Effect of Oxidized Fibrinogens on Blood Coagulation

E. V. Roitman, O. A. Azizova*, Yu. A. Morozov, and A. V. Aseichev*

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We studied the effect of UV-irradiated fibrinogen on blood coagulation. Fibrinogen with oxidation degree of 10% moderately activated the intrinsic pathway, but inhibited the extrinsic pathway of blood coagulation. Fibrinogen with oxidation degree of 20% inhibited both the extrinsic and intrinsic blood coagulation pathways. We revealed disturbances in the formation of fibrin clot with oxidized fibrinogen, suppression of platelet aggregation mediated by collagen receptors, and inhibition of aggregation associated with von Willebrand factor activity. ADP initiated platelet aggregation, which was in direct proportion to the degree of fibrinogen oxidation. Our results indicate that oxidized fibrinogen produces a dysregulatory effect on platelets.

Key Words: *oxidized fibrinogen; blood coagulation system; platelet aggregation*

The development of pathological states and diseases (*e.g.*, ischemia, diabetes mellitus, hypertension, and tumors) is accompanied by activation of free radical processes. This phenomenon received the name "oxidative stress" [9]. Attention was paid to the mechanism of changes accompanying oxidative stress and resulting in the development of diseases. Studies showed that the pathogenesis of diseases is related to the interaction of products formed during oxidation of lipids, proteins, and nucleic acids with cell components. These products initiate the reactions affecting cell metabolism and genetic apparatus induce expression of genes for foreign proteins, and lead to transformation of cells.

Fibrinogen (FG) is an independent risk factor of atherosclerosis and its complications [7,8]. This compound is most sensitive to oxidation with plasma proteins. Probably, oxidized FG modulates the development of atherosclerosis, its complications, and complications of inflammatory diseases.

Recent studies showed that the intensity of thrombus formation increases in the presence of oxidized FG [10]. Experiments performed in Russia over the last 2 years showed that oxidized FG induces platelet aggregation [1]. The mechanism of platelet aggregation induced by oxidized FG remains unclear.

Here we studied the effect of oxidized FG on blood coagulation and rheological properties of the blood.

MATERIALS AND METHODS

The blood was taken from healthy donors (hematocrit 40.40±0.22%) and stabilized with 3.8% sodium citrate.

The experiments were performed with human plasma FG (Sigma). The solution of FG in phosphate buffered saline (pH 7.4, 3 mg/ml) was oxidatively modified by UV-irradiation on a DRK-120 ultrahigh-pressure mercury arc lamp with natural cooling (operating current 1.2 A). Oxidative modification of FG was controlled by recording the decrease in tryptophan fluorescence of the protein. The solution of FG (100 μl) was placed in a cuvette. The volume was brought to 4 ml with distilled water. The intensity of fluorescence was measured on a Perkin-Elmer LS50 spectrofluorimeter at excitation and emission wavelengths of 280 and 340 nm, respectively. We used oxidized FG (10 and 20% oxidation).

The solution of oxidized FG (0.5 ml) was added to tubes with 10 ml citrate blood, mixed, and imme-

Russian Research Center for Surgery, Russian Academy of Medical Sciences; *Laboratory for Biophysical Bases of Pathology, Institute of Physicochemical Medicine, Russian Ministry of Health, Moscow. ***Address for correspondence:*** oazizova@mail.ru. O. A. Azizova

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diately assayed. The blood from healthy donors served as the control.

Samples were analyzed by routine clotting methods for studying the blood coagulation system (activated partial thromboplastin time, prothrombin and thrombin time, and FG concentration). Thromboelastogram (TEG) was recorded. We measured the count of platelets, studied platelet aggregation induced by ADP, ristocetin, and collagen, and estimated the content of soluble fibrin monomer complexes (SFMC) [4].

The results were analyzed by nonparametric Dunn test, Kruskal test, and χ^2 test. The differences were significant at $p<0.05$.

RESULTS

Addition of 0.5 ml oxidized FG to 10-ml blood samples had no effect on FG concentration in the plasma and major physicochemical characteristics of the blood (pH). This treatment did not decrease the hematocrit and platelet count.

The solution of oxidized fibrinogen (10%) moderately activated the intrinsic pathway, but inhibited the extrinsic pathway of blood coagulation (Fig. 1). Fibrinogen with an oxidation degree of 20% inhibited both the extrinsic and intrinsic pathways of blood coagulation ($p<0.05$). Deceleration of thrombin formation was accompanied by a decrease in the rate of clotting.

The increase in the amount of SFMC depended on the degree of FG oxidation. Treatment with FG oxidized by 10 and 20% was followed by an increase in the content of SFMC by one-third ($p>0.05$) and 1.5 times ($p<0.05$), respectively, compared to the control.

Oxidized FG probably suppresses factors of blood coagulation that have the phospholipid structure (extrinsic pathway) and readily undergo oxidation.

Lengthening of the thrombin time suggests that oxidized FG primarily affects the final stage of coagulation. The addition of oxidized FG was accompanied by accumulation of SFMC in the plasma, decrease in the amplitude of TEG ($p<0.05$), and reduction of rigidity and density of the clot ($p<0.05$). These changes reflect disturbances in the formation of normal fibrin clot.

Similar results were reported previously [5]. The observed changes indicate that oxidized FG is less accessible to thrombin. Under these conditions, polymerization of fibrin monomers was decelerated. It should be emphasized that the effect of FG directly depended on the degree of oxidation.

Oxidized FG had no effect on fibrinolytic activity. However, oxidized fibrinogen significantly modified platelet aggregation. For example, oxidized FG suppressed platelet aggregation induced by ristocetin and collagen (Fig. 2). These changes were most pronounced after treatment with FG oxidized by 10% ($p<0.05$).

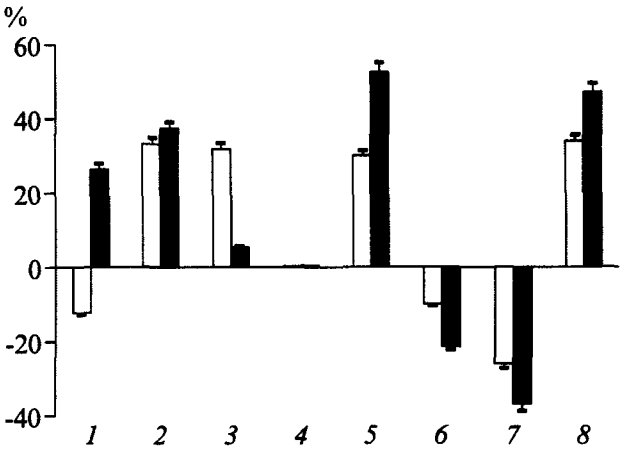

Fig. 1. Blood coagulation in the presence of fibrinogen oxidized by 10 (light bars) and 20 % (dark bars): activated partial thromboplastin time (*1*), thrombin time (*2*), international normalized ratio (*3*), reaction time in thromboelastogram (TEG, *4*), TEG time between the start and end of clotting (*5*), maximum amplitude in TEG (*6*), rigidity of clot (*7*), and soluble fibrin monomer complexes (*8*).

By contrast, ADP initiated platelet aggregation, which was in direct proportion to the degree of FG oxidation. The intensity of platelet aggregation in samples containing highly oxidized FG was 35% higher compared to the control and 2-fold exceeded that observed under the influence of FG with oxidation degree of 10%.

Our results indicate that oxidized FG has different effects on platelets. The compound suppresses adhesion mediated by collagen receptors and inhibits aggregation associated with von Willebrand factor activity. It is surprising that the observed changes were most pronounced after addition of low oxidized FG (10%). This phenomenon requires further investigations. The sharp increase in the intensity of ADP-induced platelet aggregation illustrates activation of platelet degranulation. These data show that oxidized fibrinogen has a dysregulatory effect on platelets.

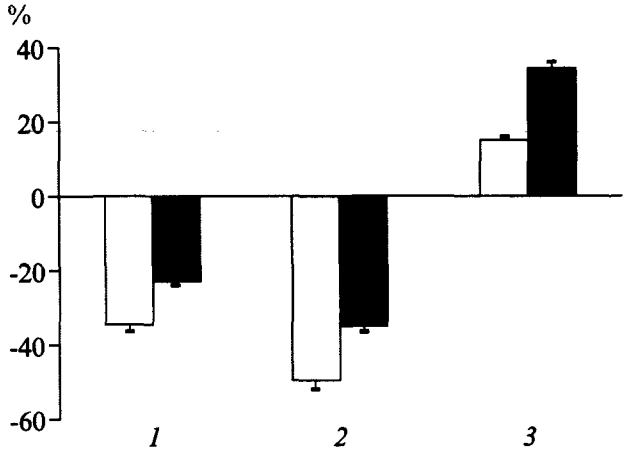

Fig. 2. Platelet aggregation induced by ristocetin (*1*), collagen (*2*), and ADP (*3*) in the presence of fibrinogen oxidized by 10 (light bars) and 20 % (dark bars).

It can be hypothesized that oxidized FG has a direct effect or mediates free radical attack on receptor regions in the phospholipid membrane of platelets. Under these conditions oxidized FG modifies membrane structures of platelets, which violates intracellular signal transduction and formation of fibrinogen bridges between cells. Other mechanisms underlying the effects of oxidized FG cannot be excluded.

The influence of oxidized FG on the blood coagulation system depends on the degree of oxidation. Since the concentration of FG in samples differed insignificantly, the observed changes primarily depended on the degree of FG oxidation. The action of oxidized FG was similar to the effect of oxidized low-density and very-low-density lipoproteins on the blood coagulation system [2,3].

Fibrinogen with oxidation degree of 10% produced different effects, which was probably compensated by the pool of natural antioxidants. By contrast, highly oxidized FG violated all pathways of blood coagulation. Published data show that similar changes in hemostasis accompany pathological hyperactivation of disseminated intravascular blood coagulation [6].

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