Evaluation of Fibrinolytic Therapy by Measuring Cross-Linked Fibrin Derivatives and Plasmin-α₂-Plasmin Inhibitor Complex in Plasma

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Takahashi, H., Takizawa, S., Hanano, M., Tatewaki, W., Nagasaki, Y., Sasagawa, Y. and Shibata, A. Evaluation of Fibrinolytic Therapy by Measuring Cross-Linked Fibrin Derivatives and Plasmin-α₂-Plasmin Inhibitor Complex in Plasma. Tohoku J. exp. Med., 1987, 153 (4), 295–302 — Plasma levels of α₂-plasmin inhibitor (α₂PI), plasmin-α₂PI complex and cross-linked fibrin derivatives (XDP) were measured in 8 patients (12 episodes) with thromboembolic disorders on the initial administration of urokinase. In conjunction with a decrease in plasma α₂PI activity and antigen, plasmin-α₂PI complex increased following urokinase infusion in all cases except one who received a low dose (60,000 units) of urokinase. However, changes in XDP were variable among the patients. Plasma XDP level increased markedly in one, moderately in 4, slightly in one, and remained unchanged in 6 cases (episodes). The increment of plasma XDP correlated (r=0.804, p=0.003) with the dose of urokinase administered, but was independent of changes in plasmin-α₂PI complex. The plasma XDP elevation was associated with clinical improvement. These results suggest that simultaneous measurements of XDP and plasmin-α₂PI complex in plasma would be valuable for the pharmacological or hemostatic assessment of thrombolytic therapy.

In fibrinolytic therapy, in addition to the angiographic evaluation, plasma fibrinolytic activity has been monitored by measuring fibrinogen, α₂-plasmin inhibitor (α₂PI), plasminogen and fibrin/fibrinogen degradation products (FDP) (Samama et al. 1985). Currently used FDP assays which use polyclonal antibodies against fibrinogen or its fragments discriminate neither between degradation products of fibrinogen and fibrin nor between fibrin degradation products which are cross-linked or are not. Fibrinolytic therapy with urokinase or strepto-
kinase (or tissue-type plasminogen activator [t-PA]) induces breakdown of both fibrin and fibrinogen. By employing these FDP assays, we can not differentiate the fibrinolysis from fibrinogenolysis. Recently, it has become possible to measure specifically cross-linked fibrin derivatives (XDP) by using monoclonal antibodies which react with cross-linked fibrin degradation products including D-dimer (Elms et al. 1983, 1986; Rylatt et al. 1983; Whitaker et al. 1984). Elevated plasma (or serum) XDP levels have been observed in patients with thromboembolic disorders, disseminated intravascular coagulation and after streptokinase infusion (Whitaker et al. 1984; Hunt et al. 1985; Elms et al. 1986; Lew et al. 1986). Furthermore, sensitive enzyme-linked immunosorbent assays (ELISA) of plasmin-α2PI complex have been developed (Harpel 1981; Mimuro et al. 1987).

In this paper, we describe the measurements of plasma levels of XDP and plasmin-α2PI complex simultaneously during urokinase infusion in patients with various thromboembolic disorders. The results indicate that measurement of XDP would be potentially useful for monitoring thrombolysis during fibrinolytic therapy.

**Materials and Methods**

**Patients**

Eight patients with thromboembolic disorders in this study consisted of one with aortic stenosis complicated by acute pulmonary embolism, 2 with acute myocardial infarction, one with thrombotic prosthetic heart valves, 2 with deep vein thrombosis, and 2 with cerebral artery occlusive diseases. For 12 thromboembolic episodes in these patients, urokinase was infused at an initial dose of 60,000 to 960,000 units (U), mostly by a bolus infusion followed by a drip infusion for 1 to 2 hr (Table 1).

**Preparation of plasma samples**

Venous blood was collected from the patients immediately before and just after urokinase infusion into a polypropylene syringe and quickly transferred into siliconized tubes containing one-tenth volume of either 0.129 M trisodium citrate or a mixture of 0.11 M trisodium citrate, 1,000 U/ml heparin and 1 trypsin inhibitor unit (TIU)/ml aprotinin. The blood samples were centrifuged at 2,000 g for 20 min at 4°C, and the supernatant plasma was separated. Fibrinogen, plasminogen and α2PI activity were measured using citrated plasma, and other assays were performed using plasma prepared in the presence of citrate, heparin and aprotinin.

**Assay methods**

Plasma fibrinogen was measured by the method of Clauss (1957), and its normal range is between 2.0 and 3.5 g/liter. α2PI activity and plasminogen activity were assayed using chromogenic substrates, PS-994 (Sankyo, Tokyo) and S-2251 (KabiVitrum, Stockholm, Sweden), with normal values of 0.71-1.22 U/ml and 0.76-1.18 U/ml, respectively. Free (not-complexed) α2PI antigen was quantitated by an ELISA sandwich method (TD-80; Teijin Ltd., Tokyo) using two monoclonal antibodies to α2PI (JTPI-1 and JTPI-2), one of which (JTPI-1) was directed against the reactive site of α2PI (Mimuro et al. 1987). Plasmin-α2PI complex was measured by an ELISA sandwich method (TD-80C; Teijin) using polyclonal rabbit antibody against human plasminogen and peroxidase-conjugated
RESULTS

A case of pulmonary embolism (Patient 1)

A 58-year-old woman with aortic stenosis and angina pectoris suddenly complained of chest pain and dyspnea. Pulmonary perfusion scintigraphy performed 2.5 hr later demonstrated multiple defects in the lung field, confirming the diagnosis of multiple pulmonary infarction, and then fibrinolytic therapy with urokinase was started in conjunction with heparin infusion (Fig. 1). Before any specific therapy (3 hr after the initial symptoms), the patient had a markedly elevated XDP (5.2 mg/liter) and a moderately increased plasmin-α2PI complex in plasma, suggesting the presence of enhanced endogenous fibrinolysis. Both α2PI activity and antigen had been within the normal ranges. After the initial

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**Table 1. Patient profiles**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Thromboembolic episode</th>
<th>Initial dose of urokinase (units)</th>
<th>Outcome (measures for assessment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>F</td>
<td>Acute pulmonary embolism</td>
<td>420,000</td>
<td>Effective (scintigraphy, blood gas analysis)</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>F</td>
<td>Thrombotic prosthetic heart valves</td>
<td>(a) 60,000</td>
<td>Ineffective</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b) 360,000</td>
<td>Effective (fluoroscopy, echocardiography)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(c) 360,000</td>
<td>Ineffective</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(d) 360,000</td>
<td>Ineffective</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>F</td>
<td>Deep vein thrombosis with pulmonary infarction</td>
<td>(a) 180,000</td>
<td>Ineffective</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b) 360,000</td>
<td>Ineffective</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>M</td>
<td>Left ventricular thrombosis following acute myocardial infarction</td>
<td>360,000</td>
<td>Effective (angiography, echocardiography)</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>F</td>
<td>Acute myocardial infarction (PTCR)</td>
<td>960,000</td>
<td>Effective (coronary angiography)</td>
</tr>
<tr>
<td>6</td>
<td>74</td>
<td>M</td>
<td>Cerebral infarction</td>
<td>420,000</td>
<td>Undetermined, early death</td>
</tr>
<tr>
<td>7</td>
<td>79</td>
<td>F</td>
<td>Vertebro-basilar artery insufficiency</td>
<td>240,000</td>
<td>Ineffective</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>F</td>
<td>Venous thrombosis</td>
<td>240,000</td>
<td>Effective (physical signs)</td>
</tr>
</tbody>
</table>

PTCR, percutaneous transluminal coronary recanalization.

monoclonal anti-α2PI antibody (Mimuro et al. 1987). Normal values of free α2PI antigen and plasmin-α2PI complex are 44-77 mg/liter and below 0.8 mg/liter, respectively. Plasma XDP levels were quantitated by an ELISA kit (Dimertest-EIA; MAbCo., Ltd., Springwood, Queensland, Australia), with a normal upper limit of 0.15 mg/liter.
urokinase infusion (420,000 U), a further increase in XDP (up to 8.0 mg/liter) was observed with a marked decrease in $\alpha_2$PI and an elevation of plasmin-$\alpha_2$PI complex. The magnitude of these response to the subsequent infusions was modest (Fig. 1). Urokinase was discontinued on the 4th day with clinical improvements ascertained by a marked improvement in the pulmonary scintigram and no recurrence of chest pain. Plasma fibrinogen level changed from the preinfusion value of 3.2 g/liter to 2.8 g/liter after the initial infusion.

*Other cases of thromboembolic disorders (Patients 2 through 8)*

Urokinase was infused into 7 patients for 11 thromboembolic episodes (Table 1 and Fig. 2). Before fibrinolytic therapy, plasma plasmin-$\alpha_2$PI complex was moderately elevated in one, marginally elevated in 4, and within the normal limits in 6 cases (episodes), and plasma XDP was slightly elevated in 7 cases and below the upper limit of normal in other 4 cases (Table 2). Immediately after the initial dose of urokinase, both $\alpha_2$PI activity and antigen decreased and plasmin-$\alpha_2$PI complex was elevated in all cases except one who received only 60,000 U of urokinase. Changes in plasma plasminogen level was less marked than those in $\alpha_2$PI. Mean plasma fibrinogen concentration was decreased from 2.70 ± s.d. 0.79
Cross-Linked Fibrin Derivatives and Urokinase

Fig. 2. Changes in plasma $\alpha_2$PI, plasmin-$\alpha_2$PI complex and XDP during the initial urokinase (UK) therapy in Patient 2 (A-D), Patient 3 (E and F), Patient 4 (G), Patient 5 (H), Patient 6 (I), Patient 7 (J) and Patient 8 (K). The dosage of urokinase infused was indicated by tens of thousands units.

Fig. 3. Correlation among the urokinase (UK) dose, a decrease in $\alpha_2$PI, an increase in XDP and an increase in plasmin-$\alpha_2$PI complex during the initial urokinase therapy in 7 patients (Patients 2 through 8).
Plasma XDP increased apparently in 4 cases, and slightly in one. In 4 of these 5 cases, the elevation of XDP was associated with clot dissolution ascertained by angiography or echocardiography and physical examination. Thrombus dissolution could not be confirmed in one patient (patient 6), because he died early before re-evaluation. Plasma XDP was never elevated in the remaining 6 cases without clinical improvement (Table 2 and Fig. 2).

Relationship between the urokinase dose and changes in XDP, α₂PI and plasmin-α₂PI complex was analyzed by the method of least squares (Fig. 3). The decrease in α₂PI and the elevation of XDP correlated positively with the dosage of urokinase administered. The generation of plasmin-α₂PI complex did not significantly correlate with the urokinase dose; the correlation coefficient (r) was still weak (r=0.590, p=0.073) even when one case with 960,000 U urokinase was omitted from the analysis. The elevation of XDP did not correlate with the changes in α₂PI or plasmin-α₂PI complex. The generation of plasmin-α₂PI complex correlated with the decrease in α₂PI activity (Fig. 3). Only 4 of 10 cases with an increase in plasmin-α₂PI complex showed a significant elevation of XDP, and XDP never changed or only minimally elevated in the remaining 6 cases (Table 2 and Fig. 2).

DISCUSSION

It is desirable to assess the clinical effectiveness of fibrinolytic therapy by confirming the thrombus dissolution by angiography. However, it is not always
practical to perform the angiographic re-examination. Pharmacological or hemostatic estimation of thrombolytic therapy would be important in this situation. This study was performed to evaluate the usefulness of the new fibrinolytic parameters such as XDP and plasmin-α2PI complex when urokinase was infused to promote the recanalization of the thromboembolic lesions. On the whole, α2PI was decreased and plasmin-α2PI complex was elevated following the infusion. Changes in plasmin-α2PI complex will reflect more directly the intravascular plasmin generation than a decrease in α2PI. The generation of plasmin-α2PI complex was demonstrated when urokinase was infused at a dose of 180,000 U or more (Table 2 and Fig. 2). The magnitude of endogenous fibrinolysis was minimal in most patients as compared with the fibrinolysis induced by urokinase administration, as assessed by the level of plasmin-α2PI complex and XDP in the circulating plasma. It is noticeable that XDP was not elevated in all cases, even when plasmin-α2PI complex was increased. The generation of XDP was independent of changes in either plasmin-α2PI complex or α2PI levels (Fig. 3). This would be related to the fact that urokinase given intravenously activates circulating plasminogen to plasmin, and only some free plasmin molecules adsorb to fibrin polymers and proteolyze them into XDP. The XDP increment positively correlated with the urokinase dose. These findings suggest that, in cases of no or minimal elevation of XDP, the dosage of urokinase was not sufficient to induce fibrinolysis (but not fibrinogenolysis) and should be increased in the subsequent infusions, or the mass of thrombus which can be dissolved might be too small to produce a detectable increase in the circulating plasma level of XDP.
studies would be necessary in order to assess the relationship between the elevation of XDP and clot lysis (reperfusion) documented by angiography in large numbers of patients.

We conclude that measurements of XDP and plasmin-α2PI complex, in addition to widely used FDP, α2 PI and fibrinogen determinations, would be useful for monitoring thrombolysis during fibrinolytic therapy.

References


