Effect of Fibrin Polymerization on Flow Properties of Coagulating Blood

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Accepted in final form 28 March 1997

Abstract. The evolution of stress in coagulating blood is described by a Maxwell-like constitutive model. The evolution is essentially affected by conversion of fibrinogen to fibrin monomer, fibrin polymerization and its crosslinking. The modifying effects of the process result from the active constituents of blood plasma, blood cell concentration and intensity of flow. The interrelation of stress evolution and the kinetics of polymerization determines these effects in terms of the order of the kinetics of polymerization and degree of fibrin polymerization and values of the constitutive coefficients. The results are presented for normal blood, the average of the effect of blood constituents and blood platelets on coagulation.

Key words: Blood coagulation, Fibrin polymerization, Blood rheology

1. Introduction

The process of blood coagulation and formation of a blood clot is a complex biochemical phenomenon consisting of enzymatic chain reactions of plasma constituents affected by endothelial and blood cells. The process is initiated when the balance of anticoagulant and procoagulant mechanisms is lost. The initiation may originate from mechanical injuries and tissue releases but also from the impediment of blood flow or alternations in the blood composition.

The clot formed in the blood vessel is an obstacle for flow and thus is a resistance to a mechanical load induced by flowing blood. Consequently, the clot deforms and may fracture when deformation exceeds a critical limit. Rigidity of the clot thus play a significant role both in cases where clot modifies and even blocks blood flow, as well as in cases in which there is the fracture of the fibrin network and disintegration of the clot.

Conversion of fibrinogen to fibrin and covalent cross-linking of fibrin polymer by the transglutaminase Factor XIII plays a pivotal role in blood clot formation [1]. Modifying the effects of the process resulting from the active constituents of blood plasma, blood cell concentration and intensity of flow then form clots of various structures and thrombogenic properties. These properties may be evaluated in terms of the kinetics of clot formation and the variation of blood flow properties during coagulation. Thus changes of the above mentioned modification effects and their

consequences may be studied in detail throwing light on the biochemical events, the role of cellular elements and the effects of flow.

A rotational viscometer proved to be suitable for studying the above stated aspects of blood coagulation and clot formation. The initiation of coagulation in a rotational viscometer enables the monitoring of the evolution of the stress response to shearing [2] and thus variation of blood viscosity in the course of fibrin polymerization and cross-linking, as well as the measurement of the viscoelastic properties of the fully formed clot [3]. In the process of polymerization, the molecular weight increases and at the stage of crosslinking and forming of a three-dimensional network, the blood transforms from a liquid to a porous solid. A liquid-to-solid transition leads to adhesion of the clot to the wall and eventually to the breaking up of the solidity of the clot. In the first case, the imposed external forces are small enough so that clot deformation corresponds to deformation of an elastic solid. When the external forces increase successively later, the induced elastic strain exceeds a critical level and the fibrin network breaks up. The rupture of network fibres is an irreversible process. When broken up, the network loses the ability to form again the original crosslinked structure.

The rheological behaviour of normal blood and coagulating blood corresponds to the behaviour of a viscoelastic liquid. However, the response to deformation is different, depending on the organization of the inner structure and the bonds between structure units. When only interparticle forces, due to a natural tendency of red blood cells (RBC) to aggregate, are affecting viscous and elastic deformation, the breaking up of the structure by shearing is easy and imposed shear stress is of the order of the magnitude of several mPa [4]. During fibrin polymerization and cross-linking the stress increases up to hundreds of Pa [2, 3]. Though blood rheological behaviour is generally viscoelastic, the constitutive properties change significantly.

The aim of this paper is to analyze and describe the evolution of shear stress during blood coagulation in steady shear flow affected by the kinetics of fibrin polymerization and crosslinking. The use of this description for evaluation of the coagulating process of different blood samples, both normal and pathological, may provide parameters suitable for sample comparison.

2. Theory

The evolution of stress of a viscoelastic material can be described by a Maxwell-like equation

$$\boldsymbol{\tau} + \lambda(\xi)\boldsymbol{\tau}_{(1)} = 2\eta(\xi)\mathbf{D}, \quad (1)$$

where $\boldsymbol{\tau}$ is the stress tensor and $\boldsymbol{\tau}_{(1)}$ its convected time derivative [5], $\mathbf{D}$ denotes the rate of the deformation tensor, η the viscosity and λ the relaxation time, and which are considered to be dependent on the internal structure parameter ξ which is, in general, a function of position and time. Introducing ξ into Equation (1), it

is proposed that ξ conveys all structural information and governs the rheological properties of blood. When the rheological effects of flowing blood are described by Equation (2), ξ is the variable which describes the state of red blood cell aggregation [6–8]. The evolution for ξ is proposed in the form of the reversible kinetic equation,

$$d\xi/dt = k_A(1 - \xi) - k_D\xi, \quad (2)$$

where k_A and k_D are the rate constants for RBC aggregation and disaggregation, respectively.

When blood is coagulating and consequently fibrin is polymerizing, the internal structure parameter ξ may represent the concentration of network segments, i.e., the concentration of macromolecular chains joining two network junctions. Since fibrin polymerization is an irreversible process, the r th order irreversible kinetic equation is assumed to describe the evolution of fibrin polymerization,

$$d\xi/dt = k_A^*(1 - \xi)^r. \quad (3)$$

where k_A^* is the rate constant of fibrin polymerization.

For steady shearing flow to which blood is subjected during experimental coagulation, the closed form solution of the system of Equations (1) and (4), describing stress evolution, is

$$\tau_{12} = 2\eta(\xi)D_{12}, \quad (4)$$

$$\xi = 1 - [k_A^*(r - 1)(t - t_0) + 1]^{1/(1-r)}. \quad (5)$$

The initial condition is $\xi = 0$ at $t = t_0$ where t_0 is the initial time of fibrin polymerization and crosslinking, i.e., the time of the previous process of conversion of fibrinogen to fibrin monomer.

3. Experimental

Materials. Fresh human blood in healthy donors (Etablissement de Transfusion Sanguine, Nancy) was used for the experiments. The blood was withdrawn by venipuncture, anti-coagulated with sodium citrate and its concentration of red blood cells was adjusted. Platelet concentration was determined by using a Coulter counter. The average value for 5 blood samples was 20.8×10^4 platelets/ml. Average fibrinogen concentration in plasma was 3.1 g/l. To initiate coagulation, an aqueous solution of 2% calcium chloride was added to samples (0.1 ml of solution to 1 ml of sample).

Methods. A rotational viscometer Contraves LS 30 with coaxial cylinders was used for the measurement of viscosity of coagulating blood. The measurement of viscosity was commenced immediately after the addition of the recalcifying agent

Figure 1. The effect of the concentration of red blood cells on growth of shear viscosity in shear flow at shear rate 0.0175 sec. Experimental data are denoted by symbols, the predictions, Equation (7), by lines.

to blood, rapid mixing and placing the sample in the instrument. The structure changes in coagulating blood owing to polymerization of fibrin macromolecules was monitored by a light back scattering technique in the erythro-aggregometer (Regulest Co., Florange, France) (see [9, 10]). The changes were evaluated in terms of the non-dimensional light intensity ϕ defined $\phi = 1 - (r - r_0)/(r_{\max} - r_0)$, where r_0 and $r_{\max}$ are minimum and maximum measured intensities, respectively. All experiments were carried out at 37° C.

4. Results

Typical experimental data denoted by the symbols in Figs. 1 and 2 were compared with the results of the description introduced in Section 2. The viscosity dependence on structural organization in Equation (5), $\eta(\xi)$, is assumed to be of the form

$$\eta(\xi) = \eta_0 \exp(a\xi), \quad (6)$$

where η_0 is the viscosity of normal non-coagulated blood and a is a parameter.

The rate constant in Equation (5), k_A^* , is considered to be affected by the value of a critical viscosity η_c . This critical viscosity value corresponds to the degree of conversion ξ_c at time t_0 when the fibrin network starts to break up. The values η_c and t_0 are determined from the experimental data presented in Figures 1 and 2. Determination of a critical degree of change transition, ξ_c , requires supplementary measurement of the time dependence of the structural change of coagulating blood by the erythro-aggregometer.

The results of the structural change measurement are shown in Figure 3 as the time dependent variation of the back scattered light intensity from the blood sample confined in the gap between cylinders of the erythro-aggregometer. Though the

Figure 2. The effect of the intensity of flow on growth of shear viscosity of coagulating blood for a concentration of red blood cells 0.40. For symbols and lines, see Figure 1.

shear rate 5 sec is the minimal possible one for the instrument, it is well above shear rates affecting coagulation processes presented in Figures 1 and 2. However, for blood of the same concentration of red blood cells (0.40), the intensity of flow affects above all the initial time t_0 of fibrin polymerization, i.e., time of the process of conversion of fibrinogen to fibrin monomer, as can be seen from Figure 3. The time of polymerization, i.e., the time to form the stage when light intensity starts to increase up to the maximum value, is for coagulating blood at rest 16 min and 12.5 min for blood in shear flow of intensity 5 sec. The difference is approximately 20%. Thus it may be considered that the kinetics of fibrin polymerization and crosslinking in flows with intensity lower than 5 sec can be approximated by the kinetics manifested in blood subjected to flow with intensity 5 sec. Consequently, the degree of polymerization at the critical time t_c is $\xi_c = 0.05$, the initial time is $t_0 = 342$ sec, the critical time $t_c = 420$ sec and the critical viscosity $\eta_c = 22$ Pas at shear rate 0.2/sec; $\xi_c = 0.16$, $t_0 = 375$ sec, $t_c = 505$ sec and $\eta_c = 132$ Pas at 0.032/sec and $\xi_c = 0.45$, $t_0 = 385$ sec, $t_c = 665$ sec and $\eta_c = 225$ Pas at 0.0175/sec.

It follows from the data presented in Figure 1 for equal intensity of flow and different concentration of red blood cell, when normal physiological values are 0.40–0.45, the concentration affects primarily the initial time of fibrin polymerization and crosslinking. The initial time is $t_0 = 140$ sec, the critical time $t_c = 315$ sec and the critical viscosity $\eta_c = 228$ Pas (RBC concentration 0.2); $t_0 = 385$ sec, $t_c = 665$ sec and $\eta_c = 255$ Pas (RBC concentration 0.40); $t_0 = 455$ sec, $t_c = 795$ sec and $\eta_c = 235$ Pas (RBC concentration 0.60). On the other hand, nearly the same values of critical viscosity suggests that the polymerization is also of the same degree. Consequently, it follows from experiments, Figure 3, that $\xi_c = 0.45$ for all samples shown in Figure 1 irrespective of different red blood cells concentrations.

Figure 3. The experimental data of the time dependent variation of back scattered light intensity from aggregating blood at rest and coagulating blood at rest and shear flow, shear rate 5/sec, concentration of red blood cells 0.40.

Introducing the specified values of t_0 , t_c , η_c and ξ_c , together with the viscosity of noncoagulated normal blood $\eta_0 = 4$ mPas, when its shear rate dependent variation can be neglected, to Equations (4) and (6), the following values of the constitutive parameters determining kinetics of the polymerization are obtained. In relation to the data in Figure 1, the parameters are in case of blood coagulation in shear flow of intensity 0.0175/sec and RBC concentration 0.20, $k_A^* = 0.02$, $r = 6.04$ and $a = 24.4$; RBC concentration 0.40, $k_A^* = 0.005$, $r = 3.2$ and $a = 24.6$; RBC concentration 0.60, $k_A^* = 0.003$, $r = 2.90$ and $a = 24.4$. In relation to the data in Figure 2, the parameters are with respect to the intensity of flow 0.2 sec, $k_A^* = 0.001$, $r = 32.7$ and $a = 172.5$; intensity of flow 0.032/sec, $k_A^* = 0.003$, $r = 9.4$ and $a = 65.05$; intensity of flow 0.0175/sec, $k_A^* = 0.005$, $r = 3.2$ and $a = 24.6$. As follows from the calculations, the order of the kinetic equation for fibrin polymerization and crosslinking in which we are interested, besides the evaluation of factors such as ξ_c and η_c , is increasing with decreasing concentration of red blood cells and with increasing intensity of flow in the investigated range. This suggests the mechanism of coagulation in flow which is discussed in the following section.

5. Discussion

The experimental observations were carried out in this study using normal human blood. This means that the effect of active constituents of blood plasma on blood coagulation was not explicitly considered. The effect was averaged using different normal blood samples so that the portions of plasma constituents, as well as blood platelets, are in the normal physiological range. Thus RBC concentration and the intensity of flow of coagulating blood may be varied to show a trend in the effects of these variables on coagulation through simple viscosity measurements. The effect

is specified in the time domain by the initial viscosity η_0 and time t_0 and final critical viscosity η_c and time t_c . The link of viscosity changes with the kinetics of polymerization determines the viscosity function in the time domain as well as the critical degree of polymerization ξ_c and the constitutive characteristics of the kinetics of polymerization, namely, the order of the polymerization r and the rate constant k_A^* .

From the point of view of enzymatic chain reactions during coagulation, it is believed that red blood cells have minimal impact on this process. Thus increased initial time of polymerization t_0 as well as the critical time t_c suggests that red blood cells slow down conversion of fibrinogen to fibrin due to their role as a mechanical obstacle for the contact of blood platelets on which surface the reaction takes place.

It has been found [11] that RBCs cause an increase of the pore size of the fibrin network. Consequently, the number of crosslinks of the fibrin network is decreased and the thickness of fibrin fibres is increased. Relating this result to the rate of polymerization specified by the order r of the kinetics of polymerization and crosslinking, the rate of formation of a network with large pores is lower than one dense with small pores. On the other hand, the conversion is substantially affected by the increasing intensity of flow due to the likely increased frequency of contacts of blood platelets on which surface the conversion takes place.

From the above it follows that the measurement of shear viscosity of coagulating blood and the description of its time evolution enables the quantification of the process not only in the time domain but also by the parameters k_A^* and r of the kinetics of polymerization and formation of the fibrin network which is a base of blood clots. The description of polymerization thus provides the parameters suitable for the comparison of different samples. Its use may contribute to characterization of blood coagulation in both normal and pathological blood samples.

However, the kinetics of coagulation is only one aspect of the coagulation process. Another is, for example, the efficiency in preventing blood loss, as well as the elasticity and deformability of the clot. The clot formed in the blood vessel is an obstacle for flow and has to resist a mechanical load induced by flowing blood. Consequently, the clot deforms and may fracture when deformation exceeds a critical limit. Our recent preliminary measurements revealed that the maximal physiological stress, which is about 6 Pa at normal conditions and about 40 Pa at pathological ones [12], is approximately 10 times lower than necessary for the rupture of the clot. Thus only inhomogenities and/or a disturbance in the complex network of the procoagulant factors, which may decrease clot elasticity as well as deformability and adhesion of the clot to the vessel wall, can lower the disruptive stress level. The inhomogenities and disturbance may be of a different origin, associated, for example, with the platelet quantity or disorders due to their activation and, consequently, may result in hemostatic defects.

Acknowledgments

The authors wish to thank the Program of the Scientific Cooperation BARRANDE. Our thanks are also expressed to a referee for helpful commentary and for drawing our attention to the work on clinical aspects of coagulation.

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