CHARACTER OF THE MODIFYING ACTION OF POLYUNSATURATED FATTY ACIDS ON GROWTH OF TRANSPLANTABLE TUMORS OF VARIOUS TYPES

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UDC 616-006-092.9-092-02:615.31:
577.117.2

KEY WORDS: polyunsaturated fatty acids; modifying action; transplantable mouse tumors; inhibitor of prostaglandin biosynthesis.

Diets with a high content of essential polyunsaturated fatty acids (PUFA) are known to stimulate growth of some induced and transplantable animal tumors [6, 10, 13]. Meanwhile evidence has been obtained of the antitumor activity of PUFA when injected parenterally into animals, and also in experiments in vitro [1, 7]. It is not yet known to what the contradictory effect of PUFA on different experimental models can be attributed. An important role in the realization of the modifying action of PUFA may be played by their metabolites and, in particular, by prostaglandins (PG). Clinical and experimental investigations in recent years have demonstrated the intensification of PG biosynthesis during malignant growth, their participation in antitumor immunity, and their influence on the proliferation and differentiation of tumor cells [5, 9, 12, 14]. The aim of the present investigation was to study the character of the modifying action of PUFA on growth of various types of transplantable tumors in mice kept on a standard diet and with inhibition of PG biosynthesis.

EXPERIMENTAL METHODS

Experiments were carried out on 805 C57Bl, DBA/2, and noninbred mice obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. All the animals received a standard pellet diet throughout the experiment, containing 5% of linoleic acid. Subcutaneous transplantation of mouse tumors – leukemia L-1210, mammary gland adenocarcinoma Ca-755, sarsoma 180, melanoma B-16, and Lewis lung carcinoma – was carried out by the usual method [4]. The sodium salts of linoleic, arachidonic, and α-linolenic acids and also a sucrose ester of α-linolenic acid were used. PUFA were isolated from lipids of various natural sources and purified by low-temperature crystallization, by adductive crystallization with urea, and chromatography on silica-gel, impregnated with silver nitrate. The purity of the samples thus obtained, determined by gas–liquid chromatography of methyl esters of the acids, was 95%. Subcutaneous or intraperitoneal injection of PUFA began 24 h after transplantation of the tumor and continued daily for the next 5-16 days. The quantity of added antioxidants was calculated as 0.7-1.0% of the dose of PUFA for α-tocopherol and 7-10% allowing for the weaker antiradical activity of its analog: 2,2,5,7,8-pentamethyl-6-hydroxychromane [2, 3]. Indomethacin (Sigma, USA), an inhibitor of PG biosynthesis, was given to the animals with their drinking water (10 μg/ml) ad libitum [8], and in individual cases the daily dose of the drug was injected parenterally. Control mice were given isotonic sodium chloride solution. The modifying action of PUFA and the effect of antioxidants and indomethacin on this process were estimated from the data for kinetics of tumor growth and survival of the mice. As the kinetic parameters we used the weight, volume, and average diameter of the tumor, and in the case of leukemia L-1210, the number of leukemic cells in the liver and spleen of the animals also. The statistical significance of the results was determined by Student's t test.

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Laboratory of Endogenous Modifying Factors of Carcinogenesis, Research Institute of Cardinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Department of Chemistry and Technology of Fine Organic Compounds, M. V. Lomonosov Moscow Institute of Fine Chemical Technology. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 471-474, April, 1988. Original article submitted December 18, 1986.

0007-4888/88/0004-0567$12.50

TABLE 1. Modifying Action of Sucrose Ester of α-Linolenic Acid on Growth of Mammary Gland Adenocarcinoma Ca-755 and Leukemia L-1210

Strain	Dose of prepn., mg/kg	Inhibition (−) or stimulation (+) of tumor growth, %		
		11th day	14th day	17th day
Ca-755	333	+127**		
	100	+113**	+72*	+75**
	33	+230***	+125***	+124***
	10	+147*	+96**	+71*
	3	+78*	+35	+1
		5th day	6th day	7th day
L-1210	333	−67***	−56**	−31*
	100			−46**
	33			−35*
	3			−33*

Legend. Ten animals in each group.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

EXPERIMENTAL RESULTS

Water-soluble forms of PUFA investigated had low toxicity for the mice of the above-mentioned lines. LD_{50} for the sodium salts of PUFA, given as a single intraperitoneal injection, was 75-90 mg/kg, and for the ester of α-linolenic acid and sucrose it was 600-650 mg/kg body weight.

The investigations revealed a varied character of action of the water-soluble forms of PUFA on growth of the transplantable mouse tumors. Depending on the type of tumor, a stimulating or inhibitory effect of PUFA, or no effect at all, was observed. Under these circumstances all the PUFA studied, which are precursors of different series of PG, had a similar direction of action, but their relative activity differed on different tumor strains.

A marked modifying effect of the sodium salts of PFA on growth of the transplantable mouse tumors was exhibited in doses of 7.5 to 75 mg/kg (the maximal doses were injected subcutaneously only). In the case of mammary gland adenocarcinoma Ca-755, PUFA salts in effective doses stimulated tumor growth by 40-250%. The maximal effect was observed with linoleate, which in a dose of only 8.3 mg/kg increased the rate of growth of the adenocarcinoma 10 days after transplantation by 143% ($p < 0.01$; Fig. 1). It is important to note that arachidonate, the principal endogenous precursor of PG, gave side effects only when the dose was increased to 25 mg/kg. Marked stimulation of growth of adenocarcinoma Ca-755 under the influence of PUFA was observed simultaneously with a strong tendency toward shortening of the mean life span of the animals (19-25%; $p > 0.05$).

A different character of modifying action of sodium salts of PUFA was observed with leukemia L-1210 and sarcoma 180. The PUFA tested, in a dose of 7.5-75 mg/kg, inhibited growth of these tumors by 40-85%. The greatest activity was found with α-linolenate which, in the maximal dose, increased the mean duration of survival of mice with leukemia L-1210 by 37.5% ($p < 0.01$) and inhibited growth of the subcutaneous tumor in these animals by 85% ($p < 0.01$; Fig. 2a). Besides inhibition of growth of the L-1210 tumor, under the influence of salts of PUFA leukemic infiltration of the spleen and liver was less intensive than in the control (Fig. 2b).

During the investigation of the modifying action of the sucrose ester of α-linolenic acid, dependence of the stimulating or inhibitory effect of PUFA on the type of transplanted tumor was confirmed. Administration of the α-linolenic acid ester in a dose of 10-100 mg/kg stimulated growth of adenocarcinoma Ca-755 by 71-230%, and inhibited development of leukemia L-1210 by 31-67% (Table 1). The action of the ester in adenocarcinoma Ca-755 differed from that on leukemia L-1210 by being clearly dose-dependent in character. Even when a subtoxic dose of the α-linolenic acid ester (333 mg/kg) was given, the modifying effect of this PUFA characteristic of the above-mentioned tumors was preserved.

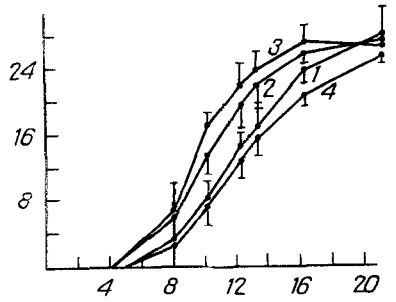

Fig. 1

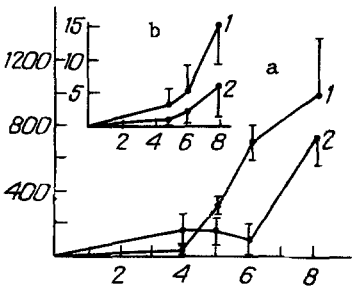

Fig. 2

Fig. 1. Stimulation of growth of mammary gland adenocarcinoma Ca-755 (4 – control) under the influence of sodium salts of arachidonic (1), α-linolenic (2), and linoleic (3) acids. Ordinate, mean diameter of tumor, mm; abscissa, time after transplantation of tumor, days. Preparations injected in a dose of 8.3 mg/kg body weight daily for 16 days. $P_{1-4} < 0.02$ (16 days); $P_{2-4} < 0.05$ (10-13 days), <0.01 (16 days); $P_{3-4} < 0.01$ (10-16 days).

Fig. 2. Inhibition of growth of subcutaneous tumor (a) and leukemic infiltration of the liver (b) in mice with leukemia L-1210 (1 – control) under the influence of sodium α-linolenate (2). Ordinate, weight of tumor, mg (a); content of leukemic cells, % (b); abscissa, time after transplantation of tumor, in days. Preparation injected in a dose of 75 mg/kg body weight for 6 days. *p < 0.01. No asterisk given in original text.

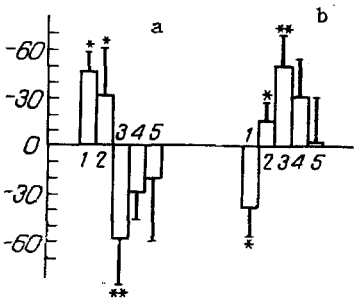

Fig. 3. Effect of indomethacin and α-tocopherol on modifying effect of PUFA in leukemia L-1210 (a) and mammary gland adenocarcinoma Ca-755 (b). Ordinate, stimulation or inhbition of tumor growth (in %) (0 – control) on 7th day (L-1210) and 14th day (Ca-755) after transplantation. 1) Indomethacin (10 μg/ml in drinking water); 2) indomethacin + sodium α-linolenate; 3) sodium linoleate (40 mg/kg – a; 9.2 mg/kg – b); 5) α-tocopherol (0.4 mg/kg – a; 80 mg/kg – b). *P < 0.05; **P < 0.01 (P for 1, 3, and 5 relative to control, for 2 and 4, relative to 3).

Lewis lung carcinoma and melanoma B-16 exhibited relative resistance to the action of PUFA when injected subcutaneously and intraperitoneally. Inhibition of growth of these tumors after injection of water-soluble forms of PUFA in the above doses did not exceed 11 and 16%, respectively.

Indomethacin, an inhibitor of PG biosynthesis, when given perorally and parenterally to animals with tumors, had the opposite effect to PUFA. In the case of leukemia L-1210 indomethacin considerably stimulated growth of the subcutaneous tumor (+46%; p < 0.05) and

increased the intensity of leukemic infiltration in the liver (+56%; $p < 0.01$), and also completely suppressed the inhibitory action of PUFA (Fig. 3). Meanwhile, the prostaglandin synthetase inhibitor significantly inhibited growth of adenocarcinoma Ca-755 (−37%; $p < 0.05$), and when used in combination with PUFA, it reduced their growth-stimulating effect. The effect of antioxidants on the modifying action of PUFA was less marked. Addition of α-tocopherol to PUFA only partially abolished their modifying effect on leukemia L-1210 and adenocarcinoma Ca-755 (Fig. 3). The α-tocopherol analog 2,2,5,7,8-pentamethyl-6-hydroxychromane had virtually no inhibitory effect on the action of PUFA.

The varied character of the modifying action of PUFA on growth of transplantable mouse tumors can evidently be attributed to differences in the metabolism of these compounds in tumors of different histogenesis. Normal cells of different types are known to synthesize different eicosanoids: protaglandins, thromboxanes, prostacyclines, leukotrienes [11]. The opposite direction of action of indomethacin and PUFA on growth of transplantable tumors and abolition of PUFA by the prostaglandin synthetase inhibitor in the present investigation point to an essential role of prostaglandins in the realization of the modifying action of PUFA.

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