Influence of Primary Coronary Intervention on Myocardial Collagen Metabolism and Left Ventricle Remodeling Predicted by Collagen Metabolism Markers

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SUMMARY

The aims of the present study were to analyze cardiac collagen metabolism changes in vivo during acute and nonacute phases of ST elevation myocardial infarction (STEMI) in patients who were treated with primary coronary intervention (PCI) only, and to determine the predictive significance of collagen I and III synthesis markers (PICP, PIIINP) as well as the collagen I degradation marker (ICTP) on left ventricular function and volume changes after STEMI. Serum levels of the carboxy-terminal propeptide of type I procollagen (PICP) and amino-terminal propeptide of type III procollagen (PIIINP) assessed on the 30th day and the carboxy-terminal telopeptide located at the C end of collagen type I (ICTP) assessed on the 7th day after STEMI were significantly higher (P = 0.01, P = 0.019, P = 0.04, respectively) in the PCI unsuccessful group than in the PCI successful group. These findings support the theory that early and successful PCI not only limits the amount of muscle necrosis but also protects cardiac collagen from ischemia-related injury. PICP and PIIINP levels assessed on the fourth day after acute STEMI enables us to predict the development of left ventricular function (EF) and end-diastolic volume changes over the course of 6 months, irrespective of the initial EF or revascularization success. (Int Heart J 2005; 46: 949-959)

Key words: Myocardial infarction, Left ventricle remodeling, Collagen metabolism, Coronary revascularization

The extent and location of a myocardial infarction (MI) together with the process of morphological and protein changes in the extracellular matrix (ECM) determine healing after a myocardial infarction. All of these factors are independent and influence the extent of left ventricular remodelling,1,3 the function of the left ventricle, and the prognosis of a patient after MI. The ECM is composed of

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various types of collagens (major ones are collagen I and III), proteoglycans, and glycoproteins. Collagen type I is synthesized in the form of procollagen with additional sequences of amino acids at the N and C ends of its molecule. The carboxyterminal propeptide of type I procollagen (PICP) is released into the bloodstream during collagen type I synthesis. On the other hand, the carboxyterminal telopeptide located at the C end of the molecule of collagen type I (ICTP) is eliminated from collagen type I during its degradation. Collagen type III is synthesized in the form of its procollagen and N-terminal propeptide (PIIINP) is released into the blood circulation during the conversion into collagen. It seems PIIINP is also released during collagen III degradation; some PIIINP remains bound on the fibres of collagen III even after its synthesis.4)

Nevertheless, the synthesis of collagen III is substantially activated during the cardiac remodeling (eg, myocardial hypertrophy, ischemic myocardium). However, the level of PIIINP can also be influenced by thrombolysis.5) The only possible way to detect \textit{in vivo} the synthesis and degradation of different types of collagens in human heart after MI is by estimation of the levels of their propeptides in serum.

The primary aim of the present study was, therefore, to evaluate the dynamism of collagen metabolism in ischemic myocardium by means of both synthesis and degradation of the collagen markers in ST segment elevation MI (STEMI) patients treated using direct percutaneous coronary intervention (PCI). Another goal was to assess the functional and volume changes of the left ventricle in these patients by means of echocardiography. Furthermore, the biochemical, functional, and volume values were correlated and the predictive significance of cardiac collagen metabolism markers on the left ventricular function and volume changes were estimated in the acute and subacute phases of STEMI.

\textbf{METHODS}

\textbf{Patients and protocol:} Forty-five patients (age, 66 \(\pm\) 8.27) underwent biochemical and functional analysis for cardiac collagen metabolism (groups A, B, and C). Thirty-five had been consecutively admitted to the coronary care unit with their first acute STEMI within 12 hours from the start of symptoms. All 35 of these patients were treated by primary PCI only; PCI was successful in 30 (group A) and unsuccessful (group B) in 5 (TIMI flow O, residual stenosis 100%). The baseline characteristics of groups A and B are presented in Table I. Eighty-six percent of these patients were treated with beta-blockers; 93.3\% received metoprolol (25-100 mg/day), and 6.7\% carvedilol (12.5 mg/day). Treatment was started within 24 hours of admission in most patients. Beta-blockers were administered to 76.2\% of the patients in group I (see below) and 64.3\% of the patients
in group II. The difference was not statistically significant. Group C (control group; \( n = 10 \), age, \( 63 \pm 4.68 \)) consisted of stable patients without a history of MI who only underwent elective diagnostic coronary angiography. STEMI (in groups A + B) was defined as typical chest pain persisting for more than 20 minutes, ST elevation above 0.1 mV in limb leads, and/or above 0.2 mV in precordial leads simultaneously in at least two leads in a 12-lead ECG, and significant increases in both creatine kinase (CK) and creatine kinase - MB fraction (CKMB) or troponin I more than twice the normal upper limit. Exclusion criteria included any disease that could potentially influence the level of the biochemical markers of collagen metabolism (skeletal and articular disease, type I diabetes mellitus, renal insufficiency, liver disease, or a malignant tumor). Blood samples for collagen analysis were obtained immediately after admission (day 1) and on days 2 (for all groups), 4, 7, and 30 (groups A + B) following admission; all samples were centrifuged and the serum was stored at \(-25^\circ C\). CK and CKMB levels were assessed at admission and then after 8, 16, and 24 hours after admission.

**Echocardiography:** The patients were evaluated by means of echocardiography on days 1, 4, and 30, and 6 months after STEMI using an HP Sonos 2500. The function of the left ventricle, expressed by ejection fraction (EF,\%), and the left ventricular end-diastolic volume index (LVEDVi, mL/m\(^2\)) were evaluated using an endocardium border automated detection method. The size of the left ventricle

### Table I. Clinical Characteristics of Groups A and B

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>65 ± 9.96</td>
<td>69 ± 10.16</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>16</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial hypertension, n</td>
<td>13</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>16</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Initial EF, mean ± SD</td>
<td>42 ± 9.1</td>
<td>31 ± 11.03</td>
<td>( P = 0.05 )</td>
</tr>
<tr>
<td>( \Delta \text{EF} \leq 10% / \Delta \text{EF} &gt; 10% ) in 6 months</td>
<td>18</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>TIMI before PCI 0-1 / 2-3, n</td>
<td>21</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>TIMI after PCI 0-1 / 2-3, n</td>
<td>0</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Number of diseased vessels: 1/2/3, n</td>
<td>11</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Anterior/inferior + lateral MI, n</td>
<td>17/13</td>
<td>3/2</td>
<td></td>
</tr>
<tr>
<td>ACE-inhibitors at discharge, n</td>
<td>14</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>( \beta )-Blockers at discharge, n</td>
<td>25</td>
<td>5</td>
<td></td>
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</tbody>
</table>

EF indicates ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; and NS, nonsignificant.

\( P = 0.05, P = 0.02, P = 0.0001 \).
and the development of ventricular aneurysm or mitral insufficiency were also evaluated. In the case of a suboptimal echocardiographic window, EF and volume were evaluated by the area-length method from the apical two-chamber view. The mean of 3 measurements was used and records were stored on videotape.

**Laboratory method:** Frozen serum samples were consequently analyzed for collagen metabolism marker kits; furthermore, both CK and CKMB were assessed. Sample thawing was always performed as a part of measuring the collagen concentration by RIA (ORION kits, Finland, detection of the isotope $^{125}$I) according to the instructions provided by the kit supplier. All measurements were analyzed by someone blinded to the clinical data.

Four different kits were used:

a) collagen synthesis:

1) PINP – N terminal propeptide of type I procollagen
2) PICP – C terminal propeptide of type I procollagen
3) PIIINP – N terminal propeptide of type III procollagen

b) collagen degradation

4) ICTP – C terminal telopeptide of type I collagen

**Statistical analysis:** Data are presented as the mean ± SD or as a percentage where appropriate. ANOVA was used to analyze the time-dependent changes of continuous variables, an unpaired $t$-test for continuous variables between groups, a paired $t$-test for within-group differences, Fisher's exact test for categorical variables, the Mann-Whitney test for independent two samples, and the chi-square test for dichotomous data. Correlations were calculated using the Spearman rank

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**Figure 1.** Comparison of PICP on days 1, 2, 4, 7, and 30 in groups A versus B according to the results of PCI. PICP was significantly higher ($P = 0.01$) on day 30 in group B with failed PCI.

PCI and PICP indicate percutaneous coronary intervention and C-terminal propeptide of type I procollagen, respectively.
correlation coefficient. The composite of major events during follow-up were analysed by the Kaplan-Meier method. The differences between the event-free survival curves were compared using the log-rank test. Values of $P < 0.05$ were considered statistically significant. Values of $P < 0.05$ were considered statistically significant (*$P$), $P < 0.01$ highly statistically significant (****$P$).

The study was conducted after receiving approval from the institutional review (ethical) committee of the 3rd Medical School Charles University issued on 19/May/1999 (reg. No. 134/99) and informed consent from each participant. The presence of heart failure, need for new coronary angiography, new hospitalisation, recurrence of MI, angina pectoris, and death were recorded during a one-year clinical follow-up.

**RESULTS**

**A) Analysis of cardiac collagen metabolism:**

**A1) Changes in PICP and PIIINP in groups A + B (STEMI patients) versus control group - influence of catheterization.**

The level of PICP was already significantly higher in group A + B on day 2 ($A + B = 91.79 \pm 32.85$ and $109.99 \pm 26.6 \mu g/L; P = 0.001$ on the first and the second day, respectively). No such increase was observed in group C ($C = 118 \pm 39.3 \mu g/L$ and $105.63 \pm 24.31 \mu g/L; P = 0.017$ for the first and second day, respectively). Similar results were observed for the serum level of PIIINP (data not shown).

**A2) PICP in groups A, B - effect of revascularisation on collagen I.**

A significant difference in PICP, assessed on day 30, was observed between group A (successful revascularisation, $147.65 \pm 34.46 \mu g/L$) and group B (unsuccessful revascularisation, $211 \pm 66.04 \mu g/L$) ($P = 0.01$) (Figure 1).

The MI patients were divided into two subgroups (subgroup I, $n = 21$: improvement of EF $\leq 10\%$ or worsening; and subgroup II, $n = 12$: improvement of EF $> 10\%$) on the basis of the maximal change in left ventricular EF during the 6-month follow-up. The baseline characteristics of subgroups I and II are shown in Table II. There was a significant increase in serial PICP concentrations between days 1 and 30 in both subgroups as well (subgroup I = $95.65 \pm 43.08 \mu g/L$, subgroup II = $151.03 \pm 32.09 \mu g/L$, for days 1 and 30, respectively, subgroup II = $84.51 \pm 14.06 \mu g/L$, $138.56 \pm 30.45 \mu g/L; P = 0.005$ for both subgroups). PICP levels on day 2 ($116.76 \pm 29.67$ and $95.46 \pm 19.61 \mu g/L$ for subgroups I and II, respectively, $P = 0.005$), on day 4 ($137.49 \pm 49.16$ and $93.65 \pm 14.46 \mu g/L, P = 0.02$) and on day 7 ($151.03 \pm 57.39$ and $138.56 \pm 30.45 \mu g/L, P = 0.01$) were significantly lower in patients whose EF improved more than 10% (subgroup II) compared to those whose did not (subgroup I). A significantly higher ($P = 0.01$)
incidence of PICP on day 4 (PICP4) above 110 µg/L was observed in subgroup I. This finding was independent of initial EF. The increase in LVEDVi during 6 month follow-up was significantly higher in the group with PICP4 > 110 µg/L than in the group with PICP4 ≤ 110 µg/L (ΔLVEDVi 8.93 ± 1.68 mL/m² versus 3.96 ± 1.01 mL/m²; P = 0.02). Furthermore, PICP4 > 110 µg/L on day 4 showed a sensitivity of 86% (specificity 64%) with respect to identifying patients of subgroup I, regardless of the initial EF. There were no significant differences between patients with anterior MI and MI in other locations with respect to the changes in PICP levels on days 1 to 30 (data not shown).

A3) PIIINP in groups A, B - effect of revascularisation on collagen III.

The time-dependent increase in serial PIIINP concentrations from day 1 to day 30 was statistically significant (A + B = 3.54 ± 1.39 and 4.84 ± 2.67 µg/L; P = 0.001 for day 1 and day 30, respectively). PIIINP levels on day 4 (PIIINP4) were significantly lower in patients in subgroup II compared to patients in subgroup I (see above), regardless of the initial EF (4.22 ± 1.68 versus 3.17 ± 1.17 µg/L, P = 0.002). A significantly higher (P = 0.002) occurrence of PIIINP4 above 4 µg/L was observed in subgroup I. This finding was independent of initial EF (data not shown). Furthermore, PIIINP > 4 µg/L on day 4 showed high sensitivity (100%), but specificity of 57% to identify patients of subgroup I.

The probability of predicting an EF improvement of less than 10% (subgroup I) during follow-up was increased by the combination of PICP > 110 µg/L and PIIINP > 4.0 µg/L both assessed on day 4. The sensitivity was 83% and the specificity 75% to identify these patients.

The increase in LVEDVi during the 6 month follow-up was significantly higher (Figure 2A) in the group of patients with PIIINP4 > 4.0 µg/L than in the group with PIIINP4 ≤ 4.0 µg/L (ΔLVEDVi 12.84 ± 1.23 mL/m² versus 4.67 ± 0.95 mL/m²; P = 0.0001).

A4) Relationship between the changes in PICP concentration and CKMB max level.

There was no significant correlation between the levels of PICP on days 1, 4, or 30 and CKMB maximal levels, although a significant correlation was observed for day 7 (P = 0.01, Spearman's correlation coefficient r = 0.45, data not shown).

A5) Effect of ACE inhibitors on cardiac collagen metabolism and left ventricle remodeling.

Seventeen study patients (49%) were treated with ACE inhibitors (ACE-i): 7 (41.2%) of whom received perindopril (2-4 mg/day), 8 (47.1%) ramipril (1.25-5 mg/day), and 2 (11.7%) trandolapril (2 mg/day). ACE-i treatment was started between days 1 and 4 in all patients. There was no statistically significant difference between subgroups I and II (see above) with respect to the number of
patients treated with ACE-i. Eight patients (78.6%) in group II had a basal EF above 40% so there was no absolute indication for ACE-i treatment in the years the study was conducted.

The mean level of ICTP on day 30 only was significantly higher in MI patients treated with ACE-i as compared to patients without ACE-i [mean ICTP on day 30 was 5.91 ± 0.88 in the ACE-i (+) group versus 3.93 ± 0.37 in the ACE-i (-) group (P = 0.043)]. The mean levels of PICP and PIINP on days 7 and 30 were not significantly different in MI patients treated with ACE-i as compared to patients without ACE-i.

Figure 2. A: Comparison of the maximal increase in left ventricular end-diastolic volume index during 6 month follow-up and PIINP level on day 4 (PIINP4) ** P = 0.0001. B: Comparison of the maximal increase in left ventricular end-diastolic volume index (LVEDVi) during 6 month follow-up between groups without and with ACE inhibitor (ACE-i) treatment. *P = 0.039. PIINP indicates N-terminal propeptide of type III procollagen.
We observed a significantly lower progression of EDVi during the 6 month follow-up in MI patients treated with ACE-i than in the non-ACE-i group (mean delta EDVi was 4.04 ± 1.00 versus 7.93 ± 1.52; *P* = 0.039) (Figure 2B). The change in the left ventricle EF during the 6 months after MI was not significantly different between the groups with and without ACE inhibitor treatment.

**A6) ICTP in groups A,B - effect of revascularisation on collagen I degradation.**

A significant difference between group A (successful revascularisation) and group B (unsuccessful revascularisation) with respect to ICTP assessed on day 7 (A = 5.38 ± 2.70 and B = 8.78 ± 3.27 µg/L; *P* = 0.04 for day 7) was observed (Figure 3). There was a significant difference in the concentration of ICTP between subgroups I and II on days 4 and 7 (data not shown).

**B) Clinical outcome:** During the one-year follow-up there were 4 deaths in group A and no deaths in group B. Two deaths in group A were cardiac (one patient died on the 2nd day after admission of cardiogenic shock and the other died one month after MI due to heart failure) and the other 2 were noncardiac (lung tumor and stroke after 6 months of follow-up).

An initial EF lower than 45% was found in 18 patients (60%) in group A and 5 patients (100%) in group B. During a 6-month follow-up period, 11 patients in group A and only 1 patient in group B (this patient underwent bypass surgery during follow-up) had an improvement in their EF of more than 10%. The small amount of noted clinical events (new MI, heart failure, death) in both groups did not differ significantly.
not allow us to evaluate the predictive value of the changes in serum concentrations of collagen metabolism markers on these events.

**DISCUSSION**

Protein remodelling of both ECM and myofibrillar compartments has been observed in ischemic myocardium. However, these data were predominantly derived from the myocardium of animals after ligation of coronary arteries. Much less is known about the protein profile of human myocardium in patients after MI. Until now the results have been derived mainly from myocardium in sectioned material. It is generally known that biochemical analysis of troponin levels or CKMB activity is very sensitive for detecting myocardial necroses after MI, but insensitive for predicting changes in cardiac function and left ventricular remodeling after MI. Therefore, the main aim of this study was to identify *in vivo* markers reflecting MI healing and remodeling of the left ventricle during both acute and nonacute periods of MI in STEMI patients. Markers of both collagen degradation and synthesis have been used for this purpose. We have shown that changes in collagen metabolism were detected very early after MI. Increases in collagen I and collagen III synthesis were observed within the first 48 hours after MI. This process might persist even 1 month after MI; however, collagen synthesis was much less pronounced in patients after successful PCI compared to patients where this treatment was unsuccessful. Therefore, unsuccessful PCI may significantly elevate the concentration of collagen in the human myocardium and accelerate remodeling of cardiac ECM after MI.

It has been shown that ACE inhibitors influence ventricular remodeling not only due to their effects on pre- and afterload reduction, but also due to their interference with the increase in DNA synthesis of fibroblasts as normal adaptive responses of the heart to the loss of muscle. The result of this interference is a decrease in the amount of collagen in noninfarcted myocardium after ACE-i treatment, as well as a probable effect of ACE-i on scar collagen. Our study was not primarily designed to assess the influence of ACE inhibitors on cardiac collagen metabolism, therefore, we retrospectively evaluated their influence on cardiac collagen metabolism. Our findings suggest that the manner in which ACE inhibitors may have decreased the amount of collagen in noninfarcted and infarcted myocardium could also be an increase in collagen degradation.

It seems that biochemical remodeling of ECM induced by ischemia is more complex. The correlation of biochemical data (PICP, PIIINP) with functional (EF) and volume changes of the left ventricle after STEMI yields some predictive values; absolute values of both PICP (marker of collagen I synthesis) and PIIINP (marker of collagen III synthesis) on the fourth day after MI have met this
requirement. The extent of EF improvement in subgroup I and II patients (up to 10% and above 10%, respectively) was impossible to explain based on the differences in basic characteristics (Table II) and thus the predictive values of PICP4 and PIIINP4 are even higher. We have proved that heart catheterization itself does not affect PICP and PIIINP levels. It seems that the markers of collagen metabolism could expand the group of biochemical markers assessing the prognosis of the MI patients. Further studies are required to determine which of the collagen metabolism markers should be incorporated into the routine management of patients with MI.

**Conclusion:** Unsuccessful coronary revascularization leads to significantly higher collagen synthesis and degradation in the myocardium of patients with acute MI. Cardiac catheterization itself does not affect collagen metabolism. The analysis of collagen metabolism markers in blood enables us to predict remodeling of the left ventricle after MI in vivo. The PICP and PIIINP serum levels on the fourth day after MI predict the changes in left ventricular function and end-diastolic volume in the 6 month period following STEMI, irrespective of the initial EF or revascularization success.

### Table II. Clinical Characteristics of Two Subgroups Divided on the Basis of Maximal Increase in LV Ejection Fraction During 6-Month Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Subgroup I: ∆EF ≤ 10%</th>
<th>Subgroup II: ∆EF &gt; 10%</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>66 ± 11.06</td>
<td>65 ± 8.55</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, M/F, n</td>
<td>10 11</td>
<td>9 5</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial hypertension, n</td>
<td>9</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Nicotine abuse, n</td>
<td>12 5</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Initial EF, mean ± SD (%)</td>
<td>41 ± 11.3</td>
<td>41 ± 6.6</td>
<td>NS</td>
</tr>
<tr>
<td>∆EF / 6 month (%)</td>
<td>4.5 ± 0.83</td>
<td>17.46 ± 1.66</td>
<td><strong>P = 0.0001</strong></td>
</tr>
<tr>
<td>∆LVEDVi / 6 month (mL/m²)</td>
<td>7.89 ± 1.44</td>
<td>3.34 ± 0.78</td>
<td><strong>P = 0.03</strong></td>
</tr>
<tr>
<td>Time pain - PCI ≤ 6hrs / &gt; 6 hrs, n</td>
<td>11 10</td>
<td>8 6</td>
<td>NS</td>
</tr>
<tr>
<td>TIMI before PCI 0-1/ 2-3, n</td>
<td>15 6</td>
<td>10 4</td>
<td>NS</td>
</tr>
<tr>
<td>TIMI after PCI 0-1/ 2-3, n</td>
<td>4 17</td>
<td>1 13</td>
<td>NS</td>
</tr>
<tr>
<td>Number of diseased vessels: 1/2/3, n</td>
<td>5 6 9</td>
<td>6 2 5</td>
<td>NS</td>
</tr>
<tr>
<td>Anterior MI, n</td>
<td>9 10</td>
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<td>NS</td>
</tr>
<tr>
<td>Inferior, lateral MI, n</td>
<td>11 5</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>ACE inhibitors at discharge, n</td>
<td>12 2</td>
<td></td>
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</tr>
<tr>
<td>β-Blockers at discharge, n</td>
<td>16 9</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

EF indicates ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; LVEDVi, left ventricular end-diastolic volume index; and NS, not significant. Significance: **P = 0.03, P = 0.0001**
ACKNOWLEDGEMENTS

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REFERENCES