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Altered membrane free unsaturated fatty acid composition in human colorectal cancer tissue

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Abstract

Polyunsaturated free fatty acids (PUFAs) participate in normal functioning of the cell, particularly in control intracellular cell signalling. As nutritional components they compose a human diet with an indirect promoting influence on tumourogenesis. The PUFAs level depends on the functional state of the membrane. This work is focused on changes only of free unsaturated fatty acids amount (AA – arachidonic acid, LA – linoleic acid, ALA – α-linolenic acid, palmitoleic acid (PA) and oleic acid) in cell membranes of colorectal cancer of pT3 stage, G2 grade without metastasis. Qualitative and quantitative composition of free unsaturated fatty acids in the membrane was determined by high-performance liquid chromatography. It was shown that the malignant transformation was accompanied by a decrease in amount of LA and ALA while arachidonic and oleic acids increased. It is of interest that free AA levels are elevated in colon cancer, as AA is the precursor to biologically active eicosanoids.

Key words: colorectal cancer, free unsaturated fatty acids, HPLC

Abbreviations: AA – arachidonic acid; ALA – α-linolenic acid; LA – linoleic acid; PA – palmitoleic acid; PUFAs – polyunsaturated free fatty acid

Introduction

A cancer transformation results in the appearance of a new cell line in the organism whose malignant activity to the organism is transmitted from one cell generation to another. Our earlier experiments show, that the phenomena connected with changes in cell membranes are suspected to play important role during the cancer transformation [1, 2].

Cell membrane is an integral part of an alive cell and it plays an essential role in life processes; it makes the cell an isolated system and it determines its specific properties and its capability of moving. The most important properties of a biological membrane are its electric charge and its potential drop between the membrane and surrounding solution. Electric properties of the membrane are determined by acid-base and complex formation equilibria of membrane and solution components [3, 4]. Most membrane components – proteins, phospholipids and fatty acids – are involved in those equilibria.

Immune cell activation (cell proliferation, phagocytosis) and tumour growth (malignancy) result in an increased rate of novo synthesis and turnover of membrane phospholipids [5].

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These processes require a constant supply of fatty acids, the main supply being those consumed in the diet. It is well established that both the amount and type of fat consumed in the diet influence the lipid composition of immune and tumour cell membranes [6, 7]. Changes in membrane composition would affect growth, interaction with other cells (immune system), and the function of proteins and other components that are in the membrane. The function of the immune system depends on interactions between different cell types and through effects on membrane composition [8]. The dietary fatty acids, for example: palmitoleic 16:1, oleic 18:1, linoleic 18:2 (9,12), α-linolenic 18:3 (9,12,15) or γ-linolenic 18:3 (6,9,12) can the potential influence these interactions. Considerable evidence supports this mechanism for *n*−3 fatty acids [8]. *N*−3 and *n*−6 fatty acids are Polyunsaturated free fatty acids (PUFAs) with two or more double bonds in the carbon atom chain. *N*−3 and *n*−6 fatty acids are named after the position of the first double bond from the methyl end of the molecule. Population-based human studies show little or no association between *n*−6 and *n*−3 PUFA intake and colorectal cancer [9]. A couple of studies examine *n*−3 fatty acid incorporation into lipid rafts, offering a logical yet unexplored link between changes in the conjugated linoleic acid (LA) content of cell membranes and changes in cellular function [5].

Changes in plasma membrane structural characteristics in mammalian cells can change the activity of proteins that serve as ion channels, transporters, receptors, signal transducers or enzymes [10–12]. Dietary lipids were demonstrated to influence the pattern of fatty acids released from lymphocytes (i.e., arachidonic acid), which would ultimately influence the synthesis of eicosanoids (prostaglandins, leukotrienes and thromboxanes) [13]. In addition to their role in regulation of immune and inflammatory responses, eicosanoids may also be needed to sustain growth of tumour cells [8, 14].

The unsaturated fatty acids play similar role such as fatty acids, additionally having a large influence on properties, structure and function of membrane. We aimed to assess quantities of main free unsaturated fatty acids in cases of the most often occurring adenocarcinoma of large intestine.

Materials and methods

Tissue samples were obtained from nine patients (three men and six women) who underwent surgical resection because of colorectal cancer. The age of patients ranged from 45 to 80 years old. Our study included colorectal cancers in G2 grade and pT3 stage without distant and lymph node metastases, classified histopathologically as adenocarcinoma. Tumour samples and normal colon mucosa were collected immediately after tumour removal. The segments from macroscopically disease free intestinal mucosa were taken in the distance not smaller than 10 cm from the neoplastic lesions.

Isolation and analysis of free unsaturated fatty acids by HPLC method

The tissues (about 0.2−0.5 g) were homogenized in 1 mM-$NaHCO_3$ (pH = 7.6)−0.5 M $CaCl_2$ in a loose – fitting Dounce homogenizer. Membrane fragments were separated from nuclei and mitochondria by rate-zonal centrifugation of the 'low-speed' pellet as described by Evans (1970). The sediment was washed and partially separated in two following centrifugationing at $1000 \times g$. The sediment was homogenated in saccharose of 1.22 density and in the next step was covered with saccharose of 1.16 density. The cell membranes were separated by centrifugation at $2000 \times g$ for 25–35 min [2].

The cell membrane was homogenized in 2% acetic acid in ethyl ether of 2:1 volume ratio. The solution was then filtered out with degreased paper filters. The suspensions were centrifuged at $500 \times g$ for 2 min, the organic and the aqueous phases were separated, the aqueous phase was shaken again with 2% acetic acid in ethyl ether of 2:1 volume ratio and the phases were separated [15]. The organic phases were combined and were evaporated to dryness. The extract was dissolved in 200 μl acetonitrile [16].

The HPLC analysis was then carried out. The isolated free unsaturated fatty acids were separated by group analysis in RP 18 column using RP-HPLC (liquid chromatography in reversed phase system); acetonitrile–water mixture in 70:30 volume ratio in isocratic elution at 1 ml/s flow rate and 214 nm wave length [17].

Statistical methods

The data obtained in this study are expressed as mean ± SD. The data were analysed using Wilcoxon Matched-Pairs Signed-Ranks Test (from standard statistical programme SPSS 8.0 PL) for comparisons between control and cancer samples. The values of $p < 0.05$ were considered significant.

Results

We developed typical chromatographic separation only of free unsaturated fatty acids: arachidonic acid (AA, 20:4*n*−6), linoleic acid (LA, 18:2*n*−6), α-linolenic acid (ALA, 18:3*n*−3), palmitoleic acid (16:1) and oleic acid (18:1), the

last one was identified only for cases 5, 6, 7 and 8. The other PUFAs like 22:5 and 22:6 were not identified in our research. These lipid agents originated from normal and tumour human large intestine tissue cells and results of our chromatographic measurements are presented in Figs. 1 and 2, respectively. AA is eluted first, closely followed by LA, PA and ALA. Amounts of separated free fatty acids were determined from peak area.

Table 1 presents the content of fatty acids and their membrane concentration in normal human large intestine and cancer cells. The content of LA and ALAs decreased significantly and the content of arachidonic and oleic acids increased significantly in the patients at pT3 stage, G2 grade without lymph node metastases compared to the unaffected cells. The AA is more significant than other fatty acids, both in control and in cancer tissues. Generally, the content of all free fatty acids decreased.

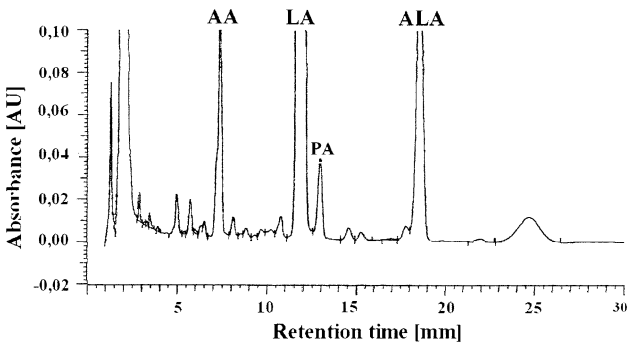

Fig. 1. Typical separation of free unsaturated fatty acids (AA – arachidonic acid, LA – linoleic acid, ALA – α-linolenic acid, PA – palmitoleic acid) in normal colon mucosa.

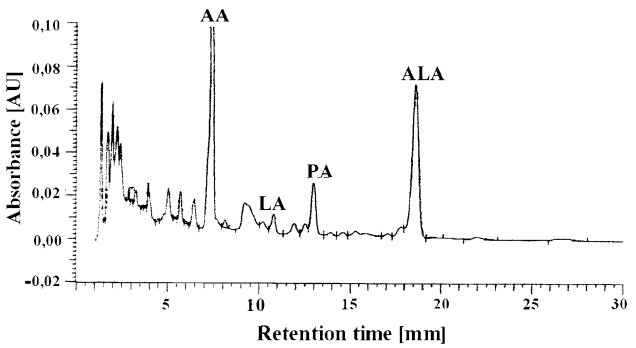

Fig. 2. Typical separation of free unsaturated fatty acids (AA – arachidonic acid, LA – linoleic acid, ALA – α-linolenic acid, PA – palmitoleic acid) in colorectal adenocarcinoma of grade G2, stage pT3, and without distant and lymph node metastases (N0 M0).

Discussion

Free fatty acids occur in cell membranes in very small quantities. The PUFAs level depends on the functional state of the membrane. Long-chain $n-3$ fatty acids were shown in immune cells to alter cell surface costimulatory and activation markers or molecules, calcium signalling and protein kinase C translocation in the membrane [18–20]. Similarly, in other cell types, membrane incorporation of $n-3$ fatty acids can alter membrane permeability, membrane fluidity and hormone and growth factor binding [21, 22].

The results of our research show changes in the amount of free unsaturated fatty acids of human large intestine cell membrane. In the all colorectal cases amount of arachidonic, oleic acids increased and amount of linoleic, α-linolenic acids decreased (Table 1). The decrease of LA and ALAs were reported in plasma and in erythrocytes from blood of colorectal cancer patients [23, 24]. These changes are probably due to metabolic alteration that is caused by the illness per se but not by malnutrition [23]. Two clinical investigations provided reports on a significant increase in plasma and tissue concentrations of AA in colorectal cancer patients compared with the controls [25, 26]. Increased AA concentrations may be related to enhancement of lipid peroxidation, which is a feature of rapidly growing cells [27, 28]. Alternatively, the increased AA values could be due to elevated desaturase activity upon LA and ALA (Fig. 3), leading perhaps to intensified formation of prostaglandins and other lipoxygenase products [29]:

After ingestion or conversion from LA, AA is preferentially incorporated into the S$n-2$ position of membrane phospholipids, where it contributes to the maintenance of normal structure and membrane fluidity [30]. The concentration of free AA in the tumour cell intensifies the cell signalling and regulates apoptosis. AA increased in cell proliferation or decreased apoptosis [31, 32]. In most studies, LA and ALA showed no effect on cell proliferation [33–36].

The other classes of unsaturated fatty acids are the palmitoleic ($n-7$) and the oleic ($n-9$) family, both of which can be produced by most of the cells in man and, thus, are not essential [37]. The results of our research show that oleic acid was identified only for cases 1, 2, 3, 4 and 9 and amount of oleic acids was increased in colon cancer. Another clinical investigation determined a significant elevation of oleic acid concentration in the plasma of colorectal cancer patients [23]. An almost statistically significant increase of oleic acid intake was found in another study of high-risk subjects for colorectal cancer [38]. The results may have been due to changes in oleic acid metabolism attributed to the pathogenic process. It has been shown that

Table 1. Content of free unsaturated fatty acids in colorectal adenocarcinoma and normal colon mucosa

		Concentration (μg/ml)		Content of fatty acids of plasmalemma (mg/g tissue)		Surface concentration of fatty acids (10^{-7}mol/m^2)	
No. of patients	Fatty acids	Control	Tumour	Control	Tumour	Control	Tumour
1.	18:2*n*−6	132.61 ± 10.60	26.92 ± 5.34[a]	0.059 ± 0.005	0.014 ± 0.002[a]	0.762 ± 0.060	0.154 ± 0.011[a]
	18:3*n*−3	104.49 ± 9.85	73.49 ± 8.60[a]	0.045 ± 0.002	0.032 ± 0.005[a]	0.601 ± 0.010	0.424 ± 0.080[a]
	16:1	68.72 ± 2.56	60.74 ± 9.57	0.032 ± 0.009	0.027 ± 0.007	0.434 ± 0.090	0.383 ± 0.012
	20:4*n*−6	81.75 ± 3.20	112.15 ± 11.34	0.036 ± 0.008	0.050 ± 0.010	0.431 ± 0.097	0.591 ± 0.090
2.	18:2*n*−6	42.38 ± 8.64	22.66 ± 2.34[a]	0.028 ± 0.005	0.014 ± 0.005[a]	0.381 ± 0.065	0.203 ± 0.057[a]
	18:3*n*−3	117.36 ± 11.64	100.89 ± 15.34	0.086 ± 0.010	0.071 ± 0.011	1.062 ± 0.254	0.912 ± 0.189
	16:1	35.52 ± 1.06	39.37 ± 1.94[a]	0.021 ± 0.005	0.028 ± 0.005	0.353 ± 0.069	0.390 ± 0.091
	20:4*n*−6	88.00 ± 4.65	99.04 ± 5.34[a]	0.064 ± 0.007	0.071 ± 0.008	0.730 ± 0.153	0.818 ± 0.111
3.	18:2*n*−6	65.09 ± 9.04	16.20 ± 2.88[a]	0.033 ± 0.006	0.011 ± 0.003[a]	0.455 ± 0.095	0.113 ± 0.023[a]
	18:3*n*−3	104.71 ± 10.34	70.70 ± 8.75[a]	0.055 ± 0.005	0.039 ± 0.008[a]	0.736 ± 0.105	0.498 ± 0.057[a]
	16:1	36.95 ± 8.64	40.01 ± 2.04	0.022 ± 0.003	0.022 ± 0.004	0.285 ± 0.065	0.301 ± 0.061
	20:4*n*−6	83.01 ± 5.07	109.76 ± 10.64[a]	0.044 ± 0.009	0.061 ± 0.007[a]	0.534 ± 0.089	0.710 ± 0.098[a]
4.	18:2*n*−6	68.01 ± 8.98	10.45 ± 1.07[a]	0.022 ± 0.004	0.003 ± 0.001[a]	0.267 ± 0.034	0.041 ± 0.020[a]
	18:3*n*−3	102.79 ± 9.67	91.68 ± 4.98	0.034 ± 0.006	0.028 ± 0.005	0.407 ± 0.087	0.363 ± 0.054
	16:1	47.25 ± 5.57	53.20 ± 7.89	0.016 ± 0.003	0.016 ± 0.003	0.205 ± 0.027	0.230 ± 0.023
	20:4*n*−6	87.91 ± 3.98	104.02 ± 9.37[a]	0.028 ± 0.005	0.031 ± 0.006	0.319 ± 0.031	0.377 ± 0.040
5.	18:2*n*−6	42.94 ± 3.87	18.49 ± 2.99[a]	0.014 ± 0.004	0.007 ± 0.001[a]	0.186 ± 0.032	0.080 ± 0.010[a]
	18:3*n*−3	96.74 ± 7.49	53.80 ± 7.08[a]	0.034 ± 0.006	0.017 ± 0.003[a]	0.423 ± 0.076	0.235 ± 0.032[a]
	16:1	32.71 ± 7.84	37.04 ± 2.59	0.010 ± 0.002	0.014 ± 0.002	0.156 ± 0.021	0.177 ± 0.029
	20:4*n*−6	79.47 ± 5.02	117.48 ± 10.01[a]	0.027 ± 0.004	0.041 ± 0.006[a]	0.318 ± 0.029	0.470 ± 0.046[a]
	18:1	68.65 ± 8.04	91.55 ± 5.75[a]	0.058 ± 0.007	0.075 ± 0.008[a]	0.296 ± 0.034	0.395 ± 0.052[a]
6.	18:2*n*−6	57.45 ± 4.67	11.81 ± 1.79[a]	0.016 ± 0.004	0.003 ± 0.001[a]	0.195 ± 0.042	0.040 ± 0.006[a]
	18:3*n*−3	90.12 ± 9.99	68.25 ± 6.99[a]	0.024 ± 0.005	0.019 ± 0.004	0.309 ± 0.025	0.234 ± 0.041[a]
	16:1	23.78 ± 6.32	27.36 ± 3.65	0.005 ± 0.001	0.008 ± 0.001[a]	0.090 ± 0.009	0.102 ± 0.026[a]
	20:4*n*−6	87.24 ± 5.97	131.17 ± 11.02[a]	0.024 ± 0.004	0.035 ± 0.005[a]	0.273 ± 0.031	0.411 ± 0.047[a]
	18:1	44.04 ± 4.87	96.93 ± 10.01[a]	0.011 ± 0.002	0.027 ± 0.004[a]	0.149 ± 0.043	0.327 ± 0.035[a]
7.	18:2*n*−6	55.17 ± 5.01	15.10 ± 3.01[a]	0.009 ± 0.002	0.002 ± 0.001[a]	0.131 ± 0.035	0.036 ± 0.008[a]
	18:3*n*−3	98.01 ± 9.99	52.63 ± 5.73[a]	0.019 ± 0.004	0.009 ± 0.002[a]	0.235 ± 0.041	0.126 ± 0.032[a]
	16:1	32.44 ± 5.01	27.14 ± 3.47	0.005 ± 0.001	0.005 ± 0.001	0.085 ± 0.008	0.071 ± 0.007
	20:4*n*−6	83.12 ± 4.87	141.71 ± 11.08[a]	0.015 ± 0.003	0.026 ± 0.005[a]	0.182 ± 0.051	0.310 ± 0.045[a]
	18:1	50.57 ± 6.03	98.15 ± 9.87[a]	0.009 ± 0.002	0.019 ± 0.004[a]	0.119 ± 0.024	0.232 ± 0.029[a]
8.	18:2*n*−6	81.74 ± 8.05	12.68 ± 3.08[a]	0.057 ± 0.008	0.007 ± 0.001[a]	0.570 ± 0.045	0.089 ± 0.009[a]
	18:3*n*−3	103.76 ± 10.51	50.95 ± 6.75[a]	0.071 ± 0.009	0.036 ± 0.005[a]	0.938 ± 0.095	0.462 ± 0.085[a]
	16:1	48.28 ± 4.95	58.22 ± 5.95[a]	0.028 ± 0.005	0.043 ± 0.004[a]	0.478 ± 0.055	0.575 ± 0.093
	20:4*n*−6	86.08 ± 5.38	103.17 ± 9.04[a]	0.064 ± 0.007	0.071 ± 0.007	0.715 ± 0.095	0.855 ± 0.110
	18:1	27.89 ± 7.05	95.96 ± 8.99[a]	0.019 ± 0.004	0.056 ± 0.006[a]	0.249 ± 0.028	0.855 ± 0.101[a]
9.	18:2*n*−6	36.80 ± 5.01	9.16 ± 2.90[a]	0.017 ± 0.003	0.003 ± 0.001[a]	0.257 ± 0.045	0.064 ± 0.009[a]
	18:3*n*−3	77.76 ± 6.08	46.15 ± 4.98[a]	0.033 ± 0.005	0.021 ± 0.004[a]	0.411 ± 0.055	0.244 ± 0.035[a]
	16:1	43.22 ± 4.84	49.17 ± 4.43	0.017 ± 0.003	0.021 ± 0.004	0.250 ± 0.028	0.284 ± 0.029
	20:4*n*−6	60.23 ± 5.93	91.19 ± 8.95[a]	0.025 ± 0.004	0.037 ± 0.005[a]	0.291 ± 0.034	0.441 ± 0.085[a]

The adenocarcinomas were of grade G2, stage pT3, and without distant and lymph node metastases (N0 M0).
18:2*n*−6, linoleic acid; 18:3*n*−3, α-linolenic acid; 16:1, palmitoleic acid; 20:4*n*−6, arachidonic acid; 18:1, oleic acid.
Statistically significant differences for $p < 0.05$.
[a]In comparison with control.

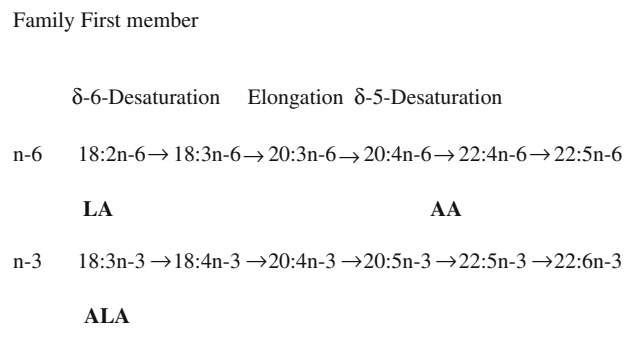

Fig. 3. Metabolism of $n-6$ and $n-3$ PUFAs.

human colon tumour growth is promoted by oleic acid [39] through mechanisms of an increase in fatty acid oxidation and disturbance of membrane enzymes [40].

The fact that they showed an overall reduction in free unsaturated fatty acids in cancer membranes is interesting, considering the recent report in the literature of elevated proportion of saturated to unsaturated total fatty acids in colonic adenocarcinoma [41], which has been attributed to elevated levels of the enzyme fatty acid synthase in colon cancer [42]. It has been suggested that the saturated fatty acids (synthesized in excess by the overexpressed fatty acid synthase) in cancers may be targeted to lipid raft microdomains [43]. Lipid rafts are membrane microdomains rich in cholesterol, sphingolipids and phospholipids with saturated fatty acid side chains [41]. It has recently been shown that increased dietary $n-3$ fatty acids seem to decrease sphingomyelin, cholesterol and caveolin-1 content collectively, suggesting that $n-3$ fatty acids can modulate lipid rafts composition [44]. Polyunsaturated fatty acids have been suggested to play a role in cancer therapy and have also been shown to perturb membrane lipids rafts, thereby affecting cell functions [45, 46].

Reduction of general contents of PUFAs in the large intestine cell membrane is reached as a result of the cancer transformation (Table 1). Moreover, levels of the normal PUFAs metabolism enzymes were limited in the tumour tissues [47]. In addition increased amount of phospholipids enhanced surface density of negatively charged groups of large intestine cell membrane at low pH values and that of positively charged ones at high pH; it has been confirmed by the results of our previous work [2]. Electrophoretically determined functional group surface concentrations of human large intestine normal and cancer cell membrane is of the order of 10^{-7} mol/m^2 whereas functional group surface concentration calculated from the amount of all fatty acids as determined by the HPLC method amounts about 2×10^{-7}. The functional groups of proteins are disclosed only in the electrophoretic studies. However this problem does not explain in our work.

To sum up, the cell membrane structure and the function are modified during cancer transformation. It is reflected by changes in the amount of phospholipids and free fatty acids of human large intestine cell membrane. The results obtained in this study and taking into consideration that free unsaturated fatty acids can perturb membrane lipids rafts, thereby affecting cell functions. Certainly cancer alterations also refer to proteins of biological membranes. It might lead to the reconstruction and functional rearrangement of the cell membrane, for example: the permeability, electric properties, fluidity etc. Therefore, the further studies are to be developed on fatty acids associated membrane proteins.

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