radicals is activated microglia [8]. Myeloperoxidase (MPO) is a key enzyme in the generation of oxidizing species in activated microglia [8]. MPO expression by resident microglia in normal brain is negligible but its upregulation has been reported in the midbrains of PD patients and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice [6]. Additionally, levels of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1beta (IL-1β) and IL-6 that expressed by glial cells, are elevated markedly in the serum, brain and cerebrospinal fluid (CSF) of patients with PD [5,17,19]. Drugs that act through the dopaminergic system are the routine treatments for PD; however, modulation of neuroinflammation is important in order to modify disease progression.

Silymarin, is a polyphenolic flavonoid derived from the seeds and fruits of the milk thistle plant (*Silybum marianum*), routinely used to treat liver diseases and is known to own antioxidative [35], anti-apoptotic properties [16], anti-inflammatory [10], neuroprotective effects [1] and also has been demonstrated to decrease lipid peroxidation [4]. Furthermore, it has been founded that its anti-oxidative activity is related to the scavenging of free radicals [21]. Silymarin could also protect dopaminergic neurons from neurotoxicity induced by lipopolysaccharide, by inhibiting microglia activation and reducing production of inflammatory mediators [37]. Current study was carried out in attention to protective effects of silymarin in several experimental models of neuronal injury. In particular, silymarin protects neurons from oxidative stress associated damages in focal cerebral ischemia in rats [28], protect the spinal cord against lipopolysaccharide induced neuroinflammation [36] and prevent from cerebral ischemia–reperfusion-induced brain injury in rats [12]. However, less information is available about its effect on catalepsy and inflammatory parameters associated with Parkinson disease. Considering all these previous findings we attempted to investigate anti-cataleptic effect of pre-treatment with silymarin in 6-OHDA-lesioned rats and striatal MPO activity as well as CSF levels of TNF-α and IL-6.

2. Materials and methods

2.1. Animals

Study was carried out on male Wistar rats (200 ± 20 g). The animals were given food and water ad libitum and were housed in standard polypropylene cages, four per cage at a controlled ambient temperature of 25 ± 2 °C under a 12-h light/12-h dark cycle. Animals were acclimated to the testing conditions for 2 days before the behavioral investigations were conducted. Procedures were carried out in accordance with the ethical guidelines for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz, Iran (National Institutes of Health Publication No. 85-23, revised 1985).

2.2. Chemicals

All chemicals were purchased from Sigma Chemical Co. (USA). Solutions were made freshly on the days of experimentation by dissolving drugs in physiological saline (0.9% NaCl) except for silymarin which was dissolved in 50% polyethylene glycol (PEG). The drugs were injected intraperitoneally (i.p.) except for 6-hydroxydopamine (6-OHDA) which was injected into substantia nigra pars compacta (SNc).

2.3. Surgical procedures

The animals were anesthetized by i.p. injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). After the rats were deeply anaesthetized (loss of corneal and toe pad reflexes), they were fixed in a stereotaxic frame (Stoelting, Wood Lane, IL, USA) in the flat position. The scalp hairs were completely shaved, swabbed with povidone iodine 10% and a central incision made to reveal skull. A 0.7 mm bar hole was drilled and 23 gauge sterile stainless steel cannula, as a guide cannula inserted for subsequent injection of 6-OHDA into SNc. The coordinates for this position were determined according to the rat brain in stereotaxic coordinates [26] anteroposterior from bregma (AP) = −5.0 mm, mediolateral from the midline (ML) = 2.1 mm and dorsoventral from the skull (DV) = −7.7 mm. Desipramine (25 mg/kg, i.p.) was injected 30 min before intra-nigral injection of 6-OHDA to avoid degeneration of noradrenergic neurons. Then 6-OHDA (8 μg/per rat in 2 μl saline with 0.2% ascorbic acid) was infused by infusion pump at the flow rate of 0.2 μl/min into the right substantia nigra. At the end of injection, guide cannula was kept for an additional 2 min and then slowly was withdrawn. Sham-operated animals were submitted to the same procedure except 2 μl vehicle of 6-OHDA (0.9% saline containing 0.2% (w/v) ascorbic acid) was infused into the SNc.

2.4. Cannula verification

For confirmation of placement of the cannula in the SNc of the brain, at the end of experiments all rats with guide cannula were euthanized by deeply anaesthetized with a high dose of ether and decapitated. The brains with the injecting tube in situ were removed and placed in a formaldehyde (10%) solution. After 1 week, the tissues were then embedded in paraffin. Then serial sections (3 μm) were cut with a microtome (Leitz, Germany), and the placement of the tip of the cannula in the SNc was microscopically controlled. Data from rats with an incorrect placement of the cannula were excluded from the analysis.

2.5. Catalepsy assay test

Catalepsy was assessed by using a standard bar test. Anterior limbs of rat gently extended on 9 cm high bar (0.9 cm in diameter) and the duration of retention of rats in this imposed posture was considered as the bar test elapsed time. The end point of catalepsy was designated to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. The cut-off time of the test was 600 s. All observations were made between 9.0 a.m. and 4.0 p.m. The animals were trained to maintain their anterior limb on the high bar for 2 consecutive days before main test. Catalepsy was assessed three weeks after neurotoxin injection in four consecutive times with 1-h interval.

2.6. CSF sampling

At the end of experiments, the anesthetized rats were mounted in a stereotaxic frame. The surface of the neck region were shaved and swabbed with ethanol (70%). The position of the animal's head was sustained downward at almost 45°. A needle (scalp vein-23) which connected to a draw syringe was put horizontally and centrally into the cisterna magna for CSF collection with no incision at this region. The colorless CSF sample was slowly drawn into the syringe in a volume of 100 μl. The CSF samples were kept frozen at