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Dietary fish oil suppresses tumor growth and metastasis of Lewis lung carcinoma in mice

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In this study we examined the influence of different polyunsaturated fatty acid (PUFA) diets on the tumor growth and metastatic dissemination of the well-characterized Lewis Lung Carcinoma (3LL) in C57BL/6J mice. The tumor-bearing mice were fed ad libitum with three different diets of 5% oil; either soybean oil (SO), which is rich in omega-6 (ω-6); perilla oil (PO), which is rich in omega-3 (ω-3) 18:3; and fish oil (FO), which is rich in ω-3, 20:5 and 22:6 PUFA. A significantly slower growth of primary tumor, lower mortality rate, and lower metastatic spread were observed in mice fed FO. This trend was also observed in mice fed first with SO and after tumor inoculation transferred to the FO diet. Indomethacin reduced significantly the metastasis growth in the SO-fed group, and exerted only a small effect in the FO and PO fed counterparts, suggesting that eicosanoid derivatives of ω-6 fatty acids support the process of tumor growth. In addition, the long chain PUFA of fish oil, which are sensitive to oxidation, could act as targets for membrane perforation and eventual elimination of the proliferating tumor cells. (J. Nutr. Biochem. 8:619–622, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Polyunsaturated fatty acids (PUFA) have been reported to be involved in tumor growth and metastatic processes such as detachment from the primary growth, lodgement in small blood vessels, invasion through the extracellular matrix, arrest in distant organ capillary bed, adhesion at site of arrest, and rate of proliferation.[1–5] PUFA operate on two distinct physiologic levels. When incorporated into the membrane phospholipids they contribute to membrane fluidization and may thus support tumor growth.[3,6–8] However, the contribution to membrane fluidity of the various types of PUFA, e.g., ω-3 versus ω-6 is of similar magnitude[3,6] and thus cannot account for the differences observed in tumor development. On the other hand, various PUFA have markedly different influences on a series of metabolic processes including insulin secretion, glucose metabolism,[9,10] eicosanoid formation[3] and lipid metabolism, in particular the synthesis of triglycerides.[11,12] Each of these could, in principle, affect tumor progression.

In this study we examined the effect of three oils enriched with different PUFA on the rate of growth and dissemination of Lewis Lung Carcinoma in mice, as an assessment of dietary recommendation for cancer patients.

Methods and materials

Mice, diets, and handling

C57BL/6J male mice, 20 to 22 weeks old, with an average weight of 30 g, were bred at the Weizmann Institute Animal Breeding Center. The mice were kept in filter-covered plastic cages (10 mice per cage) and fed ad libitum with a basal oil-free standard diet supplemented and pelleted with 5% of either soybean oil, SO, perilla oil, PO, (donated by Professor Yu Hua Cheng, Jilin University, China) or fish oil FO (Inter-Harz GmbH, Elmshorn,

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Table 1 Fatty acid contents (in % weight) and ω-3/ω-6 fatty acid ratios in the final experimental diets

Fatty acid	SO	PO	FO
16:0 Palmitic	0.79	0.70	1.19
16:1 Palmitoleic	0.04	0.04	0.61
18:0 Stearic	0.22	0.27	0.20
18:1 Oleic	1.61	1.13	1.11
18:2 Linoleic ω-6	3.05	1.69	1.30
18:3 Linolenic ω-3	0.26	2.15	0.02
20:1 Eicosenoic	ND	ND	0.08
20:4 Arachidonic ω-6	ND	ND	0.07
20:5 Eicosapentaenoic ω-3	ND	ND	0.89
22:5 Docosapentaenoic ω-3	ND	ND	0.07
22:6 Docosahexaenoic ω-3	ND	ND	0.44
ω-3/ω-6	0.085	1.27	1.0

SO, soybean oil; PO, perilla oil; FO, fish oil; ND, not detactable

Germany). The fatty acid contents in the final diets are given in *Table 1*.

Diet regimen and treatment

Ad libitum feeding with the different diets started two weeks before tumor inoculation. In two groups, diet was changed after tumor inoculation; SO diet replaced FO in one group (FO/SO) and SO replaced the FO in the other (SO/FO).

Mice in each of the five dietary groups were divided, after inoculation, in two major groups: treated or not by indomethacin (IND), and further subdivided in leg-amputated bearing tumor or not. So that, in addition to control mice, each dietary group had five groups with 10 mice per group. This procedure was repeated for three times.

Tumor line

A highly metastatic clone (D122) of the 3LL Lewis Lung Carcinoma,[13] (isolated and kindly provided by Professor L. Eisenbach, of our Institute), was maintained in vitro in RPMI medium supplemented with 10% heat-inactivated fetal calf serum, combined antibiotics, sodium pyruvate, and nonessential amino acids.

Tumor growth and spontaneous metastasis

Mice were inoculated in the footped with 5×10^5 cells per mouse in 50 μL PBS. Tumor size was monitored with a Varnier caliper. In accord with previous studies,[13,14] when local tumor reached a diameter of 8 to 9 mm, (28 to 32 days after inoculation in the SO-fed mice), the tumor-bearing leg, was removed by amputation after ligation above the knee joint. Experiments were terminated 24 days after amputation because of high mortality rate in the SO groups. The surviving mice were killed, and lungs were assessed for metastatic load by weighing and histologic examination.

Indomethacin

A 8 mg/mL stock solution of indomethacin (IND) (Sigma) in absolute ethanol was prepared and stored at 4°C. The solution was diluted to a concentration of 20 μg/mL into the drinking water. The average water intake was 3 mL/mouse/day, accounting for approximately 60 μg IND/mouse/day.

Histologic examination

For light microscopic examination, the lungs were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin according to Lillie.[15]

Results

Higher body weight and reduced mortality rate characterized the FO-fed mice. Their tumor reached 10 mm diameter at an average of 10 days later than in the SO-fed mice. IND administration had a small effect of slowing tumor growth in the SO-fed group. Intermediate effects of IND were observed in the PO, FO/SO, and in the SO/FO-fed groups. Amputated mice presented significantly higher lung weight and metastatic load in all dietary groups in comparison to the nonamputated counterparts. However, significantly higher metastatic load was observed in lungs of the amputated mice of the SO or FO/SO groups in comparison with mice under the FO or SO/FO diets. Amputated mice under PO diet presented intermediate results. A summary of lung weights is presented in *Figure 1*. IND reduced the increase

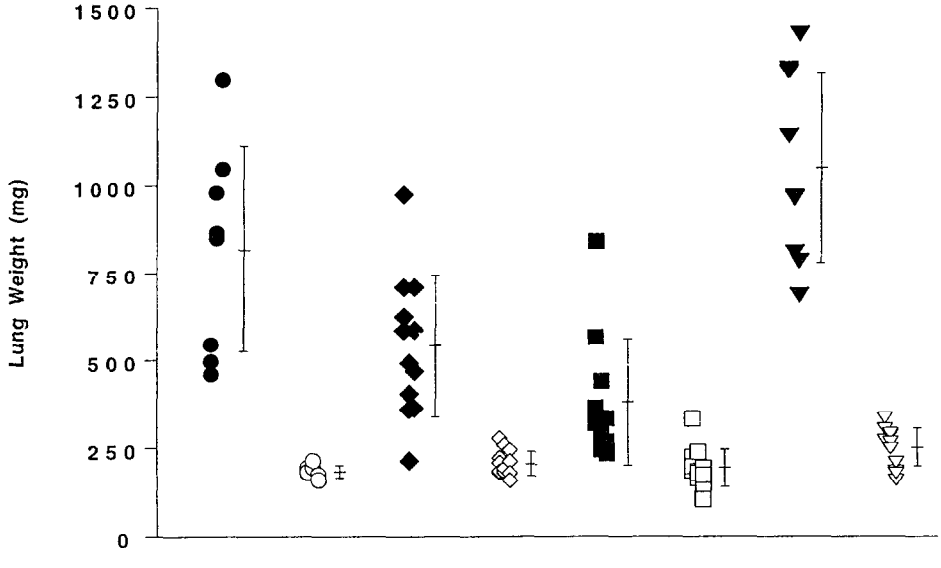

Figure 1 Lung weight of mice bearing 3LL carcinoma inoculated in the footpad under SO diet (●, ○); FO diet (◇, ◆); SO/FO diet (■, □), and FO/SO diet (▼, ▽). Dark symbols, mice with resected primary growth; open symbols, mice without resection of primary growth (control groups). For details see text.

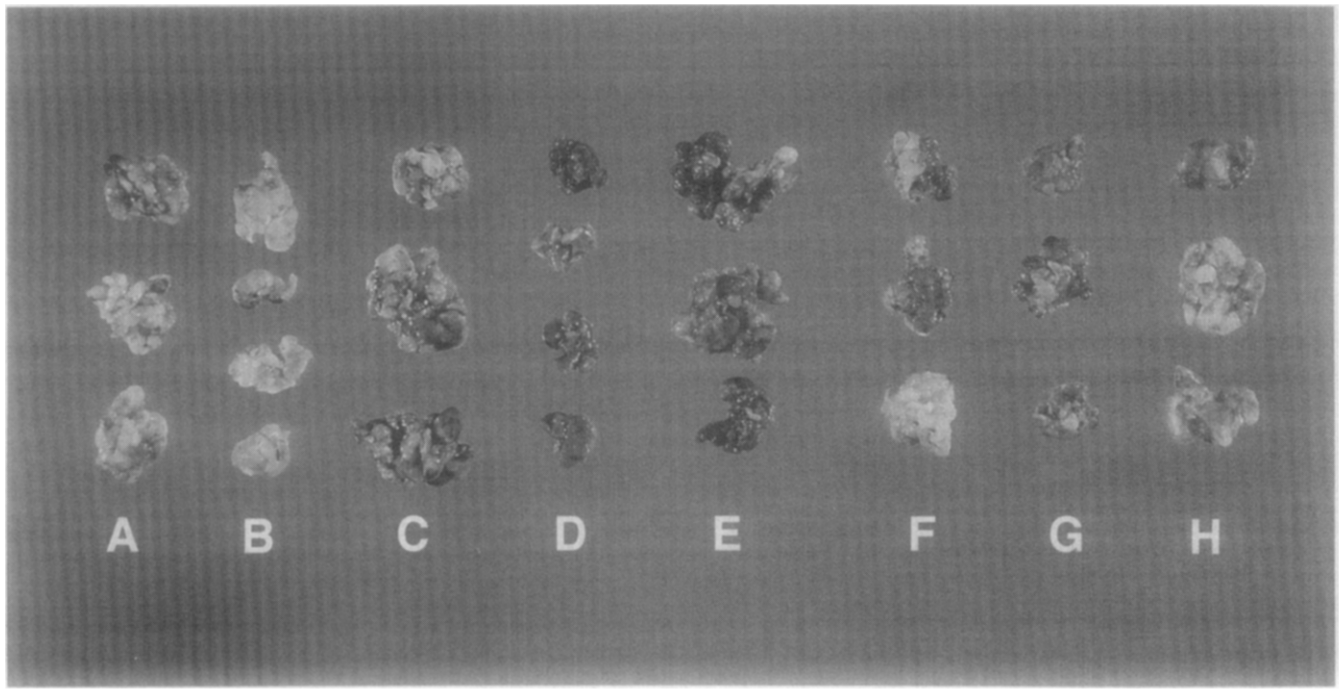

Figure 2 View of representative isolated lungs from 3LL bearing mice under various oil diets. Mice with primary growth: A-SO diet, B-FO diet. Mice with resected primary growth: C–SO diet, D–FO diet, E–FO/SO diet, F–SO/FO diet, G–SO diet + indomethacin, H–FO diet + indomethacin.

in lung weight in the SO and FO/SO groups, but had no effect on mice of the other groups. *Figure 2* presents the view of the lungs removed from the mice under the different diets.

Pathologic examination clearly indicated that the resected lungs belonging to the SO fed mice were significantly higher in weight and dimensions than in the FO-fed mice. In the SO-fed mice the pleural surfaces, were occupied by numerous well-circumscribed white gray nodules. Only a few small nodules were found in the lungs of the FO-fed mice. Histologically, the tumor in the lungs of both groups were composed of spindle- or polygonal-shaped cells presenting numerous mitotic patterns. However, in the SO group the lungs presented marked atypical bizzare giant cells, extensive necrosis, and vessels invaded by cancer cells. Lungs of mice belonging to SO/FO group presented similar features to those found in the FO group, whereas lungs of mice fed PO or IND presented intermediate adverse features.

Discussion

Attenuation of tumor growth by diets rich in long ω-3 PUFA were observed previously in rodent carcinomas[16–22] as well as human breast cancer cells in vitro[23] or when grown in athymic nude mice.[24–27] However, the effect of PUFA on metastasis ensuing the removal of the primary growth, has not been addressed previously. We have shown here that after resection of the primary growth, the FO-fed groups presented a significantly lower level of lung metastasis manifested in a lower lung weight, better lung histologic and morphologic profiles, higher body weight, and lower mortality rate as compared with mice fed PO, and to a greater extent to mice fed SO. It is important to note that these beneficial effects were also observed in mice fed initially with SO diet and after tumor inoculation were placed on the FO diet (see *Figure 1*).

The different modes of action of dietary lipids in cancerous processes are not yet fully elucidated. Several studies suggested a different influence on host' insulin, glucose, and lipid metabolism[9–12] and that the incorporation of dietary fatty acids into the phospholipids of the tumor cell membranes affect their fluidity.[3,6–8] However, the magnitude of the acquired membrane fluidity under the SO, PO, or FO diets used in this study is expected to be very similar and therefore cannot account for the marked differences observed in tumor growth and metastasis observed in our study. On the other hand, many studies have attributed metabolic advantages to tumor cells rich in ω-6 fatty acids[4,12] as they enhance cell division and serve as precursors for eicosanoid metabolites.[16,19] Such eicosanoids may enhance tumor development and act as immunosuppressants in addition to their angiogenic activity. Our observation that IND administration reduced tumor progression in the SO and FO/SO groups supports the trend where ω-3 PUFA can counteract the proposed support for tumor progression provided by the ω-6 PUFA.[16–22,27] An additional mechanism for the putative antitumor activity of the ω-3 PUFA could be associated with their high sensitivity to oxidation, in particular in the longer ones 20:5 and 22:6, which are essentially present in FO only. One may assume that on integration into the tumor membranes they become immediate targets for oxidation, which can lead to their disintegration. This interesting mechanism, where a natural metabolite can induce a cytolytic effect, is now being investigated in our laboratory.

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