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Undercarboxylated matrix Gla protein in patients with ST-segment elevation myocardial infarction and chronic coronary syndromes

Niedokarboksylowane białko macierzy Gla u pacjentów z zawałem serca z uniesieniem odcinka ST oraz przewlekłymi zespołami wieńcowymi

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Abstract

Introduction. Matrix Gla proteins (MGPs) are usually considered as natural inhibitors of soft tissue calcification in chronic inflammatory disorders. However, MGP levels in acute inflammation related to myocardial ischemia have been poorly investigated.

This study aimed to compare the serum concentrations of uncarboxylated MGPs (ucMGPs) between patients with ST-segment elevation myocardial infarction (STEMI) vs. with chronic coronary syndromes (CCS) and to investigate the association between ucMGP concentration and an increased risk of major adverse cardiovascular events (MACE) in STEMI patients.

Material and methods. 155 consecutive patients were enrolled (mean ± standard deviation age, 64 ± 13 years), including 80 patients with a first STEMI and 75 ones with CCS as controls. Blood samples were obtained within the first 24 h from hospital admission to evaluate ucMGP levels. Combination of MACE [all-cause mortality, heart failure (HF) within the first 30 days after myocardial infarction] was evaluated.

Results. ucMGP levels were higher in patients with STEMI than in controls (2929 ± 96.5 ng/mL vs. 67.3 + 32.3 ng/mL; $p < 0.0001$). A significant positive correlation between ucMGP and high-sensitivity C-reactive protein, troponin levels was found.

Multivariate analysis showed that ucMGP was an independent associate of STEMI [odds ratio (OR) 1.39; confidence interval (CI): 0.78–2.14, $p = 0.01$]. Although ucMGP did not predict the combined MACE, however it was an independent associate of HF occurrence 30 days after STEMI (OR, 1.20; 95% CI: 1.07–1.30, $p = 0.04$).

Conclusion. Elevated ucMGP levels in patients with STEMI indicate that some MGPs may be involved in disorders related not only to chronic but also acute inflammatory states.

Key words: undercarboxylated matrix Gla protein, myocardial infarction, chronic coronary syndrome

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Introduction

Matrix Gla proteins (MGPs) are extrahepatic vitamin K-dependent proteins produced not only by chondrocytes and fibroblasts but also by vascular smooth muscle cells in the arterial media and other tissues such as some cancer cells [1–3]. The biological function of MGPs remains the subject of research, the proteins are usually perceived as natural inhibitors of the arterial wall and soft tissue calcification [1–3]. They protect tissues against calcification by binding newly formed hydroxyapatite crystals (and thereby preventing their accumulation in tissues) as well as by stimulation of vascular macrophages to promote phagocytosis and apoptosis of the MGP-hydroxyapatite complexes [2–6]. MGPs also inhibit the binding of bone morphogenetic protein 2 (BMP-2) to its receptor, which impairs protein function. Expression of BMP-2 in endothelial foam cells of atherosclerotic plaques leads to chondrogenesis and osteogenesis, and thus to vascular calcification [5].

For biological activation, MGPs have to undergo carboxylation of the Gla residues, followed by phosphorylation of the serine residues. Both carboxylation and phosphorylation depend on vitamin K_2 (especially menaquinone 7). Only the active form of MGP is both phosphorylated and carboxylated, while uncarboxylated MGP (ucMGP) and carboxylated but not phosphorylated MGP, as well as phosphorylated but uncarboxylated MGP, are partially active forms. Finally, uncarboxylated and dephosphorylated MGP is a fully inactive form [3].

The biological role of different forms of MGPs as markers of cardiovascular disease remains controversial. Serum ucMGP concentrations were reported to correlate with arterial calcification in patients with hypertension [3]. Low plasma ucMGP levels were independently associated with mortality and cardiovascular complications in patients with coronary artery disease [4]. High plasma levels of carboxylated but not phosphorylated MGP were independently associated with mortality in patients with chronic cardiovascular disorders [1, 6, 7].

Although previous studies have confirmed a positive association between elevated circulating serum levels of MGPs in cardiovascular disorders related to chronic inflammation, there are limited data on MGP levels in inflammatory states related to acute ischemia. Therefore, the present study aimed to evaluate serum ucMGP levels in patients with ST-segment elevation myocardial infarction (STEMI) compare to those with chronic coronary syndromes (CCS) and also to investigate the association between serum ucMGP concentration and an increased risk of major adverse cardiovascular events (MACE) in STEMI patients.

Material and methods

Study population

We enrolled 155 consecutive patients, including patients with the diagnosis of a first STEMI (N = 80) and patients with CCS (N = 75) who served as controls. All participants were hospitalized at the Department of Coronary Disease and Heart Failure at Jagiellonian University Medical College in Kraków, Poland. After coronary angiography, all participants were referred for percutaneous coronary intervention (PCI). The inclusion criteria were as follows: diagnosis of a first STEMI or CCS (both groups referred for PCI), glomerular filtration rate (GFR) of 60 mL/min or higher and left ventricular ejection fraction (LVEF) of 50% or higher on echocardiography at hospital admission. Patients with a GFR of less than 60 mL/min, myocardial infarction at previously, LVEF lower than 50% on admission, severe valvular calcification, current treatment with vitamin K antagonists, disorders of calcium and phosphorus metabolism, autoimmune disorders, acute infection on admission, and current malignancies were excluded.

The Thrombolysis in Myocardial Infarction (TIMI) Risk Score (STEMI) was used for estimation of 30-day mortality in patients with STEMI [8]. After hospital discharge patients with STEMI underwent 30 days follow-up. During this time, participants were contacted by phone and 30 days after infarction the patients were assessed at the outpatients' department.

The primary endpoint was the occurrence of a difference in ucMGP concentration between STEMI patients when compared to CCS. **Secondary endpoints** were the relationship between levels of ucMGP in STEMI and a combination of MACE including all-cause mortality and heart failure (HF) within the first 30 days after MI.

Laboratory measurements

Serum concentrations of ucMGP were measured with a sandwich enzyme-linked immunosorbent assay, double antibody kit (MyBioSource, Inc., San Diego, California, United States), with a detection range of 0.156 to 10 ng/mL, a sensitivity of 0.094 ng/mL, and intra- and interassay coefficients of variation of less than 8% and 10%, respectively. Blood samples were obtained within 24 hours from hospital admission, after the PCI procedure.

Other laboratory examinations included complete blood count, renal function tests, lipids profile, and the measurement of high-sensitive C-reactive protein (hsCRP) levels were measured in patients with STEMI.

Complete echocardiography was performed using the VIVID S-6 ECHO unit (GE Medical System), equipped with a multifrequency harmonic transducer (2.5–4 MHz) before hospital discharge and at the end of follow-up. The systolic

function of the left ventricle was estimated with LVEF using the Simpson method. The average values of three consecutive measurements were recorded.

Important definitions

STEMI was defined by electrocardiographic ST-segment elevation in patients with chest discomfort/pain or equivalent) with positive biomarkers of necrosis [troponin T or/and creatine kinase myocardial band (CK-MB)]. Hypertension was defined as arterial blood pressure equal to or more than 140/90 mm Hg or drugs treatment for hypertension. CCS was defined as typical angina induced by exertion or emotional stress with positive results of treadmill exercise stress or stress echocardiography, perfusion scintigraphy. Hyperlipidaemia was defined as low-density lipoprotein (LDL)-cholesterol ≥ 1,7 mmol/L or/and elevation triglycerides ≥ 1,7 mmol/L or treatment with hypolipemic therapy/ /diet. Diabetes mellitus was diagnosed in line with applicable guidelines [9].

The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of Jagiellonian University (KBET; 122.6120.323.2014). Each study participant provided written informed consent before enrolment.

Statistical analysis

All continuous variables were expressed as mean [standard deviation (SD)], and categorical variables were expressed as percentages. The Mann–Whitney test was used to compare normally and non-normally distributed continuous variables. The analysis of variance or the Kruskal–Wallis test was applied as appropriate. The χ^2 test was used to evaluate the differences in categorical variables between study groups. All statistical tests were 2-sided. The relationships between continuous variables were assessed by the Spearman rank correlation. A multiple regression analysis was used for identifying associates of STEMI and with MACE as a dependent variable. Statistical significance was accepted at a p-value of less than 0.05. Statistical analysis was performed using the STATISTICA 13.0 PL software (StatSoft, Poland).

Results

A clinical characteristic of study patients has been provided in Table 1.

A total of 155 participants aged 48 to 76 years [mean standard deviation (SD) age, 64 (13) years; men, 72%] were enrolled. The mean ± SD TIMI risk score in the STEMI patients with STEMI were 3.68 ± 2.67. The baseline characteristics of the study population have been presented in Table 1.

Serum levels of ucMGP were higher in patients with STEMI than in those with CCS (2929 ± 96.5 ng/mL; range, 689–16,310 ng/mL vs. 67.3 ± 32.3 ng/mL; range, 19.1–520.3 ng/mL, p < 0.0001). There were no differences in ucMGP levels between patients with single-vessel and those with the multivessel disease either in the STEMI or controls.

In patients with STEMI, ucMGP levels correlated with peak troponin (r = 0.35, p = 0.03), peak CK-MB_{mass} (r = 0.41, p = 0.02), and hsCRP levels (r = 0.34, p < 0.001), as well as with LVEF (r = –0.37, p = 0.01). There was no correlation between ucMGP levels and the levels of low-density lipoprotein cholesterol, triglycerides, high-density lipoprotein cholesterol, haemoglobin, and fasting glucose in either group. In patients with CCS, ucMGP levels correlated only with hsCRP levels (r = 0.42, p = 0.04). In both STEMI and controls the levels of ucMGP were higher in patients with diabetes compare to non-diabetic ones (STEMI: p = 0.03; controls: p = 0.04; respectively).

In multivariate analysis ucMGP, hsCRP concentrations, hypertension, diabetes, age, waist circumference were independent associates of STEMI (Table 2). During the post-infarction follow-up, 3 deaths occurred, 10 patients revealed HF. Thus, combined MACE was observed in the 6.25% STEMI patients.

When the participants were compared concerning the occurrence of MACE within the first month after the STEMI, the ucMGP of 2832 + 92.2 ng/mL was observed in the group presenting with the endpoint and 2794 ± 89.7 in the remaining (p = 0.05). Among those who developed HF, the ucMGP was 2937 ± 92.4 ng/mL, while in patients without HF the ucMGP was 2889 ± 87.2 ng/mL in the group without HF (p = 0.03). When ucMGP was assessed in relation to death, there was no significant association (p = 0.07). Multivariate analysis of ucMGP in relation to HF, after adjusting the confounders: age below 40 years or equal and above 75 years old at admission, sex, diabetes mellitus, LVEF bellow 50% at hospital discharge, anterior ST elevation, time to primary PCI treatment above 4 hours showed an OR of 1.20 (95% CI: 1.07–1.30, p = 0.04).

Discussion

To the authors' knowledge, this is the first study that not only compares ucMGP concentrations between patients with STEMI and those with CCS but also assesses the relationship between levels of ucMGP in the acute phase of STEMI and combination of MACE including all-cause mortality, HF within the first 30 days after MI. Higher serum ucMGP levels in patients with STEMI than in controls, as well as the significant positive correlation between ucMGP and CR, troponin and CK-MB levels in these patients, suggests that some MGPs may be involved in acute inflammation

Table 1. Baseline characteristics of the study population (N = 155)

Variable	STEMI N = 80	CCS N = 75	p-value
Male sex, N [%]	62 (76)	50 (67)	0.13
Age, years	58.34 (11.33)	53.23 (10.57)	0.09
Hypertension, N [%]	75 (94)	68 (91)	0.47
Diabetes mellitus, N [%]	34 (42)	42 (56)	0.09
BMI [kg/m^2]	25.3 (4.45)	25.9 (3.98)	0.06
Waist circumference [cm]	97.4 (4.67)	94.7 (4.85)	0.07
Smoking, N [%]	14 (18)	10 (13)	0.47
LVEF [%]	54.21 (3.41)	64.32 (4.33)	0.002
Coronary angiography, N [%]			
Single-vessel disease	35 (44)	21 (28)	0.04
Two-vessel disease	43 (54)	48 (64)	0.19
Three- or multivessel disease	2 (3)	6 (8)	0.23
LDL-C [mmol/L] (SD)*	2.60 (0.42)	2.82 (0.2)	0.09
Triglycerides [mmol/L] (SD)*	1.31 (0.5)	1.23 (0.3)	0.06
HDL-C [mmol/L] (SD)*	1.13 (0.73)	1.24 (0.5)	0.08
Haemoglobin [g/dL] (SD)*	14.51 (0.78)	14.32 (1.3)	0.65
hsCRP [mg/dL] (SD)*	16.41 (4.21)	2.73 (1.24)	< 0.0001
eGFR [mL/min] (SD)*	81 (13.52)	78 (12.51)	0.56
Baseline CK-MB [U/l] (SD)*	23.51 (3.45)	–	–
Peak CK-MB [U/l] (SD)*	45.52 (2.73)	–	–
Baseline hsTnT [ng/mL] (SD)*	0.76 (0.45)		
Peak hsTnT [ng/mL]	6.56 (1.34)	–	–
Treatment on admission, N [%]			
ACEI	60 (75)	54 (72)	0.67
ARB	7 (9)	9 (12)	0.50
Beta-adrenolytics	37 (46)	45 (60)	0.08
CCB	17 (21)	24 (32)	0.12
Diuretics	15 (19)	25 (33)	0.03
Antiplatelet agents	80 (100)	75 (100)	1.00
Statins therapy	39 (49)	48 (64)	0.05

*Data are shown as mean ± standard deviation (SD); STEMI — ST-segment elevation myocardial infarction; CCS — chronic coronary syndromes; BMI — body mass index; LVEF — left ventricular ejection fraction; LDL-C — low-density lipoprotein cholesterol; HDL-C — high-density lipoprotein cholesterol; eGFR — estimated glomerular filtration rate (CKD-EPI formula); hsCRP — high-sensitivity C-reactive protein; CK-MB — creatine kinase-myocardial band; hsTnT — high-sensitivity troponin T; ACEI — angiotensin-converting enzyme inhibitor; ARB — angiotensin receptor blocker; CCB — channel calcium blocker

related to the size of myocardial necrosis, plaque rupture and thrombosis in the course of acute myocardial ischemia.

We hypothesized that MGP secretion may be stimulated by inflammatory cytokines, cell adhesion molecules, and acute-phase proteins, which are released in STEMI. In an experimental animal study, Yao et al. [10] demonstrated that MGP was bound by serum-soluble heat shock protein 70 (HSP-70), which is also released in myocardial infarction and expressed in atherosclerotic lesions. This may result in elevation of the plasma levels of the partially active form, namely, ucMGP. In human studies, elevated HSP-70 levels correlated with ischemic myocardial damage [11]. In the presented study, the size of infarct (evaluated based on troponin and CK-MB levels as well as LVEF) positively correlated with CRP levels as a marker of an acute inflammatory reaction. The CRP synthesis is influenced by interleukin 6, which enhances vascular calcification in vitro [12], an action that is opposite to the function of the active MGP form. The activation of MGP may play a multifaceted protective role in artery integrity also in acute injury.

Table 2. Univariate and multivariate regression analysis for identifying associates of ST-segment elevation myocardial infarction

Variables	OR	95% CI	p-value
Univariate analysis			
Male sex	1.5	0.30–4.05	0.02
Age (years)	3.02	0.56–4.9	0.003
Hypertension	3.09	1.0–5.20	< 0.0001
Diabetes mellitus	2.15	0.52–3.83	0.001
BMI [kg/m^2]	1.45	0.42–3.39	0.01
Waist circumference [cm]	1.98	1.02–3.78	0.03
ucMGP [ng/mL]	1.92	1.15–3.15	0.001
hsCRP [ng/mL]	3.45	1.23–3.46	< 0.00001
LDL-C [mmol/L]	1.15	1.02–2.90	0.09
Triglycerides [mmol/L]	1.27	0.89–1.98	0.53
HDL-C [mmol/L]	0.92	0.34–1.72	0.39
Multivariate analysis			
Age (years)	1.47	0.78–5.15	< 0.0001
Waist circumference > 100 cm	1.54	0.72–2.32	0.04
Hypertension	2.45	0.78–4.78	< 0.0001
Diabetes	2.32	1.01–3.98	0.002
hsCRP [mg/dL]	1.45	0.81–2.78	0.02
ucMGP [ng/mL]	1.39	0.78–2.14	0.01

OR — odds ratio; CI — confidence interval; BMI — body mass index; ucMGP — uncarboxylated matrix Gla proteins; hsCRP — high-sensitivity C-reactive protein; LDL-C — low-density lipoprotein cholesterol; HDL-C — high-density lipoprotein cholesterol

Dahlberg et al. [13] observed increased concentrations of dp-ucMGP in surgical patients in the early postoperative period, and the expression of this protein was particularly high in patients at cardiovascular risk. The authors postulated poor levels of vascular vitamin K in those patients and suggested next research about interactions between perioperative corrective treatment with different types of vitamin K supplements and cardiovascular complications. Meanwhile, human studies on changes in ucMGP levels in patients with myocardial infarction are very limited. In contrast to the presented results, Margonato et al. [14] did not reveal any differences in the serum concentrations of MGP between patients with STEMI and CCS; however, in the presented and their studies MGP concentrations were higher in patients with diabetes compared with nondiabetic individuals.

In another study, Buyukterzi et al. [15] compared patients with acute coronary syndromes (ACS) and those with normal coronary arteries on angiography. The levels of ucMGP were significantly higher in patients with ACS than in those with normal epicardial coronary arteries. Similar to this study, the ucMGP concentrations correlated with CRP levels and LVEF, but in contrast to this study findings, the authors did not report the association of ucMGP with troponin and CK-MB levels. Is possible that those differences between studies arise from the larger size of MI and secondary higher values of troponin, CK-MB levels in the presented study (included only STEMI patients) compare to previously published work (included also patients with unstable angina, non -STEMI and STEMI).

Although in the presented work ucMGP levels (in addition to ageing, diabetes, hypertension, abdominal obesity and hsCRP levels) were independent associates of STEMI however ucMGP was not predictive of combined MACE within 30 days after STEMI treated with primary PCI. On the other hand, it has been demonstrated that ucMGP was significantly higher in patients who developed HF within 30 days of the STEMI. The association remained also after adjustment for age, sex diabetes, LVEF (at discharge), anterior ST elevation, time to primary PCI treatment. Results suggest the potential role of matrix Gla proteins as a marker of HF development in short term follow-up after STEMI.

The available studies have assessed the mechanism of MGP expression only in disorders associated with chronic low-grade systemic inflammation. Although the presented study does not elucidate the role or the mechanism of MGP expression in acute MI, elevated MPG levels in patients with STEMI and its predictive value in STEMI and HF in short term follow-up indicate the need for further research on the biological function of MGPs.

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Conflict of interest

The authors declare no conflict of interest.

Streszczenie

Wprowadzenie. Białka macierzy Gla (MGP) uważa się za naturalne inhibitory wapnienia tkanek miękkich w przewlekłych stanach zapalnych. Jednak stężenie MGP w ostrym procesie zapalnym towarzyszącym zawałowi serca zostało słabo zbadane.

Celem tego badania było porównanie stężeń niedokarboksylowanego MGP (ucMGP) w surowicy u pacjentów z zawałem serca z uniesieniem odcinka ST (STEMI) ze stężeniami ucMGP u osób z przewlekłymi zespołami wieńcowymi (CCS) oraz zbadanie związku między stężeniem ucMGP a podwyższonym ryzykiem poważnych niepożądanych zdarzeń sercowo-naczyniowych (MACE) u pacjentów ze STEMI.

Materiał i metody. Do badania włączono 155 kolejnych pacjentów (średnio ± odchylenie standardowe 64 ± 13 lat), w tym 80 pacjentów ze STEMI jako pierwszą manifestacją choroby wieńcowej oraz 75 pacjentów z CCS jako grupą kontrolną. Próbki krwi pobrano w pierwszych 24 h od przyjęcia do szpitala w celu oceny stężenia ucMGP. Oceniono MACE złożony ze śmiertelności ogólnej oraz niewydolności serca (HF) w pierwszych 30 dniach po zawale serca.

Wyniki. Stężenie ucMGP były istotnie wyższe u pacjentów ze STEMI niż w grupie kontrolnej (2929 ± 96,5 ng/ml *vs.* 67,3 ± 32,3 ng/ml; p < 0,0001). Stwierdzono istotną dodatnią korelację między ucMGP, białkiem C-reaktywnym oznaczanym metodą wysokoczułą oraz stężeniem troponiny sercowej. Analiza wieloczynnikowa wykazała, że ucMGP był niezależnie związany z wystąpieniem STEMI (iloraz szans [OR] 1,39; przedział ufności [CI]: 0,78–2,14; p = 0,01). Jednakże stężenie ucMGP nie było predyktorem złożonego MACE, ale wiązało się niezależnie z wystąpieniem HF 30 dni po STEMI (OR, 1,20; 95% CI: 1,07–1,30; p = 0,04).

Wnioski. Podwyższone stężenie ucMGP u pacjentów ze STEMI wskazuje, że niektóre MGP mogą uczestniczyć także w ostrych stanach zapalnych.

Słowa kluczowe: niedokarboksylowane białko macierzy Gla, zawał serca, przewlekły zespół wieńcowy

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