Inhibitor of the Thrombin Time in Systemic Amyloidosis: A Common Coagulation Abnormality

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Patients with primary systemic amyloidosis (AL) often experience bleeding, and we report a newly recognized coagulation abnormality in AL. Of 103 patients with primary systemic AL studied over 2 years, 41 had prolongation of the thrombin time (range, 25 to 46 seconds; normal, <22 seconds) and reptilase time (range, 17 to 39 seconds; normal, 14 to 16 seconds). The fibrinogen from the plasma of 36 patients was precipitated by β-alanine and diluted to a concentration of approximately 200 mg/dL. The thrombin times of the precipitated fibrinogens were normal in 34 patients, implying that an inhibitor was responsible for the abnormal tests. The addition of patient fibrinogen-free plasma to normal plasma prolonged the thrombin times, and this result confirmed the presence of an inhibitor. The inhibitor is more likely to be present in patients with nephrotic syndrome (20 of our patients) and congestive heart failure (six). A circulating monoclonal protein (24 patients), the presence of amyloid liver involvement (eight), and the presence of amyloid neuropathy (nine) were not predisposing factors. Only one patient had deficiency of factor X. We conclude that inhibition of fibrinogen conversion to a fibrin clot rather than dysfibrinogenemia is the cause of the prolonged thrombin time in primary systemic AL.

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plasma and allowed to incubate at room temperature for 30 minutes, and thrombin times were determined.

Plasma was depleted of fibrinogen by heating in a 56°C water-bath for 4.5 minutes. Samples were centrifuged and tested for the presence of inhibitor as described above.

**Interpretation.** The mean thrombin time of the normal PPP was 20.9 seconds (2 SD range, 17.7 to 24.1 seconds; 1 SD, 1.6 seconds; \( n = 35 \)). The mean thrombin time of the normal isolated fibrinogen was 17.4 seconds (2 SD range, 14.9 to 19.9 seconds; 1 SD, 1.25 seconds; \( n = 35 \)).

A normal result is a purified fibrinogen thrombin time less than or equal to the normal PPP thrombin time (generally 18 to 21 seconds). A normal isolated fibrinogen thrombin time and prolonged PPP thrombin time are consistent with the presence of an inhibitor. The prolongation of both clotting times is consistent with a dysfibrinogenemia.

### RESULTS

The fibrinogen from the plasma of 36 patients with abnormal thrombin times was precipitated by \( \beta \)-alanine and diluted to a concentration of approximately 200 mg/dL. The thrombin times of the precipitated fibrinogens were normal in 34 of the 36 patients, implying the presence of an inhibitor.

Three patients with known dysfibrinogenemia (one plasma sample was kindly provided by Dr Douglas Triplett, Muncie, IN) were studied. The isolated fibrinogens of these patients produced prolonged thrombin times (Fig 1). All were substantially more prolonged than the only two amyloid-associated fibrinogens that did not correct to the normal range.

Normal and amyloid PPPs were depleted of fibrinogen using 0.5% bentonite or heating to 56°C for 4.5 minutes. After centrifugation, the supernatants were mixed with normal PPP and thrombin times were determined. Supernatant from the three amyloid plasmas prolonged the thrombin time (Table 1).

The thrombin time was the only coagulation test result that was consistently abnormal: prolonged in 36 of the 41 patients studied (Table 2). Clottable fibrinogen levels (biuret method) generally were elevated (range, 210 to 1,190 mg/dL; median, 636 mg/dL) in patients whose thrombin time was prolonged. No patient had hypofibrinogenemia. Consistently negative protamine gel tests indicated the absence of active disseminated intravascular coagulation. The reptilase time was consistently elevated (range, 17 to 39 seconds; median, 24 seconds; control, 15 seconds) in direct proportion to the prolongation of the thrombin time, suggesting that a heparin-like inhibitor was not present.

The clinical abnormalities are listed in Table 3. The most common findings were abnormal echocardiograms and nephrotic syndrome. Although these were the most common, no correlation with the degree of prolongation of the thrombin time was found.

A circulating monoclonal protein was seen in 24 of the patients. The results of immunoelectrophoresis are shown in Table 4. The serum monoclonal protein was generally small in amount and exceeded 1 g/dL in only nine of the patients and exceeded 2 g/dL in only one. Nine patients had a serum monoclonal protein and did not have associated congestive heart failure or nephrotic syndrome. The thrombin time in these nine patients ranged from 19.7 to 41.8 seconds (median, 22.3 seconds). In the five patients without a monoclonal serum protein and without heart failure or nephrotic syndrome, the thrombin time ranged from 20 to 26.3 seconds (median, 23.4 seconds). Only one patient had a prolonged prothrombin time (22.2 seconds), and this patient had a factor X level of 6%. The factor X levels were not depressed below 40% in any of the other patients.

Figure 1 shows the results of the thrombin time before and after the fibrinogen was purified. Of the 36 patients whose thrombin time was prolonged 2 seconds greater than control, the thrombin time normalized in 34. The thrombin time was shortened a median of 8 seconds in the 36 whose thrombin time was abnormal. In a control group of normals, purification of the fibrinogen resulted in a median shortening of the thrombin time by 4 seconds.

Neither increasing the protein concentration with bovine serum albumin nor adding fibrin split products (Dade) resulted in prolongation of the thrombin time (data not shown).

![Fig 1. Thrombin times of PPP (○) and isolated fibrinogen (●) in normals (\( n = 25 \)), patients with dysfibrinogenemia (\( n = 3 \)), and patients with AL (\( n = 36 \)).](image-url)
The thrombin time can be prolonged for several reasons, including (1) the presence of heparin, (2) hypofibrinogenemia, (3) dysfibrinogenemia, (4) circulating anticoagulants, and (5) the presence of fibrin or fibrinogen split products. Differentiation of most of these causes is not difficult through the quantitative determination of the fibrinogen value and of the reptilase time to exclude heparin-type anticoagulants and through assays for fibrin degradation products and fibrin monomers.

However, generally dysfibrinogenemia is diagnosed through exclusion of other causes because no simple in vitro test exists to confirm the presence of a structurally abnormal fibrinogen. The cause of prolonged thrombin times in AL has been previously attributed to the presence of an abnormal fibrinogen. These studies report prolongation of the thrombin times were not different between those amyloid patients with and those without a serum monomeric protein. The thrombin time abnormality did not have any effect on the likelihood of clinical bleeding or thrombosis.

Hypofibrinogenemia may result from defective production of fibrinogen, as may be seen in severe liver disease. Total fibrinogen concentrations were preserved in our patients despite significant hepatomegaly in six. In fact, all patients tested had fibrinogen values greater than 200 mg/dL.

Circulating anticoagulants of the lupus-type were not specifically sought, and although some patients with monoclonal proteins have a lupus-type activity, these patients are rare, comprising only 10 of 219 such patients in our laboratory. None of these patients had amyloidosis.

Butler and Baldwin described a patient with factor X deficiency who also had a prolonged thrombin time, and in a review of the literature they found that eight of 14 patients had either a thrombin time greater than 1.5 times control or a prolonged thrombin time that was not corrected in vitro when mixed with normal plasma. They postulated, as have others, that inhibitors may be present,
but they did not demonstrate direct evidence of inhibition or the presence of normal fibrinogen.

In this study we identified the presence of normal fibrinogen in patients who had AL with prolonged thrombin times and demonstrated the normal action of thrombin on this isolated fibrinogen. An inhibitor or inhibitors must exist in the plasma of patients with primary systemic AL. These inhibitors are separated from the fibrinogen isolation procedure.

The prolonged thrombin time in AL is not due to dysfibrinogenemia but to an inhibitory activity that remains in the supernatant after fibrinogen is precipitated.

REFERENCES

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