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Research Report

Escin attenuates behavioral impairments, oxidative stress and inflammation in a chronic MPTP/probenecid mouse model of Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder that results mainly due to the death of dopaminergic neurons in the substantia nigra (SN), and subsequently has an effect on one's motor function and coordination. The current investigation explored the neuroprotective potential of escin, a natural triterpene-saponin on chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid (MPTP/p) induced mouse model of PD. Administration of MPTP led to the depleted striatal dopamine content, impaired patterns of behavior, enhanced oxidative stress and diminished expression of tyrosine hydroxylase (TH), dopamine transporter (DAT) and vesicular monoamine transporter-2 (VMAT-2). The expressions of interleukin-6 and -10, glial fibrillary acidic protein (GFAP), ionized calcium-binding adaptor protein-1 (IBA-1), tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS) in SN were also enhanced. Oral treatment of escin significantly attenuated MPTP/p induced dopaminergic markers depletion, physiological abnormalities, oxidative stress and inhibit neuroinflammatory cytokine expressions in SN. The result of our study confirmed that escin mediated its protection against experimental PD through its antioxidant and anti-inflammatory properties.

1. Introduction

PD is a progressive neurodegenerative disease that facilitates dopaminergic neuronal loss in the SN resulting in striatal dopamine depletion and leads to a well known decline of motor functions. Motor impairment includes rigidity, tremor, bradykinesia and postural instability. Although abnormal oxidative stress and mitochondrial dysfunction are known to be associated with the pathological processes underlying PD, the exact etiology of PD remains unclear (Jenner, 2003). Neurotoxin based experimental models of PD have been used to demonstrate the pathogenesis and to test the potential

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neuroprotective agents. MPTP is a mitochondrial complex-1 inhibitor that causes parkinsonian features in various animal models and humans and is used as a tool to study the molecular events that lead to the degeneration of dopaminergic neurons (Jackson-Lewis et al., 2012). MPTP is metabolized into 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase-B (MAO-B) and this active metabolite MPP^+ causes the loss of dopaminergic neurons by entering into the dopaminergic neurons via DAT and inhibiting mitochondrial complex I and depleting the neuronal's ATP stores (Zang and Misra, 1993). Progressive inhibition of electron transport chain; ETC-I, also leads to the shunting of electrons through the ETC-II, which may generate the production of ROS 5–7 times more. Nigrostriatal MPTP neurotoxicity is also dependent on p53 (Trimmer et al., 1996) and nitric oxide synthase (NOS) (Przedborski et al., 1996) expressions, which forms nitric oxide (NO·) a potential precursor to the toxic peroxynitrite anion (ONOO) and hydroxyl free radical (·OH) (Beckman et al., 1993). These oxidative stress are likely to contribute to the death of vulnerable neurons.

It is reported that the brain is abnormally sensitive to oxidative damage because 20% of the total oxygen consumption in the body is solely from the brain; and it is enriched in the more easily peroxidizable fatty acids, while its anti-oxidant defences (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH) and catalase) are relatively sparse (Floyd, 1999). Within the midbrain, the SN appears to be among the most vulnerable regions primarily because it operates under a pro-oxidative state in comparison to the other parts of the brain, even in healthy individuals. Specifically, the SN has a high metabolic rate combined with a high content of oxidizable species, including DA, DA-derived ROS, neuromelanin, polyunsaturated fatty acids, iron and a low content of antioxidants (glutathione in particular) – all of which render this region of the brain to be highly vulnerable to the effects of peroxynitrite and sulfite (Marshall et al., 1999); when combined with the high levels of ascorbate in the brain, the iron/ascorbate mixture is a potent pro-oxidant for brain membranes (Floyd, 1999).

Neurotoxic mechanism of MPTP involves the generation of free radicals which thereby elicits the oxidative stress dependent neuroinflammatory cascade, and activates the glial cells especially microglia (Halliwell, 1992; Jackson-Lewis and Smeyne, 2005). Activated microglia stimulates the production of proinflammatory mediators such as cytokines, transcription factors which in turn further regulates the genes required for the expression of COX and iNOS (Yokoyama et al., 2008). All these factors drive the dopaminergic neurotoxicity, which results in a vicious, progressive and self-propelling cycle (Halliwell, 1992; Murray et al., 2003). Furthermore, MPP^+ can be packaged into vesicles by the efficient VMAT-2, thereby displacing vesicular DA into the cytoplasm (Liu et al., 1992).

At present, common pharmacological medication provide some relief to the symptoms of PD but fail to abrogate the disease development. Unfortunately, efforts to find effective agents that provide protection against this neurodegeneration have been rather unsuccessful. Phytochemicals such as alkaloids, terpenes, flavonoids, isoflavones, saponins *etc.* are known to exhibit protective activity against many neurological diseases (H. Li et al., 2013; X. Li et al., 2013). A number of reports have indicated that the Horse chestnut (*Aesculus hippocastanum*) possesses therapeutic properties for complaints ranging from anti-fever to anti-haemorrhoidal actions (Sirtori, 2001). Lorenz and Marek (1960) concluded that the anti-oedemigenous, anti-exudative and vasoprotective activities of horse chestnut are exclusively due to escin. This triterpene has been described to exhibit antioxidant (Xin et al., 2011), anticancer (Patlolla et al., 2006) and anti-inflammatory (Matsuda et al., 1997) properties. Wang et al. (2012) demonstrated that escin can attenuate acute omethoate induced cerebral edema by ameliorating the permeability of the blood–brain barrier. Zhang et al. (2010) indicated the neuroprotective property of escin through its anti-inflammatory function in the hippocampal neurons of mice. It also suppressed traumatic brain injury (Xiao and Wei, 2005) and inhibited brain ischemia injury in rats by its anti-apoptotic property (Hu and Zeng, 2004).

In our previous manuscript (Anandhan et al., 2012a, 2012b, 2012c), we have reported that the theaflavin a potent antioxidant offered neuroprotective effect, by means of bringing back the relative normal level of physiological impairments, expression of dopaminergic markers and caspases. Furthermore, findings have shown that oxidative stress, mitochondrial dysfunction and neuroinflammation might be involved more in the pathogenesis of PD along with the dopaminergic neurodegeneration (Monahan et al., 2008) and attenuation of neuroinflammation and oxidative stress might be the prime target to halt the progression of the disease. In this study, the neuroprotective effect of escin, a potent antioxidant and anti-inflammatory agent is evaluated by analyzing various behavioral changes, protein expression studies of dopaminergic markers that involved in DA metabolism, uptake and release, oxidative stress and inflammation related indices against MPTP/p induced dopaminergic neurodegeneration. So this study has dealt with the combined role of oxidative stress mediated neuroinflammation and behavioral impairments in MPTP/p induced model of PD as target for neuroprotection of escin.

2. Results

2.1. *Escin attenuates the MPTP/p induced dopamine depletion*

Mice that received chronic MPTP/p injection with an interval of 3.5 days for 35 days showed a substantial diminution of DA in ST tissue (Table 1). Pre-oral treatment of escin dose did enhance the levels of striatal DA in MPTP/p treated PD mice when compared to the escin untreated PD mice. However there is no significant difference in the levels of DA between 4 and 8 mg/kg escin treated PD mice. So 4 mg/kg of escin was chosen as the effective dose and further experiments were carried out. No notable changes were observed between escin alone treated and control groups ($p<0.05$).

2.2. *Escin improved the MPTP/p induced behavior abnormalities*

Chronic treatment of MPTP/p induced motor impairment was measured by means of retention time in rotating rod, swimming ability in swim test, motor coordination and