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Up-regulated synthesis of mature-type adrenomedullin in coronary circulation immediately after reperfusion in patients with anterior acute myocardial infarction☆

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Abstract

*Objective*Levels of adrenomedullin (AM), a potent vasodilatory peptide, have been shown to increase in the early stage of acute myocardial infarction (AMI). The purpose of this study was to determine whether coronary sinus-aortic step-up of mature forms of AM is accelerated in patients with AMI after reperfusion.

Methods: The subjects were 29 consecutive patients with a first episode of anterior AMI and 10 normal controls. All patients with AMI underwent balloon reperfusion therapy within 24 h after symptom onset. Plasma levels of two molecular forms of AM (an active, mature form [AM-m] and an intermediate, inactive glycine-extended form [AM-Gly]) in the aorta and coronary sinus (CS) were measured by specific immunoradiometric assay after reperfusion.

Results: Plasma levels of AM-m and AM-Gly in the aorta and CS were higher in AMI patients than in controls. CS-aortic step-up of AM-m, which is an index of myocardial production of AM-m, was significantly greater in AMI patients than in controls (1.7 ± 1.4 vs. 0.4 ± 0.3 pmol/L, $P < 0.01$). However, there was no significant difference in CS-aortic step-up of AM-Gly ($P = 0.30$). AMI patients with left ventricular dysfunction ($n = 10$) had a significantly higher CS-aortic AM-m step-up than AMI patients without left ventricular dysfunction ($n = 19$). AM-m in the aorta and CS negatively correlated with the left ventricular ejection fraction ($r = -0.50$, $r = -0.48$, $P < 0.01$).

Conclusions: Myocardial synthesis of AM-m is accelerated in patients with reperfused AMI, especially in patients with critical left ventricular dysfunction. Increased myocardial synthesis of active AM may protect against cardiac dysfunction, myocardial remodeling, or both after the onset of AMI.

Keywords: Acute myocardial infarction; Adrenomedullin; Reperfusion

1. Introduction

Human adrenomedullin (AM) has various physiological functions, including vasodilatation, diuresis, natriuresis, inhibition of aldosterone secretion, and inhibition of fibroblast proliferation and cardiomyocyte hypertrophy, [1–3] suggesting that this peptide protects against cardiovascular disease. Indeed, recent studies have shown that long-term AM treatment and AM gene therapy provide

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cardiovascular protection by reducing oxidative stress via activation of an intracellular cAMP signaling pathway or nitric oxide signaling pathway [4–7]. We previously demonstrated that plasma AM levels increase in the early phase of acute myocardial infarction (AMI) in proportion to the severity of myocardial damage [8,9]. We and other groups have also shown that a sharp rise in the plasma AM level during AMI indicates poor prognosis [10,11]. In addition, it has been reported that components of the myocardial AM signaling system, including AM and the AM receptor, are up-regulated in rat models of AMI [12]. These findings suggest that myocardial production of AM is involved in the pathophysiology of AMI.

AM is produced from the AM precursor by a two-step enzymatic pathway. First, the AM precursor, which consists of 185 amino acids, is converted to glycine-extended AM (AM-Gly), a 53-amino-acid peptide that is an inactive intermediate form of AM. Subsequently, AM-Gly is converted to active mature AM (AM-m), a 52-amino-acid peptide with a C-terminal amide structure, by enzymatic amidation [13]. Two molecular forms of AM (AM-m and AM-Gly) circulate in human plasma, and levels of both forms increase in patients with AMI [14]. Furthermore, recent studies have shown that the ratio of AM-m to total AM (AM-m/AM-T ratio) is higher in myocardial tissue than in plasma, and that the AM-m/AM-T ratio of myocardial tissue is increased in cases of cardiac hypertrophy or heart failure [15,16]. However, little information is available about the cardiac synthesis of the two molecular forms of AM in humans. The purpose of the present clinical study was to determine whether CS-aortic step-up of the active mature form of AM is accelerated in patients with AMI after reperfusion, and, if so, to investigate the pathophysiological role of AM-m in this condition.

2. Methods

2.1. Subjects

The subjects were 29 consecutive Japanese patients with a first episode of anterior AMI (24 men and 5 women; mean age, 65 ± 12 years) and 10 control patients (9 men and 1 woman; mean age, 59 ± 5 years) with chest pain symptom, normal LV function and no fixed coronary arterial stenosis. All of the patients with AMI underwent successful balloon reperfusion therapy within 24 h after symptom onset. The mean time between the onset of AMI and reperfusion was 9.1 ± 7.0 h. Diagnosis of anterior AMI was based on the following criteria: typical chest pain lasting longer than 30 min, ST segment elevation of >0.1 mV in precordial leads, and a subsequent rise in serum creatine kinase and its myocardial band isoenzyme to at least twice the upper limit of normal. Patients with chronic renal disease (serum creatinine level >1.4 mg/ml), chronic lung disease or inflammatory disease on admission were excluded from the study.

Occluded lesions in the proximal left anterior descending arteries were successfully recanalized in all AMI patients by conventional direct balloon angioplasty. Stents were implanted in 26 of the 29 AMI patients. After angioplasty, left ventriculography was performed. Left ventriculograms from the 30° right anterior oblique view were analyzed to determine left ventricular ejection fraction (LVEF) and left ventricular (LV) volume, using the area-length method. A 5-French catheter (Terumo, Tokyo, Japan) was positioned in the CS via the femoral vein, and its position was confirmed by injection of contrast dye. Blood samples were simultaneously drawn from the ascending aorta and the CS 20 min after reperfusion. Each sample was immediately put into a tube containing disodium EDTA (1 mg/ml) and aprotinin (500 units/ml), and the contents were centrifuged at 4 °C for 10 min at 3000 ×g.

The procedures used in this study conformed to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from each patient, and the protocol was approved by the ethical committee of our institute.

2.2. Assay of molecular forms of AM

Plasma samples were immediately frozen and stored at -70 °C until assayed. Both AM-m and total AM (AM-T) in the ascending aorta and CS were measured using recently developed specific immunoradiometric assay kits (AM mature RIA SHIONOGI, AM RIA SHIONOGI, Shionogi , Osaka, Japan) [17,18]. These assay systems use two monoclonal antibodies against human AM. One antibody recognizes the ring structure of human AM in both kits. The other antibody recognizes the carboxy-terminal sequence in the AM-m kit, and recognizes AM (25–36) in the AM-T kit. The assays measure human AM-m or AM-T by sandwiching it between the two antibodies without the extraction of plasma. The detection limit of human AM-m or AM-T is 0.5 pmol/L with each kit. AM-Gly was calculated using the following formula: (AM-Gly)=(AM-T)-(AM-m). The intra-assay and inter-assay coefficients of variation for the AM-m kit were 4.4–8.2% and 5.5–8.3%, respectively, and those of the AM-T kit were 3.4–7.3% and 5.3–9.0%, respectively. The concentration of brain natriuretic peptide (BNP) in plasma was measured using the Shiono RIA BNP assay kit (Shionogi) as previously reported [4].

2.3. Statistical analysis

All data are expressed as means ± SD, unless otherwise indicated. Mean values were compared between two groups using the unpaired Student's *t*-test or paired Student's *t*-test, as appropriate. Log transformation was used to normalize the distribution of plasma peptide levels, where appropriate. Mean values were compared among three groups using analysis of variance, followed by Bonferroni's multiple comparison test. Correlation coefficients were calculated