Atherosclerosis 239 (2015) 343–349

Contents lists available at ScienceDirect

Atherosclerosis

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

Increased monomeric CRP levels in acute myocardial infarction: A possible new and specific biomarker for diagnosis and severity assessment of disease

Junhong Wang [a, 1], Bo Tang [b, 1], Xinjian Liu [c], Xin Wu [d], Hui Wang [f], Di Xu [a], Yan Guo [a, *]

[a] Department of Gerontology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, PR China
[b] Vazyme Biotechnology Company, Nanjing 210004, PR China
[c] Department of Pathogen Biology, Nanjing Medical University, Nanjing 210029, PR China
[d] Department of Clinical Laboratory Medicine, BenQ Medical Center, Nanjing 210019, PR China
[f] Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, PR China

ARTICLE INFO

Article history:
Received 23 October 2014
Received in revised form
28 December 2014
Accepted 16 January 2015
Available online 28 January 2015

Keywords:
Acute myocardial infarction (AMI)
Monomeric CRP (mCRP)
Diagnosis
Prognosis

ABSTRACT

Background: Monomeric CRP (mCRP) plays an important role in the process of atherosclerotic plaque rupture. Recently, it has been reported that mCRP was associated with acute myocardial infarction (AMI).
Objectives: The aim of this study was to examine whether mCRP is increased in AMI patients and to investigate the possibility of using circulating mCRP as a biomarker for AMI diagnosis and severity assessment of disease.
Methods: A mCRP monoclonal antibody was generated and verified for its specificity. Immunofluorescence was used to assess the localization of mCRP in the infarcted myocardium. Furthermore, 101 AMI, 38 unstable angina pectoris (UAP) and 41 stable angina pectoris (SAP) patients were enrolled, and 43 healthy volunteer were recruited as controls in the study. Venous blood samples were collected to measure the circulating mCRP, cardiac Troponin T and hs-CRP levels.
Results: Significantly increased mCRP levels were observed in the infarcted myocardium of model mice. In addition, significantly increased plasma mCRP levels were also detected in AMI patients (20.96 ± 1.64 ng/ml) compared to those with UAP, SAP or in control patients (all 0 ng/ml, $p < 0.001$). ROC analysis revealed that circulating mCRP had considerable diagnostic accuracy for AMI with an AUC of 0.928 (95% confidence interval 0.887–0.969). Furthermore, nine patients (9/101, 8.91%) in AMI group died before the 30-day follow-up, and their plasma mCRP concentration was significantly higher than those in surviving patients (36.70 ± 10.26 vs. 19.41 ± 1.43 ng/ml, $P = 0.002$).
Conclusions: These results indicate that mCRP is increased in AMI and that circulating mCRP might be a potential biomarker for diagnosis and severity assessment of disease in AMI.

1. Background

Traditionally, C-reactive protein is recognized as a cyclic, disc-shaped pentamer (pentameric CRP, pCRP) in human plasma that consists of five noncovalently linked subunits of 23 kDa. This protein is an acute phase reactant, which responds to tissue injury, infection and inflammation, with plasma levels increasing from a baseline level of 1–2 mg/l up to 100- to 1000-fold. Furthermore, small increases in the baseline level of plasma CRP have been associated with an increased risk for cardiovascular events, such as acute coronary syndrome (ACS) and stroke [1–3]. pCRP has emerged as an important predictor of cardiovascular risk in a variety of clinical settings. However, as a highly sensitive and nonspecific indicator for systemic inflammation, increased pCRP was observed in many inflammatory diseases, which makes it difficult for this protein to be a specific biomarker for diagnosing and predicting the disease severity of ACS.

Recently, it has been postulated that pCRP can undergo dissociation, thereby acquiring distinct functionality [4,5]. This alternative conformation of CRP has been termed modified or

* Corresponding author. Department of Gerontology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, PR China.
E-mail address: cnjjh2000@aliyun.com (Y. Guo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.atherosclerosis.2015.01.024
0021-9150/

monomeric CRP (mCRP), with antigenicity-expressing neo-epitopes differing from native pCRP epitopes. The dissociation process of pCRP to mCRP can occur spontaneously under a variety of conditions, including low pH, absence of calcium, increased temperature and urea chelation [4,6]. Recently, a new mechanism of the structural change in CRP that augments inflammation in ACS patients was proposed whereby the pCRP dissociation to mCRP is mediated by cell membranes on activated platelets [7–9]. mCRP has previously been detected in atherosclerotic plaques [7] and infarcted myocardium [10]. However, there are no reports of mCRP being easily detected in the peripheral blood because of its presumably limited solubility. In addition, previous studies have demonstrated that circulating pCRP levels rise consistently in association with the size of myocardial infarction following AMI [11,12] and the conversion of pCRP to mCRP is further demonstrated to exist and be involved in the progress of AMI [13,14]. We, therefore, investigated whether mCRP is released in the peripheral circulation and could be easily detected in patients after AMI. In addition, we evaluated the possibility of circulating mCRP as a potential diagnostic biomarker for acute myocardial infarction.

2. Materials and methods

2.1. Monomeric CRP preparation

The mCRP was prepared according to the method of Potempa et al. [15]. Briefly, native CRP (pCRP, 1 mg/ml) was chelated with 10 mM EDTA and incubated in 8 M urea for 2 h at 37 °C. Urea was removed by dialysis against TBS (0.01 M Tris–HCl and 0.05 M NaCl, pH 7.3).

2.2. mCRP monoclonal antibody preparation

Animal handling and use complied with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996) and this entire study was approved by the Animal Care and Use Committee of Nanjing Medical University. The C-terminal octapeptide of pCRP (the mouse and human sequence is the same) was synthesized and its sequence is Phe-Thr-Lys-Pro-Gly-Leu-Trp-Pro (this C-terminal octapeptide of pCRP is a neoepitope only expressed on mCRP [4]). KLH or BSA protein was then coupled to this synthesized peptide(Chinese peptide Co. Ltd, Hangzhou, China). Monoclonal antibody against mCRP was produced according to a previously elsewhere described protocol [16].

2.3. Reactivity of monoclonal antibody (mAb) to mCRP

The monoclonal antibody to mCRP was purified using protein G affinity chromatography. Denatured/non-reduced SDS-PAGE followed by Western blotting analysis with pCRP- and mCRP-specific antibodies was applied. Briefly, commercial human pCRP (Merck, Germany, Cat: AG723, purified from human pleural fluid) was resuspended in the loading buffer without β-mercaptoethanol (reducing agent), boiled, and electrophoresed on 12% SDS-PAGE. After being electrotransferred, the membranes were then incubated for 2 h at 37 °C with the purified mAb (2 mg/ml) or commercial pCRP antibody (Abcam, MA, USA, cat: ab50861), washed three times in PBS-T and again incubated with a 1:3000 dilution of goat anti-mouse IgG peroxidase conjugate (Vazyme, Nanjing, China) for 1 h. Following additional washes, bound antibodies were detected using an ECL Western blotting Kit (Pierce, IL, USA).

2.4. Immunofluorescent detection of mCRP in infarcted mice hearts

Twenty-four 8- to 12-week-old male C57BL/6J mice were used for myocardial infarction experiments. Mice were anesthetized by isoflurane inhalation (isoflurane 2–3% vol/vol). Myocardial infarction was induced using a closed-chest mouse model of 1 h of ischemic/reperfusion as previously described [17]. After 24 h, 3 days and 7 days of reperfusion, the chest was opened and the heart was immediately excised for histological studies.

The excised hearts were fixed in zinc-formalin and embedded in paraffin. Next, the sections were cut and incubated for 20 min in 95 °C non-boiling citrate buffer to allow antigen retrieval. Subsequently, the primary antibody to mCRP we prepared (concentration: 5.3 mg/ml, dilution: 1:100) or mouse IgG control (Santa Cruz, cat: sc-2025, 1:100) were incubated with the sections for 1 h at room temperature. After being washed with PBS for three times, the sections were incubated with biotinlyated anti-mouse IgG (cat: MKB-2213, Vector laboratories, CA, USA) for 20 min. Then, the sections were incubated with Alex Fluro 488 conjugated streptavidin (cat: S32354, life technologies, OR, USA) for 30 min, and nuclei were stained with DAPI (cat: D1306, life technologies, OR, USA). Stained sections were scanned using a Zeiss Axio Immager M2 microscope equipped with a Zeiss digital camera.

2.5. Patients enrollment and study design

This study protocol was carried out in accordance with the principles of the declaration of Helsinki and had been approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University. The ethics committee of the First Affiliated Hospital of Nanjing Medical University approved that only verbal informed consent was required because all data were going to be analyzed anonymously. Blood samples were collected at presentation from all patients, and the lithium heparin anticoagulated blood sample of all patients was collected for plasma mCRP and cardiac Troponin T analysis. All sample aliquots were immediately stored at −80 °C until assays were performed.

From Feb. 2012 to Dec. 2012, consecutive, patients with symptoms suggestive of ACS admitted to the Chest Pain Unit of the first affiliated hospital of Nanjing Medical University were screened. All patients received a 12-lead ECG recording on admission and after 6 h. Patients with advanced kidney dysfunction (estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m^2, calculated using Chronic Kidney Disease Epidemiology Collaboration (CKDEPI) equation) were excluded from the study, as were patients who refused to participate or with early discharge/transfer. In total, 139 ACS patients and 41 consecutive stable angina pectoris (SAP) patients from the cardiology department were enrolled in our study. In the ACS group, 101 were acute myocardial infarction patients and 38 had unstable angina pectoris (UAP). ACS was defined as acute onset of prolonged chest pain or chest discomfort accompanied by ST-segment elevation or depression evolving into pathological Q waves or inverted T wave, as well as increased levels of laboratory biomarkers (including cardiac troponin T (cTn-T)) on the basis of current guidelines [18–20]. Patients with cTnT concentrations at presentation below the diagnostic cut-off (0.1 ng/ml) received a final diagnosis of UAP or evolving NSTEMI depending on the presence of an elevated cTnT concentration in at least one of the consecutive samples collected within 24 h after index event. Of the AMI patient subgroup, 55 were STEMI and 46 NSTEMI. In addition, from the patients with the diagnosis of AMI, 13 patients' blood samples were consecutively obtained at 6, 12 and 24 h after the onset of chest pain, and then once daily until the seventh day. All AMI patients were followed up 30 days after initial hospitalization. Information about cause of death was obtained from hospital or