Diagnostic Use of Serum Deoxyribonuclease I Activity as a Novel Early-Phase Marker in Acute Myocardial Infarction

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Background—The delayed release of serum cardiac markers such as creatine kinase isoenzyme MB and equivocal early electrocardiographic changes have hampered a diagnosis of acute myocardial infarction (AMI) in the early phase after its onset. Therefore, a reliable serum biochemical marker for the diagnosis of AMI in the very early phase is desirable.

Methods and Results—Serum samples were collected from the patients with AMI, unstable angina pectoris, stable angina pectoris, and other diseases. Levels of serum deoxyribonuclease I (DNase I) activity in the patients were determined. An abrupt elevation of serum DNase I activity was observed within approximately 3 hours of the onset of symptoms in patients with AMI, with significantly higher activity levels (21.7±5.10 U/L) in this group compared with the other groups with unstable angina pectoris (10.4±4.41 U/L), angina pectoris (10.8±3.70 U/L), and other diseases (9.22±4.16 U/L). Levels of the DNase I activity in serum then exhibited a marked time-dependent decline within 12 hours and had returned to basal levels within 24 hours.

Conclusions—We suggest that serum DNase I activity could be used as a new diagnostic marker for the early detection of AMI. **(*Circulation*. 2004;109:2398-2400.)**

Key Words: myocardial infarction ■ enzymes ■ diagnosis

In patients with acute myocardial infarction (AMI), the infarct size is an important determinant of both mortality and morbidity.[1] When revascularization and thrombolytic therapies are initiated rapidly, there is a greater potential for reduction in the infarct size. The release of cardiac proteins from injured cardiac tissue into plasma has been used as a diagnostic marker for the exclusion or confirmation of AMI.[2] However, it is often difficult to make a diagnosis of AMI in the very early phase, ie, within 3 hours of onset. This is partly due to a delay in the appearance in the serum of the biochemical markers specific for myocardial damage.[2,3]

Deoxyribonuclease I (DNase I, EC 3.1.21.1), one of the well-known enzymes, was the first enzyme to be recognized as specific for DNA.[4] One of its proposed roles is DNA breakdown during apoptosis.[5] DNase I has been detected in human myocardium, and it has been reported that the activity level increases in heart failure due to idiopathic dilated cardiomyopathy.[6] However, the association between serum DNase I activity level and coronary heart disease (CHD) has not yet been clarified. In the present study, we assessed the serum DNase I activity in patients with AMI and related CHD and found that there is a specific elevation of serum DNase I activity in the very early stages of AMI.

Methods

Patients and Sample Collection

We assessed 53 consecutive Japanese patients with AMI admitted to our hospitals between September 2002 and May 2003. The clinical diagnosis of AMI and unstable angina pectoris (UAP) was made according to the European Society of Cardiology/American College of Cardiology Committee criteria.[7] The mean lapse time between the onset of symptoms and hospital admission was 10.7±13.8 hours. Emergent coronary angiography was performed in all of these patients. In addition, we assessed 15 patients with UAP, 43 patients with stable angina pectoris (AP), 9 patients with chest pain syndrome (CPS), 75 patients with other diseases (OD), and 72 healthy volunteers. In the OD group, trauma (n=13), acute heart failure (AHF, n=14), chronic heart failure (CHF, n=10), old myocardial infarction (n=6), chronic renal failure (CRF, n=9), stroke (n=5), and others were included. Serum samples were collected from the patients as soon as possible after admission and 3, 6, 12, and 24 hours later.

The human ethics committees within the study institutes approved this study protocol, and all patients gave written informed consent.

Biochemical Assessment

The level of the DNase I activity in serum samples was determined using the single radial enzyme diffusion method, as described previously.[8,9] Serum creatine kinase isoenzyme MB (CK-MB) and

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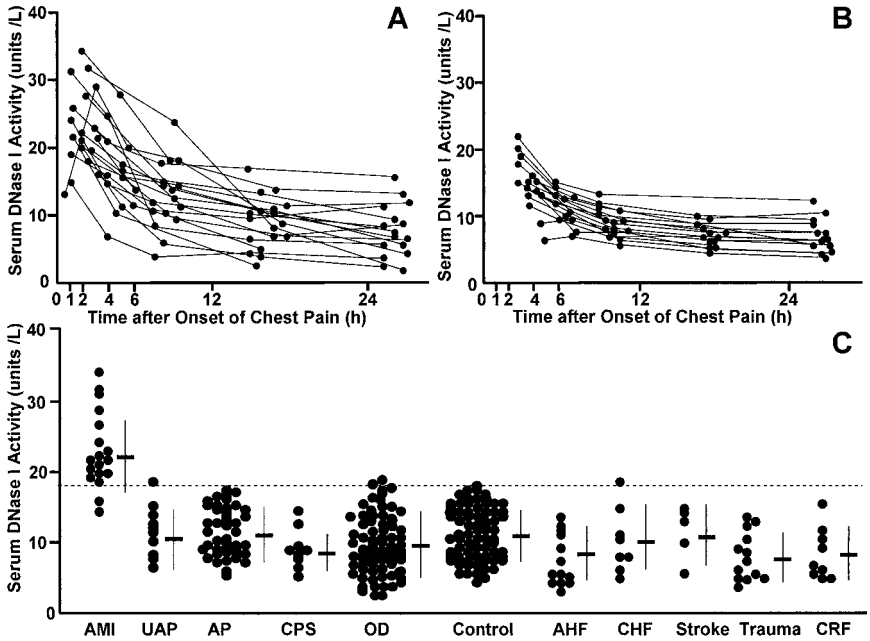

A and B, Changes in the serum DNase I activity level as a function of time from onset in 18 and 15 patients with AMI admitted to our hospital within 3 hours and within 3 to 6 hours, respectively, of the onset. Several patients confirmed that before their admission to the hospital and before the onset of acute chest pain, their outpatient serum DNase I activity levels were within the normal range. C, Scatter-diagram of serum levels of DNase I activity in the AMI, AP, UAP, CPS, and OD groups, together with those of the patient groups with AHF, CHF, stroke, trauma, and CRF among the OD group. Bars and broken line represent mean±SD and the upper limit of the normal range, respectively.

troponin T concentrations were determined using conventional methods. In this study, the cutoff levels for concentration of CK-MB and troponin T were 5.20 and 0.01 μg/L, respectively.

Statistical Analysis

Data were expressed as mean±SD. Statistical comparison between patient groups was performed by ANOVA using StatView software. Differences were considered significant at $P<0.05$.

Results

Serum DNase I activity in healthy subjects was determined to be 10.7±3.60 U/L, and the upper limit of the normal range of serum DNase I activity was considered to be 17.9 U/L (mean+2SD). When the serum DNase I activity level was measured periodically in the AMI group, a marked increase in serum DNase I activity was observed immediately after the onset of chest pain, with the maximal level being reached within approximately 3 hours and a gradual decline to basal levels within 24 hours (Figure, A and B). Thus, there was an abrupt and transient elevation in serum DNase I activity in patients with AMI in the early stage soon after the onset of chest pain.

In 18 patients with AMI admitted to our hospitals within 3 hours of the onset of chest pain, the mean serum DNase I activity level was significantly higher than that found in the UAP, AP, or OD group (Table and Figure, C); levels of the activity in the patients with AHF, CHF, CRF, stroke, and trauma were 7.91±3.31, 10.0±4.10, 8.00±3.75, 10.9±3.70, and 7.41±3.50 U/L, respectively. Furthermore, the serum DNase I activity level (8.50±2.80 U/L) in the 9 patients with CPS who were initially considered to have AMI on clinical grounds but found to have no evidence of myocardial infarction on follow-up assessment was within the normal range. Therefore, this elevation in the serum activity level seems to be specific for AMI when compared with other forms of CHD in which onset of acute chest pain is a feature.

When we examined the relationship between serum DNase I activity level in the AMI group and the time delay between symptom onset and hospital admission, the mean serum DNase I activity subsided over time and reached basal levels within 24 hours; the mean value between 0 and 3 hours was 21.7±5.10 U/L (n=18); between 3 and 6 hours was 14.1±2.50 U/L (n=15); between 6 and 12 hours was 10.5±4.20 U/L

Baseline Characteristics and Mean Serum DNase I Activity in AMI, UAP, AP, and OD Patients and Controls

Characteristic	AMI (n=18)	UAP (n=15)	AP (n=43)	OD (n=75)	Control (n=72)
Sex, male/female, n	12/6	11/4	27/16	42/33	40/32
Age, y	68.4±9.80	65.0±14.9	70.2±7.50	62.1±17.3	40.1±21.6
Diabetes	47.9	36.4	54.4	25.3	0
Current smoking	55.1	60.0	54.5	49.3	39.0
Hypertension	67.8	45.5	58.1	39.4	0
Hypercholesterolemia	64.2	45.5	58.1	21.1	0
Serum DNase I activity, U/L	21.7±5.10*	10.4±4.41	10.8±3.70	9.22±4.16	10.7±3.60

Values are mean±SD or percentages.
*$P<0.0001$ vs other groups.

(n=11); and between 12 and 24 hours was 8.30±2.10 U/L (n=9). This relationship reflected the intra-individual time-dependent changes in the serum DNase I activity seen in all patients in the AMI group after hospital admission. The serum levels of DNase I activity exceeded the cutoff levels in 88% of the patients. By contrast, in the 18 patients with AMI admitted within 3 hours of onset, only 8 were CK-MB–positive and 9 were troponin T–positive on admission. Thus, we were able to detect an elevation in the serum DNase I activity within approximately 3 hours of the onset of symptoms and before accurate CK-MB and troponin T results.

Discussion

DNase I has been postulated to be responsible for internucleosomal DNA degradation during apoptosis.[5] Apoptosis is induced by ischemic injuries to myocardium in AMI,[10,11] and myocyte death attributable to apoptosis in the failing heart has been demonstrated.[12] Yao et al[6] reported significant levels of DNase I to be detectable in the human myocardium. Therefore, it is plausible that the ischemic injury may recruit DNase I from the myocardium of patients with AMI. Additional clarification of the mechanism responsible for DNase I elevation and the physiological significance of DNase I in AMI will undoubtedly have important biological and clinical implications.

Serum DNase I activity was significantly elevated within 3 hours of the onset of acute chest pain in the patients with AMI, whereas the serum levels of CK-MB and troponin T were slightly elevated and exceeded the cutoff levels in approximately 45% of those patients; we were thus able to detect the elevation of the serum DNase I activity earlier than that of CK-MB and troponin T. As an early marker for myocardial necrosis, the serum myoglobin level is a sensitive test but lacks cardiac specificity, because there may be an elevation in the serum levels of myoglobin secondary to musculoskeletal injury.[13] However, the serum DNase I activity level does not rise after trauma, and surgical trauma has been reported to induce no elevation of the activity,[14] suggesting that DNase I may be a more specific marker for AMI than myoglobin. Furthermore, there is a slight overlap between the levels of activity in patients with AMI and patients with OD (Figure, C). Because the latter patients do not suffer from chest pain, this overlap would not lower the diagnostic accuracy of serum DNase I activity testing. Thus, the serum DNase I level may be a useful clinical tool in the early diagnosis of AMI within approximately 3 hours of the onset of symptoms.

The results of the present study will facilitate future studies in larger numbers of patients to evaluate the enzyme as a useful biochemical marker with regard to specificity and sensitivity in comparison with the other cardiac markers, such as myoglobin and heart-type fatty-acid binding protein.[15]

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