OXIDATIVE AND ANTIOXIDATIVE SYSTEMS OF THE BLOOD IN MECHANISMS FOR PROTECTING AND INJURING ERYTHROCYTES IN MICE INFESTED WITH *Plasmodium berghei*

V. D. Ponoinetskii, V. V. Pokrovskii, L. S. Kladukhina, and V. A. Yurkiv

UDC 616.936-022.1-07:616.155.1-008.97:576.893.192.6]-092:612.118.223

KEY WORDS: lipid peroxidation; glutathione peroxidase; superoxide dismutase.

Introduction of the malarial plasmodium into the erythrocyte and the subsequent vital activity of the parasite lead to a disturbance of the homeostatic mechanisms of the cell, culminating in its death. Investigations [5, 10] have shown that the erythrocytic forms of various species of malarial plasmodia actively utilize many compounds and cofactors of the host cell: NADPH, ATP, glutathione, riboflavin, pantothenic acid, and so on. Etkin and Eaton [6] showed by indirect methods that hydrogen peroxide accumulates in the erythrocyte, catalase activity is reduced, and hemoglobin is converted into methemoglobin. These and other facts served as grounds for the hypothesis [4] that lipid peroxidation (LPO) is activated during the development of malarial plasmodia in erythrocytes.

The object of this investigation was to study total activity of the enzymes glutathione peroxidase and superoxide dismutase in the blood and the dynamics of accumulation of LPO products (products reacting with 2-thiobarbituric acid — TPA-active products) in the blood stream depending on growth of the parasitemia during the development of malarial infection in mice.

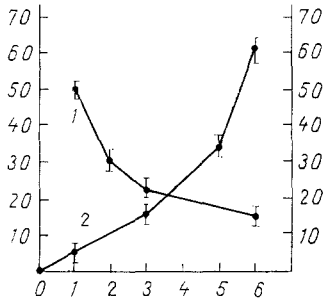

Fig. 1

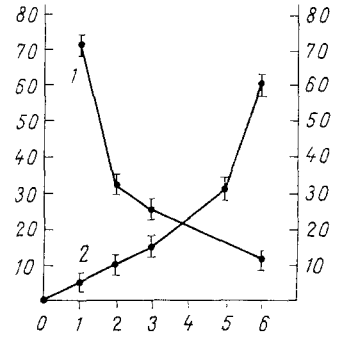

Fig. 2

Fig. 1. Dynamics of changes in blood superoxide dismutase activity (1) and parasitemia (2). Abscissa, time (days); ordinate, left — percent of affected erythrocytes, right — enzyme activity (in percent of normal).

Fig. 2. Dynamics of changes in blood glutathione peroxidase activity (1) and parasitemia (3). Abscissa, time (days); ordinate, left — percent of affected erythrocytes (2), right — enzyme activity (in percent of normal).

Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR K. V. Bunin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 307-309, September, 1981. Original article submitted February 11, 1981.

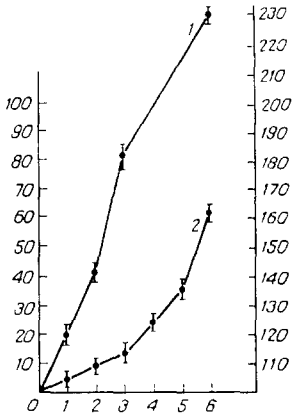

Fig. 3. Dynamics of blood concentration of TBA-active products (1) and parsitemia (2). Abscissa, time (days); ordinate, left — percent of affected erythrocytes (2), right — blood concentration of TBA-active products (in percent of normal).

EXPERIMENTAL METHOD

Male BALB/c mice weighing 20 ± 2 g were infected by intraperitoneal injection of 10^5 parasites of a virulent strain of *Plasmodium berghei*. Animals of the experimental group (10 mice at each point), after preliminary heparinization (200 i.u. per mouse) were decapitated on the 1st-6th day after the beginning of infection. The collected blood was used to determine the percentage of affected erythrocytes in thin films and for quantitative determination of TBA-active products [1]. After precipitation of hemoglobin with a cold (−20°C) mixture of chloroform and ethanol, total glutathione peroxidase [2] and superoxide dismutase [7] activity was determined in the hemolyzed blood.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that superoxide dismutase activity fell sharply during development of the disease (by 75-80%) and correlated with the percentage of affected erythrocytes. Gluthathione peroxidase activity fell by 83-87%, in the same relationship (Fig. 2). Direct correlation could be seen between the dynamics of parasitemia and the quantity of TBA-active products (Fig. 3).

The results confirm to some extent the hypothesis [4] that vital activity of the plasmodia is accompanied by activation of LPO reactions in the erythrocyte, and also by a sharp decline in activity of the enzyme system (studied in the present investigation) responsible for detoxication of peroxides and active forms of oxygen. We know from data in the literature [8, 9] that disturbance of the activity of these enzymes leads to a reduction in the life span of the erythrocyte and to rupture of its membrane. It has also been suggested [3] that LPO products and superoxide radicals formed in excess may, in turn, inactivate the enzymes protecting the host cell.

Disturbance of the integrity of the erythrocyte membranes may thus depend both on the intensity of these reactions in the cell and on the concentrations of substrates and cofactors necessary for the normal functions of enzymes of the host cell. To determine the degree of participation of each of the above factors in stabilization and destabilization of the erythrocyte membranes further investigations are needed.

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EXTRUSION OF IONIZED CALCIUM AS A MARKER OF PLATELET RELEASE

P. S. Grybauskas, M. Z. Puodžuikyniene, and J. J. Rudžoniene

UDC 611.018.52+612.115.3

KEY WORDS: platelets; ionized calcium; secretion.

The platelet release reaction (PRR) of biologically active substances is unchanged by the action of a critical concentration of aggregating substances on the platelets, of which thrombin gives the most complete natural effect. The study of PRR is interesting in order to understand the mechanisms of prevention of formation of platelet aggregates in the circulation and the development of vascular thrombosis.

Aggregating platelets release a number of pharmacologically active substances [1], simultaneously with which they also release ionized calcium (Ca^{++}). Quantitative determination of extruded ATP, ADP, serotonin, various enzymes, and other substances requires the use of complex techniques, whereas no great difficulty arises during precise measurement of Ca^{++} by the use of an ion-selective technique [7].

The method of atomic absorptiometry has shown [3] that the total calcium content in human platelets may reach 260 ± 62 nmoles/10^9 cells. Under the influence of thrombin, depending on the functional activity of the cells, up to 58-85% of the Ca^{++} is released from the platelets [8]. The exceptional importance of this ion in the activity of all cells of the body is confirmed by the latest convincing evidence [2, 4, 6]. Accordingly the possibility of monitoring PRR by Ca-selective electrodes can facilitate the study of this phenomenon in pathological states associated with the risk of disturbances of blood coagulation, and also during treatment with various drugs.

This paper gives the results of an investigation of the release of Ca^{++} from platelets under the influence of thrombin, using an ion-selective electrode.

EXPERIMENTAL METHOD

Platelet-enriched plasma was obtained from citrated human plasma in the usual way. Platelets were isolated by repeated centrifugation for 15 min at 1000 g, then washed three times with buffer consisting of 0.9% NaCl, 0.5% glucose, and 0.02 M Tris-HCl, pH 7.4. The platelets were then resuspended in the same buffer up to a concentration of $2.2 \cdot 10^8$ cells/ml. To unify the conditions of measurement the calcium concentration in the suspension was adjusted to 10^{-5} M.

Commercial thrombin, manufactured by the Kaunas Bacterial Preparations Factory, was dialyzed for 48 h against Tris-HCl, pH 7.0, to remove as much as possible of the calcium contained in it. Thrombin with activity of 100 units/ml and with a residual calcium content of 0.1 mmole/liter was used in the experiment.

Research Institute of Physiology and Pathology of the Cardiovascular System, Kaunas Medical Institute, Lithuanian Ministry of Health, Kaunas. (Presented by Academician of the Academy of Medical Sciences of the USSR Z. I. Januškievičius.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 309-310, September, 1981. Original article submitted January 19, 1981.

