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 polyphenolic 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.

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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].

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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.
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, tracheostomy 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 lungs were dissected out, followed by perfusion fixation with 4% paraformaldehyde. The lung tissues were embedded in paraffin, and sections were prepared. 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 wall thickness = medial wall area + lumen area. The RVSP 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. 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 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.

![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.](image)
thickness (%) = medial wall thickness/external diameter × 100. For quantitative analysis, 20 randomly selected vessels from each rat were counted, and the average was calculated.

2.4. Enzyme-linked immunosorbent assay for BNP in serum

BNP is a 32 amino acid polypeptide secreted by myocytes in response to excessive stretching of ventricles. It is used for the diagnosis and staging of heart failure [23]. Of the range of biomarkers investigated in PAH to date, only BNP and its N-terminal cleavage product have been included as prognostic parameters in PAH treatment guidelines [24, 25]. Their levels elevate in proportion to the degree of RV dysfunction and RV remodeling in PAH. Blood samples from the RV were stored at room temperature for 1 h and then centrifuged at 3000 rpm at 4 °C for 15 min. Serum samples were collected and stored at −80 °C. BNP levels were analyzed using a commercial EIA kit (RayBiotech, Norcross, GA, USA) according to the manufacturer's instructions.

2.5. Measurement of oxidative stress in lung

Lung tissues were homogenized in saline at 4 °C. The homogenates were centrifuged at 3000 rpm at 4 °C for 10 min. The protein concentrations of the supernatants were measured with the BCA Protein Assay kit (Beyotime Institute of Biotechnology, Jiangsu, China). In lung homogenate supernatants, superoxide dismutase (SOD) activity was determined using SOD assay kits (Jiancheng Bio-Technology, Nanjing, China) and is expressed as U/mg protein. Furthermore, the lipid peroxide product malondialdehyde (MDA) as an index of lipid peroxidation and a stable indicator of OS, was determined with the TBA assay using an MDA kit (Jiancheng Bio-Technology, Nanjing, China) and expressed as nmol/mg protein.

2.6. The Bio-Plex Pro™ rat cytokine immunoassay

We determined the levels of IL-1β, IL-2, IL-4, IL-6, IL-10, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and macrophage inflammatory protein 1 alpha (MIP-1α) in serum using the commercially available BPLX Pro Reagent Kit (Bio-Rad, Hercules, CA, USA). The Bio-Plex Pro™ rat cytokine immunoassay was performed according to the manufacturer’s instructions.

2.7. Western blotting

Lung tissues were homogenized in ice-cold lysis buffer (Cell Signaling Technologies, Beverly, MA) and then centrifuged at 10,000 × g for 20 min. The protein concentrations...
of the supernatants were measured with the BCA Protein Assay kit (Beyotime Institute of Biotechnology, Jiangsu, China). The total proteins were incubated in boiling water for 10 min. Equal amounts of total protein were separated on 8–12% SDS-PAGE gels and electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, U.S.) pre-activated by methanol in the transfer buffer. The membranes were blocked with 5% skimmed milk for 1 h and incubated with specific primary antibodies in 3% BSA at 4 °C with gentle shaking, overnight. β-actin (Cell Signaling Technologies, Beverly, MA) was used as a loading control. Immunoreactive bands were detected using HRP-conjugated goat anti-rabbit IgG as the secondary antibody (1:2000) (Cell Signaling Technologies, Beverly, MA) pre-activated by methanol in the transfer buffer. The membranes were biotinylated using streptavidin–HRP (1:5000) (Cell Signaling Technologies, Beverly, MA, U.S.) pre-activated by methanol in the transfer buffer. The membranes were then incubated with avidin–HRP (1:5000) (Vector Laboratories, Burlingame, CA) for 30 min. Immunoreactive bands were detected using enhanced chemiluminescence (Millipore, Bedford, MA). Primary antibodies included rabbit anti-rat polyclonal antibodies against caspase-1 (1:500) (Abcam Ltd. Hong Kong), NLRP3 (1:500) (LifeSpan Biosciences, Seattle, WA), IL-1β (1:1000) (Abcam Ltd. Hong Kong) and rat β-actin (1:1000) (Cell Signaling Technologies, Beverly, MA).

2.8. Statistical analysis

All above assessments were carried out in a blinded fashion. All data were expressed as the mean ± standard deviation (SD). Differences in measured variables between groups were determined by one-way analysis of variance followed by the Student–Newman–Keul test for multiple comparisons. p-Values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS software (version 16.0).

3. Results

3.1. EA prevents the MCT-induced increase in RVSP, right ventricular hypertrophy, and pulmonary artery remodeling

Only two rats in MCT group died during the course of this study. Neither MCT nor EA affected systemic arterial pressure. MCT injection led to lower body weight than Control rats (328.7 ± 19.6 g vs. 363.7 ± 19.2 g, p < 0.05). EA administration didn’t improve the decreased weight growth caused by MCT (p > 0.05). MCT-exposed rats developed right ventricular hypertrophy (RV/(LV + S) ratio: 49.3 ± 7.4% vs. 27.8 ± 1.5%, p < 0.05) and PAH (RVSP: 69.5 ± 5.7 mm Hg vs. 36.5 ± 4.2 mm Hg, p < 0.05) four weeks after MCT injection. EA treatment reduced the RVSP with a dose-dependent manner. EA-high (50 mg/kg/day) group showed lower RVSP than EA + MCT (30 mg/kg/day) group (47.8 ± 2.8 mm Hg vs. 56.4 ± 3.7 mm Hg, p < 0.05), but still higher than Control group (47.8 ± 2.8 mm Hg vs. 36.5 ± 4.2 mm Hg, p < 0.05) (Fig. 1A). EA + MCT group got lower RV/(LV + S) ratio than MCT group (37.1 ± 2.2% vs. 49.3 ± 7.4%, p < 0.05). However, EA-high group didn’t show further improvement of RV hypertrophy than EA + MCT group (p > 0.05) (Fig. 1B).

MCT-exposed rats showed pulmonary artery remodeling (Fig. 2B). Compared with controls, MCT-exposed rats demonstrated an increased medial thickness of pulmonary arteries (20.9 ± 3.7% vs. 11.3 ± 2.9%, p < 0.05) (Fig. 2E). These lung pathological changes were ameliorated by EA treatment (EA + MCT vs. MCT: 15.9 ± 2.8% vs. 20.9 ± 3.7%, p < 0.05; EA-high vs. MCT: 14.9 ± 2.9% vs. 20.9 ± 3.7%, p < 0.05). However, there was no dose-dependent manner found (Fig. 2E).

3.2. EA suppresses the MCT-induced increase in serum BNP

Plasma BNP is used for the diagnosis and staging of heart failure[23]. Four weeks after MCT administration, serum BNP levels were markedly higher in MCT-injected rats than in Controls (876.3 ± 194.9 pg/ml vs. 278.3 ± 41.5 pg/ml, p < 0.05). This increase was suppressed by EA (30 mg/kg/day) (141.1 pg/ml ± 142.2 pg/ml, p = 0.05) (Fig. 3).

3.3. EA attenuates OS in the lungs

The attenuation of OS by EA (30 mg/kg/day) was associated with the preservation of SOD activity as well as the down-regulation of MDA (Fig. 4). Increasing evidence indicates that OS plays a causal role in the development of PAH[13]. SOD activity was inhibited by MCT (3.9 ± 1.8 U/mg protein vs. 10.4 ± 1.3 U/mg protein, p < 0.05), whereas EA treatment reversed this inhibition (8.6 ± 2.4 U/mg protein vs. 3.9 ±

Fig. 3. EA decreases serum BNP levels in MCT-induced PAH rats. Enzyme-linked immunosorbent assay showed that MCT-treated rats had higher BNP levels compared with Controls, whereas EA supplementation reduced the MCT-induced increase in BNP levels. Data represent means ± SD. n.s., not significant; *p < 0.05 versus Control group; ▲p < 0.05 versus MCT group. n = 8 per group. EA, ellagic acid; MCT, monocrotaline; PAH, pulmonary artery hypertension; BNP, B-type natriuretic peptide.

Fig. 4. EA suppresses OS in the lungs of MCT-treated rats. Data represent means ± SD. SOD activity in the MCT group was lower than that in the Control group, but in the EA group, it was higher than in the MCT group. The MDA level in the lungs of the MCT group was higher than that in the Control group, and in the EA group, it was lower than in the MCT group. n.s., not significant; *p < 0.05 versus Control group; ▲p < 0.05 versus MCT group. n = 8 rats per group. EA, ellagic acid; MCT, monocrotaline; SOD, superoxide dismutase; MDA, malondialdehyde.
3.4. EA attenuates inflammatory cytokine levels in serum

Development of pulmonary artery hypertension was associated with the up-regulation of inflammatory cytokines IL-1β (801.3 ± 277.9 pg/mg vs. 90.0 ± 1.6 nmol/mg protein, p < 0.05), IL-2 (801.8 ± 234.1 pg/mg vs. 99.3 ± 44.2 pg/mg, p < 0.05), IL-4 (339.1 ± 127.5 pg/mg vs. 15.8 ± 5.5 pg/ml, p < 0.05), IL-6 (1000.1 ± 565.2 pg/mg vs. 20.3 ± 6.8 pg/ml, p < 0.05), IL-10 (1810.9 ± 717.8 pg/mg vs. 514.1 ± 31.7 pg/ml, p < 0.05), MIP-1α (186.3 ± 58.2 pg/mg vs. 30.5 ± 18.1 pg/ml, p < 0.05) and IFN-γ (226.5 ± 113.3 pg/mg vs. 16.0 ± 4.7, p < 0.05) in the serum of MCT-treated rats. EA (30 mg/kg/day) treatment reversed these changes (p < 0.05) (Fig. 5A-G). For TNF-α, there was no statistical significance, despite a downward trend after EA treatment (Fig. 5H).

3.5. EA inhibits inflammasome activation in rats with MCT-induced PAH

Western blot analysis demonstrated that the lung NLRP3 (Fig. 6A, B) and caspase-1 (we analyzed the active subunit p20) (Fig. 6C, D) were up-regulated in MCT-treated rats compared with the control group. Activated caspase-1 (p20) contributes to the maturation of IL-1β [8,9]. Consistent with the increased ratio of active caspase-1 (p20)/β-actin, increased expression of IL-1β (the active subunit p17) was also observed in the lungs of MCT-treated rats (Fig. 6E, F). These results suggest a role of NLRP3 inflammasome activation in lung inflammation under the pathologic condition of PAH in rats. In MCT-exposed rats treated with EA (30 mg/kg/day), we found an attenuation of lung NLRP3, caspase-1 (p20), and IL-1β (p17) expressions (Fig. 6).

4. Discussion

The major findings of this study were as follows: 1) EA improved hemodynamics (dose-dependent), right ventricular hypertrophy and pulmonary vascular remodeling in rats with MCT-induced PAH. 2) MCT up-regulated oxidative stress, NLRP3 signal pathway and increased inflammatory activity in the lungs and serum. 3) Pathophysiology improvements with EA treatment were associated with a downregulation of NLRP3 signal pathway.

Progressive increases in pulmonary vascular resistance and pressure impair the performance of the right ventricle, resulting in right-heart failure, and ultimately death [17]. The RVSP, which well reflected the pulmonary artery pressure, decreased after EA treatment. The right ventricular response to increased pressure load is recognized as critical to survival [26]. MCT-treated rats developed right heart failure with increased RV/(LV + S) ratio and medial hypertrophy relative to the control group. EA prevented MCT-induced PAH and subsequent right heart failure. BNP is an ideal biomarker reflecting RV dysfunction and disease severity in PAH. It has been considered a prognostic parameter in PAH treatment guidelines [25]. The BNP level increased in MCT-treated rats, indicating infarcted right ventricle dysfunction due to PAH. EA improved impaired right ventricle dysfunction and reduced BNP levels.

Although, several studies have demonstrated the potential benefits of natural antioxidants in PAH, the underlying mechanism has not been fully determined. Genistein and resveratrol have been demonstrated to prevent MCT-induced PAH in rats [7,27]. In present study, we identified EA as another potential treatment for PAH, and found the common mechanism of action for antioxidants in PAH.

Inflammation plays a key role in human PAH as well as in experimental models [28,29]. In response to injury and stress, lung vascular cells produce inflammatory mediators, thereby recruiting inflammatory cells. Inflammatory cells might perpetuate the release of cytokines and growth factors, forming positive feedback loops. These processes could lead to vascular remodeling by matrix remodeling, collagen deposition, and vascular cell proliferation and migration in PAH [28,29] and, finally, to increased pulmonary resistance and right heart failure.

IL-1β is a prototypic multifunctional cytokine involved in inflammation. Many studies have indicated that IL-1β plays a pivotal role in the pathogenesis of PAH [2–7]. Consistent with previous findings, we found that serum and lung IL-1β levels are elevated in MCT-treated rats, along with the up-regulation of other pro-inflammatory cytokines (Fig. 5). Additionally, EA can attenuate MCT-induced PAH while inhibiting inflammation in rats. These results further demonstrate the
pivotal role of inflammation, especially IL-1β, in the pathogenesis of PAH.

In addition to the previously mentioned inflammatory reaction, OS is another characteristic of PAH. OS reflects an imbalance between the systemic manifestation of ROS and the antioxidant ability to eliminate these reactive intermediates. A major role for the increased generation of ROS in the pathogenesis of experimental PAH is indicated by antioxidant intervention studies performed in fetal lambs [30], hypoxia-exposed mice [15] and rats [31], permanent high-flow exposed rats [32], and monocrotaline-exposed rats [14]. Additionally, PAH patients present increased OS [33]. SOD acts as the first line of defense against ROS by dismutating superoxide to H$_2$O$_2$. Kamezaki et al. reported that gene transfer of extracellular SOD ameliorates MCT-induced PAH in rats [14]. ROS-reducing strategies induced by an SOD mimetic improve chronic hypoxia-induced PAH [15]. In the present study, we detected increased OS in the lungs of rats in MCT group. EA-treated rats presented improved vascular remodeling coupled with decreased OS. However, the exact mechanism by which antioxidants prevented PAH and inflammatory status remained elusive until the discovery of the NLRP3 inflammasome.

The NLRP3 inflammasome is activated in several types of lung injury. Wu et al. suggested that the alveolar macrophage NLRP3 inflammasome may sense lung alveolar stretching to induce the release of IL-1β [11]. NLRP3-deficient mice display a suppressed inflammatory response and blunted lung epithelial cell apoptosis in response to hyperoxia-induced acute lung injury [34]. The generation of ROS is central to NLRP3 activation [8,12], which in turn, is critical to the activation of caspase-1 and IL-1β [8,9]. The roles of inflammation, caspase-1, and IL-1β in the pathogenesis of experimental PAH have been shown by several specific antagonist intervention studies performed in rats with MCT-induced PAH respectively [2,35,36]. Our findings are in accordance with previous studies implying that polyphenolic antioxidants can

Fig. 6. EA inhibits lung NALP3 inflammasome activation in MCT-treated rats. Representative Western blot results and graph showed lung NALP3, caspase-1 and IL-1β expression 4 weeks after MCT injection in different groups of rats. Relative protein levels of NALP3, caspase-1(p20) and IL-1β(p17) were determined after normalization with β-actin. Data are the means ± SD. The NLRP3 inflammasome pathway was activated in MCT-induced PAH rats, further EA treatment can down-regulate this signal pathway. *p < 0.05 versus Control group; ▲ p < 0.05 versus MCT group; #p < 0.05 versus Control group. n = 6 per group. EA, ellagic acid; MCT, monocrotaline; IL-1β, interleukin-1beta.
attenuate MCT-induced pulmonary hypertension in rats [7,27]. Since Villegas et al. reported that the NLRP3 inflammasome pathway was activated in hypoxic PAH in mice [15], this result contradicted the finding that IL-1ra cannot inhibit the development of PAH in chronically hypoxic rats but can in MCT-treated rats [2]. Those two researches suggested the pivotal role of IL-1β in MCT-induced PAH but chronic hypoxic PAH. Here, we showed that the MCT can induce increased OS, IL-1β elevation and NLRP3 inflammasome activation in the lungs. Considering the respective roles of OS, ROS, inflammation and IL-1β in the pathogenesis of PAH, as well as the interactions between ROS, the inflammasome and IL-1β, we propose that the effect of antioxidants on PAH may contribute to the inhibition of the NLRP3 inflammasome by inhibiting OS.

EA inhibited the NLRP3 inflammasome in MCT-treated rats, primarily by exerting its antioxidant effects to reduce OS, which has been demonstrated in other studies. In ischemia–reperfusion (I/R) injury, EA can ameliorate lung injury and OS after intestinal I/R injury in rats [37]. In lung disease, increased ROS production activates inflammatory cells in the pulmonary defense system, causing pulmonary fibrosis in the bleomycin (BLM)-induced lung fibrosis model [38]. EA attenuates BLM-induced pulmonary toxicity in rats [39]. Pulmonary hypertension, and exercise capacity reduction are also present in BLM-treated rats. These findings aided our understanding of that EA treatment provides benefits in MCT-treated PAH rats.

Limitations of our study include the absence of specific NLRP3 antagonist interference or NLRP3 gene deficiency animal analysis to confirm the role of NLRP3 in PAH, additional experiments if EA can reverse the established PAH.

5. Conclusion

The pathophysiological changes in inflammation-related PAH may involve the activation of the NLRP3 inflammasome pathway. EA can attenuate MCT-induced PAH, pulmonary vascular remodeling and right ventricular hypertrophy through exerting inhibiting NLRP3 inflammasome signal pathway. It was demonstrated that EA was a promising treatment candidate for PAH and established the role of the NLRP3 inflammasome pathway in the pathogenesis of PAH.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2014.11.161.

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