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Research article

Total ginsenosides suppress monocrotaline-induced pulmonary hypertension in rats: involvement of nitric oxide and mitogen-activated protein kinase pathways

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ABSTRACT

Background: Ginsenosides have been shown to exert beneficial pharmacological effects on the central nervous, cardiovascular, and endocrine systems. We sought to determine whether total ginsenosides (TG) inhibit monocrotaline (MCT)-induced pulmonary hypertension and to elucidate the underlying mechanism.

Methods: MCT-intoxicated rats were treated with gradient doses of TG, with or without N^G-nitro-L-arginine methyl ester. The levels of molecules involving the regulation of nitric oxide and mitogen-activated protein kinase pathways were determined.

Results: TG ameliorated MCT-induced pulmonary hypertension in a dose-dependent manner, as assessed by the right ventricular systolic pressure, the right ventricular hypertrophy index, and pulmonary arterial remodeling. Furthermore, TG increased the levels of pulmonary nitric oxide, endothelial nitric oxide synthase, and cyclic guanosine monophosphate. Lastly, TG increased mitogen-activated protein kinase phosphatase-1 expression and promoted the dephosphorylation of extracellular signal-regulated protein kinases 1/2, p38 mitogen-activated protein kinase, and c-Jun NH2-terminal kinase 1/2.

Conclusion: TG attenuates MCT-induced pulmonary hypertension, which may involve in part the regulation of nitric oxide and mitogen-activated protein kinase pathways.

1. Introduction

Pulmonary hypertension is a progressive disease associated with increased constriction and remodeling of the pulmonary vasculature, ultimately leading to right heart failure. However, the pathological mechanism of pulmonary hypertension at the molecular level remains unclear. Nitric oxide (NO) is produced from L-arginine (L-arg) by endothelial NO synthase (eNOS) and causes smooth muscle relaxation by the activation of soluble guanylate cyclase, followed by the accumulation of cyclic guanosine monophosphate (cGMP) [1]. Evidence suggests that impaired NO production may lead to pulmonary hypertension [2]. Furthermore, daily treatment with an NO donor attenuates monocrotaline (MCT)-induced pulmonary hypertension and pulmonary vascular remodeling [3].

The mitogen-activated protein kinases (MAPKs)—including three members p38 MAPK, c-Jun NH2-terminal kinase 1/2 (JNK1/2), and extracellular signal-regulated protein kinase 1/2 (ERK1/2)—are a family of central signaling molecules that respond to numerous stimuli by phosphorylating a variety of substrates including

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transcription factors, enzymes, and other kinases, thereby orchestrating cellular proliferation, differentiation, survival, apoptosis, and inflammation. Recent evidence supports the notion that MAPKs may be involved in the remodeling of vasculature [4,5].

Ginsenosides are the main active ingredients in ginseng (*Panax ginseng* Meyer), a well-known and popular herbal medicine used in China. To date, at least 30 different ginsenosides have been extracted from the roots [6], stems, or leaves of *P. ginseng* [7]. Several cell culture and animal studies show that ginsenosides confer beneficial effects on the cardiovascular system through various mechanisms including adjusting blood pressure, modifying vasomotor function, stimulating NO production, and influencing ion channels [8,9]. We have shown previously that total ginsenosides (TG) can inhibit vascular smooth muscle cell proliferation, carotid neointimal hyperplasia, and ventricular hypertrophy both *in vivo* and *in vitro* [10–12]. However, little is known regarding the effect of TG on pulmonary hypertension. Therefore, we sought to investigate whether TG alleviates MCT-induced pulmonary hypertension in rats and to explore whether TG affects signaling pathways previously implicated in pulmonary hypertension.

2. Materials and methods

2.1. Materials

TG extracted from *P. ginseng* Meyer leaves and stems was received from Professor Rui Zhao (Beijing Naturally Occurring Drugs Research Institute, China) and quantified in our laboratory as described previously [13]. Briefly, TG was refluxed with 70% ethanol and then separated by column chromatography with D101 resin (Xi'an Jiaotong University School of Medicine, China). For quality control, TG chemical fingerprints were established on a Phenomenex ODS column using high-performance liquid chromatography at 203 nm. Ten ginsenosides (Rg1, Re, Rb1, Rb2, Rc, Rg2, Rb3, Rg3, Rf, and Rd) were identified from TG by comparing retention times with authentic compounds. The final product consisted of 21.60% Re, 18.24% Rg2, 15.36% Rb2, 13.65% Rd, 8.4% Rc, 5.26% Rb1, 5.20% Rg1, 4.64% Rb3, 2.15% Rg3, and 1.97%Rf, accounting for 96.47% of the TG, and other minor ginsenosides.

MCT was obtained from Sigma Chemical Co. (St. Louis, MO, USA). L-Arg and NG-nitro-L-arginine methyl ester (L-NAME, an eNOS inhibitor) were purchased from Alexis Biochemicals Company (Lausanne, Switzerland). Bicinchoninic acid protein assay kit was purchased from Merck (Whitehouse Station, NJ, USA). The NO detection kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and the cGMP assay kit was purchased from Amersham (UK). The reverse transcription-polymerase chain reaction (RT-PCR) kit was purchased from TaKaRa (Dalian, China). The primer of eNOS was synthesized by TaKaRa Biological Engineering Company (TaKaRa, Dalian, China). Antibodies recognizing eNOS, phospho-ERK1/2 (p-ERK1/2), phospho-p38MAPK (p-p38MAPK), phospho-JNK1/2 (p-JNK1/2), mitogen-activated protein kinase phosphatase-1 (MKP-1), and horseradish peroxidase-conjugated secondary antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and the catalog numbers were sc-650, sc-23759-R, sc-17852-R, sc-6254, and sc-370, respectively. All other chemicals were of reagent grade.

2.2. Animals and experimental design

All animal procedures were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. The protocol was approved by the Committee on Animal Research and Ethics of Xi'an Jiaotong University (Xi'an, China).

8 to 12-wk-old male Sprague–Dawley rats weighing 180–220 g were purchased from the Fourth Military Medical University, Xi'an, China. Two animals per cage were housed under constant temperature (25°C) and humidity (50%) and maintained on a 12-h light/dark cycle. Animals were maintained on standard rodent chow, and food and water were available *ad libitum*. Prior to the initiation of the experiment, rats were acclimatized to the environmental conditions for 1 wk.

All rats were given a single intraperitoneal injection (i.p.) of MCT (60 mg/kg) except for the control group (an equal volume of 0.9% saline). Each group consisted of 8–10 rats. Rats injected with MCT were randomly divided into seven groups: (1) MCT group—rats were administered with vehicle (i.p.); (2–4) TG group—rats were administered (i.p.) with TG at 20, 40, and 80 mg/kg/d (TG was dissolved in 0.9% saline, and the concentration of TG was 0.4%, 0.8%, and 1.6% (w/v), respectively); (5) L-arg group—rats were given L-arg (200 mg/kg/d, i.p.); (6) TG + L-N group—rats were given intragastric (i.g.) administrations of L-NAME at 20 mg/kg/d and i.p. administration of TG at 40 mg/kg/d; (7) L-a + L-N group—rats were administered with L-NAME (20 mg/kg/d, i.g.) and L-arg (200 mg/kg/d, i.p.). Control group rats were given i.p. with vehicle. All treatments continued for 18 d. TG doses were chosen according to our preliminary experimental results and previous reports [10,14].

2.3. Right ventricular systolic pressure and right ventricular hypertrophy index measurements

Eighteen days after MCT/saline injections, all surviving rats were anesthetized with sodium pentobarbital solution (40 mg/kg), and right ventricular systolic pressure (RVSP) was measured by right heart catheterization. The right jugular vein was isolated, and a small polyethylene catheter (PE-50 tube; American Health & Medical Supply International Corp., Scarsdale, New York, USA) was inserted through the right jugular vein via a small transverse cut and then advanced into the right ventricle (RV) under the guidance of the pressure waveform. The catheters were filled with heparinized saline (10 U/ml heparin in 0.9% saline). The other end of the catheter was connected to a biosignal acquisition processor, and RVSP was directly measured using PowerLab monitoring hardware and software (AD Instruments, Colorado Springs, CO, USA). Next, rats were killed by cervical dislocation under anesthesia. The chest was opened, and the whole lungs and hearts were excised.

The lungs and body were weighed, and the lung weight (LW)/body weight (BW) was calculated. The left lungs were removed for histological analysis, and the right lungs were excised and divided by the upper and lower lobes. The lower lobe of each group was used for NO and cGMP measurement, and the upper lobe was frozen in liquid nitrogen for real-time PCR and Western blot analysis.

The RV and left ventricle (LV) plus septum (LV + S) were weighed, and the weight ratio of RV/(LV + S) was calculated to assess the right ventricular hypertrophy index (RVHI).

2.4. Assessment of pulmonary artery remodeling

Left lungs were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned at 5 μm. Hematoxylin and eosin (HE) and elastic Van Gieson (EVG) stainings were performed according to common histopathological procedures. Pulmonary vascular remodeling was evaluated by determining wall thickness (WT) according to the method described by Barth et al [15]. External diameters (EDs) and WTs of at least 15 pulmonary arteries (50–100 μm in diameter) in each rat in control, MCT, TG 40 mg/kg, L-arg 200 mg/kg, TG + L-N, and L-a + L-N groups were assessed under 400× magnification using a computerized morphometric system (Qwin; Leica, Wetzlar, Germany) by two pathologists blinded to