Assessment of fibrin degradation products during fibrinolytic therapy for acute myocardial infarction

CHARLES W. FRANCIS, M.D., D. GERARD CONNAGHAN, M.B., B.CH., AND VICTOR J. MARDER, M.D.

ABSTRACT In a group of 39 patients who received fibrinolytic therapy for acute myocardial infarction, serum crosslinked fibrin degradation products (XLDP) were quantitated by an enzyme-linked immunosorbent assay (ELISA) using an antibody reactive with a site near the γγ crosslink of fibrin, and characterized by a gel electrophoretic method to distinguish fibrinogen degradation products (FDP) from XLDP. After coronary artery reperfusion, 63 of 81 (69%) serum samples showed XLDP by gel analysis, whereas the incidence of positive samples before reperfusion, 53 of 144 (37%), was significantly less (p < .0001). The first appearance of serum XLDP by gel analysis was most often in the 15 min interval immediately before or after angiographic documentation of reperfusion, and the elapsed treatment time required to produce a positive test was shorter with more intensive treatment regimens. However, the appearance of serum XLDP was not a specific indicator of reperfusion in individual patients, since one or more serum samples was positive in five of eight patients who did not show reperfusion as well as in 27 of 29 patients who did show reperfusion. Furthermore, the concentration of serum XLDP as measured by ELISA showed no significant difference in samples from patients who did or did not have reperfusion or between samples taken before or after reperfusion. There was a close temporal correlation between the first appearance of serum XLDP (gel analysis) and the initial decrease in plasma fibrinogen (systemic lytic state), and the degree of elevation of serum XLDP (ELISA) was also correlated with the intensity of the systemic lytic state. In addition, electrophoretic analysis of pretreatment plasma samples demonstrated crosslinked fibrin polymers that disappeared during fibrinolytic therapy coincident with the appearance of serum XLDP and in parallel with fibrinogen conversion to degradation products (fragments X, Y, and D). Two patients without a lytic state showed no change in plasma fibrin polymers during therapy, and XLDP were not present in serum despite coronary reperfusion in one patient. Thus the results indicate that XLDP appearing in the blood during fibrinolytic therapy for acute myocardial infarction are not predictive of successful fibrinolytic therapy, but rather may reflect degradation of circulating fibrin polymers associated with the fibrinogenolysis of the systemic lytic state.


THE GOAL of fibrinolytic therapy is the dissolution of occluding thrombi in order to restore vascular patency and reperfusion of ischemic tissue. The molecular mechanism by which this is achieved is the binding of plasminogen activator to the fibrin matrix of the thrombus, conversion of bound plasminogen to plasmin, and proteolytic degradation of the fibrin. Since the degradation products of crosslinked fibrin (XLDP) retain factor XIII-mediated crosslinks between γ chains, they reach macromolecular size and their identification in the circulation during fibrinolytic therapy could be theoretically used as an indicator of thrombus dissolution. At the same time, fibrinolytic therapy with streptokinase or urokinase, tissue-type plasminogen activator (t-PA), or acylated plasminogen:streptokinase activator complex (APSAC) is regularly accompanied by hypofibrinogenemia and circulating fibrinogen degradation products. Such derivatives of fibrinogen are not crosslinked by factor XIII and are all smaller than fibrinogen, namely fragments X, Y, D, and E.

Methods in clinical use for measuring serum fibrinogen and/or fibrin degradation products (FDP), such as the tanned red cell hemagglutination inhibition im-
munoassay,\(^\text{14}\) latex agglutination test,\(^\text{15, 16}\) or staphylococcal clumping test,\(^\text{17, 18}\) identify nonclottable derivatives of both fibrinogen and crosslinked fibrin and therefore are not useful in distinguishing thrombolysis (fibrinolysis) from the lytic state (fibrinogenolysis). However, the structural and immunologic differences between XLDP and FDP have been exploited in developing methods for their specific identifications.\(^\text{19-26}\)

Thus the potential exists for laboratory evaluations that could detect the presence of small quantities of XLDP in blood even with the concomitant presence of large quantities of FDP.

In this study, we have applied two such techniques to measure the XLDP in blood samples from patients who received fibrinolytic therapy with either streptokinase or APSAC for acute myocardial infarction. An enzyme-linked immunosorbent assay (ELISA) utilizing a monoclonal antibody that recognizes an epitope near the \(\gamma\) chain crosslink site quantitates the total concentration of \(\gamma\) chain crosslinked derivatives of fibrin in blood.\(^\text{23}\) A gel electrophoretic method\(^\text{26}\) identifies the type of fibrinogen or fibrin derivatives in each sample, distinguishing specific fragments of fibrinogen from those of crosslinked fibrin and allows comparison of changes occurring in both plasma and serum. The changing presence of these specific derivatives before, during, and after fibrinolytic therapy is correlated with the results of serial coronary angiography and plasma fibrinogen concentration to determine the probable source of XLDP found in the blood.

**Methods**

**Blood samples.** Blood was collected before therapy and at 15 min intervals during catheterization from the femoral artery catheter (after discarding the first 10 ml) or from a peripheral vein into sodium citrate (0.4\% final concentration) and \(\alpha\)-amino caproic acid (EACA) (0.1M final concentration). Plasma was separated from red cells by centrifugation of blood at 3500 g for 15 min at 4\(^\circ\) C. To prepare serum, blood was collected into thrombin (10 U/ml final concentration) and EACA (0.1M final concentration) and allowed to clot for 1 hour at 37\(^\circ\) C, then centrifuged as for plasma. To determine whether the EACA was effective in inhibiting proteolysis during serum preparation, streptokinase at concentrations up to 500 U/ml was added to blood containing 0.1M EACA and serum was prepared. Gel analysis of these samples showed no XLDP and no FDP corresponding to fragments X, Y, or D. Fibrinogen concentration was determined as clottable protein by the method of Ratnoff and Menzie.\(^\text{27}\) A “systemic lytic state” was defined as a decrease in plasma fibrinogen of 10\% or more. Plasma and serum were stored at \(-20^\circ\) C prior to testing. Plasma and serum samples were obtained from patients with acute myocardial infarction who were treated with fibrinolytic therapy; the selection criteria, details of treatment, and clinical results have been previously published.\(^\text{12, 28}\) All patients underwent cardiac catheterization and angiography, and fibrinolytic therapy was instituted in those with complete occlusion of the appropriate artery. Fibrinolytic therapy consisted of intracoronary streptokinase in low or standard dose\(^\text{29}\) or intravenous APSAC followed by intracoronary streptokinase as needed.\(^\text{12}\) Coronary angiography was performed every 15 min until transfer from the catheterization laboratory and at 24 hr.

**ELISA.** Serum XLDP were measured with an ELISA method (Dinertest, American Diagnostica, Greenwich, CT). A monoclonal antibody (DD/3B6) reactive with a site near the factor XIIIa-mediated \(\gamma\) chain crosslink was bound to wells of a microtiter plate (Immulon II, Dynatech Laboratories, Alexandria, VA). After washing with phosphate-buffered saline containing polysorbate 20 (Tween 20), dilutions of serum or standards were incubated for 1 hr at 25\(^\circ\) C and washing was then repeated. An enzyme-linked second monoclonal antibody (DD/4D2) reactive with fibrinogen and its core domains was then added to the wells and incubated for 1 hr at 25\(^\circ\) C and the plate was washed. The substrate was added to the wells, color developed for 20 min, the reaction stopped with sodium fluoride, and the optical density measured with a microtiter plate reader (Titretek Multiscan, Flow Laboratories, McLean, VA). Concentrations were determined by comparison with a standard curve relating the logarithm of the concentration of standard absorbency. The purified standard provided by the manufacturer showed bands migrating as DD and E by gel electrophoresis. The assay was sensitive to 10 ng/ml and was not reactive with fibrinogen at up to 1 mg/ml.

**Electrophoretic analysis.** Fibrinogen and fibrin derivatives were identified in plasma or serum after sodium dodecyl sulfate (SDS) 2\% agarose electrophoresis.\(^\text{26}\) Plasma or serum was prepared for electrophoresis by diluting 1:20 in 0.01M phosphate buffer, pH 7, containing 1.7\% SDS and incubating for 1 hr or at 100\(^\circ\) C for 5 min. Samples of 10 \(\mu l\) were electrophoresed at 150 volts on a flatbed electrophoretic apparatus (Pharmacia Fine Chemicals, Piscataway, NJ) with cooling to 10\(^\circ\) C. Electrophoresis proceeded until the tracking dye (bromphenol blue) had migrated 10 cm, after which the gels were fixed, washed, and overlayered with \(\text{\textsuperscript{125I}-labeled antifibrinogen immunoglobulin G, then washed and dried for autoradiography. Gels were interpret-}

**Results**

A total of 225 blood samples were tested, with an average of 6.1 per patient. The patients studied included 30 men and seven women with a mean age of 56.6 years and a ratio of diaphragmatic to anterior infarction of 25:12 (table 1). The mean interval from the onset of pain to start of therapy was 247 min, and the longest interval was 490 min. Treatment regimens included 17 of intracoronary streptokinase at various dosages, nine of 30 mg of intravenous APSAC, and 11 of intravenous APSAC followed by intracoronary streptokinase. Reperfusion was documented angiographically in 29 of 37 patients after a mean interval of 53 min.

The electrophoretic and ELISA results for a single patient are shown in figure 1. No electrophoretically identifiable fibrinogen or fibrin derivatives were found.
TABLE 1
Clinical characteristics, type of fibrinolytic therapy, and treatment outcome

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Sex (male/female)</td>
<td>30/7</td>
<td></td>
</tr>
<tr>
<td>Age (yr, mean ± SD)</td>
<td>56.6 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>Location of infarction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragmatic/anterior</td>
<td>25/12</td>
<td></td>
</tr>
<tr>
<td>Duration from onset to therapy (min, mean ± SE)</td>
<td>247 ± 82</td>
<td></td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracoronary streptokinaseA</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Intravenous APSACB</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Intravenous APSAC plus intracoronary streptokinaseC</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Angiographic result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reperfusion/no reperfusion</td>
<td>29/8</td>
<td></td>
</tr>
<tr>
<td>Duration from onset of therapy to reperfusion (min, mean ± SD)</td>
<td>53 ± 34</td>
<td></td>
</tr>
<tr>
<td>Samples per patient (mean ± SD)</td>
<td>6.1 ± 1.9</td>
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</table>

*Total dose 32,000 to 534,000 U over 67 to 130 min.

B30 mg as a 5 min intravenous bolus.

CInitial therapy with a 5 min intravenous bolus of APSAC, 5 to 30 mg, followed after 16 to 64 min by intracoronary streptokinase at a total dose of 40,000 to 194,000 U over 6 to 56 min.

In the serum before treatment. After 12 min of therapy, bands corresponding to both FDP and XLDP were present and these increased in intensity throughout the course of therapy. A systemic lytic state was evident by the time of the 12 min sample with a fall of plasma fibrinogen to 49% of original concentration, coincident with the appearance in serum of fibrinogen fragments X, Y, and D. Reperfusion was documented by angiography at 90 min. The concentration of XLDP measured by ELISA was below the sensitivity of the assay in the pretreatment sample, increased to a peak of 1220 ng/ml at 40 min, then decreased to between 400 and 700 ng/ml until the last sample at 180 min. In this patient both the ELISA and gel analysis indicated a rise in serum XLDP, although the results with the two methods were somewhat discrepant, since the gel was most strongly positive for XLDP when the concentration by ELISA was decreasing.

To clarify the relationship between the finding of XLDP by gel analysis and the concentration determined by ELISA, the results on all samples were compared. In general, the ELISA results paralleled those obtained by gel analysis (figure 2). The mean concentration in samples with a positive gel for XLDP was 15,003 ± 2057 (SE) ng/ml, significantly higher than the level in the samples with a negative gel, 403 ± 74 ng/ml (p < .001). The frequency of positive gels increased progressively in samples with higher XLDP concentrations, from 23% below 100 ng/ml to 100% above 10,000 ng/ml. Only seven of 84 (8.3%) samples with XLDP concentrations greater than 1000 ng/ml were associated with a negative gel.

Electrophoretic analysis identified XLDP in at least one serum sample from 27 of 29 patients who achieved reperfusion, but also in five of eight without reperfusion (table 2), indicating that the appearance of serum XLDP was not a specific predictive marker of reperfusion in an individual patient. However, the proportion of samples that were positive after the start of therapy (not shown) in patients with reperfusion was 95 of 146 (65%), significantly higher than that in patients who did not show reperfusion, 23 of 50 (46%) (p < .05). The appearance of XLDP was further correlated with coronary clot lysis, in that XLDP were identified in 53 of 144 (37%) samples taken before reperfusion or in patients never achieving reperfusion, but in 63 of 91 (69%) samples taken after reperfusion (p < .0001) (table 2).

In patients who achieved reperfusion, the time of initial gel identification of XLDP correlated closely with the timing of vascular reperfusion (figure 3). During the 15 min interval immediately before the angiogram documenting reperfusion, 10 of 26 (38%) of patients showed XLDP for the first time, and 12 (46%) showed the new appearance of serum XLDP in the 15 min intervals before and after clot lysis. In nine patients (35%), XLDP appeared more than 15 min before reperfusion and five patients (19%) had the first appearance of serum XLDP more than 15 min after reperfusion.

Similarly, there was a temporal relationship between the elevation of XLDP measured by ELISA and angiographic analysis. Figure 4 shows a rapid rise to a peak concentration at 30 min before lysis that was maintained through the time of reperfusion, followed by a decline to concentrations below 1000 ng/ml by 50 min after reperfusion. However, serum XLDP concentrations did not distinguish between patients who did or did not achieve reperfusion (table 3). In the 29 patients who achieved reperfusion, the mean serum concentration in samples taken after lysis was 10,372 ng/ml, which was not significantly higher than the level before reperfusion, 7370 ng/ml. These concentrations were similar to the mean in samples from patients who did not develop reperfusion, 8422 ng/ml. Also, the mean peak serum concentrations during therapy were not significantly different in patients with or without reperfusion, indicating that the results obtained by this test do not serve as a useful marker to specifically indicate coronary artery reperfusion.

During therapy, the proportion of patients showing one or more positive gel analyses for XLDP increased
FIGURE 1. Electrophoretic analysis and ELISA in serum samples from a single patient. The patient was treated with intracoronary streptokinase as a 10,000 U bolus followed by a continuous infusion of 4000 U/min for 131 min; reperfusion was initially demonstrated at 90 min. Electrophoresis was performed in SDS 2% agarose gels, toward the anode (bottom). Samples of plasmic digests of fibrinogen and crosslinked fibrin were run concurrently and bands corresponding to specific fragments are labeled.

progressively so that 50% of patients had a positive test by 40 min and 86% were positive at 120 min (figure 5). Five patients did not show XLDP at any time during the course of therapy, and a single patient had a positive gel analysis before therapy. The rate of appearance of XLDP differed in the 14 patients receiving 30 mg of APSAC compared to those receiving intracoronary streptokinase or a lower dose of APSAC. By 45 min all 14 patients receiving 30 mg of APSAC had identifiable serum XLDP, while the cumulative percentage of patients with a positive gel analysis who received other

TABLE 2
Identification of XLDP by gel analysis in relation to reperfusion

<table>
<thead>
<tr>
<th>XLD concentration (ng/ml)</th>
<th>Present</th>
<th>Absent</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1160</td>
<td>1220</td>
</tr>
<tr>
<td>5</td>
<td>690</td>
<td>465</td>
</tr>
<tr>
<td>10</td>
<td>480</td>
<td>680</td>
</tr>
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</table>

FIGURE 2. Serum concentrations of XLDP by ELISA in samples that are positive or negative by gel analysis. Values noted at the right indicate the percentage of samples within each bracketed concentration range that was positive for XLDP by electrophoretic gel analysis.

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therapy increased more slowly, reaching a maximum of 76% at 120 min. Further evidence of the influence of the amount of fibrinolytic therapy on the elevation of serum XLDP was shown in the analysis of peak concentrations measured by ELISA (figure 6). Patients treated with low-dose intracoronary streptokinase (200 to 400 U/min) had the least elevation of XLDP, with only 13% of patients achieving a serum concentration greater than 1000 ng/ml. This degree of elevation was found in 92% of patients treated with 30 mg of APSAC and in 63% of patients treated with intermediate-intensity regimens. No patient receiving low-dose intracoronary streptokinase therapy had XLDP concentrations above 10,000 ng/ml, whereas 42% of patients receiving 30 mg of APSAC and 13% of patients receiving other regimens developed serum concentrations in excess of 50,000 ng/ml in at least one sample during therapy.

Comparison of the gel electrophoretic results on plasma and serum samples obtained concurrently allowed correlation between the degradation of plasma fibrinogen and fibrin polymers and the appearance of FDP and XLDP in serum (figures 7 and 8). For example, the pretreatment serum from patient A (figure 7) showed no FDP and XLDP, while the pretreatment plasma showed a predominant fibrinogen ("monomer") band, bands corresponding to fibrin dimer, trimer, and quadramer, and trace amounts of FDP (fragments Y and D) migrating further than fibrinogen. At 37 min after reperfusion, the plasma fibrinogen had fallen 24% of the baseline concentration and the plasma sample at this time showed heavy bands corresponding to fragments Y and D. The fibrinogen band was little changed, although some conversion to fragment X with a slightly faster mobility may have occurred. In addition, the intensity of the dimer band was markedly decreased, and bands were noted in positions consistent with crosslinked fibrin derivatives XX, XY, and XD. The nonclottable derivatives in the serum sample confirmed the presence of fragments X, Y, and D (FDP) as well as the higher molecular weight of XLDP (XD, XY, XX) noted in the plasma.

Patient B did not achieve reperfusion of the coronary artery. The electrophoretic patterns of plasma and serum, however, were very similar to those for patient A, who did achieve reperfusion. The pretreatment plasma showed fibrinogen, and fibrin dimer and trimer bands, all of which disappeared during therapy, at the same time that the fibrinogen concentration dropped. The serum showed new bands of both FDP and XLDP during therapy. Therefore similar changes in plasma and serum may occur in patients who develop the lytic state, namely reduction of fibrinogen, fibrin dimer and trimer, and appearance of both FDP and XLDP, whether reperfusion occurs (A) or not (B).

By contrast, XLDP did not appear in the serum of some patients who did not develop a lytic state regardless of whether reperfusion occurred (figure 8). The pretreatment plasma sample from patient C showed fibrinogen and fibrin polymer bands that were un-
changed after reperfusion, and neither FDP nor XLDP were detected in serum (not shown). The plasma fibrinogen was 97% of initial after reperfusion, indicating that a systemic lytic state had not developed and the absence of XLDP was confirmed by ELISA (320 ng/ml). Patient D, who also failed to develop the lytic state, had similar electrophoretic results, even though reperfusion did not occur. Fibrinogen and fibrin polymers were present before treatment, and also in the plasma sample taken 81 min after the start of therapy ("during therapy") at which time the plasma fibrinogen was 98% of original concentration. No FDP or XLDP were detected in the plasma (figure 8) or serum (not shown) samples, and the serum concentration of XLDP by ELISA was not elevated (260 ng/ml). Thus the gels for patients A and B demonstrate that in the presence of a systemic lytic state, both fibrinogen and fibrin polymer bands are degraded and serum FDP and XLDP appear. On the other hand, two patients who did not develop hypofibrinogenemia and the systemic lytic state showed no loss of fibrinogen or fibrin polymers from the plasma and no appearance of FDP or XLDP in the serum, despite reperfusion in one of these (patient C).

The association between the appearance of XLDP by gel analysis and the development of a systemic lytic state is reinforced by the temporal correlation between the first appearance of XLDP on gels and the time of decrease in plasma fibrinogen, indicative of the lytic state (figure 9). Among the 31 patients in whom this correlation could be made, 19 (61%) had the first appearance of fibrin products in the 15 min interval just before documentation of the systemic lytic state, and in three additional patients this occurred within 15 min after the decrease in plasma fibrinogen. Thus a total of 22 of 31 (71%) patients had the first appearance of XLDP by gel analysis within 15 min of first documentation of a decrease in plasma fibrinogen. This association is also demonstrated by the negative correlation between fibrinogen concentration during therapy and the XLDP concentration measured by ELISA (figure 10). Fibrinogen concentrations less than 80% of pretreatment values were associated with progressive elevations of XLDP concentration.

Table 3: Serum concentrations of XLDP in patients who did or did not achieve reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Before therapy</th>
<th>Before reperfusion</th>
<th>After reperfusion</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reperfusion (n = 29)</td>
<td>596 ± 168</td>
<td>7,370 ± 2,280</td>
<td>10,372 ± 1,884</td>
<td>17,738 ± 4,448</td>
</tr>
<tr>
<td>No reperfusion (n = 8)</td>
<td>268 ± 92</td>
<td>8,422 ± 2,727</td>
<td>—</td>
<td>22,062 ± 14,623</td>
</tr>
</tbody>
</table>

*Concentration measured by ELISA in ng/ml (mean ± SE).
Discussion

During fibrinolytic therapy for acute myocardial infarction, XLDP were identified by a gel electrophoretic technique and their appearance was correlated temporarily with coronary reperfusion as demonstrated angiographically (figure 3). In addition, a significantly higher proportion of serum samples contained XLDP after reperfusion than before (table 2). However, the identification of serum XLDP was not a specific marker of clot lysis in individual patients, since five of eight patients without reperfusion also had a positive assay (table 2). In addition, the proportion of patients with a positive test increased progressively during therapy regardless of reperfusion outcome (figure 5) and some patients with documented reperfusion showed neither XLDP nor FDP in the blood (figure 8, patient C).

Similar results were obtained for the serum concentration of XLDP, as measured by ELISA using a

![Figure 7](image-url)

**FIGURE 7.** Electrophoretic results in two patients who developed a systemic lytic state. Coronary artery reperfusion occurred in patient A but not in patient B. Plasma and serum samples were obtained simultaneously at the indicated points before or during therapy.

![Figure 8](image-url)

**FIGURE 8.** Electrophoresis of plasma from two patients who did not develop a systemic lytic state. Coronary artery reperfusion occurred in patient C but not in patient D.

![Figure 9](image-url)

**FIGURE 9.** Appearance of XLDP by gel analysis in relation to the time that a systemic lytic state occurred. Zero time indicates the first development of a systemic lytic state, defined as a decrease in plasma fibrinogen concentration below 90% of initial. Patients not included in the analysis include two in whom a systemic lytic state did not develop (see figure 8) and three in whom a systemic lytic state developed but no XLDP were detected. In these latter three patients, the decrease in fibrinogen appeared in only the final sample, to 77%, 84% and 68% of original.
monoclonal antibody directed toward a site at or near the γγ isopeptide crosslink.\textsuperscript{25,29} Values increased markedly during fibrinolytic therapy (table 3), as reported previously by Hunt et al.\textsuperscript{31} with peak levels achieved in the 30 min period preceding clot lysis (figure 4). However, as with the gel findings, results were not different in patients who did or did not show lysis, nor were levels higher in samples obtained after reperfusion compared with those obtained before (table 3). Thus the serum concentration of XLDP as measured by ELISA also was not a useful indicator of coronary clot lysis. Just as was noted for the gel analyses (figure 5), the degree of elevation of the serum concentration by ELISA appeared to correlate with the intensity of fibrinolytic therapy (figure 6), as reflected also by a strong negative correlation with plasma fibrinogen concentration (figure 10).

Nonspecificity of the gel and ELISA tests in identifying and measuring XLDP is an unlikely explanation for their failure to accurately reflect coronary clot lysis. By gel analysis, the migration positions of crosslinked fibrin fragments DD and DY overlap with those of fibrinogen fragments Y and X. However, XLDP that are larger than fibrinogen can be identified distinctly.\textsuperscript{30} The ELISA is unreactive with fibrinogen at concentrations as high as 1 mg/ml and is at least 10,000-fold more sensitive to crosslinked fibrin fragment DD than to a mixture of fibrinogen degradation fragments X, Y, D, and E.\textsuperscript{25,29} Although some reactivity with very high concentrations of serum FDP is possible, 10 mg/ml would be required to equal the reaction obtained with a DD level of only 1000 ng/ml. Since the highest pretreatment fibrinogen concentration in our patients was 6.7 mg/ml, such crossreactivity is not a sufficient explanation for the elevated concentrations of serum XLDP.

An alternative explanation for nonspecificity of serum XLDP as a reflection of coronary artery thrombolysis is that incomplete clot lysis, insufficient to result in vascular reperfusion, could have produced an elevated serum XLDP concentration without noticeable change in angiographic appearance. Evaluation of such a possibility would require knowledge of the full size of the clot before and after therapy, and techniques to make this determination are still unavailable. Additionally, fibrinolysis and release of XLDP into the circulation could occur at sites of thrombus formation other than the coronary artery, as in ulcerated atheromatous plaques or endocardial mural thrombi. The arterial catheterization procedure itself is associated with thrombin generation and fibrin formation on the catheter\textsuperscript{32} and lysis of this fibrin could also contribute to elevated XLDP unrelated to coronary artery clot lysis. Formation of extravascular fibrin is a universal accompaniment of inflammation and its degradation at sites of coincidental trauma or infection could elevate serum XLDP, although its extravascular location may be less accessible to the fibrinolytic agent.

Although all of these alternative explanations are feasible, the data shown in figures 7 to 10 suggest that during fibrinolytic therapy serum XLDP derives from degradation of circulating crosslinked fibrin polymers. Evidence that qualitative changes in plasma fibrinogen occur in patients with acute myocardial infarction has been demonstrated by several methods. Increased plasma fibrinopeptide A levels reflecting thrombin action on fibrinogen and the formation of fibrin 1 has been noted in patients with acute myocardial infarction on presentation.\textsuperscript{33-36} Using gel exclusion chromatography, Fletcher et al.\textsuperscript{37} identified an increased plasma concentration of high molecular weight complexes of fibrin with XLDP or FDP in patients with acute myocardial infarction, representing 22% of total plasma fibrinogen in the first 5 days. Reinicke et al.\textsuperscript{38} used fibrin sepharose chromatography to demonstrate an increased plasma fibrin concentration of up to 3% of circulating plasma fibrinogen in patients presenting with acute myocardial infarction.

The pretreatment plasma of four patients, shown in figures 7 and 8, showed bands migrating as fibrin dimer, trimer, and quadramer, which represent covalently crosslinked molecules because they persist after
electrophoresis in SDS. It is known\textsuperscript{39, 40} that factor XIII, can form $\gamma$ chain crosslinked polymers of fibrinogen and hybrids of fibrinogen and fibrin or of fibrin alone, and the polymers that are demonstrated may therefore contain fibrinogen as well as fibrin lacking fibrinopeptides A or both fibrinopeptides A and B. Degradation of such $\gamma\gamma$ crosslinked dimers would yield plasmic derivatives retaining the crosslinked portion such as fragments DD, DY, and YY, and these would be identified as XLDP by either the electrophoretic or ELISA method. Disappearance of fibrin polymers was demonstrated in patients who developed a positive electrophoretic assay for XLDP (figure 7), but no change occurred in patients with a negative electrophoretic assay (figure 8), suggesting that a likely source of XLDP is the degradation of circulating plasma fibrin polymers. The appearance of XLDP by gel was closely associated with development of the systemic lytic state, regardless of whether reperfusion occurred or not (figures 7 and 8). In the only three patients in whom XLDP were not identified in the presence of a lytic state, the decrease in fibrinogen concentration was small (77%, 84%, and 68% of original) and occurred only in the final sample before the patient left the catheterization laboratory, suggesting that the gel technique was not sufficiently sensitive in these cases.

It is possible that the results obtained do not accurately reflect processes in vivo but that significant crosslinking or plasmic degradation of fibrinogen or crosslinked fibrin occurred during venipuncture, blood processing, and storage. Control experiments indicate that the EACA used in blood collection was an effective inhibitor of proteolytic degradation during serum preparation, making it unlikely that significant FDP or XLDP were generated during blood processing. In plasma, the proportion of polymer may be increased during processing and storage, since blood was drawn from a catheter and citrated plasma analyzed after storage at $-20^\circ$ C. However, using the same technique as employed in this study but with blood collected into EDTA to inhibit factor XIII, action, Connaghan et al.\textsuperscript{26} identified a dimer band that represented 7% of total autoradiographic density. In this study no attempt has been made to determine the proportion of polymers present in comparison with normal, and an increase during storage should not affect the correlation between degradation of polymers seen in plasma and the appearance of serum XLDP such as shown in figures 7 and 8.

The ELISA measurements of serum XLDP concentrations are also compatible with their origin from degradation of plasma fibrin polymers. Considering only 2% of the clottable fibrinogen as fibrin dimer, a fibrinogen concentration of 3 mg/ml, would translate to a plasma dimer concentration of 60,000 ng/ml. Since the fragment DD portion of a fibrin dimer represents about 30% of the total mass, complete plasmatic degradation would generate approximately 18,000 ng/ml of fragment DD. The mean peak concentrations of XLDP measured by ELISA (table 3) are compatible with this value. On the other hand, if the origin of the serum XLDP were from a thrombus, the peak concentration of XLDP in serum would require degradation of approximately 70 mg of crosslinked fibrin, assuming recovery of 75% of its mass as XLDP.\textsuperscript{41} Assuming a whole blood fibrinogen concentration of 2 mg/ml, this quantity of crosslinked fibrin would require an original volume of 35 ml of whole blood (hematocrit 40%). Although the fibrin content of coronary artery thrombi is not known, this volume of blood is much larger than that of a coronary artery and thereby also argues against the source of serum XLDP from coronary artery thrombi.

Thus, although a test that is specific for XLDP could partly reflect coronary artery thrombolysis, our results indicate that measurement of such unique FDP in the blood by means of sensitive and specific methods of electrophoretic analysis or antibody-specific ELISA is not a reliable indicator for predicting or detecting the success of therapy. The small amount of XLDP derived from coronary artery thrombolysis cannot be detected by the techniques used because of a larger amount originating from other sources during fibrinolytic therapy. Elevations of serum XLDP also may result from degradation of crosslinked fibrin present at unknown or undetected vascular or extravascular sites. However, the studies presented here indicate that elevated serum XLDP may be expected in patients who develop the systemic lytic state, which regularly accompanies streptokinase therapy\textsuperscript{8} but which also occurs in most patients receiving t-PA\textsuperscript{9, 10} or APSAC\textsuperscript{11, 12} for the treatment of acute myocardial infarction. Most likely, the XLDP result from degradation of circulating crosslinked fibrin polymers, in parallel with the hyperplasminemic degradation of plasma fibrinogen and independent of the occurrence of vascular reperfusion.

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