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Basic nutritional investigation

Vitamin E content in fish oil emulsion does not prevent lipoperoxidative effects on human colorectal tumors

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ABSTRACT

Objective: The anticancer action exerted by polyunsaturated fatty acid peroxidation may not be reproduced by commercially available lipid emulsions rich in vitamin E. Therefore, we evaluated the effects of fish oil (FO) emulsion containing α -tocopherol 0.19 g/L on human colorectal adenocarcinoma cells and tumors.

Methods: HT-29 cell growth, survival, apoptosis, and lipid peroxidation were analyzed after a 24-h incubation with FO 18 to 80 mg/L. Soybean oil (SO) emulsion was used as an isocaloric and isolipidic control. In vivo, nude mice bearing HT-29 tumors were sacrificed 7 d after an 11-d treatment with intravenous injections of FO or SO $0.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ FO or SO to evaluate tumor growth, necrosis, and lipid peroxidation.

Results: The FO inhibited cell viability and clonogenicity in a dose-dependent manner, whereas SO showed no significant effect compared with untreated controls. Lipid peroxidation and cell apoptosis after treatment with FO 45 mg/L were increased 2.0-fold ($P < 0.01$) and 1.6-fold ($P = 0.04$), respectively. In vivo, FO treatment did not significantly affect tumor growth. However, immunohistochemical analyses of tumor tissue sections showed a decrease of 0.6-fold ($P < 0.01$) in the cell proliferation marker Ki-67 and an increase of 2.3-fold ($P = 0.03$) in the necrotic area, whereas malondialdehyde and total peroxides were increased by 1.9-fold ($P = 0.09$) and 7.0-fold ($P < 0.01$), respectively, in tumors of FO-treated compared with untreated mice.

Conclusion: These results suggest that FO but not SO has an antitumor effect that can be correlated with lipid peroxidation, despite its vitamin E content.

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Introduction

Epidemiologic and experimental data have suggested that consumption of fish oil (FO) rich in ω -3 polyunsaturated fatty acids (PUFAs) may play a role not only in cancer prevention but also in cancer therapy [1,2]. Although different mechanisms of action have been discussed in the literature [3], the anticancer properties of ω -3 PUFAs have been primarily ascribed to their ability to downregulate proinflammatory eicosanoid synthesis from cyclooxygenase-2 (COX-2) [4–6]. Such a mechanism of

action may be of particular value in colorectal cancer, where the ectopic overexpression of COX-2 is a typical feature [7], which promotes tumor growth and vascularization by the induction of vascular endothelial growth factor and the antiapoptotic proteins, Bcl-2 and Bcl-X_l [8,9]. However, it is probably not the only one involved. Other observations have suggested that the underlying mechanism may also be related to COX-independent pathways [10]. We previously reported that arachidonic acid can exert a cytotoxicity similar to that of ω -3 PUFAs, such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), in several human colorectal adenocarcinoma cell lines [11,12]. This anticancer effect was furthermore abolished by the coadministration of the lipophilic and antioxidant vitamin E, suggesting that lipid peroxidation was involved [11]. Others have suggested that the anticancer potential of long-chain ω -3 PUFAs is proportional to their degree of unsaturation, which make them

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highly peroxidizable in the presence of free radicals [13]. Resulting free radical chain reactions may cause membrane phospholipid disruption, in particular in cancer cells, where protecting mechanisms against intracellular oxidative stress have been reported to be altered [14]. In addition, endproducts of lipid peroxidation, such as malondialdehyde (MDA), may induce cell apoptosis by the formation of DNA adducts, such as pyrimido [1,2- α]purin-10(3H)-one, and subsequent DNA misrepair [15,16]. In clinical conditions, however, it is not certain that the administration of commercially available lipid emulsions can produce the same cytotoxic effect on cancer cells as free ω -3 PUFAs. Indeed, for stability, FO emulsions mostly contain significant amounts of vitamin E, which could compromise the anticancer effect by blocking the lipid peroxidation cascade in membrane phospholipids [17]. Therefore, we aimed to evaluate the peroxidative and cytotoxic effects of a FO emulsion containing ω -3 PUFAs and vitamin E compared with an isocaloric and isolipidic soybean oil (SO) emulsion on human colorectal adenocarcinoma cells in culture and in xenografted nude mice.

Materials and methods

Reagents

Lipid emulsions composed of glycerol 25 g/L, (3-sn-phosphatidyl)-choline (egg lecithin) 12 g/L, D,L- α -tocopherol 0.19 g/L, and FO 113 g/L (Omegaven) and/or SO 212 g/L (Lipovenoes) were kindly supplied by Fresenius Kabi (Stans, Switzerland). Their fatty acid composition is presented in Table 1. As sham treatment, the SO emulsion was diluted two times compared with the FO emulsion to administer a similar fatty acid and energy dose (1000 kcal/L). All other chemicals were obtained from Sigma-Aldrich (Buchs, Switzerland) unless otherwise stated. EPA and DHA stock solutions (10 g/L) were dissolved and stored in ethanol at -20°C under argon.

Cell line and treatment

Human colorectal adenocarcinoma HT-29 cells (HTB-38TM; ATCC, Rockville, MD, USA) were maintained in an exponential growth phase (37°C , 5% CO_2) by subculturing twice a week in Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated fetal bovine serum and penicillin–streptomycin 0.1 g/L (all from Invitrogen, Zug, Switzerland). Cells were not treated (NT) or treated for 24 h with culture medium enriched with SO or FO emulsion 18 to 80 mg/L or the corresponding concentration of EPA or DHA (20–90 $\mu\text{mol/L}$).

Table 1

Fatty acid compositions of the intravenous lipid emulsions used according to the certificate of analysis

Content	FO (g/L)	SO (g/L)
Total fat	113	106
ω -3 Polyunsaturated fatty acids		
α -Linolenic acid (C18:3)	1.6	6.5
Stearidonic acid (C18:4)	4.6	0.0
Eicosapentaenoic acid (C20:5)	20.6	0.0
Docosapentaenoic acid (C22:5)	2.2	0.0
Docosahexaenoic acid (C22:6)	19.0	0.0
ω -6 Polyunsaturated fatty acids		
Linoleic acid (C18:2)	3.3	52.2
Arachidonic acid (C20:4)	2.6	0.0
Monounsaturated fatty acids		
Palmitoleic acid (C16:1)	7.6	0.0
Oleic acid (C18:1)	11.8	23.8
Gadoleic acid (C20:1)	1.4	0.0
Erucic acid (C22:1)	1.4	0.0
Saturated fatty acids		
Myristic acid (C14:0)	4.3	0.1
Palmitic acid (C16:0)	10.5	11.3
Stearic acid (C18:0)	2.3	3.9
pH	8.1	8.2

FO, fish oil; SO, soybean oil

Cell growth and viability

Cell growth and viability were evaluated 120 h after treatment using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The sample absorbance was measured at 595 nm after a 4-h incubation with MTT 0.5 mg/mL using an enzyme-linked immunosorbent assay microplate reader (model 680, Bio-Rad, Reinach, Switzerland; $n = 3/\text{condition}$).

Cell survival

Long-term cell survival was evaluated 14 d after treatment using a clonogenic assay. Colonies containing at least 50 cells were counted after cell fixation and staining with 0.5% crystal violet in methanol/acetic acid (3:1, v/v). The survival fraction was calculated compared with untreated controls (S/S_0 ; $n = 3/\text{condition}$).

Cell apoptosis and nuclear abnormalities

Apoptosis was quantified 48 h after treatment using an annexin V detection kit according to the manufacturer's instructions (BD Biosciences, Allschwil, Switzerland). A two-parameter fluorescence-activated cell sorting (FACS) analysis was performed after cell staining with 5 μL of annexin V conjugated with fluorescein isothiocyanate and 5 μL of propidium iodide (PI) using a FACSFit flow cytometer (BD Biosciences). PI staining was also used to examine morphologic nuclear abnormalities by confocal laser scanning LSM510 microscopy (Carl Zeiss, Feldbach, Switzerland) according to a published protocol [18] ($n = 3/\text{condition}$).

Animal model

All in vivo experiments were performed according to the Swiss legislation and approved by the official committee on the surveillance of animal experiments. A suspension of 1×10^6 HT-29 cells/0.1 mL was inoculated subcutaneously in female NMRI Rj (Han) mice 7 to 8 wk old (Janvier, Le Genest St. Isle, France). After tumor appearance, mice were assigned to the different treatment groups by selecting the similar mean tumor volume per group ($n = 6/\text{group}$).

In vivo treatment

Depending on the treatment group, mice were treated with or without 11 consecutive intravenous injections of the SO or FO emulsion 0.2 g/kg of body weight per day. Tumor volume was measured repeatedly with a caliper according to the following formula: volume = $4\pi/3 \times (\text{length}/2 \times \text{width}/2 \times \text{height}/2)$. Mice were inspected daily and weighed at the beginning and the end of treatment. When the average tumor volume reached 1 cm^3 , mice were euthanized by CO_2 inhalation and approximately 0.5 mL of blood serum was collected from the vein cava and stored at -80°C for high-performance liquid chromatographic (HPLC) analyses. Tumors and healthy tissues were weighed, frozen in liquid nitrogen or fixed with 4% paraformaldehyde, and embedded with paraffin for histologic analyses.

Immunohistochemistry

Tissue sections of 5 μm were deparaffinized and incubated with 3% H_2O_2 to block endogenous peroxidase activities. The sections were heated for 10 min in sodium citrate buffer (pH 6.0) 0.01 mol/L in a microwave oven to retrieve antigens. Non-specific bindings were blocked with tris-buffered saline tween-20/5% normal goat serum at room temperature for 60 min. Mouse anti-Ki-67 (1:10; MIB-1, Immunotech, Prague, Czech Republic) or rabbit anti-MDA (1:100; Abcam, Cambridge, MA, USA) antibody was added and tissue sections were incubated overnight in a humidified chamber at 4°C . A universal LSAB-HRP system (Dako, Baar, Switzerland) was used to detect the signals according to the manufacturer's instructions. After a slight counterstaining with hematoxylin, the tissue sections were dehydrated, mounted onto slides, and then digitized using a Mirax Scanner (Carl Zeiss, Feldbach, Switzerland). Positively Ki-67 stained nuclei and MDA intensity of at least three different fields per tumor were quantified to determine tumor cell proliferation and lipid peroxidation, respectively, using Metamorph imaging software. The percentage of necrotic area in tumor tissue sections was also measured with the help of a pathologist using Panoramic Viewer software (3D Histech, Budapest, Hungary) after simple hematoxylin and eosin staining ($n = 6/\text{group}$).

Lipid peroxidation

The formation of lipid peroxidation products in cell lysate was evaluated 24 h after treatment using a thiobarbituric acid-reactive species assay kit according to the manufacturer's instruction (Cayman Chemical, Basel, Switzerland; $n = 3/\text{condition}$). The MDA concentration was also measured using high-pressure liquid chromatography according to a published protocol, with minor

modifications [19]. Briefly, 50- μ L samples containing cell lysate or mouse serum and standard and methyl-MDA as an internal standard were analyzed with a HPLC system (Series 1100, Agilent, Morges, Switzerland) equipped with an autosampler, a quaternary pump, an ultraviolet diode-array detector, and a C18 column (Nucleodur C18 Pyramid 3 μ m, Macherey-Nagel, Oensingen, Switzerland; $n = 6$ /group).

Total peroxide production in the tumor tissue extract was quantified based on an Fe(III)-xylenol orange complex formation [20]. Briefly, 150 mg of frozen tissue sample was homogenized in 900 μ L of methanol containing butylated hydroxytoluene 5 mmol/L using a homogenizer (Qiagen, Düsseldorf, Germany). The homogenates were sonicated for 10 s and centrifuged at $15\,000 \times g$ (4°C). Two supernatant fractions of 500 μ L were collected, one being mixed with 50 μ L of methanol, the other with 50 μ L of triphenyl phosphine/methanol 10 mmol/L. After a 30-min incubation at room temperature, 550 μ L of the following reagent mixture was added to the two samples: xylenol orange 0.2 mmol/L, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ 0.5 mmol/L, H_2SO_4 50 mmol/L, and butylated hydroxytoluene 4 mmol/L dissolved in methanol. The absorbance was read at 595 nm after 1 h at room temperature. The specific signal was calculated as the difference between the two samples. Hydroperoxide (Merck, Altdorf, Switzerland) was used as a standard ($n = 6$ /group).

Data analysis

The variables were expressed as proportion or mean \pm standard deviation, as appropriate. For cell viability and the clonogenic assay, intergroup comparisons were performed with two-way analysis of variance and the mean comparison between two specific conditions with Student's *t* test. One-way analysis of variance was used for the other experiments. Analysis of variance followed by post hoc Tukey test was performed with SPSS 11.5 (SPSS, Inc., Chicago, IL, USA). A significance level of $P \leq 0.05$ was considered statistically significant.

Results

FO but not SO emulsion decreases cell viability

Colorimetric MTT assays were carried out 120 h after treatment with the FO or SO emulsion or the corresponding concentrations of EPA and DHA to highlight a possible cytotoxic effect of these emulsions on cancer cells. The type and dose of treatment were shown to significantly affect cell viability ($P < 0.01$). Although the SO emulsion had no significant effect regardless of the dose used, the dose-response curve showed that the administration of FO 18 to 80 mg/L decreased the cell viability from 14.6% to 35.7%, respectively. However, the cytotoxic effect of the FO emulsion was lower than the administration of an equivalent concentration of free ω -3 PUFAs, with cell viability decreased up to 75.5% and 83.7% for EPA and DHA 90 μ mol/L, respectively (Fig. 1A). These results were confirmed by a clonogenic assay showing that a 24-h treatment with the FO emulsion 27 to 45 mg/L decreased the long-term cell survival from 57% ($P < 0.001$) to 64% ($P < 0.001$), respectively, whereas the administration of an equivalent dose of the SO emulsion had no significant effect compared with the NT cells (Fig. 1B).

FO but not SO emulsion increases cell apoptosis

Two-parameter FACS analyses with PI and annexin V-fluorescein isothiocyanate were carried out to assess if FO or SO emulsion could induce cancer cell apoptosis. A 24-h treatment with the FO emulsion 45 mg/L increased cell apoptosis by 1.6-fold ($P = 0.04$) compared with NT cells. In contrast, the SO emulsion did not exhibit any significant effect (Fig. 2A). Confocal microscopy with PI staining confirmed this observation by showing an overall increase in chromatin condensation in the nuclei of cells treated with the FO emulsion compared with NT cells, whereas the effect appeared to be less pronounced with the SO emulsion (Fig. 2B).

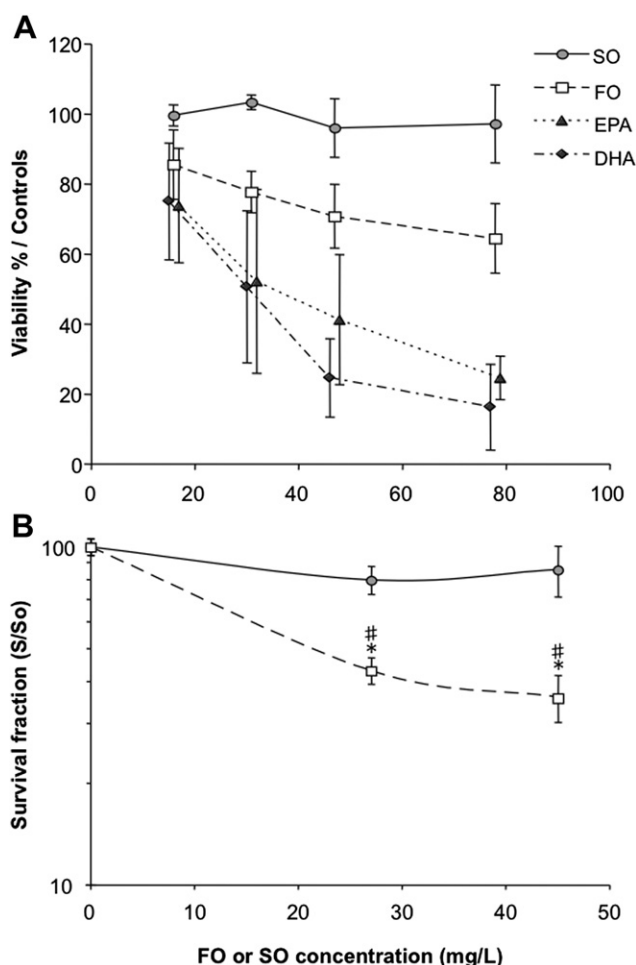


Fig. 1. Effect of FO and SO emulsions on colorectal cancer cell viability and clonogenicity. (A) HT-29 cell viability was measured 120 h after a 24-h incubation with SO (circles) or FO (squares) 18 to 80 mg/L or with the corresponding concentrations of EPA (triangles) and DHA (diamonds) using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. Each point represents the mean \pm SD of the cell viability (percentage) compared with untreated controls ($n = 3$). (B) The clonogenic potential of HT-29 cells was evaluated 14 d after a 24-h incubation with SO (circles) or FO (squares) 27 to 45 mg/L using a clonogenic assay. Each point represents the mean \pm SD of survival fractions (percentage) compared with untreated controls in a logarithmic y scale ($n = 3$). Symbols indicate difference between two groups (Student's *t*-test, * $P < 0.05$ versus untreated; # $P < 0.05$ versus SO). DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; SO, soybean oil; S/So, survival fraction compared with untreated controls.

FO but not SO emulsion increases lipid peroxidation in cell culture

Thiobarbituric acid-reactive species assays were carried out to evaluate whether lipid peroxidation products are involved in the cytotoxic effect induced by a 24-h treatment with the FO emulsion 45 mg/L. The formation of MDA-thiobarbituric acid was indeed increased by 2.0-fold ($P < 0.01$) and 1.7-fold ($P = 0.02$) in FO-treated compared with NT or SO-treated cells, respectively. The SO emulsion did not exhibit any significant effect (Fig. 2C).

FO but not SO emulsion inhibits tumor cell proliferation

The effect of the intravenous administration of FO and SO emulsion was evaluated in nude mice xenografted with the same human colorectal adenocarcinoma cell line used in vitro. Tumor-volume monitoring failed to show any significant effect of

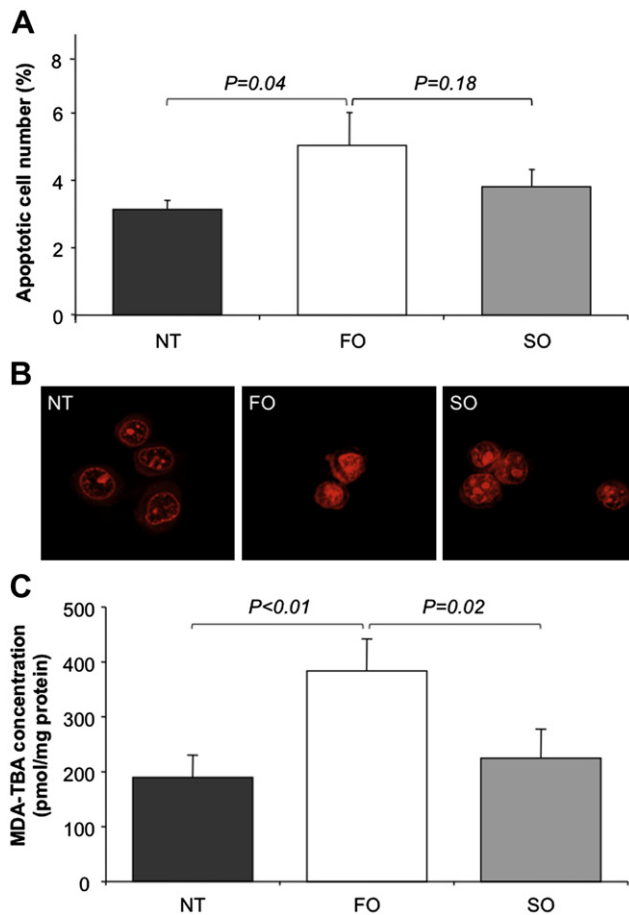


Fig. 2. Effect of FO and SO emulsions on colorectal cancer cell apoptosis and lipid peroxidation. (A) HT-29 cell apoptosis was measured 48 h after a 24-h incubation with FO (white bars) or SO (gray bars) 45 mg/L or NT (black bars) using two-parameter fluorescence-activated cell sorting analysis with propidium iodide and annexin V-fluorescein isothiocyanate. Each column represents mean \pm SD (percentage) of the apoptotic compared with the total cell number ($n = 3$). (B) Confocal microscopy with propidium iodide staining was performed 24 h after the different treatments to display nuclear abnormalities (magnification 63 \times). (C) TBA-reactive species were measured colorimetrically in an HT-29 cell culture after a 24-h incubation with FO (white bars) or SO (gray bars) 45 mg/L or NT (black bars) using an enzyme-linked immunosorbent assay microplate reader. Each column represents mean \pm SD of MDA-TBA concentration (picomoles per milligram of protein; $n = 3$). *P* values were calculated with one-way analysis of variance followed by the Tukey test. FO, fish oil; MDA, malondialdehyde; NT, not treated; SO, soybean oil; TBA, thiobarbituric acid.

the two lipid emulsions on tumor growth during the 11 d of treatment. The mean tumor weights did not differ significantly between groups when animals were euthanized 1 wk after the end of treatment (Fig. 3A). Immunohistochemistry analysis was performed to assess if FO or SO emulsion could affect in vivo tumor cell proliferation (Fig. 3B). The percentage of Ki-67-positive nuclei was decreased by 0.6-fold ($P < 0.01$) and 0.5-fold ($P = 0.04$) in tumors of FO-treated mice compared with NT or SO-treated mice, respectively, whereas there was no significant difference between NT and SO-treated mice ($P = 0.71$; Fig. 3C).

FO but not SO emulsion induces tumor necrosis

Tumors of FO-treated mice appeared overall more purplish than those of NT or SO-treated mice, suggesting the induction of

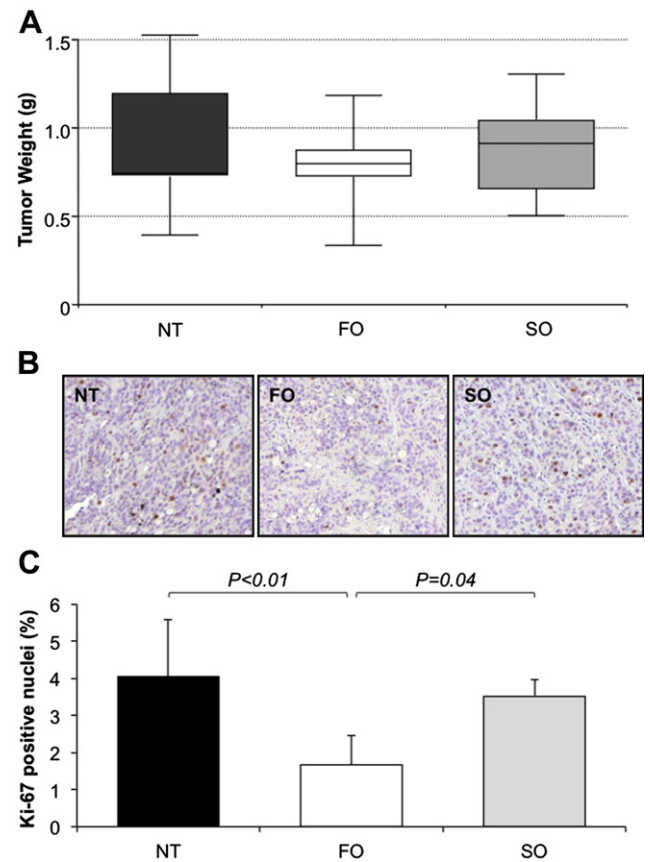


Fig. 3. Effect of FO and SO emulsions on colorectal tumor growth. (A) HT-29 tumors were weighed after the mice were NT (black bars) or treated for 11 d with daily intravenous injections of FO (white bars) or SO (gray bars) 0.2 g/kg and then sacrificed by CO₂ inhalation 7 d later. Each column represents mean \pm SD of tumor weight ($n = 6$). (B) Immunohistochemical analysis of the proliferation marker Ki-67 (dark dots) in tumor of mice NT or treated with FO or SO (magnification 20 \times). (C) Quantification of Ki-67-positive nuclei was performed in at least three fields/tumor by six tumors/group using Metamorph imaging software. Each column represents mean \pm SD (percentage) of Ki-67-positive compared with total nuclei ($n = 6$). *P* values were calculated with one-way analysis of variance followed by the Tukey test. FO, fish oil; NT, not treated; SO, soybean oil.

a necrotic process (Fig. 4A). This was supported by histologic examination (Fig. 4B) and confirmed by area measurement with the image processing software, showing a significant increased necrosis in the tumor tissue sections of FO-treated mice ($13.1 \pm 7.8\%$) compared with NT mice ($5.8 \pm 6.0\%$, $P = 0.03$; Fig. 4C).

FO but not SO emulsion increases lipid peroxidation in blood and tumors

The HPLC analyses were performed to verify if lipid peroxidation could also be increased in vivo by FO emulsion. The MDA serum level was increased by 1.2-fold and 1.6-fold in FO-treated mice compared with NT ($P = 0.37$) and SO-treated ($P = 0.04$) mice (Fig. 5A). Immunohistochemical analysis was performed to further examine MDA levels in tumor tissues using a specific anti-MDA antibody (Fig. 5B). The intensity of MDA protein detection tended ($P = 0.09$) to be increased in tumors of FO-treated mice ($50.4 \pm 44.2\%$) compared with NT ($26.9 \pm 21.1\%$) and SO-treated ($20.8 \pm 21.1\%$) mice. This observation was supported by the measurement of total peroxides in tumor tissues

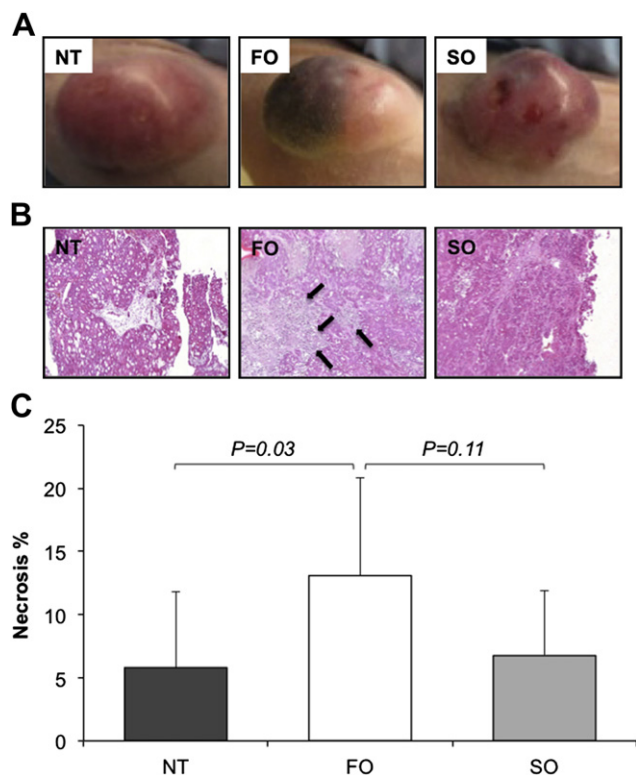


Fig. 4. Effect of FO and SO emulsions on colorectal tumor necrosis. (A) Representative appearance of tumor of mice NT (black bars) or treated for 11 d with daily intravenous injections of FO (white bars) or SO (gray bars) 0.2 g/kg. (B) Hematoxylin and eosin staining of tumor sections most commonly observed in the different treatment groups (magnification 10 \times). Arrows point to necrosis. (C) Quantification of necrotic areas was performed in tissue sections of six tumors/group using Panoramic Viewer software. Each column represents mean \pm SD (percentage) of necrotic compared with the total area ($n = 6$). P values were calculated with one-way analysis of variance followed by the Tukey test. FO, fish oil; NT, not treated; SO, soybean oil.

showing a significant increase in FO-treated (18.9 ± 4.9 pmol/mg) compared with NT (2.7 ± 9.9 pmol/mg, $P < 0.01$) or SO-treated (3.5 ± 3.2 pmol/mg, $P < 0.01$) mice (Fig. 5C).

Discussion

We previously showed in several human colorectal adenocarcinoma cell lines that the cytotoxic action of free ω -3 PUFAs was caused at least in part by a mechanism involving lipid peroxidation [11]. However, it was not certain that this anticancer effect could be reproduced in vivo, because ω -3 PUFAs are usually administered to patients in the form of a FO emulsion containing significant amounts of the antioxidant vitamin E. Therefore, we aimed to assess if a commercially available FO emulsion had anticancer effects similar to free ω -3 PUFAs in vitro and in vivo in colorectal tumor xenografted mice, which could be related to lipid peroxidation. Our results showed that the administration of a FO emulsion could effectively induce apoptosis and decrease HT-29 cell survival compared with an isocaloric and isolipidic SO emulsion. Because these two emulsions differ mainly in their fatty acid composition, it is likely that the presence of long-chain ω -3 PUFAs (i.e., EPA and DHA) in the FO emulsion played a role in its cytotoxic effect. Others have described a growth inhibitory effect of different commercially available FO emulsions on Caco-2 and HT-29 cells [21,22].

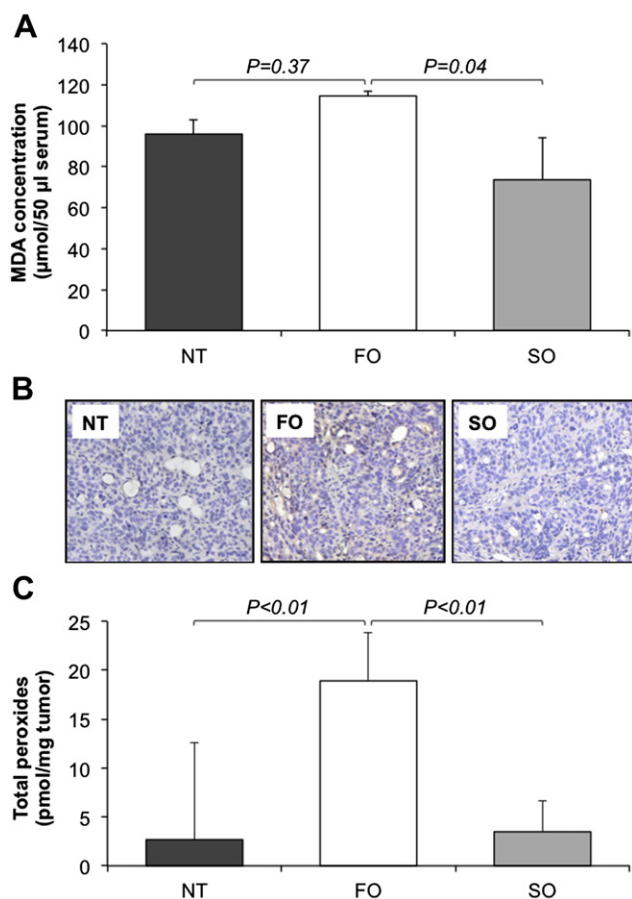


Fig. 5. Effect of FO and SO emulsions on the formation of lipid peroxidation products. (A) MDA serum concentration of mice NT (black bars) or treated for 11 d with daily intravenous injections of FO (white bars) or SO (gray bars) 0.2 g/kg was measured using high-performance liquid chromatographic analysis. Each column represents mean \pm SD of MDA concentration (micromoles per 50 μ L of serum; $n = 6$). (B) Immunohistochemical analysis of MDA in tumors of mice NT or treated with FO or SO (magnification 20 \times). (C) Total peroxide measurement in tumors of mice NT or treated with FO or SO was quantified based on Fe(III) xylene orange complex formation. Each column represents mean \pm SD of total peroxides in picomoles per milligram of tumor ($n = 6$). P values were calculated with one-way analysis of variance followed by the Tukey test. FO, fish oil; MDA, malondialdehyde; NT, not treated; SO, soybean oil.

However, they did not observe, as we did, an increased percentage of apoptotic cells after exposure to the FO emulsion, probably because their FACS analysis with only PI staining was not discriminant enough to identify apoptotic cells compared with our measurement with PI and annexin V stainings [22]. Assuming that the hydrolysis of the FO emulsion in the culture medium allows cell exposure to bioactive compounds, our results are consistent with others having shown with the 4, 6-diamidino-2-phenylindole assay that the incorporation of free EPA and DHA into HT-29 cells induces apoptosis [23]. In the present study, the presence of vitamin E 0.43 mmol/L in the FO emulsion did not prevent the formation of lipid peroxidation products, thus demonstrating that induction of free radical chain reactions could be involved in the cytotoxic effect of the FO emulsion. However, the effect of the FO emulsion was less pronounced than the administration of an equivalent dose of ω -3 PUFAs as free fatty acids. These results appear consistent with our previous observation, where the dose of vitamin E required to abolish the cytotoxic effect of ω -3 PUFAs 50 μ mol/L was

proportionally higher (10 $\mu\text{mol/L}$) than that (0.3 $\mu\text{mol/L}$) present in the FO emulsion [11]. All these results were obtained in vitro and it may be that the bioavailability of long-chain ω -3 PUFAs in the FO emulsion differs in vivo depending on the host metabolism and lipid clearance in the bloodstream. That is why we evaluated the effect of daily intravenous injections of the FO emulsion on the growth of colorectal tumors xenografted in nude mice. Tumor volume and weight measurements were not conclusive in showing a modulation of tumor growth by the FO emulsion. Nevertheless, there was a body of evidence that the FO emulsion could still exert an antitumor action by inducing a necrotic process and decreasing the expression of the cell proliferation marker Ki-67 in tumor tissue sections of FO-treated mice compared with NT mice. Less impressive results than those obtained in vitro could be explained by a lower efficacy of the bolus injections because of a short peak concentration of FO in the blood compared with a continuous infusion or dietary supplementation with consequently prolonged exposure to FO [24]. It is also possible that the presence of vitamin E in the FO emulsion, in association with endogenous defense mechanisms against free radicals, was more efficient than in vitro to prevent a lipid peroxidation cascade in membrane phospholipids. Nevertheless, MDA plasma levels and total peroxides in tumor were increased in the FO-treated mice compared with the other treatment groups, showing that lipid peroxidation can still occur in vivo and might be involved in the increase in necrotic areas that we observed in tumor tissue sections of FO-treated mice compared with NT mice. This observation is consistent with a clinical study having shown that the presence of vitamin E in FO capsules did not prevent the increased formation of plasma lipid peroxides after 3 mo of oral supplementation [25]. Because EPA, DHA, and antioxidant contents can vary greatly among natural and commercially available FO supplements [26], further investigations are needed to optimize the ratio of vitamin E to ω -3 PUFAs in emulsions dedicated to treatment. For example, γ -tocopherol could be used as vitamin E in lipid emulsion because it has been reported, in contrast to α -tocopherol, to induce growth reduction and apoptosis in different colorectal cancer cell lines, including HT-29 cells [27]. It has been reported that the modulating effect of ω -3 PUFAs on proinflammatory prostaglandin synthesis from COX-2 could play a role in minimizing side effects and increasing the efficacy of conventional radio-/chemotherapies [28]. We further speculate that ω -3 PUFA peroxidation may play a significant role in cancer treatment, especially because cancer cells have been described to be more sensitive to oxidative stress than normal cells because of altered protecting mechanisms against free radical and reactive oxygen species [29]. However, further studies are needed to confirm this hypothesis, especially because our work shows some limitations, such as the evaluation of only one FO emulsion and cancer cell line. The mode of administration (i.e., route, dose, and timing) of FO could also affect the in vivo stability and thus the bioavailability of ω -3 PUFAs. Unfortunately, we did not measure the rate of incorporation of EPA and DHA in the tumor, but rather their peroxidation product, MDA, and total peroxides. Moreover, the clinical use of FO products or algae is still under debate, especially because Roodhart et al. [30] recently reported that the release of specific PUFAs by endogenous mesenchymal stem cells could induce tumor resistance to different chemotherapeutic drugs. Although phospholipases A2, thromboxane synthase, and COX-1 are involved in this chemoresistance [30], our results showed that the modulation of eicosanoid synthesis is not the only mechanism involved and that others have to be taken into account, such as the lipoperoxidative effects on tumor growth.

This observation provides support to the other numerous studies suggesting a beneficial effect of FO in cancer therapy [31].

Conclusion

Vitamin E content in an FO emulsion does not prevent lipid peroxidation and the concomitant induction of human colorectal adenocarcinoma cell apoptosis and death. The lipoperoxidative effects were confirmed in vivo by decreased tumor cell proliferation and increased necrosis. These observations suggest that an FO emulsion could be considered adjuvant cancer therapy.

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