Validity of Enzyme-Linked Immunosorbent Assays of Cross-Linked Fibrin Degradation Products as a Measure of Clot Lysis

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Concentrations of cross-linked fibrin degradation products (XL-FDPs) in plasma, measured by enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies (MAbs) raised against fragment D-dimer of cross-linked fibrin, increase when patients are given fibrinolytic agents. Whether XL-FDPs derive from circulating cross-linked fibrin polymers in plasma, compared with clot-associated fibrin, has been questioned because increases in XL-FDP are measured by some assays after fibrinolysis in vitro in the absence of clot. We characterized the source of XL-FDP immunoreactivity in plasma of patients with acute myocardial infarction and ischemic heart disease and the response to plasminogen activation in vitro induced by pharmacological concentrations of tissue-type plasminogen activator (t-PA) and streptokinase. XL-FDPs were measured with two different ELISA. One, “pan-specific tag ELISA,” was based on a capture MAb specific for XL-FDP and a tag MAb that recognizes an epitope exposed in the fragment D region of both fibrin and fibrinogen, whereas the other, “fibrin-specific tag ELISA,” was based on a capture and tag MAb both specific for fibrin. After plasminogen activation was induced in vitro in plasma from patients with myocardial infarction, increased concentrations of XL-FDP were measured by the pan-specific tag ELISA; however, concentrations measured with the fibrin-specific tag ELISA were not increased. To determine the mechanism for this discrepancy, plasma was subjected to immunoadsorption with a MAb specific for fragment D-dimer before and after in vitro activation of the fibrinolytic system and immunoblotting with a fragment D-dimer–specific MAb and with the pan-specific MAb. Increased concentrations of fragment D-dimer, as well as fibrinogen fragment D at high concentrations, were recognized by the specific MAb. Non–cross-linked fragments were also shown by immunoblotting with the pan-specific MAb to coprecipitate with cross-linked fibrin fragments. This suggested the increased concentrations of XL-FDP measured by the pan-specific tag ELISA after in vitro activation of the fibrinolytic system were due to detection of non–cross-linked fibrinogen fragments. However, fibrin fragment D-dimer concentrations were found to increase in plasma of 15 patients given t-PA for acute myocardial infarction. We conclude fragment D-dimer in plasma of patients during thrombolysis does not originate from circulating soluble cross-linked fibrin but rather is a marker of solid-phase fibrin dissolution, which may be quantitated with assays based on capture and tag antibodies that do not detect fibrinogen or its degradation products. (Circulation 1990;82:1159–1168)

Assay of cross-linked fibrin degradation products (XL-FDPs) in plasma may be a sensitive and specific approach for the assessment of fibrinolysis in vivo. Recently, assays have been developed based on monoclonal antibodies (MAbs) that recognize epitopes specific for cross-linked γ-polypeptide chains in the fragment D region of fibrin.1–5 These assays were intended to measure fragment D-dimer, the principal product of plasmin digestion of cross-linked fibrin in vitro, but higher-

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Supported in part by SCOR in Ischemic Heart Disease grant HL-17646 and Thrombosis SCOR grant HL-35058, National Institutes of Health, Bethesda, Maryland.

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Received January 17, 1990; revision accepted May 22, 1990.
molecular-weight cross-linked fibrin polymers that contain the fragment D-dimer region are recognized also.5-9 In plasma fibrin, cross-linked fibrin polymers and fragment D-dimer may associate noncovalently with the central aminoterminal domain of other fibrin molecules or fragments to form complexes of higher molecular weight that contain cross-linked fibrin. For example, during fibrinolysis in vivo, fragment D-dimer is found associated with fragment E, a fragment of the fibrinogen.10,11 The extent to which elevations of XL-FDP measured by assays based on specific MAbs are due to increased concentrations of fragment D-dimer in vivo, compared with increased concentrations of higher-molecular-weight fibrin polymers, is unknown.

Plasma levels of XL-FDPs measured by immunoassays based on specific MAbs are elevated in some patients with deep venous thrombosis, pulmonary embolism, acute myocardial infarction, or disseminated intravascular coagulation, presumably due to physiological fibrinolytic activity induced by acute thrombosis.4,12-14 Measurement of XL-FDP may provide a more specific and sensitive measure of increased fibrinolytic activity than traditional methods of measuring fibrinogen degradation products.15 Plasma concentrations of XL-FDPs are also consistently increased after administration of fibrinolytic agents to patients with acute myocardial infarction, pulmonary embolism, or deep venous thrombosis.16-18 Whether these elevations in XL-FDP specifically reflect clot lysis is not clear because XL-FDP immunoreactivity also increases with some assays after administration of tissue-type plasminogen activator (t-PA) to normal volunteers.19 Francis et al20-22 have suggested XL-FDP such as fragment D-dimer may derive from lysis of circulating cross-linked fibrin polymers of high molecular weight that are present in plasma of patients with acute myocardial infarction as well as in plasma of other acutely ill patients.

Lawler et al23 found XL-FDP immunoreactivity increased after incubation of citrated pooled plasma with t-PA when measured with an enzyme-linked immunosorbent assay (ELISA) based on an MAb (3B6) specific for fragment D-dimer combined with a tag antibody that recognized the fragment D region in fibrin or fibrinogen ("pan-specific tag ELISA"). Francis et al24 have suggested the increased XL-FDP immunoreactivity measured with the pan-specific tag ELISA after in vitro activation of the fibrinolytic system results from an increase in the concentration of fragment D-dimer due to lysis of soluble, higher-molecular-weight cross-linked fibrin polymers containing multiple fragment D-dimer regions. However, Lawler et al23 found the concentration of XL-FDP did not change after activation with t-PA in vitro when measured with ELISA based on capture and tag MAbs specific for fibrin ("fibrin-specific tag ELISA"). We hypothesized that in the presence of extensive fibrinogen degradation, increased XL-FDP immunoreactivity with ELISAs that used pan-specific tag antibodies was due to detection of non-cross-linked fibrinogen fragments, which would not occur with a fibrin-specific tag ELISA. Interpretation of elevated XL-FDP in patients would be improved if the mechanism for these elevations occurring in vitro, and the specificity of the MAbs for different species of XL-FDP in vivo, were defined. Accordingly, we compared XL-FDP immunoreactivity with the pan-specific tag ELISA and the fibrin-specific tag ELISA before and after plasminogen activation in plasma from patients with acute myocardial infarction in vitro and characterized the XL-FDP recognized by the MAb specific for fragment D-dimer by immunoadsorption and immunoblotting with either a fragment D-dimer-specific or a pan-specific antibody. The immunoadsorption and immunoblotting method was also used to characterize the XL-FDP in plasma from patients with acute myocardial infarction after induction of thrombolysis with t-PA to determine the fibrin fragments that may contribute to XL-FDP immunoreactivity in vivo.

Methods

Plasma Samples

Plasma was obtained from patients with acute myocardial infarction or other acute ischemic heart disease immediately on admission to the coronary care unit of Barnes Hospital. Samples were similarly obtained from healthy nonhospitalized volunteers. Blood samples were collected through nontraumatic venipuncture by trained phlebotomists into precooled tubes containing 3.8% sodium citrate (9:1 vol:vol) (Becton-Dickinson, Rutherford, N.J.) and were cooled to 4°C. Platelet-poor plasma was immediately separated by centrifugation at 1,500g, stored at -20°C for no more than 4 hours, and then stored at -70°C.

Preparation of Cross-Linked and Non–Cross-Linked Fibrin and Fibrinogen Degradation Products

Non–cross-linked fibrinogen degradation products were prepared by incubating 1.0 mg/ml of fibrinogen (Kabi L, Kabi-Vitrum/Beaumont, Tex.) with 2.5 caseinolytic units (CU)/ml of human plasmin (Sigma Chemical Co., St. Louis, Mo.) in 0.15 M NaCl, 0.02 M K2PO4, and 0.05 M EDTA (pH 7.4) at 37°C. Aliquots were obtained after 0.5, 1, 5, 10, 15, 30, and 45 minutes of proteolysis with plasmin. Fibrinogen degradation products were characterized by 3-12% SDS-PAGE under reducing (4 mM β-mercaptoethanol added) and nonreducing conditions and by comparison with the known molecular weights for each type of fibrinogen degradation product.24 Cross-linked fragment D-dimer was provided by Dr. D. Rylatt (AGEN, Brisbane, Australia); this preparation of fragment D-dimer migrated as a single band at approximately 190 kDa.

Incubation of Citrated Plasma With Plasminogen Activators

The citrated plasma was rapidly thawed to 37°C in a water bath, and 0.05 M EDTA was added to each
aliquot of plasma to inhibit factor XIII activity. The samples were then incubated for 1 hour at 37°C with either 0.5, 2.5, or 5.0 μg/ml t-PA (lot no. 5254-25, predominantly single chain, Genentech, San Francisco, Calif.) or 100 or 1,000 IU/ml streptokinase (Kabiniase Kabi-Vitrum Inc., Alameda, Calif.). The preparations of t-PA and streptokinase were those used for clinical administration. After incubation, 200 KIU/ml aprotinin (Sigma Chemical Co.) and 5 μM D-phenylalanyl-l-prolyl-l-arginine chloromethylketone (PFACK, Calbiochem, La Jolla, Calif.) was added to the plasma.25,26 The sample was frozen at −70°C until it was assayed.

ELISA of XL-FDP

XL-FDPs were assayed by two ELISAs of different design. One assay, the pan-specific tag ELISA, was based on capture of XL-FDP by a MAb (3B6; AGEN, Brisbane, Australia) that recognizes the cross-linked fragment D region of fibrin,1,2 which was covalently bound to a 96-well microtiter plate supplied by the manufacturer. Plasma or standards diluted at least 1:5 with the dilution buffer supplied with the kit or standards were added in duplicate to the microtiter plate. The samples were incubated for 1 hour at room temperature. The plate was washed twice with the phosphate buffer supplied, and a second pan-specific antibody (4D2), which recognizes an epitope in the fragment D region of fibrin or fibrinogen, that was conjugated with horseradish peroxidase was added. After an additional hour, the plate was washed twice, and bound tag antibody was quantitated by adding the substrate 2,2′-azino-di-(3-ethyl-benzthiazoline) sulphonate. In healthy nonhospitalized volunteers, the concentration of XL-FDP in plasma was 67±54 ng/ml (n=8); the upper limit of normal for this assay in plasma is 300 ng/ml.13,14

The second assay for XL-FDP, the fibrin-specific tag ELISA, was based on a capture antibody specific for fibrin fragment D-dimer and a tag antibody specific for fragment D of cross-linked or non–cross-linked fibrin.4 The capture MAb (15CS), at a concentration of 32 μg/ml, was noncovalently coated onto 96-well polystyrene microtiter plates (Costar, Cambridge, UK) at 4°C in 0.04 M K2HPO4 and 0.13 M NaCl (pH 7.4; phosphate-buffered solution [PBS]) for 18 hours. The plates were washed and blocked with a 10-μg/ml solution of bovine serum albumin (BSA) for 2 hours at 22°C. The plates were then washed with PBS and stored at −20°C in a solution of 10 g/l mannitol and 20 g/l saccharose. Before use, the plates were washed with PBS. Samples were diluted in PBS, tween 80 (0.002%), 0.05 mM EDTA, and 10% BSA (assay buffer); pipetted into the wells; and incubated for 16 hours at 4°C. After the plate was washed three times in PBS, a second monoclonal tag antibody (8D3) conjugated with horseradish peroxidase was added, and the plate was incubated for 2 hours at 22°C. The plate was washed three times; 150 μl of a substrate solution of 200 μg/ml o-phenylenediamine and 0.003% hydrogen peroxide in 0.1 M citrate and 0.2 M NaH2PO4 (pH 5) was added and incubated at 22°C for 1 hour. The reaction was stopped with 50 μl of H2SO4. In healthy nonhospitalized volunteers, the concentration of XL-FDP in plasma was 191±72 ng/ml (n=18); the upper limit of normal for this assay in plasma is 500 ng/ml. By both assays, concentrations of XL-FDP were determined by comparison of the absorbance of sample wells to a concurrently obtained standard curve for purified human fragment D-dimer.

Immunoblotting of XL-FDP

Plasma samples (100 μl) were incubated for 1 hour at room temperature with a 2.6% suspension of 3 μm polystyrene microspheres (Kirkgaard Perry, Gaithersburg, Md.) to which the MAb 3B6 had been covalently coupled. The microspheres were separated from the plasma by centrifugation; washed twice with 0.15 M NaCl and 0.02 M K2HPO4 (pH 7.4); resuspended into 40 μl of 0.05 M Tris, 2% SDS, 10% glycerol, and 0.002% bromphenol blue; and heated for 5 minutes in a boiling water bath to dissociate XL-FDP bound to 3B6. The microspheres were separated by centrifugation, and the supernant was subjected to SDS-polyacrylamide electrophoresis with a 4–12% linear gradient and a 3% stacking gel (acrylamide-to-bisacrylamide ratio of 29:1) with a minigel apparatus (Mini Protean II, BioRad, Richmond, Calif.).27 The proteins were transferred from the electrophoresis gel to nitrocellulose membranes (0.45-μm pore size; Schleicher and Schuell, Keene, N.H.) by the method of Towbin et al.28 The membranes were immersed in a solution of 1% BSA and 1% mouse serum overnight and then incubated for 1 hour with a biotin-conjugated MAb specific for XL-FDP (1C3 generously provided by D. Rylatt, AGEN, Brisbane, Australia) or the biotin-conjugated pan-specific tag antibody 4D2 used in the ELISA assay. After a 15-minute incubation with avidin conjugated to horseradish peroxidase (BioRad), the membranes were washed exhaustively with 0.15 M NaCl and 0.02 M K2HPO4 (pH 7.4). A saturated solution of the substrate 3,3′-diaminobenzidine was added to detect bound antibody.

Statistics

Values for XL-FDP concentrations were analyzed by t test. Values are given as mean±SEM.

Results

XL-FDP Immunoreactivity in Plasma Before and After Activation With t-PA or Streptokinase

XL-FDPs were measured in plasma from 10 patients with myocardial infarction, from four patients who were admitted to the coronary care unit but did not have a myocardial infarction, and from two healthy nonhospitalized adult volunteers. The concentration of XL-FDP was increased in two of the 16 samples (normal, <0.3 μg/ml) when measured with the pan-specific tag ELISA (Figure 1A), com-
pared with seven of the 16 samples (normal, <0.5 µg/ml) when measured with the fibrin-specific tag ELISA (Figure 1B). All but one of the increases of XL-FDP were observed in patients with myocardial infarction; the exception was an increase measured with the fibrin-specific tag ELISA in plasma from a patient with unstable angina. Control plasma samples prepared with known concentrations of purified fragment D-dimer were tested with both assays, and the values obtained were similar. Thus, differences in individual values in patient plasmas were not due to differences in the standardization of the assays.

The concentration of XL-FDP as measured by the pan-specific tag ELISA increased in plasma incubated with either 2.5 µg/ml t-PA or 100 IU/ml streptokinase (Figure 1A). However, there was no change in the concentration of XL-FDP in these samples when tested with fibrin-specific tag ELISA.

**XL-FDP Immunoreactivity of Non–Cross-Linked Fibrinogen Degradation Products**

Non–cross-linked fibrinogen degradation products prepared by incubation of fibrinogen with plasmin for varying intervals were added to pooled citrated plasma, and the concentration of XL-FDP was measured with the pan-specific tag ELISA. Increased concentrations of XL-FDP were noted when fibrinogen fragment D was present in the fibrinogen digest (Figure 2A) and when concentrations of non–cross-linked fibrinogen degradation products exceeded 7.8 µg/ml (Figure 2B). An increase in concentration of XL-FDP was not detected with the fibrin-specific tag ELISA when non–cross-linked fibrinogen degradation products were assayed in a similar manner. Nonspecific binding of non–cross-linked fibrinogen degradation products to the microtiter plates did not account for the increased immunoreactivity with the pan-specific tag ELISA, judging from the lack of binding of fibrinogen degradation products to a plate coated with an MAb against an unrelated protein. Fragment D-dimer was not detected in the fibrinogen degradation product digest when subjected to SDS-PAGE under nonreducing conditions and immunoblotted with an MAb that has been shown to be specific for D-dimer-1C3 (Figure 3A). However, with immunoblotting this antibody was only relatively specific for cross-linked fragment D-dimer, judging by the detection of non–Cross-linked fragment D in the fibrinogen degradation product preparation. The loss of specificity for cross-linked fragment D-dimer may be due to the denaturing conditions of SDS-PAGE.

**Characterization of XL-FDP With Immunoblotting After Fibrinolysis In Vitro**

To characterize XL-FDP immunoreactivity, plasma was immunoadsorbed with the fibrin frag-

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Figure 1. Plots of cross-linked fibrin degradation products (XL-FDP) and response to plasminogen activation induced by tissue-type plasminogen activator (t-PA) or streptokinase (SK) in plasma obtained from 10 patients with acute myocardial infarction (MI), four patients with unstable angina, and two nonhospitalized volunteers as measured by two different enzyme-linked immunosorbent assays (ELISAs). XL-FDP at baseline (panel A) are increased (nl<0.3 µg/ml) in plasma from only two of 10 patients with MI, but increases are noted in all but one of the plasma samples after incubation with t-PA or SK as measured by pan-specific tag ELISA. Baseline plasma levels (panel B) of XL-FDP are increased (nl<0.5 µg/ml) in seven samples, including one from patient with unstable angina, when measured by fibrin-specific tag ELISA. There is no change after fibrinolysis with this ELISA.
ment D-dimer–specific MAb 3B6 covalently coupled to polystyrene microspheres. The XL-FDPs were eluted from the antibody-coated microspheres, subjected to SDS-PAGE, and identified by immunoblotting with the MAb 1C3. Purified fragment D-dimer added to plasma could be detected at concentrations of 0.3 μg/ml or more; in the absence of fibrinolysis, other plasma proteins were not specifically immunoadsorbed by the 3B6 antibody. In one of the two patients with myocardial infarction with XL-FDP of more than 0.3 μg/ml as measured by the pan-specific tag ELISA, a fragment D-dimer band was detected with the immunoadsorption and immunoblotting procedure before activation of the plasma with t-PA and streptokinase (Figure 3B, patient 5, lane 2). Faint bands migrating at the top of the 4–12% SDS-PAGE gradient gel were also observed in this patient’s plasma and in plasma from several other patients with myocardial infarction but not in the normal volunteers. These higher–molecular-weight bands may represent fibrin polymers specifically immunoadsorbed by the 3B6 antibody (Figure 3B, three bands migrating at top of lane 2). In plasma in which XL-FDP measured less than 0.3 μg/ml, a fragment D-dimer band was either not observed or only faintly detected (Figure 3B, patient 2).

After incubation of plasma from patients with acute myocardial infarction with 2.5 μg/ml t-PA or 100 IU/ml streptokinase, neither fragment D-dimer nor fibrin polymers appeared as defined by immunoadsorption and immunoblotting unless they were present before thrombolysis (Figure 3B). In patients whose fragment D-dimer was present in plasma before incubation with t-PA or streptokinase, the band persisted after fibrinolysis but did not change in relative intensity (Figure 3B, patient 5). Although the faint bands suggestive of fibrin polymers disappeared after fibrinolysis, there was no relation between their presence and subsequent disappearance and the extent to which XL-FDP increased as measured by the pan-specific ELISA. However, a band at 100 kDa consistent with a non–cross-linked fibrinogen D fragment species appeared in some samples (Figure 3, patient 5), generally those with the greatest increase in measured XL-FDP after fibrinolysis. Thus, binding of non–cross-linked fragment D at high concentrations by the 3B6 antibody may account for the
increase in XL-FDP measured by pan-specific ELISA after fibrinolysis in vitro.

Characterization of XL-FDP After t-PA in Humans

XL-FDPs recognized by the 3B6 antibody were characterized by immunoadsorption and immunoblotting of plasma from 15 patients given t-PA for acute myocardial infarction. The results from two representative patients are shown in Figure 4. After t-PA was given, the intensity of the fragment D-dimer band increased in parallel to the concentration of XL-FDP, as measured by the pan-specific ELISA (Figure 4A, patients A and B). Concentrations of XL-FDP were increased for more than 24 hours after the infusion of t-PA was stopped and paralleled the presence of fragment D-dimer in plasma (Figure 5). Fibrinogen, non-cross-linked fibrinogen degradation products, and higher-molecular-weight fibrin(ogen) complexes specifically immunoadsorbed by 3B6 but not specifically immunoblotted by 1C3 were detected when immunoblotting was performed with the 4D2 MAb used in the pan-specific tag ELISA that recognizes the fragment D region in both fibrinogen and fibrin (Figure 4B). Thus, it appears non-cross-linked fibrin(ogen) degradation products and higher-molecular-weight polymers may be noncovalently associated with fragment D-dimer or other XL-FDPs in plasma and therefore indirectly bound by 3B6. In some patients, particularly after extensive fibrinogenolysis, a faint band was detected after immunoadsorption and

FIGURE 3. Cross-linked fibrin degradation products (XL-FDP) characterized by immunoblotting with monoclonal antibody, 1C3, specific for fragment D-dimer after immunoadsorption with cross-linked fibrin-specific capture monoclonal antibody 3B6 used in pan-specific tag enzyme-linked immunosorbent assay (ELISA) (see "Methods"). Migration of protein–molecular-weight standards is shown. Purified non–cross-linked D fragments (panel A) in digest of purified fibrinogen (same as in Figure 2) were detected by immunoblotting method, at very high concentrations (>250 μg/ml FDP). D-dimer was not detected in non–cross-linked fibrinogen degradation product preparation despite increased immunoreactivity of preparation with pan-specific tag ELISA (see Figure 2). Results from two patients (panel B) with myocardial infarction whose plasma was characterized by immunoblotting before and after incubation with either 2.5 μg/ml of tissue-type plasminogen activator (t-PA) or 1,000 IU/ml of streptokinase (SK). Concentrations of XL-FDP measured by pan-specific tag ELISA are shown at bottom of figure. Lane 1 shows detection of purified human fragment D-dimer (D–D) added to plasma from healthy volunteer. Faint band below fragment D-dimer is due to nonspecific detection of murine IgG, small amount of which is eluted from microspheres in immunoadsorption step. Lane 2 is plasma from patient with myocardial infarction and elevated XL-FDP measured by pan-specific tag ELISA. Fragment D-dimer is detected in plasma of this patient before treatment. Fragment D-dimer band does not change after incubation of plasma with t-PA (lane 3) or SK (lane 4), but band at 100 kDa consistent with non–cross-linked D species is detected. Lanes 5–7 show plasma from another patient with no elevation of XL-FDP at baseline; fragment D-dimer does not appear after fibrinolysis despite increased XL-FDP measured by pan-specific tag ELISA after streptokinase.
immunoblotting, consistent with binding of non-cross-linked fragment D by 3B6 as shown by patient B in Figure 4B and similar to results in vitro (Figure 3B). Nonspecific binding of fibrinogen degradation products to the 3B6-coupled polystyrene beads was excluded by the lack of binding of these fragments to beads coupled with an MAb directed against a non-fibrinogen-related plasma protein (Figure 5, lane 9).

Discussion

Our results indicate that both before and after thrombolysis in patients with acute myocardial
infarction, fragment D-dimer is the principal XL-FDP in plasma detected by currently available MAbs specific for cross-linked fibrin. However, measurement of the XL-FDP concentration with ELISA based on these specific MAbs may be critically affected by recognition of noncovalent complexes of D-dimer with non-cross-linked fibrinogen fragments in plasma by tag antibodies that are not specific for fibrin and by the extent to which fibrin fragments other than fragment D-dimer are recognized. Thus, in the presence of extensive fibrinogen degradation, such as that induced by incubation of plasma with streptokinase or high concentrations of t-PA, concentrations of XL-FDP may be significantly overestimated with an assay based on a pan-specific tag antibody due to detection of non-cross-linked fibrinogen degradation products. The results of immunoblotting confirm that increased concentrations of XL-FDP measured by ELISA in patients given t-PA are always associated with increased fragment D-dimer in plasma. However, in patients with marked fibrinogen degradation after t-PA, an almost fourfold overestimation of the XL-FDP concentration with the pan-specific tag ELISA compared with the fibrin-specific tag ELISA has been found, consistent with our results in vitro.23

Specificity of Assays for XL-FDP

Digestion of cross-linked fibrin by plasmin in purified systems produced a variety of XL-FDPs, including fragment D-dimer and higher–molecular-weight cross-linked polymers.6–9 Cross-linked fibrin and fragment D-dimer bind in a noncovalent manner with fragment E (the fragment that contains the central domain of fibrin or fibrinogen) to form the D-dimer/E complex or larger polymers of fibrin containing the fragment E region.11 Lysis of clot in plasma has been shown to produce a variety of molecular species of XL-FDP and complexes.8,9 Similar species of cross-linked fragments have been observed with electrophoretic methods in plasma from patients with disseminated intravascular coagulation or after administration of fibrinolytic agents.10 The MAbs used were intended to recognize only the cross-linked fragment D region of fibrin or fragment D-dimer.1–4 However, 3–4% crossreactivity with non-cross-linked fragment D regions was found with MAb 3B6, presumably because the epitope recognized is not the specific cross-linked site.1 The concent-

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**FIGURE 5.** Characterization of cross-linked fibrin degradation products (XL-FDP) with immunoblotting method (see Figure 3 and text) in another patient given tissue-type plasminogen activator (t-PA). Concentrations of XL-FDP measured by pan-specific tag enzyme-linked immunosorbent assay (ELISA) are shown below times indicating when sample was obtained. Fragment D-dimer is not present before t-PA but appears in plasma after t-PA and persists for more than 24 hours. Lane 8 illustrates appearance of 1.0 μg/ml of purified fragment D-dimer added to pooled normal plasma for comparison.
tration of XL-FDP in serum or plasma measured by assays based on these MAbs is elevated in a variety of thrombotic and fibrinolytic disorders, including deep venous thrombosis, pulmonary embolism, myocardial infarction, and disseminated intravascular coagulation or after administration of fibrinolytic agents.3,4,12-14,16-18 The extent to which the immunoreactivity of XL-FDP detected by these antibodies in vivo is due to fragment D-dimer, higher–molecular-weight XL-FDP, or XL-FDP in complexes with other fibrin(ogen) fragments has not been previously demonstrated. Judging from the results of immunoblotting after immunoadsorption of plasma with the MAb 3B6, the capture antibody of the most widely used ELISA, fragment D-dimer is the predominant cross-linked fibrin degradation product recognized by this antibody in plasma from patients with myocardial infarction in whom physiological fibrinolytic activity is increased. However, particularly after thrombolysis, fragment D-dimer may be associated with fibrin or fibrinogen fragments in plasma. This appears to account for overestimation of the XL-FDP concentration with the pan-specific tag ELISA compared with the fibrin-specific tag ELISA when there is extensive fibrinogen degradation. Assays of XL-FDP based on agglutination of latex particles coupled with the 3B6 MAb should also be less susceptible to measuring non–cross-linked fibrin(ogen) fragments.

Implications of Increased XL-FDP in Patients With Acute Myocardial Infarction

The significance of elevated XL-FDP in plasma from patients with myocardial infarction has not been defined. We previously found increased concentrations of XL-FDP in plasma from patients with myocardial infarction who presented with complications (e.g., severe heart failure, arrhythmias) but in only 20% of patients with uncomplicated infarction.13 These data are similar to our current results: only two of 10 patients had increased concentrations of XL-FDP measured by the pan-specific tag ELISA. However, XL-FDPs were increased in 60% of these patients when measured by the fibrin-specific tag ELISA. Whether this is due to better detection of cross-linked fibrin fragments other than fragment D-dimer or to differences related to the assay design will need to be defined in a larger study population. Other investigators have found high–molecular-weight fibrin(ogen) complexes in plasma of most patients with infarction.20 These complexes may form when there is increased thrombin activity and formation of soluble fibrin.21,29,30–32 Francis et al20,21 have found cross-linked gamma chains in high–molecular-weight fibrin(ogen) products in serum and plasma from patients with myocardial infarction and suggested these high-molecular-weight species represented soluble cross-linked fibrin polymers containing cross-linked fragment D regions. However, they did not find a correlation between elevations in XL-FDP measured with the pan-specific tag ELISA and the presence of cross-linked fibrin polymers.

Sources of XL-FDP In Vivo

After administration of t-PA or streptokinase to patients with myocardial infarction or pulmonary embolism, concentrations of XL-FDPs in plasma increase.16–18,34 Because high–molecular-weight cross-linked polymers may occur in such patients, Francis et al22 have suggested lysis of these polymers may be a major source of increased concentrations of XL-FDP after thrombolysis. Increased XL-FDP immunoreactivity after incubation of plasma in vitro with t-PA was thought by these investigators to be due to release of D-dimer fragments from fibrin polymers. However, we have shown that concentrations of fragment D-dimer do not increase in plasma from patients with acute myocardial infarction in response to plasminogen activation in vitro. Increases in XL-FDP immunoreactivity under these conditions reflects detection of non–cross-linked fibrin(ogen) degradation products. Thus, our results suggest the increases in XL-FDP measured by ELISA in patients given fibrinolytic agents predominantly reflect increased fragment D-dimer due to lysis of noncirculating cross-linked fibrin.

Clinical Implications

We have shown that increased concentrations of XL-FDP in plasma, measured by currently available assays based on MAbs specific for cross-linked fragment D regions of fibrin, reflect increased concentrations of fragment D-dimer and, to a lesser extent, high–molecular-weight cross-linked fibrin polymers or complexes, which should be specific markers of increased fibrinolytic activity. However, in the presence of extensive fibrinogen lysis, the results of such assays must be interpreted with caution because, in addition to XL-FDP, non–cross-linked fibrin(ogen) degradation products may be measured by ELISA that use a pan-specific tag antibody. After induction of pharmacologic fibrinolysis in humans, fragment D-dimer is the predominant species of XL-FDP in plasma detected by these antibodies. Although the source of fragment D-dimer remains to be defined, it is unlikely to originate from circulating soluble fibrin.

Acknowledgments

The authors acknowledge the expert technical assistance of Mary Jane Eichenseer and Dognia Thorton.

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**Key Words**: fibrin degradation products • thrombolysis • D-dimer • enzyme-linked immunosorbent assay
Validity of enzyme-linked immunosorbent assays of cross-linked fibrin degradation products as a measure of clot lysis.

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_Circulation._ 1990;82:1159-1168
doi: 10.1161/01.CIR.82.4.1159

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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