Steroids 113 (2016) 103–112

Contents lists available at ScienceDirect

Steroids

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

Diosgenin attenuates the brain injury induced by transient focal cerebral ischemia-reperfusion in rats

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ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 6 July 2016
Accepted 13 July 2016
Available online 16 July 2016

Keywords:
Diosgenin
Ischemia-reperfusion injury
Middle cerebral artery occlusion
Anti-apoptosis
Anti-inflammation
NF-κB

ABSTRACT

The aim of the present study is to explore the potential cerebroprotection of diosgenin against the transient focal cerebral ischemia-reperfusion (I/R) injury and its possible underlying mechanisms. The diosgenin at two dose levels, namely 100 and 200 mg kg^{-1}, was intragastrically administrated once daily for 7-day period prior to the surgery. Then, the rats were subjected to middle cerebral artery occlusion (MCAO) using the intraluminal thread for 90 min. After 24 h reperfusion, several diagnostic indicators were evaluated and all animals were sacrificed to harvest their brains and blood for subsequent biochemical analyses. The results indicated that diosgenin treatment significantly inhibited the death rate and improved the impaired neurological functions along with neurological deficit scores and cerebral infarct size as compared with the rats exposed to I/R insult without agents administration. The increase in the number of apoptotic cells determined by TUNEL in the hippocampus CA1 and cortex was also apparently attenuated in the diosgenin treatment group, which was closely correlated with suppression of Caspase-3 activity and Bax/Bcl-2 ratio. In addition the elevated concentrations of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in blood serum of the I/R treated rats were reduced almost to their normal level. Further results obtained from the Western blotting analysis revealed that the protein expression of IκBα in the injured brain was up-regulated, while the p65 subunit of NF-κB was down-regulated in nucleus after the treatment. Collectively, this neuroprotection of diosgenin against I/R injury may be attained through its anti-apoptosis, anti-inflammation and intervening the NF-κB signal pathway properties. Due to the satisfactory findings, diosgenin might be a powerful therapeutic agent to combat the similar disease in future clinic.

1. Introduction

Cerebrovascular diseases (CVD), including ischemic and hemorrhagic strokes [1], are becoming more and more prevalent in modern population according to recent epidemiological investigations. Blockage of the blood flow to a specific area of the brain is called ischemic stroke [2]. Due to its characteristics of high incidence and recurrence rate, ischemic stroke accounting for more than 50 percent of the total CVD remains as the second main cause of adult disability and mortality in the worldwide [3]. When this disease occurs, immediate restoration of the adequate blood supply to ischemic regions, i.e. reperfusion, is the best strategy of treatment in clinic [4]. Although reperfusion relieves the cerebral ischemia and brain injury to some extent, it causes further brain damage called cerebral ischemia-reperfusion (I/R) which couldn't be negligible, since it is even severer than the damage caused by ischemia under certain conditions [5]. Thus, it is crucial to prevent the brain from I/R insult during the therapy of ischemic stroke. Multiple pathophysiological mechanisms including toxicity of the amino acid, imbalance of intracellular calcium, reactive oxygen species, apoptosis, and inflammation observed in pathogenesis of I/R injury make this pathological process extremely complex according to numerous scientific researches [6]. Therefore, a clear and acceptable theory has not been developed until now. Because extensive researches are carried out in laboratory, various neuroprotective drugs and strategies have been emerged [7]. However, to our disappointment, almost no treatments could be successfully used in clinic after translation from basic research. Therefore, it is highly

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http://dx.doi.org/10.1016/j.steroids.2016.07.006
0039-128X/

desirable to discover potentially therapeutic agents and gather evaluable experimental data for their subsequent pharmacological use.

Natural bioactive compounds from Traditional Chinese Medicine (TCM) that are used to cure various diseases have increasingly concerned in recent decades. They deserve reasonable investigations because of minimum side effects and weak toxicities [8]. Diosgenin (25R-spriost-$\Delta^{5(6)}$-en-3β-ol) is an important intermediate substance used to synthesize different steroid hormones, contraceptives, steroids, and cortisone in the pharmaceutical industry [9]. It is usually present in many plant species such as *Dioscorea*, *Costus* and *Trigonella* in the form of steroid saponins which are composed of diosgenin as the aglycone with various sugar moieties attached through glucosidic bonds [10]. Steroid saponins existed in *Dioscorea zingiberensis* C.H. Wright, whose rhizomes were reconsidered as a major food source that the local residents used to solve the hunger problem in 1980s in Ankang city of Shaanxi Province in China, are the preferred source to extract the diosgenin after acid hydrolysis. Most of these compounds derived from diosgenin could be efficiently used for the treatments of cardiovascular, coronary diseases, neurotoxicity, and Alzheimer's disease [11–13]. Besides being used as an important starting material in synthesis, diosgenin reveals various other biological activities such as anti-cancer, anti-inflammation, anti-malarial, and controlling the cholesterol metabolism effects according to the modern pharmacological investigations [14]. However, to our knowledge there is no report on the effect of diosgenin against I/R injury. Therefore, our present experiment was conducted to evaluate the therapeutic efficacy of diosgenin on the transient focal I/R injury in rats using the animal model induced by middle cerebral artery occlusion (MCAO) with the intraluminal thread, and its possible underlying mechanisms.

2. Material and methods

2.1. Materials and chemicals

The diosgenin (UC-000080, over 98% purity, Fig. 1) was supplied by Nanjing Spring & Autumn Biological Engineering Co., Ltd (Nanjing, PRC). The 2, 3, 5-triphenyltetrazolium chloride reagent (TTC, 1014795, 99% purity) and Nimodipine tablets (140851, 98% purity) were purchased from Xiya Reagent (Chengdu, PRC) and Yabao Pharmaceutical Group CO., Ltd (Shanxi, PRC), respectively. The ELISA available assay kits (TNF-α, IL-1β and IL-6) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, PRC). The primary anti-bodies (rabbit anti-rat) of Caspase-3, Bcl-2, Bax, NF-κB and GADPH were offered by Zhongshan Glodenbridge Biotechnology Co., Ltd (Beijing, RPC) except for the IκBα from Sigma-Aldrich chemical Co. (St. Louis, MO., USA). The secondary anti-bodies were provided by Wuhan Boster Biological Technology Co., LTD (Hubei, PRC), and the other relevant test kits including TUNEL, DAB and BCA were obtained from Beyotime Institute of Biotechnology Co., Ltd. (Haimen, China). The squeezing corn oil of Luhua was purchased from Renrenle Supermarket (Shandong, RPC). The Millipore Milli-Q water system (Milford, MA, USA) was used to produce distilled water (18 MΩ cm^{-1}). All other regents were of analytical grade with high purities.

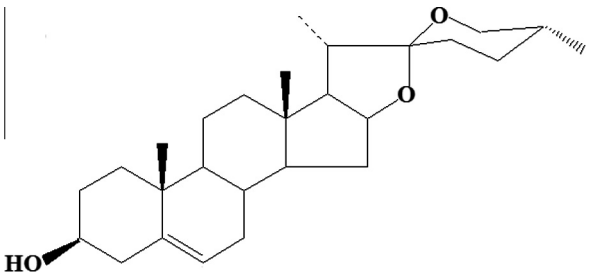

Fig. 1. The chemical structure of diosgenin.

2.2. Animals

The present study used healthy adult male Sprague-Dawley rats 8–10 weeks old weighing 250–280 g obtained from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, RPC). The rats were kept five per cage in a constant SPF (Specific pathogen Free) conditions where the temperature, relative humidity, and light-dark cycle in a day were controlled at 22 ± 1 °C, 45–55% and 12 h/12 h (lights from 7:00 am to 19:00 pm). Meanwhile, these rats were allowed free access to the quantitative standard food while drinking enough purified water ad libitum. The animals were housed for seven days to acclimatize in this environment prior to the surgery. The experimental protocols were made according to the guidelines approved by the Animal Experimental Committee of Xi'an Jiaotong University (NO. XAJTU 2014-03). In all the animal experiments the number of animals was reduced as much as possible and operations were performed under deep anesthesia to alleviate the suffering that were strictly in agreement with the local Animal Ethical Committee (2012, NO. 3245/2012).

2.3. Drug administration and induction of transient focal I/R model

The animals were randomly divided into 6 groups each containing 13 rats and received corresponding drugs as follows sham operated group (SG), model control group (I/R), Nimodipine group (NG, 20 mg kg^{-1}), diosgenin at the low dose (DL, 100 mg kg^{-1}), and diosgenin at the high dose (DH, 200 mg kg^{-1}). All these preparations were intragastrically administrated once daily for 7 days prior to the surgery.

Thirty minutes after the seventh treatment, all the rats except for the SG group were underwent the I/R surgery induced by MCAO using intraluminal filament based on the previously established method with minor modifications [15]. During this entire process, the room temperature was always maintained at about 25 °C with an air conditioner to avoid cerebral hypothermia. In brief, anesthesia was elicited by intraperitoneal injection of 3.5% (3.5 g was dissolved in 100 mL physiological saline) choral hydrate at the dose of 350 mg kg^{-1} to each animal. Under a table lamp, the right common carotid artery (CCA), internal carotid artery (ICA) and external carotid artery (ECA) were all bluntly and carefully separated from the vagus nerve and adjacent tissues through a midline incision made on the neck of the rats fixed in the supine position. The distal end of ECA and the origin of ECA were both ligated with 4-0 silk suture, and a loose silk suture was placed around the root of ICA at the same time. After a small puncture of CCA was made at 5 mm away from the bifurcation, a nylon monofilament about 40 mm in length and 0.24–0.26 mm in diameter, whose tip was polished round with abrasive paper and coated with nail oil tip, was transiently introduced from this cut on CCA into the lumen of the ICA. When the gentle resistance was felt, which indicated that this thread had reached the anterior of cerebral artery to enough occlude the entrance of middle cerebral artery (MCA) causing the cerebral ischemia, the previous suture was immediately fasten and tied to prevent bleeding and the neck skin was also sutured. The distance from the bifurcation to the origin of MCA was in the range of 18–20 mm depending on the weight of animals. After 90 min, the reperfusion was established by restoring the blood flow via carefully withdrawing this inserted filament by about 10 mm. All the surgical procedure except for making a small incision on CCA and