BIOPHYSICS AND BIOCHEMISTRY

Megakaryocytopoiesis under Hypoxic Conditions

E. V. Lebedeva, B. G. Yushkov, and V. A. Chereshnev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 12, pp. 627-630, December, 2003
Original article submitted December 11, 2002

Acute and chronic hypoxia led to acceleration of proliferation and differentiation of bone marrow megakaryocytes and increase in their functional activity. The count of young cells in the peripheral blood increased during acute hypoxia. Chronic hypoxia led to increase in the number of old platelets with lower functional activity, which was accompanied by thrombocytosis.

Key Words: *megakaryocytic stem; hypoxic hypoxia; peripheral blood; hemopoietic organs*

It is well established that megakaryocytes adjacent to bone marrow sinuses form and release platelets into the sinusoidal space, while circulating megakaryocytes migrate into the lungs and are responsible for pulmonary thrombocytopoiesis [6]. The medium into which platelets are released is characterized by higher oxygen concentration than bone marrow tissue containing stem cells and precursors. It was hypothesized that megakaryocytopoiesis depends on pO_2. The decrease in atmospheric oxygen concentration decreases the count of megakaryocytes and degree of ploidy, decelerates maturation and apoptosis [9], and changes expression of cytokine receptors on megakaryocytes [10]. Previous studies showed that thrombocytosis develops during high-mountain breath holding and "climb" or "descent" in an altitude chamber [3,5]. These changes are related to activation of megakaryocytopoiesis, release of large platelets into the circulation (including giant cells of delicate structure) [3,5], and increase in blood thrombocytopoietic activity [5]. Adhesive and aggregation activity of platelets increases under hypoxic conditions. It contributes to the development of thromboembolic complications during high-altitude hypoxia that determine the severity and outcome of pathological processes [3,5].

Institute for Ecology and Genetics of Microorganisms, Ekaterinburg Branch. ***Address for correspondence:*** yushkov@cranium.ural.ru. Yushkov B. G.

The total number of platelets and megakaryocytes in mice maintained in a chamber at normal atmospheric pressure (atmospheric oxygen concentration 6-7%) undergoes biphasic changes. The count of these cells increases on days 2-4, but decreases on days 6-14 of hypoxia [8]. Experiments of N. N. Petrishchev showed that the redistribution of platelets occurs in the initial stage of hypoxia (similarly to stress conditions), while the follow-up period is characterized by stimulation of thrombocytopoiesis [5].

MATERIALS AND METHODS

Male outbred albino rats weighing 150-200 g were maintained in an altitude chamber with combined extract-and-input ventilation at reduced air pressure (40.98 kPa) for 6 h. Hypoxia was modeled once or repeatedly (5 days) at 9.00-15.00 [4].

The total number of peripheral blood platelets was measured immediately after "descent" (acute hypoxia) or last session (chronic hypoxia) using a Microx SX blood analyzer (Hoffman La Roche). Platelets were counted in smears stained with hematoxylin and eosin. We estimated the number of young, mature, old, and activated platelets [2].

Platelet adhesion was evaluated by the percent of cells adhered to glass surface during blood passage through standard Amersham columns filled with glass beads.

0007-4888/03/1366-0554$25.00

TABLE 1. Number and Mean Diameter of Bone Marrow Megakaryocytes in Rat Femur during Hypoxic Hypoxia (*M*±*m*)

Period	Megakaryoblasts	Promegakaryocytes	Megakaryocytes	Total number
Cells, 1000/100 g				
Control	0.28±0.15	6.87±1.02	62.91±1.14	70.06±12.51
Acute hypoxia	0.25±0.13	3.72±0.67*	80.64±0.68*	84.62±8.46
Chronic hypoxia	0.70±0.25	4.51±0.70	94.98±0.68*	100.19±9.45
Mean diameter of cells, μ				
Control	12.38±1.11	18.92±0.44	21.97±0.1	21.63±0.18
Acute hypoxia	10.70±0.83	20.99±0.60*	24.20±0.15*	24.02±0.15*
Chronic hypoxia	9.96±0.65	22.38±0.58*	25.06±0.17*	24.84±0.17*

Note. *$p<0.01$ compared to the control.

Aggregation properties were studied in platelet-rich plasma (PRP) stabilized with heparin on a 120 LA laser aggregometer (Biola). Epinephrine (100 μg/ml) and ADP (2.5 and 10.0 μg/ml) served as aggregation inductors. The initial light transmission of PRP and light transmission of platelet-poor plasma (PPP) were taken as 0 and 100%, respectively. We constructed the curves for the mean size of aggregates and light transmission. The degree of aggregation was estimated by the maximum mean size of aggregates (R_{max}) and maximum rise in light transmission (LT_{max}). The rate of aggregation was determined by the maximum slope of each curve (rel. units/min and %/min).

Platelet disaggregation was studied by disintegration of conglomerates formed after spontaneous aggregation.

The total count of bone marrow myelokaryocytes in rat femur was measured by the method of Mantz [7]. The density of megakaryocytes, partial megakaryocytogram, and mean number of nuclear segments in megakaryocytes were estimated in histological preparations of the bone marrow and spleen stained with hematoxylin and eosin using an Avtandilov grid [1]. The diameter of cells was measured with an ocular micrometer.

The results were analyzed by parametric tests of variational statistics (Microsoft Excel software). We calculated the arithmetic mean, error, dispersion, and coefficient of variation. The differences between arithmetic means were estimated by Student's *t* test. The probability of error was determined (*p*). The differences between arithmetic means were significant at $p<0.05$.

RESULTS

Acute hypoxia accelerated transition of promegakaryocytes into megakaryocytes and increased the size of these cells and stem cells (Table 1). The mean number of nuclear segments in megakaryocytes remained unchanged.

The count of peripheral blood platelets remained at the initial level, while the number of young cells increased (Table 2). Spontaneous aggregation resulted in the formation of less stable aggregates. The maximum time of disaggregation under hypoxic and control conditions was 3.57±2.19 and 13.57±1.91 min, respectively ($p<0.01$). We revealed an increase in the degree and acceleration of ADP-induced aggregation. Increasing the concentration of ADP was accompanied by acceleration of aggregation. These changes were

TABLE 2. Thrombocytogram, Number, and Size of Peripheral Blood Platelets from Rats during Hypoxic Hypoxia (*M*±*m*)

Period	Young	Mature	Old	Activated cells	Total number
Platelets, g/liter					
Initial level	27.93±5.59	596.34±8.03	72.62±2.51	1.40±1.26	698.29±31.56
Acute hypoxia	86.43±6.96*	568.37±25.49	77.64±20.44	—	732.44±28.70
Chronic hypoxia	29.68±8.91	605.23±22.10	184.70±21.69*	4.95±4.45	824.57±42.23*
Mean diameter of platelets, μ					
Initial level	2.28±0.05	1.38±0.01	0.76±0.01	2.87±0.01	1.35±0.01
Acute hypoxia	2.10±0.05*	1.27±0.01*	0.79±0.01	—	1.33±0.01
Chronic hypoxia	2.45±0.15	1.17±0.01*	0.75±0.01	1.68±0.16*	1.13±0.01*

Note. *$p<0.01$ compared to the initial level; —, absence of activated cells.

TABLE 3. Aggregometry of Aortic Blood Platelets from Rats during Hypoxic Hypoxia (*M*±*m*)

Period	R_{max}, rel. units	Maximum slope, rel. units/min	LT_{max}, %	Maximum slope, %/min
ADP, 2.5 μg/ml				
Control	13.75±1.10	53.64±8.12	36.10±4.48	74.44±8.51
Acute hypoxia	22.15±1.95*	111.41±17.98*	43.70±5.76	109.64±11.96
Chronic hypoxia	14.95±1.82	69.60±5.35	46.45±8.77	137.14±23.09*
ADP, 10 μg/ml				
Control	12.95±1.36	47.12±7.88	39.95±3.97	71.02±9.53
Acute hypoxia	21.24±1.19*	123.33±5.90*	40.00±5.74	133.23±18.64*
Chronic hypoxia	16.03±2.44	92.55±15.07*	54.94±4.31*	135.00±8.31*

Note. *$p<0.01$ compared to the control.

followed by an increase in the maximum slope of light transmission curves (Table 3).

The count of promegakaryocytes returned to the initial level, while the number of megakaryocytes remained high with prolonging the exposure. Megakaryocytes were large (Table 1). The development of thrombocytosis in the blood was related to accumulation of old and small platelets (Table 2).

Morphological changes in platelets were not accompanied by shifts in spontaneous aggregation. The degree of ADP-induced aggregation during chronic hypoxia was lower than that during acute hypoxia. The maximum radius of aggregates after chronic hypoxia did not differ from the control. However, in rats exposed to chronic hypoxia ADP-induced aggregation developed more rapidly than in intact animals. It was manifested in an increase in the maximum slope of light transmission curves. Increasing the ADP concentration accelerated and potentiated aggregation. LT_{max} of PRP and maximum slope of the curve for aggregate radius increased (Table 3). Aggregation inductor epinephrine had no effect on rat platelets. The intensity of platelet adhesion did not differ from the control.

Hypoxia was followed by an increase in the density of bone marrow cells. This index in rats exposed to acute and chronic hypoxia and control animals was 50±5, 53±5, and 28±5 cells/mm^2, respectively ($p<0.01$). The observed changes were most pronounced in the proximal (acute hypoxia, 18±4 cells/mm^2; chronic hypoxia, 56±7 cells/mm^2; control, 5±3 cells/mm^2) and distal epiphyses (acute hypoxia, 56±7 cells/mm^2; chronic hypoxia, 83±7 cells/mm^2; control, 20±6 cells/mm^2, $p<0.01$). Splenic cells also increased in size during acute and chronic hypoxia (27.05±71.17 and 28.44±1.33 m, respectively, compared to 21.72±1.05 μ in the control).

Our results show that acute hypoxia is followed by stimulation of megakaryocyte differentiation and increase in the size of cells. Blood platelet content remains unchanged due to recruitment of young functionally active platelets into the circulation. Lengthening of the exposure is accompanied by stimulation of differentiation and acceleration of maturation of the cytoplasm. These changes contribute to the development of thrombocytosis; the count of small old platelets also increases. The study of ADP-induced aggregation showed that during chronic hypoxia functional activity of platelets is lower than during acute hypoxia. Acute and chronic hypoxia is accompanied by an increase in the size of spleen megakaryocytes. The density of bone marrow megakaryocytes most significantly increases in epiphyseal zones.

REFERENCES

1. G. G. Avtandilov and V. G. Aleksandrov, *Basics of Quantitative Pathoanatomy* [in Russian], Moscow (2002).
2. G. I. Kozinets, V. V. Vysotskii, V. M. Pogorelov, *et al*., *Blood and Infection* [in Russian], Moscow (2001).
3. A. A. Markosyan, *Physiology of Platelets* [in Russian], Leningrad (1973).
4. V. S. Novikov, A. V. Dergunov, and O. T. Kuttubaev, *Hypoxia. Adaptation, Pathogenesis, and Clinics*, Ed. Yu. L. Shevchenko [in Russian], St. Petersburg (2000), pp. 24-94.
5. N. N. Petrishchev, *Resistance of Vessels to Thrombosis* [in Russian], St. Petersburg (1994).
6. J. Dzieciol, W. Debek, L. Chyczewski, *et al*., *Rocz. Akad. Med. Bialymst*., **40**, No. 1, 88-93 (1995).
7. J. M. Mantz, *O. P. Soc. Biol*., **151**, 1957-1960 (1957).
8. T. P. McDonald, M. B. Cottrell, S. A. Stweward, *et al*., *Exp. Hematol*., **20**, No. 1, 51-56 (1992).
9. S. S. Mostafa, W. M. Miller, and E. T. Papoutsakis, *Br. J. Haematol*., **111**, No. 3, 879-889 (2000).
10. S. S. Mostafa, E. T. Papoutsakis, and W. M. Miller, *Exp. Hematol*., **29**, No. 7, 873-883 (2001).