Journal of Ethnopharmacology 134 (2011) 147–155

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Tanshinone IIA attenuates neuronal damage and the impairment of long-term potentiation induced by hydrogen peroxide

Wei Wang[a,1], Li-Li Zheng[a,1], Fang Wang[a,b,c], Zhuang-Li Hu[a,b,c], Wen-Ning Wu[a], Jun Gu[a], Jian-Guo Chen[a,b,c,*]

[a] Department of Pharmacology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[b] The Key Laboratory of Neurological Diseases, Ministry of Education of China, Wuhan, Hubei 430030, China
[c] Institutes of Biomedicine and Drug Discovery, Huazhong University of Science and Technology, Wuhan 430030, China

ARTICLE INFO

Article history:
Received 5 July 2010
Received in revised form 25 October 2010
Accepted 28 November 2010
Available online 4 December 2010

Keywords:
Tanshinone IIA
Hydrogen peroxide
Cortical neuron
Apoptosis
Long-term potentiation
Hippocampus

ABSTRACT

Aim of the study: Tanshinone IIA (Tan IIA) is one of the key components of *Salvia miltiorrhiza Bunge* that has been widely used for various cardiovascular and cerebrovascular disorders in Asian countries. Many studies have reported that Tan IIA has antioxidative properties, but whether Tan IIA can rescue neurons from oxidative insult has never been reported. The present study was undertaken to evaluate the possible neuroprotective effects of Tan IIA on hydrogen peroxide (H_2O_2)-induced oxidative stress in rats.
Materials and methods: H_2O_2-induced cytotoxicity was evaluated by the cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay and flow cytometry with PI staining. Calcium imaging experiments were carried out to measure intracellular free calcium concentration. Western blotting was used to determine the expression of Bax and Bcl-2 protein. Electrophysiological studies in hippocampal slices were performed to investigate the effect of Tan IIA on synaptic function and cognitive impairment caused by H_2O_2.
Results: It was found that pretreatment with Tan IIA protected primary rat cortical neurons against H_2O_2-induced cytotoxicity. Furthermore, Tan IIA markedly reduced the elevation of $[Ca^{2+}]_i$ evoked by H_2O_2. Western blot analysis indicated that pretreatment with Tan IIA prevented the increase in Bax/Bcl-2 ratio induced by H_2O_2. In addition, preincubation of Tan IIA 20 min prior to H_2O_2 exposure could reverse H_2O_2-induced hippocampal LTP impairment, but without significant alteration in basal synaptic transmission and LTP induction.
Conclusions: These findings demonstrate that Tan IIA might serve as a novel promising therapeutic agent for oxidative stress injury in neurodegenerative diseases.

1. Introduction

Formation of reactive oxygen species (ROS) has been proposed to be an important step leading to neuronal death related to a variety of neurodegenerative diseases, such as stroke, Alzheimer's disease (AD) and Parkinson's disease (PD) (Olanow, 1993; Zhou et al., 2008; Zawia et al., 2009). Although ROS play a major role in biological processes (Esposito et al., 2004), excessive ROS can damage cellular components such as lipid, protein, and DNA and initiate subsequent cell death via necrosis or apoptosis (Andersen, 2004). Oxidative damage produced by excessive ROS is thought to cause neuronal injury and underlie cognitive impairment and neurodegeneration. Therefore, therapeutic strategies aimed at alleviating or preventing ROS injuries might be a reasonable choice for the treatment of these neurodegenerative diseases.

Long-term potentiation (LTP), which is a manifestation of activity-dependent synaptic plasticity, has been demonstrated in the hippocampus and other brain regions of rodent and has increasingly been the leading candidate for the studies on learning and memory (Malenka and Bear, 2004). Studies from several laboratories have suggested that ROS are critical for LTP and excessive ROS can inhibit the induction of LTP in the hippocampal CA1 region. Our previous work has revealed that the membrane-permeable

Abbreviations: Tan IIA, Tashinone IIA; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; MTT, methyl thiazolyl tetrazolium; PI, propidium iodide; ACSF, artifical cerebrospinal fluid; LTP, long-term potentiation; HFS, high frequency stimulation; fEPSPs, field excitatory postsynaptic potentials; fura-2/AM, fura-2-acetoxymethyl ester.

* Corresponding author at: Department of Pharmacology, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, Hubei 430030, China. Tel.: +86 27 83692636; fax: +86 27 83692608.
E-mail addresses: chenj@mails.tjmu.edu.cn, chenjg@mail.hust.edu.cn (J.-G. Chen).
[1] These authors contributed to this work equally.

0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.11.069

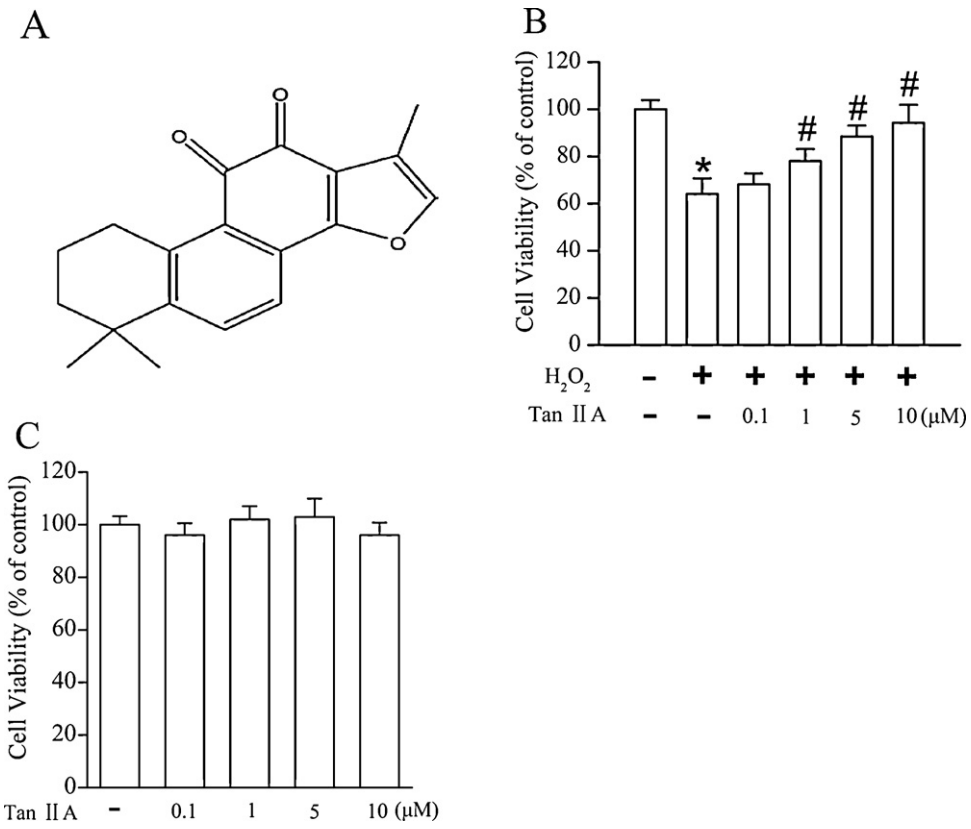

Fig. 1. Protective effect of Tan IIA against cytotoxicity induced by H_2O_2 in cultured rat cortical neurons. (A) The chemical structure of Tan IIA. (B) The cells were treated with different concentrations of Tan IIA for 24 h and followed by incubation with 120 μM H_2O_2 for 10 h. Cell viability was measured by MTT assay. Results were presented as mean ± S.E.M. The data were obtained from four independent experiments performed in duplicate. $^*P < 0.05$ *vs* control. $^\#P < 0.05$ *vs* H_2O_2. (C) Pretreatment with different concentrations of Tan IIA did not affect the viability of cortical neurons under basal conditions ($n = 4$, $P > 0.05$ *vs* control). Data were presented as mean ± S.E.M.

oxidizing agent chloramine-T (Ch-T) inhibits the induction of LTP (Cai et al., 2008). In addition, exposure of the hippocampal slices to 20 μM H_2O_2 prevented induction of new LTP, suggesting a link between H_2O_2 and LTP (Kamsler and Segal, 2003). Since excessive ROS play such detrimental roles in hippocampal LTP, some natural antioxidants have been shown to protect against ROS-induced LTP impairment in vitro (Wang et al., 2006).

Recently, there has been intense interest in the antioxidant properties of natural products due to their neuroprotective effects in patients (Zhu et al., 2004). Among these natural products, the dried roots of *Salvia miltiorrhiza Bunge* (Danshen), which belongs to the *Labiatae* family, are widely used in the treatment of cardiovascular and cerebrovascular disorders in Asian countries (Li et al., 2008a). Tanshinone IIA (Tan IIA), which is one of the major lipid-soluble pharmacologic constituents of Danshen (Fig. 1A), is the most abundant form of Tanshinone extracted from Danshen and possesses the most characteristic structure. Recently, Tan IIA has been shown to exhibit antioxidant properties which prevents the oxidation of low-density lipoproteins (LDLs) (Niu et al., 2000), reduces cellular damage caused by free radicals (Wang et al., 2003), and alters the expression and/or activity of specific antioxidant enzymes to protect cells from oxidative damage (Li et al., 2008b). Additionally, Tan IIA has the potential to penetrate the blood–brain barrier and acts as an inhibitor of acetylcholinesterase (AchE), which support the traditional application of Tan IIA to alleviate cognitive dysfunction (Ren et al., 2004). All of these observations prompted us to explore whether Tan IIA could be used to protect against oxidative stress injury in the central nervous system. In the present study, we investigated the protective effects of Tan IIA on H_2O_2-induced neuronal apoptosis in primary cultured cortical neurons and LTP impairment in hippocampal slices as well as the underlying mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

H_2O_2 was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, and B27 supplement were purchased from Gibco Invitrogen (Carlsbad, CA, USA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) was obtained from Amresco (Amresco, USA). Antibody to β-actin was purchased from Upstate Biotechnology (Lake Placid, NY, USA). Mouse monoclonal antibody against Bax and polyclonal antibody against Bcl-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Fura-2/AM was obtained from Biotium (Hayward, CA, USA). Cell apoptosis PI detection kit was purchased from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China). Other general agents were purchased from commercial suppliers.

2.2. Preparation of Tan IIA

The dried roots of *Salvia miltiorrhiza Bunge* (Danshen) were collected from Weifang, Shandong, China and authenticated by Professor Jia-Ning Wang of Department of Pharmacology, Tongji Medical College, Huazhong University of Science and Technology. A voucher specimen has been deposited in Department of Pharmacology, Tongji Medical Center, Huazhong University of Science and Technology. Tan IIA used in this study was prepared as