Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Protection effect of piperine and piperlonguminine from *Piper longum* L. alkaloids against rotenone-induced neuronal injury

*Hao Wang, Jia Liu, Ge Gao, Xia Wu, Xiaomin Wang, Hui Yang**

Center of Parkinson's Disease Beijing Institute for Brain Disorders, Key Laboratory for Neurodegenerative Disease of the Ministry of Education, Department of Neurobiology Capital Medical University, Beijing 100069, China

ARTICLE INFO

Article history:
Accepted 20 July 2015
Available online 29 July 2015

Keywords:
Parkinson's disease
Mitochondrial permeability transition pore
Treatment

ABSTRACT

Currently available treatment approaches for Parkinson's disease (PD) are limited in terms of variety and efficacy. *Piper longum* L. (PLL; Piperaceae) is used in traditional medicine in Asia and the Pacific Islands, with demonstrated anti-inflammatory and antioxidant activities in preclinical studies, and alkaloid extracts of PLL have shown protective effects in PD models. The present study investigated the mechanistic basis for the observed protective effects of PLL. Rats treated with PLL-derived alkaloids showed improvement in rotenone-induced motor deficits, while reactive oxygen species (ROS) production was decreased, mitochondrial membrane potential was stabilized, and the opening of the mitochondrial permeability transition pore (mPTP)—which is involved in ROS production—was inhibited. In addition, rotenone-induced apoptosis was abrogated in the presence of these alkaloids, while a pretreatment stimulated autophagy, likely mitigating neuronal injury by the removal of damaged mitochondria. These findings provide novel insight into the neuroprotective function of PLL as well as evidence in favor of its use in PD treatment.

This article is part of a Special Issue entitled SI: Neuroprotection.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) that results in depletion of dopamine (Olanow, 2007). The pathological hallmarks of this disease have been extensively described, while the etiology remains ambiguous (Olanow and Stern, 2008).

There are currently few treatments available for PD, and most of these only alleviate the symptoms (Lees et al., 2009); thus, the development of new drugs with fewer side effects and improved biocompatibility are needed. Among possible

Abbreviations: 6-OHDA, 6-hydroxydopamine; MMP, mitochondrial membrane potential; mPTP, mitochondrial permeability transition pore; MRI, magnetic resonance imaging; PD, Parkinson's disease; PIP, piperine; PLG, piperlonguminine; PLL, *Piper longum* L. (Piperaceae); PLLE, PLL extract; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase

*Corresponding author.
E-mail address: huiyang@ccmu.edu.cn (H. Yang).

http://dx.doi.org/10.1016/j.brainres.2015.07.029
0006-8993/

preventive strategies, naturally derived compounds have been promising, for instance in enhancing mitochondrial function and reducing oxidative damage (Olanow, 2004; Schapira and Olanow, 2004); specifically, this effect has been shown by *Piper longum* L. (PLL; Piperaceae) (Kumar et al., 2011), a Piper plant species used in traditional medicine in Asia and the Pacific Islands (Shoba et al., 1998). Various protective properties of PLL have been reported, including anti-inflammatory and antioxidant activities in preclinical studies (Kumar et al., 2009; Das et al., 2012; Nabi et al., 2013). Previous reports have described the isolation of the alkaloids piperine (PIP) and piperlonguminine (PLG) from PLL extracts (PLLE), and were identified as the major constituents and active ingredients in our previous work (Liu et al., 2011) (Patent number: CN101791336A). PIP is a known inhibitor of monoamine oxidase (Al-Baghdadi et al., 2012) and has anti-apoptotic and -inflammatory activities in 6-hydroxydopamine (6-OHDA)-induced PD models (Shrivastava et al., 2013). PIP was reported to suppress the production of tumor necrosis factor-α and interleukin-6, as well as the activation of nuclear factor-κB and extracellular signal-regulated kinases 1/2 by lipopolysaccharide (Lee et al., 2013), and also inhibits the levels of amyloid-beta precursor protein and amyloid beta 40 and 42 peptides in SK-N-SH cells (Qi et al., 2009). Previous work from this laboratory suggested that PLLE could mitigate rotenone-induced PD-like symptoms, although the underlying mechanism was unknown.

Several mechanisms have been proposed in the pathogenesis of PD, including oxidative stress, mitochondrial dysfunction, impairment of the ubiquitin–proteasome system, and neuroinflammation (Olanow, 2007). Examination of postmortem brain tissue, cell culture studies, and animal models of PD as well as genetic analyses of patients have provided evidence supporting a significant role for oxidative damage and mitochondrial dysfunction in the development and progression of PD (Parker and Swerdlow, 1998; Mandemakers et al., 2007; Surendran and Rajasankar, 2010; Hauser and Hastings, 2013). Thus, therapeutic strategies that target mitochondrial components involved in the oxidative stress response could potentially be effective in the treatment of PD (Reale et al., 2012; Schapira, 2012). The opening of mitochondrial permeability transition pore (mPTP) is central to mitochondrial structure and function, including reactive oxygen species (ROS) production. A mutation in the mitochondrial gene *PTEN-induced kinase 1* leads to selective opening of the mPTP (Gautier et al., 2012), and the pharmacological agent rasagiline, which is prescribed to PD patients, exerts its effect in part through the mPTP (Youdim et al., 2005), indicating that changes in mPTP opening can be a causal factor in PD (Martin, 2010; Mashayekhi et al., 2014). These findings also suggest that the mPTP could serve as a viable drug target (Penna et al., 2013; Rao et al., 2014).

The present study examined the effects of PIP and PLG on rotenone-induced neurodegeneration in rats. These two compounds were found to inhibit apoptosis while increasing autophagic activity by blocking the opening of the mPTP. These findings might indicate that alkaloid extracts of PLL exert neuroprotective effects in a rat model of PD by directly affecting mitochondrial function.

2. Results

2.1. *Rotenone-induced motor impairment is ameliorated by PLLE*

After ipsilateral intracranial infusion of rotenone to induce SNpc injury, the time on the rotarod was decreased compared to sham-operated controls, an effect that was reversed by pretreatment with 12.5 mg/kg PLLE at 2 and 4 weeks after lesioning; In rats receiving 25 mg/kg PLLE, the effect persisted till to 6 and 8 weeks (Fig. 1A). We also included the group of saline, sham+12.5, sham+25, and DMSO group because the PIP/PLG was mainly dissolved by DMSO. The result showed there were no significant differences among groups of saline, sham+12.5, sham+25, and DMSO (Supplement 1).These results might indicate that rotenone-induced impairment of motor coordination was mitigated by treatment with PLLE. In the second week after lesioning, the midbrain of rats was examined by immunohistochemistry (ICH) for the expression of TH by dopaminergic neurons. Results showed that rotenone treatment reduced the number of TH-positive neurons compared to controls, but this decrease was less pronounced in animals pretreated with PLLE (Fig. 1B), TH-positive neurons count number analysis showed that rotenone injury induced obvious decrease of the TH-positive neurons in midbrain while pretreatment of PLLE could significantly reverse the neuron deletion of the TH-positive neurons ($P<0.01$) (Fig. 1C). At last the detection of TH protein levels by western blotting matched well with IHC result, PLLE treatment could effectively reverse the TH level in midbrain compared to rotenone injury (Fig. 1D). Taken together, results suggested that PLLE protects against the loss of dopaminergic neurons resulting from rotenone exposure.

The catwalk was used to evaluate changes in gait associated with brain injury. The footsteps of the rotenone treatment group, including the paw print area (print length and width) and rhythm (stride length and width), were decreased with respect to control rats (Fig. 1E–G), reflecting the rigidity of the muscles and decreased motor capability, which is similar to what is observed in PD. In rats pretreated with PLLE the footsteps were less indistinguishable from those of the sham-operated controls, with no differences in paw print area or rhythm. Taken together, the results might indicate that PLLE treatment can mitigate the behavioral and neuronal degeneration induced by the administration of rotenone.

2.2. *PLLE suppresses cell death induced by rotenone and stabilizes the MMP*

According the behavioral test and immunohistochemistry analysis result we further analyze the protection effect at the cellular level to pave a way for the mechanism investigation of PLLE. To examine the cellular mechanisms underlying the protective effects conferred by PLLE, PI and Hochest stain for cell death analysis was used firstly to assess the protection effect on cell level. In SK-N-SH cells, rotenone (100 nM) increased the cell death level (PI stain positive under the Hochest-stain background) compared to vehicle-treated cells. In cells pretreated with CoQ and PLLE, cell death level was suppressed to levels