Gamma Globulin Inhibition of Fibrin Clot Formation

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ABSTRACT

Myeloma gamma globulins have been reported to interfere with fibrinogen-fibrin conversion. A patient with multiple myeloma is described with a gamma globulin IgG, concentration of 11 g per dl, prolonged thrombin time and poor clot retraction. Purified gamma globulin from the patient’s serum and from normal serum caused prolongation of the thrombin time and reptilase clotting time assays in both normal plasma and in solutions of bovine fibrinogen. In addition, fibrin clots formed during the thrombin time assays were found to be ultrastructurally abnormal. This data suggests that the interaction of gamma globulin in the polymerization of fibrin may, in at least some cases, be due to non-specific protein interaction.

A variety of abnormalities of blood coagulation have been reported in patients with malignant paraproteinemias. Deficiencies and inhibitors of plasma coagulation factors as well as platelet defects have been recorded. One of the most frequently observed abnormal laboratory tests is a significantly prolonged thrombin time. A recent report indicated that the thrombin time is prolonged in plasma from 71 percent of patients with IgG myeloma, 57 percent of patients with IgA myeloma, 25 percent of patients with Bence Jones myeloma and 15 percent of patients with IgM paraproteinemia. Previous studies suggested that the paraprotein may interfere with fibrin monomer aggregation. A possible mechanism involves the binding of Fab fragments of the myeloma immunoglobulin to fibrin monomer inhibiting its normal polymerization and aggregation.

The present report describes a patient with multiple myeloma and a series of studies designed to elucidate in part the mechanism by which myeloma proteins inhibit the normal fibrin monomer aggregation following the action of thrombin.

Case Report

The patient was a 66 year old woman who had multiple myeloma for three years prior to our studies. Bone marrow showed an infiltrate of plasma cells and x-ray studies revealed diffuse osteoporosis and compression fractures of D9 and L12. Hemoglobin was 8.4 g per dl, leukocyte count was 3,900 per ftl and platelet count was 100,000 per μl. Uric acid level was 5.6 mg per dl and BUN was 28 mg per dl. Protein electrophoresis showed a paraprotein in the gamma globulin region. There was 11 g per dl of IgG, 85 mg per dl of IgA, and 52 mg per dl IgM. The IgG paraprotein was monoclonal for lambda chains and was of a Gm subtype. The relative plasma viscosity was 3.1.

The standardized Ivy bleeding time was normal at 5 min, prothrombin time was 10.9 seconds with a control of 9.5 seconds and the activated partial thromboplastin time was 42.5 seconds with a control of 41 sec. The clot was of poor quality and did not retract but remained insoluble in a solution of 5M urea. The fibrinogen was 490
mg per dl, euglobulin clot lysis was complete at 2.5 hours and the serum fibrin degradation products were measured at 10 µg per ml. Factor V was 188 percent and factor VIII was 164 percent of normal. Multiple thrombin times were significantly prolonged at 40 to 43 sec with a control of 20 to 23 sec. When patient’s plasma was diluted 1:1 with normal plasma the thrombin time remained significantly prolonged at 40 sec with a control of 20 sec.

Materials and Methods

COAGULATION STUDIES

Whole blood was obtained by clean venipuncture employing a two syringe technique and diluted 9:1 with 3.8 percent sodium citrate. Platelet poor plasma was obtained by centrifugation for 30 minutes at 12,000 g. Routine coagulation studies were performed using standard methods. The thrombin time was measured according to the method of Rapaport and Ames and fibrinogen was assayed by the method of Ellis and Stransky.

GAMMA GLOBULIN PREPARATION

Gamma globulin was prepared from the patient’s serum and from pooled normal serum by the precipitation with 50 percent saturated ammonium sulfate. The precipitate was washed several times in 50 percent saturated ammonium sulfate, resolubilized in a minimum volume and extensively dialyzed at 4° against 0.01 M phosphate 0.14 M NaCl pH 7.8 solution (PBS). Dialysis was terminated when BaSO₄ was no longer detectable in the dialysate following the addition of BaCl₂. Protein electrophoresis and quantitative immunodiffusion demonstrated that the protein fractions employed in these studies consisted of 99 percent pure IgG. Protein solutions were adjusted to a concentration of 4.2 g per dl with PBS.

CLOT INHIBITION STUDIES

The clot inhibition studies were performed using the thrombin time assay. For the purpose of these studies, control thrombin times were set normally between 18 to 23 sec. The following experiments were performed: (1) Thrombin time assays of patient’s plasma were diluted with pooled normal plasma; (2) Thrombin time assays were performed on pooled normal plasma diluted with myeloma protein. Pooled normal plasma diluted with normal IgG served as a control; (3) Thrombin time assays of bovine fibrinogen in PBS at 3 mg per ml with various amounts of added myeloma IgG. Controls were bovine fibrinogen (3 mg per ml) with various concentrations of normal IgG and albumin; and (4) In addition, reptilase clotting times were performed on various mixtures of myeloma IgG and normal IgG in a bovine fibrinogen PBS solution (3 mg per ml).

ELECTRON MICROSCOPY

Experimental and control fibrin clots were fixed in cacodylate-buffered 2 percent gluteraldehyde, pH 7.4 and post fixed in veronal-buffered osmium tetroxide con-

*Phosphate buffered solution.
ADDED GAMMA GLOBULIN Gm/dl

FIBRINOGEN mg/dl

Figure 2. Comparison of thrombin time assays on pooled normal plasma mixed either with various concentrations of normal IgG or myeloma IgG. Fibrinogen concentrations determined as a function of dilution of the samples are given on the abscissa together with the concentrations of added IgG.

taining 0.2 M sucrose. The specimens were then dehydrated in graded alcohol solutions, impregnated with propylene oxide and embedded in Araldite. Thin sections were cut with a LKB Ultratome, stained with uranyl acetate and lead citrate and examined with a Philips EM 300 electron microscope.

Results

CLOT INHIBITION STUDIES

The thrombin time of normal control plasma was 24 sec and for the patient’s plasma was 43.5 sec. A prolongation of the thrombin time in normal plasma was noted by its progressive dilution with increasing concentrations of patient’s plasma (figure 1).

When increasing concentrations of myeloma protein and concentrated normal gamma globulin were added to pooled normal plasma, a prolongation of the thrombin time was observed (figure 2). No significant difference was observed between the inhibitory effects observed with myeloma IgG or with normal pooled IgG. There was only a minimal prolongation of the thrombin times when normal plasma was diluted with PBS. When fibrinogen concentration (3 mg per ml) was held constant, a similar increase in the thrombin time was noted as a function of total added immunoglobulin (figure 3).

The reptilase assays showed prolongation of clotting time as the concentration of both

Figure 3. Results of thrombin time assays on fibrinogen in phosphate buffered solution (3 mg per ml) with various amounts of concentrated normal IgG, concentrated myeloma IgG and albumin.

Myeloma protein

Normal concentrated gamma globulin

Figure 4. Reptilase clotting assays performed on bovine fibrinogen phosphate buffered solution (3 mg per ml) mixed either with various concentrations of myeloma IgG or normal IgG.
myeloma and normal IgG increased in bovine fibrinogen solution (figure 4).

ELECTRON MICROSCOPIC STUDIES

Ultrastructural examinations were performed on fibrin clots obtained from (1) incubation of myeloma IgG with pooled normal plasma and normal concentrated IgG in pooled normal plasma and (2) incubation mixtures of myeloma IgG in bovine fibrinogen solution or normal concentrated IgG with bovine fibrinogen solution. Although the ultrastructural morphology of the fibrin clots varied somewhat from area to area, in general two consistent abnormalities were apparent in those clots formed in high concentrations of myeloma and normal IgG. The fibrin strands appeared smaller, narrower and less compactly formed as compared to clots from incubation mixtures containing no or low concentrations of gamma globulin (figures 5 and 6). In addition, large amounts of amorphous granular material, which formed irregular aggregates along the length of the narrow fibrin strands were observed (figure 7).

Discussion

The thrombin time assay measures the conversion rate of fibrinogen to a fibrin clot through the enzymatic action of thrombin. Prolongation of the thrombin time has been
associated with insufficient fibrinogen concentrations (less than 1 mg per ml), abnormal fibrinogen activity (dysfibrinogenaemia), presence of antithrombins (heparin), elevated concentrations of fibrin degradation products and paraproteinemic states.

In this report and other similar cases, the prolongation of the thrombin time in patients with multiple myeloma was directly related to the presence of a pathologic protein.

Early reports suggested that myeloma proteins may act as an antithrombin. Reptilase converts fibrinogen to a fibrin clot through the enzymatic cleavage of fibrinopeptide A. Thus antithrombins do not cause a significant prolongation of the reptilase clotting time. In this and other reports, the reptilase clotting time was prolonged by added concentration of myeloma protein suggesting that the inhibition of clot formation does not result from a direct anti-thrombic effect but probably relates to a defect in fibrin polymerization.

Coleman et al found that the inhibitory effect of IgG myeloma proteins occurred at lower concentrations (3 mg per ml) than those reported herein. Fab fragments, moreover, possessed an inhibitory effect equal to the intact protein. These data suggested that the myeloma inhibitor proteins may interact with fibrin monomer through an immunologic reaction or at least by way of the Fab end of the molecule. In contrast, the same investigators noted that protein concentrations more comparable to in vivo levels (30 mg per ml in one case and 80 mg per ml in another case) were needed before an inhibitory effect was seen with IgA myeloma proteins. Although the myeloma protein described in this current report was typed as IgG, its inhibitory effect was seen only at in vivo levels (30 mg per ml). In addition, pooled normal concentrated gamma globulin also showed an inhibitory effect in clot formation at equivalent concentrations of immunoglobulin protein. These data suggest that the myeloma protein and the normal concentrated gamma globulin inhibited clot formation through a nonspecific protein interference.

The electron microscopic findings of abnormally narrow fibrin strands coated by amorphous granular material in clots formed in the presence of high concentrations of myeloma proteins is in agreement with earlier findings of other investigators who have described the same abnormal clot structures using myeloma proteins. Those authors proposed that high concentrations of myeloma proteins impeded the normal lateral aggregation of fibrin monomer. The current studies demonstrated that the identical morphologic alterations occurred in clots formed in the presence of high concentrations of normal gamma globulin.

The discordance between data presented in this report and those recorded by Coleman et al may be reconciled by the possibility that some IgG, myeloma proteins in low concentrations inhibit normal fibrin aggregation because of their high affinity for fibrinogen monomers. While it is tempting to speculate that these reactions may be primary immunologic events, the complementary structural configuration of the immunoglobulin necessary to interact...
with the highly reactive sites on fibrinogen may merely be a reflection of the multiple binding functions demonstrated in homogenous immunoglobulins. In contrast, other myeloma proteins and indeed normal gamma globulin with low affinity for fibrinogen monomers may interfere with fibrin clot formation only at very high in vitro concentrations.

References