Serum deoxyribonuclease I activity can be used as a sensitive marker for detection of transient myocardial ischaemia induced by percutaneous coronary intervention

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Aims Cardiac markers such as troponin T (c-TnT) have proven unsuitable for the detection of early and transient myocardial ischaemia. We recently reported that abrupt elevation of serum deoxyribonuclease I (DNase I) activity in the early stage of acute myocardial infarction could be used as a diagnostic marker. To evaluate whether serum DNase I could be used as a marker of early myocardial ischaemia, we investigated alterations in its levels after transient ischaemia induced during percutaneous coronary intervention (PCI).

Methods and results In 24 consecutive patients with stable angina undergoing elective PCI and 12 patients undergoing coronary angiography (CAG), serum samples were tested for DNase I, creatine kinase isoenzyme MB (CK-MB), and c-TnT before, soon after, and 3 and 12–24 h after completion of the procedures. Serum DNase I activity had risen significantly from baseline by 3 h after PCI in 21 of the 24 PCI patients. The mean percentage difference from baseline in serum DNase I activity 3 h after PCI was 35.9 ± 37.5%. Even among the 16 PCI patients whose levels of CK-MB and c-TnT were within the normal range, 13 showed elevation of serum DNase I activity from baseline after PCI. In the CAG patient group, DNase I activity levels remained unchanged at all times after CAG.

Conclusion Elevation of serum DNase I activity can be used as a sensitive marker for detection of transient myocardial ischaemia.

Keywords
Angioplasty; Ischaemia; Deoxyribonuclease I

Introduction

Owing to a lack of a rapid and reliable tests, it is often difficult to make a diagnosis of acute and transient myocardial ischaemia in patients with acute coronary syndromes (ACS). Markers of cardiac necrosis such as creatine kinase isoenzyme MB (CK-MB), myoglobin, and cardiac troponin T (c-TnT), particularly when measured in the first 2–6 h after the onset of myocardial ischaemia, have proven unreliable for detection of myocardial ischaemia.⁴⁻⁵ Furthermore, use of these markers is controversial in the detection of transient myocardial ischaemia occurring during percutaneous coronary intervention (PCI).⁶⁻⁷ To improve the process of triage of patients with ACS, we need useful diagnostic markers that can exclude or confirm myocardial ischaemia. In this context, biochemical markers that exhibit a rapid response to myocardial ischaemia (rather than injury) and are easily assessed are desirable and should be developed.

Deoxyribonuclease I (DNase I, EC 3.1.21.1) is an endonuclease that preferentially attacks double-stranded DNA in a Ca²⁺-dependent manner to produce oligonucleotides with 5’ phospho and 3’ hydroxy termini.⁸ DNase I is postulated to be responsible for DNA breakdown during apoptosis.⁹ Recently, we demonstrated that serum DNase I activity could be used as a novel diagnostic marker for the early detection of acute myocardial infarction (AMI); abrupt elevation of serum DNase I activity levels occurs within ~3 h of the onset of symptoms in patients with AMI, permitting the diagnosis of AMI before accurate CK-MB and c-TnT results become available.¹⁰ These findings suggested...
to us that serum DNase I may be sensitive and suitable for the detection of myocardial ischaemia before abnormal levels of the conventional cardiac markers can be detected. PCI offers an in vivo model of mild transient myocardial ischaemia–reperfusion in humans. In this clinical study, we investigated whether the levels of activity of DNase I in the serum were altered in response to transient myocardial ischaemia developing during PCI. We then evaluated DNase I as a sensitive marker for the detection of myocardial ischaemia and compared its usefulness with that of CK-MB and c-TnT.

**Methods**

**Subjects**

We performed elective PCI in 46 consecutive Japanese patients presenting with stable angina between March and November 2003. Among them, 14 patients were excluded from the study because of the multi-vessel disease. In addition, eight patients were excluded because they had one or more signs or symptoms of acute ischaemic conditions, including stroke, peripheral vascular disease, aortic dissection, trauma and shock, or objective evidence of AMI (unequivocal electrocardiographic abnormalities, serial cardiac marker elevations, or angiographic evidence) in the 2 weeks before catheterization. Therefore, the total study population comprised 24 patients who had >70% stenosis in a major coronary artery with no evidence of useful collateral coronary artery circulation around the single stenotic vessel (PCI group). On the other hand, 66 patients underwent diagnostic coronary angiography (CAG) for a typical chest pain during the same study period. Forty-eight patients were excluded because they had conditions such as >25% stenosis in a major coronary artery, valvular disease, myocarditis, and any signs or symptoms of acute ischaemic conditions as described earlier; six patients declined to take part in the study. Ultimately, 12 patients were enrolled as the control group and we recruited 12 healthy volunteers from laboratory workers in our institute for assessment of intra-individual variation in serum DNase I activity. These volunteers, aged 40.1 ± 21.6 years, had no physiological or biochemical features indicative of ischaemic heart disease.

The study protocol conformed to the Declaration of Helsinki and was approved by the Human Ethics Committee of the institutes; each subject included in the study gave written informed consent before study participation.

**PCI procedures and sample collection**

All coronary angioplasties were performed according to a standard technique described previously. All PCIs were done by a radial approach using a 6-French sheath, and a 4- or 5-French sheath was used for CAG. Decisions on the number of balloon inflations, whether or not to use an intraluminal stent, and total inflation time were based entirely on the clinical judgement of the interventional cardiologists. All patients received 200 mg ticlopidine twice a day at least 24 h before PCI. Intra-arterial heparin, 8000 and 3000 U, was administered before introduction of the guide wire in PCI and CAG, respectively. A 12-lead electrocardiogram (ECG) was recorded at baseline and after balloon inflation to assess ST segment shift and ventricular rhythm disturbances. Experience of chest pain during balloon inflation was heard from the patient.

Blood samples were taken from the radial sheath before the procedure (baseline) and soon after the end of the last balloon inflation in the PCI group, or after diagnostic CAG in the control group. Follow-up blood samples were obtained from the antecubital vein 3 and 12-24 h after completion of the procedure. Also, blood samples were collected from several patients (n = 11) 6 h after the procedure. Furthermore, to assess intra-individual variations in the activity, blood samples were obtained periodically, according to the same time schedule used for the PCI group, from the antecubital veins of each volunteer. Serum samples were prepared from each blood sample and stored at −80°C until assay.

**Measurement of DNase I activity and cardiac markers in serum of patient**

Levels of DNase I activity in serum samples were measured by the single radial enzyme diffusion (SRED) method, as described previously. The assay method can determine picogram to femtogram quantities of DNase I in 1 μL serum samples within 30 min. One unit of enzyme assayed corresponds to 0.6 ng of purified human DNase I. Daily variation in serum DNase I activity has been postulated to occur, and we determined the mean intra-individual per cent difference in activity levels using healthy volunteers (n = 12) to be 7.0 ± 2.7%. The upper limit of the normal range of intra-individual per cent differences was estimated to be 12.4% (mean ± 2SD) as a tentative cutoff value. When the per cent difference in serum DNase I activity levels from baseline in each patient exceeded the cutoff level, elevation of activity levels was considered positive. To clarify whether the activity measured by this method was entirely derived from DNase I, we employed an inhibition assay using anti-human DNase I antibody, as described previously. Serum CK-MB concentration was determined with an automated chemiluminescence system (Ciba Corning Diagnostics Corp., Medfield, MA, USA) and serum c-TnT with an electrochemiluminescence immunoassay system (Elecsys 1010 System, Roche Diagnostics Corp., Mannheim, Germany), in accordance with the manufacturer's instructions. Cutoff levels of CK-MB and c-TnT indicative of positive activity were taken as 5.20 and 0.01 μg/L, respectively.

**Statistical analysis**

Biochemical data in the PCI and control groups were expressed as mean value ± SD. Per cent differences from baseline at each time interval after completion of the procedure were used to clearly reflect the within-patient variability in serum DNase I activity levels. The values were calculated as follows:

\[
\frac{\text{assay level after PCI or CAG} - \text{baseline assay level before PCI or CAG}}{100} \times 100
\]

Categorical variables in the clinical background were compared using the χ² test or the unpaired t-test. The Tukey-Kramer multiple comparison test was used to assess differences in values measured before, and at various time intervals after, PCI or CAG. Correlations between variables were evaluated using Pearson's correlation coefficient. Data analysis was performed with StatView 5.0 (SAS, Cary, NC, USA). Data differences were studied by using a two-sided t-test with a significance level of 0.05. On the basis of our previous study for the 12 patients used as a control group, the power to detect a 46% difference in serum DNase I activity levels for a two-sided test with a level of significance of P = 0.05 was 83.3%.

**Results**

**Study group patient characteristics**

The clinical and procedural characteristics of all the patients included in the PCI and control groups are presented in Table 1. Although the mean age in the PCI group was significantly lower than that in the control group (P < 0.05), no significant differences were found in the prevalence of coronary risk factors such as hypertension,
high cholesterol levels, diabetes mellitus, smoking habits, or obesity between the two groups. In the PCI group, 21 patients (88%) had exhibited a transient ischaemic ST segment shift and 12 patients (50%) experienced chest pain during the procedure, but none of the patients had ventricular rhythm disturbance. No patients in the control group had ECG changes, chest pain, or ventricular rhythm disturbance during CAG. Of the 24 PCI patients, 8 patients had minor complications; 5 (20%) had small side branch occlusion (<0.5 mm diameter); 1 (4%) had a possible distal embolization; 2 (8%) had major side branch occlusion.

Measurement of serum DNase I activity levels

In the PCI group, a marked elevation in serum DNase I activity was generally observed 3 h after completion of PCI; the activity level then tended to return to baseline by 12–24 h (Figure 1). Obvious alterations in activity level were not found in the control group. All the enzyme activity detected in the serum samples by the SRED method was completely abolished by anti-human DNase I antibody, confirming that the activity was entirely derived from authentic DNase I.

Table 2 shows the per cent differences from baseline of serum DNase I activity levels after the procedures in both groups. In the PCI group, the mean per cent difference at 3 h after PCI compared with baseline was 35.9 ± 37.5% (n = 24). The levels of serum DNase I activity at 3 h after PCI were significantly higher than the baseline levels. The mean per cent difference from baseline in serum DNase I activity had declined at 6 h after PCI (n = 11) (11.8 ± 32.9%) and had returned to baseline by 12–24 h (n = 24) (−3.80 ± 28.3%). However, no significant elevation of the activity, even at 3 h after CAG, was observed in the control group.

There was no correlation between the highest postprocedural serum DNase I activity and distribution of the occluded coronary artery, number of inflations, total occlusion time, or maximum balloon pressure in the PCI group.

Association of DNase I elevation with that of other cardiac markers

No elevation of c-TnT and CK-MB levels was observed in 16 (67%) and 17 (71%), respectively, of the 24 patients who underwent PCI; PCI patients could be classified into c-TnT-positive and -negative groups. Among the 16 c-TnT-negative patients whose levels of both CK-MB and c-TnT remained within the normal range at all times after PCI, elevation of serum DNase I activity 3 h after PCI occurred in 13 (81%). In contrast, all eight c-TnT-positive patients (100%) whose marker levels exceeded their respective cutoff levels exhibited significant increments in serum DNase I activity (Figure 2). All of these patients had minor complications such as small side branch occlusion, possible distal embolization, or major side branch occlusion. Although the extent of the rise in serum DNase I activity observed after PCI was relatively low compared with that in AMI patients, the levels of DNase I activity exceeded the threshold value estimated as being diagnostic for AMI in 13 of the PCI patients (Figure 3).

Discussion

Major findings

This clinical study is the first to demonstrate that a transient coronary arterial occlusion occurring during PCI can induce elevation of serum DNase I levels; we propose this enzyme as a novel and sensitive marker for the detection of transient myocardial ischaemia. Serum DNase I levels rose significantly from baseline by 3 h after PCI in the PCI patients, whereas none of the control patients who underwent CAG exhibited any alterations of serum DNase I activity at any time after angiography. These findings indicate that alterations in activity observed in the PCI group are a result of...

### Table 1 Clinical and procedural characteristics of the study patients

<table>
<thead>
<tr>
<th></th>
<th>PCI</th>
<th>Control</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Total populations, n</td>
<td>24</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>67.5 ± 10.4</td>
<td>76.7 ± 8.0</td>
<td>0.012</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>21 (88)</td>
<td>9 (75)</td>
<td>0.35</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>11 (46)</td>
<td>3 (25)</td>
<td>0.23</td>
</tr>
<tr>
<td>Coronary risk factor, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>13 (54)</td>
<td>3 (25)</td>
<td>0.15</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14 (58)</td>
<td>3 (25)</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (54)</td>
<td>6 (50)</td>
<td>0.81</td>
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<tr>
<td>Hypercholesterolaemia</td>
<td>14 (58)</td>
<td>5 (42)</td>
<td>0.34</td>
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<tr>
<td>Obesity</td>
<td>9 (42)</td>
<td>3 (25)</td>
<td>0.45</td>
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<tr>
<td>Clinical treatment</td>
<td></td>
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<tr>
<td>Stent insertion, n (%)</td>
<td>18 (75)</td>
<td>—</td>
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<tr>
<td>Mean No. of balloon inflation, n (± SD)</td>
<td>5.21 (1.91)</td>
<td>—</td>
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<tr>
<td>Mean total balloon</td>
<td>211 (110)</td>
<td>—</td>
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<td>Inflation time, s (± SD)</td>
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<td></td>
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<tr>
<td>Mean inflation pressure, atm (± SD)</td>
<td>13.2 (4.37)</td>
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<tr>
<td>Culprit lesion, n (%)</td>
<td></td>
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</tr>
<tr>
<td>Left anterior descending</td>
<td>12 (50)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Left circumflex</td>
<td>5 (19)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Right coronary artery</td>
<td>7 (26)</td>
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transient myocardial ischaemia induced by transient balloon inflation, not of coronary catheterization alone. It has been reported that, in AMI patients, levels of serum DNase I rise significantly to maximum levels until 3 h after onset of AMI, then gradually decline and returns to baseline within 24 h.\textsuperscript{10} In our study group, most patients whose levels of transient myocardial ischaemia induced by transient balloon inflation, not of coronary catheterization alone. It has been reported that, in AMI patients, levels of serum DNase I rise significantly to maximum levels until 3 h after onset of AMI, then gradually decline and returns to baseline within 24 h.\textsuperscript{10} In our study group, most patients whose levels of transient myocardial ischaemia induced by transient balloon inflation, not of coronary catheterization alone. 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both CK-MB and c-TnT had not increased after PCI showed a transient and definitive elevation of serum DNase I activity. In light of these findings, we can suggest that myocardial ischaemia, rather than injury, induces such alterations in serum DNase I activity. Although it remains unknown why the onset induces a transient rise in serum DNase I activity in AMI patients, these findings indicate that this elevation of serum DNase I activity may be due mainly to the myocardial ischaemia that occurred during AMI. Sensitive, early detection of myocardial ischaemia with serum DNase I makes DNase I suitable as an early-phase marker of AMI.

DNase I and other cardiac markers of myocardial ischaemia

Elevation of the cardiac marker of myocardial injury CK-MB occurs in only 6–20% of successful PCIs, and elevation of c-TnT in 13–53%. Furthermore, CK-MB and c-TnT levels do not routinely increase in the 2–6 h after ischaemic injury or PCI. Therefore, these cardiac markers are unreliable as diagnostic tests for the presence of mild, reversible myocardial ischaemia, as supported by the fact that their levels did not rise after PCI in 67% of PCI patients. In contrast, elevation of serum DNase I activity occurred 3 h after PCI in 88% of PCI patients and preceded the changes in CK-MB and c-TnT levels. Furthermore, even in 81% of the PCI patients whose levels of both markers remained within the normal range, DNase I was elevated 3 h after PCI. These findings indicate that serum DNase I is superior to both CK-MB and c-TnT for detection of transient myocardial ischaemia in the early phase. Recently, ischaemia-modified albumin (IMA), measured by the albumin cobalt binding test, was shown to be a marker of myocardial ischaemia in the PCI setting; 83–95% of patients with transient coronary occlusion during PCI showed a statistically significant rise in IMA concentration. On the basis of the comparison across studies, serum DNase I (88%) may not be inferior to IMA with regard to sensitivity for detection of myocardial ischaemia. ACS, a well-known life-threatening disorder, is often confirmed in accordance with ACC/AHA guidelines. However, absence of typical chest pain, ST segment changes, regional wall motion abnormalities, and cardiac marker elevation is frequently observed, making definite diagnosis of ACS difficult. The general concept of ACS encompasses unstable angina (UA) and AMI, both of which induce myocardial ischaemia. Accordingly, assessment of serum DNase I could help make a diagnosis of not only AMI, but also UA, and may prove to be a tool for risk stratification.

Proposed mechanism of DNase I elevation by myocardial ischaemia

The mechanism responsible for DNase I elevation induced by PCI or AMI remains to be clarified. Furthermore, the possibility that mild necrosis, which merely goes undetected with c-TnT and CK-MB, induces transient elevation of serum DNase I activity could not be ruled out in this study. However, we have demonstrated in preliminary studies that both the levels of DNase I activity and DNase I gene expression in cultured human cells increases under hypoxia compared with normoxia, suggesting that hypoxia allows serum DNase I activity to rise (Kominato et al., unpublished data). Transcriptional activation by hypoxia-induced factor (HIF-1) is known to be involved in the early response of the myocardium to ischaemia. In the 5' upstream region of the human DNase I gene, several hypoxia-response elements that may bind to HIF-1 have been found to be present. Therefore, it seems plausible to postulate that ischaemia induced by PCI or AMI causes elevation of serum DNase I levels through the effects of HIF-1 on up-regulation of gene expression.

Limitations of this study

First, the biological significance of elevation of serum DNase I activity during myocardial ischaemia remains to be clarified. DNase I is considered responsible for apoptosis. Experimental evidence suggests that cardiomyocytes are able to undergo apoptosis during hypoxia, myocardial infarction, and ischaemia–reperfusion. Therefore, the possible involvement of the enzyme in apoptosis may provide a clue to the mechanism and biological significance of its elevation. Experimental trials need to be performed to demonstrate the relationship between DNase I and apoptosis during transient myocardial ischaemia. Secondly, our sample size was small. Nevertheless, significant differences in the accurate and rapid diagnosis of myocardial ischaemia could be drawn between the use of serum DNase I activity and other cardiac markers, implying that our findings are robust. Further studies in larger numbers of patients are warranted to evaluate whether serum DNase I activity is a clinically useful diagnostic marker for detection of transient myocardial ischaemia.

Conclusion

Transient myocardial ischaemia occurring during PCI induces a significant elevation of serum DNase I activity. Increased activity of this enzyme may prove useful as a sensitive marker for detection of transient myocardial ischaemia.
Acknowledgement
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References