Peptides 42 (2013) 84–88

Contents lists available at SciVerse ScienceDirect

Peptides

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

Increased plasma levels of intermedin and brain natriuretic peptide associated with severity of coronary stenosis in acute coronary syndrome

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ARTICLE INFO

Article history:
Received 7 September 2012
Received in revised form 21 January 2013
Accepted 21 January 2013
Available online 4 February 2013

Keywords:
Acute coronary syndrome
Intermedin
Brain natriuretic peptide

ABSTRACT

Intermedin (IMD) is a newly discovered peptide with increased levels in plasma and cardiac tissue in mice with ischemia/reperfusion. Continuous administration of low dose IMD markedly elevated the mRNA abundance of myocardial BNP in rats. Plasma BNP levels may reflect the severity of degree of coronary stenosis in patients with acute coronary syndrome (ACS). However, the role of circulating IMD in coronary heart disease remains unclear. We aimed to examine the plasma content of IMD and brain natriuretic peptide (BNP) and its clinical significance in patients with ACS. We collected plasma samples from 41 patients with ACS and 31 controls and measured IMD and BNP levels by radioimmunoassay. The severity of coronary artery stenosis for patients with ACS was measured by coronary angiography. Plasma IMD and BNP levels were markedly higher in ACS patients than that in controls ($P < 0.05$). The increased plasma IMD and BNP were positively correlated with degree of coronary stenosis in ACS patients ($r = 0.263$ and $r = 0.238$, respectively, both $P < 0.05$). In addition, plasma levels of IMD were positively correlated with BNP levels.

1. Introduction

Acute coronary syndrome (ACS) represents heterogeneous disorders ranging from unstable angina pectoris without evidence of myocardial necrosis to myocardial infarction associated with significant increase in levels of troponin and the isoenzyme creatine kinase MB [6]. Because of the heterogeneous clinical presentation, ACS encompasses a wide range of events and different prognoses in terms of plaque type lesions and coronary atherosclerosis diffusion. Risk assessment is based on clinical history and examination, electrocardiography (ECG) characteristics and markers of myocardial damage but remains relatively inaccurate [1].

Recently, several biomarkers have been purposed to identify patients at high risk and requiring aggressive therapy or early angiography to detect their risk profile and optimize treatment [19]. Biomarkers may be useful for the immediate diagnosis of disease; they can assist in risk stratification and can direct therapeutic decisions by reflecting the disease course and giving some prognostic information [1]. By using a number of biomarkers, clinicians can risk stratify patients over a broad range of short- and long-term cardiac events. Nevertheless, which biomarker combination is best for risk prediction remains unclear [25].

Intermedin (IMD), also called adrenomedullin 2 (ADM2), is a novel member of the calcitonin/calcitonin gene-related peptide family [27,31]. It exists extensively in the heart, kidney, blood vessels, hypothalamus, gastrointestinal tract, lung, spleen, pancreas, skin, thymus and ovary. It is a potent systemic and pulmonary vasodilator, influences regional blood flow, and augments cardiac contractility [2,10]. IMD can dose-dependently augment cardiac perfusion [13] and inhibit cardiac fibroblast activation induced by angiotensin II [37]. It ameliorated impaired cardiac function both in an *ex vivo* and *in vivo* model of myocardial ischemia/reperfusion injury and isoproterenol-induced myocardial ischemia injury [16,29,38,44]. Recently, we discovered that IMD protects against myocardial injury by inhibiting endoplasmic reticulum and oxidative stress [32,45]. Therefore, IMD is considered a potential endogenous protector of the heart.

IMD is a paracrine/autocrine factor secreted by myocardium and upregulated during the pathophysiologic process in some animal models. IMD/ADM2 content in blood plasma and right ventricular tissue was increased in rats with chronic hypoxia-induced

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http://dx.doi.org/10.1016/j.peptides.2013.01.011

pulmonary hypertension [12]. IMD mRNA expression was upregulated in myocardium and aorta of spontaneously hypertensive rats [3,42]. IMD mRNA levels were significantly increased in the atrium, right ventricle, non-infarcted part of the left ventricle and infarcted part of the left ventricle of rats with chronic heart failure [14]. In NG-nitro-L-arginine methyl ester (a nitric oxide synthase inhibitor) treated rats, IMD levels were increased both in plasma and left ventricular cardiomyocytes [4]. The mRNA and protein expression of IMD in plasma and cardiac tissue was upregulated in a mice *in vivo* ischemia/reperfusion model [44]. The role of adrenomedullin has been proved that it is a good marker of prognosis and survival in patients with coronary aretery disease or heart failure [7,23]. The effects of IMD generally resemble those of ADM, but it is sometimes more potent and appears to have some unique actions.[2,15]. So, we supposed that IMD might be a good marker of diagnosis or prognosis in such patients. It was originally introduced that plasma brain natriuretic peptide (BNP) is a useful biomarker for diagnostic and prognostic value in ACS [9,17]. Plasma levels of BNP may reflect the severity of degree of coronary stenosis and can be used to predict the severity of coronary stenosis in patients with ACS. Meta-analysis suggests that elevated BNP levels were associated with an increased risk of death in patients with ACS [43]. It was reported that continuous administration of low dose IMD 1-47 via mini-osmotic pumps markedly elevated the mRNA abundance of myocardial BNP in spontaneously hypertensive rats [41]. The future of cardiac biomarker testing may be in multimarker testing to better characterize each patient and therapy accordingly. Combined detection of the biochemical markers could be helpful for risk stratification of the patients with ACS [21,33]. In this study, we measured the plasma levels of IMD and BNP in patients with ACS, and analyzed the correlation of IMD and brain natriuretic peptide (BNP) and degree of coronary stenosis.

2. Materials and methods

2.1. Subjects

The study was approved by the Ethics Committee of Anzhen Hospital, Beijing, and complied with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from subjects before their inclusion.

Patients were admitted to our hospital with suspected ACS. Diagnostic criteria for unstable angina and acute myocardial infarction were taken from the related guidelines from American College of Cardiology/American Heart Association [36]. All subjects underwent coronary angiography (Innova 3100, GE, Fairfield, CT) to measure degree of coronary stenosis. Angiography was conducted by cardiologists using standard techniques. The angiograms were blindly done by two different cardiologists regarding patient identity and etiology. Patients with ketoacidosis, acute infection, severe liver or renal disease, congestive heart failure, malignant tumors, and sodium disorders were excluded. Thirty one healthy subjects with no clinical problems (1 men; mean age 33 years, range 25–42 years) who were undergoing a health checkup in our hospital served as controls.

Coronary angiography was performed in multiple views according to the standard Judkins technique by using the number of involved coronary branches and the sum of Gensini scores to assess severity of coronary artery stenosis. The Gensini score system [18] yields a qualitative evaluation of coronary angiogram, which grades the narrowing of the coronary artery lumen as 1 for 1%–25% narrowing, 2 for 26%–50% narrowing, 4 for 51%–75% narrowing, 8 for 76%–90% narrowing, 16 for 91%–99% narrowing, and 32 for total occlusion. This score is then multiplied by a factor that takes into account the importance of the lesion position in the coronary arterial tree, e.g., 5 for the left main coronary artery, 2.5 for the left anterior descending branch or circumflex artery, 1.5 for the midregion, 1 for the distal left anterior descending branch, and 1 for the mid-distal region of the circumflex artery or the right coronary artery. The observer that measures the degree of coronary stenosis is blind to the intermedin and BNP plasma levels.

2.2. Sample collection

Blood samples from all subjects were placed in tubes containing disodium ethylenediamine tetraacetic acid (1 mg/mL) and aprotinin (500 KIU/mL; Sigma, St. Louis, MO) that were centrifuged immediately at $3500 \times g$ for 10 min at 4 °C; plasma was stored at −80 °C.

2.3. Radioimmunoassay of plasma levels of IMD and BNP

Plasma samples extracted through a Sep-Pak C18 cartridge were assayed by use of a radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, USA). The IC50 of human IMD was 31 pg per tube, and the cross-reactivity with human IMD was 100%. No cross-reactivity was found with human calcitonin gene-related peptide (CGRP) and ADM. The within- and between-assay coefficients of variation for the immunoreactive IMD assay were less than 5% and 10%, respectively. The IC50 of the BNP assay was 28 pg per tube and the reactivity with human BNP 100%. No cross-reactivity was found with rat somatostatin, β-endorphin and P-substance. The within- and between-assay coefficients of variation for the immunoreactive BNP assay were less than 5% and 10%, respectively.

2.4. Measurement of other variables

The levels of fasting blood glucose (FBG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), total cholesterol (TC), and high sensitivity C-reactive protein (hs-CRP) were assessed by routine biochemistry. Left ventricular ejection fraction (LVEF) was measured by echocardiography on day 2 of hospitalization. Intra- and interassay coefficients of variation were <5%.

2.5. Statistical analysis

Continuous data such as serum IMD levels were expressed as mean ± standard deviation because were normally distributed and as median and interquartile range values when non-normally distributed, such as plasma BNP levels and Gensini score. Natural logarithmic transformation of data was used for statistical analysis when it was not normally distributed. Chi-square test, Student's *t* test and nonparametric Mann-Whitney test were used for statistical analyses. Correlations between variables were tested by Pearson and nonparametric Spearman correlation analysis. A $P < 0.05$ was considered statistically significant.

3. Results

3.1. Subject profiles

We included 41 patients (30 males; mean age 59.3 ± 11.4 years) and 31 healthy controls (1 male; mean age 33.4 ± 8.7 years) (Table 1). Fisher's exact test indicated that the selection of patients with a history of smoking and gender differences showed no significant differences between ACS and control groups. ACS patients differed from controls only in significantly higher systolic blood pressure, diastolic blood pressure, plasma levels of hs-CRP and creatine kinase-MB (all $P < 0.05$).