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Coronary Artery Disease

Study on Connective Tissue Growth Factor Expressed in Patients with ST-Segment Elevation Myocardial Infarction

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Background: This study measured the change in connective tissue growth factor levels after the onset of unstable angina and ST- segment elevation myocardial infarction, and studied its correlation with peak creatine kinase-MB (CK- MB) enzyme. It also discussed the significance of myocardial fibrosis after myocardial infarction. To detect the serum levels of connective tissue growth factor in patients with ST-segment elevation myocardial infarction and its relationship with the maximum level of CK-MB.

Methods: We selected 50 patients with ST-segment elevation myocardial infarction and 50 patients with unstable angina. Connective tissue growth factor levels were examined 24 h, 2 d, 7 d, and 14 d after the onset of ST-segment elevation myocardial infarction, and within 24 h for unstable angina, using enzyme-linked immunosorbent assay (ELISA). The maximum level of CK-MB was detected by immunosuppression.

Results: The serum level of connective tissue growth factor in the unstable angina patients was 10.34 ± 2.00 ng/mL, and the levels in the ST-segment elevation myocardial infarction patients were 16.76 ± 3.17 ng/mL at 24 h, 29.87 ± 4.90 ng/mL at 2 d, 45.02 ± 8.35 ng/mL at 7 d, and 31.61 ± 4.40 ng/mL at 14 d. Compared with the unstable angina patients, the connective tissue growth factor levels in the ST-segment elevation myocardial infarction patients were significantly higher since day 1 ($p < 0.01$). The maximum level of CK-MB was correlated with connective tissue growth factor levels at 7 d ($r = 0.859$, $p = 0.000$).

Conclusions: Connective tissue growth factor was significantly expressed in the ST-segment elevation myocardial infarction patients, indicating that it might play an important role in myocardial fibrosis.

Key Words: Angina patients • Connective issue growth factor • Creatine kinase-MB • ST-segment elevation myocardial

INTRODUCTION

Connective tissue growth factor (CTGF) was first cloned from human umbilical vein endothelial cells by Bradham in 1991.[1] CTGF has a variety of biological activities, and it is believed that CTGF can promote cell proliferation, extracellular matrix deposition, cell phenotype transformation, mediate cell adhesion, and angiogenesis, stimulate cell migration, and induce apoptosis.[2-7] With regards to cardiovascular disease, CTGF can promote the formation of atherosclerotic plaque, participate in the inflammatory response with myocardial infarction,[8] lead to left ventricular hypertrophy with hypertension,[9]

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These author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

and promote myocardial fibrosis with chronic heart failure.[10] This study measured the change in CTGF levels in different time periods after the onset of unstable angina (UA) and ST-segment elevation myocardial infarction (STEMI), and studied its correlation with peak creatine kinase-MB (CK-MB) enzyme. It also discussed the significance of myocardial fibrosis after myocardial infarction and its correlation with the range of myocardial infarction.

MATERIALS AND METHODS

Study population

This study collected 50 patients hospitalized due to initial acute ST-elevation myocardial infarction without direct percutaneous coronary intervention (PCI) or thrombolytic indications from February 2014 to February 2015. The general situations of the patients are shown in Table 1. All patients were diagnosed with initial acute ST-elevation myocardial infarction and confirmed by both electrocardiography and coronary angiography. The exclusion criteria were previous severe valvular disease, cardiomyopathy, severe chronic heart failure, and persistent atrial fibrillation. These STEMI patients included 16 cases of wide range myocardial infarction (including 13 cases of acute extensive anterior myocardial infarction, 2 cases of inferior wall combined right ventricular myocardial infarction, and 1 case of inferior wall combined posterior wall myocardial infarction), 18 cases of mid-range myocardial infarction (all of anterior myocardial infarction), and 16 cases of small range myocardial infarction (including 11 cases of anterior myocardial infarction, 3 cases of inferior wall myocardial infarction, and 2 cases of limitation anterior myocardial infarction). These 2 cases were small range myocardial infarction. All of the patients received conventional intensive medical treatment and elective PCI after hospitalization. The door-to-balloon time was 278.30 ± 43.20 h for these 50 patients from chest pain to open infarction-related artery (IRA).

The present study was approved by the ethical committees of The Second Affiliated Hospital of Xuzhou Medical College, and all participants provided written informed consent.

Specimen collection

There were 50 hospitalized patients with myocardial infarction and 50 patients with unstable angina. Five ml of venous blood were extracted from the patients with myocardial infarction to detect CTGF at 24 h, 2 days, 7 days, and 14 days after disease onset. Five ml of venous blood were extracted from the patients with UA to detect CTGF at the time of hospitalization. Five ml of venous blood were extracted from the patients with STEMI every two hours to detect CK-MB and to obtain the CK-MB enzyme peak value.

Experimental methods

We pre-studied a certain amount of the sample (< 3 months) to determine the best ratio of dilution. Then we used unified enzyme-linked immunosorbent assay (ELISA) to detect both CTGF and N-terminal pro-B-type natriuretic peptide (NT-proBNP) contents in serum. The ELISA kits for CTGF and NT-proBNP were bought from PeproTech Company in America, and all of the operations were conducted in strict accordance with their instructions. Quality control and calibration results were all in required ranges. Immune suppression and a Roche automatic biochemical analyzer were used to detect serum CK-MB levels, with the reference value being 0-25

Table 1. Clinical data comparison between two groups of patients

Variable	UA group (n = 50)	STEMI group (n = 50)	p value
Age (yr)	61.80 ± 6.30	62.00 ± 7.60	0.785
Sex (male/female)	36/14	39/11	0.491
Combined with hypertension	44	46	0.507
Combined with diabetes	26	25	0.842
Creatinin (umol/L)	69.00 ± 8.80	71.00 ± 7.90	0.325
LVEF (%)	55.40 ± 7.60	51.20 ± 6.80	0.038
NT-proBNP (pg/ml)	170.40 ± 53.70	632.80 ± 186.90	0.012

LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

U/L. All reagents were purchased from Roche Company. The left ventricular ejection fraction (LVEF, %) was determined by echocardiographic measurements.

Statistical analysis

We used SPSS 17.0 software for data analysis, and data are presented as mean ± standard deviation ($\bar{x} \pm s$). We used the independent samples *t* test for comparisons between two groups, and the paired *t* test within certain groups. Pearson linear correlation analysis was used to analyze the quantitative relationship between indicators. A p value < 0.05 was considered to be statistically significant.

RESULTS

General comparison between two groups of patients

With respect to age, sex, and comorbidities, there were no statistically significant differences between the patients with UA and STEMI. It has been reported that serum concentrations of CTGF are related to renal function,[11,12] however there was no statistically significant difference in creatinine between our two groups of patients. The LVEF (%) values and NT-proBNP levels of the STEMI patients were significantly higher than those of the UA patients (p < 0.05, Table 1).

CTGF test results

There was a statistically significant difference between the patients with UA and those with STEMI in CTGF. In addition, compared to the patients with angina, those with myocardial infarction had higher CTGF levels (Table 2, p < 0.01).

CTGF levels were detected in the patients with STEMI at 24 h, 2 days, 7 days, and 14 days after disease onset. The results were statistically significant, with a gradual increase from 24 h to 7 days after disease onset, and then a gradual decrease (Figure 1; Table 2, p < 0.01).

Correlation between CTGF and the peak of CK-MB enzyme

Pearson correlation analysis between CTGF and the peak value of CK-MB enzyme in the STEMI patients at different time points indicated an unobvious correlation between CTGF and peak CK-MB enzyme at 24 h, 2 days and 14 days after disease onset, while a correlation between CTGF and peak CK-MB enzyme was noted 7 days after the onset (Table 3).

Table 2. Serum CTGF and CK-MB content changes of two groups of patients

Group	CTGF (ng/mL)	Peak value of CK-MB(U/L)
Angina group	10.34 ± 2.00	-0.90 ± 0.30
STEMI patients		
24h	16.76 ± 3.17*	103.45 ± 28.64*
2d	29.87 ± 4.90*‡	158.37 ± 39.41*‡
7d	45.02 ± 8.35*†	187.10 ± 57.43*†
14d	31.61 ± 4.40*#	162.21 ± 42.53*#

* p = 0.01 vs. Angina group; # p = 0.01 vs. 7 days; † p = 0.01 vs. 2 days; ‡ p = 0.01 vs. Myocardial infarction 24 h. Data were presented as mean ± SD or as number. p < 0.05 was considered statistically significant.

CK-MB, creatine kinase-MB; CTGF, connective tissue growth factor; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction.

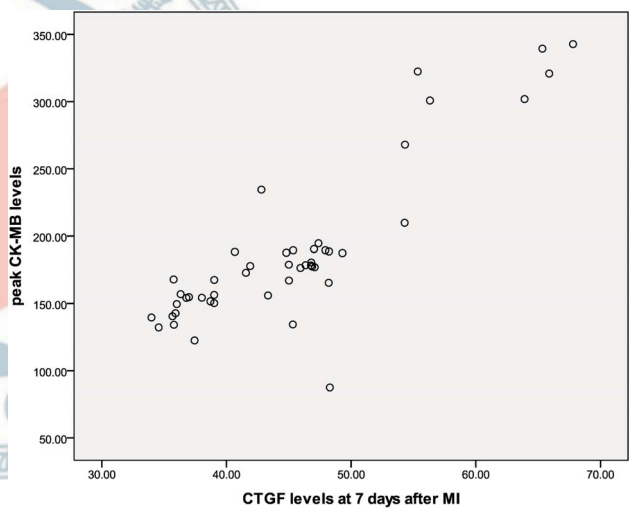

Figure 1. *Connective tissue growth factor (CTGF) levels at 7 days after myocardial infarction (MI) and peak creatine kinase-MB (CK-MB) levels in patients of ST-segment elevation myocardial infarction (STEMI).*

Table 3. Pearson correlation between CTGF and the peak value of CK-MB enzyme of myocardial infarction patients in different periods of time

Item	Hospitalization time			
	24h	2d	7d	14d
r	-0.071	-0.103	0.859	0.227
p	0.625	0.477	0.000	0.113

CK-MB, creatine kinase-MB; CTGF, connective tissue growth factor.

DISCUSSION

CTGF is a C-terminal cysteine-rich secreted polypeptide containing 349 amino acids with a relative molecular weight of 30888. It is a member of the highly conserved CTGF, Cef10/cyr61 and Nov polypeptide family.[13] It is widely presented in a variety of tissues and organs, such as the heart, brain, kidney, lung, liver, placenta, pancreas, and connective tissue. Under physiological conditions, a variety of tissues and cells may secrete a basic amount of CTGF; while, under pathological conditions, it is closely related to skin scarring, atherosclerosis, organ fibrosis, wound healing and other diseases.[14] Studies have shown that CTGF is closely related to myocardial fibrosis, which plays an important role in cardiac remodeling after myocardial infarction.[8]

In this study, there was a statistically significant difference in CTGF content in the serum of the myocardial infarction patients compared with the UA patients 24 h after disease onset ($p < 0.01$), and it significantly increased with time, indicating that the inflammatory response caused by myocardial infarction can lead to the release of a variety of inflammatory mediators, including CTGF, from regional myocardial tissue. The CTGF levels of patients with STEMI as 24 h, 2 days, 7 days, and 14 days after disease onset showed a statistically significant difference ($p < 0.01$), with a gradual increase from 24 h to 7 days after disease onset. Compared with the result on the 7th day, the CTGF level significantly decreased after 14 days ($p < 0.01$). However, this level was still significantly higher than that of the UA patients ($p < 0.01$). These findings indicated that the expression of CTGF after STEMI lasted from the start phase until the late reconstruction phase of myocardial infarction. CTGF is known to be involved in the process of tissue repair after injury, and it can also promote the transformation of cardiac fibroblasts to myofibroblasts, and stimulate fibroblast proliferation. In addition, it can induce cardiac fibroblasts to secrete collagen and sticky protein and increase the expression levels of myocardial infarction markers.[15] This function of CTGF can lead to myocardial fibrosis,[9,10] cardiac remodeling, and a decline in cardiac function after myocardial infarction.

In this study, correlation analysis showed that the peak level of CK-MB enzyme was positively correlated with the CTGF level at 7 days after disease onset ($r = 0.859$, $p < 0.01$), but was poorly correlated at other time periods. This indicated that peak CTGF was related to peak CK-MB enzyme after myocardial infarction, and that peak CK-MB enzyme was positively correlated with the range of myocardial infarction.[16-18] Therefore, peak CTGF might be associated with the range of myocardial infarction, i.e., a greater range of myocardial infarction results in a higher expression of CTGF in infarct myocardial tissue, more apparent of cardiac remodeling, and greater impact on cardiac function. In this study, there was no long-term follow-up of the patients with cardiac function, so it was not clear whether CTGF was negatively correlated with cardiac function in the patients after myocardial infarction.

The present study also had several limitations. First we only analyzed the correlation of CTGF levels with the maximum value of CK-MB. Second, deeper insights into how CTGF influences the development of myocardial infarction were also unclear. Further studies are needed to better understand these results.

CONCLUSIONS

This study demonstrated that CTGF might be related to cardiac remodeling after myocardial infarction, although further investigations are needed to elucidate its precise role and mechanism on myocardial infarction, as well as on its relationship with other neuroendocrine activation after myocardial infarction. CTGF may be considered to be an early diagnostic indicator and therapeutic target of myocardial infarction to prevent myocardial remodeling after myocardial infarction, and to avoid the development of refractory heart failure.

DECLARATION OF CONFLICT OF INTEREST

All the authors declare no conflict of interest.

REFERENCES

1. Bradham DM, Igarashi A, Potter RL, Grotendorst GR. Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. *J Cell Biol* 1991;114:1285-94.

2. Parada C, Li J, Iwata J, et al. CTGF mediates Smad-dependent transforming growth factor β signaling to regulate mesenchymal cell proliferation during palate development. *Mol Cell Biol* 2013; 33:3482-93.
3. Ma B, Kang Q, Qin L, et al. TGF-β2 induces transdifferentiation and fibrosis in human lens epithelial cells via regulating gremlin and CTGF. *Biochem Biophys Res Commun* 2014;447:689-95.
4. Kiwanuka E, Andersson L, Caterson EJ, et al. CCN2 promotes keratinocyte adhesion and migration via integrin α5β1. *Exp Cell Res* 2013;319:2938-46.
5. Zhen Y, Ye Y, Yu X, et al. Reduced CTGF expression promotes cell growth, migration, and invasion in nasopharyngeal carcinoma. *PLoS One* 2013;8:e64976.
6. Charrier A, Brigstock DR. Regulation of pancreatic function by connective tissue growth factor (CTGF, CCN2). *Cytokine Growth Factor Rev* 2013;24:59-68.
7. Oka M, Kubota S, Kondo S, et al. Gene expression and distribution of connective tissue growth factor (CCN2/CTGF) during secondary ossification center formation. *J Histochem Cytochem* 2007;55:1245-55.
8. Gravning J, Ørn S, Kaasbøll OJ, et al. Myocardial connective tissue growth factor (CCN2/CTGF) attenuates left ventricular remodeling after myocardial infarction. *PLoS One* 2012;7:e52120.
9. Szabó Z, Magga J, Alakoski T, et al. Connective tissue growth factor inhibition attenuates left ventricular remodeling and dysfunction in pressure overload-induced heart failure. *Hypertension* 2014;63:1235-40.
10. Wu CK, Wang YC, Lee JK, et al. Connective tissue growth factor and cardiac diastolic dysfunction: human data from the Taiwan diastolic heart failure registry and molecular basis by cellular and animal models. *Eur J Heart Fail* 2014;16:163-72.
11. Tachaudomdach C, Kantachuvesiri S, Changsirikulchai S, et al. Connective tissue growth factor gene expression and decline in renal function in lupus nephritis. *Exp Ther Med* 2012;3:713-8.
12. Metalidis C, van Vuuren SH, Broekhuizen R, et al. Urinary connective tissue growth factor is associated with human renal allograft fibrogenesis. *Transplantation* 2013;96:494-500.
13. Lau LF, Lam SC. The CCN family of angiogenic regulators: the integrin connection. *Exp Cell Res* 1999;248:44-57.
14. Moussad EE, Brigstock DR. Connective tissue growth factor: what's in a name? *Mol Genet Metab* 2000;71:276-92.
15. Dean RG1, Balding LC, Candido R, et al. Connective tissue growth factor and cardiac fibrosis after myocardial infarction. *J Histochem Cytochem* 2005;53:1245-56.
16. Rakowski T, Dziewierz A, Legutko J, et al. Creatine kinase-MB assessed in patients with acute myocardial infarction correlates with cardiac magnetic resonance infarct size at 6-month follow up. *Hellenic J Cardiol* 2014;55:4-8.
17. Wang TD. Our stance towards the 2017 ACC/AHA high blood pressure clinical practice guideline: has the pendulum swung too far? *Acta Cardiol Sin* 2018;34:1-3.
18. Chen YH, Huang SS, Lin SD. TIMI and GRACE risk scores predict both short-term and long-term outcomes in Chinese patients with acute myocardial infarction. *Acta Cardiol Sin* 2018;34:4-12.