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Ratios of Substrates and Inhibitors of Prostaglandin Synthesis in Blood Plasma of Patients with Heart Ischemia

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ABSTRACT

To check the proposed hypothesis that the relative content of individual polyunsaturated fatty acids (PUFAs)—substrates and inhibitors of prostanoid synthesis—in plasma can be regarded as a quantitative risk factor of blood clotting, a test was conducted on free fatty acids content in blood plasma of healthy people (group 0) and patients with heart ischemia before (group 1) and after (group 2) they were treated for a month with a food additive called "Eiconol," enriched with PUFA ω3. Different proportions of PUFAs have been calculated in all cases, accounting for the contribution of each acid to the process of primary clotting. Comparison of PUFA rations among the three groups showed significant differences of means between groups 0 and 1 and also group 1 and 2 for 6 out of 7 proposed coefficients, which disappeared after "Eiconol" treatment (comparison of groups 0 and 2). The results led to the conclusion that out of the proposed PUFA proportions, the coefficients describing the relative content of arachidonic acid in blood plasma may be the most informative for diagnosis and treatment efficiency in evaluation of heart and vascular diseases.

Index Entries: Polyunsaturated fatty acids; prostacyclin; thromboxane; prostanoids; thrombosis.

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INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are abundant components of the mammalian cell membranes. There are many published data mostly concerning their effects on lipid metabolism, eicosanoid synthesis, and prevention of coronary heart disease. The main precursor of prostanoids (PG) in humans is arachidonic acid (AA), and a number of morbid conditions and diseases were associated with an imbalance in the prostacyclin (PGI_2)/thromboxane (TxA_2) system (*1*).

It is known that in the situation with a tendency for thrombosis, TxA_2 production is elevated, PGI_2 production is reduced, or both occur. The opposite situation is found in some morbid conditions associated with an increased bleeding tendency (*1,2*). However it was found that only 0.93% among more than 3000 examined persons had the defects in the prostanoid system when 20% among them had cardiovascular disorders (*3*).

Previously, we investigated the ratio of PUFAs (*4*), which are substrates (*5,6*) and inhibitors (*7*) of prostanoid synthesis. It was shown that the ratios correlated with acuteness of cardiovascular disorders (*4*). Therefore we supposed the influence of the plasma PUFA ratios on the disorders if defects in the systems of prostanoid synthesis and stabilization were absent.

The present study was designed to investigate the ratio of free PUFAs in plasma of patients with heart ischemia (HI) before and after their treatment with a food additive called "Eiconol," enriched with PUFAs ω3.

METHODS

The primary HI group was comprised of 43 patients (men and women), aged 55 ± 7 (group 1). These patients had no history of myocardial infraction within a year. Nineteen patients among them were also examined after 1 mo of treatment with a food additive "Eiconol," enriched with PUFAs ω3 (group 2).

Control subjects were 26 volunteers aged 38 ± 15, who had no medication and no past history of ill health (group 0). Plasma samples were obtained from blood collected by venipuncture in sodium citrate solution (3.8%). Free fatty acids (FFAs) were extracted from plasma by the method described elsewhere (*8*) with their additional extraction with hexane (*9*).

FFAs were esterified by methanol in the presence of 14% BF_3 and analyzed using the gas chromatograph "Varian" Model 3700 with a capillar column (30 m × 0.25 mm). Pentadecaenoid acid was an internal standard.

Statistic analysis was performed using SYSTAT program. The mean FFA concentrations and their ratios (coefficients K_1-K_7) and standard deviation were calculated for groups 0, 1, and 2. The mean values among the groups were compared, and their significant differences evaluated by Student's *t*-test.

Table 1
The Content of FFAs in Blood Plasma
of Healthy People (Group 0) and Patients with HI Before
(Group 1) and After (Group 2) 1 Mo of Treatment with "Eiconol"

FFAs	FFA in plasma, μM		
	Group 0, n = 26	Group 1, n = 43	Group 2, n = 19
14:0	9 ± 3	28 ± 16	20 ± 14
16:0	256 ± 43	283 ± 127	186 ± 52
16:1ω9	16 ± 8	28 ± 16	14 ± 4
18:0	158 ± 39	148 ± 72	98 ± 31
18:1ω9	198 ± 52	227 ± 138	142 ± 50
18:2ω6	196 ± 41	201 ± 81	131 ± 43
20:3ω6	13 ± 5	18 ± 11	9 ± 4
20:4ω6	82 ± 24	79 ± 56	39 ± 16
20:5ω3	24 ± 16	8 ± 7	7 ± 4
22:6ω3	33 ± 12	16 ± 14	12 ± 11

"Eiconol" as a food additive prepared from fish oil (PUFA ω3/ω6 = 0.12) was a product of "Trinity" (Moscow, Russia).

RESULTS AND DISCUSSION

Numerous studies showed that dietary PUFAs ω3 were useful in cardiovascular disease prevention and treatment. "Eiconol" is used in clinics as a food additive enriched with PUFAs ω3. PUFAs are incorporated into tissue phospholipids and are the immediate precursors of prostanoids. Dietary administration of "Eiconol" was shown to increase the content of PUFAs ω3 in the membrane phospholipids (*10*).

We investigated the FFAs composition in human blood plasma of healthy volunteers (group 0) and patients with HI before (group 1) and after (group 2) 1 mo of their treatment with "Eiconol" (Table 1). The results were analyzed using the coefficients K_1-K_7, which reflected the contribution of PUFAs in synthesis of prostanoids with opposite effects on platelet aggregation.

The coefficient K_1 = C20:4/C20:3 + C20:5 reflects the content of TxA_2 and PGI_2 precursor vs the content of the concurrent inhibitors of arachidonic acid conversion and precursors of prostanoids preventing or limiting thrombosis (5). The coefficient K_2 = C20:4/C22:6 characterizes the ratio of TxA_2 and PGI_2 content vs the most potent inhibitor of their synthesis (7). The coefficient K_3 = C20:3 + C20:5/C22:6 reflects the contribution of C22:6 in inhibition of PGs possessing vasodilatory and antithrombotic properties. The coefficients K_4, K_5, K_6, and K_7 show the ratio of

Table 2
The PUFA Ratios (*K*) in Blood Plasma
of Healthy People (Group 0) and Patients with HI Before
(Group 1) and After (Group 2) 1 Mo of Treatment with "Eiconol"

K	Group 0, n = 26	Group 1, n = 43	Group 2, n = 19
K_1	2.39 ± 0.70	3.28 ± 1.45	2.61 ± 1.06
K_2	3.17 ± 2.96	6.03 ± 3.15	4.03 ± 1.94
K_3	1.47 ± 1.85	2.10 ± 1.43	1.67 ± 0.87
K_4	0.09 ± 0.03	0.16 ± 0.05	0.13 ± 0.04
K_5	0.54 ± 0.07	0.65 ± 0.07	0.58 ± 0.09
K_6	0.15 ± 0.07	0.06 ± 0.03	0.11 ± 0.06
K_7	0.22 ± 0.07	0.13 ± 0.06	0.18 ± 0.09

C20:3, C20:4, C20:5, and C22:6 vs their summary concentrations in plasma. The coefficients K_1-K_7 were calculated for all plasma samples. The results of statistical analysis of the data are shown in Table 2. Table 3 displays the significant differences of the mean values for K_1-K_7 among groups 0, 1, and 2.

The data (*11,12*) showing the abnormal capacities for exchange of C20 and C22 PUFAs between media and platelets and endothelial cells were the background for this analysis. From these data, we assumed that the PUFA ratios in plasma at any time reflected their ratios in membrane phospholipids of these cells (*13*).

As a result of the "Eiconol" application, K_1 significantly decreased. This diminution could reflect both the decrease in the relative C20:4 content (K_5) and the increase in C20:5 in the pool of PUFAs (K_6). The increase in C20:5 content could occur owing to both the elevated content of the acid in "Eiconol" and the quick transformation of C22:6 into C20:5 in humans (*14*). The relative C20:3 decrease (K_4) did not much affect K_1. The "Eiconol" application led to a significant diminution of the absolute (Table 1) and relative (K_5) contents of AA, being the only precursor of thrombogenic thromboxane in plasma. As a result of treatment with "Eiconol," the content of C20:5 in the PUFA pool in plasma (K_6) doubled. C20:5 is known as PGI_3 and a nonthrombogenic TxA_3 precursor as well as a competitive inhibitor of AA conversion. The relative content of C22:6 (K_7) being the inhibitor of all prostanoid synthesis was also raised. The coefficient K_2 after "Eiconol" treatment decreased significantly, but the change in K_3 was not significant. The comparison of the K_2 and K_3 behavior together with the known positive clinical effect of "Eiconol" allowed us to suggest that C22:6 inhibited the TxA_2 synthesis in platelets more strongly than the prostacyclin synthesis in endothelial cells. The elevation of the ratio 6-keto-$PGF_{1\alpha}$/TxB_2 was shown in rat plasma as a result of "Eiconol" diet (*10*).

Table 3
Significant Differences in Mean K Values
Among Groups of Healthy People (Group 0) and Patients with HI
Before (Group 1) and After (Group 2) 1 Mo of Treatment with "Eiconol"

Comparison of groups	PUFA ratios						
	K_1	K_2	K_3	K_4	K_5	K_6	K_7
0 and 1	+	+	−	+	+	+	+
0 and 2	−	−	−	+	−	+*	+*
1 and 2	+	+	−	+	+	+	+*

(+) Significant difference ≥ 0.95.
(+*) Significant difference ≥ 0.9.
(−) No difference.

Comparison of K_1-K_7 among the examined groups of people (Table 3) showed significant differences between healthy people (group 0) and patients with HI (group 1) in all coefficients but K_3. The same conclusions follow from comparison between patients before and after their treatment with "Eiconol" (groups 1 and 2).

Thus, although the plasma FFAs themselves may not be a risk factor of cardiovascular disorders, their ratios could be an informative risk indicator. From our results, the most informative ratios reflect the relative contents of AA in plasma, namely the coefficients K_1, K_2, and K_5. Likewise, a month-long treatment with "Eiconol" made the K_1, K_2, and K_5 indistinguishable on average in groups 0 and 2.

We have examined a possible influence of PUFA ratios on prostanoid synthesis only. Our results did not contradict the published data (*15*), showing the inhibition of TxA_2/PGH_2 receptors of platelets by C20:5 and 22:6.

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