Oxidatively Modified Fibrinogen Modulates Blood Rheological Parameters

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

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We studied the effect of UV-oxidized fibrinogen with oxidation degrees of 10 and 20% on rheological parameters of the blood. The effect of fibrinogen with 10% oxidation degree was moderate and variable, which attests to its partial compensation with the pool of natural antioxidants. The effect of fibrinogen with 20% oxidation degree was more pronounced. It dramatically decreased deformability of erythrocytes, delayed formation of linear aggregates, accelerated formation of 3D-aggregates, enhanced the total hydrodynamic strength of aggregates, but decreased stability of the largest aggregates. It did not increase plasma viscosity, but enhanced viscosity of the blood at all shear rates. At both degrees of oxidation, suspension stability of the blood decreased, the Caisson viscosity did not change, and the difference between the values of Caisson and asymptotic viscosities markedly increased. On the whole, oxidative fibrinogen produces negative changes in blood rheological parameters, and its effect depends on the degree of oxidation.

Key Words: *oxidized fibrinogen; rheological parameters; erythrocyte deformation*

The free radical processes and oxidative stress [10] play an important role in the development of atherosclerosis and most prevalent cardiovascular diseases such as CHD and hypertension. Oxidative modification of LDL is the key factor in atherogenesis [5]. Apart from lipoproteins, blood fibrinogen (FG) undergoes free radical oxidation and is the most oxidizable plasma protein [9].

FG is an independent risk factor of the development of atherosclerosis and its complications [7]. High level of FG in patients with established coronary disease aggravates the course of the disease [6]. High content of FG correlates with increased blood viscosity [8]. The role of FG in erythrocyte aggregation and in the formation of blood rheological properties is well documented [1].

However, we found no papers on the effect of oxidized fibrinogen (OFG) on rheological parameters of the blood. Our aim was to study the effect of OFG on these parameters.

MATERIALS AND METHODS

Experiments were carried out on the blood of healthy donors (hematocrit 40.4±0.22%) stabilized with heparin microdoses.

Human plasma FG (Sigma) was used in the study. FG (3 mg/ml phosphate-saline buffer, pH 7.4) was subjected to oxidative modification by UV-irradiation using a DRK-120 ultrahigh pressure arc mercury lamp with natural cooling (working current 1.2 A). Oxidative modification of FG was assessed by the drop in tryptophan fluorescence of the protein. FG solution (100 μl) was placed into a cuvette and diluted to 4 ml with distilled water. Fluorescence was measured on an LS50 (Perkin-Elmer) spectrofluorimeter at excitation wavelength of 340 nm and emission wavelength of 340 nm. Two solutions of OFG with different oxidation degree were used: 10% (OFG-10) and 20% (OFG-20).

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

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OFG (0.5 ml) was added to tubes with 10 ml heparinized blood, stirred, and immediately analyzed. The control samples were drawn from healthy donors.

The macrorheological studies included measurements of viscosity on an AKR-2 rotation viscosimeter (shear rate ranged from 5 to 300 sec^{-1}), assessment of suspension stability, and calculation of Caisson viscosity and fluidity limit.

Aggregation of erythrocytes was examined with an ADE-5 automated erythrocyte aggregometer by measuring light backscattering (λ=630 nm) from Couette blood flow with a thickness of 1 mm. This approach made it possible to assess the time of linear (T_1, sec) and 3D (T_2, sec) aggregate formation, the final size of aggregates (Ampl), the total hydrodynamic strength of aggregates (β, sec^{-1}), and the strength index of the largest aggregates.

Deformability of erythrocytes was studied using the method of rigidometry. We also used rating methods to assess aggregation and deformability of erythrocytes by the corresponding indices of aggregation and deformability of red cells [4].

The hemorheologic indices were compared to the control values taken as zero.

The results were analyzed statistically using the nonparametric Mann's, Kruskal's, and the χ^2 tests. The differences were significant at $p<0.05$.

RESULTS

The effect of OFG-10 manifested in a pronounced decrease in erythrocyte deformability ($p<0.05$), which evidently resulted from modification of physicochemical structure of the membranes: increase in lipid phase viscosity and decrease in elasticity and mechanical strength (Fig. 1).

At the same time, the processes of erythrocytic aggregation and disaggregation also changed: T_1 and T_2 increased, while Ampl decreased ($p<0.05$). While the total hydrodynamic strength of the aggregates (β) decreased, the largest aggregates became more tolerant to shear destruction. Probably, the pool of natural antioxidants partially eliminated the effect of OFG.

The effects of OFG-20 somewhat differed from those of OFG-10 and were more pronounced. OFG-20 drastically decreased erythrocytic deformability (by 75%, $p<0.05$). T_1 increased by 37% ($p<0.05$), although T_2 decreased. The parameter β of aggregates increased, although the largest aggregates became less resistant to shear-caused destruction ($p<0.05$). Overall, OFG-20 exerted a negative effect on microrheological characteristics of the blood, because it accelerated the formation of middle-size 3D-aggregates resistant to hydrodynamic destruction. In living organism, these changes frequently promote disturbances of blood microcirculation due to occlusion of microvessels with these cell aggregates.

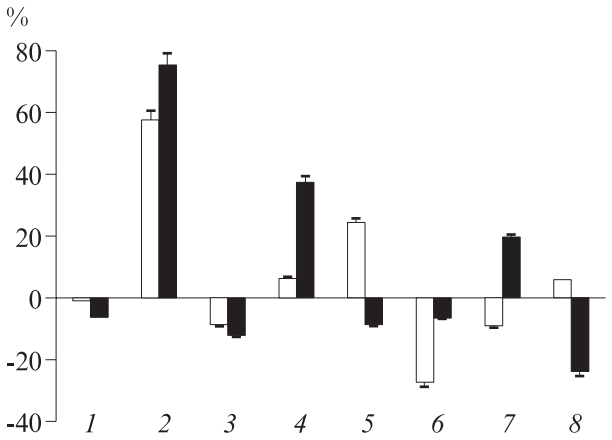

Fig. 1. Effect of fibrinogen with 10% (open bars) and 20% (filled bars) oxidation degree on microrheological properties of the blood. *1*) erythrocyte deformability index; *2*) erythrocyte rigidity index; *3*) erythrocyte aggregation index; *4*) rate of linear aggregate formation; *5*) rate of 3D-aggregate formation; *6*) aggregate final size; *7*) total hydrodynamic strength of aggregates; *8*) hydrodynamic strength of the largest aggregates.

OFG-10 produced no significant changes in blood and plasma viscosity at all values of shear rate (Fig. 2). Similarly, OFG-20 did not change the plasma viscosity, although it increased the blood viscosity at all shear rates ($p<0.05$).

In both cases, OFG decreased suspension stability of the blood. In addition to the observed changes in erythrocytic aggregation/disaggregation, these data suggest that OFG molecules are not entirely involved in the formation of cell aggregates. Our data are more consistent with the depletion model [11] than with the routine views on the bridge mechanism of erythrocyte

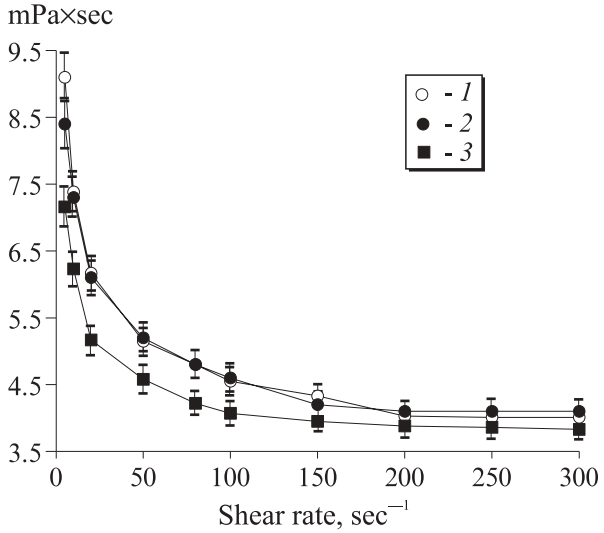

Fig. 2. Effect of oxidized fibrinogen on blood viscosity. *1*) control; fibrinogen with 10% (*2*) and 20% (*3*) oxidation degree.

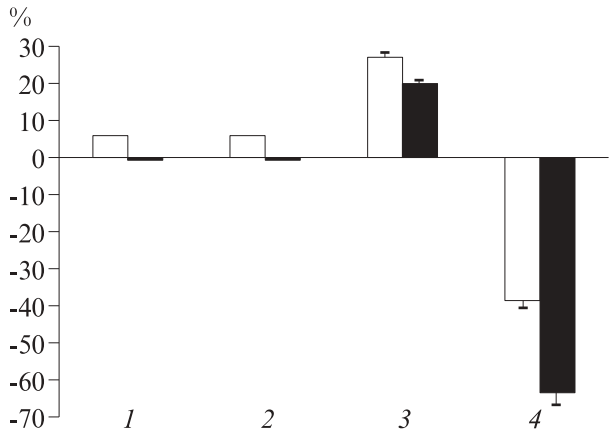

Fig. 3. Effect of fibrinogen with 10% (open bars) and 20% (filled bars) oxidation degree on macrorheological properties of the blood. *1*) plasma viscosity; *2*) Caisson viscosity; *3*) difference between asymptotic and Caisson viscosities; *4*) fluidity limit.

aggregation. However, this hypothesis needs further verification.

It is noteworthy that OFG virtually did not change Caisson viscosity, but considerably increased the difference between this parameter and asymptotic viscosity ($p<0.05$, Fig. 3).

The latter observation suggests that erythrocyte membrane is the major target for OFG. It this respect, this effect of OFG is similar to the effect of oxidized LDL and VLDL on rheological properties of the blood [2,3,12]. However, the prevailing effect was a decrease in suspension stability of the blood, as indicated by diminished blood fluidity limit in both experiments.

It can be concluded that the effects of OFG on rheological properties of the blood are related to the degree of its oxidation. Probably, this parameter is the most important for the development of the disclosed changes, because in our experiments the difference in OFG concentrations was minimum.

The effect of OFG-10 was variable, which probably resulted from partial compensatory action of the pool of natural antioxidants. By contrast, the effect of OFG-10 on all rheological properties of the blood was negative.

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