European Journal of Pharmacology 725 (2014) 23–31

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

A role for diallyl trisulfide in mitochondrial antioxidative stress contributes to its protective effects against vascular endothelial impairment

Li-Li Liu [b,1], Li Yan [b,1,2], Yuan-Hong Chen [b,1,3], Guo-Hua Zeng [b], Ying Zhou [b], He-Ping Chen [b], Wei-Jie Peng [a,b], Ming He [a,b,**], Qi-Ren Huang [a,b,*]

[a] Key State Laboratory of Food Science & Technology, Nanchang University, Nanchang 330047, PR China
[b] Key Laboratory of Basic Pharmacology in Jiangxi Province, Nanchang University, Nanchang 330006, PR China

ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form
23 December 2013
Accepted 8 January 2014
Available online 17 January 2014

Keywords:
Diallyl trisulfide
Endothelial cells
Diabetes mellitus
Oxidative stress

ABSTRACT

Persistent hyperglycemia increases a systemic oxidative stress, causing the onset of vascular endothelial dysfunction and atherosclerosis. Diallyl trisulfide (DAT), a natural organosulfur compound in garlic, has been reported to have actions of dilating blood vessels and antibacteria, etc. In this study, models of obese diabetic rat *in vivo* and high glucose concentration (HG)-induced endothelial cell injury *in vitro* were used to investigate the protective effects of DAT on vascular endothelial injury and its underlying mechanisms. In the *in vivo* model, the obese diabetic rats were injected venously with DAT (5.0 mg kg^{-1} d^{-1}) and Vitamin E (1.0 mg kg^{-1} d^{-1}) respectively, once daily for 7 consecutive days. In the *in vitro* model, HG-injured HUVEC were treated with or without DAT (25 μmol L^{-1}, 50 μmol L^{-1}, 100 μmol L^{-1}) or Vitamin E (25 μmol L^{-1}) respectively for 24 h. The extents of vascular endothelial injury and protective effects of DAT were evaluated. The results both *in vivo* and *in vitro* displayed that DAT-treatment significantly attenuated the endothelial cell impairments. Besides, DAT-treatment markedly decreased the levels of malondialdehyde (MDA) and reactive oxygen species, whereas elevated the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in mitochondrium. Moreover, DAT-treatment considerably improved mitochondrial respiration function. Taken together, our results suggest that DAT protects vascular endothelium from HG or hyperglycemia induced-injury by reducing mitochondrial oxidative stress. The findings provide a novel insight for DAT to potentially treat the oxidative stress diseases, *i.e.*, atherosclerosis, diabetes, and neurodegenerative diseases.

1. Introduction

Diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia, often accompanied by the structural and functional abnormalities of heart, brain and kidney. Increasing evidence has demonstrated that diabetes increases the risk of cardiovascular diseases, which is the overwhelming causes of morbidity and mortality in developed countries (Brown et al., 2010; Tandon et al., 2012). Hyperglycemia is considered as a basic pathological change in diabetes and plays a critical role in the development of diabetic vascular complications, such as atherosclerosis (Reusch and Wang, 2011). Vascular endothelial cells are the first barrier of vessels and are insulted by the hyperglycemia, but the underlying mechanism is not fully understood. During diabetes, the hyperglycemia generates a large amount of reactive oxygen species and causes the endothelial injury (Folli et al., 2011). Previous studies have shown that hyperglycemia-induced endothelial dysfunction is often resulted from overproduction of reactive oxygen species and considered as an initiating process of vascular complications in diabetes (Brouwers et al., 2010). An imbalance of reactive oxygen species metabolism induced by the hyperglycemia (overproduction or/and decreased clearance of reactive oxygen species) leads to oxidative stress damage through

Abbreviations: DAT, diallyl trisulfide; GSH-Px, glutathione peroxidase; HUVEC, human umbilical vascular endothelial cells; HG, high glucose concentration; LDH, lactate dehydrogenase; MDA, malondialdehyde; SOD, superoxide dismutase

* Corresponding author at: Key Laboratory of Basic Pharmacology in Jiangxi Province, Nanchang University, 461 Ba-Yi Street, Nanchang 330006, PR China. Tel./fax: +86 791 86361839.
** Co-Corresponding author at: Key Laboratory of Basic Pharmacology in Jiangxi Province, Nanchang University, 461 Ba-Yi Street, Nanchang 330006, PR China. Tel.: +86 791 86362231; fax: +86 791 86361839.
E-mail addresses: jxhm56@hotmail.com (M. He), qrhuang@ncu.edu.cn (Q.-R. Huang).
[1] Equal contributions to this article.
[2] Present address: Department of Pharmaclology, Yichun University, Yichun 336000, Jiangxi Province, PR China.
[3] Present address: Department of Internal Medicine, People's Hospital of Xinjian County, Nanchang 330100, PR China.

0014-2999/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2014.01.010

peroxidation of lipids, proteins, and DNA. Therefore, the antioxidative effect of antioxidants provides an avenue for prevention and therapy of diabetic vascular complications (Rocha et al., 2010).

Garlic is world-widely used as a flavoring and medicinal agent. Evidence from laboratory and clinical studies has revealed the biological benefits of the garlic, including antibacteria (Casella et al., 2013), anticarcinoma (Zeng et al., 2013), antioxidation (Khatua et al., 2012), lipid-regulating and glycemia-lowering etc. (Kumar et al., 2013). Diallyl trisulfide (DAT, $C_6H_{10}S_3$, FW: 178) is a natural organosulfur compound from the garlic. It was reported that DAT harbors the abilities to activate eNOS and reduce adhesion molecule expression induced by ox-LDL (Predmore et al., 2012). Moreover, Liu et al. (2005) also reported that both garlic oil and DAT have a good glycemic control in diabetic rats through increasing the secretion and sensitivity of insulin. In recent years, several studies have confirmed that DAT has beneficial effects on the cardiovascular diseases, including an increase of anti-oxidative activity and a decrease of peroxidized lipids (Predmore et al., 2012; Lei et al., 2010; Chiang et al., 2013). Clinical studies have also shown that a short-term treatment with DAT may ameliorate the impaired endothelial function in patients with coronary artery disease (Qi et al., 2000; Gu and Zhu, 2011). However, it still remains unclear whether DAT improves endothelial function impaired by high glucose concentration (HG) or hyperglycemia. Therefore, the purpose of this study was to investigate the protective effects of DAT on vascular endothelial cell injury and to explore its possible mechanisms both *in vivo* and *in vitro*.

2. Materials and methods

2.1. Agents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco-BRL (NY, USA). The assay kits of lactate dehydrogenase (LDH), SOD, MDA and GSH-Px were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, CHN). DAT, Vitamin E, streptozotocin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium nitroprusside, acetylcholine, phenylephrine and dimethyl sulfoxide (DMSO) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against SOD, GSH-Px and cytochrome *C* oxidase were purchased from Santa Cruz (CA, USA). DAT was dissolved in DMSO as a stock solution at a 100 mmol L^{-1} concentration, and the stock solution was then diluted with the medium to the desired concentration prior to use.

2.2. Establishment of obese diabetic rat model

All animal procedures were approved by the Institutional Animal Care and Use Committee of Nanchang University School of Medicine and conducted in accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No.85-23, revised 1996). The model establishment was performed according to the method described by Srinivasan et al. (2005) with minor modifications. Briefly, male Sprague-Dawley (SD) rats weighing 150–180 g (provided by Department of Experimental Animals, Nanchang University, CHN) were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2 °C) and humidity ($55 \pm 5\%$) with 12:12 h light and dark cycle. All the rats were randomly allocated into two dietary regimens by feeding with either standard chow or high fat diet (HFD, 58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) *ad libitum*, respectively, for the initial period of 2 weeks. After the first 2 weeks of dietary manipulation, the HFD-fed rats were injected intraperitoneally (i.p.) with a low dose of streptozotocin (STZ, 35 mg kg^{-1}) while the control rats were given a vehicle citrate buffer (pH 4.4) in a dose volume of 1 mL kg^{-1}, i.p., respectively. Eventually, all the rats were further fed with the corresponding diet for 4 weeks. The body weight and metabolic estimations such as fasting plasma glucose (FPG), fasting serum insulin (FINS) and triglycerides (TG) were measured just before modeling and after modeling. The HFD-fed rats with the FPG of ≥ 11.1 mmol L^{-1} were considered as obese diabetic rats.

2.3. Experimental protocol in vivo

Twenty-four obese diabetic rats were randomly divided into three groups, *i.e.*, dibetes mellitus(DM), DAT (5.0 mg kg^{-1} d^{-1}) and Vit E (1.0 mg kg^{-1} d^{-1}), respectively, and additional 8 rats fed by standard chow with normal FPG were considered as control one. The rats in DAT and Vit E group were injected from tail vein with DAT (5.0 mg kg^{-1} d^{-1}) or Vit E (1.0 mg kg^{-1} d^{-1}) respectively, once daily for 7 consecutive days; while those in DM and control group were given vehicle DMSO in a dose volume of 1 mL kg^{-1}. The rats were allowed to continue to feed on their respective diets until the end of treatment. In the end, the body weight and metabolic estimations (FPG, FINS and TG) were measured. In addition, the functions of endothelial-dependent and -independent aortic relaxation and morphometric changes of aorta were examined.

2.4. Blood sample collection and metabolic parameter assays

Before blood samples collected, the rats were fasted for 12 h. The blood samples from the pre-modeling rats were collected from the retro-orbital plexus of the rats under light ether anesthesia using capillary tubes into Eppendorf tubes containing heparin (20 μL, 200 IU mL^{-1}) and heparin-free, respectively. The blood samples from the post-modeling rats were collected from the carotid artery by anaesthetizing with ketamine (70 mg kg^{-1}, i.p.). The plasma was separated by centrifugation (5 min, 5000 rpm) and was used for detecting the FPG levels, while the serum for measuring the FINS and TG levels with the assay kits according to the manufacturer's instruction.

2.5. Functional assessment of rat aortic endothelium

The functional assessment of rat aortic endothelium was carried out by a modification of the previously described method (Zhang et al., 2009). Briefly, after the last administration of DAT or Vit E, the rats were fasted for 12 h and anaesthetized with ketamine (70 mg kg^{-1}, i.p.). Immediately after, the body weight was recorded and the blood samples were collected. Then, the rats were sacrificed by cervical dislocation. The thoracic aortae were rapidly isolated and carefully freed of adhering fat, connective tissue and the accompanying vein. The segment length of the aortic preparations was approximately 3 mm. The aorta rings were isometrically mounted in 10 mL organ baths filled with pre-warmed Kreb–Henseleit (K–H) solution (82.8 mmol L^{-1} NaCl, 4.7 mmol L^{-1} KCl, 2.4 mmol L^{-1} KH_2PO_4, 1.2 mmol L^{-1} $MgSO_4$, 2.7 mmol L^{-1} $CaCl_2$, 11.1 mmol L^{-1} dextrose, and 25 mmol L^{-1} $NaHCO_3$, pH 7.4, 37 °C) continuously gassed with 95%O_2–5%CO_2 in a myograph system (model 620 M, DMT, Denmark). An initial passive tension in aorta ring was set as 9.81 mN (2 g). All preparations were then allowed to equilibrate for at least 60 min before further experimentation. The aorta rings were precontracted with 1 μmol L^{-1} phenylephrine. Dose–response curve was obtained by cumulative addition of acetylcholine (10^{-7}–10^{-4} mol L^{-1}) and sodium nitroprusside (10^{-7}–10^{-4} mol L^{-1}).