Fatty Acids of the Lipid Fraction of Erythrocyte Membranes and Intensity of Lipid Peroxidation in Iron Deficiency

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A high level of polyunsaturated fatty acids in erythrocyte membranes and an increased concentration of LPO products in the plasma and erythrocytes are observed in children with severe and moderate iron deficiency, whereas the values do not differ from the control in children with mild and latent iron deficiency. It is assumed that the excess of polyunsaturated fatty acids in erythrocyte membranes may lead to a shortening of the erythrocyte life span in severe and moderate iron deficiency.

Key Words: *fatty acids; erythrocyte membranes; iron deficiency; lipid peroxidation*

The development of iron deficiency anemia (IDA) is attended by an acceleration of imperfect hemopoiesis and by the appearance of erythrocytes with abnormal shape and reduced life span in the peripheral blood [6]. An important role in the genesis of these disturbances is allocated to impaired synthesis of the lipid component of erythrocyte membranes [14] and insufficient activity of erythrocyte enzymes (catalase, aconitase, cytochrome oxidase) and SH groups [4,13], which facilitate the susceptibility of erythrocyte membranes for oxidation [5]. *In vitro* studies of physicochemical properties of erythrocytes in adults have revealed that one of the causes of their reduced life span is a high sensitivity to glucose deficit and a low capability for deformation due to excessive rigidity [12] due not only to an altered lipid component but also to impaired functioning of membrane ATPases [14]. Fatty acids (FA) of the lipid component of erythrocyte membranes are known to largely determine their functional state and susceptibility for oxidation [2]. Despite the great number of investigations of physicochemical properties of erythrocytes in iron deficiency, the composition of erythrocyte membranes and the intensity of lipid peroxidation (LPO) in this disorder remain poorly understood.

The aim of the present investigation was to study the composition of FA of the lipid fraction of erythrocyte membranes and the intensity of LPO in children with iron deficiency of varying severity.

MATERIALS AND METHODS

FA of the lipid fraction of erythrocyte membranes and the intensity of LPO were studied in 46 children with different degrees of iron deficiency. Of these, 11 children had latent iron deficiency (LID) and 35 IDA of different severity. The children were aged from 9 to 24 months. LID and IDA were diagnosed as recommended earlier [11]. Parameters of the fatty acid composition of lipids from erythrocyte membranes and the activity of LPO in healthy children of the same age served as the control. The total lipid fraction was ex-

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TABLE 1. Fatty Acid Composition of Lipid Fraction of Erythrocyte Membranes in Iron Deficiency in Children ($M \pm m$)

FA, %	Control (n=9)	LID (n=11)	IDA		
			mild (n=14)	moderate (n=12)	severe (n=9)
C14:0	0.09±0.01	0.09±0.01	0.09±0.01	0.06±0.01*	0.03±0.01*
C15:0	0.15±0.02	0.12±0.01	0.14±0.02	0.09±0.03*	0.05±0.02*
C16:0	19.7±1.05	18.3±0.92	16.8±0.97	16.1±1.14	15.2±1.32*
C16:1	4.51±0.27	4.32±0.55	4.39±0.19	4.16±0.32	4.19±0.27
C18:0	18.1±1.63	19.4±1.14	18.1±1.15	16.1±1.17	13.4±1.41*
C18:1	22.2±2.17	23.1±2.03	23.0±1.06	17.3±1.90	15.9±1.76*
C18:2	20.4±1.15	19.6±1.30	22.9±1.06	23.7±1.39	25.2±1.40*
C20:4	14.8±0.72	15.0±0.93	15.1±0.64	22.8±1.13*	26.1±1.07*
IS	0.64±0.09	0.63±0.07	0.63±0.07	0.47±0.03*	0.40±0.02*

Note. Here and in Table 2 an asterisk denotes reliable differences in comparison with the control ($p<0.05$).

tracted from erythrocyte membranes as described previously [10] after the erythrocytes had been washed four times with buffered saline: 0.05 M Tris HCl (pH 7.4), 0.87% NaCl, 0.25 M $MgCl_2$, and erythrocyte ghosts were obtained as described elsewhere [9]. The fatty acid composition of the lipid fraction of the erythrocyte membranes was analyzed by gas chromatography using a Chrom-4 chromatograph [8]. The relative content of individual FA, the total content of saturated and unsaturated FA, and the index of saturation (IS) - the ratio of total saturated to total unsaturated FA - were determined. The level of diene conjugates (DC) and of malonic dialdehyde (MDA) in the plasma and erythrocytes was determined immediately after the blood had been drawn into cooled tubes. DC were analyzed after Plazer with some modifications [3]. MDA was determined after Staucliff with modifications [15].

RESULTS

It was found that the FA composition of erythrocyte lipids and the level of LPO products in LID and IDA did not differ from those in the control (Tables 1 and 2). Erythrocyte membranes from the patients with moderate IDA were characterized by an increased concentration of unsaturated FA due to arachidonic (C20:4) acid (30% on average) and by a reliable drop of the concentration of saturated FA: myristic (C14:0) and pentadecanoic (C15:0) acids. The revealed changes in the content of individual FA in moderate IDA were accompanied by a reliable decrease of IS of erythrocyte membranes and by an increase in the concentration of DC and MDA in erythrocytes by 34.3 and 22.6%, respectively. The increased content of polyunsaturated FA (PUFA) in moderate IDA may be considered as an adaptive reaction aimed at compensating for the "excessive rigidity" of erythrocyte membranes due to the accumulation of cholesterol [7]. The level of LPO products in the plasma of patients with moderate IDA did not differ from the control (Tables 1 and 2).

In severe IDA we observed a decreased content of all four saturated FA: myristic (C14:0) (3-fold), pentadecanoic (C15:0) (3-fold), palmitic (C16:0) (1.2-fold), and stearic (C18:0) (1.3-fold) acids. It should be noted that the concentration of linoleic (C18:2) acid increased by 20% on average, while arachidonic (C20:4) acid increased more considerably, by 80% on average. The above changes in the fatty acid composition of erythrocyte membranes resulted in a drop of IS to 0.40±0.02 vs. 0.64±0.09 in the control ($p<0.05$). At the same time in severe IDA we observed a rise

TABLE 2. Content of LPO Products in Plasma and Erythrocytes in Iron Deficiency in Children ($M \pm m$)

LPO products, mmol/liter	Control (n=9)	LID (n=11)	IDA		
			mild (n=14)	moderate (n=12)	severe (n=9)
DC in plasma	14.1±0.07	13.5±0.4	14.3±0.6	15.0±0.4	19.0±0.5*
DC in erythrocytes	16.4±0.6	14.8±0.9	16.1±0.7	19.9±0.4*	23.3±0.3*
MDA in plasma	2.0±0.1	2.0±0.3	2.0±0.1	2.2±0.1*	3.2±0.3*
MDA in erythrocytes	3.1±0.1	3.1±0.2	3.0±0.3	3.8±0.2*	4.6±0.4*

in the concentration of DC and MDA not only in erythrocytes but also in the plasma (Table 2).

Thus, latent iron deficiency and mild IDA in children are not accompanied by changes in the fatty acid composition of erythrocyte membranes or in the intensity of LPO. In moderate IDA the intensity of LPO in the plasma remains unchanged, whereas in erythrocytes we find an accumulation of DC and MDA which is apparently caused by reduced activity of superoxide dismutase [1] and other iron-containing enzymes of the antioxidant system [13]. In severe IDA we observed an accumulation of LPO products both in the plasma and in erythrocytes, which apparently resulted not only from simultaneous depletion of the antioxidant capabilities of the plasma and erythrocytes, but also from the increased content of PUFA, LPO substrates, in the erythrocyte membranes [2]. The increased content of PUFA in erythrocyte membranes may, furthermore, be a direct cause of the shortened life span of erythrocytes in severe and moderate IDA, due to the ability of PUFA to oxidize readily under conditions of activated LPO and depleted antioxidant enzymes.

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