Accumulation of Eicosapentaenoic Acid in Membranes of Erythrocytes and Leukocytes during Alterative-Exudative and Allergic Inflammation

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Children patients with alterative-exudative (gastrointestinal diseases) and allergic inflammation (bronchial asthma) exhibited similar changes in fatty acide composition of erythrocyte and leukocyte membranes. They included accumulation of considerable amounts of eicosapentaenoic acid and decrease in the content of docosahexaenoic acid (long-chain derivative of eicosapentaenoic acid). The accumulation of eicosapentaenoic acid and decrease in the content of docosahexaenoic acid in cell membranes probably play a role in the pathogenesis of chronic inflammatory diseases. It is related to a possible decrease in the formation of anti-inflammatory eicosanoids (series-5 leukotrienes) and protective compounds (resolvins and protectins) from fatty acids and changes in the physicochemical properties of cell membranes.

Key Words: *inflammation; children; membranes of erythrocytes and leukocytes; eicosapentaenoic acid; docosahexaenoic acid*

Long-chain (eicosanoid) polyunsaturated fatty acids of ω3 and ω6 families are the precursors for a variety of bioactive compounds (prostaglandins, leukotrienes, thromboxanes, *etc*.). They play a role in the regulation of various phases of the inflammatory response [8,9], enter the composition of cell membrane phospholipids, and determine structural and functional properties of cell membranes [11]. It is important to evaluate possible changes in fatty acid composition of cell membranes in children with general inflammatory diseases. These severe chronic diseases often cause disability of patients. This problem received little attention [7].

Here we studied the composition of fatty acids in membranes of erythrocytes and leukocytes from children patients with inflammatory diseases of the gastrointestinal tract (IDGIT; esophagitis, gastroduodenitis, and stomach ulcer). These diseases are induced by infectious, mechanical, or chemical agents and belong to normergic alterative-exudative inflammation. This type of inflammation is accompanied by pain and fever. Other patients with bronchial asthma (BA) had allergic inflammation. This inflammation is initiated by the antigen—antibody interaction and has a hyperergic nature. It is accompanied by bronchial obstruction, serous exudation, and bronchial edema. The pain syndrome and fever are not observed during allergic inflammation.

MATERIALS AND METHODS

The trial included 57 children aged 7-14 years. Thirty-eight children had IDGIT (esophagitis, gastroduodenitis, and peptic ulcer). IDGIT in 25 patients was accompanied by class I-III obesity. Thirteen of thirty-eight children with IDGIT had a normal body weight. Nine patients with BA (period between disease attacks) were examined at the Moscow Institute of Pediatrics

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and Children Surgery (Russian Medical Technologies) and N. F. Filatov Children's Municipal Clinical Hospital No. 13. Ten conventionally healthy children had an unconfirmed diagnosis of surgical diseases (Children's Municipal Clinical Hospital, Lyubertsy). Erythrocyte membranes were obtained as described previously [4]. Leukocyte membranes were isolated by precipitation on a BECKMAN L7 centrifuge (ROTOR Ti 50.3, r_{av}=64.2 mm) at 44,000*g* and 0°C for 30 min [6]. The isolation of lipids and methylation of fatty acids were conducted as described elsewhere [3]. The composition of fatty acid methyl esters was studied by gas-liquid chromatography on a Dani 1000 chromatograph equipped with a flame-ionization detector. A quartz capillary column (length 60 mm, diameter 0.25 mm) was filled with phase HP-23. Nitrogen served as a carrier gas (42 m/min). Temperature of the evaporator, column, and detector was 230, 100-220, and 240°C, respectively. Fatty acid methyl esters were identified by comparing with the chromatogram of a standard mixture of saturated and unsaturated fatty acids. The unsaturation index (UI) of fatty acids was calculated as follows: UI=total amount of fatty acids/total amount of saturated fatty acids. The index of metabolic efficiency (IME) was calculated as follows: IME=20:4/20:2+20:3+0:5+22:5+22:6 [2].

The results were analyzed by a microprocessor (program of internal standardization). The data are expressed in percents of total amount of fatty acids in cell membranes. Statistical treatment of the results was performed by Student's *t* test and nonparametric Mann—Whitney test (SPSS 14 software). The differences were significant at $p<0.05$.

RESULTS

The composition of fatty acids in erythrocyte membranes from healthy children was characterized by prevalence of ω6-family arachidonic acid (AA) and high content of ω3-family docosahexaenoic acid (DHA; relative to the amount of polyunsaturated fatty acids). The contents of AA (20:4) and DHA (22:6) decreased sharply, while the amount of eicosapentaenoic acid (EPA; 20:5) of the ω3 family increased significantly in IDGIT patients (Table 1; Fig. 1, *a*). These changes were observed in IDGIT patients with normal body weight and obesity (Table 1; Fig. 1, *a*). Therefore, severe metabolic changes during obesity do not affect the composition of cell membrane fatty acids under conditions of inflammation. Changes in the amount of fatty acids in erythrocyte membranes were followed by variations in some indexes for the state of membranes. The EPA/DHA ratio in membranes was significantly elevated during inflammation (12.8 *vs*. 3.2 in healthy donors). Patients with inflammation were characterized by decreased ω6/ω3 family ratio for unsaturated fatty acids (0.9-2.0 *vs*. 2.9 in healthy donors), UI (1.15 *vs*. 1.3 in healthy donors), and IME

TABLE 1. Fatty Acid Content in Erythrocyte Membranes from Healthy Children and Patients with IDGIT (% of Total Fatty Acid Content; *M*±*SD*)

Fatty acids	Healthy children	IDGIT in obesity	IDGIT in patients with a normal body weight
12:0	0.74±0.40	1.4±1.4*	1.57±0.59**
14:0	2.30±1.04	3.97±2.60**	3.8±1.6*
14:1	0.57±0.30	0.74±0.40	0.91±0.40*
16:0	22.6±4.6	26.0±4.6	26.2±7.0
18:0	15.4±3.8	13.3±4.5	11.20±3.91*
18:1	15.9±2.1	18.5±5.5*	17.0±4.7
18:2	10.0±2.1	7.8±3.4*	6.5±2.8**
18:3	0.26±0.10	0.57±0.40**	0.66±0.40**
20:3	1.2±0.5	0.8±0.5*	0.52±0.46**
20:4	9.7±3.5	4.7±3.6**	2.3±1.4***
20:5	3.2±2.5	6.7±7.5	10.3±7.6*
22:6	2.1±0.8	1.18±0.90**	0.82±0.60***
Total ω3	8.33±2.70	10.45±7.20	13.8±7.3*
Total ω6	21.3±5.8	13.7±6.2**	9.7±2.6***

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to healthy children.

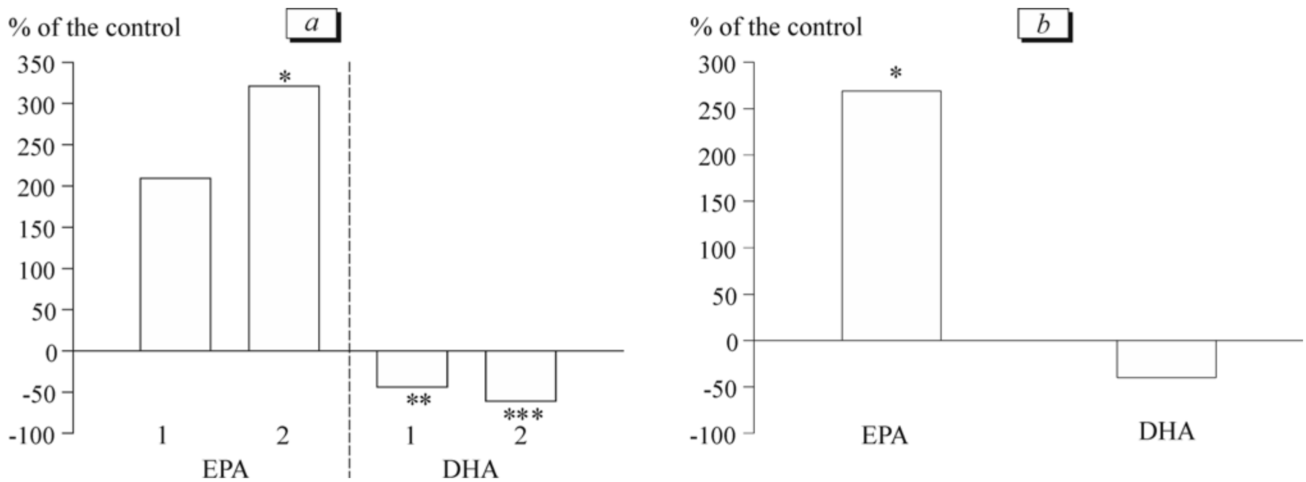

Fig. 1. Content of EPA and DHA in erythrocyte membranes from children patients with IDGIT (*a*) and in leukocyte membranes from children patients with BA (*b*). IDGIT+obesity (*1*); IDGIT (*2*). Ordinate: % of total fatty acid content in cell membranes from healthy children (control). *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control.

(0.20-0.61 *vs*. 1.2 in healthy donors) [2]. These data illustrate changes in the physicochemical properties of membranes. The observed changes are probably associated with the decrease in membrane fluidity and plasticity. It is related to reduction of membrane fatty acid unsaturation and decrease in the content of long-chain derivatives of essential polyunsaturated fatty acids (*e.g.*, AA and DHA) [5,11].

Similar changes in fatty acid composition of leukocyte membranes were observed in children with allergic inflammation (concomitant disorder in BA). The content of EPA increased significantly in leukocyte membranes. We revealed a tendency to accumulation of α-linoleic acid, increase in the total content of ω3-family polyunsaturated fatty acids, and a tendency to a decrease in the amount of DHA (22:6, ω3) and ω6 family polyunsaturated fatty acids (Table 2; Fig. 1, *b*). The ω6/ω3 ratio in leukocyte membranes from BA patients was reduced by 2 times (0.39 *vs*. 0.79 in healthy donors). By contrast, the EPA/DHA ratio increased significantly in these patients (26.8 *vs*. 6.0 in healthy donors). These changes resulted in an increase of UI (1.23 and 0.72 in BA patients and healthy donors, respectively). However, IME in BA patients was lower than in healthy donors (0.06 and 0.13, respectively).

Our results indicate that the inflammatory reaction of two types is accompanied by a sharp increase in the EPA/DHA ratio in two types of cell membranes. These changes illustrate the development of metabolic blockade of EPA conversion into DHA. This general response reflects the progression of changes, which are typical of various types of inflammation and different types of cells. Blockade of EPA conversion into DHA has a pathogenetic role. On the one hand, this state is accompanied by changes in the physicochemical properties of cell membranes and dysfunction of organs and tissues (*e.g.*, GIT) with secretory activity (gastric epithelium or pancreas). On the other hand, accumulation of EPA in cell membranes can result in reduced production of protective eicosanoids from EPA such as leukotriene D5, major type of leukotrienes in healthy children [1], and a decrease in the production of some metabolites of DHA (resolvins and protectins) that playing an important role in reparative processes during cell injury [10]. This hypothesis is confirmed by the reduced production of leukotriene

TABLE 2. Fatty Acid Content in Erythrocyte Membranes from Healthy Children and Patients with BA (% of Total Fatty Acid Content; M±SD)

Fatty acids	Healthy children	BA patients
12:0	1.15±0.30	0.6±0.3**
14:0	12.3±3.7	7.5±4.1
14:1	1.74±0.50	0.8±0.6*
16:0	34.1±8.2	26.9±9.6
18:0	8.9±2.2	8.01±2.50
18:1	15.4±3.3	17.3±4.4
18:2	5.7±1.8	5.2±2.6
18:3	0.74±0.32	0.95±0.81
20:4	0.83±0.55	0.83±0.78
20:5	4.8±4.8	12.9±8.9*
22:6	0.80±0.56	0.48±0.29
Total ω3	8.4±4.6	15.3±8.3
Total ω6	6.6±2.1	6.01±2.7

D5 during IDGIT (concomitant disease in obesity). We conclude that significant variations in fatty acid composition of cell membranes during inflammation (accumulation of EPA and decrease in the contents of DHA and AA) play an important pathogenetic role in the development and severe recurrences of inflammatory diseases.

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