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Research Article

Angiotensin-converting enzyme 2 activation ameliorates pulmonary endothelial dysfunction in rats with pulmonary arterial hypertension through mediating phosphorylation of endothelial nitric oxide synthase

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Abstract

This study aims to investigate the effect of angiotensin-converting enzyme 2 (ACE2) activation on pulmonary endothelial function in the process of preventing pulmonary arterial hypertension (PAH) in rat models and to explore the underlying mechanisms. Specific pathogen free rats were randomly divided into five groups including control group, PAH group, PAH + Resorcinolnaphthalein (Res) group (ACE2 activation), PAH + Res + MLN4760 group (ACE2 inhibition), and PAH + Res + L-NAME group (endothelial nitric oxide synthase [eNOS] inhibition). Rat PAH model was constructed using combined left pneumonectomy with a single dose of monocrotaline injection 1 week after the surgery, and the rats were then given corresponding reagents. Hemodynamics, endothelial function, and pathologic changes were evaluated 3 weeks after monocrotaline injection. The concentration of nitric oxide (NO), expression of eNOS, and phosphorylation of eNOS at Ser1177 and Thr495 in the lung tissues from rats were also investigated.The Res-induced activation of ACE2 led to decreased mean pulmonary arterial pressure (mPAP) and pulmonary artery remodeling in the PAH + Res group comparing with the PAH rats ($P < .05$). In addition, the reduction in mPAP induced by acetylcholine (Ach) was augmented in PAH + Res group ($P < .05$), but this was not observed under the treatment with sodium nitroprusside (SNP) ($P > .05$). The ratio of decrease in mPAP caused by Ach to that caused by SNP (Ach/SNP) was also increased ($P < .05$) in ACE2-activated rats. However, the protective effects of ACE2 activation on PAH were counteracted by co-administration of MLN4760, an ACE2 antagonist (all $P > .05$). The mechanistic study showed that the concentration of NO in the lung tissues was downregulated in the PAH group but upregulated in the PAH + Res group ($P < .05$), whereas the NO concentration in the PAH + Res + MLN4760 group was not obviously different from that in the PAH group ($P > .05$). Regarding the factors regulating NO release, we found that the eNOS was upregulated in the PAH group, and Res did not affect the expression of eNOS. The phosphorylation of eNOS at Ser1177 was increased but at Thr495 was reduced after Res injection, when compared with the PAH group ($P < .05$). As expected, co-injection of MLN4760 eliminated these differences ($P > .05$). The reduction in mPAP induced by Ach was attenuated in the PAH + Res + L-NAME group compared with the PAH + Res group ($P < .05$), but this was not observed in rats treated with SNP ($P > .05$). The Ach/SNP ratio of decline in mPAP was also decreased in the PAH + Res + L-NAME group ($P < .05$). Activation of ACE2 had a protective role in the development of PAH via improving the function of pulmonary arterial

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endothelium. This effect was potentially mediated by promoted NO release as a consequence of increased phosphorylation of eNOS at Ser1177 and dephosphorylation of eNOS at Thr495. J Am Soc Hypertens 2017;■(■):1–11.

Keywords: ACE2; endothelial function; NO.

Introduction

Pulmonary arterial hypertension (PAH) is a refractory disease caused by a variety of etiologies. Endothelial dysfunction, which can be manifested as a deficiency in endothelium-dependent pulmonary vasodilation, has been considered as an important pathogenesis of PAH. Angiotensin-converting enzyme 2 (ACE2) is an ACE homolog and its main function is to convert angiotensin II into angiotensin-(1-7).[1,2] In previous studies, we found that increased angiotensin-(1-7) and decreased angiotensin II induced by ACE2 activation ameliorated PAH through protecting endothelial cells in rats,[3,4] which has also been reported by others,[5–7] but the underlying mechanism remains largely unknown. Nitric oxide (NO) plays a critical role in endothelial function, and the endothelial generation of NO from L-arginine was catalyzed by endothelial nitric oxide synthase (eNOS).[8]

Phosphorylation of specific sites is an important mechanism to regulate eNOS activity and results in increase or decrease in NO production. eNOS phosphorylation at Ser1177 promotes the enzymatic activity and NO release, in contrast, eNOS phosphorylation at Thr 495 reduces the enzymatic activity and NO generation.[9] Based on our previous study,[3,4] the present study further investigated the effect of ACE2 on eNOS activation and NO release during regulating pulmonary endothelium function in the development of PAH. We found that activation ACE2 effectively downregulated the pulmonary arterial pressure (PAP) and promoted the endothelium-dependent responsive vasodilation in PAH rat models. This protective role of ACE2 was achieved through activation of eNOS by phosphorylation at Ser1177 and dephosphorylation at Thr 495 and subsequent increase in NO release.

Materials and Methods

Animals and Treatments

Specific pathogen free grade male Sprague–Dawley rats, 200–220 g in weight, were obtained from Beijing Weitong Lihua Laboratory Animal Technology Ltd. Co (Beijing, China). The PAH rat model was performed by lobectomy combined with monocrotaline injection as previously described.[3] Briefly, 1 week after left pneumonectomy, a single dose of monocrotaline (Sigma-Aldrich, St. Louis, MO, USA) at 40 mg/kg was infused subcutaneously into the abdominal wall (PAH group, n = 12). Resorcinolnaphthalein ([Res] an ACE2 activator; Cayman Chemical, Ann Arbor, MI, USA) was continuously infused at 120 μg/d[7] in PAH rats for investigation of the effects of ACE2 activation (PAH + Res group, n = 12), and MLN4760 (an inhibitor of ACE2; Millennium Pharmaceuticals, Cambridge, MA, USA) at 30 mg/kg/d plus Res at 120 μg/d were continuously infused in PAH rats to further confirm the effects (PAH + Res + MLN4760 group, n = 12). To assess whether the eNOS plays a role in ACE2 activation, L-NAME (an inhibitor of eNOS; Sigma-Aldrich) was infused intraperitoneally once a day at a dose of 50 mg/kg/d for 3 weeks in PAH rats received Res injection (PAH + Res + L-NAME group, n = 12). On the first day after monocrotaline injection, Res and MLN-4760 in separate micro-osmotic pumps (Alzet Osmotic Minipumps, Durect Corporation, Cupertino, CA, USA) were implanted subcutaneously into each rat in corresponding group. This study was approved by the Ethics Committee of Beijing Anzhen Hospital (AZ-2012-011).

Measurement of PAP and Right Ventricle Hypertrophy Index

Three weeks after monocrotaline injection, animals were anesthetized with intraperitoneal injection of 12% urethane (8 mL/kg). The pressure tube was placed to the pulmonary artery under the guidance of the guiding wire through the right external jugular vein. The position of the piezometer was determined according to the pressure waveform; the mean PAP (mPAP) was also recorded. Animals were sacrificed by high-potassium injection and lung tissues were collected. The right ventricle (RV) and left ventricle plus septum (LV + S) were also collected and weighed. The ratio of RV/(LV + S) was used to calculate the RVHI, which indirectly represented the pulmonary artery remodeling.

Measurement of ACE2 Activity in Lung Tissue

The activity of ACE2 was measured by fluorescence resonance energy transfer assays as previously described[3] in six rats per group. Briefly, the frozen lung tissues were thawed and homogenized in ACE2 assay buffer (75 mM Tris, 1 M NaCl, 0.5 μM $ZnCl_2$, pH 7.5), and then, the homogenates with lisinopril at a concentration of 50 μg/L for blocking the activity of ACE were centrifuged at 13,000 rpm for 10 minutes at 4°C. Protein concentration was determined using a standard Bradford assay. A total of 50 μg protein was then mixed well with 50 μM ACE2-specific fluorogenic substrate (R&D Systems, Inc, Minneapolis, MN, USA) at 37°C for 4 hours. The intensity