The Changes of Serum Angiotensin-Converting Enzyme 2 in Patients with Pulmonary Arterial Hypertension due to Congenital Heart Disease

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Key Words

Congenital heart disease · Pulmonary arterial hypertension · Angiotensin-converting enzyme 2

Abstract

Background: Angiotensin-converting enzyme 2 (ACE2), a primary component of the vasoprotective axis in the renin-angiotensin system (RAS), has recently been found to have regulatory actions in hypoxic pulmonary hypertension and monocrotaline-induced pulmonary hypertension. We explored the hypothesis that the level of ACE2 protein contents may be decreased in patients with pulmonary arterial hypertension (PAH) due to congenital heart disease (CHD). **Objective:** We observed the serum ACE2 protein contents in patients with PAH due to CHD (CHD-PAH), and investigated their correlation with mean pulmonary arterial pressure (mPAP). **Methods:** One hundred and four patients with CHD and 33 normal control patients (group A) were involved in the research. The patients with CHD were divided into 55 cases of nonpulmonary hypertension (group B), 25 cases of mild to moderate pulmonary hypertension (group C) and 24 cases of severe pulmonary hypertension (group D). The serum level of ACE2 protein contents were detected by enzyme-linked immunosorbent assay (ELISA), and the relation-

ship between these contents and mPAP were analyzed. **Results:** ACE2 protein contents significantly declined as mPAP increased. The mPAP was negatively correlated with the level of ACE2 protein contents. **Conclusions:** These results demonstrated that ACE2 may play an important regulatory role in CHD-PAH.

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Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease with poor survival outcome. It can be classified into 5 main categories according to the updated clinical classification. PAH due to congenital heart disease (CHD), i.e. CHD-PAH, with systemic-to-pulmonary shunt is a major subgroup [1]. The advanced stage of this is called Eisenmenger syndrome which forms a small percentage (1%) of the CHD population [2]. Eisenmenger syndrome is characterized by reversed right-to-left intracardiac shunting of blood and significant hypoxemia. Patients with Eisenmenger syndrome have a progressive

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poor exercise capacity, a reduced life expectancy and heart failure can occur. The exact pathogenesis of CHD-PAH is poorly understood. Therapeutic options for patients are limited.

Angiotensin-converting enzyme 2 (ACE2), a homolog of ACE, is a carboxypeptidase that degrades angiotensin (Ang) II to Ang-(1–7) [3, 4]. ACE2 plays an important role in the vasoprotective axis (ACE2-Ang-(1–7)-Mas axis) of the renin angiotensin system (RAS) and counterbalances the vasoconstrictive, proliferative and fibrotic axes [ACE-Ang II-Ang II type 1 receptor (AT1R) axis] of the RAS [5–7]. ACE2 is highly expressed in the lungs, is present in the circulation and is an integral membrane protein in 72 organs [8]. Recent studies demonstrated the therapeutic effects of ACE2 activation by a synthetic molecule [6], the continuous injection of resorcinolnaphthalen [9] or ACE2 gene transfer [10, 11] in a monocrotaline-induced PAH model. Recombinant ACE2 blunts the rise in pulmonary arterial pressure (PAP) that occurs in response to acute hypoxia [12]. Recombinant ACE2 attenuates arterial hypoxemia, pulmonary hypertension and the redistribution of pulmonary blood flow in a lipopolysaccharide-induced lung injury model [13]. The expression of Ang II was increased in a left-to-right, shunt-induced PAH model [14]. These observations led us to hypothesize that the imbalance between the ACE2-Ang-(1–7)-Mas axis and the ACE-Ang II-AT1R axis could participate in the pathogenesis of CHD-PAH, the serum level of ACE2 protein contents may be decreased in patients with CHD-PAH.

Materials and Methods

Study Population and Design

This study was conducted in the Department of Cardiology of the Yan'an Affiliated Hospital of Kunming Medical University between April and August 2011.

Requirements for enrollment included: an established diagnosis of isolated atrial or ventricular septal defect using the echocardiogram and an age of >14 years. Exclusion criteria included: (1) coronary artery disease, (2) hypertension, (3) heart valve diseases, (4) diabetes mellitus, (5) autoimmune diseases, (6) bleeding tendency, (7) liver or renal insufficiency, (8) pulmonary disease (e.g. chronic obstructive pulmonary disease and pulmonary embolism), (9) HIV infection and (10) refusal to participate in the study.

The CHD patients were assigned to 3 groups according to mean PAP (mPAP) which was measured by right-heart catheterization [1]: CHD with nonpulmonary hypertension (mPAP ≤25 mm Hg), CHD with mild to moderate pulmonary hypertension (50 mm Hg ≥ mPAP > 25 mm Hg) and CHD with severe pulmonary hypertension (mPAP >50 mm Hg). The ethics committee of our hospital approved this study, and written informed consent was obtained from all patients and control subjects.

Serum Samples Collection and Measurements

We collected 4-ml blood samples in glass tubes without additive and allowed them to clot at room temperature for 60 min. Serum was separated by centrifugation at 3,000 rpm for 15 min. Serum aliquots of 1.50 ml were obtained and stored at –80°C until use. The plasma ACE2 protein contents were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Shanghai Genmed Scientifics Inc., China). All steps were performed according to the instruction.

Fasting blood samples were analyzed for kidney function, total cholesterol (TC), triglyceride (TG) and liver function (e.g. alanine aminotransferase and aspartate aminotransferase) in a routine diagnostic analyzer.

Hemodynamic Measurements

All patients were hospitalized and all provided written informed consents. Right-heart catheterization was performed in the catheterization lab. With local anesthesia under continuous electrocardiographic monitoring, a 6-French pigtail catheter was advanced into the pulmonary artery through the right femoral vein by placement of a 6- or 7-French vascular sheath. The catheter was flushed with heparinized normal saline. Correct catheter positioning was verified by fluoroscopy. Transducers were positioned at the midaxillary line and zeroed at the atmospheric pressure. The patients were equilibrated for about 10 min before PAP measurements were taken.

Statistical Analysis

Data were analyzed using SPSS 17.0 software (SPSS, Chicago, Ill., USA). All data are presented as mean ± standard deviation (SD). Proportions were compared using the χ^2 test, continuous Gaussian distributed variables with ANOVA and non-Gaussian distributed variables with the Kruskal-Wallis test. The relationship between ACE2 protein contents and mPAP was assessed by the Spearman rank correlation. Statistical significance was set at $p < 0.05$.

Results

Clinical Characteristics of Participants

The study included 104 CHD patients (35 men and 69 women; 73 atrial septal defect, 31 ventricular septal defect) and 33 normal controls (group A). The 104 CHD patients were assigned to 3 groups: 55 cases of CHD with nonpulmonary hypertension (group B, mPAP ≤25 mm Hg), 25 cases of CHD with mild to moderate pulmonary hypertension (group C, 50 mm Hg ≥ mPAP > 25 mm Hg) and 24 cases of CHD with severe pulmonary hypertension (group D, mPAP >50 mm Hg). Demographic and general clinical features are presented in table 1. There were no significant differences in sex, age, alanine and aspartate aminotransferase, creatinine, TC and TG between groups.

Fig. 1. The level of ACE2 protein contents in the study population. * $p < 0.001$ vs. group A; & $p < 0.001$ vs. group B; # $p < 0.001$ vs. group C.

Fig. 2. ACE2 protein contents correlated to mPAP.

Table 1. Clinical characteristics of the study groups

Characteristics	Control (n = 33)	CHD without PAH (n = 55)	CHD with mild to moderate PAH (n = 25)	CHD with severe PAH (n = 24)
Male gender	17 (51.5)	17 (30.9)	6 (24)	12 (50)
Age, years	41.39±12.64	34.64±16.72	43.41±18.61	33.90±17.02
Patients with atrial septal defect	–	47 (85.5)	18 (72)	8 (33.3)*
Patients with ventricular septal defect	–	8 (14.5)	7 (28)	16 (66.7)*
mPAP, mm Hg	–	17.65±4.80 [#]	35.48±7.30 [#]	64.62±13.47 [#]
Alanine aminotransferase, U/l	28.6±7.69	29.9±5.93	32.4±6.13	34.7±8.32
Aspartate aminotransferase, U/l	29.9±6.93	31.5±6.47	32.1±6.04	30.2±7.01
Creatinine, μmol/l	68±14	73±8	66±14	70±12
TC, mmol/l	4.82±0.70	5.01±0.85	4.68±0.89	4.43±0.84
TG, mmol/l	1.12±0.15	0.98±0.79	1.21±0.37	1.07±0.46

Data are means ± SD or absolute numbers with percentages in parentheses.

* $p < 0.01$ vs. CHD without PAH and CHD with mild to moderate PAH groups; # $p < 0.001$ vs. CHD without PAH, CHD with mild to moderate PAH and CHD with severe PAH groups. All other comparisons between the control and the other 3 groups were insignificant.

The Level of ACE2 Protein Contents in the Study Population

The ACE2 protein contents in plasma measured by ELISA were 15.79 ± 5.03 U/l, 21.49 ± 4.19 U/l, 15.84 ± 3.80 U/l and 12.96 ± 5.98 U/l in groups A, B, C and D, respectively. The level of ACE2 protein contents in group B was significantly higher than in group A ($p < 0.001$), the level in groups C and D was significantly lower than in group

B ($p < 0.001$) and the level in group D was significantly lower than in groups A and C ($p < 0.05$). There was no significant difference between the level in group A and in group C ($p > 0.05$) (fig. 1).

Through correlation and regression analysis, there was a negative correlation between mPAP and ACE2 protein contents ($r = -0.576$, $p < 0.001$) (fig. 2).

Discussion

CHD is the most common of major congenital malformations. Blood initially shunts from the systemic to the pulmonary circulation because the resistance in the former is higher, and a large defect and chronic shunting induce PAH. Progressive increase in pulmonary vascular resistance is characterized by vascular wall remodeling with intimal fibrosis, increased medial thickness and plexiform lesions [15] which finally lead to Eisenmenger syndrome. The pathophysiological mechanisms responsible for the development of CHD-PAH are not completely known. Pulmonary vascular injury, inflammatory reaction and endothelial dysfunction [16] may all play an important role in this condition.

It has been proposed that an activated RAS causes an imbalance between the vasoconstrictive (ACE-Ang II-AT1R axis) and vasodilator (ACE2-Ang-(1-7)-Mas axis) mechanisms involving the pulmonary circulation leading to the development of PAH. ACE inhibitors and AT1R blockers can inhibit the ACE-Ang II-AT1R axis, but their primary effects are to reduce systemic blood pressure, thus they are unsuccessful in treating patients with PAH as these patients are already at a high risk of developing hypotension due to right-ventricular overload [17]. ACE2 is expressed in endothelial cells, especially in the lung microvascular endothelial cells [18, 19]. Some studies [6, 10, 11] have shown that ACE2 overexpression or activation

induce a beneficial pulmonary outcome with no adverse effects on systemic blood pressure.

The major finding of this study is that the level of ACE2 protein contents in CHD patients with nonpulmonary hypertension was significantly higher than in the group of normal controls and the level in CHD patients with pulmonary hypertension was significantly lower than in CHD patients with nonpulmonary hypertension. This phenomenon may be due to ACE2 being increased in CHD patients before the onset of PAH in order to prevent the development of PAH. However, along with the progressive endothelial damage, the expression of ACE2 was decreased.

In summary, this study is the first to demonstrate that the serum level of ACE2 protein contents is decreased in patients with CHD-PAH. We speculate that the decrease in ACE2 and the increased in Ang II shift the balance of the RAS towards the ACE-Ang II-AT1R axis, resulting in increases in vascular remodeling, fibrosis and PAH in CHD patients. So ACE2 may be a target for the treatment of CHD-PAH. Further studies are necessary to substantiate this conclusion.

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