Steroids 113 (2016) 52–63

Contents lists available at ScienceDirect

Steroids

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

Potential neuroprotection of protodioscin against cerebral ischemia-reperfusion injury in rats through intervening inflammation and apoptosis

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ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form 9 June 2016
Accepted 15 June 2016
Available online 23 June 2016

Keywords:
Protodioscin
Middle cerebral artery occlusion
Caspase-3
Bax/Bcl-2 ratio
Pro-inflammatory cytokines
NF-κB

ABSTRACT

The aim of the current research is to investigate the cerebral-protection of protodioscin on a transient cerebral ischemia-reperfusion (I/R) model and to explore its possible underlying mechanisms. The rats were preconditioned with protodioscin at the doses of 25 and 50 mg kg^{-1} prior to surgery. Then the animals were subjected to right middle cerebral artery occlusion (MCAO) using an intraluminal method by inserting a thread (90 min surgery). After the blood flow was restored in 24 h via withdrawing the thread, some representative indicators for the cerebral injury were evaluated by various methods including TTC-staining, TUNEL, immunohistochemistry, and Western blotting. As compared with the operated rats without drug intervening, treatment with protodioscin apparently lowered the death rate and improved motor coordination abilities through reducing the deficit scores and cerebral infarct volume. What's more, an apparent decrease in neuron apoptosis detected in hippocampus CA1 and cortex of the ipsilateral hemisphere might attribute to alleviate the increase in Caspase-3 and Bax/Bcl-2 ratio. Meanwhile, concentrations of several main pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in the serum were also significantly suppressed. Finally, the NF-κB and IκBa protein expressions in the cytoplasm of right injured brain were remarkably up-regulated, while NF-κB in nucleus was down-regulated. Therefore, these observed findings demonstrated that protodioscin appeared to reveal potential neuroprotection against the I/R injury due to its anti-inflammatory and anti-apoptosis properties. This therapeutic effect was probably mediated by the inactivation of NF-κB signal pathways.

1. Introduction

With the improvement of modern life standards, cerebrovascular diseases (CVD) are becoming prevalent in worldwide both in developed and developing countries, because of lacking effective and convenient medical treatment and health care system [1]. Stroke also called "Brain attack" (a kind of CVD) is primarily composed of following two forms: cerebral ischemic and hemorrhagic strokes [2].

Cerebral ischemic stroke caused by terminating the blood supply to either a part of the brain-(focal) or the whole brain (global) is a representative form accounting for 80% of stroke patients [3,4]. This leads to insufficient supply of oxygen and glucose to the brain, and interrupts its normal physiological functions, such as the ability of speech, cognition, motor activities, and memory [5]. Consequently, patients suffer from severe physical problems and heavy economic burden. Due to these characteristics of high adult morbidity such as disability and mortality as well as recurrence rate [6], it becomes the second cause of death in the world [7]. In order to inhibit the further brain damage caused by ischemia immediate reperfusion is a vital remedial treatment and considered to be the best clinical approach [8]. Although the ischemic districts of the brain regain the supply of oxygen and nutrient, the secondary impairment, i.e. ischemia-reperfusion (I/R) injury, may occur after reperfusion causing brain tissue damage which is even worse or beyond the ischemia itself under certain conditions [9]. This cerebral injury is a complicated disorder, and several major mechanisms are involved such as inflammatory response, oxidative

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http://dx.doi.org/10.1016/j.steroids.2016.06.008
0039-128X/

stress, apoptosis, excitotoxicity, ion imbalance, and acidotoxicity [10,11]. Extensive scientific researches on this I/R have been carried out, however, this pathogenesis still remains obscure, and there are no generally recognized mechanisms reported until now. These aforementioned underlying mechanisms might cooperate synergistically to cause the final severe pathogenesis. Thanks to plentiful efforts made in the laboratory after numerous studies exploring the complex cellar pathways of I/R, various safe and effective therapeutic treatments have been proposed [12]. Unfortunately, many of these strategies proved to be cerebroprotective in laboratory studies, but failed to exhibit promising efficacy in clinical trials [13]. Despite available medical agents of recombinant tissue plasminogen activator (rtPA) and tissue plasminogen activator (tPA) approved by American Food and Drug Administration (FDA) [14,15], to our disappointment, the window of time is extremely short [16], and is inconvenient to treat I/R. Therefore, development of highly potential anti-ischemic stroke agents is still urgently warranted at present.

For many centuries, the Traditional Chinese Medicine (TCM) has been used to treat human diseases. To discover ideal sources for the treatment of I/R, much more attention has been paid to the active ingredients from TCM due to its weak toxicity and multiple target spots in recent years. After systematic and detailed pharmacological investigations, enough supporting data has been accumulated. Steroid saponins consisting of spirostanol and furostanol forms are bioactive compounds abundantly present in various plants [17]. Protodioscin, also written as 25(*R*)-26-O-β-D-glucopyranosyl-furost-$\Delta^{5(6)}$-en-3β, 22α, 26-triol-3-O-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, is a furostanol and naturally occurring steroid saponin present in the rhizome of *Dioscorea zingiberensis* C.H. Wright (*D. zingiberensis*) [18]. Its structure is shown in Fig. 1. According to the experiments performed by Gauthaman et al., Adaikan et al., and Gauthaman et al., it displays a broad range of charming bioactivities mainly including anti-cancer effects against cell lines of HL-60 cells, leukemia, and NCI's [18]. However, to our best knowledge no report on the activities of protodioscin against I/R injury is available.

Therefore, the purpose of the current study is to investigate whether or not protodioscin has the neuroprotective activity on cerebral I/R injury in rat. This model, i.e. transient focal middle cerebral artery occlusion (MCAO), is established to simulate the clinical symptoms of human cerebral ischemia-reperfusion. If it reveals satisfactorily therapeutic effect of protodioscin in rats, the possible underlying mechanisms are also assessed in this research.

2. Material and methods

2.1. Materials and chemicals

The dried rhizomes of *D. zingiberensis* were offered by Heng Xiang Biological Chemical CO., Ltd (Ankang, Shannxi) and kindly identified by Professor Wenzhe Liu (College of Life Science of Northwest University, Xi'an 710069, PRC). Its voucher specimen (NO.Drsr009) was deposited in the Key Laboratory of Resource Biology and Biotechnology in West China, Ministry of Education (Northwest University, PRC). The analytical reagents of methanol, ethanol, n-butanol, dichloromethane, and acetonitrile were purchased from Hongyan Chemical Reagent Factory (Tianjing, PRC). The distilled water (18 MΩ cm^{-1}) was obtained by a Millipore Milli-Q water system in our laboratory (Milford, MA, USA).

The nimodipine pills (140851, 98% purity) and TTC reagent (1014795, 99% purity), also named 2,3,5-Triphenyltetrazolium Chloride, were supplied by Yabao Pharmaceutical Group CO., Ltd (Shanxi, PRC) and Xiya Reagent (Chengdu, PRC), respectively. The inflammatory factor ELISA kits of TNF-α, IL-1β and IL-6 were all obtained from Roche Diagnostics (Germany). The primary antibodies (rabbit anti-rat) of Caspase-3, Bcl-2, Bax, NF-κB and IκBa were provided by the Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, PRC), while other anti-bodies (including secondary ones), TUNEL, DAB, and BCA kits by Wuhan Boster Biological Technology Co., LTD (Hubei, PRC). All other experimental materials were of analytical grade.

2.2. Preparation of protodioscin

The dried raw rhizomes of *D. zingiberensis* were cut into small pieces and homogenized in a speed disintegrator. The fine powder obtained after passing through a 40-mesh sieve was extracted with 70% ethanol (M/V = 10, i.e. the ratio of mass and volume equals 10) under reflux for 2 h in thrice cycles. The collected solution was pooled and concentrated to a proper volume under reduced pressure and vacuum in a rotary evaporator. The obtained residues were redissolved in 20-fold volume of distilled water under

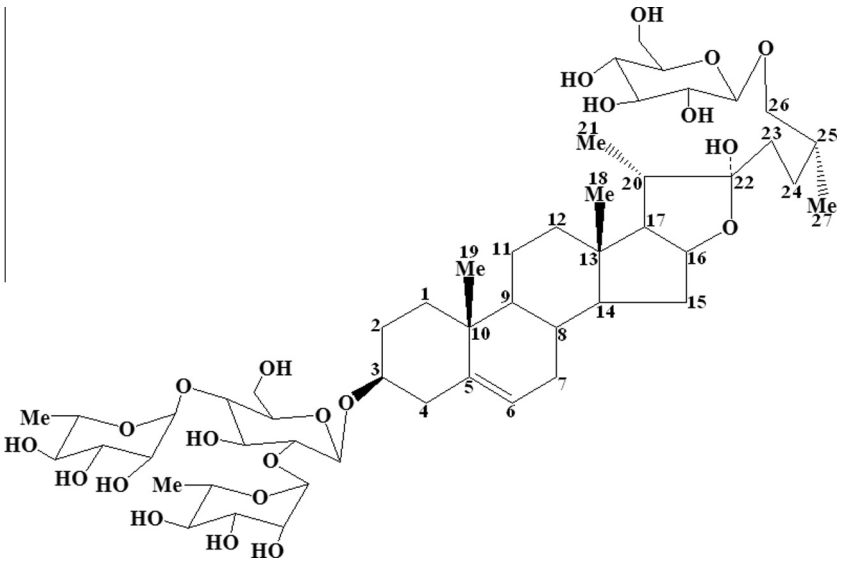

Fig. 1. The chemical structure of protodioscin with numbers marked on carbon atoms in aglycone which are consistent with the expression of the ^{1}H NMR and ^{13}C NMR in Section 3.1.