for the monocyte reaction, the MC effect is due primarily to histamine, acting via H_1-receptors, as well as to serotonin.

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Biophysics and Biochemistry

Erythropoetin Activity of Plasma in Healthy Children and Children with Iron-Deficiency Anemia

M.K.Soboleva and T.V.Manakova

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Erythropoetin (EP) - a glycoprotein hormone - is the main factor of erythropoesis regulation. EP controls proliferation and differentiation of the erythroid precursors; data are available on its stimulating influence on the proliferation of erythroblasts, hemoglobin synthesis, and the release of reticulocytes from the bone marrow into the blood [2,4,8]. In the mid-1980's recombinant erythropoetin was obtained [4], the use of which immediately yeilded promising results in iron-deficiency anemia therapy [5].

Erythropoetin therapy proved to be especially successful in the case of anemia accompanying chronic kidney failure [6] and early anemia in premature infants [7]. Recently a proposal was made by V. I. Gudim et al. [2] to use recombinant erythropoetin in iron-deficiency anemia (IDA) therapy in adults, in connection with the discovery that EP biosynthesis is reduced in moderately severe and severe IDA [2].

In the present work the activity of EP in the plasma of healthy children and children with IDA was studied.

MATERIAL AND METHODS

The method of biological testing of erythropoetin activity in vitro [9, 10] was used in modification [3] with some changes. Nucleus-containing spleen cells from anemic BALB/c mice weighing 20-30 g were used as the target cells for EP activity determination. Anemia was caused by the administration of phenylhydrazine hydrochloride at a rate of 60 mg/kg. Three days after the second administration of phenylhydrazine, a suspension of cells was prepared in the following manner: 3 ml of alfa-MEM medium

TABLE 1. Erythropoetin Activity in Plasma of Healthy Children (M±m)

Indicator	Age, month								
	2	3	6	9	12	18	24	30	36
Number of children examined	7	6	7	6	9	6	8	8	9
EP activity, mU/ml	6.3±0.7	6.9±1.4	12.5±1.7	18.3±2.5	16.9±2.8	14.8±1.9	13.9±2.6	11.8±3.7	12.5±2.6
	(5.0-10.2)	(5.5-11.6)	(6.9-19.1)	(10.2-22.	(8.8-24.8)	(8.0-22.6)	(6.2-20.8)	(5.0-22.5)	(6.4-21.5)

All-Union Scientific Center of Hematology, Moscow
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(Flow), 4 ml embryonic calf serum (Flow), 0.2 ml of 0.2 mM glutamine, 0.2 ml of 0.1 mM 2-mercaptoethanol, 0.4 ml of 20 mM HEPES buffer, and 0.2 ml of kanamycin sulfate in a concentration of 100 μg/ml were added to 2 ml of cell suspension. The medium with the cells was introduced into 96-well plates (Linbro) in a quantity of 50 μl per each hole with the addition of 50 μl of plasma of varying concentration or the EP standard. The commercial preparation "Epoetin-ALFA" (Epogen, USA) with an activity of 2000 U/ml was used as the standard. Five to seven points, each including three wells, were used for the investigation of EP activity.

Culturing was performed at 37°C in 5% CO_2 in the air and 100% humidity. After 20-22 hours tritium-thymidine was intruduced into the culture (1 μCi in 10 μl for each well) and culturing was continued for two hours. The cells were collected on Titertek nitrocellulose filters with the aid of a 12-well vacuum cell collector (Millipore); the radioactivity of each sample was measured with a liquid scintillation counter (Contron).

The indicators of iron metabolism (plasma iron, total and latent iron-binding capacity of the serum) were estimated by the bathophenanthroline method with a Bio-La-Test kit (Lachema). Ferritin concentration was determined by the radioimmunochemical method with standard IRMO-Ferritin kits. Erythropoetin activity was measured in the plasma of 66 healthy children and 41 children with "typical" IDA [1] aged 2-36 months. The IDA diagnostics was performed according to the recommendations of Pizarro [10].

RESULTS

The investigations established that EP activity in healthy young children lies in the range 5 - 24.8 mU/ml,

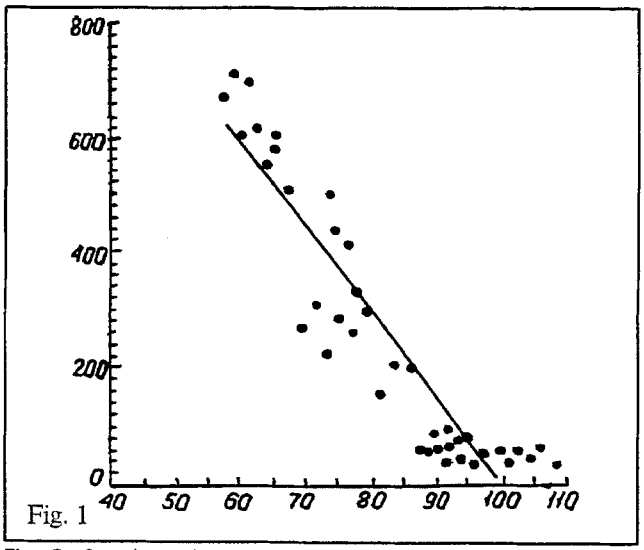

Fig. 1

Fig. 1. Correlation between erythropoetin activity and hemoglobin concentration in the case of iron-deficiency anemia in children. Abscissa: hemoglobin concentration (g/liter); ordinate: erythropoetin activity (mU/ml).

TABLE 2. Erythropoetin Activity and Indicators of Iron Metabolism in IDA in Children (M±m).

Indicator	IDA		
	Mild	Moderate -Severe	Severe
Number of children examined	17	15	9
Hemoglobin concentration, g/liter	97.2±1.4	78.9±1.5	63.2±1.0
Erythropoetin activity, mU/ml	59,9±1,4	270,3±31,2	619,6±20,9
	(40.5-88.4	(62.9-501.2)	(511.6-715.0
Iron in serum, uM	9.2±2.6	7.3±1.9	5.4±2.3
Total iron-binding capacity of serum, uM	63.1±3.9	69.5±4.1	76.8±3.6
Ferritin, ng/ml	9.2	6.9	2.6
	(5.1-9.5)	(3.5-7.5)	(0-3.5)

the mean level being 13.6±2.9 mU/ml. The lowest EP activity was found in the plasma of 2-3-month-old patients: 6.3±0.7 and 6.9±1.4 mU/ml, respectively. During the period from 2 to 9 months a slender increase of EP activity was observed in healthy children (Table 1). At that age a close correlation is revealed between the age of the examined patients and EP activity: the correlation coefficient r for the two parameters was +0.76. After 9 months the correlation between the age of the children and EP activity in their plasma drops significantly: r=+0.31. The same weak correlation was found between the hemoglobin concentration and EP activity in healthy young children: r=-0.41. After 9 months up to 3 years EP activity does not undergo any significant changes (see Table 1) and on the whole corresponds to normal indications in healthy adult donors [11]. The results of testing EP activity at an early age entirely agree with earlier findings on the normal activity of the hormone in children [12].

The results of studying EP activity in children suffering from IDA are presented in Table 2. Activity in the case of IDA in children fluctuated in a wide range: from 40.5 to 715 mU/ml and clearly depended on the severity of anemia. Our attention was attracted by the fact that in the case of IDA in children the level of EP activity rose on the average 4-fold, whereas in adults with mild IDA a 6-fold and greater increase of EP activity was found by Gudim et al. [2]. On the other hand, in the cases of moderate-severe and severe IDA in children we discovered an increase in EP activity of 20 and 46 times, respectively, while in adults a less significant (6.7 and 12.5 times) increase of EP activity was revealed [2]. Such a different reaction to IDA in adults and children could

be explained by the different duration of anemia development (in children over several months, in adults up to 10 years), the wide age range of the adults examined (from 17 to 63 years), and the variation of accompanying diseases in them.

A close correlation (r=-0.93) was discovered between EP activity and the hemoglobin concentration in children suffering from IDA (see Fig. 1). The level of EP activity in the examined patients correlated clearly with the indicators of iron metabolism depicting the degree of deficiency. Thus, the correlation coefficient between the level of EP activity and the concentration of the total iron-binding capacity of the blood serum in the case of IDA in children was +0,74. The same close correlation was revealed between the ferritin content and the activity of EP (r=-0.71). The results obtained give evidence that an adequate response to developing anemia is provided by EP production in young children.

It seems to us that the use of recombinant erythropoetin for the correction of IDA at an early age is hardly justified.

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Erythropoietic Activity in The Serum of Mice during Postnatal Ontogeny

A.K. Baskuryan, T.E. Manakova, and A.G. Kartashov

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During postnatal development of mice, changes occur both in the cellular composition of the hematopoietic tissues and in the hematopoiesis-regulating system. Neonatal mice develop transient anemia [5] that lasts for 6 weeks and is accompanied by variations in parameters of the peripheral blood [1] and functional characteristics of the bone marrow [2, 4] and by a rise in the level of the factor stimulating erythropoiesis in the plasma [9, 15]. Several authors have described hematopoietic disturbances in old mice [8, 14]. It has been suggested that the erythropoiesis-regulating mechanisms in young mice are distinct from those in older animals [6]. Examination of various hematological parameters throughout the period of postnatal ontogeny is therefore necessary if the mechanisms regulating hematopoiesis in general and erythropoiesis in particular are to be understood.

The purpose of the present study was to examine changes in erythropoiesis in mice with age by comparing some peripheral blood parameters and serum erythropoietic activity in neonatal (aged 2-25 days), sexually mature (aged 1-12 months), and old (aged 15-18 months) animals.

All-Russian Hematological Research Center, Moscow.
(Presented by A.I. Vorob'ev, Academician of the Russian Academy of Medical Sciences)