Clinica Chimica Acta 413 (2012) 691–695

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

The clinical implications of increased cyclophilin A levels in patients with acute coronary syndromes

Jinchuan Yan *, Xuan Zang, Rui Chen, Wei Yuan, Jie Gong, Cuiping Wang, Ying Li

Department of Cardiology, The Affiliated Hospital of Jiangsu University, Zhenjiang, 212001, China

ARTICLE INFO

Article history:
Received 17 August 2011
Received in revised form 10 December 2011
Accepted 12 December 2011
Available online 20 December 2011

Keywords:
Cyclophilin A
Acute coronary syndromes
Atherosclerosis

ABSTRACT

Background: Cyclophilin A is a secreted molecule that has a physiological and pathological role in cardiovascular diseases. However, limited information is available on the relationship between cyclophilin A concentration and acute coronary syndromes (ACS). We investigated whether cyclophilin A concentration is related to the stability of coronary atherosclerotic plaque in patients with ACS.
Methods: This study included normal controls (n = 50), patients with stable angina (SA) (n = 60) and patients with ACS, including unstable angina (UA) (n = 60) and acute myocardial infarction (AMI) (n = 90). Serum soluble cyclophilin A, matrix metalloproteinase 9 (MMP-9), MMP-3 and C-reactive protein concentrations (CRP) were measured. All coronary stenosis were assessed by angiographic coronary stenosis morphology.
Results: Serum cyclophilin A concentration in ACS (UA and AMI) subjects were significantly higher than those in patients with SA and controls ($p<0.05$). Serum cyclophilin A correlated positively with serum MMP-3 and MMP-9 and CRP in ACS patients($r_1 = 0.69$, $r_2 = 0.52$, $r_3 = 0.49$ $p<0.0001$), but not in control. Furthermore, the increased cyclophilin A concentrations was associated with the number of complex coronary stenoses ($r_1 = 0.63$, $p<0.0001$), but not smooth lesions or stenosis severity, in coronary artery disease patients. Logistic regression analysis also demonstrated that serum cyclophilin A concentration was an independent predictor factor for ACS(OR, 2.721, 95% CI 1.563–4.042, $p = 0.001$).
Conclusion: Patients with ACS showed that increased concentrations of cyclophilin A may be a valuable marker for predicting the severity of ACS.

1. Introduction

Atherosclerosis is now considered to be a chronic(auto)-inflammatory disease [1–3]. Clinically, acute coronary syndromes (ACS) refer to a class of clinical syndromes due to acute myocardial ischemia and include patients with unstable angina (UA), non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI). Since integrity of the extracellular matrix has been considered as a critical determinant in the stability of coronary atheromata, disruption of vulnerable atheromatous plaque and secondary coronary thrombosis are the most common pathogenic mechanism of ACS. Previous studies have shown that several members of the matrix metalloproteinases (MMP**s**) family including interstitial collagenase (MMP-1), stromelysin (MMP-3) and gelatinase B (MMP-9), are involved into the degradation of fibrillar collagen and thus have a critical role in the development and stability of coronary plaques. Accumulating clinical evidence has demonstrated that the presence of complex coronary plaques is associated with rapid disease progression and adverse prognosis in patients with coronary artery disease. Further, complex lesions are thought to reflect a tendency toward thrombogenesis or further plaque disruption [4].

Cyclophilin A (CyPA) is a secreted protein and plays a role in the pathogenesis of various cardiovascular diseases such as vascular stenosis, atherosclerosis, and abdominal aortic aneurysms [5]. For example, CyPA has been shown to function as an inflammatory mediator that promotes atherosclerosis in ApoE-deficient mice [6]. Previous studies showed that CyPA was secreted from foam cells and activated matrix metalloproteinase, suggesting a role of CyPA in later stages of atherosclerosis and plaque rupture [7,8]. However, little information has addressed the potential relationship between CyPA and coronary complex stenosis morphology in patients with coronary artery disease. Therefore, the present study was designed to investigate the correlation between serum CyPA concentration and the other biochemical markers, i.e., MMPs and CRP, and the number of complex lesions in patients with coronary artery disease.

2. Materials and methods

2.1. Reagents

CyPA ELISA kit with detection limit of 0.1 ng/ml was from Shanghai Yuan ye Bio-tech Co., Ltd. MMP-3 ELISA kit with detection limit of

* Corresponding author. Fax: +86 511 85026169.
E-mail address: yanjinchuan@hotmail.com (J. Yan).

0009-8981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2011.12.009

0.312 ng/ml was from Wuhan EIAab Science Co., Ltd. and MMP-9 kit with detection limit of 0.156 ng/ml was from R&D systems (Minneapolis, MN). High-sensitive CRP (Immulyte hs-CRP, with intra-assay and CVs of <8% at different concentrations of CRP) was from Siemens Medical Solutions (Los Angeles, CA, detection limit of 0.01 ng/ml).

2.2. Patients and control subjects

Patients undergoing clinically indicated diagnostic coronary angiography in our coronary care unit were consecutively registered. Sixty patients with unstable angina (Braunwald class III) had experienced ischemic chest pain at rest within the preceding 48 h, but without evidence of myocardial necrosis by enzymatic criteria. Sixty patients with stable angina underwent coronary angiography because of signs and symptoms of clinically stable angina. There were 90 patients with AMI<6 h after the onset of symptoms. Inclusion criteria were typical chest pain and ST segment elevation or Q wave in at least 2 contiguous electrocardiography leads. For comparison, 50 healthy people served as controls. The controls were gender- and age-matched subjects hospitalized for chest distress but with normal angiography finding. Patients with infection, tumor, liver or kidney diseases were excluded (Table 1). The study was reviewed and approved by the institution review committee, and informed consent was obtained from all patients and control subjects.

2.3. Blood sampling protocol

Peripheral venous blood was obtained from patients within 1 h after hospitalization. Venous blood was drawn into pyrogen-free blood collection tubes without any additives (Becton Dickinson), immediately immersed in melting ice, and allowed to clot for 1 h before centrifugation (1500×*g* and 4 °C for 10 min). Serum was stored at −80 °C until analyzed, and samples were thawed once.

2.4. Measurements of MMP-9, MMP-3, CyPA, and hs-CRP

Levels of Serum MMP-3 and MMP-9 were determined by ELISA according to the manufacture's instructions. Levels of CyPA were determined by ELISA (CyPA detection limit, 0.1 ng/ml; with intra- and inter-assay CVs of <15% at different concentrations of CyPA) according to the manufacturer's instructions. Serum samples were frozen, respectively, and thawed only once. Specific immunoassays for high-sensitive CRP was used in triplicate as described previously [10].

Table 1
Characteristics of the study groups.

Groups	Control	SA	UA	AMI
	(n=50)	(n=60)	(n=60)	(n=90)
Age, Y	60±10	61±8	59±9	63±11
Sex, M/F	31/19	38/22	37/23	56/34
TCH(mmol/l)	4.7±0.7	4.9±0.8	5.1±0.9	5.0±1.1
HDL(mmol/l)	1.3±0.7	1.1±0.5	1.2±0.6	0.9±0.4
TG,mmol/l	1.6±0.3	1.5±0.4	1.7±0.8	1.8±0.9
LDL(mmol/l)	2.7±0.6	2.8±0.6	2.7±0.7	3.2±0.8*
Medication, %				
Calcium antagonist	0	9	11	13
Nitroglycerin	0	13	21	19
ACEI	0	9	8	12
β-blockers	0	82	78	80
HMG-COA reductase inhibitors	0	61	64	67
Aspirin	0	98	100	100

TG: triglyceride, TCH: total cholesterol, LDL: low density lipoprotein.
* p<0.05 ACS vs. UA, SA and control.

2.5. Angiographic coronary stenosis morphology

All coronary stenoses with ≥30% diameter reduction were assessed by 2 experienced cardiologists who had no knowledge of the results of serum CyPA concentrations or the identity and clinical characteristics of the patients. Stenosis morphology was assessed as reported previously in several studies [9]. Briefly, stenoses were considered to be complex or smooth. Complex lesions were defined by the following features: 1) irregular morphology or scalloped borders, or both; 2) overhanging or abrupt edges perpendicular to the vessel wall; 3) ulceration; 4) and/or the presence of filling defects consistent with intracoronary thrombus. Coronary stenoses without complex features were classified as smooth lesions.

2.6. Statistical analysis

Statistical evaluation was performed with Graph pad software (Prism3.0) and SPSS11.5 software. Normally distributed data were expressed as mean±SD. Non-normally distributed data were expressed with the median and interquartile range. Differences among the three or four groups were analyzed by Kruskal–Wallis one-way analysis. For comparisons within the same individuals over time, the Wilcoxon matched pairs test was used. Correlation was evaluated using regressive analysis. The Pearson two-way test was used to assess the relation between two quantitative variables with normal distributions. For non-normally distributed data, a Spearman correlation analysis was performed. Logistic regression was performed to assess the association between CyPA and ACS. A $P<0.05$ were considered statistically significant.

3. Results

3.1. Serum concentrations of CyPA

Serum concentrations of CyPA were significantly increased in patients with UA [12.1(3.5–21.2) ng/ml] and AMI 13.9(4.7–26.4) ng/ml compared with those obtained from control [2.2(0.8–3.2) ng/ml] and SA group [2.6(0.9–3.9) ng/ml] (Fig. 1A). In 12 patients with unstable angina, blood samples were also available from a time before the development of the unstable angina (median time between blood samplings, 11 weeks; range, 3 to 16 weeks). Notably, all these patients had a rise in CyPA concentrations concomitant with the development of unstable angina (Fig. 1B). Furthermore, serum concentrations of CyPA were analyzed in 30 patients with stable angina before and 24 h after percutaneous transluminal coronary angioplasty (PTCA). As shown in Fig. 1C, PTCA procedure resulted into a marked rise in CyPA concentrations in the patients.

3.2. Serum CRP, MMP-3 and MMP-9 concentrations in patients with ACS

The serum concentrations of MMP-3[33.2(26.4–42.3)ng/ml] and MMP-9 [59.6(48.1–75.4) ng/ml] in patients with ACS were more than twice compared with those of control and SA group(Fig. 2A). CRP concentration in patients with ACS [19.3(11.8–23.5) ng/ml] was significantly higher than in patients with SA[8.4(6.7–10.6) ng/ml] and controls[8.2(6.4–10.4) ng/ml](Fig. 2B).

3.3. Correlation between both MMP-9, MMP-3 and CRP with CyPA

The increased CyPA concentration in patients with ACS showed a positive correlation with MMP-9, MMP-3 and CRP ($r_1=0.69$ $p<0.001$, $r_2=0.52$, $r_3=0.49$. $p<0.0001$, $n=150$). However, we did not find a correlation between CyPA concentration and CRP, MMP-3, MMP-9 in non-ACS subjects.