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Echinacoside prevents the striatal extracellular levels of monoamine neurotransmitters from diminution in 6-hydroxydopamine lesion rats

Hong Chen [a,b,*], Fu Chun Jing [a,b], Chang Ling Li [c], Peng Fei Tu [d], Qiu Sheng Zheng [a,b], Zheng Hua Wang [a,b]

[a] *Ministry of Education, Key Laboratory of Xinjiang Phytomedicine Resources, Shihezi University, Shihezi 832002, China*
[b] *Department of Pharmacology, College of Pharmacy, Shihezi University, Shihezi 832002, China*
[c] *Department of Molecular Pharmacology, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China*
[d] *Peking University Modern Research Center for Traditional Chinese Medicine, Beijing 100083, China*

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Abstract

We investigated the effects of echinacoside, a phenylethanoid glycoside isolated and purified from the stems of *Cistanche salsa*, a Chinese herbal medicine, on the striatal extracellular levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in 6-hydroxydopamine (6-OHDA) lesion rats. Seven days after 6-OHDA was injected into the right striatum of rats, the striatal extracellular levels of DA, DOPAC and HVA fell significantly ($P < 0.01$ vs. vehicle), as demonstrated by the method of cerebral microdialysis and high performance liquid chromatography with electrochemical detection. However, simultaneous treatment with echinacoside (7.0, 3.5 mg/kg) attenuated the diminution of them ($P < 0.01$ vs. model). The results implied that echinacoside could protect the striatal dopaminergic neurons from injury induced by 6-OHDA and may be useful in the prevention and treatment of Parkinson's disease (PD).

Keywords: Echinacoside; Dopamine; 3,4-Dihydroxyphenylethanoid acid; Homovanillic acid; 6-Hydroxydopamine; Parkinson's disease; Brain microdialysis; Rats

1. Introduction

Parkinson's disease (PD) is a well-known chronic neurodegenerative disease and has long been believed to be associated with the abnormal loss of dopamine (DA)-rich neurons in the central nervous system (CNS) (Kurth and Adler, 1998). The ensuing diminution of DA concentration leads to the imbalance of regulations to patient's motor function, which causes the special neurological motor symptoms such as bradykinesia, rest tremor and rigidity. Though the real pathogenesis of PD is still unknown to date, previously published studies showed that oxidative stress, a cellular dysfunction between the production and scavenging of free radicals, was the main reason related to the neuronal death (Lucio et al., 2003; Kelso et al., 2001; Freyer, 1998; You and Lin, 2002). 3, 4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are two important intermediates in the metabolic course of DA and can directly reflect the change of DA in CNS. Furthermore, experiments both in animal models and in patients suggested that there were decreases of DA and its metabolites, especially DOPAC and HVA, in the process of PD (Kish et al., 1992; Wang et al., 2005).

6-Hydroxydopmine (6-OHDA) is a selective dopaminergic neurotoxin (Ungerstedt, 1968), which produces reactive oxygen species (ROS) and thus damages the nigrostriatal dopaminergic neurons through oxidative stress (Cohen and Heikkila, 1974). When injected into the striatum, 6-OHDA may produce a continuous lesion right from several minutes to 1 week or month and can make the decrease of DA and its metabolites (Carmen et al., 2005; Zhang et al., 2003) in this region, which will finally lead to the emergence of some symptoms of PD.

* Corresponding author at: Department of Pharmacology, College of Pharmacy, Shihezi University, Shihezi 832002, China. Tel.: +86 993 2302286; fax: +86 993 2057093.
E-mail addresses: chenhong57@126.com, fleming7798@163.com (H. Chen).

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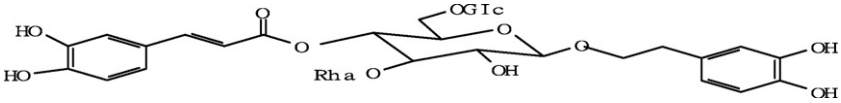

Fig. 1. The chemical structure of echinacoside.

Echinacoside (Fig. 1) is a phenylethanoid glycoside isolated and purified from the stems of *Cistanche salsa*, a parasitic plant native to northwest China, which is used as a traditional Chinese herbal medicine with antisenile and antifatigue effects (Xiong et al., 1999). It has been evident that echinacoside had neuroprotective effects and may be useful in the treatment of some neurodegenerative diseases (Deng et al., 2004a). Several other phenylethanoid glycosides were also proved to have similar actions and may be used to prevent neurons from oxidative stress-induced toxic injuries (Geng et al., 2004; Lee et al., 2006; Deng et al., 2004a; Sheng et al., 2002; Pu et al., 2003; Tian and Pu, 2005). However, the cellular and molecular mechanisms such as the changes of neurotransmitters and their metabolites that underlie the actions are not fully understood. Based on this reason, the present study aimed at the observation of the effects of echinacoside on the striatal extracellular levels of DA, DOPAC and HVA in 6-hydroxydopamine lesion rats.

2. Materials and methods

2.1. Animals and reagents

Male Wistar rats, weighing 230–270 g, were used in this study and housed individually in cages with food and water consumed ad libitum. The animals were kept under the temperature of $24 \pm 1\,^{\circ}C$ and the relative humidity of $55 \pm 5\%$ with a 12-h light:12-h-dark cycle (lights on at 7:00 a.m.). All experiments were performed in accordance with the guidelines established by the European Community for the care and use of laboratory animals and were approved by the Animal Care Committee of the Shihezi University.

Echinacoside from *Cistanche salsa* was kindly provided by Dr. Peng Fei Tu (Peking University Modern Research Center for Traditional Chinese Medicine). The purity of the compounds was shown to be more than 98% on high performance liquid chromatography (HPLC). DA, DOPAC, HVA, 1-heptanesulfonic acid sodium salt (HSA), triethylamine (TEA, ≥99%) and ascorbic acid were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (ACN, HPLC grade) was purchased from Fisher (New Jersey, USA). Phosphorate acid (PA) and EDTA tetrasodium salt were obtained from Guoyao Group Co., Ltd. (Shanghai, China). Ringer's fluid was prepared in our laboratory. All the solutions were prepared by deionized water of at least 18.2 MΩ cm specific resistance.

2.2. Experimental design

All animals were divided into five groups: vehicle, 6-OHDA, echinacoside high and low doses and medopar, among which the vehicle-treated rats were used as control, the 6-OHDA-treated as model and medopar-treated rats as positive drug group. On the operation day, rats accepted the injection of 4 μl 0.9% saline (for vehicle group) or 6-OHDA (12 μg/4 μl in 0.9% saline containing 0.1% ascorbic acid, for 6-OHDA, echinacoside and medopar groups) into the right striatum. After the operation each rat was given intraperitoneal injection of 0.9% saline (2 ml/kg, for vehicle and 6-OHDA groups), echinacoside (7.0 or 3.5 mg/kg for echinacoside high or low dose groups) or medopar (64 mg/kg), respectively and this administration was carried out one time a day at 8 o'clock a.m. during the following 7 consecutive days. At the end of the last administration, the microdialysis procedure was performed.

2.3. Surgery and microdialysis procedure

The rats were anesthetized with an intraperitoneal injection of 50 mg/kg of sodium pentobarbital and were fixed in a stereotaxic apparatus (SAS-4100, Bioanalytical Systems, Inc., West Lafayette, USA). After the skull was exposed, a burr hole was drilled for the accommodation of guide cannula (Microbiotech AB, Stockholm, Sweden). The cannula was implanted into the right striatum with the following coordinates: AP + 0.2 mm, ML −3.0 mm, DV −3.5 mm from bregma according to the brain atlas of Paxinos and Watson (1998), and was secured to the skull with screws and dental cement. Each rat was housed individually following the surgical operation.

Seven days later the dummy stylet in the guide cannula was pulled out and a microdialysis probe (MAB/6; o.d. 0.6 mm, membrane length 4 mm, cut-off 15,000 Da, Microbiotech AB, Stockholm, Sweden) was inserted into it while the rat was keeping in awake, freely moving status. The probe, which had been connected to a microinfusion pump (MD-1001 Baby Bee Syringe Drive and MD-1020 Bee Hive Controller, Bioanalytical Systems, Inc., West Lafayette, USA), was perfused at a constant flow rate of 1.5 μl/min with Ringer's solution composed of 125 mM NaCl, 3.3 mM KCl, 2.4 mM Mg_2SO_4, 1.25 mM KH_2PO_4, 1.85 mM $CaCl_2$ (pH 7.1). After an 80-min equilibrium period, dialysate samples were consecutively collected every 20 min into vials containing 5 μl saline of 0.1% ascorbic acid in order to prevent DA, DOPAC and HVA from oxidation. All samples were injected directly into the HPLC–ECD system and analyzed immediately or kept at $-70\,^{\circ}C$ until analysis.

2.4. Chemical assays

DA, DOPAC and HVA were determined by a Shimadzu (Kyoto, Japan) LC-10ADvp HPLC system with a Shimadzu L-ECD-6A amperometric detector. Separations were performed by a Hypersil GOLD C_{18} column (ODS, 150 mm × 4.6 mm i.d., 5 μm, Thermo, UK). The column and detector were placed in the compartment of Shimadzu CTO-10A column oven under the temperature maintained at 40 °C. The analytes were detected