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Protective effects of aloperine on monocrotaline-induced pulmonary hypertension in rats

Fan Wu[a,1], Yinju Hao[a,1], Jiamei Yang[a,1], Wanxia Yao[a,1], Yanping Xu[b,1], Lin Yan[c,1], Yang Niu[d,1], Tao Sun[e,1], Jianqiang Yu[a,f,1,*], Ru Zhou[a,d,f,1,*]

[a] Department of Pharmacology, College of Pharmacy, Ningxia Medical University, Yinchuan 750004, PR China
[b] Echocardiogram Room, Heart Center, General Hospital of Ningxia Medical University, Yinchuan750004, PR China
[c] College of Basic Medical Sciences, Ningxia Medical University, Yinchuan 750004, PR China
[d] Key Laboratory of Hui Ethnic Medicine Modernization, Ministry of Education, Ningxia Medical University, Yinchuan 750004, PR China
[e] Ningxia Key Laboratory of Craniocerebral Diseases of Ningxia Hui Autonomous Region, Ningxia Medical University, Yinchuan 750004, PR China
[f] Ningxia Hui Medicine Modern Engineering Research Center and Collaborative Innovation Center, Ningxia Medical University, Yinchuan 750004, PR China

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ABSTRACT

Pulmonary hypertension (PH) is serious, fatal disease which is promoted by oxidative stress. Aloperine have antioxidation effects, which effects on pulmonary arteries remain unclear. Therefore, this study is designed to investigate whether aloperine has protective effects on PH induced by monocrotaline and whether these effects are associated with oxidative stress.

PH was induced by monocrotaline (60 mg/kg), and subsequently oral administration of aloperine (25, 50, 100 mg/kg/day). At the end of the experiment, hemodynamic, pathomorphologic, electrocardiographic and echocardiographic data from the rats were obtained. At same time, oxidative stress biomarkers (superoxide dismutase, malonyldialdehyde, catalase, glutathione peroxidase, total antioxidant capacity) and the protein expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-2, NOX-4 in the lung of rat has been detected. The result shows that aloperine treatment showed significantly improvement in hemodynamic, pathomorphologic, electrocardiographic and echocardiographic data. Moreover, aloperine treatment can alleviate the changes of oxidative stress biomarkers and suppress the expression levels of NOX-2, NOX-4.

In summary, this study indicates that aloperine have protective effects on monocrotaline-induced PH. And these effects may be related to inhibit oxidative stress.

1. Introduction

Pulmonary hypertension (PH) is a life-threatening condition with a high mortality and the 5-year survival rate is only 49% [1]. PH is characterized by pulmonary vascular structural remodeling, excessive vessels constriction, and subsequently increased pulmonary pressure and eventually leading to right heart failure [2–5]. There are a number of drugs for treating PH, but these drugs have undesirable side-effects [6]. Therefore, novel drugs are required to prevent progression of pulmonary hypertension.

In recent years, accumulating evidence suggests that oxidative stress plays a vital role in the development of PH [7–9]. Pharmacological researches indicate that experimental drug treatments, such as fasudil [10], angelica dahurica [11], oxymatrine [12], could prevent progression of PH by interfering oxidative stress. In addition, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), which is a kind of superoxide generating enzyme, has been the focus of discussion due to NOX is able to generate reactive oxygen species (ROS) and is considered to be involved in pathogenesis of PH [13,14]. And many research also showed that down-regulation of NOX expression is related to the inhibition of the development of some cardiovascular disease, such as PH and hypertension [11,15,16]. Thus, antioxidation effects and interference of NOX may potentially serve as therapeutic target in inhibiting pulmonary hypertension.

Over the past decades, increasing attention was paid to Chinese medicines for its advantages, such as multi-targeted efficacy,

* Corresponding authors at: Department of Pharmacology, College of Pharmacy, Ningxia Medical University, Yinchuan 750004, PR China
E-mail addresses: yujq910315@163.com (J. Yu), zhou-ru926@163.com (R. Zhou).
[1] These authors takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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abundant resources, few side-effect, as well as lower cost [17]. Aloperine (ALO) is one of quinolizidine alkaloid extracted from traditional Chinese herb *Sophora flavescens Ait* [17–19]. Our previous study demonstrated that aloperine can attenuate neuropathic pain via its antioxidation in mice model [17]. But the protective effects of aloperine on PH have not been investigated.

Thus, we hypothesize that antioxidant effects of aloperine, as demonstrated in other study, may play a role in the treatment of pulmonary hypertension. To test this hypothesis, PH model of rats has been established by a single subcutaneous injection of monocrotaline (MCT), a pyrrolizidine alkaloid. And recent researches have shown that MCT-induced PH model imitates many characteristics of PH in patients, including oxidative stress, endothelial dysfunction, inflammatory infiltration and vascular remodeling [20–23]. Moreover, some non-invasive methods, including echocardiography, are proven to be reliable tools for analysis of the effects of MCT and therapeutic effects of potential drug candidates [24–26]. As these studies demonstrated that the changes in echocardiographic parameters, such as 'pulmonary artery acceleration time' (PAAT) and 'pulmonary artery deceleration' (PAD), are closely related to pulmonary pressure.

The experimental design of MCT-induced PH model could be divided into two categories, prevention protocol and treatment protocol. For the purpose of this study, we focused on the treatment strategy which is more relevant to the clinical practice [27,28]. Hence, we choose to use aloperine for therapy after subcutaneous injection for MCT 21 days.

In addition, in this study, sildenafil was selected as positive drug to verify the validity of experimental design and the reliability of the results. Sildenafil is a PDE-5 inhibitor and is also a widely recognized anti-PH drug. This drug can inhibit activity of PDE-5 to reduce cGMP degradation, subsequently causing relaxation of vascular smooth muscle and reversal of vascular remodeling, leading to pulmonary arterial pressure decreased.

In the present study, we tested the protective effect of aloperine on MCT-induced PH model. Afterwards, we examined the expression of NOX-2, NOX-4 and levels of oxidative stress in order to further elucidate the mechanisms of protective action of aloperine on PH

2. Materials and methods

2.1. Animals and reagents

Healthy adult male Sprague-Dawley rats with bodyweight between 180 and 220 g were obtained from the Experimental Animal Center of Ningxia Medical University (Ningxia, China). The rats were housed in a temperature-controlled environment with a 12 h light/dark cycle and allowed feed and water freely. The animal experimental protocol was approved by the Animal Experimental Committee Ningxia Medical University.

Aloperine was purchased from Dushun biological chemical company (Ningxia, China), with HPLC purity of 99%. Monocrotaline was purchased from Sigma-Aldrich Institute (MO, USA). Anti-smooth muscle alpha-actin (α-SMA, Cat NO: 55135-1-AP) and anti-β-actin (Cat NO: 20536-1-AP) polyclonal antibodies were supplied from Proteintech Group (CA, USA). Anti-NOX-2 monoclonal antibodies were obtained from ABCam Biotechnology (MA, USA, Cat NO: ab-129068), and Anti-NOX-4 polyclonal antibodies were obtained from Santa Cruz Biotechnology (CA, USA, Cat NO: sc-30141). The superoxide dismutase (SOD) determination kit, malonyldialdehyde (MDA) determination kit, catalase (CAT) determination kit, glutathione peroxidase (GSH-PX), and the determination kit of total antioxidant capacity (T-AOC) were purchased from Jiancheng Bioengineering Institute (Jiangsu, China).

2.2. Experimental design

Adult male rats were randomized to the six groups (6 rats in each group): (1) Control group; (2) Monocrotaline (MCT) group; (3) Aloperine (ALO) 25 mg/kg group; (4) Aloperine (ALO) 50 mg/kg group; (5) Aloperine (ALO) 100 mg/kg group; (6) Sildenafil group (Sildenafil at 30 mg/kg). The model of pulmonary hypertension was established by a single subcutaneously injecting monocrotaline with 60 mg/kg (control animals subcutaneously injecting saline) at day 0. Subsequently, all rats in control group and in one of MCT treatment groups (the MCT group) received vehicle intragastrically daily from day 21 to day 42; while rats in other groups received intragastrically aloperine at three doses or sildenafil from day 21 to day 42.

2.3. Echocardiographic measurement

Forty-one days after MCT administration, echocardiographic images were acquired on a GE VIVID7 ultrasonographic system (General Electric, CO, USA). The rat right ventricular wall thickness and right ventricular systolic and diastolic internal diameter were recorded by M-mode recordings. The maximum pulmonary velocity (PVmax), pulmonary artery acceleration time (PAAT) and pulmonary artery deceleration (PAD) was assessed by continuous wave Doppler recording. The calculation method of PAAT, PAD, PVmax has been previously described [24].

2.4. Hemodynamic and electrocardiographic (ECG) measurements

The day after echocardiographic measurement, the rats were weighed and anesthetized by 20% urethane (1 ml/100 g), subsequently the rats were tied to the operating table in the supine position. During the experiment, the rats were kept respiration spontaneously and freely. A positive electrode (Alcott biotech, Shanghai, China) is inserted in the left forepaw and a negative electrode was inserted into the rat left forepaw, respectively; while the ground electrode was inserted into the right hind paw to record ECG. After animal electrocardiogram was measured, the polyethylene catheter (Beijing Union Medical College, Department of Pathophysiology, Beijing, China) connected with pressure transducers (Alcott biotech, Shanghai, China) was inserted into right external jugular vein, through right atrium, right ventricle, it eventually reached pulmonary artery for measuring right ventricular systolic pressure (RVSP) and mean pulmonary arterial pressure (mPAP) by MPA-cardiac function acquisition and analysis system (Alcott biotech, Shanghai, China). The position of the catheter tip was determined by the waveform and was adjusted accordingly.

2.5. Evaluation of right heart hypertrophy

After measurement of hemodynamic parameters, heart and lung tissue of the rats in each group was completely removed. The heart tissue was than separated to evaluate right ventricular hypertrophy. The right ventricle and left ventricle plus septum were detached. Finally, weight of the dissected individually heart ventricular and left ventricle plus septum was determined for calculation of the right ventricular hypertrophy index (RVHI), the calculation formula as following: $RV/LV + S \times 100\%$.