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RESEARCH ARTICLE

Dynamic changes in serum angiopoietin-1, angiopoietin-2, and angiopoietin-2/angiopoietin-1 ratio in acute myocardial infarction patients treated with primary percutaneous coronary intervention

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Abstract

Context: Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) play divergent roles in myocardial ischemia and reperfusion injury.

Objective: To investigate serum Ang-1 and Ang-2 levels in ST-segment elevation myocardial infarction (STEMI) patients treated with primary percutaneous coronary intervention (PCI).

Methods: Serum Ang-1 and Ang-2 were measured in 85 STEMI patients in the first week after PCI.

Results: Ang-1, Ang-2 and Ang-2/Ang-1 ratio (Ang-2/1) were all increased at admission, and had dynamic changes after PCI. Ang-2 and Ang-2/1 at admission and 2 h after PCI were positively correlated with peak cardiac troponin T levels.

Conclusion: The extent of myocardial damage may be linked to circulating Ang-2 and Ang-2/1.

Keywords: Acute myocardial infarction, angiopoietin, cardiovascular disease

Introduction

Myocardial ischemia and reperfusion can injure cardiomyocytes and endothelium, leading to vascular leakage, which promotes myocardial edema and aggravates cardiomyocyte death (Bijnens et al., 2008; Turer et al., 2010). Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2), initially implicated in embryonic vasculogenesis and angiogenesis (Suri et al., 1996; Maisonpierre et al., 1997), have been shown to play divergent roles in mediating cell survival and endothelial integrity in recent years (Harfouche et al., 2002; Dallabrida et al., 2005; Lee et al., 2009; Tuo et al., 2011; van der Heijden et al., 2011). Angiopoietin-1 (Ang-1), a secreted 70-kDa glycoprotein primarily expressed by mesenchymal cells, is the major agonist for the tyrosine kinase receptor Tie-2. Angiopoietin-2 (Ang-2), also a secreted 70-kDa glycoprotein, is exclusively expressed by endothelial cells, and acts as an antagonist for Tie-2 (Suri et al., 1996; Maisonpierre et al., 1997). In animal myocardial infarction and ischemia/reperfusion (I/R) models, adenoviral vectors carrying Ang-1 could not only promote angiogenesis, but also increase cardiomyocyte survival, inhibit vascular leakage, and finally reduce infarct size (Sun et al., 2007; Tuo et al., 2008; Lee et al., 2011), whereas adenoviral vectors carrying Ang-2 have been shown to have opposite effects (Tuo et al., 2008). These results suggest that Ang-1 and Ang-2 may play important roles in myocardial infarction and I/R injury.

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Former studies showed plasma Ang-2 levels are significantly increased, and correlate with peak total creatine kinase (CK) levels in patients with acute myocardial infarction, but patients receiving urgent percutaneous coronary intervention (PCI) were excluded in these studies (Lee et al., 2004; Pannitteri et al., 2006). Therefore, circulating Ang-1 and Ang-2 levels in patients with ST-segment elevation myocardial infarction (STEMI) treated with primary PCI are unknown.

We hypothesized that circulating Ang-1 and Ang-2 levels may have similar changes in STEMI patients receiving primary PCI. Also, because Ang-1 and Ang-2 have opposite pathophysiological effects in animal models, this study will also investigate Ang-2/Ang-1 ratio (Ang-2/1) in these patients.

Methods

Patient selection

A total of 85 consecutive patients with first STEMI admitted to the Department of Cardiology, Peking University Third Hospital, from October 2010 to September 2011 were included. STEMI was diagnosed according to the 2004 American College of Cardiology/American Heart Association guideline. All patients received primary PCI within 12 h from symptom onset, and Thrombolysis In Myocardial Infarction (TIMI) flow grade was ≥2 at the end of procedure. Exclusion criteria were: age >80 years, cardiogenic shock at admission, TIMI flow grade <2 at the end of procedure, previous history of myocardial infarction, significant valvular heart disease, peripheral vascular disease, chronic heart failure, chronic inflammatory diseases, significant kidney or hepatic diseases, tumor.

During the same study period, 25 age and sex matched subjects who were admitted to the same hospital because of atypical chest pain but with normal coronary arteries confirmed by coronary angiography were included as controls.

This study was approved by the ethics review boards of Peking University Health Science Center. All patients gave their consent to use part of their blood for scientific purposes.

Treatment and procedures

STEMI patients were treated with a loading dose of aspirin 300 mg and clopidogrel 300–600 mg at admission, and a bolus of 5000 IU of heparin before PCI. The PCI procedure was then completed according to standard technique (Kolh et al., 2010). After PCI, patients received standard therapy including aspirin, clopidogrel, statins, β-blockers and angiotensin-converting enzyme (ACE) inhibitors if there were no contraindications. The control subjects received aspirin 100 mg and clopidogrel 75 mg per day before angiography.

Laboratory assays

Venous blood samples were taken from STEMI patients at admission (baseline), and at 2 h, 6 h, 24 h, 48 h and 1 week after PCI. In the control subjects, venous blood samples were obtained in the morning of the same day when angiography was performed. All samples were collected into vacuum blood collection tubes with clot activator and were immediately placed in 4°C refrigerators. Within 30 min after collection, samples were centrifuged at 3000 rpm for 10 min at 4°C, divided into aliquots, and stored at −80°C until analysis. Repeated freeze-thaw cycles were avoided.

Serum Ang-1 and Ang-2 were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instruction (ELISA kit, R&D Systems). The minimal detection limits were 156 pg/ml for both Ang-1 and Ang-2. These assays were performed by an investigator blinded to the sources of the samples. Ang-2/1 ratio was calculated.

Serum cardiac troponin T (cTnT) levels were measured at admission and every 6 h during the first and second days after PCI using a high-sensitive assay (Roche cTnT hs; cutoff ≥0.014 ng/ml).

Statistics

Clinical parameters and angiopoietins levels of STEMI patients and the controls were compared using Student's unpaired *t*-tests or χ^2-tests. Changes of angiopoietins levels of STEMI patients were analyzed by repeated measures. Spearman or Pearson correlation was used to identify the bivariate correlations. Statistical significance was defined as $p < 0.05$. All analyses were performed with SPSS for Windows version 15.0 (SPSS, Chicago, IL).

Results

Clinical characteristics

The clinical characteristics and laboratory findings at baseline, and medications before admission of STEMI patients and control subjects are summarized in Table 1. The proportion of current smoking and nitrites treatment in STEMI patients was higher than in control subjects. STEMI patients had lower high density lipoprotein cholesterol (HDL-C), higher glucose, higher high-sensitivity C-reactive protein (hs-CRP) levels, and higher white blood cell (WBC) count than control subjects. There were no significant differences in other clinical parameters such as age, sex, body mass index (BMI), concomitant illnesses, systolic blood pressure, heart rate, lipid profile other than HDL-C, medications other than nitrites between the two groups.

Presentation characteristics of STEMI patients are shown in Table 2. Median (inter-quartile range) time from symptom onset to admission and to PCI were 132 (92–263) and 218 (128–344) min separately. Serum cTnT levels were lower than 0.014 ng/ml at admission in 57 patients (67.1%). Median (inter-quartile range) peak cTnT after PCI were 4.6 (3.1–6.5) ng/ml.

Table 1. Patient clinical characteristics, laboratory findings and medications at baseline.

	STEMI patients ($N = 85$)	Controls ($N = 25$)	p Value
Age (years)	57.2 ± 11.2	62.0 ± 9.6	0.09
Male (%)	87.1	76.0	0.18
Current smoker (%)	80.0	36.0	<0.001
Hypertension (%)	44.7	64.0	0.09
Diabetes mellitus (%)	23.5	24.0	0.96
Stroke (%)	12.9	4.0	0.17
BMI (kg/m^2)	25.6 ± 4.9	25.6 ± 3.0	0.99
Systolic blood pressure (mmHg)	137.1 ± 30.6	130.7 ± 16.6	0.32
Heart rate (beats/minute)	73.7 ± 14.6	67.6 ± 10.6	0.05
TC (mmol/l)	4.4 ± 1.0	4.5 ± 1.0	0.94
LDL-C (mmol/l)	2.8 ± 0.7	2.6 ± 0.8	0.35
HDL-C (mmol/l)	0.9 ± 0.2	1.0 ± 0.2	<0.01
TG (mmol/l)	2.0 ± 1.3	1.7 ± 0.8	0.33
Creatinine (μmol/l)	74.7 ± 13.2	75.8 ± 9.5	0.69
Glucose (mmol/l)	9.1 ± 4.3	5.3 ± 0.8	<0.001
Hs-CRP (mg/l)	6.2 (2.9–15.1)	1.4 (0.8–2.2)	<0.001
WBC count (10^9/l)	10.7 ± 3.1	6.3 ± 1.2	<0.001
Medications (%)			
Antiplatelet	47.1	40.0	0.53
ACE inhibitor/ARB	41.2	44.0	0.80
β-blocker	21.2	16.0	0.57
Statin	48.2	36.0	0.28
CCB	32.9	36.0	0.78
Nitrates	28.2	8.0	0.04
Insulin	8.2	8.0	0.97

Values represent mean ± SD, median (inter-quartile range), or the percentage of STEMI patients and control subjects.
ACE inhibitor, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; STEMI, ST-segment elevation myocardial infarction; BMI, body mass index; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; Hs-CRP, high-sensitivity-CRP; WBC, white blood cell.

Table 2. Presentation characteristics of STEMI patients (n = 85).

Characteristic	Value
Time from symptom onset to admission (min)	132 (92–263)
Time from symptom onset to PCI (min)	218 (128–344)
Peak cardiac troponin T (ng/ml)	4.6 (3.1–6.5)

Values represent median (inter-quartile range) or the percentage of STEMI patients.

Dynamic changes in serum Ang-1, Ang-2 and Ang-2/1 ratio

Serum Ang-1, Ang-2 levels and Ang-2/1 ratio of STEMI patients and control subjects are shown in Table 3. STEMI patients exhibited significant higher Ang-1 and Ang-2 levels from baseline to 1 week after PCI, and higher Ang-2/1 from baseline to 48 h after PCI ($p < 0.001$). At 1 week after PCI, Ang-2/1 was not significantly different between the two groups ($p = 0.516$).

Dynamic changes in serum Ang-1, Ang-2 and Ang-2/1 in STEMI patients from baseline to 1 week after PCI were observed (analysis of repeated measures, each $P < 0.001$, Figure 1). Both Ang-1 and Ang-2 decreased during the first week after PCI. Ang-2/1 increased slightly after PCI, reached highest level at 24 h, and then decreased.

Table 3. Serum Ang-1, Ang-2 and Ang-2/1 in all subjects.

	STEMI patients ($N = 85$)	Controls ($N = 25$)	p Value
Ang-1, pg/ml			
Baseline	20076.8 ± 1767.1	3000.0 ± 431.8	<0.001
2 h	15563.2 ± 1493.7		<0.001
6 h	14372.4 ± 1561.5		<0.001
24 h	11455.7 ± 1647.4		<0.001
48 h	9402.9 ± 2011.3		<0.001
1 week	4087.1 ± 1027.3		<0.001
Ang-2, pg/ml			
Baseline	2113.2 ± 116.2	198.9 ± 25.1	<0.001
2 h	1710.6 ± 148.9		<0.001
6 h	1622.6 ± 170.4		<0.001
24 h	1357.7 ± 185.3		<0.001
48 h	1029.8 ± 255.4		<0.001
1 week	245.5 ± 74.0		<0.001
Ang-2/ 1, 10^{-2}			
Baseline	10.6 ± 0.9	6.7 ± 1.4	<0.001
2 h	11.0 ± 0.9		<0.001
6 h	11.4 ± 1.3		<0.001
24 h	12.0 ± 2.2		<0.001
48 h	10.8 ± 2.6		<0.001
1 week	6.5 ± 2.5		0.52

Values represent mean ± SD.
The data of STEMI patients were collected at baseline and 2 h, 6 h, 24 h, 48 h, 1 week after PCI. The data of control subjects were taken in the morning of the same day when angiography was performed.
Ang-2, angiopoietin-2; Ang-2/1, angiopoietin-2 to angiopoietin-1 ratio Ang-1, angiopoietin-1.

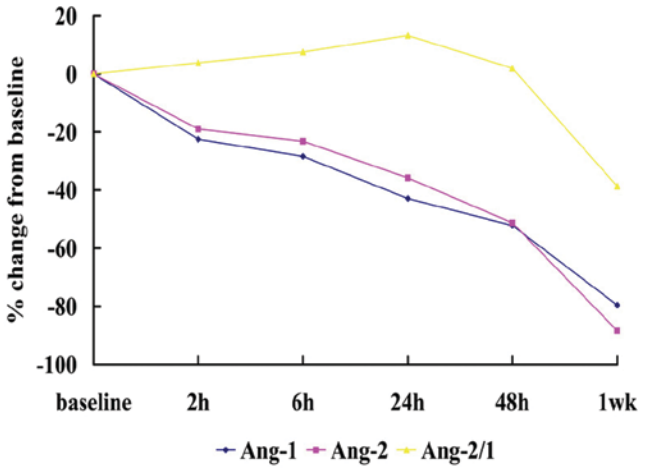

Figure 1. Changes of serum Ang-1, Ang-2 and Ang-2/ 1 in STEMI patients Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; Ang-2/1, angiopoietin-2 to angiopoietin-1 ratio. The data were collected at baseline and 2h, 6h, 24h, 48h, 1wk after PCI.

Association between angiopoietins and clinical characteristics

Serum Ang-1, Ang-2 and Ang-2/1 at all time points in STEMI patients showed no significant association with age, sex, smoking status, past history of hypertension,

diabetes mellitus or stroke, time from symptom onset to admission or to PCI, WBC count, hs-CRP, glucose or creatinine levels (data not shown).

Relationship of angiopoietins with cTnT levels

Serum Ang-1, Ang-2 and Ang-2/1 in STEMI patients were not significantly correlated with cTnT at admission (data not shown).

Ang-2 and Ang-2/1 at admission and 2 h after PCI were positively correlated with peak cTnT levels after PCI (Figure 2), but Ang-2 and Ang-2/1 at other time points, and Ang-1 at all time points were not significantly correlated with peak cTnT levels (data not shown).

Discussion

Myocardial ischemia can trigger a compensatory response to improve myocardial perfusion by the formation of new vessels (angiogenesis) and by the enlargement of pre-existing collateral vessels (arteriogenesis) (Helisch et al., 2003). Angiopoietins are major regulators of angiogenesis (Suri et al., 1996; Maisonpierre et al., 1997). The present study demonstrates that both serum Ang-1 and Ang-2 levels were increased in STEMI patients within 12 h of symptom onset. Median symptom onset to admission time was 132 min, and 67.1% of patients had cTnT <0.014 ng/ml at admission, indicating circulating angiopoietins may increase earlier than cTnT in STEMI patients. After successful primary PCI, both of the two angiopoietins decreased, indicating circulating levels of angiopoietins may reflect the myocardial ischemia state.

Different from our finding, a former study (Lee et al., 2004) showed that plasma Ang-2 but not Ang-1 levels are increased in patients with acute myocardial infarction. In Lee's study, both STEMI and non-ST-elevation myocardial infarction (NSTEMI) patients were included, and baseline blood samples were taken within 24 h of admission. Different from Lee's study, the present study included only STEMI patients, and baseline blood samples were taken at admission. Another former study (Pannitteri et al., 2006) showed a double wave of release of Ang-2 in STEMI patients receiving thrombolytic therapy. But in the present study, as mentioned before, both Ang-1 and Ang-2 decreased during the first week after PCI. Evidence has shown that STEMI patients treated with primary PCI have more effective restoration of vessel patency and less re-occlusion than patients receiving thrombolytic therapy (Kolh et al., 2010), which may be one of the reasons why this study found different changes of angiopoietins from the former one. To the best of our knowledge, the present study is the first to investigate serum Ang-1 and Ang-2 levels in patients with STEMI before and after successful primary PCI.

The present study is also the first to demonstrate that Ang-2/1 ratio in STEMI patients was increased at admission, reached highest level at 24 h after PCI, and then decreased. Ang-2 and Ang-2/1 at both baseline and 2 h after PCI were positively correlated with peak cTnT in the present study, indicating that circulating Ang-2 and Ang-2/1 ratio may predict the extent of myocardial damage.

Ang-1 and Ang-2 play divergent roles in mediating cell survival, vascular quiescence and inflammation. Ang-1 has anti-apoptosis (Harfouche et al., 2002; Dallabrida et al., 2005), anti-permeability (Lee et al., 2009; Tuo et al., 2011) and anti-inflammatory effects (Kim et al., 2001; Gu et al., 2010), while Ang-2 seems to have opposite effects (Tuo at al., 2011; van der Heijden et al., 2011). Ang-1 and Ang-2 have been shown to be involved in several kinds of cardiovascular diseases, as well as sepsis. Atherosclerotic

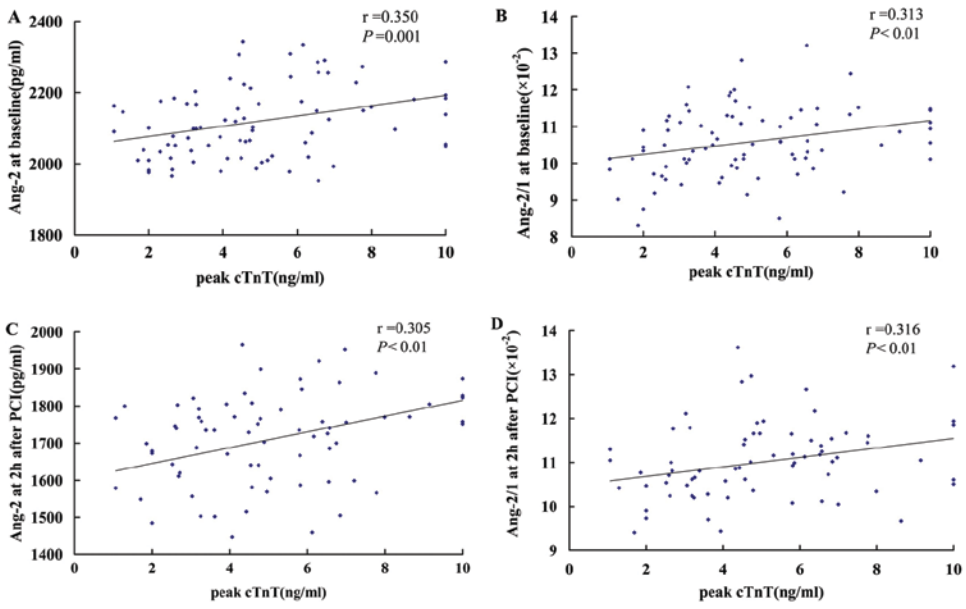

Figure 2. A. linear regression of Ang-2 at baseline with peak cTnT; B. linear regression of Ang-2/1 at baseline with peak cTnT; C. linear regression of Ang-2 at 2h after PCI with peak cTnT; D. linear regression of Ang-2/1 at 2h after PCI with peak cTnT.

plaque microvessels are associated with plaque hemorrhage and rupture (Di Stefano et al., 2009). Evidence showed that in plaques with high microvessel density, the balance between Ang-1 and Ang-2 is in favor of Ang-2, suggesting a role for Ang-2 in the development of (unstable) plaque microvessels (Post et al., 2008). Clinical studies showed that higher plasma Ang-2 levels are predictive of myocardial infarction (Patel et al., 2008; Iribarren et al., 2011) and stroke recurrence (Chen et al., 2010). In patients with severe sepsis, lower Ang-1 and higher Ang-2 levels are correlated with 28-day mortality (Ricciuto et al., 2011). In animal myocardial infarction and I/R models, shifting the Ang-2/1 ratio to favor Ang-1 by administration of adenovirus expressing Ang-1 could prevent myocardial and endothelial cell apoptosis, reduce vascular leakage and infarct size (Sun et al., 2007; Tuo et al., 2008; Lee et al., 2011), while shifting the Ang-2/1 ratio to favor Ang-2 results in a significant increase in myocardial infarct size (Tuo et al., 2008). The present study shows higher Ang-2 and Ang-2/1 ratio were associated with larger infarct size, indicating increased Ang-2 may cause myocardial damage, overcoming the protective effect of Ang-1.

The present study failed to show association of angiopoietins with risk factors such as hypertension, diabetes mellitus, or markers of inflammation such as Hs-CRP. The sample size of this study is small and, therefore, our data need confirmation in future studies.

In conclusion, serum Ang-1, Ang-2 levels and Ang-2/1 ratio in STEMI patients were significantly increased at admission, and had dynamic changes within the first week after primary PCI. Ang-2 and Ang-2/1 at admission and 2 h after PCI were positively correlated with peak cTnT levels, suggesting that the extent of myocardial damage may be linked to circulating Ang-2 and Ang-2/1. Further investigation is warranted to determine the relationship between angiopoietins and cardiac function, and the role of angiopoietins as biomarkers of reperfusion success and predictors of cardiovascular events in STEMI patients.

Declaration of interest

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