

Activation of renin–angiotensin–aldosterone system (RAAS) in the lung of smoking-induced pulmonary arterial hypertension (PAH) rats

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Abstract

Objectives: To explore the role of the renin–angiotensin–aldosterone system (RAAS) in the pathogenesis of pulmonary arterial hypertension (PAH) induced by chronic exposure to cigarette smoke.

Methods: 48 healthy male SD rats were randomly divided into four groups (12/group): control group (group A); inhibitor alone group (group B); cigarette induction group (group C); cigarette induction + inhibitor group (group D). After the establishment of smoking-induced PAH rat model, the right ventricular systolic pressure (RVSP) was detected using an inserted catheter; western blotting was used to detect the protein expression of angiotensin-converting enzyme-2 (ACE2) and angiotensin-converting enzyme (ACE); expression levels of angiotensin II (AngII) in lung tissue were measured by radioimmunoassay.

Results: After six months of cigarette exposure, the RVSP of chronic cigarette induction group was significantly higher than that of the control group; expression levels of AngII and ACE increased in lung tissues, but ACE2 expression levels reduced. Compared with cigarette exposure group, after losartan treatment, RVSP, ACE and AngII obviously decreased ($P < 0.05$), and ACE2 expression levels significantly increased.

Conclusion: Chronic cigarette exposure may result in PAH and affect the protein expression of ACE2 and ACE in lung tissue, suggesting that ACE2 and ACE play an important role in the pathogenesis of smoking-induced PAH.

Keywords

Smoking, PAH, chronic obstructive pulmonary disease, RAAS

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Introduction

Pulmonary arterial hypertension (PAH) is a disease of pulmonary vascular resistance increase caused by a variety of factors, ultimately leading to right ventricular failure.¹ Animal experiments demonstrated that chronic cigarette exposure may cause lung parenchyma and airway inflammation, and induce the reconstruction of small pulmonary vessels and small airway, resulting in pulmonary arterial hypertension.^{2,3} Previous studies have reported that activation of the renin–angiotensin–aldosterone system (RAAS) system was involved in the pathogenesis of pulmonary vascular remodeling and PAH.^{4,5} The expression of angiotensin-converting enzyme (ACE) increased in the pulmonary arterial of patients with PAH.⁵ RAAS system activation and increased AngII levels were observed in

PAH rats induced by hypoxia and monocrotaline,⁶ and the expressions of AngII and AngII type 1 receptor (AT1R) were also increased *in vivo*.⁷ In addition, treatment with ACEI or AT1R inhibitors can relieve the pulmonary

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vascular remodeling and PAH, and improve endothelial function in hypoxia- monocrotaline-treated rats.⁸

In the RAAS system, the key role of ACE is to promote Ang I to generate AngII. ACE2 is a regulating member of RAAS system discovered in recent years, with a key role in the pathway of AngII–Ang generation (1–7).⁹ ACE and ACE2 may have opposite physiological effects; ACE2 is the specific negative regulatory mechanism after RAAS system activation.^{9–11} ACE2 has been suggested to be associated with the severe acute respiratory syndrome caused by coronavirus infection, acute lung injury, pulmonary fibrosis and PAH induced by monocrotaline. A recent study showed that the antihypertensive effect of AT1R was partly due to the increased metabolic AngII by ACE2.⁸ Therefore, the present study aimed to investigate whether ACE2 and ACE have effects on the PAH induced by chronic cigarette exposure, further exploring the exact mechanism of chronic cigarette smoking-induced PAH.

Materials and methods

Experimental animals

The present study was approved by the Ethical Committee of West China Hospital, Sichuan University. 48 male Sprague–Dawley rats (200–250g) were randomly divided into four groups (12/group): control group (group A); inhibitor group (group B); cigarette induction group (group C); cigarette induction + inhibitor group (group D).

Cigarette exposure scenarios[12]: rats in cigarette exposure group passively received exposure to cigarette smoking in a standard toxicological box, igniting 15 cigarettes (each containing 14 mg tar and 1.0 g nicotine), twice per day for 30 min; the control group was exposed to fresh air in a same kind of toxicological box at the same time; inhibitor losartan was injected intraperitoneally daily at a dose of 10 mg/kg.d⁻¹. Normal control group daily received equal saline injection for six months.

Pulmonary artery pressure measurements and morphological analysis

After six months of observation, rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg) and fixed in supine position, breathing room air; the right chest was exposed to make a 'V' notch; with heparin (50 U/ml) anticoagulation, the silicone tube (0.45 mm ID, 0.8mm OD) was inserted into the right ventricle through the right atrium; the other end of the catheter was connected to a P23×L pressure sensor; after treated by the carrier amplifier, the pressure was recorded by Gould-34008/DASA4600 physiology record instrument. After detection of the right ventricular systolic pressure (RVSP), lung tissues of all rats were collected and fixed in 4% paraformaldehyde, and

then the lungs were paraffin-embedded overnight and sliced, histological staining; the left lungs were stored in a -80°C refrigerator for biochemical analysis. Paraffin sections were placed in murine ACE2 polyclonal antibody (Santa Cruz, Texas, USA) for immunohistochemical staining to observe the ACE2 protein expression. An optical microscope (H600, Nikon, and Tokyo, Japan) and a radioisotope dynamic digital camera (Nikon, Tokyo, Japan) were used to collect images.

AngII in lung measured by radioimmunoassay

AngII radioimmunoassay kit (Beijing Nonth Institute of Biological Technology, Beijing, China) was used to measure the expression levels of AngII: the lung tissue was washed with ice-saline and chopped; and then it was heated in 0.1 M HCl at 100°C for 10 min and homogenized. After centrifugation at 15,000×g for 30 min, the supernatant was freeze-dried and re-dissolved in 400μl buffer; the radioactivity was measured by a γ counter.

ACE2 and ACE protein levels in lung tissue detected by western blot

Protein expressions of ACE2 and ACE were detected by western blot. Lung homogenate solution was mixed with the tissue lysate (Roche Apphed Sciece, Indianapolis, USA) containing 50 mM of Tris-HCl, 150 mM of NaCl, 1% of NP-40, 0.5% of sodium deoxycholate, 2 mM of NaF, 2 mM of EDTA, 0.1% of SDS and protease inhibitors. The protein concentration of lung homogenates was measured using BCA assay (Pierce, Rockford, IL, USA). Equal amounts of protein samples (30μg) were separated using 10% of polyacrylamide gel and transferred to a 0.45μm of polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA). Membranes were incubated with ACE2 polyclonal antibody (1:500; Santa Cruz Biotechnologies, Santa Cruz, CA). Referring to the manual operation, western blot was performed using enhanced chemiluminescence.

Statistical methods

SPSS 17.0 was used for statistical analysis. Measurement data were expressed as mean±SD; differences between groups were compared using one-way analysis of variance. *P*<0.05 was considered statistically significant.

Results

Increased RVSP by cigarette smoke induction

After six months of cigarette exposure the RVSP of rats significantly increased (Figure 1); losartan can significantly reduce the increased RVSP caused by smoking, suggesting that losartan may relieve chronic smoking-induced PAH.

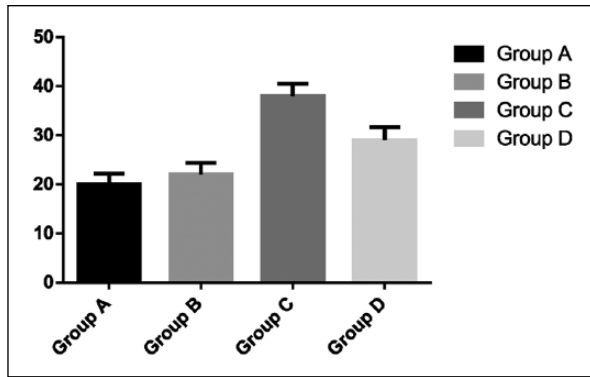


Figure 1. Smoking-induced increased right ventricular systolic pressure in rats. Y-axis: RVSP level (mmHg). Compared to group A, * $P < 0.05$; compared to group C, ** $P < 0.05$.

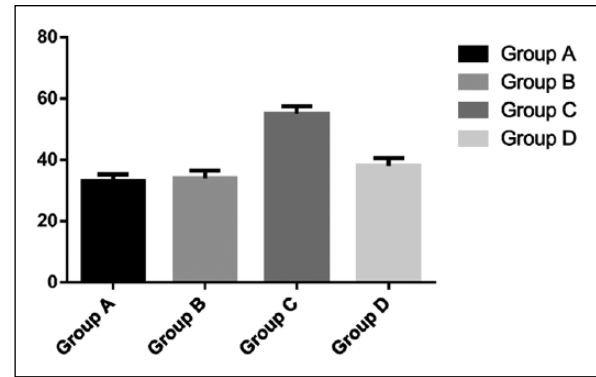


Figure 2. Smoking-induced increased Ang II concentration in rats. Y-axis: Ang II concentration (µg/mg protein). Compared to group A, * $P < 0.05$; compared to group C, ** $P < 0.05$.

Increased Ang II caused by cigarette smoke exposure and therapeutic effects of losartan

We have detected AngII concentrations in lung tissues by radioimmunoassay after six months of exposure to cigarette. As shown in Figure 2, expression levels of AngII in the lung tissues of rats in chronic cigarette exposure group were significantly increased, but was significantly inhibited by losartan ($P < 0.05$).

Increased ACE and decreased ACE2 caused by cigarette-induced PAH and the therapeutic effects of losartan

To further explore the increasing mechanism of AngII in smoking-induced PAH rats, we examined the expression levels of ACE and ACE2 in the cigarette exposure group. Western blot was used to detect ACE and ACE2 protein expressions, and the expression levels of ACE2 were also detected by a immunohistochemical staining method. Immunohistochemistry showed that compared with the control group, the lung section positive staining showed that the cigarette exposure group had a significant reduction in ACE2 expression (Figure 3).

Western blotting analysis showed that cigarette exposure significantly increased the ACE levels in lung and reduced ACE2 levels (Figure 4). Losartan treatment could significantly increase ACE2 levels, and it was also found that the expression levels of ACE decreased in cigarette exposure group and cigarette exposure + losartan group, indicating that the smoking-induced expressions of ACE2 and ACE can be reversed to some extent by losartan.

Discussion

Smoking reduces the immune response of the host defense. Smoke-mediated oxidative stress response can stimulate the release of pro-inflammatory cytokines, and induce

inflammatory response.¹² Selman et al.¹³ reported that smoking may act directly on the vascular system, affecting the release of vascular regulation factor, thus regulating vascular contraction and relaxation and the proliferation of vascular smooth muscle. Eventually, it leads to increased vascular resistance and pulmonary artery pressure which is caused by the remodeling of pulmonary arterioles, arteries and micro arteries.⁶⁻⁸

This study confirmed that chronic cigarette-smoke exposure significantly increased RVSP in rats, which was significantly reduced by losartan. Mean pulmonary arterial hypertension and increased pulmonary vascular muscle degrees were also observed in rats exposed to cigarette smoke for 16 weeks.¹⁴ Our findings were consistent with the above study; cigarette exposure for six months can significantly increase RVSP in rats. In summary, chronic cigarette smoke may directly lead to the pulmonary artery remodeling and PAH. In recent years, it has been found that exposure to nicotine (the main component of cigarette smoke) can increase both the expression and activity of ACE in human endothelial cells.¹⁵ ACE plays a key role in the RAAS system, which can convert Ang I to AngII; cigarette smoke exposure can activate the RAAS system to increase AngII levels. The major physiological and pharmacological effects of AngII are mediated by AT1R,¹⁶ and it has been confirmed that AngII plays an important role in PAH by binding to AT1R.¹⁶ This study suggested that the pathogenesis of cigarette-induced PAH also involves the activation of the RAAS system. We found that compared with the control group, PAH rats exposed to cigarette smoke for six months had a reduction in ACE2 expression and an increased expression of ACE and AngII (almost double). In the RAAS system, ACE decomposes Ang I to generate potent vasoconstrictor and ACE2 hydrolyzes Ang I to generate a negative regulatory protein Ang (1-7).⁹ In previous study, Karram et al.¹⁷ found that losartan therapy affected plasma ACE protein expression in normotensive rats. Some experiments have confirmed that losartan can

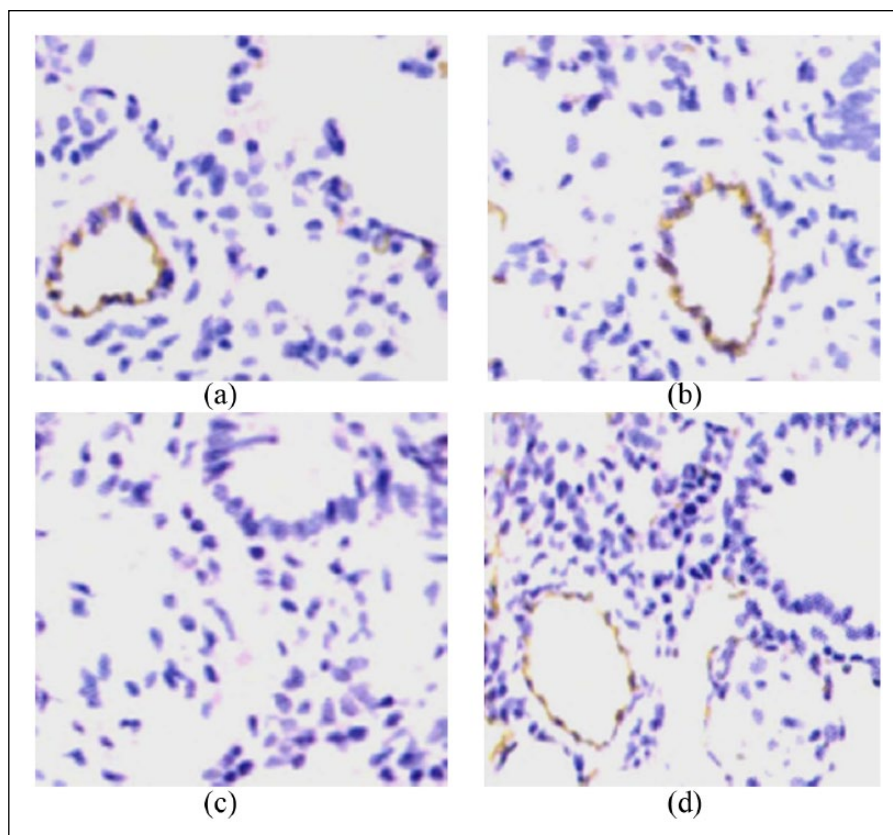


Figure 3. ACE2 expression in lung tissue sections by immunohistochemical detection ($\times 100$). (a) control group (group A); (b) inhibitor alone group (group B); (c) cigarette induction group (group C); (d) cigarette induction + inhibitor group (group D).

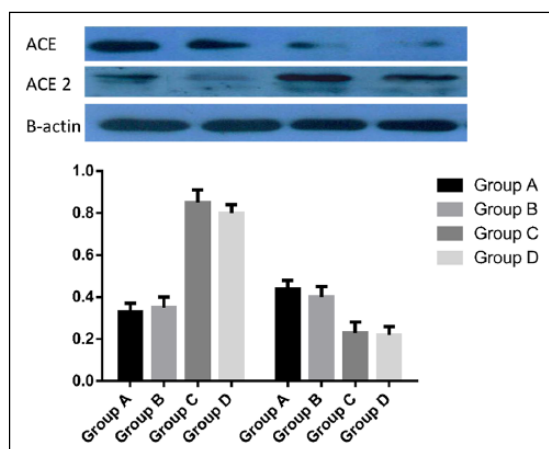


Figure 4. ACE2 and ACE expression in lung tissue by western blotting.

Compared to group A, $*P < 0.05$; compared to group C, $**P < 0.05$.

effectively increase ACE2 activity and its protein expression in rat myocardium or human kidney. Increased ACE2 can accelerate the conversion of AngII to Ang (1–7) to reduce the concentration of AngII.^{18–19} This study found that after six months of exposure to cigarette, ACE2 protein expression was significantly reduced and pulmonary artery pressure was increased significantly in rat lungs.

In conclusion, chronic cigarette smoke exposure induced a significant increasing in RVSP, AngII levels and ACE protein expression in lung tissue, but decreased ACE2 expression. Losartan treatment can effectively reduce the degree of PAH and reverse the increased AngII and ACE expression, indicating that losartan can not only inhibit the effect of AngII in the lung tissue of cigarette smoke-induced PAH rats, but can also reduce the levels of AngII. ACE and ACE2 play roles in chronic cigarette smoke-induced PAH by regulating AngII expression.

Conflict of interest

None declared.

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