Serum Aminoterminal Type III Procollagen Peptide Reflects Repair After Acute Myocardial Infarction

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In 16 patients with acute myocardial infarction and in 15 controls, procollagen type III aminoterminal peptide in serum (PIIINP) was measured consecutively. Serum PIIINP was increased on the second to third postinfarction day (p<0.01) and remained elevated for more than 4 months. Peak values were observed on the third to seventh postinfarction day. The individual peak changes were correlated to infarction size calculated from serum CK-MB and serum lactate dehydrogenase (p=0.60, p=0.02). The changes in distribution of PIIINP-related antigens in serum after gel chromatography were similar to changes observed during wound healing in humans. PIIINP is cleaved off procollagen type III during the biosynthesis of type III collagen, which characterizes the early stages of repair and inflammation. Our findings suggest that serum PIIINP reflects the repair processes and scar formation following acute myocardial infarction. The serum PIIINP alterations in acute myocardial infarction differ essentially from the changes in myocardial enzymes reflecting myocardial injury. Serum PIIINP may therefore provide new and clinically relevant information on the healing of myocardial infarction. (Circulation 1990;81:52–57)

Repair processes following acute myocardial infarction (AMI) are believed to be of importance to the postinfarction prognosis. Inadequate healing may lead to ventricular dysfunction, aneurysm formation, or cardiac rupture. Attempts have been made to trace the scar formation, but so far no methods are available for routine clinical application.

Collagen type I and type III are the major fibrillar constituents in developing granulation tissue. In human autopsy studies and experimental investigations in dogs and rats, the invasion of fibroblasts and collagen fibers in the infarction zone has been demonstrated at the second postinfarction day. From the second postinfarction day, the tissue 4-prolyl hydroxylase activity, an indicator of collagen synthesis, and the tissue hydroxyproline concentration, a marker of collagen content, remain increased in the infarction zone. The tissue hydroxyproline concentration in the infarction zone remains above the level of healthy myocardium for 6–10 weeks.

The procollagen type III aminoterminal peptide (PIIINP) is an extension peptide of the procollagen type III (Figure 1). During the conversion of procollagen type III to collagen type III, PIIINP is cleaved off in a stoichiometric fashion and liberated into extracellular fluid. Elevated serum PIIINP may reflect enhanced synthesis and deposition of fibrillar collagen or alteration in degradation and elimination of circulating PIIINP. Within recent years, radioimmunoassays detecting PIIINP-related antigens have been developed. The assays differ with respect to the antigen forms preferentially measured in serum.

During wound healing following abdominal surgery in man and during development of experimentally induced granulation tissue in rats, changes in serum PIIINP appear to reflect the locally enhanced collagen type III production.

The purpose of the present study was to analyze serum PIIINP following AMI. The correlation

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between infarction size and increase in serum PIIINP and the changes in PIIINP antigen profile following AMI were studied. The findings were compared with previously published observations on serum PIIINP in wound healing in man\textsuperscript{15,16} and in experimentally induced granulation tissue in rats.\textsuperscript{17}

Methods

Patients

In this primary study of serum PIIINP and AMI, we wanted a priori to exclude factors except AMI that might possibly affect the serum PIIINP. The exclusion criteria were as follows: symptoms for more than 24 hours, previous AMI within the last 3 months, impaired liver (prothrombin time <0.6) or kidney function (serum creatinine >120 $\mu$mol/l), malignant and metabolic diseases, inflammatory rheumatic diseases, or treatment with glucocorticoids or cytostatic drugs. For a period of 3 months, 64 patients with symptoms of AMI were admitted to the coronary care unit. Of the 27 patients with AMI (World Health Organization criteria of AMI\textsuperscript{19}), 11 were excluded because of symptoms for more than 24 hours (eight patients) or previous AMI within the last 3 months (three patients). The control group included the first 15 patients of the 37 patients suspected to have AMI at admission but who did not comply with the WHO criteria. Thirteen controls had angina, and in two patients no definite diagnosis could be established.

The 16 patients with AMI included in the study were 10 men and six women (aged, 38–85 years; median age, 62 years). The control group consisted of nine men and six women (aged, 40–80 years; median age, 67 years).

The study was performed in accordance with the Helsinki Declaration II. All patients had given their informed consent, and the local ethics committee on human research had approved the study protocol.

Blood Sampling Procedure

Blood samples were drawn immediately on admission and 1, 2, 3, 5, 7, 10, 14, 30, 60, and 90 or 120 days later. In the control group, blood samples were drawn during hospitalization (2–7 days; median, 5 days) according to the protocol. Additional follow-up samples were collected 22–24 months after the AMI.

Measurements of lactate dehydrogenase (LD), creatine kinase (CK), creatinine, and prothrombin time were carried out as routine analyses. Blood samples were collected for subsequent measurements of CK isoenzyme MB (CK-MB) and PIIINP. The blood samples were allowed to clot for 30 minutes at room temperature and then centrifuged for 10 minutes at 1,500g. Aliquots were immediately frozen at $-20^\circ$ C for subsequent analyses.

Biochemical Measurements

Analyses of serum LD and CK were made according to the recommended Scandinavian standards.\textsuperscript{19,20} Serum CK-MB determinations were made by the immunoinhibition method.\textsuperscript{21} The estimate of infarction size was calculated from sequential determinations of serum LD and CK-MB.\textsuperscript{22}

The concentration of intact and high molecular weight PIIINP antigens was determined by an equilibrium type radioimmunoassay\textsuperscript{12} (PIIINP-RIA Kit, Farmos Diagnostica, Oulunsalo, Finland) with normal serum range 2.1–5.1 $\mu$g/l. The assay is insensitive to the smaller PIIINP degradation products. An estimate of PIIINP degradation products was made by a sequential saturation radioimmunoassay\textsuperscript{13} (PIIINP-Fab assay, Hoechst AG, Frankfurt, FRG) with normal serum range 27–78 $\mu$g/l. In this assay, the antibodies have equal affinity to the intact peptide and its degradation product, the Col 1 fragment. The analyses were made as previously described.\textsuperscript{23} Samples to be compared were analyzed in the same setup. The 2-year follow-up samples were analyzed together with samples from the initial setup (days 0–120). No changes were observed in the samples analyzed twice.

The intra-assay variation (2 standard deviations), using a control reference serum, was 8% in the PIIINP-RIA Kit and 9% in the PIIINP-Fab assay.

The molecular weight distribution of PIIINP antigen holding peptides was investigated by gel chromatography in sera from five patients. Samples were applied to a Sephacryl S-300 column (1.6$\times$90 cm), equilibrated in phosphate-buffered saline (0.05% Tween 20, pH 7.2), and eluted at a flow rate of 14 ml/hr. Fractions of 1.9 ml were collected. The column was calibrated with labeled bovine intact PIIINP (Hoechst AG, Frankfurt, FRG), human intact PIIINP, and human Col 1 (kindly provided by Dr. J. Risteli, Oulu, Finland). PIIINP-related antigens in the fractions were determined in a PIIINP radioimmunoassay\textsuperscript{14} (PIIINP RIA-gnost assay, Hoechst AG, Frankfurt, FRG), which has a higher sensitivity than the assays used for the serum measurements. The antibodies in the PIIINP RIA-gnost assay have an affinity for intact propeptide (and larger PIIINP-related antigens) approximately 10 times that of the smaller Col 1 fragment. The fact that this radioimmunoassay detects all PIIINP antigen-holding peptides makes it useful for characterization of total antigen profiles after gel chromatography.
The sensitivity of the three assays to different PIIINP-holding peptides in serum is shown in Figure 2.

**Statistical Methods**

Statistical analyses were made using Pratt matched pairs signed rank test for related two samples and the Mann-Whitney signed rank test for independent two samples. Correlations were calculated using the Spearman rank correlation coefficient. $p$ values less than 0.05 were considered statistically significant. Data are expressed as medians and interquartile ranges.

**Results**

**Serum Aminoterminal Type III Procollagen Peptide**

In the control group, serum PIIINP and serum PIIINP-Fab remained stable throughout the observation period. On admission, no differences in serum PIIINP and serum PIIINP-Fab were observed between the patients with AMI and the controls (Figure 3). In the AMI group, serum PIIINP remained statistically significantly increased from the second postinfarction day. The serum PIIINP reached maximum values at day 3 with individual peaks between days 2 and 7. In the AMI group, no statistically significant changes were observed in serum PIIINP-Fab. At 22–24 months after AMI, serum PIIINP levels were not statistically different from the values on day 0.

The LD infarction size estimation was made on serum samples from days 0 to 10, and the CK-MB infarction size estimation on samples from days 0 to 3. The estimates on infarction size, calculated from serum CK-MB and LD, were significantly correlated (Spearman’s $\rho=0.84$, $p=0.001$).

A significant correlation was observed between the estimated infarction size (CK-MB and LD) and the individual peak changes in serum PIIINP during the first week after the AMI (Figure 4): serum CK-MB infarction size estimate versus peak serum PIIINP increase: Spearman’s $\rho=0.60$, $p=0.02$; serum LD infarction size estimate versus peak serum PIIINP increase: Spearman’s $\rho=0.58$, $p=0.03$.

**Elution Profiles After Gel Chromatography**

Sera from four patients with uncomplicated postinfarction recovery were separated by gel chromatography. The elution profiles at day 0 revealed a normal molecular weight distribution of the PIIINP-related

**Figure 2. Normal PIIINP antigen profile after separation of serum by gel chromatography. Sepharcl S-300 column (1.6×90 cm) equilibrated in phosphate-buffered saline (0.05% Tween 20, pH 7.2), flow rate 14 ml/hr, fraction volume 1.9 ml, 2 ml serum applied. Peaks A and B represent high molecular weight PIIINP-related antigens; peak C corresponds to the intact peptide, and peak D corresponds to the Col 1 fragment. Dashed line, PIIINP-kit (Farmos); dotted line, PIIINP-Fab (Hoechst); solid line, PIIINP RIA ghost (Hoechst); D.L., detection limit.**

**Figure 3. Serum PIIINP (S-PIIINP) in patients with acute myocardial infarction (solid line) and in controls (dashed line). The interquartile ranges and the medians are shown. S-PIIINP at follow-up compared with S-PIIINP on admission (Pratt matched pair signed rank statistics): *$p<0.05$, **$p<0.01$, NS $p=0.05$.**

**Figure 4. Correlation between peak serum PIIINP increase after acute myocardial infarction, maximum change in serum PIIINP ($\Delta$ S-PIIINP), and infarction size calculated from CK-MB. Asterisk indicates a patient who developed an aneurysm of the interventricular septum.**
antigens (Figure 5). A minor peak (A), probably representing pN-collagen type III (collagen type III still attached PIIINP), was eluted, corresponding to the void volume. The second peak from the left (B) represents aggregated intact PIIINP or procollagen degradation products. The minor third peak (C) corresponds to intact PIIINP. The largest peak (D), at the right, corresponds to the Col 1 fragment. At day 3, the high molecular weight fractions and the intact propeptide increased whereas the Col 1 fraction remained unchanged. The pattern at days 10 and 120 appeared similar to that at day 0, even though the content of pN-collagen and the intact PIIINP might be slightly increased at day 120.

No changes were observed in the Col 1 peak (D) throughout the observation period.

**Serum PIIINP During Development of Aneurysm of Interventricular Septum**

A 73-year-old man with a small anterior myocardial infarction (estimated infarction size by CK-MB

64 units/l) revealed the expected changes in serum LD and CK. The peak serum PIIINP (day 2) was higher than expected from the calculated infarction size (Figure 4).

A second increase was observed in the serum PIIINP from day 7 (Figure 6), whereas the serum CK and LD levels remained normal.

At day 20, chest pain reappeared. Serum CK and LD were slightly elevated whereas serum PIIINP was still increased. Like the patients with uncomplicated postinfarction recovery, gel chromatography confirmed that the increase in serum PIIINP was due to increased content of intact and high molecular weight PIIINP-related antigens. The diagnosis of an aneurysm of the interventricular septum was confirmed by echocardiography. The patient died 3 months after the AMI.

**Discussion**

The present study demonstrates a delayed increase in serum PIIINP as compared with serum LD and CK. Serum PIIINP increased on the second postinfarction day, and peak values were observed on the third to fifth day. These levels correspond to an increased activity of tissue prolyl 4-hydroxylase in tissue from the infarction zone on the second day after infarction with maximum activity on the third day.5

Serum PIIINP remained increased for more than 4 months after AMI, suggesting ongoing repair processes. This finding is substantiated by the increase in hydroxyproline concentration in the healing myocardium observed in human and experimental studies.1,6-9

In our prospective study, the time of observation was settled before results were available. Our 4-month observation period turned out to be too short to determine the time when serum PIIINP had leveled off. Consequently, we carried out an additional follow-up 22-24 months after the AMI and found that at that time the serum PIIINP had returned to day 0 level. The increased serum PIIINP values during the post-
infarction period were observed only in the assay detecting intact PIIINP. In the PIIINP-Fab assay no changes occurred, indicating unchanged content of PIIINP degradation products. Chromatography confirmed that the changes were limited to the intact propeptide and the high molecular weight fragments. Identical findings have been demonstrated during wound healing after surgery in humans and during experimental induction of granulation tissue in rats. The antigen pattern and the chronologic alterations in serum PIIINP are consistent with a stimulated synthesis of collagen type III following AMI.

In spite of an extensive collagen degradation in the infarcted myocardium on the first postinfarction day, no changes were observed in serum PIIINP during this period. Consequently, degradation of pN-collagen type III does not contribute to the serum PIIINP increase.

Impaired degradation and elimination of circulating PIIINP-related antigens due to hemodynamic changes after AMI may theoretically contribute to the alterations in serum PIIINP. The intact peptide is extracted in the liver, and the Col I fragment is partially metabolized and eliminated in the kidneys. In the liver, intact peptide is probably degraded by endothelial cells. Decreased liver blood flow due to backward failure may cause impaired degradation of circulating PIIINP and lead to increased serum concentration. However, we did not find any relation between liver enzymes and prothrombin time and between serum creatinine and the changes in serum PIIINP. Furthermore, the increase in serum PIIINP lasting for more than 120 days excludes alterations in the metabolism of circulating PIIINP as the cause of the postinfarction increase of serum PIIINP. On the other hand, this finding is consistent with prolonged scar formation in the myocardium.

The peak serum PIIINP values were significantly correlated to the estimated infarction sizes, based on sequential determination of serum LD and CK-MB. This correlation is in accordance with that between the rate of collagen deposition during the early stage of infarction healing (measured by tissue hydroxyproline content) and the infarction size, as demonstrated by Jugdutt et al. Collagen type III plays a dominant role in early granulation tissue formation (scars).

The patient developing an aneurysm of the interventricular septum had a high peak value of serum PIIINP and a secondary increase in serum PIIINP in spite of normal serum CK and LD concentrations. This observation may be explained by an enhanced healing process following a relative increase of the hemodynamic load on the interventricular septum weakened by infarction. A relative increase of hemodynamic load may provide continuous stimulation of the collagen synthesis, resulting in high serum PIIINP. The mechanism may be similar to the stimulated collagen biosynthesis in penicillamine-induced angiopathy. Another possibility is that the inadequate healing process leads to enhanced spill-over to the circulation of procollagen peptide not deposited in the developing scar.

Our study has demonstrated a long-lasting increase in serum PIIINP following AMI. The antigen profile in serum and the similarity to the changes in serum PIIINP during wound healing indicate that the postinfarction changes reflect collagen formation in the myocardium as part of scar formation. In contrast, changes in serum CK and LD reflect myocardial damage. The peak values of serum PIIINP are correlated to the infarction size.

Serum PIIINP may provide new information of prognostic importance in patients with AMI.

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