CREATION OF A HIGH FIBRINOLYTIC POTENTIAL IN THE VICINITY OF A THROMBUS WITH THE AID OF Fc-RECEPTORS OF STREPTASE-LOADED LEUKOCYTES

N. N. Tsybikov, B. I. Kuznik,
A. N. Lozhkina, and A. V. Kradenov

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Normalization of hemodynamic homeostasis in thrombosis is most easily achieved by the creation of a high fibrinolytic potential in the vicinity of the fibrin clot, which can be done by the use of large doses of fibrinolytics, including streptokinase, streptase, plasmin, brinase, etc. However, this method of thrombolytic therapy is restricted by a number of side effects associated with high toxicity of the preparations, their allergizing action, and the rapidity of their inactivation and elimination from the blood stream. Despite definite progress in the field of thrombolytic therapy, associated with the introduction of the new enzyme preparation streptodecase [3], the urgency of the problem still remains as high as ever. At the same time, we know that leukocytes have high fibrinolytic potential [1, 2] and that they contain unique fibrinolytic proteases [6], capable of interacting through Fc-receptors with immune complexes, and with aggregated and native immunoglobulins, and of releasing lysosomal enzymes by exocytosis [7-9, 11].

The writers have used the above-mentioned properties of leukocytes to develop a new method of creating a high local fibrinolytic potential.

This paper gives the results of experiments conducted *in vitro* and using the suggested method.

EXPERIMENTAL METHOD

Leukocytes were isolated from citrated donors' blood by the dextran method. Contaminating erythrocytes were lysed with 0.84% NH_4Cl and removed by washing twice with buffered physiological saline. The washed cells were transferred to medium 199 ($2 \cdot 10^7$ cells to 1 ml of medium) and incubated for 1, 3, 5, 10, and 20 min (37°C) with streptase (from Behringwerke, West Germany) with final activity of 100 U in 0.1 ml of cell suspension. Contact between leukocytes and streptase was interrupted by 20-fold dilution with cold medium 199 and sedimentation of the cells in a refrigeration centrifuge. The absolute quantity of streptase taken up by one leukocyte was calcuated on the basis of specific activity of the enzyme and the area of lysis of fibrin film. Streptase-loaded leukocytes (SLL) were washed off twice with medium 199. Some of the leukocytes thus obtained were incubated with antifibrin serum (from Sevac, Czechoslovakia) in a dilution of 1:100 at 37°C for 10 min, and then washed off with medium 199. The fibrinolytic activity of intact leukocytes, SLL, and SLL with antifibrin antibodies, and also of the supernatant, was determined by the method in [4]. In another series of experiments a special test system was prepared. Silk threads (No. 2) were kept for 2-3 min in 0.4% solution of human fibrinogen (from the regional blood transfusion station), then transferred for 10-15 sec into 1% thrombin solution (from the Central Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR), and washed off by immersion in buffered physiological saline. The effectiveness of washing was verified by spectrophotometric investigation of the washings. The washing was stopped when the optical density (OD_{280}) reached zero. The fibrin coated threads (FCT) thus obtained were incubated with antifibrin serum at 37°C for 20 min, after which they were washed in running water to remove unreacted components until $OD_{280} = 0$. The FCT treated in this way were added to plasma diluted with medium 199 (1:1, total volume 0.5 ml), containing $1 \cdot 10^7$ SLL or intact leukocytes, and after addition of 0.2 ml 0.277% $CaCl_2$ and clot formation, the rate of lysis of the clot was determined.

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TABLE 1. Changes in Fibrinolytic Activity of Leukocytes and Supernatant Depending on Duration of Incubation with Streptase (M ± m)

Test substrate	Number of observations	Initial data	Duration of incubation with streptase, min				
			1	3	5	10	20
Leukocytes (/mm²)	5	75±9	98±8*	92±5	102±7*	112±10*	88±4
Supernatant (/mm²)	5	369±21	246±19 †	201±19 †	197±12 †	206±14 †	

*P < 0.05; †P < 0.01.

EXPERIMENTAL RESULTS

On incubation of the leukocytes with streptase their fibrinolytic activity increased compared with that of intact cells (Table 1). This effect was exhibited after only 1 min of incubation and it could be detected after 3, 5, and 10 min of the experiment. According to our calculations, one leukocyte takes up $1.7 \cdot 10^{-12}$ g of streptase. If, however, contact between cells and streptase continued for 20 min, the activation effect was lost, evidently because of intracellular degradation of the assimilated enzyme. In the course of incubation the fibrinolytic activity of the "streptase" supernatant decreased, evidence of pinocytosis of the enzyme by the leukocytes and, consequently, of the specificity of the observed effect.

Treatment of SLL with antifibrin serum did not lead to the expected exocytosis of leukocytic enzymes and a further increase in fibrinolytic activity of the cell suspension on fibrin dishes. The reason was most probably that native antifibrin antibodies were not bound significantly, or even at all, with Fc-receptors of the leukocytes. During contact between SLL and fibrin films, exocytosis of the enzymes did not therefore take place.

However, when the experiment was conducted in another test system different results were obtained. The clot formed in plasma diluted with the addition of $CaCl_2$ did not dissolve throughout the period of observation (72 h). In the presence of SLL the clot lysed in the course of 120 ± 20 min ($P < 0.01$). If FCT treated with antifibrin serum were added to this system (plasma + SLL), the duration of lysis was shortened to 35 ± 5 min ($P < 0.01$). A more than a hundredfold increase in the velocity of lysis of fibrin was thus achieved.

What is the mechanism of the effect thus discovered? To answer this question, it must be recalled that in this experimental model FCT consists of fibrin (antigen), immobilized on threads. During incubation with antifibrin serum an antigen–antibody complex is formed directly on FCT. The addition of SLL to this system was accompanied by interaction of the Fc-receptors of the leukocytes with Fc-fragments of antifibrin antibodies and by exocytosis both of lysosomal enzymes and of the pinocytosed streptase, which ultimately stimulated fibrinolysis. The facts discovered demonstrate once again the ability of phagocytic cells not only to interact with immune complexes, but also to release enzymes by exocytosis [7-9, 13].

This method of enhancing the fibrinolytic potential in the immediate vicinity of a thrombus, developed by the present writers, has many advantages over others. Incubation of leukocytes in a solution of streptase leads to activation of endogenous fibrinolytic enzymes. The total fibrinolytic potential of the leukocytes is certainly enhanced by pinocytosed and, possibly, adsorbed streptase. The reason why the method on the whole is promising is as follows. In the first stage leukocytes behave as a transporter of fibrinolytic agents, in the second stage they accumulate in the region of the thrombus, and in the third stage, by releasing enzymes, they increase the rate of lysis of the clot by more than 100 times. Success in the next step, the use of SLL in experiments *in vitro*, will be determined by the obtaining of antifibrin antibodies, hopefully of monoclonal origin. It must be emphasized that the antibodies must distinguish between specific fibrin determinants, and when introduced into the blood stream, they must not interact with fibrinogen. There is no reason to doubt that such antisera and antibodies can be obtained [6, 10].

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SERUM AND LIVER ALCOHOL DEHYDROGENASE LEVELS IN RATS DIFFERING IN ALCOHOL MOTIVATION

M. A. Petrova

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Research in the last decade in the field of enzyme diagnosis of alcoholism has led to the use of enzyme tests as additional diagnostic and prognostic criteria in patients with alcohol-induced lesions of organs and tissues, especially the liver [2]. Research in this direction also encourages the hope that specific biochemical markers of alcoholism will be discovered; enzymes of ethanol and acetaldehyde metabolism and their numerous molecular forms are of great interest from this point of view [6]. With the appearance of new and highly sensitive methods of determining activity of ethanol-metabolizing enzymes in material so readily available as blood [3, 7], on the one hand our ideas on the role of these enzymes in the pathogenesis of alcoholism have been widened and, on the other hand, the need has arisen for the place of these tests in clinical practice to be assessed.

The aim of this investigation was to study alcohol dehydrogenase (ADH; EC 1.1.1.1.) in the blood serum and liver of rats with a preference for drinking ethanol or water, and also in animals during chronic ethanol poisoning and after withholding ethanol.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-250 g, with free choice between water and 15% ethanol solution, were divided into three groups: those preferring water (PW), an intermediate group (IG), and those preferring ethanol (PE), by the method described previously [4]. The IG rats were subjected to chronic alcoholization, with an initial consumption of ethanol solution equivalent to 16-35% of the total quantity of liquid drunk. For 4 months the animals received only 15% ethanol solution to drink, and for the next 11 months the animals were given the choice between water and 15% ethanol solution. At the end of alcoholization, animals whose ethanol consumption had increased to not less than 50% of the total volume of fluid drunk, evidence of the formation of addiction to alcohol, were used in the experiments. Control animals were given water for 15 months.

ADH activity was determined in the blood serum by the method in [7] and in the postmitochondrial supernatant of the liver by the method in [5], and expressed in μ moles NAD/min/liter of serum and μ moles NADH/min/g of liver tissue (subsequently described as activity units), respectively. The histological investigations were carried out in the Department of Biochemistry, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, by Professor V. A. Nagornev.

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