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Effect of Chia oil (*Salvia Hispanica)* rich in ω-3 fatty acids on the eicosanoid release, apoptosis and *T*-lymphocyte tumor infiltration in a murine mammary gland adenocarcinoma ☆

C.E. Espada[a], M.A. Berra[a], M.J. Martinez[b], A.R. Eynard[a], M.E. Pasqualini[a,*]

[a]*Iα Cátedra de Biología Celular, Histología y Embriología, Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Ciudad Universitaria. Casilla de Correos 220, 5000, Córdoba. Argentina*
[b]*Laboratorio de Calidad de Granos. EEA-INTA Manfredi. Ruta Nac. 9 km 636. Córdoba, Argentina*

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Abstract

We investigated the effects of certain dietary polyunsaturated fatty acids (PUFAs) and related eicosanoids on the growth and metastasis formation of a murine mammary gland adenocarcinoma. *Salvia hispanica* (ChO) and *Carthamus tinctorius* (SaO) vegetable oil sources of ω-3 and -6 PUFAs and a commercial diet as control (CO), were used. We analysed fatty acids of neoplastic cells (NC) membranes by GLC; the eicosanoids 12- HETE and 12-HHT (LOX and COX metabolites) by HPLC and apoptosis and *T*-lymphocyte infiltration by flow cytometry and microscopy. NC from ChO groups showed lower levels of arachidonic acid and of both eicosanoids compared to SaO and CO ($p<0.05$). The ChO diet decreased the tumor weight and metastasis number ($p<0.05$). Apoptosis and *T*-lymphocyte infiltration were higher and mitosis decreased with respect to the other diets ($p<0.05$). Present data showed that ChO, an ancient and almost unknown source of ω-3, inhibits growth and metastasis in this tumor model.

1. Introduction

Dietary fat is thought to be one of the main risk factors for cancer, based on reports of positive correlations of certain fat intakes with increases of breast, colon and prostate tumors [1,2]. The ω-6/ω-3 ratio of polyunsaturated fatty acids (PUFAs) in the diet is reported to be important, but its effect still remains to be clarified. Corn and Safflower (*Carthamus tinctorius* L) oils (SaO), as well as cow meat, are rich in ω-6 PUFAs and are frequently consumed in western populations [3]. Fish oil and certain vegetables seed oils, such as those derivates from Mistol (*Zysyphus mistol* L.) and Chia (*Salvia hipanica* L.) are rich in ω-3 PUFAs and have been eaten since for pre-Hispanic cultures living in South America up to the present day [4,5].

PUFAs derived from essential fatty acids (EFAs) are precursors of the eicosanoids. These are very short-lived substances which are required for the regulatory mechanisms of almost all tissues in the body, and the manipulation of dietary fatty acid (FA) intake significantly affects eicosanoid synthesis [6].

12-Hydroxyeicosatetraenoic acid (12-HETE), a 12-lypoxygenase (12-LOX) derivative arachidonic acid (AA) metabolite, is formed in excess in various tumor tissues and epithelial cancer cell lines and the ability of tumor cells to generate this eicosanoid is positively correlated to their metastatic potential [7–9] Furthermore, experimental evidence indicates that AA derivative eicosanoids from cyclooxygenase (COX) pathway contribute to cancer progression through increasing DNA damage and mutation. The likely mediators of

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*Corresponding author. Tel.: +54 351 4334020; fax: +54 351 4810404.
E-mail address: epasqual@cmefcm.uncor.edu (M.E. Pasqualini).

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this DNA damage are malondialdehyde (MDA) and 12-hydroxyheptadecatrienoic acid (12-HHT) being both of these products formed in equimolecular proportions [10].

PUFAs and their metabolites exert antitumoral effects, perhaps affecting the gene expression or activating signal transduction molecules involved in the control of cell growth, differentiation, apoptosis, angiogenesis, metastasis, immune system and inflammation [11,12]. In addition, inflammatory cells produce a highly complicated mixture of eicosanoids, reactive oxygen species and free radicals, as well as growth and differentiation cytokines, which affect the carcinogenic process [13]. *T*-lymphocytes play a critical role in defining the type and magnitude of the immune response, and PUFAs influence the production of a range of lipid-derived secondary messengers in lymphocytes and leucocytes. Nevertheless, the mechanisms involved in the effects of PUFAs on *T*-cell functions remain to be elucidated [14].

ω-6 PUFA rich diets increment the cell production for PGE_2, an AA-COX- derivative eicosanoid, which in turn inhibits lymphocyte proliferation, interferon gamma (INF-γ) and interleukin-2 (IL-2) production by *T*-helper cells (Th) [15]. On the other hand, the ω-3 PUFA rich diets increase lymphocyte proliferation and Th1-type cytokine production such as IL-2 and IFN-γ [16]. However, other studies indicated that ω-3 dietary supplementation decreased lymphocyte proliferation and also reduced production of IL-2 and IFN-γ [17]. Therefore, there is a discrepancy between the biological response of the ω-3 and -6 PUFA diet supplements on the lymphocyte proliferation and the eicosanoid synthesis, which may affect tumor growth and metastasis.

The purpose of the present study was to investigate by comparison, the effect of an ancient rich vegetable rich source of ω-3 PUFAs, *Salvia hispanica* (Chia) derived oil, and a rich source of ω-6 PUFAs, *Carthamus tinctorius* (Safflower) derived oil, on the LOX and COX derivative eicosanoids 12 (S)-HETE and 12 (S)-HHT, respectively, and on several parameters linked to the neoplastic progression and host immunology using a transplantable murine mammary gland adenocarcinoma.

2. Materials and methods

2.1. Animals feeding and diets

Since weaning, 60 male and female BALB/c mice were randomly distributed among three dietary groups. The experimental diets were prepared by replacing the 6% fat of the commercial diet adding this percentage with different fats to a basic semisynthetic diet.

Table 1
Fatty acids composition of Chia (*Salvia hipanica* L.) and Safflower (*Carthamus tinctorious* L.) oils and control diet (commercial)

Diets	14:0	16:0	18:1	18:2	18:3
CO	6.74	30.09	47.69	11.89	3.56
ChO	6.47	2.51	6.71	21.36	63
SaO	7.09	2.61	14.27	75.80	0.24

Fatty acids were determined as indicate in Materials and methods. Values are the average of at least three determinations (SEM were less than 6% in all cases).

As shown in Table 1, the fats were provided by Chia oil (ChO), which contained high percentage of 18:3 ω-3 (5), or by Safflower oil (SaO), which contained elevated quantity of 18:2 ω-6. The final composition of the diets was 6% tested oil, 17% casein, 33% sucrose, 38% corn starch, 2% fiber, 2% salts mixture and 0.5% vitamin mixture. The control group (CO) was maintained on a commercial diet (Cargill, S.A.C.I., Argentina) whose composition was: 20% of proteins, 64% carbohydrates, 4% fibers and 6% of fats containing 10% of 18:2, ω-6 and 3% of 18:3,ω-3. Per gram weight diets did not differ significantly in caloric value [18]. Food and water were provided ad libitum. Animals were kept in a light and temperature-controlled room and according to the Institutional Animal Care Guidelines (Animal Care Committee from National University of Córdoba, Argentina). To avoid rancidity within the food containers, each oil was stored in dark sealed glass recipients at 15 °C until being used, with fresh food being offered daily.

2.2. Tumor

Three months after the experimental diets were initiated, a murine transplantable mammary gland adenocarcinoma having moderate (M3) metastatic capability was inoculated subcutaneously [19].

Animals were sacrificed at 45–50 days after inoculum. During necropsy, tumor weight, size and number of metastasis were recorded. Macroscopic metastasis were recorded in all organs of the host fed animals with the aid of a magnifying lens.

Neoplastic cell (NC) suspensions, isolated from primary tumors, were obtained as published elsewhere [18].

2.3. Plasma membrane separation and fatty acid determination

Plasma membrane purification from NC was achieved as described elsewhere [20]. Briefly, total homogenates obtained in a hipotonic hepes–manitol buffer were treated with 10 mM $ClCa_2$. The suspensions were centrifuged at 3000 g for 15 min and the pellet discarded.

The supernatant was then centrifuged at 48000 g for 30 min, and the pellet containing the plasma membrane fragments was collected in PBS buffer at 20 °C overnight and used the following day. As a control of plasma membrane purity, membrane marker enzymes were determined as previously described [20].

Total lipids were extracted from plasma membrane fractions and partitioned according to Folch. Methyl esters were obtained from the lower phase containing phospholipids, then methanolisis was performed and analyzed by using gas chromatography on a capillary column HP-INNOWAX (polyethylene glycol 30 m × 320 μm × 0.50 μm) on a Hewlett Packard 6890 with an FID detector. FA methyl esters were identified by comparison of retention times with those of the commercial standards [20].

2.4. AA metabolite analysis

Representative AA (20:4 ω-6) metabolites, the 12(S)-HHT and 12(S)-HETE, released after stimulation by NC suspensions, were analyzed. Washed tumor cell suspensions (1.0×10^7/ml), were treated with Ca^{2+} and Mg^{2+} and stimulated with the ionophore A23187 (2 M) for 15 min at 37 °C. 12(S)-HHT and 12(S)-HETE were extracted and quantified by HPLC in an Agilent 1100 Series. The spectrums were read between 240 and 400 nm with a Photo Diode Array (PDA), and the eicosanoids were detected at 235 nm. The gradient began with 50% of solvent (A): 0.02% acetic acid: water, (v/v), and 50% solvent, (B): 0.02% acetic acid: acetonitrile falling to 0% of water solution 25 min later. Quantification of 12(S)-HHT, 12 and (S)-HETE were obtained by means of standard curves containing the same synthetic compounds (Biomol Co.) [18,21,22].

2.5. Apoptosis and mitosis

Apoptosis of NC in tumor cells suspension was analyzed by flow cytometry (FC) (Coulter® XL EPICS® Flow Cytometer), using Annexin V-Fitc kit (SIGMA Co.) based on the binding properties of conjugated Annexine V-Fitc to plasma membrane phosphatidylserine (PS) in combination with propidium iodide (PI) staining [23]. A complementary approach was the counting of apoptotic figures in slides of tumor tissue, fixed in 10% neutral formalin, dehydrated and embedded in paraffin, and stained with hematoxylin and eosin (H&E). Apoptotic figures were recognized by characteristic morphological changes as shrinking, condensation and fragmentation of nuclei. Apoptotic and mitotic figures were counted from 10 animals for each dietary condition *per* 10 high-power fields, using a light microscope (LM) Olympus BH2 [18].

2.6. Tumor leukocytic infiltration

The leukocytic infiltration in tumor cell suspensions was evaluated by flow cytometry (Coulter® XL EPICS® Flow Cytometer) using CD3 monoclonal antibody fluorescein-isothiocyanate (FITC)-conjugated hamster antimouse (1:100 PBS) (BD Biosciences Pharmigen), as the CD3 complex is expressed on mature *T*-lymphocytes and NK-T cells [24]. We also evaluated the leukocyte infiltration by immunocytochemistry: tumor samples were fixed in a mixture of glutaradehyde 1% and paraformaldehyde 4% in sym-collidine buffer 0.1 M ph 7.2 for 4 hs at room temperature. Then, they were included in Unicryl (Biocell, UK) polymerized at 360 nm UV light at 4 °C for 4–6 days. Semithin sections (1 μm) were cut with a Reichert E ultramicrotome and incubated with CD3 monoclonal antibody anti-mouse (1:100 PBS)(BD Biosciences Pharmigen). The secondary antibody was then IgG conjugated with 5 or 10 nm particles of colloidal gold (1:50 PBS) (Sigma-Aldrich USA). Silver enhancement was carried out according to the suppliers instructions (Biocell, UK). Immunolabeling was evaluated by LM measuring the gold-silver/mm^3 particles in sections [25].

2.7. Statistical analysis

Data were analyzed using the ANOVA test. The LSD test was used to compare the mean differences between groups, with $p < 0.05$ being considered significant. For all the analyses the InfoStat program (2005) was used.

3. Results

3.1. Fatty acid composition of NC membranes

Profiles of main FAs of NC membranes, isolated from hosts fed with different PUFAs, are shown in Table 2. The FA composition of the membranes correlated fairly well with the lipid source of the diet. As expected, the tumor membranes obtained from SaO (ω-6 rich) hosts showed a higher percentage of AA (C20:4) with respect to ChO (ω-3) and Control (CO) (Table 2). Samples

Table 2
Fatty acid profile of NC membranes from host bearing M3 adenocarcinoma fed on different diets

Diets	14:0	16:0	18:1	18:2	18:3	20:4	20:5
CO	0.85	24.65	13.83	31.19	9.67	18.85	0.94
ChO	1.26	24.99	16.07	35.55	7.78	11.25	3.07
SaO	0.90	22.61	14.04	26.47	13.53	21.54	0.89

Fatty acids composition of NC membrane was determined as indicate in Materials and methods. Values represents the mean in percentage of at least three determinations from NC suspensions of 10^8 cells/ml (SEM were less than 6% in all cases).

isolated from hosts fed on ChO diet showed the highest percentage of eicosapentaenoic acid (EPA, 20:5 ω-3) in comparison with the SaO and CO groups (Table 2).

3.2. AA-derived eicosanoids

The three varieties of diets affected in different ways the COX, 12 (S)-HHT and LOX, 12 (S)-HETE derived eicosanoids released from NC after stimulation with ionophore A 23187 (Figs. 1a and b).

Compared with NC obtained from SaO and CO diets, samples from hosts fed on ChO diet released the lowest levels of 12 (S)-HETE (11.59 ng/10^7 cells) ($p<0.05$) (Fig. 1a). Also, ChO host CN produced the lowest ($p<0.05$) amounts of 12 (S) HHT (85.91 ng/10^7 cells) with respect to the other diets (SaO = 135.58 ng/10^7 cells; CO = 160.37 ng/10^7 cells) (Fig. 1b).

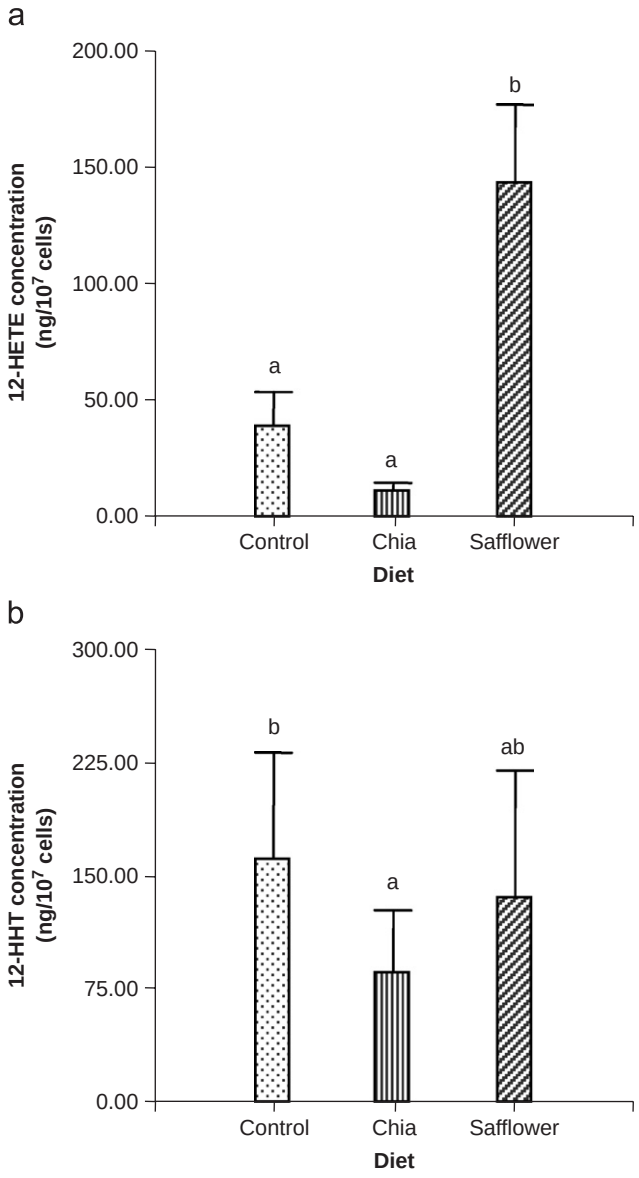

Fig. 1. Eicosanoids released from M3 hosts fed on different diets. (a) 12-HETE and (b) 12-HHT production after stimulation with ionophore A 23287 (2 M). Values represent the mean ± SD of 10 samples. Different letters represent significant differences ($p<0.05$).

3.3. Tumor growth parameters and metastasis number

There were not significantly differences in mice weight among the three experimental diet groups (data not shown). The diets affected the tumor weight and the metastasis number. Tumor weight from the ChO group was significantly lower (12.86 ± 0.21 g) than the CO group (18.29 ± 0.63 g) but not from SaO (14.27 ± 0.10) (Fig. 2a) ($p<0.05$).

ChO animals had the lowest number of metastasis (0.5 ± 0.33) with respect to the SaO (2.25 ± 0.16) and CO

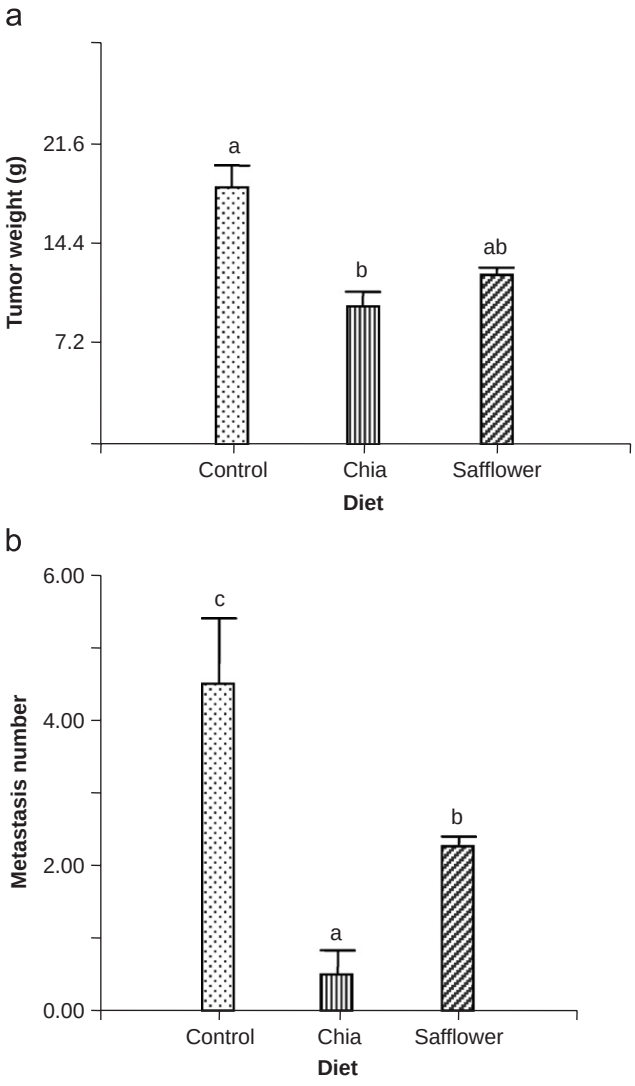

Fig. 2. (a) Tumor weight from host fed on Chia, Safflower and Control diets, recorded during necropsy at 45–50 post inoculum; (b) metastasis number in M3 hosts fed on different diets. Values represent the mean ± SD of 10 samples on each group. Different letters represent significant differences ($p<0.05$).

mice groups (4.5±0.91) ($p<0.05$) (Fig. 2b). The metastasis were located in the lung, liver and pancreas.

3.4. Dietary PUFA effect on NC apoptosis and mitosis

Dietary PUFA composition of the different diets significantly affected both the number of apoptosis and also the quantity of mitosis (Table 3) and (Figs. 3a, b and c)

Cell samples obtained from ChO experimental group and measured by FC with conjugated Annexin V-Fitc (Fig. 3a), exhibited higher percentages of apoptosis (17%) compared with SaO (12.8%) and CO (6.30%).

The apoptotic cell counting evaluated by LM correlated fairly well with the results obtained using Annexin V-Fitc (Table 3). The apoptotic figures counted on ChO tumor sections were significantly higher ($p<0.05$) than SaO and CO groups (Table 3). The mitotic figures in ChO were significantly lower ($p<0.05$) compared to SaO and CO treatments (Table 3)

3.5. Tumor leukocytic infiltration

Dietary PUFAs significantly affected the lymphocyte *T* and NK-T cell infiltration in the tumor (Fig. 4 and Table 4). The results obtained by FC using CD3 monoclonal (FITC)-conjugated were: ChO, 19; SaO, 3.87; and CO, 0.89 ($p<0.05$). ChO CN suspensions showed the highest percentage of *T*-lymphocytes. Similar results were obtained from CD3 inmunolabeled tumor sections, with ChO host fed diet showing 35.5% marked positively, respect to SO and CO groups that had values of 23 and 4.23%, respectively (Table 4).

4. Discussion

This work adds original results concerning to the differential effects of seed oils rich in ω-3 and -6 PUFAs on the growth and metastasis of a murine mammary gland adenocarcinoma which may be relevant to human breast cancer tumorigenesis. As source of ω-3, we used a native oil isolated from Chia seeds (*Salvia hispanica* L.) and Safflower seed oil (*Carthamus tinctorious* L.) was used as an unusually rich ω-6 source [3,5]. It is well known that dietary PUFAs are selectively incorporated into cellular membranes. In our study NC membranes PUFAs were modified according to the different dietary lipids offered to the host (Table 2). The Chia oil diet increased the levels of EPA and decreased the AA in comparison to Safflower oil and Control diets (Table 2). These results agree with previous experimental data showing that ω-3 membrane enrichment was achieved by feeding hosts with lipid sources rich in ω-3 [4,26].

In our experimental model, the metabolites derived from AA, such as 12-HETE and 12-HHT were significantly reduced in the NC ChO samples with respect to the SaO and CO groups (Figs. 1a and b). These changes could be linked to: (a) decreasing availability of AA in the membrane, thus (b) favoring the biosynthesis of EPA-derived 3-series prostanoids and 5-series leukotrienes and (c) reducing desaturation and elongation of LA to AA due to the higher affinity of ω-3 FAs to elongases and desaturases [27].

We also observed that ChO diet significantly reduced the tumor weight (Fig. 2a) linked to an increase in apoptosis and a reduction in mitosis found in tumor tissues (Fig. 3a, Table 3). The apoptotic NC observed in the ChO could be caused by IL-2 and TNF γ released by *T*-lymphocytes in accordance to the increment in the tumor leukocyte infiltration showed in this group (Fig. 4 and Table 4) [28]. Indeed, EPA itself might induce apoptosis by stimulating caspase activity leading to DNA fragmentation. These speculations fit in with recent studies in cultured human breast cancer cell lines treated with a combination of ω-3 PUFAs [29].

On the other hand, SaO group exhibited higher levels of AA (Table 2) in the cell membranes and release of its metabolite 12-HETE respect to ChO (Fig. 1a) which were correlated to a higher number of metastasis (Fig. 2b). In agreement with these data results from other group showed that increased expression of 12-LOX in human prostate carcinoma cells stimulate tumor metastasis by enhancing cell adhesiveness, spreading, motility and invasiveness [30]. In addition, recent studies about the effects of ω-6 and -3 PUFA on human lung cancer cells demonstrated that ω-3 PUFA reduced the cells invasion potential probably by downregulating the cell adhesion/invasion- related molecules, as MMP-1, integrin-α2 and nm23-H4 [31]. The highest number of metastasis in CO group (Fig. 2b) could be associated with the lower ω-3 and -6 PUFAs amount of this diet respect to ChO and SaO (Table 1).

PUFAs *per se* and their metabolites provided cellular microenvironments that confers a switch on/off at the initiation and/or promotion stages of a wide range of cancers, with new implications for prevention,

Table 3
Number of apoptosis and mitosis recorded in tumour sections from hosts fed on the different dietary conditions.

Diets	M3 tumour	
	Apoptosis	Mitosis
CO	15.75±2.18 (a)	31.38±4.36 (b)
ChO	25.88±3.00 (b)	16.88±2.17 (a)
SaO	12.50±1.88 (a)	28.25±1.99 (b)

Values represent the mean±SEM of apoptotic and mitotic figures counted from 10 animals of each dietary condition (10 high-power fields). Different letters represent significant differences ($p<0.05$).

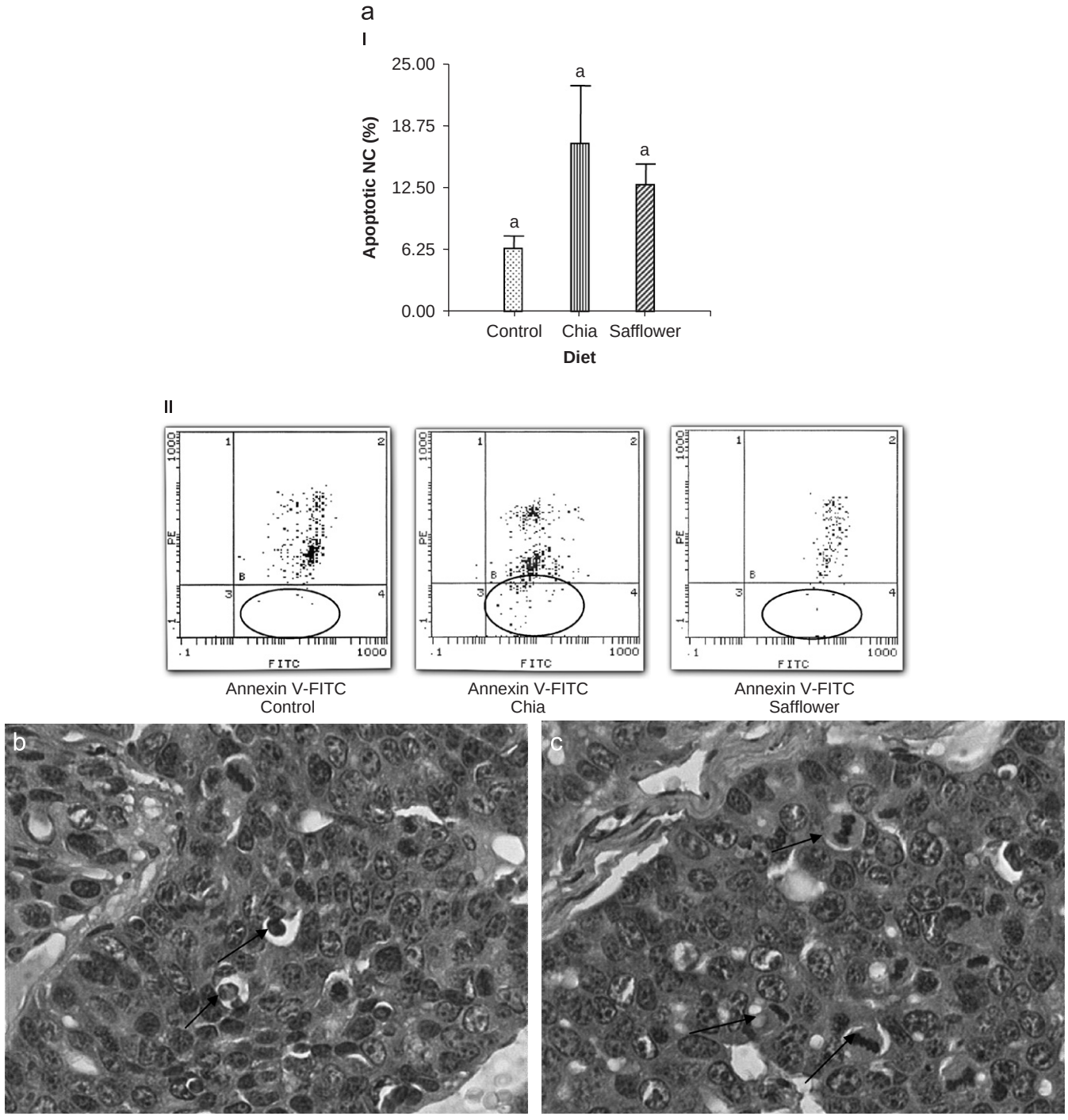

Fig. 3. (a-I) Apoptotic neoplastic cells in tumor cell suspension as determined by Flow Citometry using Annexin V/Propidium iodide double staining. Values represent the mean ± SD of three independent assays. Different letters represent significant differences ($p < 0.05$). (a-II) Flow citometry graphics show the apoptotic cells populations in tumor cells suspensions in the different diet conditions (circle areas). (b) Apoptotic (arrows) and (c) mitotic figures on neoplastic tumor tissue fixed in 10% neutral formalin, dehydrated and embedded in paraffin and stained with hematoxylin and eosin (arrows) (H&E) (400 ×).

monitoring, treatments and the possibility of readjustment of the immune profile. Nevertheless, it is still unclear how the PUFAs and eicosanoids exert their pro- or anti-tumorigenic actions. The mechanisms may be complex and probably involve multiple pathways. In this work, we show that a diet rich in ω-3 PUFAs (ChO) was able to inhibit mammary gland tumor growth and metastasis by enhancing membrane ω-3 EFAs, thus decreasing the AA-derived LOX and COX eicosanoids and increasing NC apoptosis.

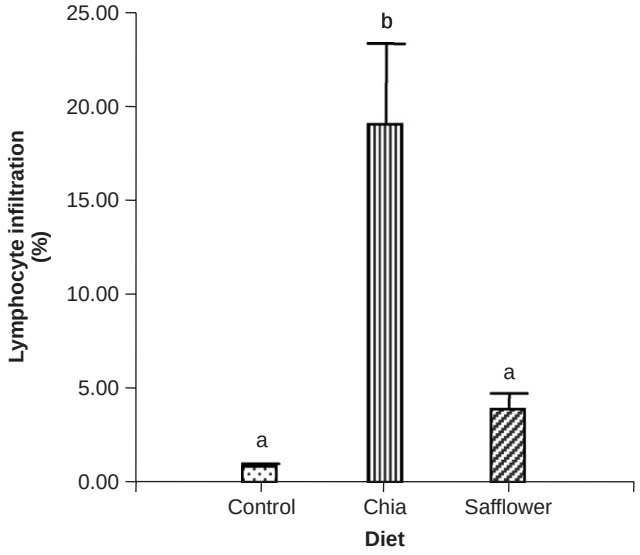

Fig. 4. Lymphocyte infiltration in NC M3 suspensions. NC suspensions of the primary tumor were analyzed by flow cytometry for the expression of CD3-FITC-conjugated as described in Materials and methods section. Different letters represent significant differences ($p < 0.05$).

Table 4
Percentage of positive immunolabeling for CD3-T-lymphocyte and NK-T cell infiltration in tumor tissue

Diet	Percentage of lymphocytes infiltration
Control	4.23 ± 0.15 (a)
Chia oil	35.5 ± 1.23 (b)
Safflower oil	23.06 ± 5.46 (c)

The value indicates immunolabeling and represents the mean ± SEM of gold-silver particles with positive labeling of CD3 in 15 fields evaluated in 10 tumor sections of each dietary treatment. Different letters represent significant differences ($p < 0.05$).

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References

[1] A.R. Eynard, Potential of essential fatty acids as natural therapeutic products for human tumors, Nutrition 19 (2003) 386–388.
[2] U. Das, Essential fatty acids: biochemistry, physiology and pathology, Biotechnol. J. 1 (2006) 420–439.
[3] L. Dajuel, H.H. Mündel, Safflower: *Carthamus tinctorius*, International Plant Genetic Resources Institute, 1996, pp: 1–83.
[4] S.E. Muñoz, M. Piegari, C. Guzmán, A.R. Eynard, Differential effects of dietary *Oenothera*, *Zizypus mistol* and Corn oils and essential fatty acid deficiency on the progression of a murine mammary gland adenocarcinoma, Nutrition 15 (1999) 208–212.
[5] R. Ayerza, W. Coates, M. Lauria, Chia seed (*Salvia hispanica* L.) as an ω-3 fatty acid source for broilers: influence on fatty acid composition, cholesterol and fat content of white and dark meats, growth performance, and sensory characteristics, Poult. Sci. 81 (2002) 826–837.
[6] H. Tapiero, G.N. Ba, P. Couvreur, K.D. Tew, Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies, Biomed. Pharmacot. 56 (2000) 215–222.
[7] W.G. Tong, X.Z. Ding, T.E. Adrian, The mechanisms of lipoxygenase inhibitor-induced apoptosis in human breast cancer cells, Biochem. Biophys. Res. Commun. 296 (2002) 942–948.
[8] X.Z. Ding, W.G. Tong, T.E. Adrian, 12-lipoxygenase metabolite 12(S)-HETE stimulates human pancreatic cancer cell proliferation via protein tyrosine phosphorylation and ERK activation, Int. J. Cancer 94 (2001) 630–636.
[9] D. Nie, S. Krishnamoorthy, R. Jin, K. Tang, Y. Chen, Y. Qiao, A. Zacharek, Y. Guo, J. Milanini, G. Pages, K.V. Honn, Mechanisms regulating tumor angiogenesis by 12-lipoxygenase in prostate cancer cells, J. Biol. Chem. 281 (2006) 18601–18609.
[10] J.P. Plastaras, F.P. Guengerich, D.W. Nebert, L.J. Marnett, Xenobiotic metabolizing cytochromes P450 convert prostaglandin endoperoxide to hydroxyheptadecatrienoic acid and mutagen, malondialdehyde, J. Biol. Chem. 275 (2000) 11784–11790.
[11] K. Nishimura, H. Tsumagari, A. Morioka, Y. Yamauchi, K. Miyashita, S. Lu, M. Jisaka, T. Nagaya, K. Yokota, Regulation of apoptosis through arachidonate cascade in mammalian cells, Appl. Biochem. Biotech. 102–103 (2002) 239–250.
[12] M. Rodriguez-Cruz, A.R. Tovar, M. del Prado, N. Torres, Molecular mechanisms of action and health benefits of polyunsaturated fatty acids, Rev. Invest. Clin. 57 (2005) 457–472.
[13] H. Harizi, N. Gualde, Pivotal role of PGE2 and IL-10 in the cross-regulation of dendritic cell-derived inflammatory mediators, Cell Mol. Immunol. 3 (2006) 271–277.
[14] A. Bonin, N.A. Khan, Regulation of calcium signaling by docosahexaenoic acid in human T-cells: implication of CRAC channels, J. Lipid. Res. 41 (2000) 277–284.
[15] S.S. Nair, J. Leitch, M.L. Garg, N-3 polyunsaturated fatty acid supplementation alters inositol phosphate metabolism and protein kinase C activity in adult porcine cardiac myocytes, J. Nutr. Biochem. 12 (2001) 7–13.
[16] R. Verlengia, R. Gorjao, C.C. Kanunfre, S. Bordin, T.M. de Lima, E.F. Martins, R. Curi, Comparative effects of eicosapentaenoic acid and docosahexaenoic acid on proliferation, cytokine production and pleiotropic gene expression in Jurkat cells, J. Nutr. Biochem. 15 (2004) 657–665.
[17] P. Purasiri, A. Mxkechnie, S.D. Heys, O. Eremi, Modulation in vitro of human natural cytotoxicity, lymphocyte proliferative response to mitogens and cytokine production by essential fatty acids, Immunology 92 (1997) 166–172.
[18] M.E. Pasqualini, M.A. Berra, R.O. Calderón, D.C. Cremonezzi, C. Giraudo, A.R. Eynard, Dietary lipids, eicosanoid release and apoptosis of cells of a murine lung alveolar carcinoma, Prost. Leuk Essent Fatty Acids 72 (2005) 235–240.
[19] E. Bal de Kier Joffé, I. Puricelli, M. del CC Vidal, E.S. de Lustig, Characterization of two murine mammary adenocarcinoma tumors with different metastatic ability, J. Exp. Clin. Cancer Res. 2 (1983) 51–160.
[20] R.O. Calderón, A.R. Eynard, Fatty acids specifically related to the anisotropic properties of plasma membrane for rat urothelium, Biochim. Biophys. Acta 1483 (2000) 174–184.
[21] M.E. Pasqualini, C.E. Mohn, J.P. Petiti, A.R. Eynard, COX and LOX eicosanoids modulate platelet activation and procoagulation induced by two murine cancer cells, Prost. Leuk Essent Fatty Acids 63 (2000) 377–383.

[22] M.E. Pasqualini, V.L. Heyd, P. Manzo, A.R. Eynard, Association between E-catherin expression by human colon, bladder and breast cancer cells and the 13-HODE: 15-HETE ratio, a possible role of their metastatic potential, Prost. Leuk Essent Fatty Acids 68 (2003) 9–16.

[23] I. Vermes, C. Haanen, C.P.M. Reutelingsperger, A novel assay for apoptosis: flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled annexin V, J. Immunol. Methods 180 (1995) 38–52.

[24] R.C. Duke, J.J. Cohen, S.A. Boehme, M.J. Lenardo, C.D. Surh, H. Kishimoto, J. Sprent, Morphological, biochemical and flow cytometric assays of apoptosis, in: J. Coligan, A.M. Kruisbeek, D. Margulies, E.M. Shevach, W. Strober (Eds.), Current Protocols in Immunology, Wiley, New York, NY, 1995, pp. 3.17.1–3.17.33.

[25] M. Brunotto, A.M. Zárate, A. Cismondi, M.C. Fermandez, R.I. Noher de Halac, Valuation of exfoliative cytology as prediction factor in oral mucosa lesions, Oral. Med. Pathol. 10 (2005) 92–102.

[26] A.J. Hulbert, N. Turner, L.H. Storlien, P.L. Else, Dietary fats and membrane function: implications for metabolism and disease, Biol. Rev. 80 (2005) 155–169.

[27] S.C. Larsson, M. Kumlin, M. Ingelman-Sunderg, A. Wolk, Dietary long-chain n-3 fatty acids for prevention of cancer: a review of potential mechanisms, Am. J. Clin. Nutr. 79 (2004) 935–945.

[28] M. Ikeguchi, K. Oi, Y. Hirooka, N. Kaibara, $CD8^+$ lymphocyte infiltration and apoptosis in hepatocellular carcinoma, Eur. J. Surg. Oncol. 30 (2004) 53–57.

[29] P.D. Schley, H.B. Jijon, L.E. Robinson, C.J. Field, Mechanisms of omega-3 fatty acid-induced growth inhibition in MDA-MB-231 human breast cancer cells, Breast Cancer Res. Treat. 92 (2005) 187–195.

[30] D. Nie, J. Nemeth, Y. Qiao, A. Zacharek, L. Li, K. Hanna, K. Tang, G.G. Hillman, M.L. Cher, D.J. Grignon, K.V. Honn, Increased metastatic potential in human prostate carcinoma cells by overexpression of arachidonate 12-lipoxygenase, Clin. Exp. Metast. 20 (2003) 657–663.

[31] S.H. Xia, J. Wang, J.X. Kang, Decreased n-6/n-3 fatty acid ratio reduces the invasive potential of human lung cancer cells by downregulating of cell adhesion/invasion-related genes, Carcinogenesis 26 (2005) 779–784.