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ICARISIDE II, A NOVEL PHOSPHODIESTERASE-5 INHIBITOR, ATTENUATES STREPTOZOTOCIN-INDUCED COGNITIVE DEFICITS IN RATS

CAIXIA YIN, YUANYUAN DENG, JIANMEI GAO, XIAOHUI LI, YUANGUI LIU AND QIHAI GONG*

Department of Pharmacology and Key Laboratory of Basic Pharmacology of Ministry of Education, Zunyi Medical University, Guizhou 563000, China

Abstract—Beta-amyloid (Aβ) deposition and neuroinflammation are involved in Alzheimer's disease (AD)-type neurodegeneration with cognitive deficits. Phosphodiesterase-5 (PDE5) inhibitors have recently been studied as a potential target for cognitive enhancement by reducing inflammatory responses and Aβ levels. The present study was designed to investigate the effects of icariside II (ICS II), a novel PDE5 inhibitor derived from the traditional Chinese herb *Epimedium brevicornum*, on cognitive deficits, Aβ levels and neuroinflammation induced by intracerebroventricular-streptozotocin (ICV-STZ) in rats. The results demonstrated that ICV-STZ exhibited cognitive deficits and neuronal morphological damage, along with Aβ increase and neuroinflammation in the rat hippocampus. ICS II improved cognitive deficits, attenuated neuronal death, and decreased the levels of $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$ and PDE5 in the hippocampus of STZ rats. Furthermore, administration of ICS II at the dose of 10 mg/kg for 21 days significantly suppressed the expression of beta-amyloid precursor protein (APP), beta-secretase1 (BACE1) and increased the expressions of neprilysin (NEP) together with inhibited interleukin-1β (IL-1β), tumor necrosis factor (TNF)-α, cyclooxygenase-2 (COX-2) and transforming growth factor-β_1 (TGF-β_1) levels. In addition, ICS II exerted a beneficial effect on inhibition of IκB-α degradation and NF-κB activation induced by STZ. Taken together, the present study demonstrated that ICS II was a potential therapeutic agent for AD treatment. © **2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: icariside II, Alzheimer's disease, streptozotocin, phosphodiesterase-5, beta-amyloid, neuroinflammation.

INTRODUCTION

Alzheimer's disease (AD), a progressive neurodegenerative disease affecting the life quality of patients, is characterized by the loss of neurons and synapses, as well as the decline of cognitive function. The pathophysiological hallmarks of AD include neurofibrillary tangles (NFTs) and senile plaques (SP) in the hippocampus and cerebral cortical neurons (Chu et al., 2014; Song et al., 2014). The formation of NFTs comes from intraneuronal accumulation of abnormally hyperphosphorylated microtubule-associated protein tau, while the SP is composed primarily of extracellular depositions of beta-amyloid (Aβ) (Watanabe et al., 2015). Although the etiopathogenetic mechanism of AD remains unknown, increasing studies have indicated the occurrence of AD was closely related to Aβ deposition and neuroinflammation (Yu et al., 2015; Zhu et al., 2015b). Aβ is a degradation product of beta-amyloid precursor protein (APP) hydrolyzed by beta-secretase (BACE-1) and γ-secretase. The accumulation and aggregation of Aβ in brain activate astrocytes and microglia, initiating the neuroinflammation through releasing of various inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2). In turn, these inflammatory cytokines promote the Aβ generation and eventually lead to memory impairment (Joo et al., 2008; Yoon et al., 2010; Jin et al., 2014; Xu et al., 2014). Until now, various agents are applied in clinic to treat AD, such as tacrine, donepezil, galantamine and rivastigmine, as well as memantine. Most of them are acetylcholinesterase (AChE) inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists. However, their applications only partially control the symptoms of AD and always bring adverse effects. Thus, discovery and development of new agents for AD treatment is still on the way.

Phosphodiesterase-5 (PDE5) is a cGMP-specific hydrolase. PDE5 receive attention as their inhibitors are used to treat erectile dysfunction (ED). However, PDE5 inhibitors are also active for cognitive enhancement (Dell'Agli et al., 2008; García-Osta et al., 2012; Sakamoto et al., 2015). Selective PDE5 inhibitors, such as sildenafil and vardenafil, mitigated memory deficits in AD by reducing inflammatory responses and Aβ levels (Zhang et al., 2013a; Zhu et al., 2015a), indicating PDE5 inhibitors might be potential neuroprotective agents for the treatment of AD.

*Corresponding author. Address: Department of Pharmacology and Key Laboratory of Basic Pharmacology of Ministry of Education, Zunyi Medical University, Zunyi 563000, China. Tel: +86-851-286-096-23; fax: +86-851-286-097-26.
E-mail address: gqh@zmc.edu.cn (Q. Gong).

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; APP, beta-amyloid precursor protein; Aβ, beta-amyloid; BACE1, beta-secretase 1; BCA, bicinchoninic acid; COX-2, cyclooxygenase-2; ELISA, enzyme-linked immunosorbent assay; ICS II, icariside II; ICV, intracerebroventricular; IDE, insulin degrading enzyme; IL-1β, interleukin-1β; IκB, inhibitory κB; NEP, neprilysin; NF-κB, nuclear factor κB; PDE5, phosphodiesterase-5; STZ, streptozotocin; TGF-β_1, transforming growth factor-β_1; TNF-α, tumor necrosis factor-α.

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Herba Epimedii is a traditional Chinese herb applied for cardiovascular diseases and osteoporosis, as well as sexual and neurological dysfunctions (Chen et al., 2014; Li et al., 2015a). Icariin and icariside II (ICS II) are the two major active ingredients of *Herba Epimedii* with similar structures. Studies have shown that icariin improves cognitive ability and inhibits memory impairment *in vitro* and *in vivo* (Urano and Tohda, 2010; Li et al., 2015b; Zhang et al., 2015). In addition, it is well known that ICS II is one of the primary metabolites of icariin (Cai et al., 2011), and has been proven to possess a wide range of efficacy including anti-inflammatory, anti-osteoporosis, anti-hypoxia and anti-cancer activities (Kang et al., 2012; Khan et al., 2015). It was confirmed that ICS II was a specific PDE5 inhibitor and was used to treat diabetic ED (Zhang et al., 2013b; Bai et al., 2014). It is implied that, as an effective PDE5 inhibitor, ICS II could be potentially used to improve AD-associated cognitive deficits.

Therefore, this study was designed to investigate the therapeutic effect of ICS II on AD-type cognitive deficits and the underlying mechanisms. As intracerebroventricular (ICV) injection of streptozotocin (STZ) resembles molecular, pathological, and behavioral features of AD and is used for preclinical testing of pharmacological therapies for AD (Labak et al., 2010; Grieb, 2015), STZ induced AD model was used in the present study.

EXPERIMENTAL PROCEDURES

Reagent

ICS II (purity ⩾ 98% by HPLC) was purchased from Nanjing Zelang Medical Technology Corporation Ltd. (Nanjing, China), which was dissolved by double distilled water with 30-minute ultra-sonication. STZ was purchased from Sigma Aldrich. (St. Louis, MO, USA) and dissolved in a vehicle consisting of 0.05 M citrate buffer (pH 4.2). All reagents were of analytical grade and commercially available.

Animals

Adult male Sprague-Dawley (SD) rats (250 ± 20 g) were obtained from the Animal Center of the Third Military Medical University (Chongqing, China; Certificate No. SCXK2012-0005). Rats were housed five per cage in a controlled- temperature environment (23 ± 1 °C under a natural light/dark cycle) with free access to food and water. We attempted to minimize animal pain and to reduce the number of rats used.

All animal experiments were strictly carried out in accordance with NIH guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996), and the study protocol was approved by the Animal Experimentation Ethics Committee of Zunyi Medical University.

Surgery and administration

The rats were randomly divided into 4 groups: sham group (n = 14), model (STZ) group (n = 12), ICS II-L (STZ + ICS II 3, low doses) group (n = 12), ICS II-H (STZ + ICS II 10, high doses) group (n = 11). ICV injection of STZ was established as previously described (Rodrigues et al., 2010). Briefly, rats were given anesthesia with 7% chloral hydrate (300 mg/kg, i.p), followed by a single injection of STZ (1.5 mg/kg) bilaterally into each lateral ventricle (5 μL/ventricle), and the injections were carried out at the first day and the third day. The heads of the rats were positioned according to the coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, and 3.6 mm ventral to the surface of the brain (Gibbs et al., 2011; Tota et al., 2012). Meanwhile, control animals were done with volume-matched vehicle, instead. Rats in ICS II-L and ICS II-H groups were intragastrically administered with ICS II at doses of 3 or 10 mg/kg on the first day after the second STZ injection for 21 consecutive days, while sham and model groups were administered with volume-matched double water.

Morris water maze test

The Morris water maze (MWM) was used for the evaluation of ICS II on spatial learning and memory ability of rats and was performed on the 17th day after the second STZ injection as previously described (Choi et al., 2013; Liu et al., 2013b; Wang et al., 2014). Briefly, the MWM consisted of a large circular black pool (160 cm in diameter and 50 cm in height) filled with water (24 ± 2 °C and 30 cm in depth), which was divided into four quadrants. Within the pool, a hidden platform of 12 cm diameter was located 1 cm below the water level in the center of the target quadrant. There were two steps in the procedure of the MWM test. The place navigation test is the first step, which was conducted twice per day (9:00 am and 15:00 pm) for four consecutive days. The swimming speed and escape latency of the rats to reach the hidden platform were recorded. Rats were given 120 s to find the platform by swimming, and remained on the platform for 15 s. If a rat did not find the platform within 120 s, its escape latency was recorded as 120 s, while it was gently guided to the platform and stayed on there for 15 s. The spatial probe test was the second step, which was assessed on the 5th day after taking out the platform. During the test, the swimming speed, the time spent in the target quadrant and the frequency crossing the target quadrant were measured by the TopScan-Topview Behavior Analyzing System (TopScan Version 3.00).

Morphometric analysis

After the MWM test, four rats extracted freely from each group were given anesthesia with 7% chloral hydrate, then the rats were perfused via the ascending aorta in 0.1 M phosphate-buffered saline (PBS), and followed by 4% paraformaldehyde (pH 7.4). The brains were removed and fixed with 4% paraformaldehyde for one week, and then cut into coronal sections (3 μm thick) for hematoxylin-eosin (HE) staining (Li et al., 2015b) and Nissl staining (Liu et al., 2015), respectively. The histopathological abnormalities were examined under a light microscope (KS300, Zeiss-Kontron, Germany).