Mechanisms for Elevated Fibrin/Fibrinogen Degradation Products in Acute Experimental Pulmonary Embolism

By John Cade, Jack Hirsh, and Erwin Regoeczi

The mechanism and significance of elevated levels of serum fibrin degradation products (FDP) in pulmonary embolism were investigated experimentally. Dogs were embolized with autologous blood clot-incorporating canine $^{125}$I-fibrin and were infused with either saline, heparin, or streptokinase. Serial measurements were made of total FDP by hemagglutination inhibition assay and of radioactive FDP. After saline, the peak level of total FDP was 323 μg/ml, but radioactive FDP was only 8 μg/ml. After heparin, these values were 44 and 11 μg/ml, respectively, and after streptokinase, 415 and 20 μg/ml. The results suggest that under these experimental conditions the elevated levels of FDP in pulmonary embolism are derived mainly from lysis of fibrin deposited after embolization rather than from lysis of the original embolus. Heparin inhibits both fibrin deposition and elevation of FDP levels after embolism.

ELEVATED LEVELS OF circulating fibrin/fibrinogen degradation products (FDP) have been reported in patients with acute pulmonary embolism. The mechanism for the raised levels of FDP is uncertain but could include lysis of the fibrin in the original embolus, lysis of fibrin which has been subsequently deposited, or a combination of both mechanisms. We have investigated this question in experimental pulmonary embolism and have obtained results suggesting that the raised levels of FDP were due mainly to lysis of newly accreted fibrin rather than to lysis of the original embolus itself.

MATERIALS AND METHODS

Experimental Pulmonary Embolism

Adult mongrel dogs of either sex and of an average weight of 19 kg (range, 10-29 kg) were used. A standardized massive pulmonary embolus of autologous blood clot-incorporating canine $^{125}$I-fibrin was given to each animal, as described in detail previously. In brief, sterile autologous blood (1.5 ml/kg body weight) was mixed with approximately 50 μCi of canine $^{125}$I-fibrinogen and clotted with calcium-thrombin (0.025 M CaCl$_2$ and 1 U thrombin per ml of blood) in a polythene tube of 5 mm I.D. for 18 hr at 20°C. The tube was introduced under light pentobarbital anesthesia via the femoral vein to the junction of the inferior vena cava and right atrium, and its contents were delivered as a single bolus by means of a saline-filled syringe connected to the free end of the tube.

Treatment

Saline, heparin, or streptokinase were given by continuous intravenous infusion from a syringe pump at a rate of 2 ml/hr for 24 hr, commencing at the time of embolization. Sterile isotonic

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saline was given to eight control animals. Sodium heparin (M.T.C. Pharmaceuticals, Hamilton, Ontario, Canada) was given to six animals in a loading dose of 200 U/kg immediately before embolization and a maintenance dose of 800 U/kg/24 hr. Streptokinase (Streptase, lot 401, Hoechst Pharmaceuticals, Montreal, Quebec, Canada) was given to seven animals in a loading dose of 250,000 U over 10 min immediately before embolization and a maintenance dose of 100,000 U/hr.

Assay Procedures

Blood was taken before and 1, 4, and 24 hr after embolization for measurement of the activated partial thromboplastin time, fibrin plate lysis, plasma fibrinogen, and FDP levels.

FDP were assayed by two methods, one to measure total FDP and the other to measure FDP derived from the original embolus. Total FDP were assayed in serum using a tanned red cell hemagglutination inhibition assay, with an antibody against canine fibrinogen which had been raised in rabbits and adsorbed against canine serum. Embolus-derived FDP were calculated by dividing the protein-bound radioactivity of plasma (counts per milliliter) by the initial specific radioactivity of fibrin in the embolus (counts per microgram). None of the plasma radioactivity was thrombin clottable and over 80% was protein bound.

To ensure that the results of both assay methods for FDP were directly comparable, separate matching tests were performed on serial blood samples taken from a dog which had been given a bolus intravenous injection of a liquified radioactive blood clot, prepared as described above, except that the clot was lysed with streptokinase before injection. As shown in Fig. 1, the results of both methods were closely comparable over the range of values measured ($r = 0.92$, $p < 0.001$). In addition, the peak level of FDP measured by the hemagglutination inhibition assay was about 65 µg/ml.

Measurement of Residual Embolus

This was performed as described in detail previously. In brief, after 24 hr, the animal was given 4000 U of heparin intravenously and sacrificed by exsanguination. The lungs were homogenized, and the residual radioactivity in the lungs was expressed as a percentage of the radioactivity initially injected as embolus.
RESULTS

**FDP Levels after Pulmonary Embolism**

Figure 2 shows the levels of total and radioactive FDP before and 1, 4, and 24 hr after embolization in animals given saline, heparin, or streptokinase. In both the saline- and streptokinase-treated animals, there was a marked increase in total FDP at 1 hr (271 and 415 µg/ml, respectively) and 4 hr (323 and 410 µg/ml), and the levels were still moderately elevated at 24 hr (38 and 96 µg/ml). The differences in FDP levels between saline- and streptokinase-treated animals at 1, 4, and 24 hr were not statistically significant. In contrast, the levels of total FDP in the heparin-treated animals were always significantly lower (44, 15, and 6 µg/ml at 1, 4, and 24 hr) than in either of the other two groups (p < 0.05). Also shown in Fig. 2 is the peak level of FDP (65 µg/ml) in the dog which received, instead of an embolus, a bolus intravenous injection of the corresponding amount of blood clot lysate. This peak is considerably less than the highest level of total FDP in saline- or streptokinase-treated animals (323 and 415 µg/ml, respectively).

The lowest levels of radioactive FDP at all times after embolism were found in the saline-treated animals. Radioactive FDP were not significantly greater in heparin-treated than in control animals but were significantly greater in streptokinase-treated animals (p < 0.05). In all three groups, the highest levels of radioactive FDP were seen at 1 hr after embolization, when the values were 8.1, 11, and 20 µg/ml after saline, heparin, and streptokinase, respectively. These values represented 12%, 16%, and 29%, respectively, of the total amount of embolized radioactive fibrin when corrected for the estimated circulating plasma volume.

There was a significant fall in plasma fibrinogen levels 1 and 4 hr after embolization in saline- and streptokinase-treated animals, but no significant change in heparin-treated animals (Table 1). By 24 hr, plasma fibrinogen levels
Table 1. Serial Plasma Fibrinogen Levels Before and After Pulmonary Embolism in Animals Treated With Saline, Heparin, and Streptokinase

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before</th>
<th>1 hr</th>
<th>4 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>242</td>
<td>204*</td>
<td>174*</td>
<td>340†</td>
</tr>
<tr>
<td></td>
<td>(28)</td>
<td>(24)</td>
<td>(24)</td>
<td>(37)</td>
</tr>
<tr>
<td>Heparin</td>
<td>210</td>
<td>202</td>
<td>194</td>
<td>371†</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(30)</td>
<td>(20)</td>
<td>(39)</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>216</td>
<td>191*</td>
<td>178*</td>
<td>356†</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(11)</td>
<td>(11)</td>
<td>(15)</td>
</tr>
</tbody>
</table>

Before, 1 hr, 4 hr, and 24 hr refer to time before and at intervals after embolization. Results are mean (mg/100 ml) with standard error in parentheses.

* Indicates significant decreases in plasma fibrinogen (p < 0.02).
† Indicates significant increases in plasma fibrinogen (p < 0.005).

were significantly increased in all treatment groups, presumably in response to the trauma. Plasma fibrinolytic activity was unchanged in animals given saline or heparin, the mean area of fibrin plate lysis remaining 0 sq mm throughout the experiment. In animals given streptokinase, the mean area of fibrin plate lysis increased from 0 sq mm before treatment to 39, 36, and 66 sq mm at 1, 4, and 24 hr, respectively after commencement of treatment. The partial thromboplastin time was between 2 and 2.5 times control at 1, 4, and 24 hr in heparin-treated animals.

Amount of Resolution of Emboli

The percentage of the original pulmonary embolus that was still present at 24 hr was 49% after saline, 28% after heparin, and 6% after streptokinase. These values were highly significantly different (p < 0.01).

DISCUSSION

In this study, two different methods were used to measure the amount of circulating FDP during the early course of acute experimental pulmonary embolism. One method was sensitive to total FDP and the other was specific only for FDP derived from the original embolus. The striking discrepancy in the results of the two methods suggests that the elevated levels of FDP after pulmonary embolism were derived mainly from sources other than the original embolus. Thus, in control animals, there was only minor elevation of radioactive FDP derived from the original embolus, but there was a marked increase in the total FDP.

The standardized massive embolus represented an average fibrin mass of approximately 50 mg, and the amount of resolution by 24 hr was 51%, 72%, and 94% after saline, heparin, and streptokinase, respectively. This amount of resolution occurred without giving rise to radioactive FDP levels greater than 20 μg/ml. The maximum obtainable concentration of embolus-derived FDP, derived after a bolus intravenous injection of the in vitro lysate of the experimental embolus, was 65 μg/ml. By contrast, peak levels of total FDP in control animals were greater than 300 μg/ml. These differences suggest that the amount of fibrin accretion accompanying untreated experimental pulmonary embolism is considerably greater than the fibrin mass of the original embolus itself. This
conclusion is also supported by the observation of a significant early decrease in plasma fibrinogen in control animals, the average decrease by 4 hr representing a net loss of approximately 500 mg of fibrinogen.

In heparin-treated animals, total FDP was only minimally elevated, although there was increased resolution of the original embolus. In addition, the early fall in plasma fibrinogen seen in control animals was not found in heparin-treated animals. These findings suggest that the lower levels of total FDP in these animals were due to inhibition of new fibrin deposition by heparin and support the conclusion that the higher levels of FDP in control animals were derived largely from newly accreted fibrin. While some degree of fibrinogenolysis may have contributed to the elevated levels of total FDP in streptokinase-treated animals, this is unlikely in control or heparin-treated animals, since in these animals the systemic fibrinolytic state remained unchanged. Any possible contribution to circulating levels of FDP by non-plasmin-mediated fibrinogenolysis or by lysis of fibrin deposited in sites other than the lungs was not explored in the present study.

The levels of total FDP obtained in our experiments were similar to those reported after clinical pulmonary embolism in man. Thus, Rickman and associates 7 reported that nine out of 19 patients with angiographically proven pulmonary embolism had FDP levels greater than 100 µg/ml, and in four patients the levels were greater than 300 µg/ml. Wilson et al.3 reported that, in patients with clinical pulmonary embolism, FDP disappeared more rapidly after heparin therapy. Our findings of high levels of total FDP in control animals and of low levels in heparin-treated animals are consistent with these clinical observations.

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REFERENCES


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