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Ellagic acid prevents monocrotaline-induced pulmonary artery hypertension via inhibiting NLRP3 inflammasome activation in rats

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ABSTRACT

Background: Pulmonary artery hypertension (PAH) is characterized by vascular remodeling, high pulmonary blood pressure, and right ventricular hypertrophy. Oxidative stress, inflammation and pulmonary artery remodeling are important components in PAH. Ellagic acid (EA) is a phenolic compound with anti-oxidative, anti-inflammatory, and anti-proliferative properties. This study aimed to investigate whether EA could prevent the development of monocrotaline (MCT)-induced PAH in rats.

Methods: Male Sprague-Dawley rats received EA (30 and 50 mg/kg/day) or vehicle one day after a single-dose of monocrotaline (MCT, 60 mg/kg). Hemodynamic changes, right ventricular hypertrophy, and lung morphological features were assessed 4 weeks later. Activation of the NLRP3 (NACHT, LRR, and PYD domain-containing protein 3) inflammasome pathway in the lungs was assessed using Western blot analysis.

Results: MCT induced PAH, oxidative stress, and NLRP3 inflammasome activation in vehicle-treated rats. EA reduced the right ventricle systolic pressure, the right ventricular hypertrophy and the wall thickness/external diameter ratio of the pulmonary arteries compared with vehicle. EA also inhibited the MCT-induced elevation of oxidative stress, NLRP3, and caspase-1, IL-β in the lungs and the elevated levels of brain natriuretic peptide (BNP) and inflammatory cytokines in serum.

Conclusions: Ellagic acid ameliorates monocrotaline-induced pulmonary artery hypertension via exerting its anti-oxidative property inhibiting NLRP3 inflammasome signal pathway in rats.

1. Introduction

The understanding of pulmonary artery hypertension (PAH) has undergone a paradigm shift in recent years. PAH was once thought to be caused by increased vasoconstrictor tone; however, vasodilators did not have the expected satisfactory clinical outcome. The characteristic vascular abnormalities of PAH include abnormal muscularization of distal precapillary arteries and medial thickening of large pulmonary muscular arteries [1]. PAH is now seen as a vasculopathy in which structural changes driven by excessive vascular cell growth and inflammation, along with the recruitment and infiltration of circulating cells, play a major role.

The pro-inflammatory cytokine interleukin 1-beta (IL-1β) has been implicated in PAH [2–7]. The NLRP3 inflammasome, comprising the NLR protein NLRP3, the adapter ASC, and pro-caspase-1, is central to the activation of IL-1β [8,9] and plays a key role in innate immunity [9] and lung injury [10,11].

The generation of reactive oxygen species (ROS) is the central element regulating NLRP3 activation [8,12]. Coincidentally, multiple studies implicate oxidative stress (OS) in the development of PAH [13–16]. OS has been associated with alterations in the ROS and nitric oxide signaling pathways. Dysregulation of the oxidant/antioxidant balance impairs vascular tone and contributes to the pathological activation of antiapoptotic and mitogenic pathways, leading to cell proliferation and obliteration of the vasculature [17]. Antioxidant intervention shows protective effects in experimental PAH [14,15]. However, the underlying mechanism has never been fully understood. As a polyphenolic compound, ellagic acid (EA) possesses multiple biological activities such as radical scavenging [18], antioxidant [19,20] and anti-proliferative activities [21]. The aim of the present study was to advance the understanding of the detailed interaction between antioxidants, OS, the NLRP3 inflammasome, IL-1β and PAH.

Considering the critical role of the NLRP3 inflammasome in innate immunity and the multiple biological activities of EA, we hypothesized that the NLRP3 inflammasome pathway may be activated in PAH and that EA may prevent the progression of PAH. To test our hypothesis, we investigated the chronic efficacy of EA treatment in monocrotaline (MCT)-treated rats. We particularly addressed the question of whether EA can exert beneficial effects on PAH through inhibition of the NLRP3 inflammasome.

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2. Methods

2.1. Animal model

Male Sprague-Dawley rats (200–220 g) were provided by the Sun Yat-sen University Laboratory Animal Center (Guangzhou, China). The animals received humane care in compliance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Rats were randomly divided into four groups as follows: (1) Control group (n = 8) received normal saline (NS) + vehicle, (2) MCT group (n = 10) received MCT + vehicle, (3) EA + MCT group (n = 8) received MCT + EA at 30 mg/kg/day and (4) EA-high group (n = 8) received MCT + EA at 50 mg/kg/day. The PAH model was established by a single dose of 60 mg/kg of MCT injected intraperitoneally, while the Control group was injected with NS only. MCT (Sigma-Aldrich, St. Louis, MO, US) was dissolved in 1 N HCl neutralized with 1 N NaOH and diluted with NS. EA (Sigma-Aldrich, St. Louis, MO, US) dissolves poorly under natural aqueous environments. Methyl-beta-cyclodextrin (Me-β-CD) was applied to increase bioavailability of EA [22]. MCT-treated rats received, by gavage, either EA (30 and 50 mg/kg body weight, in 10% Me-β-CD). 10% Me-β-CD was used as vehicle. The EA treatments started one day after the MCT injection and were maintained daily for 4 weeks. Body weight was measured weekly to adjust the dose accordingly.

2.2. Hemodynamic measurements

Rats were anesthetized with pentobarbital (60 mg/kg, i.p). Right ventricle systolic pressure (RVSP) was measured by right heart puncture [14]. Systemic arterial pressure was monitored by cannulating the right carotid artery. Heart rate was monitored by electrocardiography. The RVSP was measured using 22-gauge I.V. catheter that was inserted into the right ventricle without regular thoracotomy, reducing blood loss and hemodynamic changes. Briefly, tracheotomy was performed, and rats were mechanically ventilated with room air (Harvard Rodent Ventilator, model 683; Harvard Apparatus Co., Millis, MA) using a tidal volume of 8 ml/kg and a respiratory rate of 80 breaths per min. After the thoracic cavity was opened using an upper abdominal incision approach by freeing the xiphoid process and opening the diaphragm, a 22-gauge I.V. catheter (Insyte-W, Becton Dickinson, Utah, U.S.) was inserted directly into the right ventricle. Systemic arterial pressure and RVSP were recorded using a miniature pressure transducer (TSD104A, BIOPAC Systems Inc., U.S.) digitized by a BIOPAC MP100 data acquisition system. After hemodynamic analysis, blood from the RV were stored at room temperature for 1 h and then centrifuged at 3000 rpm at 4 °C for 15 min. Blood serum was collected and stored at −80 °C, and the rats were euthanized by exsanguination. Lungs and hearts were excised for Western blotting (stored at −80 °C) and histological analysis.

2.3. Right ventricular hypertrophy and morphological measurements

The RV wall was separated from the LV wall and the ventricular septum and then weighed. The weight ratio of the right ventricle to the left ventricle plus the septum (RV/(LV + S) ratio) was calculated as an index of right ventricular hypertrophy. Lung tissue was flushed with cold saline through the pulmonary artery. The lung tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. After hematoxylin and eosin (HE) staining was performed, these sections were examined using light microscopy (Inverted Fluorescence Microscope, NIKON Eclipse Ti-E, NIKON, Japan). Morphometric analysis was performed in the pulmonary artery with an external diameter of 25–100 μm. Medial wall thickness was calculated with the following formula: medial

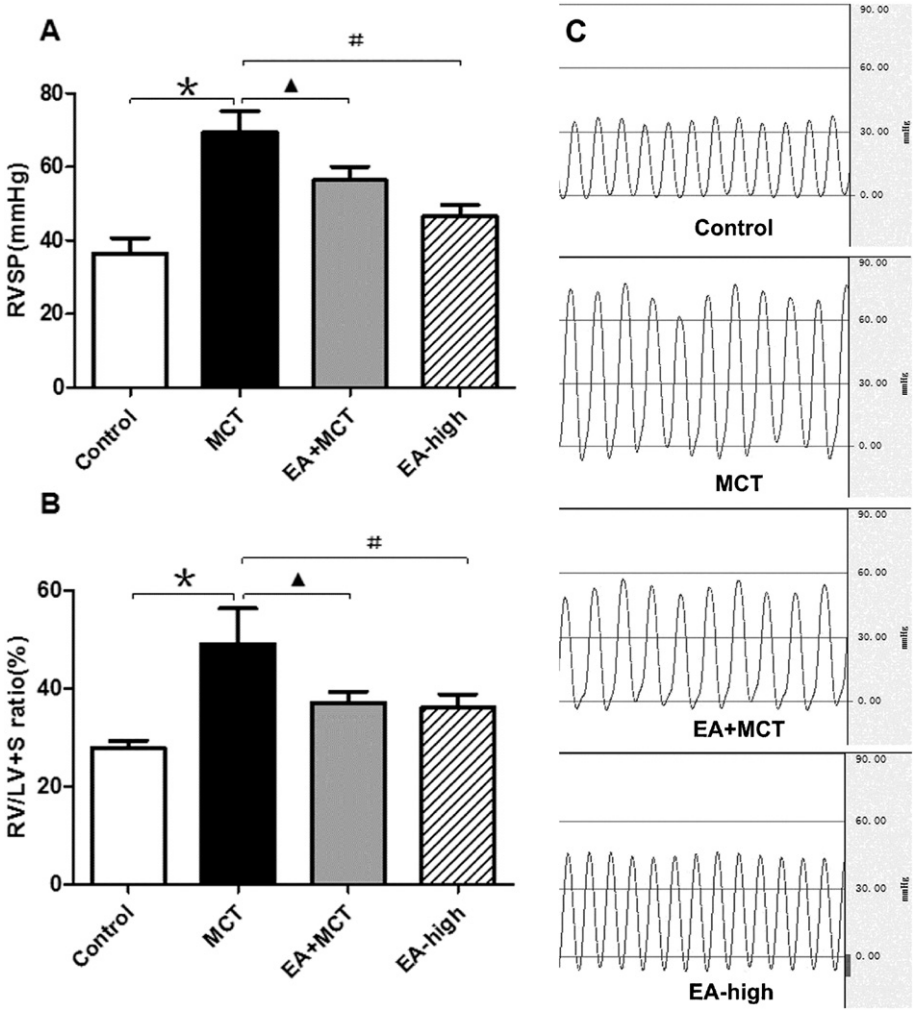

Fig. 1. EA alleviates hemodynamic changes and right ventricular hypertrophy in MCT-induced PAH 4 weeks after MCT exposure. EA decreased RVSP in MCT-treated rats. Further a dose-dependent manner presented, the RVSP of EA-high group was lower than EA-MCT group ($p < 0.05$). EA also reduced the RV/(LV + S) ratio in MCT-treated rats. Representative hemodynamic data (RVSP) from BIOPAC MP100 data acquisition system were showed in Fig. 1C. Data represent means ± SD. $^{*}p < 0.05$ versus Control group; $^{▲}p < 0.05$ versus MCT group; $^{\#}p < 0.05$ versus Control group. n = 8 per group. MCT, monocrotaline; PAH, pulmonary artery hypertension; EA, ellagic acid; RVSP, right ventricle systolic pressure; RV/LV + S ratio, the right ventricular weight to left ventricular plus septal weight ratio.