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Involvement of IGF-1 receptor signaling pathway in the neuroprotective effects of Icaritin against MPP$^+$-induced toxicity in MES23.5 cells

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ABSTRACT

Icaritin, a natural derivative of Icariin, is the major bioactive component of Epimedium Genus. The present study tested the hypothesis that the neuroprotective effects of Icaritin against 1-Methyl-4-phenylpyridinium ion (MPP$^+$)-induced toxicity involved activation of the insulin-like growth factor-1 receptor (IGF-1R) signaling pathway in MES23.5 cells. Our results revealed that Icaritin pretreatment attenuated the MPP$^+$-induced decrease of cell viability in a dose-dependent fashion. Co-pretreatment with phosphatidylinositol 3-kinase (PI3-K) inhibitor LY294002, mitogen-activated protein kinase (MEK) inhibitor PD98059 or IGF-1 receptor antagonist JB-1 could completely block the protective effects of Icaritin. Moreover, Icaritin pretreatment down-regulated MPP$^+$-induced increase of Bax/Bcl-2 ratio transcriptionally and post-transcriptionally. Further study revealed that Icaritin pretreatment could restore the decreased protein expression of Akt and extracellular signal-regulated kinase 1/2 (ERK1/2) induced by MPP$^+$ and these effects could be completely abolished by LY294002, PD98059 or JB-1. Additionally, Icaritin treatment alone time-dependently enhanced the phosphorylation of Akt and ERK1/2 in MES23.5 cells. The activation of Akt and ERK1/2 by Icaritin could be completely blocked by JB-1, LY294002 or PD98059. Taken together, our data demonstrate that IGF-1 receptor mediated activation of PI3K/Akt and MEK/ERK1/2 signaling pathways are involved in the protective effects of Icaritin against MPP$^+$-induced toxicity in MES23.5 cells.

1. Introduction

Epimedium Genus, a traditional Chinese herbal medicine, has been widely used for the improvement of sexual dysfunction and neurological function, anti-osteoporosis and cardiovascular diseases for thousands of years (Chan et al., 2014; Sze et al., 2010). Icariin, isolated from Herba Epimedii, is the major active ingredient of flavonoids. 19 metabolites of Icariin could be discovered in rat plasma after oral administration of Icariin (Qian et al., 2012). Icaritin is one prenylflavonoid metabolite of Icariin (Fig. 1). Many studies have revealed the anti-cancer property of Icaritin for different type of cancers, such as prostate cancer, bladder cancer, colorectal cancer and hepatocellular carcinoma (Pan et al., 2016; Sun et al., 2015; Zhao et al., 2015, Zhou et al., 2015). For the neuroprotection, Icaritin had been reported the protective effect on amyloid β-peptide induced neurotoxicity in cortical neurons (Wang et al., 2007). Wang et al. reported that Icaritin could promote neuronal differentiation from mouse embryonic stem cells (Wang et al., 2009). Recently, it has been shown that Icaritin could protect against lipopolysaccaride-induced inflammation both in vivo and in vitro (Lai et al., 2013). However, the neuroprotective effect of Icaritin against MPP$^+$-induced toxticity on dopaminergic neurons and the possible mechanism is unclear.

Several lines of evidence indicate that Icaritin is a potent phytoestrogen, which possesses estrogenic and anti-estrogenic activity. Ye and Lou's research have shown that Icaritin could induce the cell proliferation of human breast cancer MCF-7 cells and this

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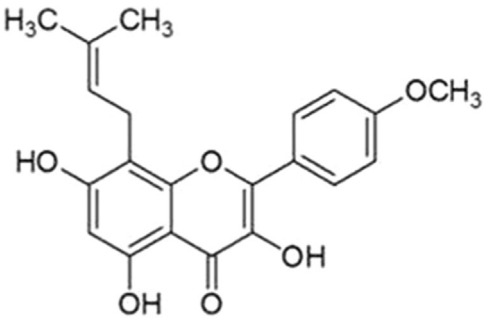

Fig. 1. Chemical structure of Icaritin.

effect could be blocked by estrogen receptor antagonist ICI182780 (Ye and Lou, 2005). Using carbon tetrachloride-induced acute liver injury model, Liu et al. have demonstrated that the anti-oxidative and anti-apoptotic effect of Icaritin was mediated by estrogen receptor (Liu et al., 2014). In contrast, several studies have demonstrated that Icaritin could provoke apoptosis of HepG2 cells and smooth muscle cells in an estrogen receptor-independent fashion (Chen et al., 2010; He et al., 2010). Mechanism study further showed that anti-apoptosis effect of Icaritin might be mediated by MEK/ERK and PI3-K/Akt (Chen et al., 2010; Li et al., 2013; Tong et al., 2011) as well as JNK1 signaling pathways (He et al., 2010). The above information indicates that the mechanism involved in the biological activities of Icaritin might be tissue and cell selectivity.

The cross-talk between IGF-1 receptor and estrogen receptor signaling pathways plays a key role in mediating the neuroprotective action of IGF-1 and estrogen (Gao et al., 2009). It has been revealed that Icariin could extend healthspan via IGF mediated pathway in C. elegans (Cai et al., 2011). Many studies have demonstrated that IGF-1 receptor mediated signaling pathway exerted protective functions on neurons against various neurotoxic damages by PI3K/Akt and MEK/ERK pathways (Bondy and Cheng, 2004; Laviola et al., 2007). In the present work, we hypothesize that the neuroprotective effects of Icaritin against MPP^+-induced toxicity in MES23.5 cells might be mediated by IGF-1 receptor signaling pathway.

2. Materials and methods

2.1. Reagents

Icaritin was purchased from Shanghai Tauto Biotech Co., Ltd (Shanghai, China). The purity of Icaritin is over 98%. Primary antibody against Akt, IGF-1 receptor and secondary antibody were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Primary antibodies against Bcl-2, pAkt, pERK1/2 and ERK1/2 were purchased from Cell Signaling Technology Inc (Danvers, MA, USA). Primary antibody against Bax and β-actin were supplied by Abcam (Cambridge, UK). LY294002 and PD98059 were purchased from Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). JB-1 was obtained from Sigma (St. Lous, MO, USA). High-Capacity cDNA Reverse Transcription Kit was from Roche (Manheim, Germany). QuantiFast™ SYBR Green PCR Kit was provided by Qiagen (Germantown, MD, USA). All other chemicals were obtained from commercial sources.

2.2. Cell culture

The MES23.5 dopaminergic neuronal cells supplied by Dr. Weidong Le (Baylor College of Medicine, Houston, USA) were routinely cultured in DMEM/F12 medium, which contains Sato's components growth medium, 5% fetal bovine serum (FBS), penicillin 100 IU/ml and streptomycin 100 μg/ml (Invitrogen, Carlsbad, CA, USA) as previously described (Ge et al., 2010). The cells were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.3. Cell viability assay

For analysis of the neuroprotective properties, MES23.5 cells were treated with Icaritin (10^{-10}–10^{-6} M) or vehicle for 24 h after which it was replaced with media containing MPP^+ (100 μM) and Icaritin for another 24 h. For antagonist treatment, LY294002 (5 μM), PD98059 (10 μM) or JB-1 (1 μg/ml) was added to each group for 1 h before Icaritin treatment. The 3-[4, 5-dimethylthiazol 2-yl] 2, 5-diphenyltetrazolium bromide (MTT) assay was employed to evaluate cell viability. Briefly, after treatment, 20 μl of tetrazolium (5 mg/ml, Sigma, St. Louis, MO, USA) in phosphate-buffered saline was added and incubated at 37 °C for 4 h. After that, 100 μl dimethyl sulfoxide was added in and the plates were shaken for 15 s. Signal detection was implemented by microplate reader at the wavelength of 595 nm.

2.4. Quantitative real time-PCR

MES23.5 cells were processed with Icaritin (10^{-7} M) or vehicle for 24 h. Subsequently, medium was replaced with which containing MPP^+ (100 μM) and Icaritin (10^{-7} M) for another 12 h. Then, total RNA was extracted by using Trizol reagent and reversely transcribed in 20 μl of a reaction mixture using the reverse transcription kit as previously implemented (Chen et al., 2013), at 60 °C for 10 min, 55 °C for 30 min and 85 °C for 5 min. Quantitative real time-PCR was performed by using a QuantiFast™ SYBR Green PCR Kit. The PCR primer sequences for Bcl-2, Bax and GAPDH were 5′-AAGCTGTCACAGAGGGGCTA-3′ (Bcl-2 forward) and 5′-CAGGCTGGAAGGAGAAGATG-3′ (Bcl-2 reverse), 5′-TCTGACGGCAACTTCAACTG-3′ (Bax forward) and 5′-CCTCCCA-GAAAAATGCCATA-3′ (Bax reverse), 5′-TGTGTCCGTCGTGGATCTGA-3′ (GAPDH forward) and 5′-TTGCTGTTGAAGTCGCAGGAG-3′ (GAPDH reverse). The PCR was performed in an Eppendorf Mastercycler PCR machine using the following cycle parameters: 1 cycle of 95 °C for 5 min, and 40 cycles of 95 °C for 10 s, 60 °C for 30 s. A comparative threshold cycle (CT) method was used, and the PCR data was calculated by the equation $2^{-\Delta\Delta CT}$. Each sample was conducted in duplicate.

2.5. Western blotting

(10^{-7} M) for 5, 10, 20, 30 min. For neuroprotection study, MES23.5 cells were processed with Icaritin (10^{-7} M) or vehicle for 24 h, then the medium was replaced with which containing MPP^+ (100 μM) and Icaritin (10^{-7} M) for another 24 h. For antagonist treatment, LY294002 (5 μM), PD98059 (10 μM) or JB-1 (1 μg/ml) was added to each group 1 h before Icaritin treatment. Cells were processed with lysis buffer consisting of 20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1 mM dithiothreitol (DTT), 0.5% SDS, and 1 mM phenylmethylsulfonyl fluoride (PMSF) as previously implemented (Chen et al., 2013; Gao et al., 2012). Then, lysates were centrifuged at 4 °C at 12,000 rpm for 20 min and protein concentrations were analyzed by the BCA method (Thermo Scientific). After protein electrophoresis and transbloting, the blots were blocked with 10% skimmed milk for 1 h and then probed with primary antibody against Bax, Bcl-2, IGF-1 receptor, pAkt, Akt, pERK1/2, ERK1/2 (1:1000) and β-actin (1:10000) overnight at 4 °C. Then, the membranes were incubated with the secondary antibody which conjugated with horseradish peroxidase (1:10,000) for 1 h at room temperature. The enhanced chemiluminescence (ECL) reagent