Phytomedicine 25 (2017) 93–99

Contents lists available at ScienceDirect

Phytomedicine

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

Original Article

Neuroprotective properties of icariin in MPTP-induced mouse model of Parkinson's disease: Involvement of PI3K/Akt and MEK/ERK signaling pathways

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ARTICLE INFO

Article history:
Received 20 October 2015
Revised 15 December 2016
Accepted 28 December 2016

Keywords:
Icariin
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Phosphatidylinositol 3-kinase
Mitogen-activated protein kinase kinase
Dopamine
Parkinson's disease

ABSTRACT

Background: *Epimedium sagittatum* is a traditional Chinese herb normally which is used to treat the osteoporosis, cardiovascular dysfunction, and to improve neurological and sexual function in China, Korea and Japan. Icariin is the major active ingredient in *Epimedium sagittatum*. In the present research, we examined the neuroprotective effects of icariin on dopaminergic neurons and the possible mechanisms in a mouse model of Parkinson's disease (PD).

Methods: Ovariectomized PD mice were treated with vehicle or icariin (3 days before MPTP injections) with or without the phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 or mitogen-activated protein kinase kinase (MEK) inhibitor PD98059. The dopamine (DA) content in the striatum was studied by HPLC. Western blot was used to determine the protein expressions of Bcl-2, Bax and Caspase 3 in the striatum. The numbers of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the substantial nigra pars compacta (SNpc) were assessed by immunohistochemistry. The activation of Akt and ERK by icariin were detected in doparminergic MES23.5 cells.

Results: Icariin pretreatment could ameliorate the decreased striatum DA content and the loss of TH-IR neurons in the SNpc induced by MPTP. The MPTP-induced changes of Bcl-2, Bax and caspase 3 protein expressions in the striatum could be reversed by icariin pretreatment. Blockade of PI3K/Akt or MEK/ERK signaling pathway by LY294002 or PD98059 could attenuate the increase of DA content in the striatum and TH-IR in the SNpc induced by icariin in PD mice model. Additionally, icariin treatment alone significantly induced the phosphorylation of Akt and ERK in a time dependent pattern in dopaminergic MES 23.5 cells. These effects were abolished by co-treatment with LY294002 or PD98059.

Conclusion: These data demonstrated that icariin has neuroprotective effect on dopaminergic neurons in PD mice model and the potential mechanisms might be related to PI3K/Akt and MEK/ERK pathways.

Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease which is related to the dysfunctions of nigrostriatal dopaminergic systems (Dauer & Przedborski, 2003). Clinical studies support that the incidence of PD in male is higher than that in women, suggesting the potential neuroprotective effects of estrogen on nigrostriatal system (Miller & Cronin-Golomb, 2010; Smith & Dahodwala, 2014). In animal models of PD, estrogen treatment could protect against toxic insults and exert apparent neuroprotective effects on the dopaminergic neurons (Campos et al., 2012; Cordellini et al., 2011). Even though, the undesirable side effects of hormone

Abbreviations: DA, dopamine; IGF-1R, insulin-like growth factor-1 receptor; IRS-1, insulin receptor substrate-1; MAPK, mitogen activated protein kinase; MEK, mitogen-activated protein kinase kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTT, 3-[4, 5-dimethylthiazol 2-yl] 2, 5-diphenyltetrazolium bromide; PD, Parkinson's disease; PI3K, phosphatidylinositol 3-kinase; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-IR, tyrosine hydroxylase-immunoreactive.

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http://dx.doi.org/10.1016/j.phymed.2016.12.017
0944-7113/

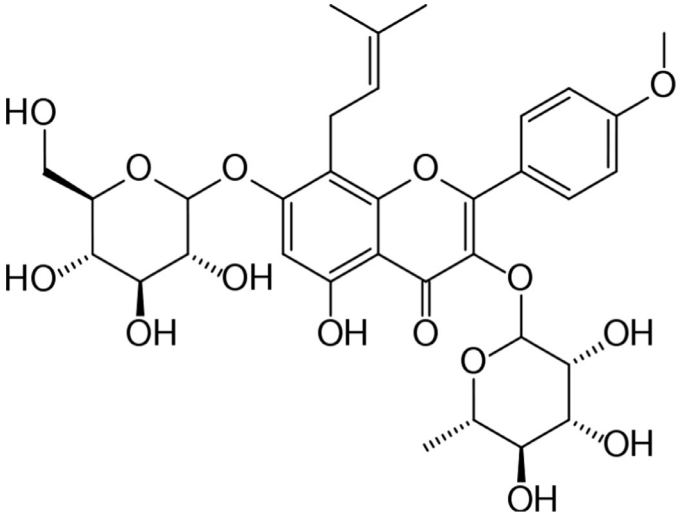

Fig. 1. Formular structure of icariin.

replacement therapy limited the use of estrogen in clinic. Many women turn to natural products due to the few side effects.

Epimedium sagittatum is a traditional Chinese herb which is commonly used to treat the kidney-yang or yin deficiency, rheumatoid arthritis, osteoporosis and cardiovascular diseases (Chan et al., 2014; Chen et al., 2011; Sze et al., 2010). Icariin, the major bioactive compound of *Epimedium sagittatum*, is considered to be a potential drug to treat major age-related diseases (Li et al., 2015). Our previous study showed that icariin could stimulate bone formation and suppress bone resorption in ovariectomy-induced osteoporosis (Mok et al., 2010). Zhu's research group indicated that icariin could protect against brain injury in experimental stroke (Zhu et al., 2010). Using the mice model of senescence-accelerated mouse prone 8 (SAMP8), researchers have demonstrated that orally administered icariin could prevent learning and memory impairment (He et al., 2010). Furthermore, several lines of evidence suggest that icariin could improve the learning and memory deficits in aging rats (Wu et al., 2012) and APP transgenic Alzheimer's disease mice (Zhang et al., 2014). However, there are no previous studies referring to the neuroprotective effects of icariin on dopaminergic neurons.

Icariin exerts its biological effects via several relevant pathways including insulin-like growth factor (IGF), mitogen activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), transforming growth factor-β (TGF-β), nitric oxide synthase (NOS) and others. The IGF signaling system plays an important role in regulating growth and development. Binding of IGFs to IGF-1 receptor (IGF-1R) will induce IGF-1R autophosphorylation, insulin receptor substrate (IRS)-1 phosphorylation, and subsequent activation of PI3-K/Akt pathway and MAPKs pathway (Valentinis & Baserga, 2001). MAPK pathway and PI3K pathway play important roles in cell proliferation, survival, differentiation and adaptation. It was reported that icariin could stimulate angiogenesis by activating the PI3K/Akt/eNOS and MEK-ERK-dependent signal pathways (Chung et al., 2008). Icariin was also shown to inhibit amyloid β-induced apoptosis in PC12 cells and corticosterone-induced apoptosis in hypothalamic neurons by the Akt pathway (Zhang et al., 2015; Zhang et al., 2012). It is unclear if the neuroprotective actions of icariin on dopaminergic neurons are mediated through PI3K/Akt and MEK/ERK signaling pathways.

In the present study, we aim to characterize the protective effects of icariin on 1- methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-damaged nigrostriatal system in the mice as well as to elucidate the mechanism of actions involved in mediating its neuroprotective effects in vivo and in vitro.

Materials and methods

Materials

Icariin was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). The purity of icariin (>98%) was determined by HPLC. Fig. 1 shows the formular structure of icariin. It was dissolved in DMSO and dilute with saline (saline containing 1% DMSO) before intragastric administration. LY294002 was purchased from cell signaling Technology Inc (Danvers, MA, USA). PD98058 was purchased from Calbiochem (La Jolla, CA, USA). Primary antibody of tyrosine hydroxylase (TH) was purchased from Millipore (Bedford, MA, USA). Primary antibodies of Bcl-2, Bax and secondary antibody were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Primary antibody of caspase-3 and monoclonal mouse anti-β-actin was supplied by Cell Signaling Technology, Inc. (Hertfordshire, England). All other chemicals were obtained from commercial sources.

Animals and treatment

10–12 week-old female C57BL/6 mice (18–22 g) were obtained from Vital River Experimental Animal Center of Beijing (Beijing, China). Ovariectomy was performed as described previously (Khajuria et al., 2012) and housed for 14 days. All surgery were performed under chloral hydrate anesthesia and conducted according to the "Guide for the Care and Use of Laboratory Animals" (NIH publications No. 80-23, revised 1996). The experiment was approved by the Animal Ethic Committee of Qingdao University.

Exp 1: dose-dependent effects of icariin in ovariectomized mice

The 30 mice were randomly divided into five groups: control, MPTP, icariin 50 mg/kg (ICA50), icariin 100 mg/kg (ICA100), icariin 200 mg/kg (ICA200). Three days after the icariin pretreatment, MPTP (15 mg/kg, i.p) injection (four times with intervals of 2 h) was performed at 2 h after the intragastric administration. Oral administration of icariin lasted 8 consecutive days. The mice were sacrificed after the behavioral test and the brains were removed for HPLC analysis, immunohistochemistry and western blot analysis.

Exp 2: the blocking effects of LY294002 and PD98059 on the protective effect of icariin in ovariectomized mice

The 42 mice were classified into seven groups: (1) Control group: mice were microinjected with 1 μl 0.1% ethanol in saline into lateral ventricle. Ten min later, saline containing 1% DMSO (10 ml/kg) were orally administrated. The treatment lasted 8 consecutive days. (2) MPTP group: Three days after the vehicle treatment as described for control group, the mice were treated with MPTP as mentioned in Exp 1. (3) Icariin + MPTP group: Same as MPTP group except that icariin (100 mg/kg) instead of vehicle by intragastric administration. (4) Icariin + LY294002+MPTP group: Same as icariin group except 1 μl LY294002 (1 μg/μl) microinjection into lateral ventricle. (5) Icariin+PD98059 + MPTP group: Same as icariin group except 1 μl PD98059 (1 μg/μl) microinjection into lateral ventricle. (6) LY294002 group: Same as control group except 1 μl LY294002 (1 μg/μl) microinjection into lateral ventricle. (7) PD98059 group: Same as control group except 1 μl PD98059 (1 μg/μl) microinjection into lateral ventricle. On Day 9 two sides of the striatum were rapidly removed and used for HPLC analysis and western blot, respectively. The substantia nigra were used for immunohistochemistry studies.

Rotarod test

Motor skill performance was evaluated on the rotarod equipment. Before icariin treatment, mice were pre-trained for three consecutive days with 2 training sessions of 2 min at 4 rpm and