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High plasma levels of matrix metalloproteinase-8 in patients with unstable angina

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ABSTRACT

Matrix metalloproteinases (MMPs) play a role in collagen breakdown, leading to plaque instability. High levels of MMPs mRNA and proteins, especially MMP-1, MMP-2, MMP-8, MMP-9, and MMP-13, were shown in human atherosclerotic plaques. However, among various MMPs, only MMP-1, MMP-8 and MMP-13, so-called interstitial collagenases, can initiate collagen breakdown. To elucidate whether MMP-1, MMP-8 and MMP-13 levels in blood were high in patients with unstable angina (UAP), we measured serum MMP-1 and plasma MMP-8 and MMP-13 levels in 45 patients with UAP, 175 with stable coronary artery disease (CAD), and 45 controls. Plasma C-reactive protein levels tended to be higher in patients with UAP than in those with stable CAD and controls (median 0.94 vs. 0.69 and 0.51 mg/l). Regarding blood levels of MMPs, MMP-13 levels were above the lower detection limit in only one patient with UAP (2%), one with stable CAD (1%), and none in controls. MMP-1 levels did not differ among patients with UAP, stable CAD, and controls (median 4.8, 5.3, and 5.4 ng/ml). Notably, MMP-8 levels were higher in patients with stable CAD than in controls (median 3.5 ng/ml vs. 2.8 ng/ml, $P < 0.005$), however, MMP-8 levels in patients with UAP were much higher than those in stable CAD (3.9 ng/ml vs. 3.5 ng/ml, $P < 0.05$). In multivariate analysis, MMP-8 level was an independent factor for UAP. Thus, plasma MMP-8 levels were found to be high in patients with UAP, suggesting that MMP-8 levels in UAP may reflect coronary plaque instability and that MMP-8 is a promising biomarker for UAP.

1. Introduction

Interstitial collagen, especially type I, is a major component of atherosclerotic plaques [1–3]. Matrix metalloproteinases (MMPs) play an important role in the collagen breakdown that can cause plaque instability and rupture, thus leading to acute myocardial infarction (AMI) and unstable angina pectoris (UAP). High levels of mRNA and proteins of MMPs, especially MMP-1, MMP-2, MMP-8, MMP-9, and MMP-13, have been demonstrated in human atherosclerotic plaques, most profoundly in unstable plaques prone to rupture [4–9]. With respect to blood levels of MMPs, Kai et al. [10] reported serum MMP-2 and plasma MMP-9 levels in 11 patients with UAP to be higher than those in 17 with stable angina and 17 controls. Tziakas et al. [11] also showed serum MMP-2 and MMP-9 levels to be higher in 20 patients with UAP than in 16 controls. These findings suggest that MMP-2 and MMP-9 levels in blood would be biomarkers for UAP. However, among the various known MMPs, only MMP-1, MMP-8 and MMP-13, so-called interstitial collagenases, can initiate collagen breakdown, thereby making collagen fragments susceptible to further degradation by other MMPs, such as MMP-2 and MMP-9 [12]. Although three small studies reported serum MMP-1 levels to not be high in patients with UAP [11,13,14], there has so far been no report showing blood levels of either MMP-8 or MMP-13 in patients with UAP. Therefore, our study was carried out to elucidate if MMP-1, MMP-8 and MMP-13 levels in blood were high in patients with UAP and if these levels could be biomarkers for UAP.

2. Methods

2.1. Study patients

We measured serum MMP-1 and plasma MMP-8 and MMP-13 levels in 45 consecutive patients who had UAP at rest during the preceding 48 h, so-called class III UAP according to Braunwald's classification [15], and 175 patients with stable coronary artery disease (CAD). They underwent coronary angiography for suspected CAD at the National Defense Medical College Hospital and were

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found to have CAD which was defined as at least one coronary artery having >50% luminal diameter stenosis on angiograms. Coronary angiograms were recorded by a femoral approach using the Judkins technique and cineangiogram system (Toshiba, Tokyo, Japan). All coronary angiograms were evaluated by Y.M., who was blinded to MMP data. The results were then compared with those of 45 age- and gender-matched controls who had coronary angiography for suspected CAD but were found to have angiographically normal coronary arteries. Because MMPs have been shown to be released from the myocardium with infarction [16–18], any patients with AMI who showed a significant increase (more than the upper normal range) in serum creatine kinase levels were excluded. Patients with a history of MI within 6 months, those with a history of percutaneous coronary intervention or coronary artery bypass surgery, or those with heart failure, cardiomyopathies or valvular heart disease were also excluded. Our study was approved by the ethics committee of the hospital. After written informed consent was obtained, blood samples in patients with UAP were taken within 24 h after their admission, and blood samples in patients with stable CAD and controls were taken on the day of angiography.

2.2. Measurements of serum MMP-1 and plasma MMP-8 and MMP-13 levels

After blood samples were centrifuged at $2000 \times g$ for 15 min at 4 °C, the serum and plasma were frozen and stored at −80 °C until analyzed. Serum MMP-1 levels were measured by a one-step sandwich enzyme immunoassay using a commercially available kit (Daiichi Pharmaceutical, Toyoma, Japan). This kit measures the total concentration of the precursor form, the active form, and the TIMP-1 or -2 complex forms of MMP-1 in the serum, but it is highly specific and does not cross-react with MMP-8 and MMP-13 [19]. The lower detection limit of this assay was 0.1 ng/ml. Plasma MMP-8 levels were measured by a two-site sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (MMP-8 Human Biotrak ELISA System, Amersham Biosciences, Buckinghamshire, UK). This kit measures the total concentration of both the precursor and active forms of MMP-8 in the plasma, but it is highly specific and does not cross-react with either MMP-1, MMP-2, MMP-3, MMP-9, MMP-13 or MT1-MMP [20]. The lower detection limit was 2.5 ng/ml. Plasma MMP-13 levels were also measured by a two-site sandwich ELISA with a commercially available kit (MMP-13 Human Biotrak ELISA System, Amersham Biosciences). This kit measures the total concentration of the precursor and active forms of MMP-13 in the plasma, but it is highly specific and does not cross-react with either MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 or MT1-MMP. The lower detection limit was 0.09 ng/ml. MMP-1, MMP-8 and MMP-13 levels of all samples were measured in duplicate, and the results were then averaged. Plasma high sensitivity C-reactive protein (hsCRP) levels were also measured using a BNII nephelometer (Dade Behring, Tokyo, Japan). In patients with UAP, a rapid qualitative test for troponin T (TnT) was performed using a commercially available kit (TROP T sensitive, Roche Diagnostics, Tokyo, Japan). The lower detection limit of this test was 0.1 ng/ml, and positive TnT was defined as >0.1 ng/ml. Serum lipid levels were measured by standard laboratory methods.

2.3. Statistics

Any differences between the two groups were evaluated by the unpaired *t*-test for parametric variables, by the Mann–Whitney *U*-test for nonparametric variables, and by the chi-square test for categorical variables. Any differences among the three groups were evaluated by ANOVA with the Scheffe's test for parametric variables, by the Kruskal–Wallis test for nonparametric variables, and by the chi-square test for categorical variables. The correlation between MMP-8 and hsCRP levels was evaluated by the Spearman's rank correlation test. A forward stepwise multiple logistic regression analysis was used to elucidate the independent association between MMP-8 levels and UAP. A *P*-value of <0.05 was considered to be statistically significant. The results are presented as the mean value ±SD. Since the distributions of the measured MMP-1, MMP-8 and hsCRP levels were highly skewed, these results are presented as the median value.

3. Results

As shown in Table 1, there was no difference in age or gender among the three groups. Total cholesterol levels were lower in patients with UAP than in those with stable CAD and controls. The percentage of patients taking statin was 29% in patients with UAP, 29% in those with stable CAD, and 18% in controls, respectively (*P* = NS). The number of >50% stenotic coronary vessels was similar in patients with UAP and those with stable CAD (2.0 ± 0.8 and 1.9 ± 0.8), and 1-vessel, 2-vessel, and 3-vessel disease was present in 31%, 36%, and 33% of patients with UAP vs. 38%, 37%, and 25% of those with stable CAD (*P* = NS). Of the 45 patients with UAP, 10 (22%) showed positive TnT without a significant increase in creatine kinase levels.

Table 1
Clinical characteristics in three groups.

	UAP (*n* = 45)	UAP vs. CAD	CAD (*n* = 175)	CAD vs. control	Controls (*n* = 45)	UAP vs. control
Age (years)	67 ± 9	NS	66 ± 8	NS	66 ± 7	NS
Gender (male)	34 (76%)	NS	141 (81%)	NS	34 (76%)	NS
Hypertension	33 (73%)	NS	119 (68%)	NS	26 (58%)	NS
Systolic BP (mmHg)	138 ± 25	NS	137 ± 18	NS	132 ± 14	NS
Hyperlipidemia	24 (53%)	NS	76 (43%)	NS	15 (33%)	NS
Total cholesterol (mg/dl)	190 ± 34	< 0.02	203 ± 32	NS	206 ± 30	<0.05
HDL-cholesterol (mg/dl)	51 ± 12	NS	49 ± 14	<0.001	60 ± 15	<0.005
Statin	13 (29%)	NS	51 (29%)	NS	8 (18%)	NS
Diabetes mellitus	14 (31%)	NS	62 (35%)	<0.01	6 (13%)	NS
Current smoker	18 (40%)	NS	56 (32%)	NS	12 (27%)	NS
Number of >50% stenotic coronary vessels	2.0 ± 0.8	NS	1.9 ± 0.8			
1-Vessel disease	14 (31%)	NS	67 (38%)			
2-Vessel disease	16 (36%)	NS	65 (37%)			
3-Vessel disease	15 (33%)	NS	43 (25%)			

Data are presented as the mean value ± SD or the number (%) of patients.
UAP, unstable angina pectoris; CAD, coronary artery disease; BP, blood pressure.
Hypertension was defined as blood pressures ≥140/90 mmHg or on medication.
Hyperlipidemia was defined as total cholesterol levels >240 mg/dl or on medication.
Diabetes mellitus was defined as fasting glucose levels ≥126 mg/dl or on insulin or hypoglycemic drugs.