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Research Report

Salidroside protects against MPP$^+$-induced apoptosis in PC12 cells by inhibiting the NO pathway

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ABSTRACT

Oxidative stress plays an important role in Parkinson's disease and other neurodegenerative disorders. Salidroside, a phenylpropanoid glycoside isolated from *Rhodiola rosea L.*, has potent antioxidant properties. In the present study, we investigated the protective activity of salidroside against 1-methyl-4-phenylpyridinium (MPP$^+$)-induced apoptosis in PC12 cells. We found that incubation of PC12 cells with salidroside prior to MPP$^+$ exposure significantly reduced cell apoptosis and attenuated collapse of the mitochondrial membrane potential (MMP). Furthermore, salidroside inhibited the MPP$^+$-induced nitric oxide (NO) increase and overexpression of nNOS and iNOS and suppressed accumulation of reactive oxygen species (ROS) and intracellular free Ca^{2+}. Our results show that the protective effects of salidroside on PC12 cells are mediated, at least in part, by inhibition of the NO pathway.

1. Introduction

Parkinson's disease (PD) is an old-age neurodegenerative disease, affecting more than 1% of humans over 60 years of age (De Lau and Breteler, 2006; Navarro et al., 2009). The pathological hallmark of PD is the presence of Lewy bodies (LBs), which are mainly composed of α-synuclein, in the affected neurons (Nagao and Hayashi, 2009). While the trigger for this relatively selective neuronal vulnerability remains unknown, the cascade of degenerative events leading to cell death is beginning to be understood. The major hypotheses believed to contribute to the eventual demise of nigral dopamine-producing cells include nitric oxide (NO)-induced oxidative damage and mitochondrial dysfunction (Navarro et al., 2009; Singh and Dikshit, 2007).

MPP$^+$ (1-methyl-4-phenylpyridinium), the active metabolite of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine), is selectively taken up by dopaminergic neurons via the dopamine transporter of the plasma membrane (Chiba et al., 1984). It directly and/or indirectly inhibits mito-

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Abbreviations: DAF-FM DA, 3-Amino,4-aminomethyl-2′,7′-difluorescein; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; eNOS, endothelial cell nitric oxide synthase; EDTA, ethylenediaminotetraacetic acid; Fluo-3AM, Fluo-3 acetoxymethyl ester; iNOS, inducible nitric oxide synthase; L-NMMA, NG-methy-l-arginine acetate salt; MMP, mitochondrial membrane potential; MPP$^+$, 1-methyl-4-phenylpyridinium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; $ONOO^-$, peroxynitrite; PBS, phosphate-buffered saline; PD, Parkinson's disease; PI, propidium iodide; ROS, reactive oxygen species; 3-NT, 3-nitrotyrosine

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chondrial complex I, causing abnormal energy metabolism and increased oxidative stress. In addition, MPP^+ produces neuronal loss in substantia nigra, striatal dopamine (DA) depletion (Lee et al., 2002) and behavioral impairments in humans, primates and mice (Xu et al., 2005). It also causes a severe Parkinsonian-like syndrome with loss of dopaminergic cells. Of the sources of oxidative stress, NO plays a major role in MPTP-induced models of Parkinson's disease (Choi et al., 2002; Dehmer et al., 2000). PC12 cells, a clonal rat pheochromocytoma cell line, have many properties in common with primary sympathetic neurons and chromaffin cell cultures (Greene and Tischler, 1976) and are used primarily as a neuron model for studies of MPP^+ neurotoxicity and PD. A plethora of evidence has demonstrated that MPP^+ depletes dopamine and elicits cell apoptosis in PC12 cells (Fonck and Baudry, 2001; Xu et al., 2005).

Salidroside (p-hydroxyphenethyl-b-D-glucoside; $C_{14}H_{20}O_7$; structure is shown in Fig. 1A), which is extracted from *Rhodiola rosea L.*, has been reported to have many pharmacological effects, including antioxidative, anti-aging, anti-cancer (Diaz lanza et al., 2001; Kelly, 2001; Kucinskaite et al., 2004) and neuroprotective properties. For example, salidroside has been shown to protect against neuronal cell death induced by glutamate and hypoxia/hypoglycemia (Cao et al., 2006; Chen et al., 2008) and against mitochondrial dysfunction induced by sodium azide (Zhang et al., 2007). However, whether salidroside can provide protection against dopaminergic neuron injury induced by MPP^+ remains unknown. Clarification of the effects of salidroside on apoptosis in PC12 cells may provide new insight into its mechanism of neuroprotection.

The present study was designed to verify the potential neuroprotective effects of salidroside against MPP^+-induced apoptosis in PC12 cells and to establish whether there is a correlation between the neuroprotection of salidroside and NO activity in MPP^+ induce PC12 cell apoptosis. The fact that salidroside provides a significant neuroprotective effect, the protective effects of salidroside were not only related to the downregulation of NO, inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS), but also to restore the abnormal mitochondrial membrane potential and Ca^{2+} influx. Our result provide experimental basis for the potential use of salidroside in PD clinic.

2. Results

2.1. *Salidroside prevents MPP^+-induced apoptosis of PC12 cells*

To investigate the effect of MPP^+ on PC12 cells, we exposed the cells to a range of concentrations of MPP^+ for various periods. There was a dose- and time-dependent decrease in cell viability following MPP^+ exposure (Fig. 1B). In the group treated with 500 μM MPP^+ for 24 h, cell viability was significantly decreased (56±1.8%) as compared with the control group

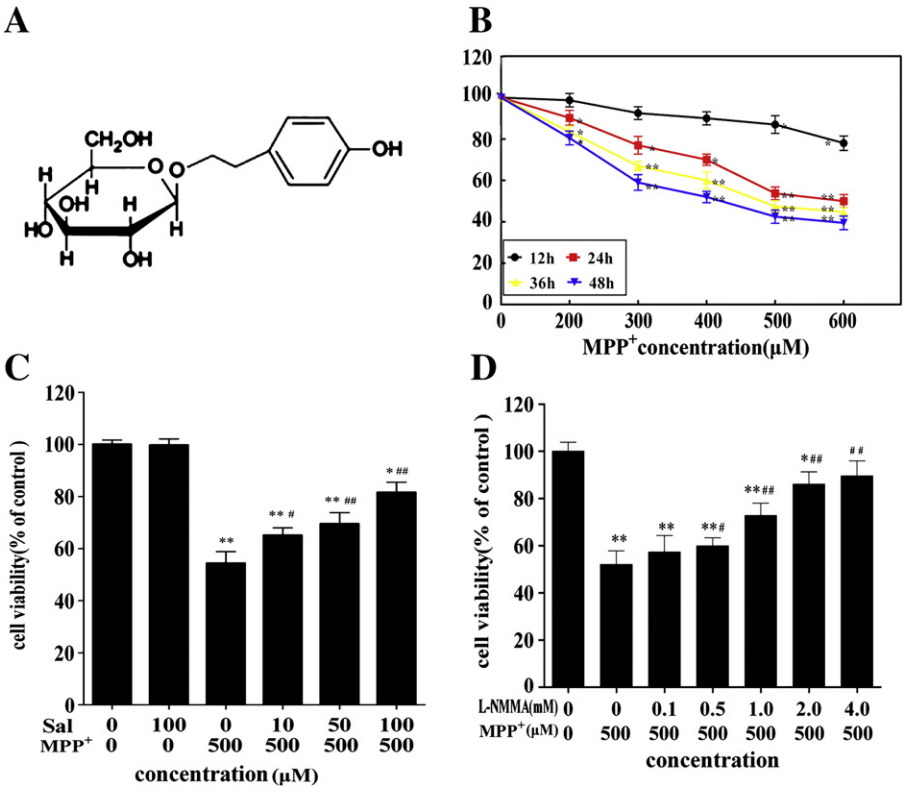

Fig. 1 – Protective effect of salidroside on MPP^+-induced cytotoxicity in PC12 cells. A: Chemical structure of salidroside. B: Dose- and time-dependent neurotoxicity of MPP^+ in PC12 cells by MTT assay. C: Effect of salidroside on MPP^+-induced neurotoxicity in PC12 cells. D: Effect of L-NMMA on MPP^+-induced neurotoxicity in PC12 cells. ($^*P<0.05$, $^{**}P<0.01$ compared with untreated control cells, $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with MPP^+-treated cells).