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Research report

Decreased Mcl-1 protein level in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice

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ABSTRACT

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a well-known neurotoxicant that can selectively destroy dopaminergic neurons and MPTP-treated animals are often used as models for studying aspects of Parkinson's disease (PD). While apoptosis has been suggested as a possible mechanism underlying MPTP-induced cell death and several apoptosis-associated proteins have been implicated in MPTP-animal models, relevant information regarding the possible involvement of Mcl-1 (myeloid cell leukemia 1) protein is missing. Mcl-l is an important member of the Bcl-2 family that is thought to be a highly regulated controller of cell death and survival. However, the expression level of Mcl-1 in response to MPTP-treatment has not been examined in any area of the brain previously. In the present study, an acute MPTP treatment regimen was utilized with male C57BL/6 mice (10 mg/kg i.p. injections, 4 times with 2 h intervals) and several protein markers were examined 24-h after the initial injection. Dramatic decreases in the immunoreactivities of tyrosine hydroxylase and dopamine transporters were observed. Western-blot analysis and immunocytochemical labeling demonstrated an MPTP-induced decrease in Mcl-1 protein levels in the striatum. In addition, the two proteins BAX and ERK, both of which are also involved in apoptosis signaling, were examined. While the total BAX levels showed no significant difference between the control and MPTP-treated groups, levels of phosphorylated ERK were significantly increased following MPTP-treatment. Since Mcl-1 is an anti-apoptotic protein, down-regulation of Mcl-1 following MPTP-treatment would be expected to lead to increased apoptotic activities processes, leading to increased neurodegeneration.

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1. Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent neurotoxicant that can selectively destroy dopaminergic neurons in the brain (Przedborski et al. 2000). When humans or animals are exposed to MPTP, clinical symptoms can be produced that closely resemble those of Parkinson's disease (Langston et al. 1983; Jenner and Marsden 1986; Markey and Schmuff 1986). The step-by-step biochemical reactions and the mode of action of MPTP have been thoroughly studied and established (Przedborski et al. 2000). MPTP is lipophilic and can easily cross the blood-brain barrier (BBB). After crossing the BBB, MPTP is oxidized by monoamine oxidase (MAO) to the intermediate product 1-methy l-4-phenyl-1,2-dihydropyridine ion ($MPDP^+$). $MPDP^+$ is then further oxidized to the toxic metabolite 1-methyl-4-phenyl-pyridinium ion (MPP^+). MPP^+ is structurally similar to the neurotransmitter dopamine and because of that, MPP^+ can be taken up by dopaminergic terminals through high-affinity binding to the dopamine transporter (DAT) expressed on dopaminergic terminals. The selective uptake of MPP^+ will result in accumulation of MPP^+ in dopaminergic neurons (Przedborski et al. 2000). MPP^+ then enters mitochondria and suppresses the activity of complex I (also known as NADH coenzyme Q reductase) in the inner mitochondrial membrane. As a consequence, oxidative phosphorylation in the mitochondria is inhibited, resulting in a rapid reduction in ATP synthesis. Insufficient synthesis of ATP leads to a loss of mitochondrial membrane potential and an increase in the production of reactive oxygen species (ROS), which can cause cell damage and ultimately cell death.

Over the past decades, MPTP-treated animal models of Parkinson's disease (PD) have been utilized in attempts to better understand the disorder and develop treatment options (Blum et al. 2001; Speciale 2002; Eberhardt and Schulz 2003; Blesa and Przedborski 2014). PD is the second most common chronic neurodegenerative disorder of the elderly, affecting about 1% of all

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people over age 60. Its symptoms include slow movements (bradykinesia), gait freezing (akinesia), limb stiffness (rigidity), and tremors of the hands and jaw. Many PD patients also suffer from accompanying symptoms such as autonomic, cognitive, and psychiatric disturbances. At the cellular level, loss of dopaminergic neurons has been attributed to be the underlying etiology of PD. This hypothesis is supported by several facts. First, there is a correlation between the degree of dopamine depletion and the severity of motor symptoms. Also, destruction of the nigrostriatal dopamine pathway or blockade of striatal dopamine receptors in either experimental animals or humans causes motor deficits similar to those seen in PD. In addition, an increase in dopamine availability or stimulating dopamine receptors reduces symptoms in PD patients (Alexander 2004). Besides abnormalities in dopaminergic transmission, significant reductions in norepinephrine, serotonin, acetylcholine and a variety of neuropeptides have also been found in PD patients (Alexander 2004). Current therapies by means of pharmacology, *e.g.* L-dopa (Connolly and Lang 2014), electrophysiology, *e.g.* deep brain stimulation (Sharma et al. 2012), and surgery, *e.g.* transplantation (Duker and Espay 2013) can alleviate some of the symptoms of PD, but there is currently no cure or prevention of the progressive course of the disease. Therefore, further elucidation of the underlying mechanisms of MPTP-induced dopaminergic toxicity may provide not only a better understanding of MPTP-induced neurotoxicity but also avenues for the development of novel treatment options.

At the molecular level, several apoptosis-associated proteins appear to be involved in MPTP-induced cell death. These include but are not limited to: B-cell lymphoma 2 (Bcl-2) (Yang et al. 1998; Vila et al. 2001); B-cell lymphoma extra-large (Bcl-xl) (Dietz et al. 2008); Bcl2-associated X (BAX) (Hassouna et al. 1996; Vila et al. 2001; Nicotra and Parvez 2002); BH3 interacting-domain death agonist (BID) (Viswanath et al. 2001); Bcl2-interacting mediator of cell death (BIM) (Wang et al. 2016); caspase 3 (Turmel et al. 2001); caspase 8 (Viswanath et al. 2001); caspase 9 (Viswanath et al. 2001); caspase 11 (Furuya et al. 2004); cytochrome c (Viswanath et al. 2001); and p53 upregulated modulator of apoptosis (PUMA) (Bernstein and O'Malley 2013). Among these signaling proteins, BAX appears to play a central role, because BAX can form pores in the mitochondrial membrane and permit the release of cytochrome c from mitochondria into cytosol, which in turn can activate down-stream caspases. BAX function can be modulated by Bcl-2 family members including the above mentioned Bcl2, Bcl-xl, BID, BIM, and PUMA. However, an important piece missing in the MPTP-model of cell death is Mcl-1 (myeloid cell leukemia 1), which plays an inhibitory role in controlling BAX function. Therefore, Mcl-1 is thought to be a highly regulated controller of cell death and survival (Michels et al. 2005; Yang-Yen 2006). In theory, a down-regulation of Mcl-1 could occur following MPTP-treatment which would consequently result in an increase in apoptosis. To test this hypothesis, an acute mouse model employing an acute MPTP-treatment regimen was utilized in the present study: the available evidence shows that dopaminergic toxicity and degeneration are well developed within 24 h in this model (Ali et al. 1993; Ago et al. 2011; Breckenridge et al. 2013; Sarkar et al. 2014).

It is well established that the MPTP-induced neurotoxicity is mediated through its active metabolite MPP^+, which, due to its structural similarity to dopamine, can be taken up by dopamine transporters which are predominantly located on presynaptic dopaminergic terminals. Because striatum, not substantia nigra, contains the most abundant dopaminergic terminals in the brain, the most profound action of MPTP-mediated neurotoxicity should occur in the striatum rather than substantia nigra. In fact, available evidence suggest that the primary site of injury in both PD and the MPTP model is the dopaminergic terminals in the striatum (Bradbury et al. 1986; Herkenham et al. 1991; Nurmi et al. 2001; Rinne et al. 2001), because neural damages such as reductions of levels of dopamine and dopamine transporters or disturbance of gene expression patterns following MPTP-treatment were much greater in the striatum than substantia nigra (Serra et al. 2002; Kühn et al. 2003; Meissner et al. 2003; Jackson-Lewis and Przedborski 2007; Ohnuki et al. 2010) and because dopaminergic terminal loss in the striatum preceded dopaminergic cell death in the substantia nigra (Herkenham et al. 1991; Serra et al. 2002; Li et al. 2009), which all suggesting retrograde degeneration of dopaminergic neurons from axons to somata (Bradbury et al. 1986; Eberling et al. 1997). Therefore, the present study was focused on the striatum and levels of Mcl-1 protein in the striatum were compared between the control and MPTP-treated groups.

2. Results

To demonstrate that the MPTP-treatment used in the present experiments was effective in causing degeneration of dopaminergic terminals, thus impairing dopaminergic transmission, TH and DAT in the striatum and substantia nigra were labeled immunocytochemically. Analyses showed that there were decreases in TH- and DAT-immunoreactivities in the striatum (Fig. 1) as well as in the substantia nigra (Fig. 2) of MPTP-treated animals compared to those in control animals, indicating effective MPTP-treatment.

To further demonstrate MPTP-induced neurotoxicity and neuronal degeneration, Fluoro-Jade C (FJ-C) labeling (Schmued et al. 2005; Sarkar and Schmued 2011; Gu et al. 2012) was performed and showed numerous FJ-C positive neurons in the substantia nigra of MPTP-treated animals with no such labeling in the control animals (Fig. 3).

Mcl-1 levels in the striatum of MPTP-treated and control animals were determined using Simon™ automated Western-blotting analyses. The results show decreased levels of Mcl-1 protein in MPTP-treated animals (Fig. 4A). The average decrease of Mcl-1 levels was 37.2% compared to those of the controls set as 100% (Fig. 4B). Statistical analyses of signal intensities revealed that the decrease in Mcl-1 protein levels following MPTP-treatment was significant at $p < .05$ level (Fig. 4). In addition,

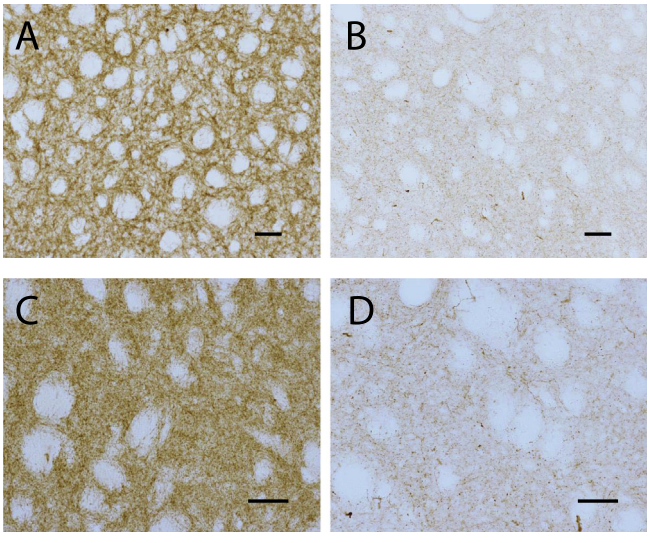

Fig. 1. Immuno-labeling of two dopaminergic markers, tyrosine hydroxylase (TH) and dopamine transporter (DAT), in mouse striatum demonstrate decreased protein levels following MPTP-treatment. Sections in Panels A and B represent TH-labeling, while those in C and D represent DAT-labeling. Tissue sections in A and C were obtained from saline-treated animals, while tissue sections in B and D were obtained from MPTP-treated animals. Scale bars = 50 μm.