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Correlation of angiotensin converting enzyme gene polymorphism with perioperative myocardial protection under extracorporeal circulation

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ABSTRACT

Objective: To observe the expression of angiotensin converting enzyme (ACE), angiotensin Ⅱ (Ang Ⅱ), cardiac troponin (cTn Ⅰ), creatine kinase isozymes (CK-MB) and muscle red protein(Myo) after cardiopulmonary bypass (CPB), and to investigate the association of polymorphisms in angiotensin converting enzyme genes and myocardial injury. **Methods:** Sixty-three patients suffered from rheumatic mitral stenosis and scheduled for mitral valve replacement with CPB, were randomly divided into three groups according polymorphisms in angiotensin converting enzyme genes: type Ⅱ, type ID, type DD (each=21). Blood samples were withdrawn from artery before operation (T1), at the beginning of CPB (T2), 30 min after CPB (T3), (T4) at the end of CPB (T5), 2 h after CPB (T6), 6 h after CPB (T7) to measure the expression of ACE, Ang Ⅱ, cTn Ⅰ, CK-MB, Myo. **Results:** The level of ACE during and after CPB were significantly higher than those before CPB ($P<0.05$). As extension of CPB time, the expression of ACE was increased. The level of cTn Ⅰ, CK-MB, Myo after CPB were significantly higher than those before CPB($P<0.05$). The level of cTn Ⅰ, CK-MB and Myo were highest at T7, T6 and T5 and T7, respectively. The level of ACE, Ang Ⅱ, cTn Ⅰ in patients with DD genotype was significantly higher than the ID and Ⅱ genotype ($P<0.05$). Besides, the level of ACE, Ang Ⅱ in patients with ID genotype was significantly higher than the Ⅱ ($P<0.05$). **Conclusions:** There is certain correlation between CPB perioperative midterm ACE and cTn Ⅰ, Myo, CK-MB. ACE DD genotype is a susceptibility gene of the CPB perioperative myocardial injury.

1. Introduction

Study on the prediction, early diagnosis and intervention of cardiovascular diseases from molecular and gene level is the hotspot in present research[1,2]. Angiotensin converting enzyme (ACE) is the important enzyme in the renin-angiotensin-aldosterone system. The major function of ACE is to catalyse the conversion of angiotensin Ⅰ to produce angiotensin Ⅱ. The regulating ACE level of ACE gene polymorphism can directly affect the generation of angiotensin Ⅱ, and angiotensin Ⅱ plays an important role in the injury of cardiac ischemia reperfusion. In recent years, there are a lot of researches on the relationship between ACE gene polymorphism and cardiovascular disease at home and abroad, and a DD type of the ACE gene has been described in association with the coronary heart disease, myocardial infarction, left ventricular hypertrophy and other cardiovascular diseases. The aim of this study is to analyse the dynamic change of cardiac pulmonary bypass (CPB), ACE and the determination results for myocardial markers in ACE gene polymorphisms and the perioperative myocardial damage under cardiac pulmonary bypass.

2. Materials and methods

2.1. Subjects

A total of 63 patients who had cardiac operational treatment due to rheumatic mitral stenosis with elective

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extracorporeal circulation in our hospital from September 2008 to December 2011 were selected. All the subjects, including 15 males and 48 females understood the experiment content, and gave their written informed consent. All patients were differentiated into separate genotype Ⅱ, DD and ID according to ACE gene polymorphism, with 21 cases in each group.

The age ranges were between 38 to 53 years: Ⅱ genotype was (43.00±3.46) years old, DD genotype was (42.00±5.57) years old, ID genotype was (44.00±2.65) years old. The average weight in Ⅱ genotype was (51.00±5.75) kg, DD genotype (52.00±3.55) kg, ID genotype was (53.00±4.62) kg. No patient had other heart diseases, the preoperative cardiac function were Ⅱ-Ⅲ degree (NYHA). Average left ventricular ejection fraction in Ⅱ genotype was (57.00±8.68)%, DD genotype was (58.00±7.56)%, ID genotype was (59.00±4.92)%. No patient had past medical history of hypertension and diabetes, and there was no obvious abnormality on preoperative complete routine biochemical examinations. No patient took related oral drugs on interfering ACE. They had conventional mitral valve replacement under CPB. There were no obvious differences ($P > 0.05$) in age, sex, weight, past medical history, preoperative cardiac function, disease composition and operation category before taking treatment.

2.2. Methods

Extracorporeal circulation machine of Terumo type and Terumo Medrenic oxygenator from Japan were adopted; the priming fluid contained hydroxyethyl starch 200/0.5 and sodium chloride injection 500-1 000 mL; heparin sodium (the heparin sodium dosage was calculated according to weight kg×3) was added to priming fluid and then the ulinastatin injection was added for 300 000 U. Under conventional general anesthesia, median sternum incision was taken and CPB (ascending aorta and the superior and inferior vena cava intubation) was established. After CPB, ascending aorta blocking was conducted after the anal temperature and nasopharyngeal temperature dropped to approximately 30 ℃. Cardiac arrest liquid (oxygenated blood: crystalloids= 4:1, 470 mL of physiological saline, 6 mL of 10% potassium chloride , 2.5 mL of 25% magnesium sulfate and 20 mL of 5% sodium bicarbonate, the initial dose was 15-20 mL/kg, then once perfusion every 30 minutes with 5-10 mL/time) was infused; at the same time, the smoothies were put into the pericardial cavity to protect cardiac muscle, drained into the left heart drainage tube through the upper right pulmonary vein to prevent left ventricle excessive expansion and avoid ventricular muscle tissue injury.

Artificial mechanical mitral valve replacement was imported in routine way, and the mean arterial pressure, heart rate, ECG, blood oxygen saturation, arterial blood CO_2 partial pressure, NPT, central venous pressure during operation was continuously monitored. NPT was maintained between 28-30 ℃, MAP＞60 mmHg in bypass process, the myocardial protection perfusion was kept in 2-2.5 L/min/m^2, the perfusion pressure was maintained in 60-80 mmHg. The blood was in moderate dilution, the hematocrit was maintained in 18%-24%, thus, CPB activated clotting time could be maintained over 750 s. After the intracardial operation, HCT was increased to 27% through diuresis and ultrafiltration after re-warming. On stabilizing circulation, CPB was stopped, then neutralization with protamineat at the end of CPB was performed. The average time for aortic blocking were: group Ⅱ (27.00±4.52) minutes, ID genotype (28.00±5.32) minutes, genotype DD (26.00±4.95) minutes; the average time for CPB were: Ⅱ genotype (49.00±6.82) minutes, genotype ID (50.00±7.32) minutes, DD genotype (48.00±8.65) minutes with no significant difference ($P > 0.05$).

2.3. Testing index

A total of 15 mL of peripheral venous blood was taken at 7 time points as before CPB, the moment of bypass, 30 minutes after bypass, the moment of aortic opening and stopping bypass, 2nd hour after operation and 24th hour after operation. Five mL blood was added to heparin lithium test tube and mixed for cTnⅠ, CK-MB and Myo measurement. Each 5 mL was added to no-additive test tube respectively for ACE and AngⅡ detection. The myocardial specimens were taken at the 10th minutes after bypass, the moment of aortic opening and the moment of heart resuscitation respectively, the samples were taken from the auricular appendix of right atrium. ACE I/D allele, ACE, and AngⅡ expression were detected respectively for the patients from each group.

2.4. Statistical analysis

The data for each of the tests were calculated using SPSS13.0 software and expressed with $\bar{x}$±SD , adopted the the single factor variance analyses for comparisons of each groups, followed by the Spearman analyses for correlation, the level of statistical significance was set at $P<0.05$.

3. Results

3.1. Comparison of each index result at perioperative CPB

The level of statistical significance ($P < 0.05$) on the ACE level that before CPB, the moment of bypass, 30 minutes after

bypass, the moment of aortic opening and stopping bypass, 2nd hour after operation and 24th hour after operation with that before CPB were compared, it was gradually increased over time. There was significant difference (P<0.05) in cTn Ⅰ among each time point before and after CPB. It was significantly (P<0.05) highest at 24th after CPB. There was significant difference (P<0.01) in CK-MB among each time point before and after CPB. It was significantly (P<0.01) highest at 2nd after CPB. Also, a significant difference in Myo (P< 0.05) was observed amongeach time point before and after CPB, it reached double peaks on stopping bypass and at 24th after CPB (Table 1).

3.2. Correlative analysis of ACE and each myocardial marker

There was significant correlation between ACE and cTn Ⅰ (r=0.651, P< 0.01). ACE also had significant correlation with Myo (r=0.194, P< 0.01) and CK-MB (r=0.137, P< 0.01).

3.3. Comparison of the related indices among different genotype

The results showed that DD genotype was significantly increased in concentration of ACE, Ang Ⅱ and cTn Ⅰ as compared to other two genotypes (P<0.05); whereas between ID genotype and Ⅱ genotype, there was significant difference in ACE and Ang Ⅱ (P<0.05) (Table 2).

4. Discussion

ACE is the core enzyme in the RAAS, its inhibitor has been widely used clinically in the treatment of related cardiac diseases. CPB can activate the complement system and release cell factors and lead to tissue injury, inflammation reaction and organ dysfunction[3]. Among them, the myocardial injury markers: cTn Ⅰ, CK-MB and Myo serum concentration can also get change accordingly[4-7]. In the present study, no association between the ACE gene polymorphism from the patients with cardiac valve replacement and the myocardial ischemia reperfusion injury at CPB perioperative term was found.

The establishment of CPB and temporary arrest of the heart during heart operation, myocardial ischemia and reperfusion can cause a certain degree of ischemic damage to cardiac muscle[8,9]. At the same time, myocardial ischemia and reperfusion can induce myocardial cell damage and lead to the increase in myocardial damage marker concentration in serum. ACE has been described as one kind of membrane-bound glycoprotein that consists of single polypeptide chain[10]. The major function of ACE is to hydrolyze the conversion of angiotensin Ⅰ to produce angiotensin Ⅱ, ACE in blood circulation originates mainly from vascular endothelial cells[11]. A number of studies identified that[12] the pressure overload could induce myocardial cell ACE mRNA and protein expression, as ACE activity is higer and Ang Ⅱ content increase more which can cause the hypertrophy and damage to myocardial cell[13]. ACE inhibitors (*eg*., ACE Ⅰ) is widely used in treating heart diseases and get obvious effect. This study showed that after CPB, ACE concentration in serum got significant increase than that before CPB, which confirmed that ACE has certain promoting action in CPB perioperative myocardial damage. Our results is opposite to other studies that found that activity of ACE gene is associated with acute lung injury, and that, the decrease of ACE concentration prompted more serious tissue damage, namely the bad prognosis[14,15].

Myocardial damage marker can be used to identify long-term and short-term heart diseases[16] in addition to the diagnosis and treatment of myocardial infarction and other

Table 1
Comparison of test result change for each index before and after CPB (n=21).

Test index	TI	T2	T3	T4	T5	T6	T7
ACE(μg/mL)	17.26±3.74	28.47±5.35	34.32±4.39	39.48±6.73*	45.39±8.23*	58.17±10.65*	94.17±10.68*
Myo(μg/mL)	65.15±5.88	132.48±18.45	149.48±19.65	215.83±20.18*	284.51±25.36	235.17±10.65*	324.59±48.12*
cTn Ⅰ (μg/mL)	0.04±0.02	0.11±0.02	0.23±0.12	0.47±0.11*	0.58±0.12*	0.78±0.12△	1.53±0.34△
CK-MB(μg/mL)	1.83±0.25	7.14±0.76	18.29±3.29	31.48±5.12△	32.18±4.32△	33.27±4.03△	1.59±3.02△

Note: comparing with that before CPB, *P<0.05, △P<0.01.

Table 2
Comparison of the related indices among different genotype.

Genotypes	ACE(U/L)	cTn Ⅰ (μg/L)	CK-MB(μg/L)	Myo(μg/L)	Ang Ⅱ (NT×mm^2)
DD	61.87±13.28△	17.27±7.24*	16.98±2.43	87.98±8.43	17.28±0.43△
ID	47.38±14.24*	11.28±5.39*	14.29±0.24	84.29±6.24	13.28±0.46*
Ⅱ	37.28±14.28	13.28±0.24	15.49±4.39	86.49±7.39	8.29±0.42

Note: comparing with that type Ⅱ, *P<0.05, △P<0.01.

monitoring. cTn Ⅰ only exist in myocardium, therefore, it is extremely high for the organic specificity. The half life is short in blood with high sensitivity. When myocardium get damaged, the myocardial cell permeability can be increased and result in elevated cTn Ⅰ in peripheral blood. CK-MB is also mainly present in myocardium with about 2%-3% of it exist in skeletal muscle, the blood test results has lesser interference, but with lower organic specificity[17], thus, CK-MB has been restricted greatly on making a distinction between tiny myocardial injury and skeletal muscle injury. So, it do have some limitations, since the skeletal muscle injury can also increase CK-MB in serum, but CK - MB test is more convenient comparing with cTn Ⅰ test and it is easy to operate[18].

In this study, it has been observed that the CK-MB was highest at 2nd after CPB. Also, there is no statistical significant difference in CK-MB at 24th hours after CPB and that before CPB, which prove that it can quickly restore to normal level and is not conducive to the long-term dynamic observation for myocardial damage and establish the diagnosis-treatment-planning. It have been shown that the application of cTn Ⅰ may play a most important role in judging the myocardial injury that caused by heart operation, for the myocardial injury markers. cTn Ⅰ could accurately reflect the degree of myocardial injury and its sensitivity was better than CK-MB, therefore, it can be useful as used as one of the independent testing index of detecting the perioperative myocardial injury degree for cardiac operation[19,20].

In patients with heart valve replacement in this study, no acute myocardial infarction happened, since the myocardial injury caused by operations, the postoperative ACE and CTn Ⅰ also had increased in various degree. It further demonstrated that ACE plays a promoting role in ischemia reperfusion injury, aortic blocking (ACC) time and the myocardial injury that caused by operational trauma and extracorporeal circulation operation. This study proved that ACE could be released with CK-MB and CTn Ⅰ simultaneously within half an hour of ischemia-reperfusion. In the contrast study, on evaluating the myocardial protection of the cold crystalloid cardioplegia with CTn Ⅰ, the postoperative CTn Ⅰ from cold crystalloid cardioplegia group was obviously higher than that cold oxygenated blood group in the control group. The postoperative auto-rebeat rate from the former was obviously lower than that in the latter, and most of patients need cardiotonic agents. This demonstrated that CTn Ⅰ can reflect the myocardial injury from microscopic view. In this study, a positive correlation between serum ACE and CTn Ⅰ was found, therefore, in certain extent serum ACE can reflect the myocardial injury from microscopic view.

The results also demonstrate certain association between ACE, Ctn Ⅰ and CPB, ACC time, and there is obvious correlation between myocardial ischemia time and the release from the patients who had valve replacement with normal coronary artery at 24th hours after operation. ACC time is the main factors that influence ACE, CTn Ⅰ release in such patients. ACE and CTn Ⅰ show linear relationship with ACC time, the myocardial injury cannot be completely eliminated for patients who had valve replacement through myocardial cardioplegia perfusion, but can only had improvement in myocardial ischemia state partially.

It has been observed through the dynamic ECG, with the increase in ACE and CTn Ⅰ concentration, ST segment obviously elevated. It suggests that there is serious injury in the myocardial tissue that corresponding ECG lead at this time. The results also confirm that ACE, cTn Ⅰ rise early after CPB and get peak at the 24th hours, which indicate that certain myocardial injury exists in the body, also, the myocardial injury at this time period has revealed may correlate with intraoperative ACC, cardiac arrest, myocardial ischemia and anoxia from CPB combined action as well as ischemia-reperfusion. Its level followed by a slight decline showed a dynamic change, maintain a relatively long, which is more suitable for early monitoring of CPB perioperative myocardial damage degree and tiny myocardial infarction. The changes of ACE and cTn Ⅰ can reflect the degree of myocardial injury to some extent[21-24], thus, it can provide better objective monitoring index for protecting perioperative myocardial, treating after operation and predicting postoperative cardiac function recovery.

In this study, the results illustrate that ACE has some significant association with cTn Ⅰ, also,it exhibits certain correlation with CK-MB and Myo. DD genotype is significantly more increase in concentration of ACE, Ang Ⅱ and cTn Ⅰ as compared to ID and Ⅱ genotype($P<0.05$). Thus we can deduce that ACE DD genotype belong to susceptibility gene, they have significant correlation with CPB perioperative myocardial injury, combine with the serum concentration change, which demonstrates the degree of myocardial injury to a certain extent. In conclusion, this study provides relevant laboratory evidence for instructing myocardial protection, and preventing, controling the clinical diagnosis, treatment, monitoring on CPB perioperative myocardial ischemia-reperfusion injury to a certain extent. These findings also suggest that it play an important directive role in improving the safety of CPB valve replacement, especially in critical and complicate heart disease operation.

Conflict of interest statement

We declare that we have no conflict of interest.

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