Toxicology Letters 243 (2016) 7–21

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Ginsenoside Rg1 attenuates motor impairment and neuroinflammation in the MPTP-probenecid-induced parkinsonism mouse model by targeting α-synuclein abnormalities in the substantia nigra

Yang Heng[a], Qiu-Shuang Zhang[a], Zheng Mu[a], Jin-Feng Hu[a], Yu-He Yuan[a,*], Nai-Hong Chen[a,b,*]

[a] State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica & Neuroscience Center, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
[b] College of Pharmacy, Hunan University of Chinese Medicine, Changsha, Hunan 410208, China

HIGHLIGHTS

- Rg1 can improve the survival rates on MPTP/p mouse model.
- Rg1 can prevent neurodegeneration induced by MPTP through attenuating glial activation and pro-inflammatory cytokine release in the SNpc.
- We firstly used 5G4 antibody on mouse to detect disease related α-synuclein.
- The neuroprotective effects of Rg1 against MPTP toxicity are probably mediated by attenuating α-synuclein abnormalities in the SNpc.

ARTICLE INFO

Article history:
Received 5 November 2015
Received in revised form 14 December 2015
Accepted 21 December 2015
Available online 23 December 2015

Keywords:
Parkinson'
s disease
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Neuroinflammation
α-Synuclein
Ginsenoside Rg1

ABSTRACT

Parkinson's disease (PD) is pathologically characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the accumulation of aggregated α-synuclein in specific central nervous system (CNS) regions. Disease development is attributed to α-synuclein abnormalities, particularly aggregation and phosphorylation. The ginsenoside Rg1, an active component of ginseng, possesses neuroprotective and anti-inflammatory effects. The purpose of the present study was to evaluate these activities of Rg1 in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)/probenecid (MPTP/p)-induced PD mouse model for the first time and to elucidate the underlying mechanisms.

Oral treatment with Rg1 significantly attenuated the high MPTP-induced mortality, behavior defects, loss of dopamine neurons and abnormal ultrastructure changes in the SNpc. Other assays indicated that the protective effect of Rg1 may be mediated by its anti-neuroinflammatory properties. Rg1 regulated MPTP-induced reactive astrocytes and microglia and decreased the release of cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the SNpc. Rg1 also alleviated the unusual MPTP-induced increase in oligomeric, phosphorylated and disease-related α-synuclein in the SNpc. In conclusion, Rg1 protects dopaminergic neurons, most likely by reducing aberrant α-synuclein-mediated neuroinflammation, and holds promise for PD therapeutics.

1. Introduction

Parkinson's disease (PD), the second most prevalent neurodegenerative disease after Alzheimer's disease (AD), is pathologically characterized by the progressive loss of nigral dopamine neurons and the presence of intraneuronal proteinaceous inclusions known as Lewy bodies (LBs) and Lewy neurites (LNs) (Schapira, 2011). PD is classified among the family of α-synucleinopathies largely

* Corresponding authors at: Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, I Xiannongtan Street, Xicheng District, Beijing 100050, China. Fax: +86 10 63165177.
E-mail addresses: yuanyuhe@imm.ac.cn (Y.-H. Yuan), chennh@imm.ac.cn (N.-H. Chen).

http://dx.doi.org/10.1016/j.toxlet.2015.12.005
0378-4274/

because misfolded α-synuclein is the main component of the LBs and LNs (Angot et al., 2010). Although the factors that initiate the misfolding of α-synuclein and which forms of α-synuclein are most toxic remain to be established (Dehay et al., 2015; Verma et al., 2015), α-synuclein is presumably present in different conformations and oligomeric states in a dynamic equilibrium regulated by factors that either accelerate or inhibit its aggregation (Vekrellis et al., 2011). Disruption of this balance may be responsible for the transformation of α-synuclein into a toxic or disease-related form.

Oligomeric α-synuclein is the most studied toxic form of α-synuclein and affects various aspects of the physiological functions of the cell, including membrane disruption, mitochondrial dysfunction, cytoskeletal changes, and proteasome impairment (Roberts and Brown, 2015; Winner et al., 2011). To investigate disease-related α-synuclein aggregation, Lachmann produced an antibody with specific reactivity against aggregated human α-synuclein with superior immunohistochemical properties (Kovacs et al., 2012). Based on the epitope this antibody recognizes, we proposed that this antibody may also function in mice. Post-translational modifications (PTMs) of α-synuclein are also thought to play an essential role in the progression of neurodegenerative diseases, including PD (Tenreiro et al., 2014). Phosphorylation at Ser129 is the most studied PTM and even serves as a potential biomarker for PD (Foulds et al., 2011). Under normal physiological conditions in vivo, only 4% of soluble, monomeric α-synuclein is phosphorylated, whereas approximately 90% is phosphorylated in LBs, suggesting a close relationship between α-synuclein phosphorylation and disease development (Tenreiro et al., 2014).

The most remarkable recent discovery related to PD is the prion-like propagation of α-synuclein. Trials of embryonic neuronal transplants were promoted in the late 1980s for improved PD treatment. Astonishingly, PD pathology, in the form of LBs and LNs, was observed in healthy embryonic dopamine neurons implanted in the striatum (STR) of PD patients, suggesting that α-synuclein can spread from diseased tissue to young, transplanted neurons (Chauhan and Jeans, 2015; Li et al., 2008). Many researchers have subsequently confirmed the prion-like propagation of α-synuclein in vitro and in vivo (Desplats et al., 2009; Luk et al., 2012). Braak established that LBs and LNs spread throughout the nervous system in a PD stage-dependent pattern (Braak et al., 2003). Based on Braak's dual-hit hypothesis, Brundin proposed that neuroinflammation promotes the prion-like behavior of α-synuclein and that novel anti-inflammatory therapies targeting this mechanism could slow disease progression (Hawkes et al., 2007; Lema Tome et al., 2013).

Currently available therapies for PD focus only on the symptoms of the disease. Drugs including levodopa, dopamine agonists and monoamine oxidase type B (MAO-B) inhibitors such as selegiline all induce various adverse reactions in long-term therapy. Furthermore, these drugs cannot slow or stop the progression of PD (Lees et al., 2009), largely because these drugs do not target the dysfunctional molecular pathways or abnormal molecules related to PD progression. Disease-related α-synuclein is considered as a potential target for experimental therapies and drug development (Dehay et al., 2015). A molecule that targets disease-related molecules or pathways with less severe adverse effects is urgently needed (Kalia and Lang, 2015).

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is extensively used to produce experimental models of PD (Jackson-Lewis and Przedborski, 2007). Three MPTP regimens are used to establish PD mouse models: acute, subacute and chronic regimens. Compared with the acute and subacute regimens, the chronic MPTP/probenecid (MPTP/p) model is considered most appropriate for studying the pathology and mechanisms of PD (Luchtman et al., 2009; Petroske et al., 2001). The neuropathology induced by MPTP/p is extremely similar to that of PD, including a loss of nigral dopaminergic neurons, a depletion of striatal dopamine, and the development of reactive microglia and astrocytes in both the SNpc and STR (Schintu et al., 2009). In addition to inducing basic PD symptoms and neuroinflammation, MPTP administration also up-regulates α-synuclein levels in mice and monkeys in the SNpc (Forno et al., 1988; Mandel et al., 2004; Vila et al., 2000). Notably, α-synuclein inclusion bodies are only found in chronic MPTP mouse model, but not in acute or subacute models (Meredith et al., 2008).

Ginsenoside Rg1, an active component of ginseng, has neurotrophic and neuroprotective effects on dopaminergic neurons against glutamate (Radad et al., 2004), 1-methyl-4-phenylpyridinium (MPP^+) (Chen et al., 2003) and rotenone (Leung et al., 2007). In animal models, Rg1 improves motor function and protects

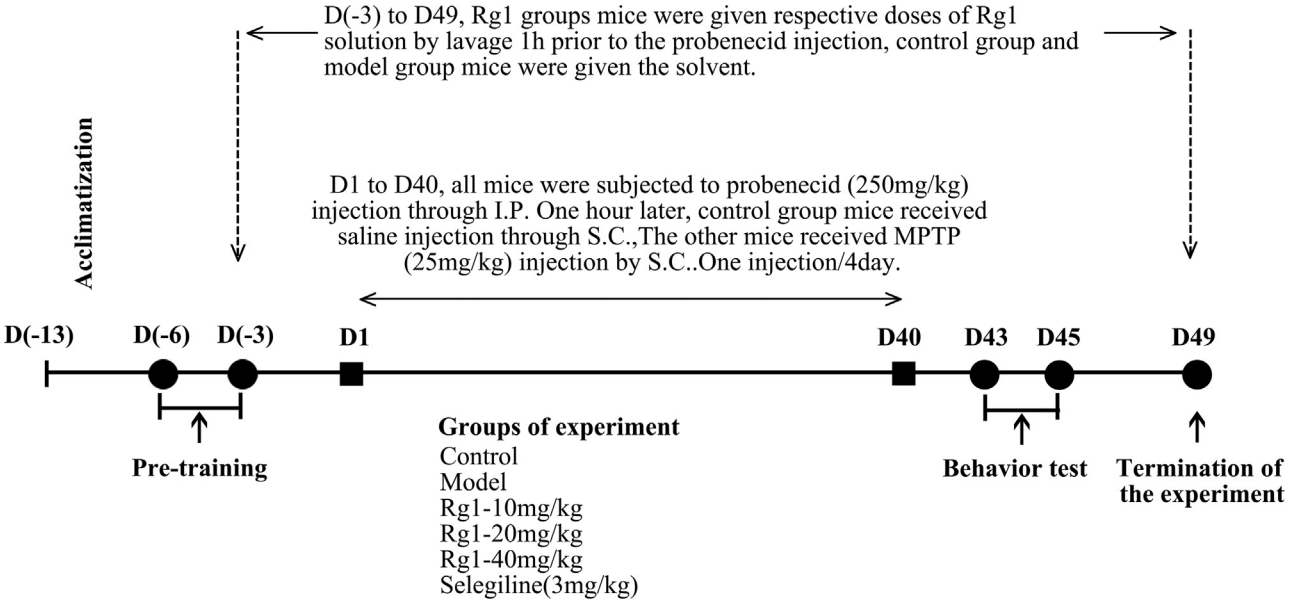

Fig. 1. Scheme of the experimental procedure.