ARTERIOVENOUS DIFFERENCE IN LEVELS OF VASCULAR AND PLATELET FACTORS OF HEMOSTASIS

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Mechanisms of thrombus formation differ in arteries and veins. Many investigations have been undertaken to discover the causes of this difference, some of which have confirmed the basic postulates of Virchow's classical theory, whereas others have opposed it. It has been found, for instance, that thrombi in veins can develop in morphologically undamaged vessels [7]. Thrombin, injected in large doses into isolated segments of veins does not cause morphological injuries to the endothelium [9]. Blood stasis of varied duration (from a few minutes to 1 h) does not impair the athrombogenic properties of the endothelium, as a result of which adhesion of platelets is not observed after restoration of the blood flow in ischemic zones [10]. The writers previously demonstrated an arteriovenous difference in antithrombin III activity and the antiaggregation properties of the vessels [1].

The aim of this investigation was to continue the study of arteriovenous differences that lie at the basis of the mechanism of thrombus formation in arteries and veins.

EXPERIMENTAL METHODS

Experiments were carried out on 72 male Wistar rats. Under pentobarbital anesthesia a midline incision was made in the anterior abdominal wall of the experimental animals. Blood was taken from the aorta of the animals of one group at its bifurcation, and in rats of the other group from the posterior vena cava. The blood was mixed with 3.14% sodium citrate solution in a ratio of 9:1. ADP-induced platelet aggregation was determined in 450 μl of blood (ADP was from Reanal, Hungary; final concentration 10^{-6} M) by counting the concentration of single platelets left in the blood 1, 3, 5, and 8 min after addition of the aggregation inducer to it, with continuous mixing at a temperature of 37°C, and counting the erythrocyte and leukocyte concentrations by the same method on a "Cellcounter" (Sweden). The remaining blood was subjected to differential centrifugation in order to obtain platelet-enriched and platelet-deprived plasma. ADP-induced platelet aggregation [5], the malonic dialdehyde (MDA) concentration [8], and the cAMP concentration, the last by radioimmunoassay with kits from Amersham Corporation (England), were determined in the platelet-enriched plasma. The antiaggregation activity of the aorta and posterior vena cava was estimated by determination of the antiaggregation properties and cAMP level in the vessel walls by radioimmunoassay with kits from Amersham Corporation. The results were subjected to statistical analysis by Student's test.

RESULTS

It will be clear from Table 1 that ADP-induced aggregation of platelets was higher in platelet-enriched plasma obtained from arterial than from venous blood ($P < 0.05$), whereas the MDA level in intact platelets from venous blood, on the other hand, was higher than in arterial blood. During thrombin-induced aggregation the accumulation of MDA increased, and more so in platelets from arterial blood ($P < 0.01$). The results demonstrate the more marked thrombogenic properties of platelets from arterial than from venous blood, in agreement with data in the literature [3]. In patients with acute myocardial infarction in the ischemic phase of the disease [2] more marked activation of platelets from arterial than from venous blood is found; this also confirms data now obtained in the study of platelet function in platelet-enriched plasma. Does the same rule apply to determination of platelet aggregation in whole

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TABLE 1. Levels of Vascular and Platelet Components of Hemostasis System in Arterial and Venous Blood (M ± m)

Parameter	Artery	Vein
	Blood	
ADP-induced platelet aggregation, %		
in plasma	53±5,6	36±4,2**
in blood	90±4,1	75±12,7
MDA concentration in platelets, nanomoles/10^9 cells		
initial at 5th minute	0,63±0,06	0,87±0,06**
thrombin-induced aggregation	1,53±0,04	1,23±0,1**
cAMP, pmoles/10^8 cells	2,48±0,42	0,77±0,27**
	Vessel	
cAMP concentration, pmoles/g	277±77,1	11,9±0,09**
6-Keto-$PGF_{1\alpha}$ level, ng/min	12,1±0,05	5,1±1,0**

Note. *P < 0.05, **P < 0.01.

blood? The study of induced platelet aggregation in blood has considerable advantages over tests in platelet-entirched plasma. These advantages are as follows: Additional procedures involving trauma to the platelets, used to obtain platelet-enriched plasma, are eliminated, the conditions of testing are the nearest possible to conditions *in vivo*, and it is possible to study changes in all cellular components of blood in response to the appearance of agents initiating blood clotting in the circulation. The results of a study of changes in cell concentrations in arterial and venous blood *in vitro* after addition of ADP to it are given in Fig. 1. ADP-induced platelet aggregation was identical in arterial and venous blood. Changes in the erythrocyte concentration likewise were the same. The leukocyte concentration in venous blood fell significantly at the 3rd minute after addition to ADP, after which it returned to its initial level, whereas it remained unchanged in arterial blood. This fact demonstrates the presence of a functional connection between the leukocytes and platelets of venous blood in the response to ADP. This connection is evidently biochemical in nature. ADP is a stimulus both for leukocytes and for platelets capable of releasing arachidonic acid from cell membrane phospholipids. Archidonic acid is oxidized in platelets by cyclo-oxygenase with the formation of thromboxane A_2, whereas in leukocytes it is oxidized by lipo-oxygenase with the formation of leukotriene B_4. Thromboxane A_2 aggregates platelets whereas leukotriene B_4 causes accumulation and chemotaxis of leukocytes. A fall in the leukocyte concentration in venous blood in response to ADP is evidently connected with the fact that leukocytes either aggregate each other or adhere to platelet aggregates. Some investigators [4] have observed adhesion of neutrophils to platelet aggregates on films prepared from blood obtained from perfusion chambers. Participation of venous blood leukocytes (by contrast with arterial blood leukocytes) in the platelet aggregation reaction is a fact which helps to explain the mechanism of formation of the red thrombus.

The study of the antiaggregation properties of arteries and veins by measuring cAMP and 6-keto-PGF_1 levels showed that their concentrations were much higher in arteries than in veins ($P < 0.01$).

Platelet function, assessed on the basis of measurement of ADP-induced aggregation in platelet-enriched plasma thus differs depending on whether the plasma is obtained from arterial or venous blood, the reason probably being a difference in the biochemical profiles of platelets in arterial and venous blood. Under the influence of thrombin, 2.5 times more MDA was formed in platelets from arterial blood than in platelets from venous blood. Most investigators are of the opinion that MDA is an indicator of synthesis of biologically active substances of the "arachidonic cascade," possessing proaggregation properties, in platelets. However, this functional potential of arterial platelets is not realized during the investigation of induced aggregation in blood, possibly due to the high cAMP level in these platelets,

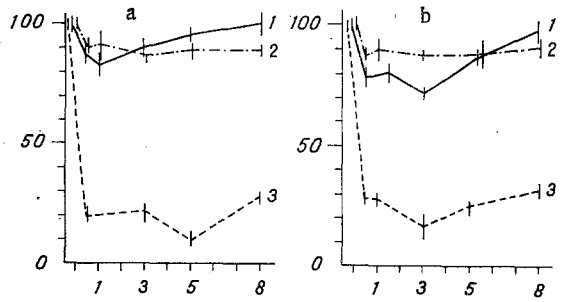

Fig. 1. Changes in cell concentrations in arterial (a) and venous (b) blood after addition of ADP (in % of initial level). Abscissa, time (in min). 1) Leukocytes, 2) erythrocytes, 3) platelets.

by contrast with platelets from venous blood (Table 1). The similar direction of the response of platelets and leukocytes from venous blood to ADP, the low anticoagulant activity and high coagulation potential of venous blood, together with the low antiaggregation activity of venous vessels and the slower rate of the blood flow in them than in arteries, are conditions that determine genesis of a red thrombus. The high functional activity of arterial blood platelets under physiological conditions is "balanced" by the high athrombogenic potential of arteries. We know that prostacycline in the vessel wall, the end product of oxidation of which is 6-keto-$PGF_{1\alpha}$, raises the cAMP level in platelets [6]. Our investigations showed that the cAMP level in platelets from arterial blood is 3.2 times higher than in those from venous blood. One probable cause of formation of a white platelet thrombus in arteries is thus evidently weakening of the athrombogenic properties of arteries, leading to disturbance of the endothelium–platelets balance and stimulating the platelets for realization of their thrombogenic function.

Consequently, great importance in the mechanism of white thrombus formation must be ascribed to disturbance of the vascular-platelet component of the hemostasis system, and in the mechanism of formation of the red thrombus — to disturbance of the coagulation component. The different interpretations of the pathogenesis of thrombi in arteries and veins give a guide to the adequate prevention and treatment of thrombus formation and its complications.

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