resistive compartment of the vascular bed may occur without any increase in capillary numbers [2,10]. In the present study, MCK activity in the skeletal muscles of rats was found to have increased after 10 days of aerobic training, but the changes in the energy metabolism of these muscles during that period were not accompanied by structural modifications either in the resistive or the metabolic compartments of the vascular bed, i.e., by improvements in the blood supply to muscle tissue.

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Parameters of Lipid Peroxidation in Erythrocytes of Children Being Treated for Acquired Severe Aplastic Anemia

V. N. Zharov, I. K. Fillipov, M. I. Bakanov, and I. V. Koshel'

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It is shown that a peroxidase-catalase mechanism of antioxidant defense predominates in the erythrocytes of children with acquired aplastic anemia, and that the high level of lipid peroxidation (LPO) processes in such children dictates the need for continued antioxidant therapy during all phases of the disease. The antioxidant effect of glucocorticoids appears to be quite sufficient for eliminating the more severe effects of LPO.

Key Words: *lipid peroxidation; anemia*

Aplastic anemia is a collective term used to designate conditions of various origin in which injury to the bone marrow results in pancytopenia. The etiology of most forms of acquired aplastic anemia remains unknown, and for this reason they are referred to as idiopathic. In idiopathic aplastic anemia, which accounts for 60-70% of cases, the reduction in erythrocyte numbers in the peripheral blood is due to a defect in the hemopoietic sys-

Research Institute of Pediatrics, Russian Academy of Medical Sciences, Moscow. (Presented by M. Ya. Studenikin, Member of the Russian Academy of Medical Sciences)

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tem [11], decreased efficiency of hemopoiesis, and erythrocyte hemolysis [6]. The critical clinical characteristic of the patient is whether or not he or she is dependent on blood transfusion. The hemic hypoxia seen in cases of transfusion dependence is a constant factor leading to dystrophic changes in the body, and in such cases the impaired lipid peroxidation (LPO) inevitably affects the antioxidant systems of the erythrocyte. Through measures that counteract the peroxidase-induced stress [9] it is possible to maintain respiration at a level necessary for the functioning of the vital systems in such a severe condition as aplastic anemia. Preservation of the structural and functional architectonics of any cell depends to a large extent on continued balance of the LPO processes. When these are disrupted, increased amounts of toxic metabolites appear in the circulation and interfere with the operation of the systems responsible for their neutralization [3,5].

The condition of the erythrocyte's antioxidant systems determines the stability of this cell and its survival while in circulation. Gaining information on LPO processes in the erythrocyte should make it easier to decide whether antioxidant therapy is indeed required and to develop improved methods for such therapy.

MATERIALS AND METHODS

The study was carried out on 78 children aged 5 to 14 years with severe aplastic anemia who were being treated in the Hematology Department of the Research Institute of Pediatrics in Moscow. The diagnosis of severe aplastic anemia was established on the basis of generally accepted criteria [11], and the disease was in all cases described as idiopathic. The patients were receiving transfusions of donor erythrocytes (the criterion of transfusion dependence) and platelets, as well as glucocorticoids and androgens. The extent of clinical and hematological compensation was evaluated while the children were being treated according to one of the protocols used in the program developed by the Institute of Pediatrics for the treatment of aplastic anemias (the initial intervention to achieve immunosuppression was splenectomy).

The anemic children comprised three groups. Children of group 1 (n=30) were in an early stage of the disease and either had not yet received any glucocorticoid therapy (GCT) or had been treated with glucocorticoids for only 2 or 3 days. Group 2 (n=28) consisted of children who had been ill for 2 to 6 months, had been on GCT for 1.5 months or more, and were totally dependent on transfusions. Group 3 children were included in the study eight or more months after the termination of GCT; they were receiving androgens (with attempts being made to discontinue them) and were not transfusion-dependent. The control group consisted of 36 healthy children.

The test cells were native peripheral blood erythrocytes. They were assayed for LPO products such as hydroperoxides [2] and malonic dialdehyde (MDA) and for the LPO enzymes superoxide dismutase (SOD) [7], glutathione peroxidase (GLP), and catalase [10], as well as for resistance to hemolysis [1].

The assays used can characterize with reasonable completeness the pathways by which LPO products are neutralized. Variations in MDA concentrations and in levels of the autohemolysis undergone by erythrocytes in the course of aplastic anemia provide direct information on the functional state of these cells.

RESULTS

The results are summarized in Tables 1 and 2. The functional state of erythrocytes, as assessed by determining the levels of their autohemolysis and of LPO products and enzymes in these cells, differed in the different periods of the disease, reflecting the severity of its clinical manifestations. In the initial period, when specific treatment was not given (or lasted for only 2 or 3 days) and so clinical and hematological characteristics of the patients were determined by the disease itself, the level of autohemolysis was elevated (indicating that destructive effects on hemopoiesis predominated), as were MDA and hydroperoxide concentrations (indicating activation of free-radical processes). During that period, SOD activity tended to rise and GLP activity was relatively high, but the activities of these enzymes were apparently not high enough to neutralize MDA, the major toxic product of impaired LPO processes.

As the disease progressed, further dystrophogenic factors were added by the treatment given. These factors included primarily the side-effects of GCT and posttransfusion immunopathological relationships. During that period, autohemolysis was at its height, but MDA concentrations tended to decrease, probably because of the antioxidant effect of the GCT and also because the catalase and GLP pathways of antioxidant defense were activated. SOD activity began to fall, while hydroperoxide levels remained consistently high.

In the period of clinical and hematological compensation (which is the equivalent of remission

TABLE 1. Levels of Autohemolysis and LPO Products in Peripheral Blood Erythrocytes from Children with Aplastic Anemia. The Values are Means±SEM

Period of disease and degree of transfusion dependence	n	Autohemolysis, %		MDA, rel. units		Hydroperoxides, rel units/ml sample
		intact	induced	intact	induced	
Early acute period prior to GCT or after short-term (2-3 days) GCT	30	3.6±0.4*	4.7±0.5*	3.5±0.3*	4.3±0.3*	5.6±0.7*
Period of well-established clinical manifestations after prolonged (>1 month) GCT. Absolute transfusion dependence	28	4.0±0.4*	5.1±0.4*	3.0±0.4*	3.9±0.4*	5.5±0.8*
Clinical and hematological compensation	20	2.9±0.6*	3.8±0.4	2.6±0.3	3.9±0.4*	6.6±0.6*
Healthy controls	36	2.7±0.1	3.4±0.8	1.8±0.2	2.7±0.3	1.8±0.3

Note. Here and in Table 2 asterisk denotes a significant difference from healthy children at $p<0.05$.

in that there is no need for transfusing donor erythrocytes), autohemolysis was much less intensive and MDA decreased, but hydroperoxides persisted at high levels. GLP and catalase activities also remained high, whereas SOD activity began to decline.

The condition of the antioxidant systems in the course of aplastic anemia depends on the degree of hypoxia and on the accumulation of toxic products from impaired LPO processes.

Erythropoiesis is the major process in hemopoiesis, and the erythrocytes are the most abundant cellular elements that govern the basic vital function of respiration. The metabolic changes occurring in erythrocytes reflect the severity of aplastic anemia, since the rates of LPO processes in these cells are similar to those in other cells or tissues. The resistance of erythrocytes to oxidant stress and hypoxia largely determines the body's ability to counteract the progression of various dystrophic changes. In aplastic anemia, the erythrocyte is unable to withstand the stress imposed on it. An indication of this is the high level of autohemolysis during acute periods of the disease. One cause of the destructive changes taking place in cells, including erythrocytes, is destabilization of their membranes due to the disordered LPO processes and the cell's inability to neutralize toxic metabolites. This is confirmed by the present findings. In the initial period, when no or only minimal treatment was given, the changes observed reflected the essence of the disease itself. As the disease progressed, LPO processes in the erythrocytes were increasingly influenced by the tissue dystrophy. The most revealing indicator of the unfavorable course taken by the disease during these two periods was the high MDA concentration, which reached its maximum in the initial period. Catalase activity was relatively low when the MDA level was high and increased subsequently as the MDA level declined. GLP activity remained high in all periods, possibly because of the need to neutralize hydroperoxides, whose concentrations also remained high during all periods, including that of clinical and hematological compensation. The peroxidase-catalse system of antioxidant defense appears to dominate in erythrocytes during aplastic anemia, and the failure of SOD activity to increase significantly in any period is evidence of the relative stability of this antioxidant system, which can probably be explained by ontogenetic features of the erythrocyte. It may be, however, that the SOD system is relatively toler-

TABLE 2. Activities of LPO Enzymes in Peripheral Blood Erythrocytes from Children with Aplastic Anemia. The Values are Means±SEM

Period of disease and degree of transfusion dependence	n	SOD, U/g Hb	Catalase before boiling, %	Thermostable catalase, %	GLP, U/g Hb
Early acute period prior to GCT or after short-term (2-3 days) GCT	30	1538.0±87.2	97.3±2.0*	19.6±2.1*	0.089±0.005
Period of well-established clinical manifestations after prolonged (>1 month) GCT. Absolute transfusion dependence	28	1464.6±67.6	97.6±0.7*	37.5±3.5*	0.106±0.006*
Clinical and hematological compensation	20	1264.7±66.3	96.6±1.6*	38.0±0.9*	0.09±0.07
Healthy controls	36	1490.5±102.8	20.6±1.0	20.4±0.9	0.073±0.005

ant to aplastic anemia. Moreover, the slight reduction in SOD activity recorded during the period of clinical and hematological compensation may be a sign of insufficiency (depletion) of this system.

The high GLP and catalase activities in the erythrocytes of children in the state of clinical and hematological compensation, on the one hand, and the failure of autohemolysis and hydroperoxide and MDA levels in these children to decrease to levels found in the controls, on the other, suggests that their clinical recovery was not a true (complete) recovery.

The high intensity of LPO processes in aplastic anemia appears to indicate a need for antioxidant therapy during all periods of the disease. The need for such therapy in acute periods is, however, debatable, for it cannot be ruled out that intensive LPO processes may play a beneficial role. The antioxidant effect of glucocorticoids may be quite sufficient to eliminate the worst effects of LPO, as is evidenced, for example, by the observed tendency toward a reduction in MDA concentration in children who had been receiving GCT for a prolonged period (group 2).

The protocols of the above-mentioned program being implemented at the Institute of Pediatrics include intravenous injections of ascorbic acid in large doses during the establishment of clinical and hematological compensation. This treatment method has been developed empirically for preventing or diminishing the severity of parodontopathy, which is one of the most common remote sequelae of aplastic anemia and of the dystrophogenic methods used for its treatment. The currently available data [8] indicate that the ascorbic acid contained in the body may completely suppress the harmful activity of free radicals (other antioxidants suppress it by 70% at most), and the introduction of additional ascorbic acid is thought to help the body's antioxidant systems to neutralize the excess toxic LPO products.

The important practical conclusion to be drawn from the present study is that the attainment of a persistent tendency toward normalization of LPO processes in the erythrocytes of patients with aplastic anemia may be looked upon as an argument in favor of discontinuing their specific treatment.

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