Thrombolytic Therapy With Streptokinase Stimulates Collagen Breakdown

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Background. Plasmin is capable of degrading extracellular matrix components such as collagen in vitro. To evaluate the significance of this for in vivo conditions, we set out to study the effect of streptokinase, which acts by converting plasminogen to plasmin, on the serum concentrations of the amino-terminal propeptide of type III procollagen (PIIINP) and the carboxy-terminal propeptide of type I procollagen (PICP).

Methods and Results. Twenty-three patients with suspected acute myocardial infarction were included in the study; 17 of them received thrombolytic therapy, and six were treated conservatively. PIIINP and PICP were assayed with radioimmunoassays. Kinetics of creatine kinase–MB release were determined to differentiate reperfusers from nonreperfusers. Composite curves of creatine kinase–MB release were constructed for different patient subgroups. During streptokinase infusion the serum concentrations of PIIINP increased rapidly, with a maximum mean increase of 50% (from 2.2±0.2 to 3.3±0.3 μg/l) in 45 minutes. A similar increase was also observed in two patients who received thrombolytic therapy but did not subsequently develop any myocardial infarction determined on the basis of enzyme release. The relative increase in PIIINP during streptokinase treatment was higher in those acute myocardial infarction patients with probable reperfusion than those with nonprobable reperfusion. Corresponding changes in PIIINP were not seen in the control group. Two days later there was a second increase in serum PIIINP for both patient groups. This change coincided with a similar increase in PICP.

Conclusions. We conclude that streptokinase, probably by activation of plasminogen to plasmin, stimulates the breakdown of type III collagen during thrombolytic therapy. This phenomenon may decrease the risk of rethrombosis of the affected artery if the exposed collagen is responsible for thrombosis formation, but it could also be involved in the development of hemorrhagic complications during thrombolytic therapy. The second increase in PIIINP levels probably indicates type III collagen synthesis of the infarcted area. This investigation represents a pilot study, and more studies on the effects of various thrombolytic agents on interstitial collagen metabolism are obviously needed. (Circulation 1991;83:1969–1975)

Coronary thrombosis is considered the final common pathway in the development of acute transmural myocardial infarction.1 In fact, there is substantial evidence that thrombolytic therapy with streptokinase is beneficial for the treatment of patients with acute myocardial infarction.2 Streptokinase is a nonenzyme protein produced by β-hemolytic streptococci indirectly activating the fibrinolytic system.3 It initially forms a complex with plasminogen, which then undergoes a transition by which the complex becomes a potent plasminogen activator.4 Consequently, plasminogen is activated to plasmin, which then degrades the fibrin clot. Nevertheless, plasmin is an unspecific proteolytic enzyme degrading circulating fibrinogen, the clotting factors V and VIII, and also the components of the extracellular matrix, including fibronectin and laminin.5–6 Moreover, plasmin has been shown to activate latent tissue collagenases capable of degrading interstitial collagens of types I, II, and III7–10 or basement membrane collagen type IV11 in vitro. It is apparently also capable of activating certain endogenous latent activators of procollagenases present in tissue fluids.8

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As parts of procollagen molecules, the carboxy-terminal propeptide of type I procollagen (PICP) and the amino-terminal propeptide of type III procollagen (PIIINP) are liberated during collagen synthesis; therefore, they can be used as markers of this process.12 PICP is considered a pure synthesis marker of type I collagen.13 However, some of the PIIINP in procollagen is not cleaved off during collagen synthesis. These procollagen molecules that still retain PIIINP are located on the surface of type III collagen fibrils,14 where they are relatively susceptible to proteolytic attack. Thus, rapid changes in serum PIIINP levels can be taken to reflect the breakdown of type III collagen, with subsequent liberation of PIIINP into blood, and more gradual changes can be indicative of changes in the synthesis rate.15,16

The main collagens of the arterial wall are types I and III; the latter may be a more potent inducer of platelet aggregation17 and, when exposed to blood elements, may play a specific role in thrombosis formation and the development of acute myocardial infarction. Changes in the structure of vessel wall collagen have been suggested to be responsible for ruptures of arterial aneurysms in certain patients,18 and the role of the subendothelial matrix in the pathophysiology of bleeding complications seen during thrombolytic therapy has been discussed.19

We set out to determine whether the activation of plasminogen to plasmin also stimulates collagen breakdown in vivo. For this purpose, circulating serum concentrations of PICP and PIIINP, which are antigens of interstitial collagens, were monitored in patients with acute myocardial infarction treated with streptokinase. To differentiate the effects of streptokinase and infarction per se, the profiles of these antigens were compared with those seen in patients treated without thrombolysis.

Methods

Patients

Patients with severe, nitroglycerin-resistant chest pain and electrocardiographic changes consistent with acute myocardial infarction were divided into two groups. Group I consisted of six patients who were treated conservatively because of contraindications to thrombolytic therapy (two had a recent history of a bleeding duodenal ulcer, a long delay from the onset of symptoms (one patient), or only ST depression and/or T wave changes in the admission electrocardiogram (three patients). Group II, who received streptokinase, consisted of one patient with only T wave changes in the electrocardiogram and 16 with ST elevations in more than two leads and no contraindications to thrombolytic therapy. No definite time limits were set for the streptokinase treatment. Some characteristics of the two patient groups are presented in Table 1.

Protocol

The patients were treated in the coronary care unit at Oulu University Central Hospital. They all received intravenous nitroglycerin and 250 mg of oral aspirin immediately on arrival. When patients were treated conservatively, one extra cannula was inserted into the cubital vein for repetitive blood sampling. The patients receiving streptokinase had two extra cannulas inserted, one for blood sampling and the other for streptokinase infusion. Streptokinase was always infused contralateral to the arm from which the blood samples were taken.

A total of 1.5 million U streptokinase was infused for 60 minutes into the patients in the thrombolytic treatment group. Any clinical signs of reperfusion (i.e., relief of chest pain, resolution of ischemic ST-T wave changes, and reperfusion arrhythmias) were recorded.

The patients were treated in the coronary care unit until they had stabilized. All drugs, including β-blockers, calcium channel blockers, nitrates, antiplatelet agents, heparin, and antiarrhythmics, were allowed because there are no previous reports of risk (or reduction) of this kind of medication affecting collagen metabolism per se. The medication was adjusted daily according to the hemodynamic status, various infarct-related complications, and contraindications.

Coronary angiography was not performed routinely in the acute setting.

The blood-collecting schedule was the same for both groups of patients. Serum samples (10 ml of blood) for procollagen propeptide assays were taken before treatment at 15-minute intervals for the first hour and then at 1.5, 4, 8, 12, 18, 24, 48, 72, and 96 hours in all and also at 120 hours in some patients. Creatine kinase (10 ml

<table>
<thead>
<tr>
<th>Table 1. Clinical Characteristics of Study Groups</th>
<th>Group I (n=6)</th>
<th>Group II (n=17)</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>60.5±3.6</td>
<td>57.1±2.1</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>5/1</td>
<td>17/0</td>
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<tr>
<td>Time delay from onset of chest pain to start of treatment (hr)</td>
<td>8.9±2.3</td>
<td>4.5±0.7*</td>
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<tr>
<td>Localization of ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Posteroinferior</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical signs†</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>CK washout criteria‡</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Negligible CK washout</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Time for CK-MBmax from onset of chest pain (hr)</td>
<td>20.3±2.0</td>
<td>15.0±1.5</td>
</tr>
<tr>
<td>AMI patients§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MBmax (IU/l)</td>
<td>158±58</td>
<td>280±52</td>
</tr>
<tr>
<td>CK-Max (IU/l)</td>
<td>1,426±427</td>
<td>2,050±290</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM. AMI, acute myocardial infarction; CK, creatine kinase; CK-MB, creatine kinase-MB.

†p<0.02 compared with group I (unpaired Student's t test).

‡Disappearance of chest pain and resolution of electrocardiographic changes or reperfusion arrhythmias.

§Criteria of both Shell et al25 (CKmax<13 hours from onset of chest pain) and Gore et al26 (CKmax<11 hours from start of treatment) fulfilled.

§Two of the patients in group II did not demonstrate CK-MB washout and were considered not to have had myocardial infarction.
blood) was assayed before treatment and at 1.5, 4, 8, 12, 18, 24, 48, 72, and 96 hours.

Electrocardiograms were taken routinely on admission to the hospital, after thrombolytic treatment, and every morning for 5 days thereafter.

**Laboratory Analysis**

Creatine kinase was analyzed in the hospital laboratory by the technique standardized according to Scandinavian requirements. Creatine kinase–MB was determined by electrophoresis. The rate of release of creatine kinase–MB in different patient subgroups was determined by constructing composite curves of its activity, as is described by Shell et al.,

The concentration of PIIINP was analyzed with an equilibrium type of radioimmunoassay based on the human antigen, which was purified from ascitic fluid of cancer patients and iodinated with 125I (Farmos Diagnostica, Oulunsalo, Finland).

Two hundred microliters of serum was used for the assay, which detects the authentic propeptide and other somewhat larger related antigens, but is not sensitive to the smaller degradation products of the propeptide. The benefit of this new assay method compared with older ones is that standards and serum samples give parallel inhibition curves, in which the intra-assay and interassay variations are about 5%. The sensitivity of the PIIINP assay, which was defined as the detectable mass equivalent to twice the mean ± SD of the zero binding value, was 0.2 µg/l. The reference interval for adults, which is based on Finnish blood donors (n=88), is 1.7–4.2 µg/l; there was no difference between women and men.

The concentration of PICP was assayed with a radioimmunoassay established recently in our laboratory. Type I procollagen was isolated from the medium of primary cultures of human skin fibroblasts; the protein was digested with bacterial collagenase, and PICP was purified by lectin-affinity chromatography, gel filtration, and ion-exchange separation on high-performance liquid chromatography. The final radioimmunoassay was established with polyclonal rabbit antibodies. This assay detects only one form of antigen in human serum with the same molecular size possessed by the isolated PICP.

**Results**

**Clinical Course of the Patients**

The average time delay between the onset of symptoms and hospital admission was 8.9 hours for group I and 4.5 hours for group II (p<0.02). Clinical signs of reperfusion were encountered in 10 of the 17 patients receiving streptokinase but not in any of those treated conservatively (Table 1).

All the patients survived and were stabilized during the 5-day period studied, and none needed immediate coronary angiography, angioplasty, or acute coronary bypass surgery.

Two patients receiving streptokinase had normal creatine kinase–MB levels throughout the treatment period, showing that no myocardial infarction occurred.

**Statistical Analysis**

For analysis of serial measurements, we basically used the principles of O’Brien and Shampoo and Matthews et al. Analysis of variance for repeated measurements was used to test the time-dependent differences in the concentrations of the procollagen propeptides. Because of the small number of patients in the two study groups, comparison between the treatments was done by determining the 95% confidence limits for the combined pretreatment values of procollagen propeptides in both patient groups and taking the subsequent values outside these limits to represent significant changes.

One-way analysis of variance followed by an unpaired t test was applied when the maximum changes in the PIIINP levels of various streptokinase-treated patient subgroups were compared with the changes seen in the conventionally treated patient group.

An unpaired t test was used when clinical characteristics between the two study groups were compared.

**FIGURE 1.** Line graph showing creatine kinase–MB (CKMB) release in acute myocardial infarction patients receiving streptokinase with probable reperfusion (▲) and non-probable reperfusion (■) or treated without thrombolysis (●). Upper-normal level for CKMB in our hospital is 25 IU/l. Time delay from onset of symptoms to treatment was 3.0±2.1, 4.9±3.1, and 8.9±5.6 hours (mean±SEM) for ▲, ■, and ● groups, respectively.
No serious bleeding or other complications were seen in the streptokinase group.

Creatine Kinase Washout

Because angiography was not performed in the acute setting, the evaluation of reperfusion must necessarily rely on enzyme washout data. In terms of the creatine kinase–MB washout criteria of Shell et al24 and Gore et al,25 only five patients in the whole streptokinase group had a high probability of reperfusion during streptokinase treatment, despite 10 patients showing clinical signs of reperfusion (Table 1). The rate of creatine kinase–MB release in the different subgroups of the acute myocardial infarction patients was evaluated by constructing composite curves for creatine kinase–MB activity, as was described earlier.24 It is evident that the patients in the conservative treatment group and those in the streptokinase group, in whom reperfusion was unlikely, demonstrated similar patterns of creatine kinase–MB washout. Conversely, the release pattern was different in those patients who were reperfused during the streptokinase treatment (Figure 1).

Amino-Terminal Propeptide of Type III Procollagen

PIIINP concentrations increased rapidly from 2.2±0.22 to 3.3±0.31 μg/l during streptokinase infusion in which the highest concentration was observed after 45 minutes of the treatment. After that the concentrations decreased somewhat, although it remained above the level seen before treatment. There was no correlation between the PIINP change and the maximum creatine kinase value attained (not shown). The PIINP levels increased again after 2–3 days (Figure 2A and Table 3).

There were two patients in the streptokinase treatment group who did not show creatine kinase–MB washout into blood and consequently did not develop myocardial infarction. Five of the 15 acute myocardial infarction patients in this group were considered to demonstrate probable reperfusion of the infarcted area, whereas 10 of them had a low probability for reperfusion (see above). All the subgroups receiving streptokinase developed significant increases in PIINP levels during the first hour of treatment compared with the conservatively treated patient group (Table 2). The maximum increase of 1.4 μg/l in PIINP was seen in the thrombolysis group with probable reperfusion. The percentage increases in the streptokinase group in which reperfusion was probable (89±27%) and the two patient groups of noninfarction with streptokinase treatment (90±3.5%) differed significantly from the percentage increase in the acute myocardial infarction group with nonprobable reperfusion (46±5.0%). However, when testing the absolute increases in the PIINP concentrations between these subgroups, no significant differences were seen (Table 2).

In group 1 (treated without thrombolysis) the PIINP levels remained constant at 2.3–2.6 μg/l for the first 2 days, after which a later increase was observed that was similar to that seen in the streptokinase group (Figure 2B).

Carboxy-Terminal Propeptide of Type I Procollagen

The PICP levels stayed constant at the level of 58–64 and 85–90 μg/l during the first few hours in the control and streptokinase treated groups, respectively. The time-dependent changes in PICP concentrations were similar in both groups. At 8–12 hours, a transient decrease of about 30% was observed. Increased levels of PICP were again noted after 2 days, coinciding with the later increase in PIINP concentrations (Figures 2C and 2D).

Fibrinogen Degradation Products

There was a rapid increase of FDP after streptokinase treatment, and the levels stayed high for 2–3 days from the start of the treatment. In the control group, FDP stayed normal throughout the observation period. The streptokinase-induced increase was much higher in FDP than PIINP (Table 3).

Discussion

The interstitial collagens are synthesized and secreted as larger precursor molecules called procollagens. These molecules contain additional collagen type-specific propeptide sequences at both the N and C-terminal ends. The propeptides are cleaved off from the collagen molecules before they assemble to form fibers. Thus, the amount of the free propeptide stoichiometrically should reflect the amount of collagen molecules deposited. This idea seems to hold for the C-terminal propeptides of interstitial collagens. However, the N-terminal propeptide of collagen type III is not completely cleaved, and many molecules retain the propeptide while already in the fibers. Such molecules are located on the surface of the fibers and seem to limit further growth of the fibril diameter. These molecules are presumably also degraded first when the fiber is dissolved. Thus, the amount of free PIINP is not directly related to the synthesis rate of type III collagen because some of the soluble antigens may be derived from degradation of type III collagens of the fibers.13,15

We hypothesized that streptokinase itself, or more probably plasmin, known to act as unspecific protease and also to possess collagenase activating properties,5–10 could be able to degrade surface molecules of interstitial type III collagen, and thus lead to very rapid changes in PIINP blood levels. To exclude rapid changes in collagen synthesis or disposal rates, PICP (a pure synthesis marker13) was assayed as a control. PIINP and PICP radioimmunoassays resemble each other,20,21 and if unspecific factors are involved they should affect both assays similarly.

Because the PICP levels did not increase here during streptokinase treatment, it is highly improbable that the very rapid increase in PIINP concentrations could be caused by increased synthesis, reduced elimination of the antigen from the blood stream, or any other unspecific factors disturbing the radioim-
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munoassay. Therefore, liberation of the PIIINP from the surface of the tissue collagen fibrils is the most probable explanation for the rapid increase in PIIINP levels during the streptokinase treatment. The facts that serum PIIINP levels increase only 2–3 days after acute myocardial infarction26 and major surgery16 (i.e., during the scar formation process) are in accordance with this idea.

PIIINP levels remained constant for the first 2 days in the patients who did not receive streptokinase, whereas all the streptokinase-treated patients, including the two who did not develop infarctions (Table 2), showed a rapid increase in PIIINP. No correlation between this and the maximum creatine kinase value was attained. These facts strongly suggest that the phenomenon is a streptokinase-mediated process and not related to myocardial infarction per se.

Types I and III collagens are widely distributed among different tissues.27 Not much is known about the transport of connective-tissue metabolites from tissues to the circulation and the contribution of various tissues to this process. However, it has been suggested that the collagen antigens of procollagen types I and III in the blood may be derived mainly from organs such as the liver and from bone marrow, in which the sinusoidal vessels are devoid of any continuous basement membrane and the connective tissue is closer to the blood vessels.15 On the other hand, collagen exposure in the coronary vessel wall is generally acknowledged as the primary event in platelet aggregation and thrombosis formation, and the heart cannot be excluded as a potential source of the increased PIIINP concentrations noted during thrombolysis for acute myocardial infarction.

Shell et al24 studied two groups of patients, one treated conventionally and the other with intracoronary fibrinolysis, and they showed that the peak of creatine kinase–MB activity occurred less than 13 hours after the onset of symptoms in all the patients in whom the infarct-related coronary artery was successfully reperfused. On the other hand, Gore et al25 observed that a peak creatine kinase value was
Results are expressed as mean±SEM. FDP, fibrinogen degradation products; PIIINP, amino-terminal propeptide of type III procollagen. Group I consisted of six patients and group II 17. A semiquantitative assay for FDP was used, and results are presented as percentage distribution at any given time point. Blood levels of FDP under 10 mg/l are considered normal. Reference interval for serum PIIINP is 1.7–4.2 μg/l.20
this collagen have been described, rendering patients liable to ruptured arteries at an early age. Most of the PICP in blood probably originates from bone matrix turnover, which is highly variable between individuals. This process explains the large variations in adult serum PICP concentrations, which are also observed here in terms of differences in the initial levels of PICP between the two groups of patients.

Serum levels of PICP, unlike those of PIIINP, reflect changes only in collagen synthesis. The PICP levels did not increase here during streptokinase infusion, but because a reliable assay for monitoring the breakdown of type I collagen remains undeveloped, it is uncertain whether streptokinase or plasmin can also stimulate type I collagen degradation. PICP levels decreased some hours after the commencement of treatment regardless of the type of treatment received, which might result from the increased blood cortisol levels encountered during acute myocardial infarction because glucocorticoids inhibit both types I and III collagen synthesis.

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References


KEY WORDS • coronary thrombosis • streptokinase • plasmin • fibrinogenolysis • collagen degradation • thrombolysis
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