Plasma Level of Mitochondrial Coupling Factor 6 Increases in Patients With Coronary Heart Disease

San Bao Chai, MD; Yong Ming Hui, MD; Xue Min Li, PhD*; Chao Shu Tang, MD**

Background The aim of the present study was to investigate alterations in the plasma level of coupling factor 6 (CF6), a novel endogenous inhibitor of prostacyclin, in patients with coronary heart disease.

Methods and Results In total, 35 patients with coronary heart disease and 20 age-matched healthy subjects were examined. Plasma levels of CF6 and 6-keto-prostaglandin (PG)F1α (a stable metabolite of prostacyclin) were measured using radioimmunoassay. The plasma level of CF6 was significantly increased in patients (254.1±29.8 pg/ml vs 219.4±36.7 pg/ml in controls, p<0.0001), whereas that of 6-keto-PGF1α was significantly decreased (23.4±2.3 pg/ml vs 26.1±4.5 pg/ml in controls, p=0.001). Moreover, after percutaneous transluminal coronary angioplasty (PTCA) and stent therapy, the level of CF6 was further increased by 30% to 330.4±26.0 pg/ml, and that of 6-keto-PGF1α was decreased by 42% to 13.5±2.0 pg/ml, compared with baseline (all p<0.01). Univariate analysis showed a significant result that the plasma level of CF6 was inversely correlated with that of 6-keto-PGF1α in the patients. The plasma ratio of CF6 to 6-keto-PGF1α was 8.4 in the control group and that in patients with coronary heart disease was increased to 24.4 after the therapy from 10.9 before therapy.

Conclusions The results suggest that an increased CF6 level may be responsible in part for the decreased prostacyclin level observed in patients with coronary heart disease, in particular after PTCA and stent therapy. As a potential risk factor for coronary heart disease, CF6 might have important clinical significance. (Circ J 2007; 71: 693–697)

Key Words: Coronary heart disease; Coupling factor 6; Percutaneous transluminal coronary angioplasty; Prostacyclin

Mitochondrial adenosine triphosphate (ATP) synthase consists of 3 domains: the extrinsic and intrinsic membrane domains, F1 and F0, respectively, joined by a stalk.1–2 Four subunits of the stalk have been identified and designated as coupling factor 6 (CF6), oligomycin-sensitivity conferral protein, and subunits b and d.3–5 CF6 is an essential component of the energy-transducing stalk of mitochondrial ATP synthase and acts as an essential component for proton ducting and ATP synthesis.6

Importantly, recent studies showed that CF6 is also localized on the surface of endothelial cells and released into the blood by shear stress,7 and that might be the only endogenous, physiologically relevant inhibitor of prostacyclin, which affects vascular function.8,9 In addition, circulatory CF6 promotes the expression of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase (NOS), in endothelial cells by enhancing its synthesis and suppressing its degradation. The plasma level of CF6, a novel vasoactive peptide, is markedly increased in patients with essential hypertension, acute myocardial infarction, end-stage renal disease and diabetes mellitus.10–13 Those studies suggest that CF6 may play an important pathogenic role in the development of atherosclerosis and cardiovascular disease; however, its role in the pathogenesis of coronary heart disease (CHD) and whether CF6 affects the metabolism of prostacyclin (PGI2) in CHD remains unclear. To investigate this, we assessed the plasma level of CF6 in 35 patients with CHD and 20 healthy subjects and analyzed the relationship between plasma level of CF6 and that of PGI2 as well as the clinical characteristics of these patients.

Methods

Subjects

The study was reviewed and approved by the Ethics Committee of Feng Tai Hospital, Beijing, and informed consent was given by each subject before the study. Subjects were consecutively recruited from among patients with CHD who were admitted to hospital from November 2005 through February 2006. The patients fulfilled the World Health Organization diagnostic criteria for CHD. In addition, all patients underwent percutaneous transluminal coronary angioplasty (PTCA) and stent therapy based on the results of coronary angiography. Patients with ketoacidosis, acute infection, severe liver or renal disease, congestive heart failure, malignant tumor were not included. Our sample included 35 patients (20 men between 43 and 82 years of age; mean age: 68.8 years of age).

Twenty healthy subjects with no clinical problems (12 men; mean age 66.9 years, range 40–78 years) who underwent a health checkup at the hospital, served as controls.
Table 1 Subjects’ Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>M/F</td>
<td>12/8</td>
<td>20/15</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.9±8.6</td>
<td>68.8±11</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.8±4.6</td>
<td>75.1±4.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>57.1±2.6</td>
<td>59.4±5.1</td>
</tr>
<tr>
<td>Males</td>
<td>1.74±0.0</td>
<td>1.74±0.0</td>
</tr>
<tr>
<td>Females</td>
<td>1.60±0.0</td>
<td>1.59±0.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Males</td>
<td>24.1±1.3</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>22.2±1.7</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>5 (25)</td>
<td>6 (17)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115.8±8</td>
<td>118.9±8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>68.7</td>
<td>70.5</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>4.8±0.5</td>
<td>4.9±0.5</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.5±0.2</td>
<td>5.0±0.2*</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.5±0.1</td>
<td>1.9±0.1*</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.7±0.1</td>
<td>2.9±0.2*</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.5±0.6</td>
<td>5.8±0.6*</td>
</tr>
<tr>
<td>LV-EF (%)</td>
<td></td>
<td>59.3±5.0</td>
</tr>
<tr>
<td>Culprit artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD (%)</td>
<td>10 (28.6)</td>
<td></td>
</tr>
<tr>
<td>LCX (%)</td>
<td>5 (14.3)</td>
<td></td>
</tr>
<tr>
<td>RCA (%)</td>
<td>7 (20)</td>
<td></td>
</tr>
<tr>
<td>LM+LAD (%)</td>
<td>2 (5.7)</td>
<td></td>
</tr>
<tr>
<td>LAD+LCX (%)</td>
<td>5 (14.3)</td>
<td></td>
</tr>
<tr>
<td>LAD+RCA (%)</td>
<td>3 (8.6)</td>
<td></td>
</tr>
<tr>
<td>LAD+LCX+RCA (%)</td>
<td>3 (8.6)</td>
<td></td>
</tr>
<tr>
<td>DES (%)</td>
<td>6 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Bare stent (%)</td>
<td>48 (88.8)</td>
<td></td>
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Data are mean±SD, unless indicated.
* p<0.05 vs controls.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; LV-EF, left ventricular ejection fraction; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; LM, left main; DES, drug-eluting stent.

Study Design
Both prior to and on the 3rd day after PTCA and stent therapy, blood samples were collected from the antecubital vein of the subjects after overnight fasting for measurement of CF6, 6-keto-prostaglandin (PG)F1α and other biochemical parameters. All subjects were given a questionnaire to gather data on smoking, family history of CHD (ie, first-degree relative ≤60 years with clinical evidence of coronary artery disease), use of medication, and past medical history (ie, essential hypertension, coronary artery disease). All subjects underwent physical examination, including measurement of height, weight, body mass index (BMI = kg/m²) and blood pressure (repeated 3 times and readings of systolic and diastolic pressure recorded). Meanwhile, all patients received routine drug therapy such as aspirin, clopidogrel sulfate, simvastatin and so on. When diagnosed with hypertension, the subjects were administered antihypertensive drugs.

Sample Collection
Blood was drawn into tubes containing disodium ethylenediamine tetraacetic acid (1 mg/ml) and aprotinin (500 units/ml; Sigma Co, St Louis, MO, USA), then centrifuged immediately at 3,500G for 10 min at 4°C; plasma was stored at −80°C until assay.

Table 2 Comparison of CF6 and 6-Keto-PGF1α Levels in Controls and Patients With Coronary Heart Disease

<table>
<thead>
<tr>
<th></th>
<th>CF6 (pg/ml)</th>
<th>6-keto-PGF1α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>219.4±36.7</td>
<td>26.1±4.5</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>254.1±29.8*</td>
<td>23.4±2.3*</td>
</tr>
<tr>
<td>After PTCA + stent</td>
<td>330.4±26.0*</td>
<td>13.5±2.0*</td>
</tr>
</tbody>
</table>

*p<0.01 vs controls; †p<0.01 vs patients with coronary heart disease.

Radioimmunoassay (RIA) for CF6
CF6 was measured using RIA as previously described. In brief, samples were extracted through a Sep-Pak C18 cartridge, then assayed using a specific RAI kit (Phoenix Pharmaceuticals Inc, Belmont, USA). The IC50 was 24.45 pg/tube and the reactivity with human CF6 was 100%. No cross-reactivity was found with human angiotensin II, urotensin II, endothelin, adrenomedullin or neuropeptide Y. The intra- 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 RIA was identical to that of synthetic human CF6.

Measurement of Other Parameters
The plasma level of 6-keto-PGF1α (a stable metabolite of prostacyclin) was assessed by RIA as previously described (Bioengineering Co Ltd Beijing, China). The intra- and inter-assay coefficients of variation for blood samples were ≤5%. The levels of fasting blood glucose (FBG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), total cholesterol (TC) and C-reactive protein (CRP) were assessed by automatic analysis. Each of the intra- and inter-assay coefficients of variation was ≤5%.

Statistical Analysis
Results are expressed as mean±SD. The chi-square test, Student’s t-test and one-way analysis of variance (ANOVA) were used for statistical analyses. Relationship between variables was tested using simple (Pearson’s correlation coefficient) linear regression analysis as indicated. A p-value <0.05 was considered significant.

Results

Subjects’ Profiles
The characteristics of the study subjects are listed in Table 1. No significant differences were found between patients with CHD and healthy controls in age, gender, smoking, BMI, FBG level, systolic blood pressure, diastolic blood pressure or HDL level. However, patients differed from controls in their significantly higher plasma levels of TC, TG, LDL and CRP (all p<0.01). Data for the left ventricular ejection fraction, culprit artery and stent are also listed in Table 1.

Alteration of Plasma Levels of CF6 and 6-Keto-PGF1α
The plasma CF6 level was markedly higher, by 16%, in patients with CHD (254.1±29.8 pg/ml) than in the controls (219.4±36.7 pg/ml, p<0.0001), and the plasma 6-keto-PGF1α level was significantly lower, by 10%, in patients...
Coupling Factor 6 With CHD

(23.4±2.3 pg/ml) than in controls (26.1±4.5 pg/ml, p=0.001). Moreover, on the 3rd day after PTCA and stent therapy, the level of CF6 (330.4±26.0 pg/ml) was increased by 30%, and that of 6-keto-PGF1α (13.5±2.0 pg/ml) was decreased by 42%, compared with baseline (all p<0.01) (Table 2). Importantly, univariate analysis showed that the plasma CF6 level was inversely correlated with the plasma 6-keto-PGF1α level in patients before and after PTCA and stent therapy (Figs 1,2). The plasma ratio of CF6 to 6-keto-PGF1α was 8.4 in the control group. In patients with CHD, the ratio of CF6 to 6-keto-PGF1α was increased to 24.4 after therapy from 10.9 before the therapy.

Univariate analysis revealed that the plasma CF6 level was not correlated with arterial blood pressure (systolic, diastolic), cholesterol content (TC, LDL, TG) or CRP level before or after PTCA (Tables 3,4).

Discussion

Mitochondrial ATP synthase is a multi-subunit membrane-bound enzyme that consists of 3 domains: extrinsic and intrinsic membrane domains joined by a stalk. Mitochondrial CF6 is an important subunit of the stalk and is reported to be essential for energy transduction. Human CF6 is synthesized as a precursor peptide in the cell cytosol, followed by the mitochondria forming the mature 78-amino acid peptide (8.9 kDa) similar to the ß and ß subunits of mitochondrial ATP synthase. Interestingly, CF6 is also present on the surface of vascular endothelial cells and released into the systemic circulation. Previous research reported that Ca2+ has a close relationship with mitochondrial function. As a systemic hormone, or as a paracrine and/or an autocrine effector, secretory CF6 inhibits the synthesis of prostacyclin in endothelial cells via the suppression of Ca2+-dependent cytosolic phospholipase activity, and therefore its physiological role is that of an endogenous vasoconstrictor. Further study showed that exogenous CF6 promotes the expression of adhesion molecules on human vascular endothelial cells and reduces the activity of NOS by increasing asymmetric dimethylarginine release. Importantly, the level of circulating CF6 is increased in patients with end-stage renal disease and is related to the occurrence of ischemic heart disease in such patients. Circulating CF6 is increased in human hypertension and modulated by salt intake, presumably via reactive oxygen species. Ding et al found that a high level of

Table 3 Pearson Correlation Coefficients Between CF6 Level and Other Risk Factors in Patients With Coronary Heart Disease

<table>
<thead>
<tr>
<th>CF6</th>
<th>SBP</th>
<th>DBP</th>
<th>FBG</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>–0.25</td>
<td>–0.25</td>
<td>0.06</td>
<td>0.01</td>
<td>–0.10</td>
<td>0.24</td>
<td>0.18</td>
<td>0.23</td>
</tr>
<tr>
<td>P</td>
<td>0.15</td>
<td>0.15</td>
<td>0.74</td>
<td>0.96</td>
<td>0.56</td>
<td>0.16</td>
<td>0.31</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Abbreviations see in Tables 1,2.

Table 4 Pearson Correlation Coefficients Between CF6 Level and Other Risk Factors in Patients After PTCA and Stent Therapy

<table>
<thead>
<tr>
<th>CF6</th>
<th>SBP</th>
<th>DBP</th>
<th>FBG</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>0.27</td>
<td>0.06</td>
<td>0.12</td>
<td>0.04</td>
<td>0.16</td>
<td>–0.09</td>
<td>–0.31</td>
<td>–0.26</td>
</tr>
<tr>
<td>P</td>
<td>0.12</td>
<td>0.74</td>
<td>0.49</td>
<td>0.84</td>
<td>0.36</td>
<td>0.60</td>
<td>0.07</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Abbreviations see in Tables 1,2.
plasma CF6 was maintained within 1 week of the onset of acute myocardial infarction, and had a close relationship with the pathologic process of myocardial tissue injury and endothelial dysfunction. Taken together, these findings suggest that circulating CF6 has a potential connection with endothelial dysfunction and may take part in the pathogenesis of cardiovascular diseases.

In the present study, we observed that the plasma CF6 level was markedly higher in patients with CHD than in the healthy controls (+16%, p<0.01). After PTCA and stent therapy, the plasma CF6 level in patients remained markedly higher than in the controls (+30%, p<0.01). CF6 expression is clearly proportional to the strength of shear stress in vascular endothelial cells5. CF6 is synthesized in an immature form in the cytosol and imported into and accumulated within the mitochondria6, which suggests that tissue injury would enhance its release into the systemic circulation. In addition, the nuclear factor-κB signaling pathway has been shown to be largely involved in CF6 release from endothelial cells20. In addition to their being involved in the mechanism of atherosclerosis, the pathologic processes of cell apoptosis, injured endothelial cells and changed shear stress may be responsible for the increased plasma level of CF6 in patients with CHD. In particular, after PTCA and stent therapy, endothelium injury is increased, which leads to a further increase in the plasma CF6 level.

Meanwhile, the plasma prostacyclin level in patients with CHD (by measuring a stable metabolite of prostacyclin, 6-keto-PGF1α) was lower then in the healthy controls (p<0.01). Previous research also found a decreased plasma level of prostacyclin in patients with CHD21. A possible mechanism involved decreased production of prostacyclin because of endothelial cell injury. Prostacyclin, a known potent vasodilator and the most potent endogenous inhibitor of platelet aggregation, is synthesized from arachidonic acid (AA) by various stimuli in many types of cells, including vascular endothelial cells and smooth muscle cells22. CF6 can suppress AA release from cellular plasma membrane and inhibits AA activity, which leads to decreased prostacyclin production. Moreover, this peptide may also counteract the biological actions of AA, such as inhibition of the voltage-gated Ca2+ current3. Interestingly, univariate analysis in our study showed a negative correlation between increased plasma CF6 level and decreased prostacyclin production in patients treated with (r=−0.61, p<0.001) or without (r=−0.76, p<0.001) PTCA and stent therapy, but was not, however, correlated with arterial blood pressure (systolic, diastolic), cholesterol content (TC, LDL, TG) or CRP level. Therefore, a high level of plasma CF6 may be responsible for the decreased PG12 level in patients with CHD. The reduced PG12 level is known to promote the adhesion of leukocytes and their migration to the inflammatory locus23 and weaken endothelium-dependent vasodilation in patients with coronary artery disease24. All these findings suggest the pathogenesis of atherosclerosis.

Importantly, the level of plasma CF6 in patients on the 3rd day after PTCA and stent therapy was higher than the level prior to therapy. The ratio of CF6 to PG12 was increased 1.3-fold compared with before therapy. We consider that the endothelium may be injured during PTCA and stent therapy, which may explain the increased CF6 level. These findings indicate that mitochondrial CF6 functions as potent hormone and may suggest a new mechanism for the treatment of CHD.

This paper reports for the first time increased plasma CF6 level in patients with CHD. The level of plasma CF6 in these patients inversely correlated with the plasma prostacyclin level. These results suggest that the circulating level of CF6 might be both an obvious marker of impaired endothelium and a novel risk factor contributing to vascular damage in CHD. As a potential intervention target for the disease, the plasma CF6 level might have important clinical significance.

Acknowledgment

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References


