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Walker-256 tumor growth is inhibited by the independent or associative chronic ingestion of shark liver and fish oil: a response linked by the increment of peritoneal macrophages nitrite production in Wistar rats

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Abstract

Fish oil (FO) is widely known by its capacity to positively modulate immune parameters and decrease the growth of some tumors. Despite the enormous number of studies addressing the effects of FO, there are few reports showing similar results using other marine sources of lipid compounds with biologic importance. This study aimed to compare the effects of shark liver oil (SLO), which is a source of omega-3 fatty acids and alkylglycerols, with those obtained with FO administration, or the association of both, on tumor growth and the innate immune system in Walker-256 tumor–bearing rats. Beginning at 21 days of age, Wistar rats were fed regular chow and/or FO and/or SLO supplement (1 g/kg body weight per day) for 14 weeks. Walker-256 tumor cells were inoculated on the 90th day. As expected, 14 days after inoculation, rats fed with FO presented tumor weights that were 50% lower than the control tumors ($P < .05$). The association of both FO and SLO and ingestion of SLO alone also reached the same reduction level. Except for adhesion, all macrophage parameters assayed were 200% higher in rats fed with FO and those supplemented with both FO and SLO compared with control rats. Only reactive nitrogen species production was increased by SLO. These results suggest that SLO might also have indirect antitumor properties. Conversely, there were no additive effects when SLO was administered with FO. Therefore, SLO is another marine compound with in vivo antitumor effects, but its action mechanisms seem not to be related to major modifications on macrophage function.
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Keywords: Wistar rats; Shark liver oil; Fish oil; Tumor cells; Macrophages; Cancer

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; LPS, lipopolysaccharide; NO, nitric oxide; PGE_2, prostaglandin E_2; PKC, protein kinase C; PMA, phorbol 12-miristate 13-acetate; PUFA, polyunsaturated fatty acids; SLO, shark liver oil.

1. Introduction

Epidemiologic studies have shown a link between total fat consumption and development of chronic degenerative diseases such as cancer [1]. Several studies have reported a positive association between high intake of fat and the incidence of breast, colon, and prostate cancer [2-4]. Reviews regarding fat ingestion and health revealed that the type of fat consumed also appears to be important in influencing cancer risk; in particular, n-3 polyunsaturated fatty acids (PUFAs) present in oily fish appear to be protective in fish oil (FO) [5-6].

We have previously reported that supplementation of the diet of young rats with FO decreases growth of the Walker-256 tumor, attenuates the cachexia associated with tumor

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bearing, and improves survival [7]. The mechanisms by which FO inhibits tumor growth and the development of cachexia are not completely known. However, we have shown that FO can act directly in the tumor, modifying the inner tumor cell environment [8], and can also act indirectly by promoting changes in host immune cell function that would result in improved host defense [8-11]. Macrophages are very important in host defense against tumors [12] because they produce several antitumor agents, including superoxide, hydrogen peroxide, nitric oxide (NO), and tumor necrosis factor α. Production of each of these agents by macrophages has been shown to be modified by dietary FO [11].

Another marine source of n-3 PUFA is shark liver oil (SLO), which also contains a group of ether-linked glycerols known as 1-*O*-alkylglycerols. These natural ether lipids have been reported to present multiple biologic activities, including inhibition of tumor growth [13-14] and enhancement of both macrophage activation [15] and specific immunity in rodents and humans [16-18]. Because of its dual content of n-3 PUFAs and alkylglycerols, SLO is of particular interest in nutrition. There are few reports of the ability of SLO to influence immune function or tumor growth in tumor-bearing rats in vivo. Also, we are not aware of studies testing the effects of the association of FO, which is rich in n-3 PUFA, with SLO, a source n-3 PUFA and 1-*O*-alkylglycerols, both with antitumor and immunomodulation properties. Therefore, we hypothesized that SLO would have anticancer activity. Our aim was to compare the effects of FO and SLO, or the association of both, on Walker-256 tumor growth and the innate immune system function in Wistar rats. Following our hypothesis, we examined the capacity of SLO to reduce tumor growth and positively modulate macrophage function of tumor-bearing rats. In addition, we expect that the association of both oils, FO and SLO, will show positive effects.

2. Methods and materials

2.1. Chemical and enzymes

Unless otherwise indicated, chemicals and enzymes used were obtained from Sigma Chemical Co (St Louis, Mo).

2.2. Study design

All studies involving animals were approved by the local animal ethics committee. Weaned Wistar rats (~20 days old) were fed a standard laboratory chow (Nuvilab CR1, Curitiba, Brazil) and received a single daily oral bolus (by pipette) of FO (Herbarium; Herbarium Laboratorio Botanico Ltda, Colombo, Brazil), SLO (Ecomer; Naturalis Alimentos Naturais, Ltda, Sao Paulo, Brazil), or FO plus SLO (1 g/kg body weight). The fatty acid composition of the rat chow and lipids is shown in Table 1. A suspension of 3×10^7 Walker-256 tumor cells, obtained from an ascitic tumor cell-bearing rat, was inoculated in the right flank of 90-day-old animals. Fourteen days after tumor inoculation, the rats were killed by decapitation, tumors were removed and weighed, and peritoneal macrophages were harvested. Tumor-bearing animals fed regular chow are referred to as W, those supplemented with SLO are referred to as WS, those supplemented with FO are referred to as WF, and those supplemented with both FO and SLO are referred to as WSF. Three independent experiments were performed. A total of 48 rats were used in this study. Eight rats were excluded from data analysis because tumor growth was not achieved (2 per group).

Table 1
Fatty acid profiles of the regular chow provided to all groups and of the SLO and FO used

Fatty acids (g/100 g of total fatty acids)	SLO	FO	Regular chow
Lauric acid (12:0)	1.0 ± 0.1	3.4 ± 0.6	–
Myristic acid (14:0)	4.8 ± 0.2	9.8 ± 0.4	–
Palmitic acid (16:0)	32.6 ± 2.0	25.3 ± 3.0	20.7 ± 3.8
Palmitoleic acid (16:1n-7)	6.5 ± 0.5	6.5 ± 0.1	–
Stearic acid (18:0)	1.6 ± 0.1	2.1 ± 0.1	2.3 ± 0.04
Oleic acid (18:1n-9)	28.2 ± 1.6	10.2 ± 0.9	19.5 ± 1.5
Linoleic acid (18:2n-6)	1.9 ± 0.4	2.0 ± 0.7	51.3 ± 4.2
α-Linolenic acid (18:3n-3)	0.6 ± 0.1	0.7 ± 0.2	5.0 ± 0.2
Arachidonic acid (20:4n-6)	0.9 ± 0.1	0.9 ± 0.2	0.1 ± 0.1
EPA (20:5n-3)	4.5 ± 0.3	20.2 ± 1.3	–
DHA (22:6n-3)	12.6 ± 1.0	18.8 ± 1.2	–

Values are means ± SEM of 3 independent measurements.

2.3. Macrophage isolation

Resident macrophages were obtained by intraperitoneal lavage with 10 mL of sterile phosphate-buffered saline (PBS). The peritoneal cells were collected by centrifugation (290 × *g*, 4°C for 5 minutes), washed, and then resuspended in RPMI 1640 medium after counting in a Neubauer chamber by optical microscopy, using a trypan blue solution (1%). Before each functional assay, macrophages were further purified by incubating peritoneal cells in tissue culture plates for 1 hour and then washing 3 times with PBS to remove the nonadherent cells [19]. Macrophage enrichment was assayed by May-Grünwald and Giemsa stains at light microscopy: more than 95% of the cells in the visual field were macrophages.

2.4. Adhesion

Macrophage adhesion was assayed according to Rosen and Gordon [20] to make the normalization of the further macrophage experiments to that variable possible. This procedure is necessary because it was previously shown that FO can alter macrophage adhesion capacity [21]. Peritoneal cells (1×10^5 cells/well) were incubated for 1 hour in an RPMI culture medium. After incubation, the plates were washed 3 times with PBS, and the adherent cells were fixed with methanol. Cells were stained with 10% Giemsa solution for 10 minutes, before thorough rinsing with

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