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Hyperfibrinogenemia and Increased Stiffness of Plasma Clots in the Active Systemic Lupus Erythematosus

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Abstract Systemic lupus erythematosus (SLE) is an autoimmune disease associated with an increased risk of thrombosis. We hypothesized that inflammation-associated hyperfibrinogenemia can contribute to the prothrombotic phenotype of fibrin clots by changing their mechanical properties. Twenty-eight SLE patients were categorized based on their disease activity scores (SLEDAI) into the groups with inactive (SLEDAI < 4, n = 14) and active (SLEDAI > 4, n = 14) forms of the disease. Clots from individual platelet-free plasma samples were probed using shear rheometry and viscoelastic properties of the fibrin gels were determined as the storage (G') and loss (G'') moduli. A significant increase of G' was revealed in the clots from the plasma of active SLE patients over inactive SLE, which correlated with elevated fibrinogen levels. Clots from the plasma of inactive SLE patients had the elasticity and fibrinogen levels indistinguishable from those in control plasma from healthy subjects. Thus, inflammatory hyperfibrinogenemia in the active SLE form makes fibrin clots stiffer which has been previously shown to be associated with a higher incidence of thrombotic disorders.

Keywords Systemic lupus erythematosus · Fibrin · Shear rheometry

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1 Introduction

In systemic lupus erythematosus (SLE), a multiorgan autoimmune disease, thrombosis makes an important contribution to mortality and long-term morbidity [1]. Although thrombotic complications are relatively common in SLE, mechanisms underlying the SLE-related thrombophilia remain unclear. A network of fibrin fibers is a major component of hemostatic blood clots and obstructive thrombi, which is responsible for their mechanical strength, integrity, and deformability in a highly dynamic environment of flowing blood [2]. Abnormal mechanical properties of fibrin clots have been shown to be associated with a higher risk of thrombosis and thromboembolism [3]. Clot mechanics depend strongly on the variable structure of fibrin that is largely determined by many environmental factors, including a level of fibrinogen, the soluble fibrin precursor in blood [4]. Because fibrinogen is an acute-phase protein that is up-regulated in response to inflammation [5], it is likely to be a pathogenic modulator of the structure and mechanical properties of clots and thrombi. All these provide a rationale for studies on the link between fibrinogen levels in blood, rheological properties of blood clots, and predisposition to thrombosis in SLE. Here we investigated the mechanical features of fibrin clots and their association with fibrinogen levels in SLE patients with respect to the disease activity.

2 Material and Methods

The study was approved by the Ethical Committee of Kazan State Medical University. Twenty-eight consecutive patients who met the revised criteria for SLE of the American College of Rheumatology [6] were enrolled in the study. Twenty-three (82%) of them were females and the mean age of the patients was 37 ± 13 years. The control group comprised 10 age- and

sex-matched healthy individuals. The disease activity was determined by the SLE Disease Activity Index (SLEDAI). Based on the score the patients were categorized into two groups: 14 patients with SLEDAI higher than 4 comprised the "active SLE" group and 14 patients with SLEDAI below 4 formed the "inactive SLE" group.

Citrated venous blood samples were centrifuged at room temperature for 15 min at 1500 g and 5 min at 10,000 g to obtain platelet-free plasma that was dispensed into aliquots and stored at − 80 °C until use. Fibrinogen was measured by clotting 400 μl of platelet-free plasma with $CaCl_2$ at 24 mM and thrombin at 5 U/ml (both final concentrations) at 37 °C. After 15 min, the clot was washed with a physiological buffer, then blotted and dissolved in 0.1 N NaOH with heating. The optical density was read at 280 nm in a Shimadzu UV-1800 spectrophotometer. Taking the specific absorption index of fibrin(ogen) equal to 1.51 for a solution with a concentration of 1 mg/ml in a 1-cm cuvette, calculations were done according to the following formula: $C = (A_{280}/1.51) \times T \times P$), where C is an actual fibrin concentration, A_{280} is the measured absorbance at 280 nm, T is a beam path or width of the cuvette (cm), and P is the multiplicity of the dilution of the sample. The amount of fibrin was equal to the amount of fibrinogen clotted in the plasma sample.

A strain-controlled rotational rheometer (DHR-2, TA Instruments, USA) was used to measure viscoelastic properties of fibrin clots. 150 μl of plasma was activated with cephalin at 1 μg/μl and calcium chloride at 24 mM (both final concentrations) and placed in the measurement gap of the rheometer at 37 °C. The sample polymerized between the plates for 30 min, while the edge was sealed with mineral oil to prevent sample drying. Measurements were done in an oscillatory shear mode using a 3% strain and 5 rad/s frequency. The extracted viscoelastic parameters were the following: a storage modulus (*G'),* reflecting reversible mechanical deformation or stiffness; a loss modulus (*G''),* characterizing slow irreversible deformation or plasticity; a loss tangent *tan* $\delta = G''/G'$ which is a measure of a relative elasticity vs. plasticity or energy loss against energy stored during deformation. The parameters were recorded for 1 min, the time interval sufficient to stabilize the measured parameters. The rheometer was equipped with a TRIOS V2.0 software (TA Instruments) used for data processing.

All experimental data were analyzed by the unpaired Student's *t* test using Excel Microsoft software package. Data are shown as a mean ± standard deviation, and *P* values are reported as follows: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

3 Results and Discussion

Significant differences in mechanical properties were revealed between clots prepared from the blood plasma of SLE patients and control clots from the plasma of healthy subjects.

The most important variations in viscoelastic characteristics were found in clots from the blood of SLE patients with distinct degrees of the disease activity (Fig. 1). In the patients at exacerbation and with a higher index of disease activity

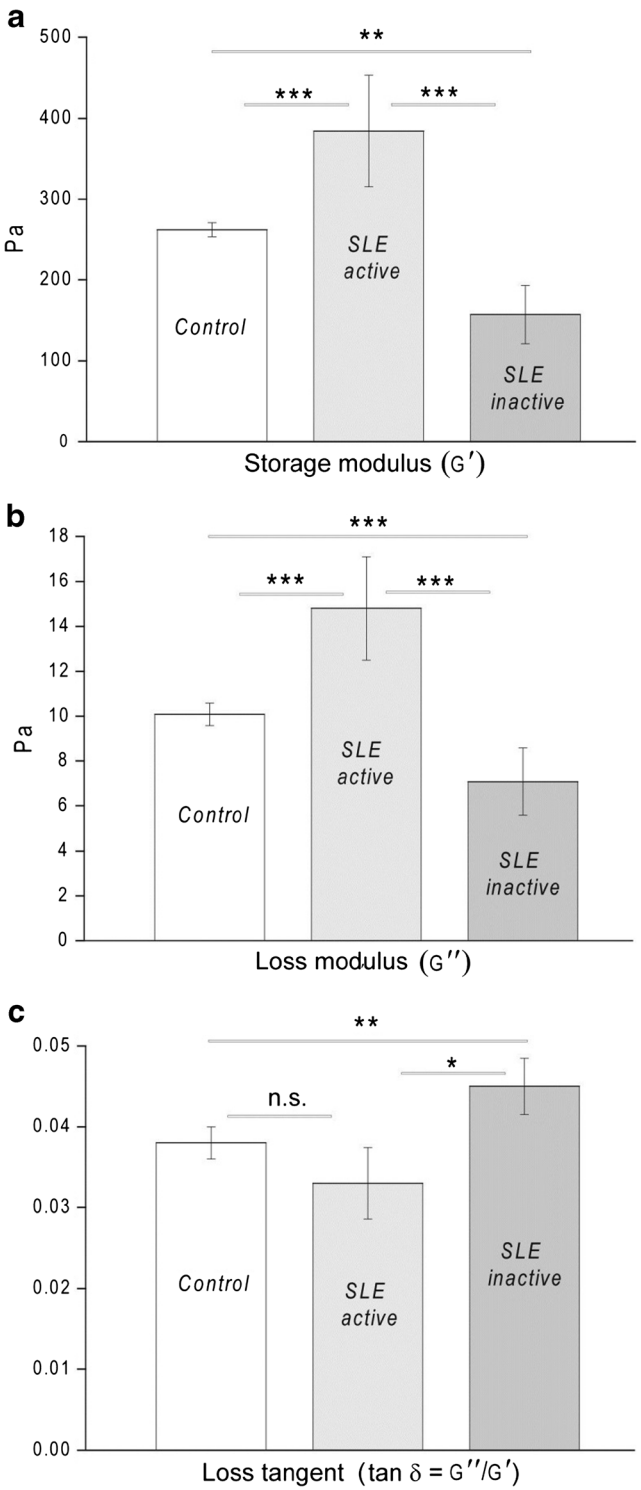

Fig. 1 Viscoelastic properties of plasma clots in the blood of patients with SLE in the active ($n = 14$) or inactive ($n = 14$) forms and of healthy subjects ($n = 10$). Average storage modulus, G' (**a**), loss modulus, G'' (**b**) and loss tangent (**c**)

(SLEDAI > 4), the fibrin clots were characterized by a significant rise of the average storage modulus G' up to 384 ± 69 Pa compared to the average value of 157 ± 36 Pa ($P < 0.001$) in patients with inactive SLE (SLEDAI < 4) and 262 ± 9 Pa ($P < 0.001$) in control, reflecting an increase of clot stiffness associated with disease severity (Fig. 1a). Changes in the loss modulus G'' followed the same trend and the values were significantly higher in the clots from the blood of patients with active SLE compared to inactive SLE and control (14.8 ± 2.3 Pa versus 7.08 ± 1.5 Pa and 10.08 ± 0.5 Pa, respectively, $P < 0.001$) (Fig. 1b). The clots showed no differences in the loss tangent (G″/G′) values between the active SLE and control (0.033 ± 0.004 versus 0.038 ± 0.02, respectively, $P > 0.05$) (Fig. 1c), implying that there was no change in the elasticity to plasticity ratio. In comparison with inactive SLE, the loss tangent values were significantly lower in active SLE (0.045 ± 0.009 versus 0.033 ± 0.004, respectively, $P < 0.05$), indicating that fibrin clots from active SLE patients are more stiff than those from inactive SLE and less prone to deformation.

Taken together, these results indicate that in the blood of patients with an active and more severe form of SLE, blood clots are stiffer than in patients with inactive SLE or healthy subjects that makes them mechanically and chemically stable and hence prothrombotic. Importantly, in 14.3% of the patients with active SLE, various clinical types of thrombosis were observed during the course of disease.

In the inactive SLE patients that did not have thrombotic complications in the history, both the storage and loss moduli were significantly smaller than in active SLE and control (Fig. 1a, b). The loss tangent values were significantly higher compared to the active SLE ($P < 0.05$) and control ($P < 0.01$) (Fig 1c) characterizing more plastic clots in the blood of inactive SLE patients, meaning that they dissipate more energy during irreversible deformation and are more deformable. Thus, in the blood of patients at remission and with minimal activity of the disease, softer fibrin networks were formed than in healthy individuals and active SLE patients.

To explain the revealed variations in viscoelasticity of plasma clots, we analyzed fibrinogen levels as the most likely determinant of fibrin mechanical properties. The average concentrations of thrombin-clottable fibrinogen in the plasma of patients with active SLE were significantly higher than in healthy controls (3.8 ± 1.2 g/l versus 2.6 ± 0.05 g/l, respectively, $P < 0.05$), while in patients with inactive SLE fibrinogen levels were the same as in control (2.5 ± 0.7 g/l and 2.6 ± 0.05 g/l, $P > 0.05$). Fibrinogen levels showed a positive correlation with G' ($R = 0.6, P < 0.01$) and G″ ($R = 0.5, P < 0.05$) in SLE patients (Fig. 2).

A higher level of fibrinogen, an acute-phase protein, at exacerbation of SLE reflects the systemic inflammatory process and at the same time serves as a predictor or marker of thrombosis. One of causative relations of the elevated fibrinogen

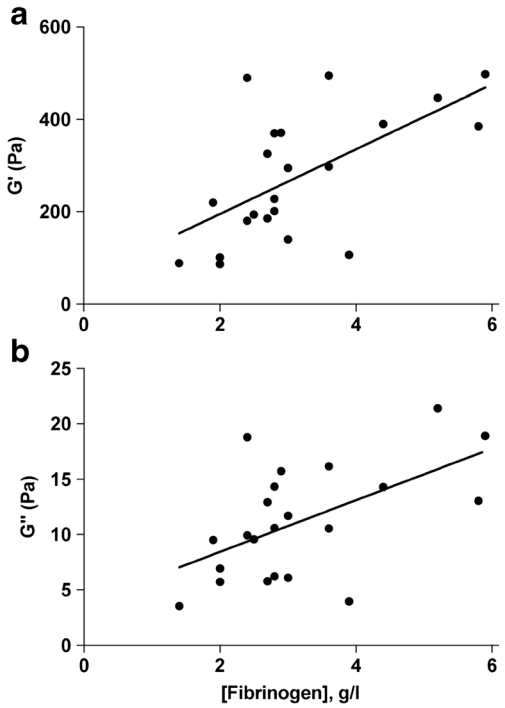

Fig. 2 Correlation plots between fibrinogen levels versus the storage modulus G' (**a**) and the loss modulus G'' (**b**) of fibrin clots in the blood plasma of SLE patients

concentration in blood to the pathogenesis of thrombosis is providing more substrate for fibrin formation and inducing abnormal architecture of fibrin networks. There is a direct relation between a fibrinogen level and fibrin clot structure with resulting changes in clot elasticity and plasticity [7, 8]. Greater fibrinogen concentrations change fiber thickness, fiber, and branch point densities and enhance clot rigidity [9]. Stiffer fibrin networks cannot properly accommodate the hydrodynamic shear flow by undergoing appropriate stress-induced structural rearrangements. In addition, platelets spread on stiffer fibrin fibers or adhered to a stiffer network have a higher degree of activation [10]. These hardened fibrin structures observed in hyperfibrinogenemia also have reduced susceptibility to fibrinolysis rendering clots and thrombi predisposed to persistent vessel obstruction and blood flow impairment, thus making rigid clots generally associated with thrombosis [3].

4 Conclusions

Fibrin clots from the blood of SLE patients in the active form of the disease differ significantly from the clots formed in the blood of SLE patients in the inactive form and in normal plasma. In the active SLE, clots are stiffer or mechanically stable, likely due to an increased level of fibrinogen, an acute-phase protein hyper-produced in response to inflammation. Our results strongly suggest that exacerbation of SLE is associated with higher levels of fibrinogen that promote

formation of stiffer fibrin networks predisposing patients to a high risk and less favorable course of thrombotic complications in SLE.

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