Clinical Nutrition 29 (2010) 663–673

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original Article

Extra-virgin olive oil-enriched diet modulates DSS-colitis-associated colon carcinogenesis in mice

S. Sánchez-Fidalgo, I. Villegas [a], A. Cárdeno, E. Talero, M. Sánchez-Hidalgo, V. Motilva, C. Alarcón de la Lastra*

Department of Pharmacology, Faculty of Pharmacy, University of Seville, Profesor García González Street, 2. 41012-Seville, Spain

ARTICLE INFO

Article history:
Received 16 June 2009
Accepted 3 March 2010

Keywords:
Extra virgin olive oil
Ulcerative colitis
Colorectal cancer
β-catenin
Inflammation parameters

SUMMARY

Background & aims: Patients with inflammatory bowel disease (IBD) are at increased risk for developing ulcerative colitis (UC)-associated colorectal cancer (CRC). Several studies have shown that extra virgin olive oil (EVOO) might possess anti-inflammatory, antiproliferative and antiapoptotic effects. We have evaluated EVOO diet effects on the severity of repeated colitis-associated CRC.
Methods: Six-week-old C57BL/6 mice were randomized into two dietary groups: sunflower oil (SFO) and EVOO diets, both at 10%. Mice were exposed to 15 cycles of 0.7% dextran sodium sulphate (DSS) for 1 week followed by distilled water for 10 days. After, the rats were sacrificed and colonic damage was both histologically and biochemically assessed.
Results: Disease activity index (DAI) was significantly higher on SFO vs. EVOO diet at the end of the experimental period. EVOO-fed mice showed less incidence and multiplicity of tumors than in those SFO-fed mice. β-catenin immunostaining was limited to cell membranes in control groups, whereas translocation from the cell membrane to the cytoplasm and/or nucleus was showed in DSS-treated groups and its expression was higher in SFO-fed animals. Cytokine production was significantly enhanced in SFO-fed mice, while this increase was not significant in EVOO-fed mice. Conversely, cyclooxigenase-2 (COX-2) and inducible nitric oxidase synthase (iNOS) expression were significantly lower in the animal group fed with EVOO than in the SFO group.
Conclusions: These results confirm that EVOO diet has protective/preventive effect in the UC-associated CRC. This beneficial effect was correlated with a better DAI, a minor number of dysplastic lesions, a lower β-catenin immunoreactivity, a proinflammatory cytokine levels reduction, a non modification of p53 expression and, COX-2 and iNOS reduction in the colonic tissue.

1. Introduction

Colorectal cancer (CRC) is one of the most common gastrointestinal tract malignancies. It is the third cause of cancer-related death in the Western world and affects about one million people every year throughout the world with a high mortality rate. CRC develops from a dysplastic precursor lesion, sporadically, in the context of high-risk hereditary conditions, or in the background of chronic inflammation. In effect, patients with inflammatory bowel disease (IBD) are among the highest risk groups for developing CRC. The risk of colorectal malignancies in colitis patients increases with the extent and duration of the disease.[1,2]

At present no genetic basis is able to explain the predisposition to CRC in those patients. Nevertheless, the main genomic instability that contribute to colon carcinogenesis is chromosomal instability, which results in damage of genetic material and consequently, loss of function of key tumor suppressor genes such as p53, which is expressed or expresses proteins that regulate growth and apoptosis. β-catenin is also an important cancer target, which plays a role in both cell adhesion and intracellular signalling. It is known to be a key component of the cadherin mediated-cell adhesion system and an important molecule in Wnt-APC signal transduction system.[3,4]

Several lines of evidence implicate chronic inflammation as a key predisposing factor to CRC in IBD. The inducible isoforms of cyclooxygenase (COX-2) and nitric oxidase synthase (iNOS) are the main enzymes involved. COX-2 is expressed after proinflammatory cytokines stimulation, growth factors, reactive oxygen species and tumor promoters. In several types of cancer, particularly, gastric

* Corresponding author. Tel.: +34 9 5 4556722; fax: +34 9 5 4556074.
E-mail address: calarcon@us.es (C. Alarcón de la Lastra).
[a] This author has to be considerate equal as the first author.

0261-5614/$ – see front matter © 2010 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2010.03.003

carcinoma and colon adenoma, COX-2 is upregulated generating protumorigenic eicosanoids, in particular, prostaglandins that can promote cell growth, angiogenesis and suppression of immunity. iNOS produces large amounts of nitric oxide (NO) implicated in initiation, promotion and progression of tumors. Besides, NO has been shown to stimulate COX-2 activity and increase p53 mutations in chronic inflammation, contributing to clonal cellular expansion and genomic instability.[5–7]

It is now becoming clear that the large amount of cytokines and growth factors released during inflammation by immune and non immune cells may influence the carcinogenesis process, in which tumors infiltrating inflammatory cells produce several cytokines. It has been proposed that pro-inflammatory cytokines including tumor necrosis factor (TNF)- α, interleukins (IL)-1, 6 and others, and interferon (INF)-γ contribute to carcinogenesis by influencing the survival, growth, mutation, proliferation, differentiation and movement of tumor and stromal cells and by regulating angiogenesis.[8]

Experimental studies have found a role of dietary lipids on cancer, particularly in colon tumor development. For instance, it was demonstrated that high fat diets rich in n-6 polyunsaturated fatty acid and saturated fatty acids promotes chemically carcinogenesis induction, while high fat diets rich in n-3 fatty acids do not.[9,10] In addition, epidemiological data have confirmed a lower colon cancer incidence in Mediterranean countries, where olive oil (rich in oleic acid, an n-9 fatty acid) is consumed, despite its caloric content.[10–12] However, there are few experimental studies addressing the protective activity of olive oil on colon cancer[13,14] and inconsistent results have also been obtained, including non-promoting,[9] weak-promoting, and even promoting effects on tumor growth.[15]

Extra virgin olive oil (EVOO), the first-pressed olive oil, contains an abundance of squalene and phenolic antioxidants including simple phenols (hydroxytyrosol, tyrosol), aldehydic secoiridoids, flavonoids and lignans (acetoxypinoresinol, pinoresinol). Recently, these components have been shown to inhibit several stages in colon carcinogenesis *in vitro*.[16,17]

In rodents, oral dextran sulphate sodium (DSS)-administration in the drinking water has been found to induce colonic inflammation with clinical and histological similarity to human UC. The DSS colitis model shows the "*inflammation-dysplasia carcinoma-sequence*" of CRC development, as well as the interplay between causative factors and background genetics. In our study, we investigated the effects of EVOO and sunflower oil (SFO) diets on the severity of chronic inflammation induced by DSS as well as the development of colitis-associated CRC by macro and microscopic techniques. Since the translocation of β-catenin from the cell membrane to the cytoplasm or nucleus is an important early event in colorectal carcinogenesis, we evaluated β-catenin involvement in our animal model. As it was mentioned above, several cytokines released during inflammation by immune and non immune cells may influence the development and growth of colitis associated CRC, thus changes in TNF-α, IL-6 and INF-γ colonic levels were investigated. Finally, p53, COX-2, prostaglandin E synthase (PGES)-1 and iNOS expression were also analyzed in the colonic mucosa.

2. Material and methods

2.1. Experimental animals

A total of 84 female C57BL/6 mice (6 weeks of age) were obtained from Charles River (Barcelona, Spain). After weaning and during the experiments, mice were placed five or six in cages and maintained in air-conditioned quarters with a room temperature of 24–25 °C, constant humidity, and an alternating 12-h light/dark cycle. Mice were randomized into two dietary groups: one standard diet elaborated with SFO and other elaborated with EVOO as lipids sources (Table 1). The components of the diet (an AIN76A diet modified in the fat component) were supplied by Harlan Iberica SA (Barcelona, Spain). The standard AIN76A diet contains 490 mg iron/kg diet, but since previous studies have confirmed that iron may increase disease activity in colitis and this is associated with oxidative stress and neutrophilic infiltration,[18–20] both diets were supplemented with two times the amount of iron in the modified control AIN76A diet (900 mg iron/kg diet).[20] The diets were prepared every week by mixing the respective compounds. The animals were fed with the corresponding diet during two weeks previous to the colitis induction and during the experiment. Body weights, food and drink consumptions were monitored once per week throughout the experiment (data not shown).

Experiments followed a protocol approved by the Animal Ethics Committee of the University of Seville and all experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

2.2. Induction of colitis

Chronic ulcerative colitis was induced by the repeated administration of DSS (0.7% w/v; MW: ≈40,000; catalogue number DB001, obtained from TdB Consultancy AB, Uppsala, Sweden), according to the method described by Yeo et al.[21] Each dietary group was divided into other two groups: one group of 20 mice was exposed to 15 cycles of DSS (DSS group) and a second group of 12 mice was administered ordinary tap water throughout the experiment (sham group). Each cycle consists of 7 days of 0.7% DSS w/v in the drinking fluid, followed by 10 days of ordinary tap water (Fig. 1). Animals were sacrificed to the end of treatment (37 weeks) by an overdose of i.p. chloral hydrate.

Table 1
Composition of experimental diets.

Ingredients	g/100 g of diet (%)
Casein	20
DL-Methionine	0.3
Cornstarch	15
Sucrose	44.91
Cellulose	5
SFO[a] or EVOO[b]	10.0
Mineral mix[c]	3.5
Vitamin mix[d]	1
Choline chloride	0.2
Fe (sulphate)	9×10^{-2}

Diet was formulated on the basis of the American Institute of Nutrition (AIN) standard reference diet with the modification of various sources of carbohydrate.

[a] SFO, sunflower oil from Ibarra SL.

[b] EVOO, extra virgin olive oil from Picual Virgin, Jaen, Spain. OLEOESTEPA (Soc Coopertiva Andaluza).

[c] Mineral mix provided the following (g/kg diet): calcium carbonate, 35.7; monopotassium phosphate, 25.0; sodium chloride, 7.4; potassium sulfate, 4.66; potassium citrate monohydrate, 2.8; magnesium oxide, 2.4; ferric citrate, 0.606; zinc carbonate, 0.165; manganese carbonate, 0.063; copper carbonate, 0.03; potassium iodate, 0.001; sodium selenate, anhydrous, 0.001025; ammonium molybdate·$4H_2O$, 0.000795; sodium metasilicate·$9H_2O$, 0.145; chromium potassium sulfate·$12H_2O$, 0.0275; boric acid, 0.00815; sodium fluoride, 0.00635; nickel carbonate, 0.00318; lithium chloride, 0.00174; ammonium vanadate.

[d] Vitamin mix provided the following (g/kg diet): nicotinic acid, 30 mg; D-calcium pantothenate, 16 mg; pyridoxine HCL, 7 mg; thiamine HCL, 6 mg; riboflavin, 6 mg; folic acid, 2 mg; D-biotin,0.2 mg; vitamin B_{12}, 25 mg; alpha tocopherol powder (250 U/g), 300 mg; vitamin A polmitate (250,000 U/mg), 16 mg; vitamin D_3 (400,000 U/g), 2.5 mg; phylloquinone, 0.75 mg.

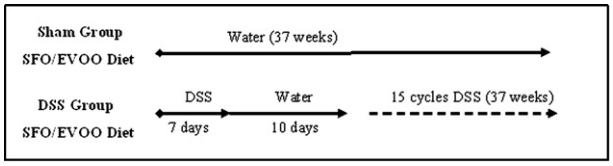

Fig. 1. Overview of experimental protocol in the experimental animal model of dextran sodium sulphate (DSS)-induced ulcerative colitis model in mice. Chronic ulcerative colitis was induced by the repeated administration of 15 cycles of DSS. Each cycle consisted of 7 days of 0.7% DSS dissolved in the drinking water *ad libitum*, followed by 10 days of ordinary tap water. Sham group mice were given ordinary tap water and they were sacrificed at the same time that DSS group.

2.3. Assessment of DSS-induced colitis

The clinical activity of colitis was evaluated during experimentation in order to determinate the disease activity index (DAI) as described by Gommeaux et al.[22] The presence of diarrhea, rectal bleeding, and weight loss were registered at the first, middle and end of DSS treatment as well as the phase of drinking water (for each cycle), and separately graded on a scale (Table 2); the average of the three values constituted the DAI.

2.4. Macroscopic and histopathological evaluation

After killing the animals, colons were removed, slightly cleaned in physiological saline to remove fecal residues, weighed and measured. The severity of macroscopic damage was evaluated by an independent observer who was blinded to the treatment. Weight/length ratio of the mice colon, as an indirect marker of inflammation was also determined. Photographs taken from colon samples were digitised using Kodak D290 Zoom camera (Eastman Kodak Co., Rochester, NY, USA). Pieces of colon were collected and frozen in liquid nitrogen to measure biochemical parameters.

Tissue samples of the region with polypoid or flat elevated lesion were excised out of every segment, fixed in 4% buffered formaldehyde, dehydrated by increasing concentrations of ethanol, and embedded in paraffin. The paraffin sections were stained with haematoxylin and eosin for histological evaluation of colonic damage. UC-associated colonic neoplasms were analyzed microscopically and diagnosed as low-grade or high-grade of dysplasia and adenocarcinoma, according to established criteria.[23] Tumor incidence was calculated as the number of tumor-bearing mice divided by the total number of mice expressed in percentage. Histopathological evaluation was determined by a pathologist who was unaware of the experimental protocol.

2.5. Immunohistochemical study of β-catenin

β-catenin staining was carried out using a streptavidin-Biotin-Peroxidase method. 5 μm thick tissue sections were dried in an oven at 55 °C for 1 h and then deparaffinized in two changes of xylene and hydrated from graded alcohols to water. A pressure-cooking antigen retrieval system was used in which the slides were boiled in citrate buffer (pH 6.0) at maximum pressure for 2 min, followed by cooling at room temperature for 20 min. The endogenous peroxidase activity was inhibited and then the sections were incubated in normal horse serum (Vectastain® Kit, Vector Laboratories, Inc., Burlingame, CA) for 20 min to reduce non-specific staining, and successively incubated with monoclonal mouse anti-β-catenin antibody (BD Transduction Laboratories TM) at dilution 1:1500 overnight at 4 °C. Subsequently, slides were treated with anti-mouse IgG antibody (Vectastain® Kit, Vector Laboratories, Inc., Burlingame, CA) for 30 min and incubated with the streptavidin-peroxidase complex (Vectastain® Kit, Vector Laborartories, Inc., Burlingame, CA) for 30 min, both steps at room temperature. The enzymatic study was developed using 3,3′–diaminobenzidine (DAB) and the sections were counterstained with haematoxylin. Negative control sections were treated as the same way omitting the primary antibody. Intensity and localization of immunoreactivity was examined on all sections using a microscope (Olympus BX61, Olympus Optical Co., Ltd., Tokyo, Japan). The pathologist evaluated β-catenin staining as percentage of cells in each group showing cell membrane or cytoplasmic and/or nucleus expression.[24]

2.6. Colonic cytokine levels

TNF-α, IL-6 and IFN-γ concentrations in colonic tissue were measured by quantitative enzyme immunoassay kits according to the manufacturer' s protocol (Diaclone, Besançon, France). Briefly, distal colon samples were weighed and homogenized, after thawing, in 0.3 ml phosphate buffer saline solution (PBS, pH = 7.2) 1% bovine serum albumin (BSA) at °C, and were centrifuged at 12,000 *g* for 10 min. The concentrations of cytokines were determined by duplicated. TNF-α, IL-6 and IFN-γ values were expressed as pg/mg tissue.

2.7. Isolation of cytoplasmic proteins and Western blot analysis

Frozen colonic tissues were weighed and homogenized in ice-cold buffer (50 mM Tris–HCl, pH 7.5, 8 mM MgCl2, 5 mM ethylene glycol bis (2-aminoethyl ether)-*N*,*N*,*N*,*N*,-tetraacetic acid, 0.5 mM EDTA, 0.01 mg/ml leupeptin, 0.01 mg/ml pepstatin, 0.01 mg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride and 250 mM NaCl). Homogenates were centrifuged (12,000 *g*, 15 min, °C) and the supernatants were collected and stored at −80 °C. Protein concentration of the homogenate was determined following Bradford colorimetric method.[25] Aliquots of supernatants containing equal amounts of protein (50 mg) were separated on 10% acrylamide gel by sodium dodecyl sulfatepolyacryamide gel electrophoresis. In the next step, the proteins were electrophoretically transferred onto a nitrocellulose membrane and incubated with specific primary antibodies: mouse anti-p53 (Ab-1) (Calbiochem, Darmstadt, Germany) at a dilution of 1:20, rabbit anti-COX-2 and rabbit anti-iNOS (Cayman Chemical, Michigan, USA) at a dilution of 1:3000 and rabbit anti-PGES-1 (Cayman Chemical, Michigan, USA) at a dilution of 1:500. Each filter was washed three times for 15 min

Table 2
Scoring of disease activity index (DAI) in DSS-induced colitis.

Score	Bleeding	Weight loss (% of initial wt)	Stool consistency
0	None	<1	Normal stools
1	Small spots of blood in stool; dry anal region	1–4.99	Soft pellets not adhering to the anus
2	Large spots of blood in stool; blood appears through anal orifice	5–10	Very soft pellets adhering to the anus
3	Deep red stool; blood spreads largely around the anus	>10	Liquid stool on long streams; wet anus

Criteria from work of Gommeaux et al.[23]

and incubated with the secondary horseradish peroxidase linked anti-rabbit for COX-2, iNOS and PGES-1 (Pierce Chemical Company, Rockford, IL, USA) or anti-mouse immunoglobulin G for p53 antibodies (Santa Cruz Biotechnology, CA). To prove equal loading, the blots were analyzed for β-actin expression using an anti-β-actin antibody (Sigma–Aldrich, MO, USA). Immunodetection was performed using enhanced chemiluminescence light-detecting kit (SuperSignal1 West Pico Chemiluminescent Substrate, Pierce, IL, USA). Densitometric data were studied following normalization to the control (house-keeping gene). The signals were analyzed and quantified by a Scientific Imaging Systems (KODAK 1D, Image Analysis Software).

2.8. Statistical analysis

All values in the figures and text are expressed as arithmetic means ± standard error (S.E.M.). Data were evaluated with Graph Pad Prism® Version 2.01 software. Comparison were done using one-way analysis of variance (ANOVA) followed by Tukey–Kramer or Dunnett's test when appropriate. P values of <0.05 were considered statistically significant. In the experiment involving histology, immunohistochemistry, or Western blot, the figures shown are representative of at least six experiments performed on different days.

3. Results

DSS administration in drinking water to the animals during the 15 cycles reproduced a chronic colitis in the two dietary groups assayed, SFO and EVOO, with evident clinic signs of diarrhea and haemorrhage rectal. The evolution of body weight and consumption of food and drink during this period did not show significant differences (data not show). On the contrary, a significant increase on weight/length of the mice colon was observed in the DSS-treated groups and fed with SFO vs. sham group ($P < 0.001$), while this difference was not statistically significant in EVOO-fed animals (Fig. 2).

Bleeding examinations were positive and accompanied by loose stools ($P < 0.01$) from cycle number 5 in SFO-fed animals group. On the contrary, visible fecal blood and diarrhea ($P < 0.05$ vs. SFO diet) were observed from cycle 12 in DSS-EVOO animals. However, DAI showed fluctuating data from one week to the other between the two groups in the course of 15 cycles of DSS treatment, although it was significantly higher ($P < 0.05$) on SFO group vs. EVOO diet at the end of the experimental period (Fig. 3).

The number, size, and location of detectable tumors were macroscopically examined. None of the animal controls, SFO or EVOO diets, which were not subject to the cycles of DSS, showed inflammation and/or injury in the colon. A total of 6 out of 20 (30%) mice that received 15 cycles of DSS and were fed with SFO diet, developed nodular, polypoid or caterpillar-like tumors[26] (Fig. 4B). These lesions ranged from 2 to 6 mm in size and appeared in the distal/rectal portion of the large intestine mainly. In contrast, none of the animals fed with EVOO diet had any macroscopic lesions.

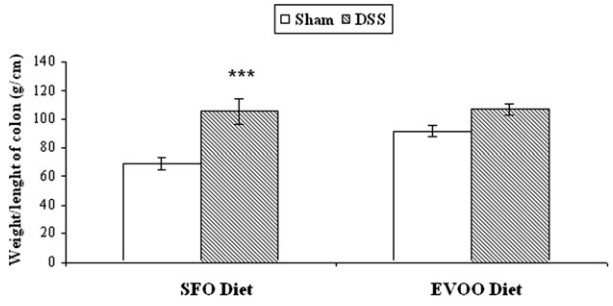

Fig. 2. Effect of a functional diet supplemented with sunflower oil (SFO diet) or extra virgin olive oil (EVOO diet) on weight/length of the colon in the experimental animal model of dextran sodium sulphate (DSS)-induced colon cancer. Data are expressed as the means ± S.E.M. ***$P < 0.001$ vs. Sham group.

Histological study of the colons isolated from the DSS-treated mice exhibited mild to moderately severe inflammation, characterized by crypt abscess and inflammatory lymphocytic infiltration with glandular destruction and regenerative atypia without differences between the groups fed with the experimental diets. However, UC-associated colonic neoplasms were analyzed and diagnosed as shown in Table 3. 55% of the 20 mice that received 15 cycles of DSS and were fed with SFO diet, developed adenocarcinomas (Fig. 5A) and 85% exhibited high-grade of dysplasia (Fig. 5C). Nevertheless, the number of adenocarcinomas and high-grade dysplasia were reduced to 22.2% and 55.6% in EVOO-fed animals (Fig. 5B and D, respectively). 100% of the animals treated with 15 cycles of DSS, independently of the diet used, experimented low-grade of dysplasia (Figs. 5E and 4F).

Since translocation of β-catenin from the cell membrane to the cytoplasm or nucleus is an important early event in human colorectal carcinogenesis, we evaluated the role of β-catenin in our animal model. Our findings show different β-catenin staining pattern between groups. Weak positivity of β-catenin immunostaining was limited to cell membrane of epithelial cells in sham groups from both diets (Table 3) (Fig. 6 A), whereas translocation from the membrane to the cytoplasm and into the nucleus was observed in the tumor tissue from all DSS-treated mice. However, cytoplasm/nuclear staining of β-catenin was higher in colonic cells from DSS animals fed with SFO diet ($P < 0.001$) (Fig. 6B) than in samples from DSS-EVOO animals. Moreover, the intensity of β-catenin expression from EVOO-fed animals was weaker (Fig. 6C) (Table 4).

In order to investigate the role of TNF-α, IL-6 and IFN-γ in colorectal carcinogenesis associated with chronic colitis, we analyzed the production of selected cytokines in colonic tissues homogenates after 15 cycles with DSS 0.7% (v/v). As shown in Fig. 7, colonic injury by DSS administration was characterized by an increase of the Th-1 cytokines levels, which was positively correlated with the inflammation and severity of the DAI. However, this enhancement only reached levels of statistical significance in the animals fed with the SFO diet ($P < 0.05$ vs sham group).

The expressions of p53, COX-2, iNOS and PGES-1 proteins were determined by Western blotting analysis in total proteins. p53 is a potent inhibitor of cell growth and tumor growth, and its inactivation, mutation or both is considered a prerequisite for tumor formation. As shown in Fig. 8A, p53 protein was diminished significantly by DSS treatment in the animals fed with the standard diet SFO ($P < 0.05$ vs. sham and vs. group fed with EVOO diet); however EVOO-fed mice group did not modified the expression of this protein, which remained at the same levels as its sham group.

We examined the expression of COX-2 and iNOS in colonic mucosa because accumulating evidences indicate an important role of both proteins in colon growth and progression (Fig. 8B and D, respectively). Our results show that COX-2 and iNOS expression significantly augmented after 15 cycles of DSS in SFO-fed group ($P < 0.05$ vs. sham and vs. group fed with EVOO diet). On the contrary, these proteins did not show significant changes between DSS-treated group fed with EVOO diet and its respective sham group.. In relation to PGES-1 expression, no significant differences between healthy and diseased mice were detected (Fig. 8C).

4. Discussion

Numerous feeding studies with different types of dietary fats, differing in fatty acid composition, have been carried out in animals

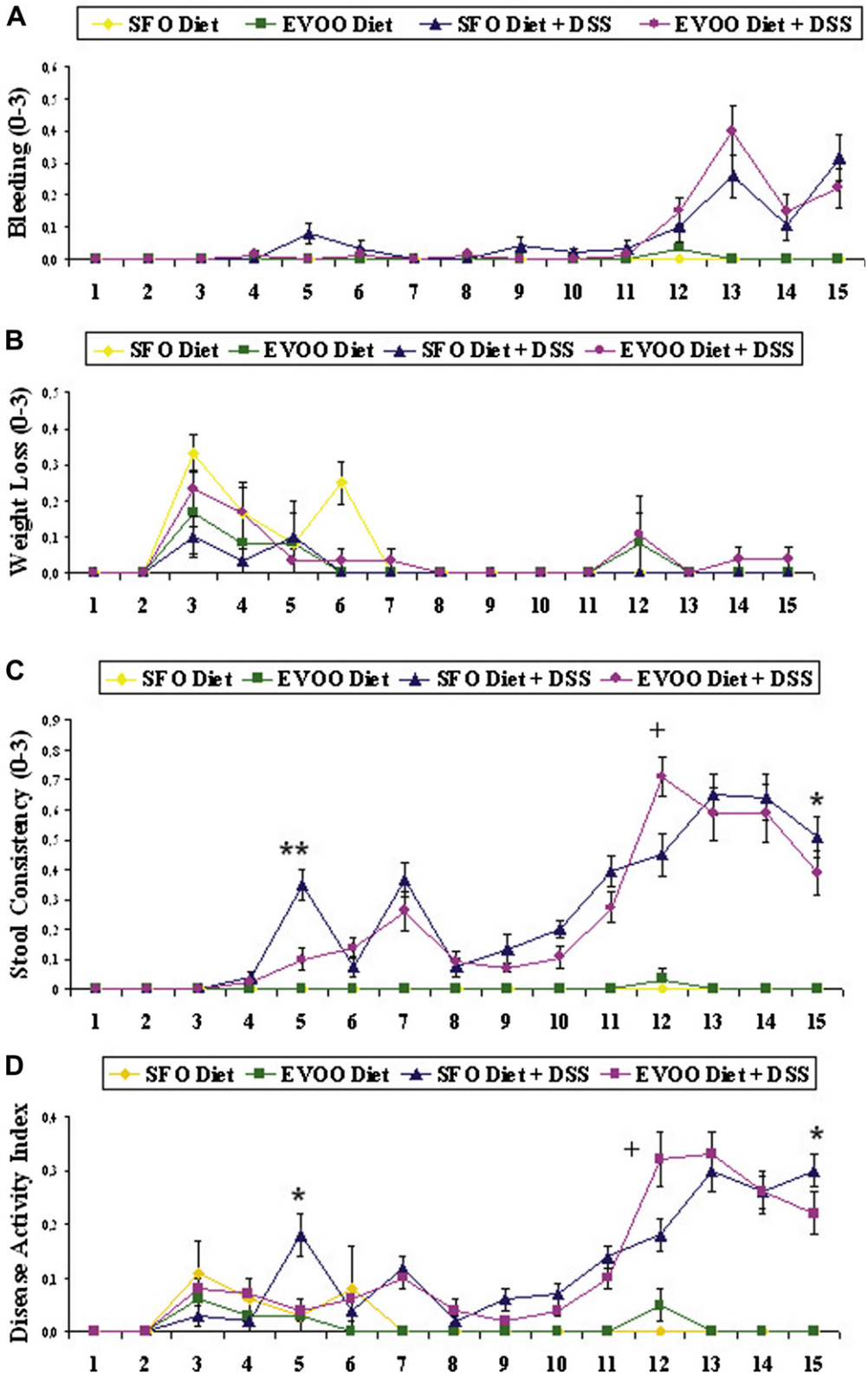

Fig. 3. Effect of a functional diet supplemented with sunflower oil (SFO diet) or extra virgin olive oil (EVOO diet) on clinical findings in the experimental animal model of dextran sodium sulphate (DSS)-induced colon cancer. Score of bleeding (A), weight loss (B) and stool consistency (C) in the course of 15 cycles. Disease activity index (D) combining the scores of bleeding, weight loss and stool consistency divided by 3. Data are expressed as the means ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. EVOO diet-DSS animals; $+P < 0.05$ vs. SFO diet-DSS animals.

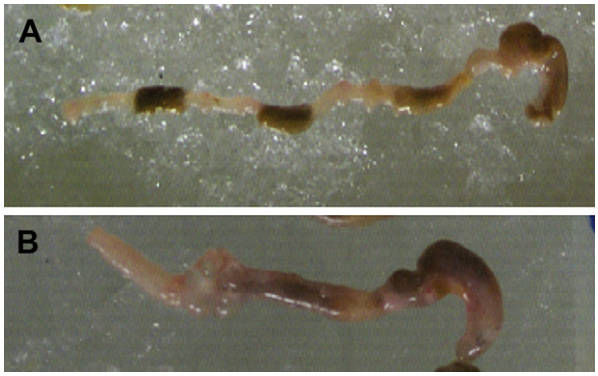

Fig. 4. Macroscopic view of the colon of an animal sham (A) and colonic tumors in animal treated with 15 cycles of 0.7% DSS and fed with functional diet supplemented with sunflower oil (B).

treated with different carcinogens evaluating the preventive or inhibitory effects on experimental carcinogenesis.[9,14,27,28] Some data have clearly demonstrated that the influence of dietary fats on cancer depends on the quantity and the type of lipids but epidemiologic evidence based on case-control, although promising, is quantitatively limited and qualitatively suboptimal.[29,30] Moreover, controversies still exist regarding to the influence of dietary fat on this pathology. Specifically, the effects of diets rich in olive oil on carcinogenesis and the molecular mechanisms involved are not conclusive, although high olive oil diets seem to have protective effects.[14,31] Despite this, olive oil appears as an example of a functional food with a variety range of constituents that could contribute to its overall chemopreventive/beneficial effects.

The focus of this study has been to demonstrate the potential protective role of dietary fat elaborated with EVOO in the development of dysplasia and/or cancer in a DSS model of mouse colitis. The results of the present work clearly indicate that a diet rich in EVOO effectively has a preventive role in the development of dysplasia and/or cancer in this experimental model. This effect was well correlated with a better DAI index, a minor number of dysplastic lesions, a lower immunoreactivity of β-catenin, a reduction of proinflammatory cytokines levels, a no modification of p53 expression and, a reduction of COX-2 and iNOS protein expression in the colonic tissue.

The pathogenesis of IBD-associated CRC is widely believed to involve a step-wise progression from inflamed and hyperplastic epithelia through flat dysplasia to finally adenocarcinoma, but the mechanism is not yet clear.[23,26] This model of UC-associated carcinogenesis where animals were subjected to long term, repeated administration of DSS with an iron supplemented diet, has demonstrated that the clinical and histopathological features are similar to those of chronic human UC and UC-associated colorectal carcinoma development. As well, CRC development in this model show the typical "dysplasia-carcinoma sequence" of UC-associated CRC, in contrast to the "adenoma-carcinoma sequence" observed in models of sporadic colon cancer.[21]

Repeated colitis with 15 cycles of DSS (0.7% w/v) administration induced dysplasia and also carcinoma in animals fed with SFO diet. On the contrary, our results show that the incidence of colonic neoplasm was diminished after EVOO diet, demonstrating that this fat seems to have protective/preventive effect. Histopathological study showed structural and cellular atypia, with alterations in tubular arrangement, allowing a diagnosis of low-grade, high-grade dysplasia or carcinoma according to the established criteria. Some previous experiments using olive oil diet have also obtained good results on the colon carcinogenesis promotion and on aberrant crypt foci (ACF) induction in models of carcinogenesis by azoxymethane (AOM).[13,14,29,32] In contrast, other work shows that olive oil does not affect ACF induction,[9] while other reports that olive oil enhances colon carcinogenesis.[15] Moreover, a recent work observed that olive oil diet does not affect 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis when compared with a corn oil-based diet.[33] However, the multiplicity of colonic tumors with this diet was quite low, which gives a protective action to the olive oil diet. These different results might be explained by the different protocols of damage induction and/or by the type and level of fat used in the diet. On the other hand, it is important to observe that our experimental model is a process of chronic inflammation, based on repeated cycles of DSS, which ultimately results in CRC. Therefore, the results obtained in the present study are very interesting since show a preventive effect in this progression of damage over time.

Disrupted regulation of the Wnt signalling pathway plays a central role in the aetiology of colon carcinogenesis.[34,35] The key molecule in this pathway is a multiprotein scaffold consisting of β-catenin, glycogen synthase kinase (GSK)-3β, and adenomatous polyposis coli (APC), and it is commonly accepted that the crucial tumor suppressor role of APC lies in its ability to destabilize cytoplasmic free -β-catenin.[36] In colorectal tumors, aberrant activation of β-catenin serves to block normal differentiation programme cells and maintains cells in a progenitor-like state.[37] Its translocation is shown frequently in both colorectal neoplasia and UC associated neoplasia in different animal models.[38–40] In the present study, β-catenin expression was stronger in those animals fed with SFO diet than in the animals fed with diet supplemented with EVOO. Moreover, translocation of this protein from membrane to the cytoplasm and into the nucleus was specially observed in the tumor tissue of DSS-treated mice fed with SFO diet.

Carcinogen treatment induced an appreciable increase in proinflammatory cytokines colonic production in mice fed the SFO diet, but not in those fed with EVOO diet. TNF-α has been proposed to be a potent mutagen based on its capacity to induce ROS generation and subsequent genetic instability in various types of cells.[41] In previous studies, TNF-α has been involved in the development of some epithelial malignancies.[42] Indeed pharmacologic blockade of TNF-α with monoclonal antibodies has demonstrated great efficacy in the treatment of colorectal carcinogenesis associated with chronic colitis.[8,43] The progression to invasive cancer requires the induction of tumor vasculature and TNF-α possesses angiogenic activities and may also enhance tumor angiogenesis by inducing the infiltration of COX-2 expressing macrophages and neutrophils.[43] Elevated TNF-α expression in colon is required for increased iNOS expression.[44] Hence, a reduction of TNF-α production may decrease tumor neovasculatization and thus the progression of colon carcinogenesis. IL-6 is produced by inflammatory and stromal cells within the tumor microenviroment that

Table 3
Incidence of ulcerative colitis-associated colonic neoplasm in animal treated with 15 cycles of DSS (0.7% v/v) and fed with different diets: sunflower diet (SFO diet) and extra virgin olive oil (EVOO diet).

Diet	Low-grade dysplasia	High-grade dysplasia	Adeno-carcinoma	Tumor incidence	Tumor multiplicity
SFO	100%	85%	55%	30%	1
EVOO	100%	55.55%	22.2%	0	0

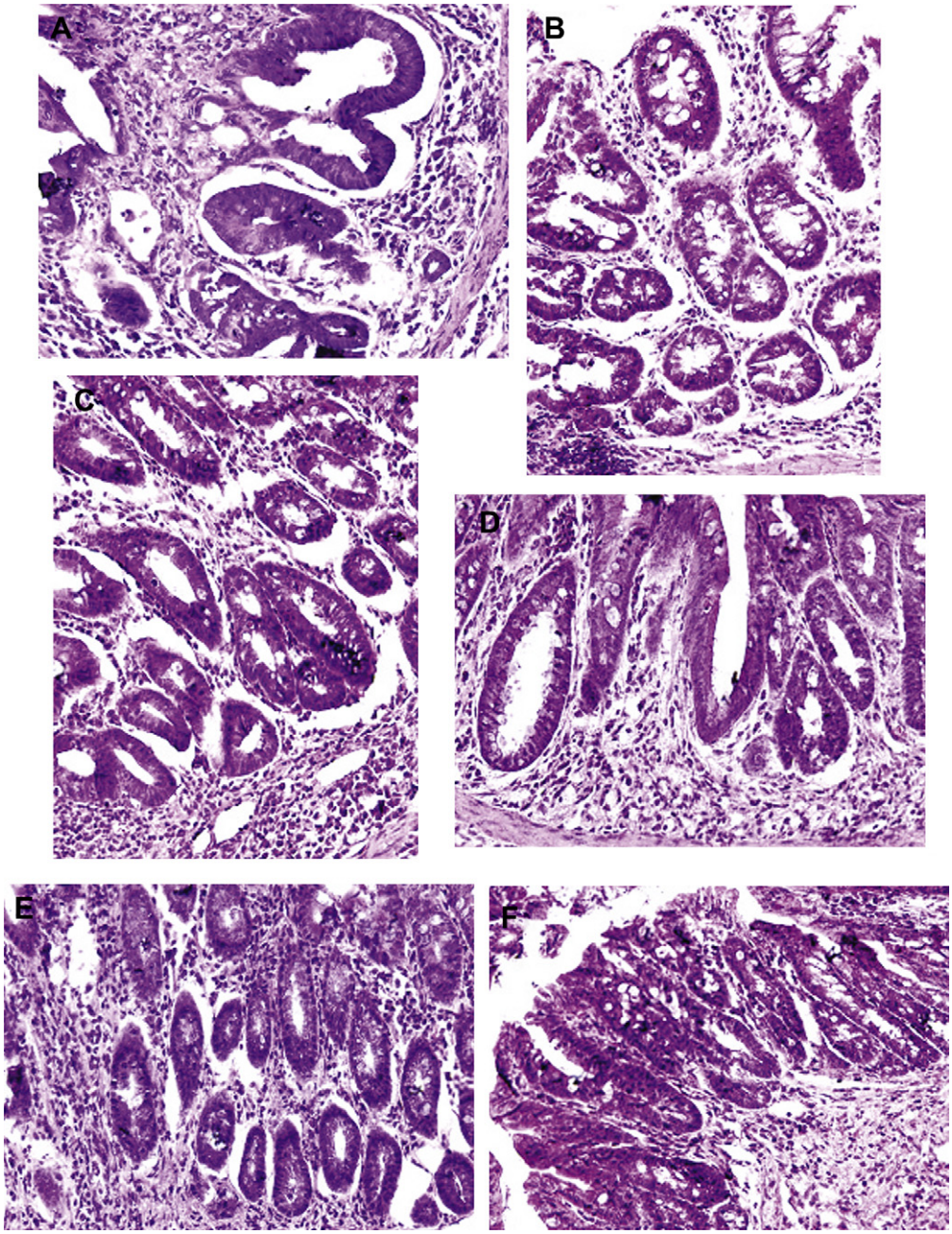

Fig. 5. Histopathology of colonic lesions in mice fed with a functional diet supplemented with sunflower oil (A, C, E) or with extra virgin olive oil (B, D, F) and treated with 15 cycles of 0.7% DSS (v/v). Adenocarcinoma (A and B), high-grade of dysplasia (C and D) and low-grade of dysplasia (E and F). Haematoxilin & eosin stain. Original magnification 200×.

binds to a common signalling receptor, gp130 leading to STAT3.[45] Our results, in agreement with those of Greten et al.,[46] showed elevated IL-6 levels in colitis-associated cancer. Additionally, exogenous administration of IL-6 to mice during tumor initiation resulted in an increase in tumor burden and multiplicity.[47] Similar data have been reported by Naugler et al.[48] in liver cancer.

In the present report, we demonstrate for the first time a strong positive correlation between colitis-associated cancer and INF-γ production. IFN-γ is a principal proinflammatory cytokine that modulates both innate and adaptive immunities and plays an important role in the pathogenesis of inflammatory diseases, including inflammatory bowel diseases.[49] IFN-γ signalling involves the types I and II IFN receptors, the receptor-associated Janus protein tyrosine kinases (JAKs), the signal transducers and activators of transcription (STATs), and members of the interferon regulatory factor (IRF) family of transcription factors. Therefore, reducing INF-γ production may contribute to retard the initiation and progression of colitis-associated cancer.

Inducible proteins as COX-2 and iNOS are increased in inflamed mucosa and remain elevated in colonic neoplasms from UC patients.[50–53] Their over expression is associated with colon tumor formation and/or promotion by a number of potential mechanisms.[28,54–59]

Studies in cancer cells, animal models of CRC and many clinical trials with both non-selective and COX-2-selective NSAIDs support an important role for the COX-2 pathway.[54] In sporadic carcinogenesis it has been shown that normal colonic mucosa does not express COX-2, but with the adenoma-carcinoma sequence, this

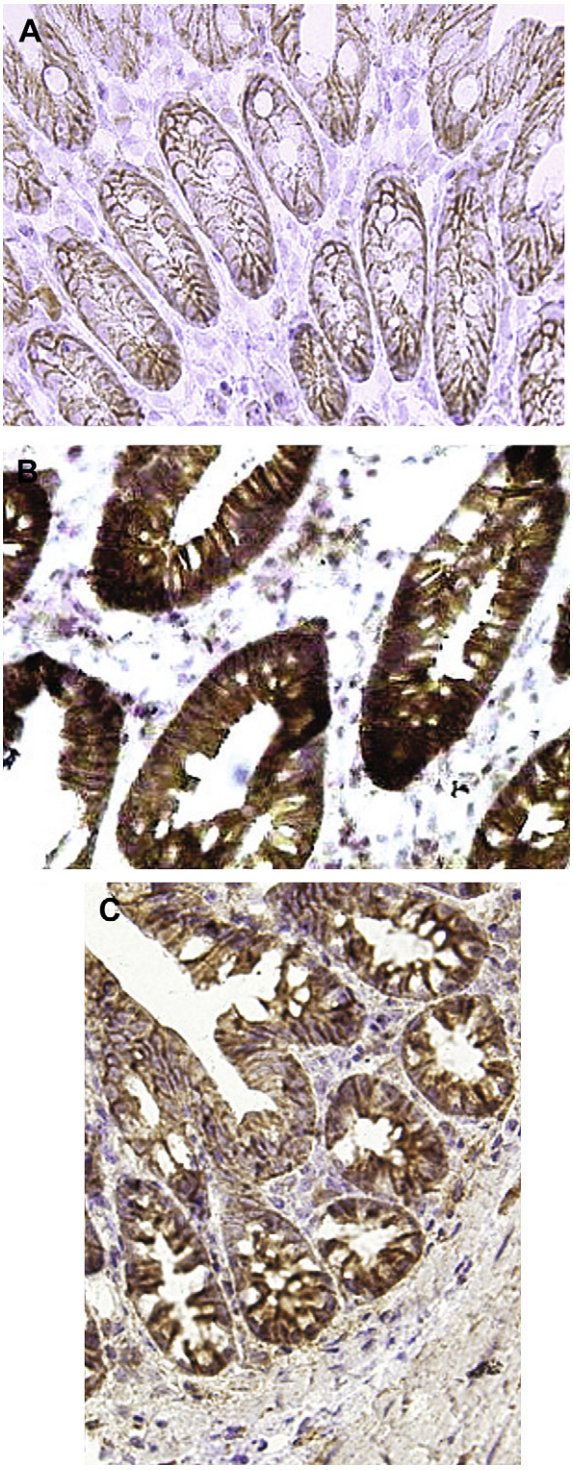

Fig. 6. Effects of functional diets on β-catenin expression by immunohistochemistry methods. Colons of sham animals fed with sunflower oil (A), colons of 0.7% DSS (v/v) treated animals during 15 cycles and fed with functional diet supplemented with sunflower oil (B) or with extra virgin olive oil (C). Original magnification 400×.

enzyme is over expressed. Among the many effects of COXs, they can catalyze the conversion of procarcinogens to carcinogens, indirectly produce free radicals, and promote angiogenesis.[60,61]

On the other hand, iNOS is also known to play a key role in colon tumor development; its excessive NO production can produce damage to DNA, either directly or indirectly by several mechanisms, interferes with DNA repair (e.g., mutation of the p53 tumor

Table 4
Inmunohistochemical evaluation of β-catenin in sham animal and treated with 15 cycles of DSS (0.7% v/v) and fed with different diets: sunflower diet (SFO diet) and extra virgin olive oil (EVOO diet).

Group		β-catenin staining pattern (%)	
		Cell membrane	Cytoplasmic and/or nucleus
SFO	Sham	80.0 ± 4.2	0
	DSS	92.3 ± 5.5	52.3 ± 3.0***
EVOO	Sham	84.2 ± 5.2	0
	DSS	80.5 ± 5.2	18.7 ± 2.7*,+++

Data are expressed as the means ± S.E.M. $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. respective sham group; $+++P < 0.001$ vs. SFO diet-DSS animals.

suppressor gene), and/or causes post-translational modification, potentially leading to tumor initiation and promotion.[57,62,63]

Our data support the concept that inhibitors of both inducible enzymes are effective chemopreventive agents against colon carcinogenesis, being an important mechanism to improve the colon tumor development. After 15 cycles of DSS treatment, EVOO diet reduced significantly both COX-2 and iNOS expression vs. SFO diet, where these proteins were appreciably incremented compared to sham group. These results are also consistent with other preclinical models where for example, diets rich in omega-3, including DHA, induce apoptosis, and inhibit COX-2 and iNOS activity in colon tumors.[29] Other *in vitro* studies have also demonstrated that this signalling pathway is involved in colon cancer growth inhibition by DHA.[51,64]

Finally, there is controversy regarding to the functions of p53 and its modulation is a complex process.[65–69] It has been hypothesized that p53 is latent in normal conditions and

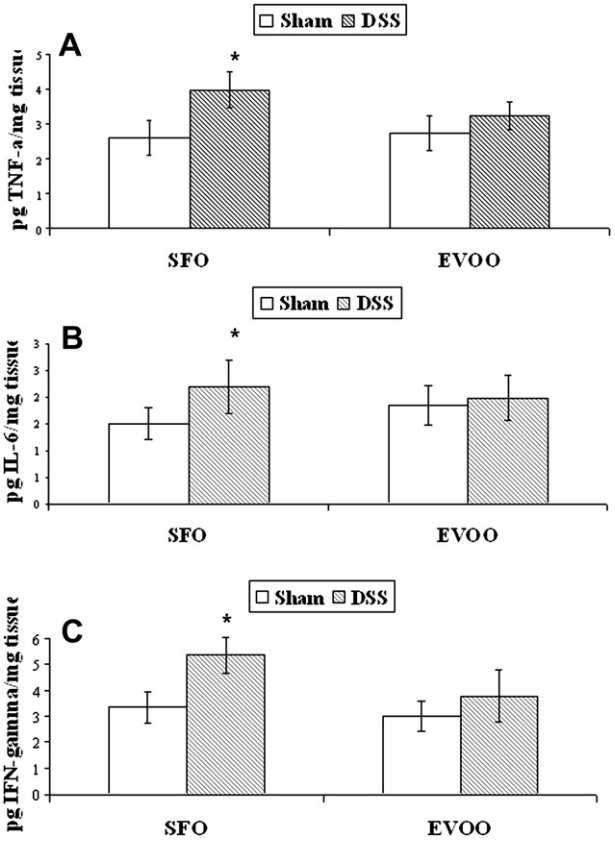

Fig. 7. Effect of functional diet supplemented with sunflower oil (SFO diet) or extra virgin olive oil (EVOO diet) on tumor necrosis factor alpha (A), interleukine-6 (B) and interferon gamma (C) in the colon tissue after 15 cycles with DSS 0,7% (v/v). Data are expressed as the means ± S.E.M. $^{*}P < 0.05$ vs. Sham group.

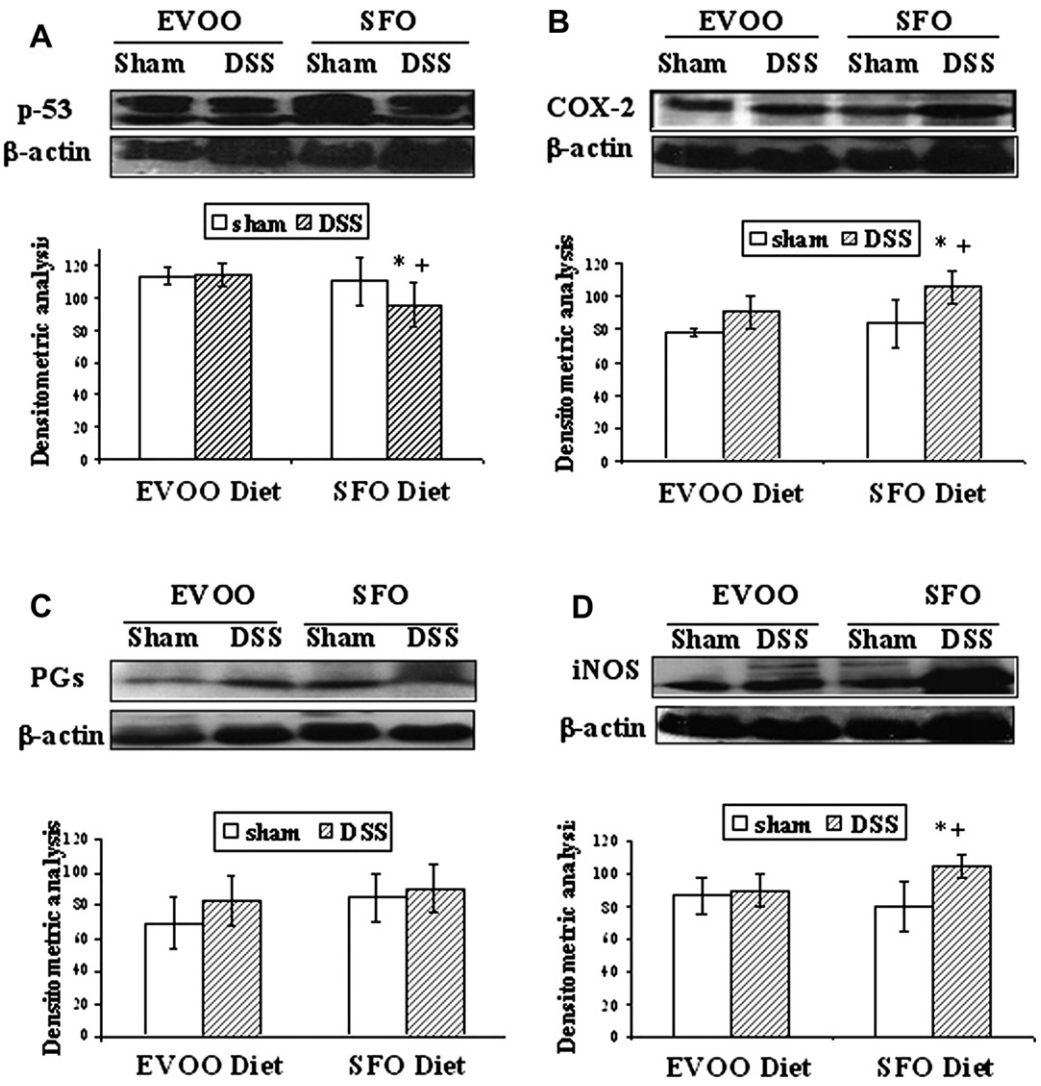

Fig. 8. Effects of functional diets supplemented with sunflower oil (SFO diet) or extra virgin olive oil (EVOO diet) on expression of different proteins in the colon tissue after 15 cycles with DSS 0.7% (v/v). Western blotting using antibodies against p53 (A), cyclooxygenase (COX)-2 (B), inducible nitric oxide synthase (iNOS) (C) and prostaglandin E synthase (PGES)-1 (D) as described in section Material and Methods. Data are expressed as the means ± S.E.M. $^{*}P < 0.05$ vs. Sham group; $+P < 0.05$ vs. EVOO diet-DSS animals.

becomes active when cells are exposed to DNA damage or other genotoxic agents, during which p53 is phosphorylated and acetylated and, in turn, accumulates in the nucleus at its target genes.[67,69–71] Loss of p53 gene function occurs late and is believed to be the defining event that drives the adenoma to carcinoma.[72] p53 inactivation is one of the most common anti-apoptotic pathway detected in cancer tissues, unprotect cells from harmful propagation of DNA damage.[73] We have observed that animals fed with EVOO did not modify p53 expression and, on the contrary, loss of p53 function was observed in animals fed with SFO diet, which may inactive apoptotic pathway driving to propagation of DNA damage. This is in accordance with other authors who have demonstrated that p53 was not immunohistochemically seen in tumor cancer lesions.[74,75]

Collectively, these results confirm that EVOO diet during DSS administration has protective/preventive effect in the progression of CRC associated to UC. This beneficial effect was correlated with a better DAI index, a minor number of dysplastic lesions, a lower immunoreactivity of β-catenin, a reduction of proinflammatory cytokines levels, a no modification of p53 expression and, a reduction of COX-2 and iNOS protein expression in the colonic tissue.

Conflict of interest

I, Catalina Alarcón de la Lastra, declare that I have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript

Acknowledgment

Supported by funds from Ministerio de Ciencia e Innovación (AGL 2005-05132) and Junta de Andalucía (Spain). S. Sánchez-Fidalgo and I. Villegas have contributed in same proportion in the developing and direction of the study.

References

1. Itzkowitz SH. Molecular biology of dysplasia and cancer in inflammatory bowel disease. *Gastroenterol Clin North Am* 2006;**35**:553–71.
2. Motilva V, Talero E, Calvo JR, Villegas I, Alarcón-de-la-Lastra C, Sánchez-Fidalgo S. Intestinal immunomodulation. Role of regulative peptides and promising pharmacological activities. *Curr Pharm Des* 2008;**14**:71–95.

3. Gavert N, Ben-Ze'ev A. Beta-Catenin signaling in biological control and cancer. *J Cell Biochem* 2007;**102**:820–8.
4. Tanaka T, Kohno H, Suzuki R, Yamada Y, Sugie S, Mori H. A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. *Cancer Sci* 2003;**94**:965–73.
5. Müller-Decker K, Fürstenberger G. The cyclooxygenase-2-mediated prostaglandin signaling is causally related to epithelial carcinogenesis. *Mol Carcinog* 2007;**46**:705–10.
6. Kawanishi S, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol Chem* 2006;**387**:365–72.
7. Mutoh M, Takahashi M, Wakabayashi K. Roles of prostanoids in colon carcinogenesis and their potential targeting for cancer chemoprevention. *Curr Pharm Des* 2006;**12**:2375–82.
8. Burstein E, Fearon ER. Colitis and cancer: a tale of inflammatory cells and their cytokines. *J Clin Invest* 2008;**118**:464–7.
9. Davidson LA, Nguyen DV, Hokanson RM, Callaway ES, Isett RB, Turner ND, et al. Chemopreventive n-3 polyunsaturated fatty acids reprogram genetic signatures during colon cancer initiation and progression in the rat. *Cancer Res* 2004;**64**:6797–804.
10. Owen RW, Haubner R, Wurtele G, Hull E, Spiegelhalder B, Bartsch H. Olives and olive oil in cancer prevention. *Eur J Cancer Prev* 2004;**13**:319–26.
11. Cottet V, Bonithon-Kopp C, Kronborg O, Santos L, Andreatta R, Boutron-Ruault MC, et al, European Cancer Prevention Organisation Study Group. Dietary patterns and the risk of colorectal adenoma recurrence in a European intervention trial. *Eur J Cancer Prev* 2005;**14**:21–9.
12. Alarcón de la Lastra C, Barranco MD, Motilva V, Herrerias JM. Mediterranean diet and health: biological importance of olive oil. *Curr Pharm Des* 2001;**7**:933–50.
13. Fujise T, Iwakiri R, Kakimoto T, Shiraishi R, Sakata Y, Wu B, et al. Long-term feeding of various fat diets modulates azoxymethane-induced colon carcinogenesis through Wnt/beta-catenin signaling in rats. *Am J Physiol Gastrointest Liver Physiol* 2007;**292**:G1150–G1156.
14. Bartoli R, Fernandez-Banares F, Navarro E, Castella E, Mane J, Alvarez M, et al. Effect of olive oil on early and late events of colon carcinogenesis in rats: modulation of arachidonic acid metabolism and local prostaglandin (E2) synthesis. *Gut* 2000;**46**:191–9.
15. Onogi N, Okuno M, Komaki C, Moriwaki H, Kawamori T, Tanaka T, et al. Suppressing effect of perilla oil on azoxymethane-induced foci of colonic aberrant crypts in rats. *Carcinogenesis* 1996;**17**:1291–6.
16. Hashim YZ, Rowland IR, McGlynn H, Servili M, Selvaggini R, Taticchi A, et al. Inhibitory effects of olive oil phenolics on invasion in human colon adenocarcinoma cells in vitro. *Int J Cancer* 2008;**122**:495–500.
17. Gill CI, Boyd A, McDermott E, McCann M, Servili M, Selvaggini R, et al. Potential anti-cancer effects of virgin olive oil phenols on colorectal carcinogenesis models in vitro. *Int J Cancer* 2005;**117**:1–7.
18. Carrier JC, Aghdassi E, Jeejeebhoy K, Allard JP. Exacerbation of dextran sulfate sodium-induced colitis by dietary iron supplementation: role of NF-kappaB. *Int J Colorectal Dis* 2006;**21**:81–387.
19. Erichsen K, Milde AM, Arslan G, Helgeland L, Gudbrandsen OA, Ulvik RJ, et al. Low-dose oral ferrous fumarate aggravated intestinal inflammation in rats with DSS-induced colitis. *Inflamm Bowel Dis* 2005;**11**:744–8.
20. Seril DN, Liao J, Yang CS, Yang GY. Systemic iron supplementation replenishes iron stores without enhancing colon carcinogenesis in murine models of ulcerative colitis: comparison with iron-enriched diet. *Dig Dis Sci* 2005;**50**:696–707.
21. Yeo M, Kim DK, Park HJ, Oh TY, Kim JH, Cho SW, et al. Loss of transgelin in repeated bouts of ulcerative colitis-induced colon carcinogenesis. *Proteomics* 2006;**6**:1158–65.
22. Gommeaux J, Cano C, Garcia S, Gironella M, Pietri S, Culcasi M, et al. Colitis and colitis-associated cancer are exacerbated in mice deficient for tumor protein 53-induced nuclear protein 1. *Mol Cell Biol* 2007;**27**:2215–28.
23. Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, et al. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. *Hum Pathol* 1983;**14**:931–68.
24. Osawa E, Nakajima A, Fujisawa T, et al. Predominant T helper type 2-inflammatory responses promote murine colon cancers. *Int J Cancer* 2006;**118**:2232–6.
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* 1976;**72**:248–54.
26. Kohno H, Suzuki R, Sugie S, Tanaka T. Suppression of colitis-related mouse colon carcinogenesis by a COX-2 inhibitor and PPAR ligands. *BMC Cancer* 2005;**5**:46.
27. Woutersen RA, Appel MJ, Garderen-Hoetmer A, Wijnands M. Dietary fat and carcinogenesis. *Mutat Res* 1999;**443**:111–27.
28. Reddy BS. Nutritional factors and colon cancer. *Crit Rev Food Sci Nutr* 1995;**35**:175–90.
29. Rao CV, Hirose Y, Indranie C, Reddy BS. Moduration of experimental colon tumorigenesis by types and amounts of dietary fatty acids. *Cancer Res* 2001;**61**:1927–33.
30. Lipworth L, Martinez ME, Angell J, Hsieh CC, Trichopoulos D. Olive oil and human cancer: an assessment of the evidence. *Prev Med* 1997;**26**:181–90.
31. Escrich E, Moral R, Grau L, Costa I, Solanas M. Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer. *Mol Nutr Food Res* 2007;**51**:1279–92.
32. Schwartz B, Birk Y, Raz A, Madar Z. Nutritional-pharmacological combinations: a novel approach to reducing colon cancer incidence. *Eur J Nutr* 2004;**43**:221–9.
33. Femia A, Dolara P, Servili M, Esposto S, Taticchi A, Urbani S, et al. No effects of olive oils with different phenolic content compared to corn oil on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. *Eur J Nutr* 2008;**47**:329–334.
34. Liu W, Dong X, Mai M, Seelan RS, Taniguchi K, Krishnandath KK, Halling KC, Cunningham JM, Boardman LA, Qian C, Christensen E, Schmidt SS, Roche PC, Smith DI, Thibodeau SN. Mutations in Axin2 cause colorectal cancer with detective mismatch repair by activating beta-c-atenin-Tcf signaling. *Nat Genet* 2000;**26**:146–7.
35. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutation in beta catenin or APC. *Science* 1997;**275**:1787–90.
36. von Kries JP, Winbeck G, Asbrand C, Schwarz-Romond T, Sochnikowa N, Dell'Oro A, Behrens J, Birchmeier W. Hot spots in betacatenin for interactions with LEF-1, conduction and APC. *Nat Struct Biol* 2000;**7**:800–7.
37. van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, et al. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 2002;**111**:241–50.
38. Fujii S, Fujimori T, Kawamata H, Takada J, Kitajima K, Omotehara F, Kaihara T, Kusaka T, Ichikawa K, Ohkura Y, Ono Y, Imura J, Yamaoka S, Sakamoto C, Ueda Y, Chiba T. Development of colonic neoplasia in p53 deficient mice with experimental colitis induced by dextran sulphate sodium. *Gut* 2004;**53**:710–6.
39. Furihata T, Kawamata H, Kubota K, Fujimori T. Evaluation of the malignant potential of aberrant crypt foci by immunohistochemical staining for betacatenin in inflammation-induced rat colon carcinogenesis. *Int J Mol Med* 2002;**9**:353–8.
40. Aust DE, Terdiman JP, Willenbucher RF, Chew K, Ferrell L, Florendo C, Molinaro-Clark A, Baretton GB, Löhrs U, Waldman FM. Altered distribution of beta-catenin, and its binding proteins E-cadherin and APC, in ulcerative colitis-related colorectal cancers. *Mod Pathol* 2001;**14**:29–39.
41. Babbar N, Casero Jr RA. Tumor necrosis factor-alpha increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: a potential mechanism for inflammation-induced carcinogenesis. *Cancer Res* 2006;**66**:11,125–11,130.
42. Scott KA, Moore RJ, Arnott CH, East N, Thompson RG, Scallon BJ, Shealy DJ, Balkwill FR. An anti-tumor necrosis factor-alpha antibody inhibits the development of experimental skin tumors. *Mol Cancer Ther* 2003;**2**:445–51.
43. Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, et al. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest* 2008;**118**:560–70.
44. Erdman SE, Rao VP, Poutahidis T, Rogers AB, Taylor CL, Jackson EA, Ge Z, Lee CW, Schauer DB, Wogan GN, Tannenbaum SR, Fox JG. Nitric oxide and TNF-alpha trigger colonic inflammation and carcinogenesis in Helicobacter hepaticus-infected, Rag2-deficient mice. *Proc Natl Acad Sci USA* 2009;**106**:1027–32.
45. Bromberg J, Wang TC. Inflammation and cancer: IL-6 and STAT3 complete the link. *Cancer Cell* 2009;**15**:79–80.
46. Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004;**118**:285–96.
47. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, et al. IL-6 and stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell.* 2009;**15**:103–13.
48. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007;**317**:121–4.
49. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003;**3**:521–33.
50. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 2004;**287**:G7–G17.
51. Narayanan BA, Narayanan NK, Simi B, Reddy BS. Modulation of inducible nitric oxide synthase and related proinflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colon cancer cells. *Cancer Res* 2003;**63**:972–9.
52. Elder DJ, Baker JA, Banu NA, Moorghen M, Paraskeva C. Human colorectal adenomas demonstrate a size-dependent increase in epithelial cyclooxygenase-2 expression. *J Pathol* 2002;**198**:428–34.
53. Rao CV, Indranie C, Simi B, Manning PT, Connor JR, Reddy BS. Chemopreventive properties of a selective inducible nitric oxide synthase inhibitor in colon carcinogenesis, administered alone or in combination with celecoxib, a selective cyclooxygenase-2 inhibitor. *Cancer Res* 2002;**62**:165–70.
54. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, Kaidi A. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumor microenvironment. *Carcinogenesis* 2009;**30**:377–386.
55. Samanta S, Swamy V, Suresh D, Rajkumar M, Rana B, Rana A, et al. Protective effects of vanadium against DMH-induced genotoxicity and carcinogenesis in rat colon: removal of O6-methylguanine DNA adducts, p53 expression, inducible nitric oxide synthase downregulation and apoptotic induction. *Mutat Res* 2008;**650**:123–31.
56. Dempke W, Rie C, Grothey A, Schmoll HJ. Cyclooxygenase-2: a novel target for cancer chemotherapy? *J Cancer Res Clin Oncol* 2001;**127**:411–7.

57. Lala PK, Chakraborty C. Role of nitric oxide in carcinogenesis and tumour progression. *Lancet Oncol* 2001;**2**:149–56.
58. Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene* 1999;**18**:7908–16.
59. Battu S, Rigaud M, Beneytout JL. Resistance to apoptosis and cyclooxygenase-2 expression in a human adenocarcinoma cell line HT29 CL.19A. *Anticancer Res* 1998;**18**:3579–83.
60. Steele VE, Hawk ET, Viner JL, Lubet RA. Mechanisms and applications of non-steroidal anti-inflammatory drugs in the chemoprevention of cancer. *Mutat Res* 2003;**523**–**524**:137–44.
61. Dannenberg AJ, Zakim D. Chemoprevention of colorectal cancer through inhibition of cyclooxygenase-2. *Semin Oncol* 1999;**26**:499–504.
62. Felley-Bosco E. Role of nitric oxide in genotoxicity: implication for carcinogenesis. *Cancer Metastasis Rev* 1998;**17**:25–37.
63. Jenkins DC, Charles IG, Thomsen LL, Moss DW, Holmes LS, Baylis SA, et al. Roles of nitric oxide in tumor growth. *Proc Natl Acad Sci USA* 1995;**92**:4392–6.
64. Narayanan BA, Narayanan NK, Reddy BS. Docosahexaenoic acid (DHA) regulated genes and transcription factors inducing apoptosis in human colon cancer cells. *Int J Oncol* 2001;**19**:1255–62.
65. Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD, et al. Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. *Mol Cell* 2001;**8**:243–54.
66. Espinosa JM, Emerson BM. Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. *Mol Cell* 2001;**8**:57–69.
67. Liu L, Scolnick D, Trievel RC, Zhang HB, Marmorstein R, Halazonetis TD, et al. p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage. *Mol Cell Biol* 1999;**19**:1202–9.
68. Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW, Appella E. DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev* 1998;**12**:2831–41.
69. Gu W, Roeder R. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 1997;**90**:595–606.
70. Takenaka I, Morin FF, Seizinger BR, Kley N. Regulation of the sequence-specific DNA binding function of p53 by protein kinase C and protein phosphatases. *J Biol Chem* 1995;**270**:5405–11.
71. Hupp TR, Lane DP. Allosteric activation of latent p53 tetramers. *Curr Biol* 1994;**4**:865–75.
72. Xie J, Itzkowitz SH. Cancer in inflammatory bowel disease. *World J Gastroenterol* 2008;**14**:378–89.
73. Fridman JS, Lowe SW. Control of apoptosis by p53. *Oncogene* 2003;**22**:9030–9040.
74. Wang JG, Wang DF, Lv BJ, Si JM. A novel mouse model for colitis-associated colon carcinogenesis induced by 1,2 dimethylhydrazine and dextran sulfate sodium. *World J Gastroenterol* 2004;**10**:2958–62.
75. Cooper HS, Murthy S, Kido K, Yoshitake H, Flanigan A. Dysplasia and cancer in the dextran sulfate sodium mouse colitis model. Relevance to colitis-associated neoplasia in the human: a study of histopathology, B-catenin and p53 expression and the role of inflammation. *Carcinogenesis* 2000;**21**:757–68.