

Complex Application of Preparation Containing Ultralow Doses of Antibodies for the Treatment of Anemia Caused by Pubertal Uterine Bleedings

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, Suppl. 1, pp. 144-147, September, 2009
Original article submitted August 1, 2008

Poetam and anaferon (pediatric formulation) administered in combination with iron preparation to patients with anemia induce pronounced positive shifts in metabolic and morphological status of mature erythrocytes: the number of sulfhydryl groups and lipoprotein complexes in cell membranes and dry weight of erythrocytes increased, while the number of forms at different stages of degeneration decreased.

Key Words: *pubertal uterine bleedings; posthemorrhagic iron-deficient anemia; erythron; poetam; anaferon (pediatric formulation)*

Iron-deficient anemia is the main and the most incident complication of pubertal uterine bleedings (UB). It has complex and mixed genesis. Long-term bleedings result in an iron-deficient state, while disturbances in hormonal homeostasis and hyperestrogen status lead to suppression of hemopoiesis. Estrogens inhibit the production of endogenous erythropoietin [5]. The use of synthetic analogs of sex hormones for the therapy of UB aggravates functional and morphological pathology of erythrocytes, which makes the therapy of anemia with iron preparations (IP) more difficult [1,4,6,8]. Combined treatment with immunomodulator preparations is also substantiated [2]. The development of anemia aggravates disturbances in hormone homeostasis and induces immunological insufficiency. The phenomenon of mutual aggravation or vicious circle is thus formed [5].

The use of preparations containing ultralow doses (ULD) of antibodies in the complex treatment of UB-associated anemia is a promising trend. Poetam

preparation contains affinity-purified antibodies to endogenous erythropoietin; anaferon (pediatric formulation, AP) contains affinity-purified antibodies to IFN- γ . Mild and rapid effects of antibodies and the absence of side effects and addiction are indisputable advantages of antibodies [7]. It should be noted that ULD of antibodies were never before used in gynecological practice.

Improvement of treatment efficiency in pubertal UB-associated anemia should be determined by reducing the duration of IP administration due to improvement of erythropoiesis parameters. The quality of treatment is based on normalization of not only peripheral erythron and ferrokinetics parameters, but also structural and metabolic status of erythrocytes.

Here we studied the effect of complex treatment with poetam and AP, preparations containing ULD of antibodies, in combination with IP on structural and metabolic status of erythrocytes in the treatment of anemia against the background of pubertal UB.

MATERIALS AND METHODS

We examined 90 adolescent girls. The main group included 60 patients aging 13-18 years with UB and

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medium severity anemia (hemoglobin 70-99 g/liter), the control group consisted of 30 age-matched healthy girls without menstrual cycle disturbances. All examinees were divided into two groups: group 1 girls received IP, poetam, and AP, and group 2 girls received IP monotherapy. Sorbifer (Egis, one tablet contains 320 mg iron sulfate) was used as IP. Sorbifer was administered in a dose of 1 tablets 2 times a day. Poetam and AP were administered in combination with IP in a dose of 1 tablet 3 times a day with equal time interval irrespective of meals.

The duration of observation was 6 weeks. Five visits of the patients were planned: before treatment (1), at the start of treatment (2), and then during the treatment with 2-week intervals (3-5).

For complex evaluation of the state of peripheral component of erythron, the number of erythrocytes and reticulocytes, hemoglobin content, color index, and hematocrit were measured using routine hematological methods. The mean erythrocyte diameter, the width of erythrocyte size distribution (degree of anisocytosis), mean hemoglobin content per erythrocyte, and mean corpuscular concentration of hemoglobin in erythrocyte were measured on an Abacus hematological analyzer (Diatron). Iron concentration in the serum and total iron-binding capacity of the serum were measured using standard diagnostic kit (Teco). Transferin and serum ferritin were assayed by a biochemical method using Orgentec Diagnostica kits.

Before and after therapy, the structural and morphological status of erythrocytes was evaluated. Sulfhydryl groups and lipoprotein complexes in peripheral blood erythrocytes were quantitatively evaluated by cytophotometry. Surface architectonics of peripheral blood erythrocytes was studied by scanning electron microscopy. The percent of each type of cells according to classification [3] was calculated.

The significance of differences between the compared parameters was evaluated using Student's *t* test (in case of normal distribution of data) and Mann-Whitney *U* test (in cases when the data did not fit to normal distribution).

RESULTS

The use of preparations containing ULD of antibodies in combination with IP significantly increased reticulocyte and erythrocyte counts in the peripheral blood, which attests to activation of the erythroid hemopoietic stem and normalized the mean erythrocyte volume, mean hemoglobin content in erythrocytes, mean concentration of hemoglobin per erythrocyte, *i.e.* reduced anisocytosis. Combined treatment with poetam, AP, and IP more rapidly ($p < 0.05$) normalized the parameters of erythron and pharmacokinetic parameters of the peripheral blood compared to IP monotherapy.

In our case, it was interesting to evaluate not only quantitative, but also qualitative characteristics of the erythron. In group 1, the concentrations of sulfhydryl groups and lipoproteins in erythrocytes significantly increased by 1.5 times ($p < 0.05$). The studied parameters in medium-severity anemia attained the values observed in the control groups (0.358 ± 0.130 and 0.724 ± 0.012 arb. units, respectively) after 4 weeks of treatment. In patients receiving IP monotherapy, the values observed in the control group were attained only by the 6th week. Thus, the use of preparations containing ULD of antibodies normalized cytochemical characteristics of erythrocyte membranes, which determined cell resistance to negative effects of hypoxia and improved their gas-transporting function (Table 1).

Disturbances in lipid spectrum of the cytolemma seriously affect the optimum morphofunctional status of erythrocytes and can induce early irreversible transformation of cells, their accelerated aging and death, as it was observed in anemia against the background of pubertal UB before treatment.

Electron microscopy showed that surface architectonics of erythrocyte membranes underwent changes in different variants of therapy and these changes modulated quantitative distribution of cell forms. The most pronounced disorganization of membranes was observed in group 2 patients, where number of normal biconcave discocytes decreased, while the number of changed and degenerative forms of erythrocytes in-

TABLE 1. Cytochemical Parameters in Patients with Medium-Severity Iron-Deficient Anemia Caused by Pubertal UB Depending on the Applied Therapy ($M \pm m$)

Parameter	Group	Before treatment	After 2 weeks	After 4 weeks	After 6 weeks
Sulfhydryl groups, arb. units	1	0.262 ± 0.120	0.288 ± 0.150	$0.374 \pm 0.180^*$	$0.383 \pm 0.120^*$
	2	0.261 ± 0.080	0.274 ± 0.110	0.315 ± 0.130	$0.338 \pm 0.130^*$
Lipoprotein complex, arb. units	1	0.564 ± 0.020	0.644 ± 0.018	$0.738 \pm 0.015^*$	$0.882 \pm 0.018^*$
	2	0.563 ± 0.012	0.628 ± 0.014	0.641 ± 0.011	$0.682 \pm 0.014^*$

Note. Here and in Table 2: $*p < 0.05$ compared to the corresponding values before treatment.

TABLE 2. Morphological Characteristics of Peripheral Blood Erythrocytes (Scanning Electron Microscopy Data) in Patients with Pubertal UB Depending on the Variant of Antianemic Treatment ($M \pm m$)

Parameter		Control group	Group 1		Group 2	
			before treatment	after 4 weeks	before treatment	after 4 weeks
Morphological forms of erythrocytes, %	discocytes	88.64±0.24	77.12±0.12	92.78±0.11*	77.12±0.12	81.99±0.11
	transitional	9.80±0.10	16.24±0.26	5.84±0.06*	16.24±0.26	13.93±0.06
	prehemolytic	1.48±0.14	5.80±0.08	1.32±0.25*	5.80±0.08	3.66±0.25
	degenerative	0.08±0.01	0.84±0.04	0.06±0.04*	0.84±0.04	0.42±0.04
Ratio of inner diameter to outer diameter of erythrocytes, %		43.46±1.56	36.16±1.24	45.02±1.16*	36.16±1.24	38.13±1.18

creased compared to the control values against the background of treatment. Additional treatment with preparations containing ULD of antibodies significantly increased the number of normal biconcave discocytes and decreased the content of transitional, prehemolytic, and degenerative forms. The best results were obtained in group 1 after 4-week therapy of medium-severity anemia (Table 2).

Thus, the use of preparations containing ULD of antibodies in combination with IP in the therapy of anemia caused by pubertal UB considerably increased the quality of treatment. Analysis of the studied parameters substantiates the obtained results determined by the use of poetam and AP in combinations with IP for stimulation of erythropoiesis processes in pubertal UB.

The increase in the content of sulfhydryl groups in peripheral blood erythrocytes is a reliable sign of inhibition of LPO processes and recovery of the function of antioxidant system [5] against the background of treatment with preparations containing ULD of antibodies, which obligatory increases the time of erythrocyte life in the circulation. Inhibition of free radical oxidation leads to strengthening of protein-lipid interaction and increases lipoprotein content in erythrocytes during therapy with poetam and AP.

This therapeutic effect improves functional characteristics of erythrocytes: activation of intracellular antiradical defense system improves cell resistance to hypoxia and therefore maintains gas-transporting function of cells; this reduces the intensity of spontaneous hemolysis of erythrocytes and prevents their early irreversible transformation. The preparations containing ULD of antibodies normalize erythrocyte architectonics, which is seen from significant increase in the number of normal erythrocyte forms (biconcave discocytes), and reduces the content of transitional, prehemolytic, and degenerative forms.

Qualitative recovery of erythropoiesis processes is confirmed by delayed clinical results. After 1 year, normal parameters of erythropoiesis and ferrokinetics were observed in 88% group 1 patients, iron-deficient state in 8%, and mild anemia in 4% patients. In group 2, normal parameters of the erythron were observed in only 2 patients, iron-deficient state in 52% and anemia in 46%.

Thus, the use of preparation containing ULD of antibodies in combination with IP in the therapy of iron-deficient anemia resulting from pubertal UB improves not only qualitative parameters of the erythron status, but also quantitative cytochemical and morphological characteristics of erythrocytes. This full-value qualitative recovery of erythropoiesis processes determines low incidence (compared to IP monotherapy) of the relapses of iron-deficient states and anemia in patients with pubertal UB.

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