Original Article

Plasma Mitochondrial Coupling Factor 6 in Patients with Acute Myocardial Infarction

Wen Hui DING, Song Yun CHU, Hong Feng JIANG, Da Yong CAI,
Yong Zheng PANG, Chao Shu TANG^{*}, and Yong Fen QI

Previous studies have shown that mitochondrial coupling factor 6 (CF6) is an endogenous peptide that inhibits prostacyclin (PGI₂) synthesis in vascular endothelial cells. In this study, we measured the plasma CF6 level of patients with acute myocardial infarction (AMI) to observe dynamic changes of CF6. All patients showed elevated plasma CF6 levels upon admission for treatment of AMI. Their CF6 levels peaked approximately 72 h after the onset of AMI and remained high for 7 days. At 7 days, their CF6 levels decreased to the level seen upon admission, but not to within a normal range. Hyperlipidemic patients had significantly greater CF6 levels at 24h after onset of AMI than patients with a normal lipid profile. On admission, the plasma CF6 level in patients with a cardiac function of Killip class $\geq$ II was higher than that in patients with a Killip class I cardiac function. At 3 days after the onset of AMI, the plasma CF6 levels of patients with a creatinine kinase (CK) peak value $\geq$ 1,500 units/l were significantly higher than those of patients with a CK peak value $<$ 1,500 units/l ($p = 0.05$). At 7 days after the onset of AMI, the plasma CF6 levels of patients who received no reperfusion were significantly higher than those of patients who received a successful reperfusion. The plasma CF6 levels of AMI patients at admission, at 24 h, and at 3 days after onset of symptoms correlated positively with the cardiac function by Killip classification, respectively. At 24 h after onset of AMI, the plasma CF6 levels correlated positively with plasma total cholesterol levels and low-density lipoprotein levels. At 3 days, the plasma level of CF6 correlated positively with the plasma CK peak value and correlated negatively with left ventricular ejection fraction. These results suggest that the plasma CF6 level was elevated in patients with AMI. (*Hypertens Res* 2004; 27: 717–722)

Key Words: coupling factor 6, acute myocardial infarction, peptide

Introduction

The alteration and interaction of various vasoactive peptides, such as angiotensin, endothelin, urotensin II, adrenomedullin, and calcitonin gene-related peptide, play a crucial role in the pathogenesis of acute myocardial infarction (AMI) (1–4). It seems reasonable to conjecture that some unknown bioactive peptides could also play an important role in AMI pathogen-

esis.

Mitochondrial coupling factor 6 (CF6) is a recently discovered endogenous vasoactive peptide (5). Mitochondrial adenosine triphosphatase synthase consists of 3 domains, namely, the extrinsic and intrinsic membrane domains, F1 and F0, respectively, joined by a stalk. Four subunits of the stalk were identified and designated as the mitochondrial CF6, oligomycin sensitivity conferral protein, and subunits b and d. Of these 4 subunits, CF6 was reported to be essential for

From the Institute of Cardiovascular Research, Peking University First Hospital, Beijing, PR China, and ^{*} Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, PR China.

This work was supported by The Major State Basic Research Development Program of the People's Republic of China (G2000056905).

Address for Reprints: Yong Fen Qi, M.D., Institute of Cardiovascular Research, Peking University First Hospital, Beijing 100034, PR China. E-mail: yongfenqi@yahoo.com

Received January 5, 2004; Accepted in revised form June 23, 2004.

energy transduction (6). In addition, CF6 is a unique inhibitor of endogenous prostacyclin (PGI₂) synthesis and therefore a novel vascular constrictor. It has been suggested that CF6 could be an important contributor to the occurrence of essential hypertension (7). CF6 exists in various tissues, among which the myocardium shows the most dense distribution in the human body. Endothelial cells are the primary source of its circulating form (6). The injury of ischemic cardiomyocytes and the drastic disturbance in endothelial function in AMI is apparent. In the present study we aimed to investigate dynamic changes of the plasma CF6 level during the AMI pathological process and to consider their possible significance.

Methods

Patients

A total of 49 Chinese patients with AMI (36 men, 13 women; average age 63.5 ± 11.9 years) who were consecutively admitted to Peking University First Hospital (from Oct. 2001 to May 2002) within 24 h of the onset of symptoms were included in the study. The diagnosis of myocardial infarction was based on the following criteria: 1) chest pain lasting longer than 30 min; 2) ST segment elevation >0.1 mV in two or more ECG leads in the same vascular territory; and 3) a subsequent rise in creatinine kinase (CK) level to higher than the upper limit of normal. Patients with chronic renal and hepatic impairment were excluded from the study.

All the patients received routine antiplatelet agents, anti-coagulant and nitrates. A total of 28 patients received reperfusion therapy (percutaneous transluminal coronary intervention [PTCI] or fibrinolytic therapy). Successful reperfusion was determined by thrombolysis in myocardial infarction (TIMI) III flow in coronary angiography in 25 patients.

Fifty healthy normotensive subjects (30 men, 20 women; average age 58.3 ± 10.7 years) who entered the hospital for a 2-day health check served as controls. Control subjects found to have cardiovascular, renal, hepatic, metabolic or endocrine diseases were excluded. All control subjects were administered a diet containing 120–170 mmol/l of sodium.

Informed consent was obtained from the participants before initiation of the study. The experimental protocol was approved by the ethical committee of our hospital.

Blood Sampling

Blood samples of the patients were drawn from the antecubital veins (with the subject in the supine position) immediately after admission and at 24 h, 3 days, and 7 days after the onset of AMI. Samples of the controls were obtained under a fasting condition in the same way as those for the patients. All the whole blood samples were received using a chilled glass tube containing disodium ethylene diamine tetraacidic

acid (1 mg/ml) and aprotinin (500 units/ml), then centrifuged immediately at $3,000 \times g$ for 10 min at 4°C, and plasma was stored at -80°C until being assayed.

Assays for CF6 in Plasma

CF6 in plasma was measured by radioimmunoassay using specific kits (Phoenix Pharmaceuticals Inc., Belmont, USA). Plasma was loaded onto a Sep-Pak C18 cartridge after being equilibrated with normal saline. The cartridge was washed with 2.5 ml of normal saline and 10% acetonitrile in 0.1% trifluoroacetic acid, and then eluted with 2 ml of 50% acetonitrile in 0.1% trifluoroacetic acid. The elution was lyophilized and subjected to radioimmunoassay (8). The IC₅₀ was 8 pg/tube, and the reactivity with human CF6 was 100%. No cross-reactivity was found with human brain natriuretic peptide, angiotensin II, calcitonin gene-related peptide, or prostacyclin. The intra-assay and inter-assay coefficients of variation for blood samples were $<10\%$. Reverse-phase high performance liquid chromatography revealed that the major peak of immunoreactive CF6 in the plasma detected by each radioimmunoassay kit was identical with that of synthetic human CF6.

Statistical Analysis

All data were expressed as the mean $\pm$ SD, unless otherwise indicated. The plasma levels of CF6 were compared over the time course of the study by use of one way ANOVA for repeated measures. The correlation between two variables was assessed by linear regression analysis. Variables were compared between two groups by unpaired Student's *t*-test. *p* values of <0.05 were considered significant.

Results

Baseline Characteristics

Coexistence(s) of Risk Factors for Coronary Artery Disease

Of the 49 patients with AMI, 29 had a history of hypertension, 5 had diabetes mellitus, 34 had hyperlipidemia, 20 had a family history of coronary artery disease, and 29 had a history of smoking.

Clinical Characteristics

Of the 49 patients with AMI, 27 patients suffered from anterior myocardial infarction, 22 had a CK peak value $\geq 1,500$ units/l, 26 had a cardiac function of Killip class I. See Table 1.

Dynamic Changes of Plasma Concentrations of CF6 in Patients with AMI

Plasma CF6 levels were significantly elevated in patients with AMI at admission in comparison to the controls. Then the level of CF6 was further elevated at 24 h and 3 days after

Table 1. Clinical Characteristics of Patients with Myocardial Infarction at Admission to Hospital

No. of patients	49
Age (years)	63.5 ± 11.9
No. of males	36
Disease history	
Hypertension	29
Diabetes mellitus	5
Hyperlipidemia	34
Smoking	29
Site of myocardium infarction	
Anterior	27
Inferior	22
Treatment in acute phase	
Successful reperfusion	25
No reperfusion or reperfusion failure	24
Peak value of plasma creatinine kinase	
≥1,500 units/l	22
<1,500 units/l	27
Cardiac function of Killip classification	
≥II	23
I	26

onset of symptoms, and reached a relative peak value at 3 days. At 7 days, the plasma CF6 concentrations dropped to the level at admission, but were still higher than those of controls. Data are shown in Fig. 1.

Relation between Plasma CF6 Level and Risk Factors for AMI and Clinical Characteristics

No significant difference of plasma CF6 level was detected between patients with and those without hypertension or diabetes mellitus, or between those with and without a family history of coronary artery disease (CAD) ($p > 0.05$). Patients with a smoking history tended to have elevated CF6, but the difference did not reach the level of statistical significance. Hyperlipidemic patients had a significantly higher CF6 level at 24 h after onset of AMI than those with a normal lipid profile ($p < 0.05$; Fig. 2).

No significant difference of plasma CF6 level was detected between patients with different infarct regions ($p > 0.05$). On admission, the plasma CF6 level in patients with a cardiac function of Killip class $\geq$ II was higher than that in patients with a Killip class I cardiac function. At 3 days after onset of AMI, the plasma CF6 levels of patients with a CK peak value $\geq$ 1,500 units/l were significantly higher than those of patients with a CK peak value $<$ 1,500 units/l ($p = 0.05$). At 7 days after onset of AMI, the plasma CF6 levels of patients who received no reperfusion were significantly higher than those of patients who received a successful reperfusion ($p < 0.05$). Data are shown in Figs. 3–5.

Fig. 1. Comparison of plasma CF6 level between AMI patients and controls. * $p < 0.05$, compared with controls. # $p < 0.05$, compared with the value at admission.

Fig. 2. Comparison of plasma CF6 level between AMI patients with and without hyperlipidemia. * $p < 0.01$, compared with AMI patients without hyperlipidemia.

Correlation of Plasma CF6 Level and Clinical Conditions

Plasma CF6 levels of AMI patients at admission and 24 h after onset of symptoms correlated positively with cardiac function by Killip classification ($r = 0.301$ and 0.552 , respectively; both $p < 0.05$). At 24 h after onset of AMI, the plasma CF6 levels correlated positively with plasma total cholesterol levels and low-density lipoprotein levels ($r = 0.464$, $p < 0.01$; $r = 0.431$, $p < 0.05$, respectively). At 3 days, the plasma level of CF6 correlated positively with the plasma CK peak value ($r = 0.332$, $p < 0.05$) and correlated negatively with left ventricular ejection fraction ($r = -0.471$, $p < 0.05$). Data are shown in Figs. 6–9.

Discussion

CF6 is a subunit of the stalk in mitochondrial ATP synthase, and acts as an essential component for energy transduction (5). Human CF6 is an 8.9 kDa peptide consisting of 76 amino acids. The homology of rat CF6 with the human

Fig. 3. Comparison of plasma CF6 between AMI patients with cardiac function of Killip I and Killip $\geq$ II. * $p < 0.05$, compared with AMI patients with cardiac function of Killip I.

Fig. 4. Comparison of plasma CF6 between AMI patients with a peak value of CK $\geq$ 1,500 units/l and < 1,500 units/l. * $p < 0.05$, compared with AMI patients with a peak value of CK < 1,500 units/l.

Fig. 5. Comparison of plasma CF6 between AMI patients with and without successful reperfusion. * $p < 0.05$, compared with patients with successful reperfusion.

amino acid sequence is 72%. As a novel vasoactive peptide, CF6 was first isolated and purified from tissues of spontaneously hypertensive rats by Osanai *et al.* in 1998 (5). CF6 reduces the production of PGI₂ by inhibiting endogenous arachidonic acid (AA) release from the plasma membrane without affecting exogenous AA and prostaglandin H₂. Its effect appears to be focused on phospholipase A₂ (PLA₂) activity independently of cyclooxygenase and prostacyclin synthase. CF6 indirectly inhibits cytosolic PLA₂ (cPLA₂) *via* mediators such as receptors, possibly because the presence or absence of its mediator(s) in the cell reduces the release of AA, which results in decreased production of PGI₂ (6). CF6 is also localized at the surface of endothelial cells (ECs) and released by shear stress, and has been implicated as an endogenous vasoconstrictor (9). CF6 may take part in regulation of vasotension as a systemic hormone or as a paracrine and/or an autocrine effector (10). The CF6 exists at high levels in various kinds of tissues, among which the myocardium is the most densely distributed throughout the human body. A large amount of CF6 is also present on the surface of ECs, and the secretion of ECs is the primary source of its circulating form. Studies of its effect in cardiovascular disease have demonstrated that a high level of plasma CF6 may contribute to the occurrence of essential hypertension, and hypertensive animals have an exaggerated response to CF6 compared with normotensive ones (6). CF6 has been found to be a novel risk factor for ischemic heart disease in end-stage renal disease, and circulating CF6 is elevated in human hypertension and modulated by salt intake, presumably *via* reactive oxygen species (11, 12).

In our study, plasma CF6 levels of AMI patients were elevated significantly compared with those of controls at the early stage of the pathologic process. This elevation may have been the result of myocardium tissue injury and necrosis of myocytes leading to the release of CF6 from mitochondria. At the same time the ECs suffered ischemia and hypoxia insults during AMI may secrete CF6 to the systemic circulation. At the same time, the ECs that suffered ischemic and hypoxic insults during AMI may have secreted CF6 into the systemic circulation. It has been suggested that angiotensin could bind with the α and β subunits of mitochondrial ATP synthase, which are also present on the surface of ECs, and inhibit the production of ATP. This makes the ECs more vulnerable to ischemic and hypoxic challenge, and ultimately to irreversible cell damage and the release of CF6 (7). Another contributor to the elevated plasma CF6 level might be the shear stress. Drastic changes of shear stress in AMI are potent stimuli of the production and release of various vasoactive peptides. It has been reported that shear stress induces a massive release of CF6 from ECs (7).

The plasma CF6 levels of patients with AMI elevated progressively from admission to day 3, reached a relative peak at day 3 that was significantly higher than the levels at admission, and then declined gradually to day 7, when they were equal to the levels at admission. This dynamic change

Fig. 6. Correlation of plasma CF6 at 24 h with plasma total cholesterol level. $r = 0.464$, $p < 0.01$.

Fig. 8. Correlation of plasma CF6 at 3 days with peak value of plasma CK. $r = 0.332$, $p < 0.05$.

Fig. 7 Correlation of plasma CF6 at 24 h with plasma low density lipoprotein. $r = 0.431$, $p < 0.05$.

Fig. 9. Correlation of plasma CF6 at 3 days with left ventricular ejection fraction. $r = -0.471$, $p < 0.05$.

could be a reflection of the progressive death of cardiomyocytes and severe endothelial dysfunction in the early phase of AMI, and the gradual return to homeostasis thereafter. A high level of CF6 in plasma could alter the balance between vasodilator factors and vasoconstrictor factors in vascular vessels by inhibiting PGI₂ synthesis, which would lead to inflammatory cell infiltration, platelet adhesion and aggregation, and microcirculation dysfunction (6). CF6 cannot inhibit the synthesis of thromboxanes A₂ (TxA₂), which could change the PGI₂:TxA₂ ratio and increase the risk of formation of thrombus. All of these routes aggravate myocardial ischemic injury.

Previous research *in vivo* has suggested that a high level of CF6 in plasma contributes to hypertension. And spontaneously hypertensive rats have higher plasma CF6 levels than normotensive Wistar Kyoto rats. In our study, however, no significant differences of CF6 concentration were found between AMI patients with and those without a history of hypertension. It might be that the severe insult of tissue injury and endothelial dysfunction in AMI influence the level of plasma CF6 far more than the chronic hypertension process.

With respect to other risk factors for coronary heart disease, we also found the following data. AMI patients with a smoking history tended to have higher plasma CF6 levels than those without. At 24 h after onset of symptoms, hyperlipidemic patients had significantly higher plasma CF6 levels than normolipidemic ones. And the concentration of CF6 correlated positively with plasma total cholesterol and low-density lipoprotein level. Given that a comparable tissue injury results from myocardium infarction, we conjectured that these differences might come from the further influence of these factors on endothelial function. Both smoking and dyslipidemia impair the endothelium and induce CF6 release. They are also risk factors leading to abnormality of endothelium-dependent vasodilatation. The constriction and spasm of vessels thereafter could affect the change of shear stress, which would also contribute to the secretion of CF6 by ECs. On the other hand, PGI₂ acts as a vasodilator *via* a direct action on vascular smooth muscle rather than the endothelium. It is suggested that the coronary dilatation induced by PGI₂ was the most important mechanism of vasodilatation during NO deficiency and also a form of compensation. The AMI patients tend-

ed to have a more dysfunctional endothelium and inadequate NO secretion (13). And the increased level of plasma CF6 could partially neutralize the compensatory effect of PGI₂. Ultimately, this would result in the further imbalance of various vaso peptides, intensified tissue injury, and acceleration of the process of AMI.

We also found that the plasma CF6 concentration of patients correlated positively with Killip classification on admission, at 24 h, and at 3 days after the onset of AMI. The CF6 levels of patients with a CK peak value $\geq 1,500$ units/l were significantly higher than those of patients with a CK peak value $< 1,500$ units/l at 3 days, and the CF6 concentrations correlated with CK peak value positively and correlated with left ventricular ejection fraction negatively. These data suggested that the release of CF6 showed a greater increase when the patients had larger infarction size or more severely disturbed hemodynamic and impaired cardiac function, which might indicate that a large number of cardiomyocytes were damaged. Further, they suggested that the plasma CF6 level might be associated with the severity of AMI. In patients who received no reperfusion therapy, the concentrations of plasma CF6 showed little decrease and remained at a high level until day 7. It was considered that the lack of reperfusion of the severely ischemic myocardium ultimately led to necrosis and the release of CF6. The accumulated CF6 could further damage the cardiovascular system by means of the mechanism mentioned above, thereby initiating a vicious cycle.

In conclusion, our study has suggested that the persistent elevation of plasma CF6 in patients within 1 week of the appearance of AMI could be the result of myocardial tissue injury and endothelial dysfunction. The concentrations of plasma CF6 were associated with the severity of AMI. As a novel vaso peptide with potential clinical significance, CF6 requires further investigation.

References

1. Ding WH, Wu FX, Ma HF, Tang CS: Dynamic changes and significance of plasma endothelin in patients with acute myocardial infarction during reperfusion. *J Chin Circ* 1998; **13**: 147–148.
2. Shi LB, Ding WH, Yang JJ: Alterations and significance of urotensin II in patients with ischemic diseases. *J Chin Med* 2001; **40**: 634–635.
3. Cao J, Zhang JH, Wang XH: Changes and its protective role of plasma adrenomedullin in patients with ischemia/reperfusion injury. *J Chin Med* 2000; **80**: 261–264.
4. Ding WH, Zhang JH, Tang CS: Calcitonine gene-related peptides and fibrinolysis. *J Chin Cardiovasc Dis* 1993; **21**: 80–82.
5. Osanai T, Kamada T, Fujiwara N, *et al*: A novel inhibitory effect on prostacyclin synthesis of coupling factor 6 extracted from the heart of spontaneously hypertensive rats. *J Biol Chem* 1998; **273**: 31778–31783.
6. Higuti T, Tsurumi C, Kawamura Y, *et al*: Molecular cloning of cDNA for the import precursor of human coupling factor 6 of H⁺-ATP synthase in mitochondria. *Biochem Biophys Res Commun* 1991; **178**: 793–799.
7. Osanai T, Okada S, Sirato K, *et al*: Mitochondrial coupling factor 6 is present on the surface of human vascular endothelial cells and is released by shear stress. *Circulation* 2001; **104**: 3132–3136.
8. Kato J, Kitamura K, Uemura T, *et al*: Plasma levels of adrenomedullin and atrial and brain natriuretic peptides in the general population: their relations to age and pulse pressure. *Hypertens Res* 2002; **25**: 887–892.
9. Osanai T, Tanaka M, Kamada T, *et al*: Mitochondrial coupling factor 6 as a potent endogenous vasoconstrictor. *J Clin Invest* 2001; **108**: 1023–1030.
10. Chang JK, Scruggs P, Yang J, *et al*: Total synthesis of human and rat coupling factor-6 amide and pressor effects in the rat. *Regul Pept* 2003; **113**: 63–69.
11. Osanai T, Sasaki S, Kamada T, *et al*: Circulating coupling factor 6 in human hypertension: role of reactive oxygen species. *J Hypertens* 2003; **21**: 2323–2328.
12. Osanai T, Nakamura M, Sasaki S, *et al*: Plasma concentration of coupling factor 6 and cardiovascular events in patients with end-stage renal disease. *Kidney Int* 2003; **64**: 2291–2297.
13. Veeravalli KK, Akula A: Involvement of nitric oxide and prostaglandin pathways in the cardioprotective actions of bradykinin in rats with experimental myocardial infarction. *Pharmacol Res* 2004; **49**: 23–29.