HEMOCOAGULATORY PROPERTIES OF KUPFFER CELLS

D. M. Zubairov, I. A. Andrushko, and V. S. Davydov

UDC 612.115-06:612.135.014.2

Experiments on rabbits have shown that the Kupffer cells possess very low thromboplastic and fibrinolytic activity but high antithrombin activity. Radiation injury leads to a decrease in the thromboplastic activity of the Kupffer cells.

Investigations have shown that the large blood vessels contain a thromboplastic substance which, as recent work in the writers' laboratory has demonstrated, is concentrated in the cell membranes [4].

Despite the fact that it is in the capillaries that the blood is in contact most with the vessel wall, no study of the coagulatory properties of the capillary endothelium has yet been undertaken.

The object of the present investigation was to study some hemocoagulatory properties of the Kupffer cells from sinusoids of the liver and also to examine their changes in the hemorrhagic diathesis developing in radiation sickness.

EXPERIMENTAL METHOD

The method used to obtain Kupffer cells from the rabbit liver was developed on the basis of previous techniques [10, 12, 13].

Rabbits weighing from 2500 to 4000 g were anesthetized with thiopental, laparotomy was performed, and 30 ml of a 15% suspension of iron carbonyl in isotonic NaCl solution containing 5% starch was injected slowly into the portal vein. The liver was perfused 3-6 h after the injection initially through the aorta, and then through the hepatic vein, with an ice-cold solution of 0.25 M sucrose containing 0.01 M EDTA solution, pH 7.4.

Liver tissue, freed from blood, was put through a stainless steel mincer. The homogenate was shaken up with 2 or 3 volumes of perfusion fluid and filtered through silk. The resulting suspension was centrifuged at 1000 rpm for 2 min. The upper, relatively translucent layer, containing injured liver cells and blood cells, was removed. The two remaining layers, an upper layer, blood red in color and containing parenchymatous cells, and a lower grayish-black layer containing reticulo-endothelial cells, were removed separately, resuspended in perfusion fluid, and recentrifuged. This procedure was repeated three times. The reticulo-endothelial cells were then collected in conical tubes, suspended in physiological saline, and placed in a solenoid magnet developing a magnetic field with an intensity of about 1000 Oe. Cells containing iron were attracted to the bottom of the tube.

The cells were inspected through a phase-contrast microscope. Reticulo-endothelial cells contained granules of iron which shadowed the details of the internal structure. The parenchymatous cells formed a frothy fraction consisting of well-preserved cells.

Two series of experiments were carried out on 37 rabbits, in which the platelets and erythrocytes were counted directly in the phase-contrast microscope, the thromboplastic activity of the blood was deter-

Department of Biochemistry and Central Scientific-Research Laboratory, S. V. Kurashov Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 12, pp. 24-26, December, 1970. Original article submitted March 16, 1970.

TABLE 1. Content of Factors Affecting Blood Clotting in Kupffer Cells

Index studied	Control animals	Irradiated animals	Statistical significance of difference between means, P
Thromboplastic activity (in %)	7.84 ± 0.87	1.99 ± 0.39	< 0.001
Antithrombin II (in %)	380.45 ± 24.25	333.45 ± 23.90	< 0.002
Antithrombin III (in %)	72.63 ± 6.46	101.50 ± 8.68	> 0.25
Fibrinolytic activity	0	0	
Thromboplastic activity of blood (in %)	100.00 ± 1.53	38.50 ± 2.36	< 0.001
Platelets (in thousands)	374 ± 22.9	123 ± 9.12	< 0.001
Erythrocytes (in thousands)	5599 ± 329.6	3930 ± 273	< 0.001
Prothrombin (in %)	100.00 ± 1.98	97.0 ± 2.06	> 0.2
Capillary resistance (in %)	100.00 ± 3.10	38.12 ± 2.18	< 0.001

mined by the method of Ulitina and Kudryashov [6], the prothrombin time was determined by Tugolukov's method [5], and the capillary resistance was determined by Borbola's method, as described by Bazaz'yan [2].

The conditions of irradiation were as described previously [1]. The exposure dose of x rays was 1200 R. After irradiation the animals developed acute radiation sickness. About 67% of the irradiated animals died during the 2 weeks before the experiment began.

The reticulo-endothelial cells of the liver of the control and irradiated rabbits were kept at −25°C. The batches of cells were thawed simultaneously and ground in a glass homogenizer. Their thromboplastic activity was determined by Perlick's method [11], their antithrombin II and III content by the method of Witte and Dirnberger [14, 15], and their fibrinolytic activity by the method of Astrup and Albrechtsen [8].

The experimental results were subjected to statistical analysis [7].

EXPERIMENTAL RESULTS

The tests showed that Kupffer cells of unirradiated rabbits possess low thromboplastic activity: on the average 7.84% of the thromboplastic activity of the blood and under 1% of the thromboplastic activity of the intima of the aorta. They also possess high antithrombin activity but do not possess fibrinolytic activity (Table 1).

The biological importance of these differences can be understood if they are compared with the low velocity of the blood flow and the low pressure, especially in the liver sinusoids, where Kupffer cells are found. If high thromboplastic activity had been concentrated in them, any injury to the capillaries would have led to their thrombosis and to disturbance of the blood flow. Comparison of the thromboplastic activity of the blood vessels revealed that the blood pressure and velocity of the blood flow in the vessels, on the one hand, were inversely proportional to the coagulatory activity of the Kupffer cells in contact with the blood, on the other hand. This relationship between the parameters of the blood pressure, blood flow velocity, and biochemical properties of the vessel wall creates optimal conditions for hemostasis in different parts of the vascular system: activation of clotting can take place instantaneously in the aorta and large arterial trunks. If the slightest injury occurs to the arterial wall, the further development of the hemostatic plug into an extensive thrombus is prevented by the high velocity of the blood flow while, on the contrary, in capillaries with a slow blood flow and low thromboplastic activity of the endothelial cells, the internal system of the blood thromboplastin evidently plays a more important role than in other parts of the vascular system in hemostasis.

In the experiments of series II, in the 10 rabbits surviving 14 days after irradiation the thromboplastic activity of the blood and the platelet count were considerably reduced (Table 1). Compared with the control animals, their capillary resistance was reduced by 62%. The changes in prothrombin activity were very slight.

Determination of the thromboplastic activity of the Kupffer cells of the irradiated rabbits showed that it was only about one-quarter that of the unirradiated animals. Activity of antithrombins II and III in

the Kupffer cells showed slight changes. Factors activating fibrinolysis could not be detected by the method of Astrup and Albrechtsen in the Kupffer cells of either irradiated or intact animals.

LITERATURE CITED

1. I. A. Andrushko, Byull. Éksperim. Biol. i Med., No. 5, 29 (1967).
2. G. G. Bazaz'yan, Vestn. Moskovsk. Univ. Seriya 6. Biol. Pochvoved., No. 2, 18 (1960).
3. D. M. Zubairov and V. I. Kurochkin, Byull. Éksperim. Biol. i Med., No. 3, 28 (1964).
4. D. M. Zubairov, G. N. Gritsuk, L. F. Vladimirova, et al., in: The Blood Clotting System and Fibrinolysis [in Russian], Kiev (1969), p. 58.
5. V. N. Tugolukov, Vrach. Delo, No. 2, 151 (1953).
6. P. D. Ulitina and B. A. Kudryashov, Lab. Delo, No. 6, 7 (1958).
7. R. A. Fisher, Statistical Methods for Research Workers [Russian translation], Moscow (1958).
8. T. Astrup and O. K. Albrechtsen, Scand. J. Clin. Lab. Invest., 9, 233 (1957).
9. K. M. Brinkhaus and S. A. Walker, Am. J. Physiol., 132, 666 (1941).
10. S. St. George, M. Friedman, and S. O. Byers, Science, 120, 463 (1954).
11. E. Perlick, in: Gefässwant und Blutplasma, Jena (1961).
12. P. Rous and J. W. Beard, J. Exp. Med., 59, 577 (1934).
13. R. Wattiaux, P. Baudhuin, A. M. Berleur, et al., Biochem. J., 63, 608 (1956).
14. S. Witte and P. Dirnberger, Klin. Wschr., 31, 598 (1953).
15. S. Witte and P. Dirnberger, Schweiz. Med. Wschr., 84, 800 (1954).