



In vivo cytogenetic effects of multiple doses of dietary vegetable oils

Cristina Márcia Wolf Evangelista^{1,3}, Lusânia Maria Greggi Antunes^{1,2} and Maria de Lourdes Pires Bianchi¹

¹*Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.*

²*Departamento de Ciências Biológicas, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil.*

³*Curso de Ciências Farmacêuticas, Universidade de Ribeirão Preto, Ribeirão Preto, SP, Brazil.*

Abstract

Polyphenols are potent antioxidants that are particularly abundant in the Mediterranean diet, with olive oil being the main fat source. A number of investigations have reported that phenolic compounds found in dietary oils are antioxidants and could provide protective effects by inhibiting DNA oxidative damage. However, few studies have been published on the biological activity of vegetable oils, including their possible mutagenic/antimutagenic effects. The objective of the current study was to investigate the cytogenetic effects of multiple doses of four vegetable oils in rat bone marrow cells and to examine the possible antimutagenic effects of these oils in chromosomal damage induced by the antitumor drug cisplatin. These oils are consumed by humans and commonly used as drug vehicles. The rats received treatment with multiple doses of canola oil, olive oil, virgin olive oil, and corn oil (5 mL kg⁻¹) alone or combined with the antitumor drug cisplatin (5 mg kg⁻¹). Treatments with vegetable oils alone did not increase the percentage of cells with chromosomal aberrations ($p > 0.05$). Olive, virgin olive and canola oils showed protective effects against cisplatin-induced chromosomal damage ($p < 0.05$). A rational mechanism for the protective effects of vegetable oils is that their phenolic compounds have antioxidant and antimutagenic properties *in vivo*.

Key words: antimutagenesis, chromosomal aberrations, vegetable oils, Wistar rats.

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Introduction

Epidemiological studies have shown that populations consuming the traditional Mediterranean diet exhibit lower frequencies of chronic diseases and enjoy good health (Wahle *et al.*, 2004). The Mediterranean diet, rich in fruits, vegetables, fish, wine and olive oil can contribute to a lower incidence of coronary heart disease and cancer (Tuck and Hayball, 2002).

Fruits, vegetables and polyphenols, potent antioxidants, are particularly abundant in the Mediterranean diet, with olive oil being the main fat source. A review concerning olive oil and breast and pancreatic cancer risk has demonstrated that increased dietary intake of olive oil is associated with a small decreased risk, or no increased risk of cancer. Data obtained with experimental animals suggests a tumor-inhibiting role for squalene, which is proposed as the most important factor in the cancer-risk reducing effect of olive oil (Newmark, 1999). A number of

investigations have reported that phenolic compounds, such as hydroxytyrosol, present predominantly in *Olea europea*, found in olive and virgin olive oils, are strong antioxidants and could provide protective effects by inhibiting oxidative damage (Tuck and Hayball, 2002). Cell culture experiments have demonstrated that olive oil phenolic compounds have anti-atherogenic and antioxidant effects, and could exert cardioprotective effects *in vivo* (Turner *et al.*, 2005).

The effects of edible oils on hypertension and myocardial remodeling were investigated in spontaneously hypertensive rats. Canola oil and palm oil were effective in decreasing blood pressure, with greater cardiomyocyte vessel indices. Soybean oil and olive oil had mild effects on myocardial structure (Aguila *et al.*, 2004). Recently, a potent antioxidant named canolol was isolated from crude canola oil and its potency was found to be greater than that of some well-known antioxidants, including α -tocopherol, vitamin C and quercetin (Wakamatsu *et al.*, 2005).

The investigation of the inter-relation between free radicals and antioxidant dietary oils is a field of great interest for elucidating mechanisms of mutagenesis/carcinogenesis (Owen *et al.*, 2000). Previous studies have shown

Send correspondence to Lusânia Maria Greggi Antunes. Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto Universidade de São Paulo, Av. do Café, s/n; 14040-903 Ribeirão Preto, SP, Brazil. E-mail: lusania@fcrp.usp.br.

that the mutagenic activity of food mutagens can be modulated by vegetable oils (Perez *et al.*, 2002). Kensese *et al.* (1989), observed weak mutagenic activity in several commercially edible palm and corn oils using liquid incubation bioassays with *Salmonella typhimurium* TA1537. Phenolic compounds present in virgin olive oil moderated the formation of carcinogenic/mutagenic heterocyclic amines in a model system (Monti *et al.*, 2001). Seven vegetable oils consumed by humans were tested for genotoxic activity in the *Drosophila* somatic mutation and recombination test. Sunflower and olive oils gave inconclusive results, and virgin olive oil was clearly non-genotoxic (Rojas-Molina *et al.*, 2005).

There is evidence in the literature suggesting that olive and canola oils have antimutagenic properties in animals (El-Nahas *et al.*, 1993; Antunes and Takahashi, 1999; Evangelista *et al.*, 2004). However, mutagenicity/antimutagenicity assays with dietary oils are limited to a few studies. The objective of the current study was to investigate the cytogenetic effects of multiple doses of four vegetable oils on rat bone-marrow cells and to examine the possible antimutagenic effects of these oils in chromosomal damage induced by the antitumor drug cisplatin. These oils are consumed by humans and commonly used as drug vehicles.

Material and Methods

Chemical agents

Commercially available oils, canola oil (CAO), olive oil (OLO), virgin olive oil (VOO), and corn oil (COO) were purchased in a local grocery store. Cisplatin (cDDP; *cis*-diamminedichloroplatinum II); CAS n. 15663-27-1, Platinil[®]) was obtained from Quiral Química do Brasil. All other chemicals and reagents used were of analytical grade.

Chromosomal aberrations assay

Healthy male and female Wistar rats were obtained from the Animal Center of the Prefeitura do Campus Administrativo de Ribeirão Preto (Universidade de São Paulo, Brazil). The rats were 6-7 weeks old and were housed in polycarbonate cages with steel wire tops. Animals weighed 100 ± 5 g and were maintained at 23 ± 2 °C in a controlled environment under a 12 h light/dark cycle and had free access to standard rat chow and fresh water *ad libitum*. This study was approved by the Animal Ethics Committee, Campus de Ribeirão Preto da Universidade de São Paulo, Brazil.

The rats were divided into experimental groups consisting of six animals, and negative and positive control groups. To assess the cytogenetic effects of multiple doses of dietary oils, the animals of the oil groups were treated with CAO, OLO, VOO or COO by gavage at a dose of 5 mL kg^{-1} b.w. 48 h, 24 h or 30 min before intra-peritoneal (i.p.) saline or cDDP. The dose of the oils tested was selected on the basis of preliminary results with single admin-

istration (Evangelista *et al.*, 2004). The dose of cDDP was the same as used by Antunes *et al.* (2000). In addition to these groups, one set of rats was treated with three administrations of distilled water and 5 mg kg^{-1} b.w. of cDDP i.p. alone and used as a positive control. A negative control group was treated with three administrations of distilled water and saline i.p. All animals were injected intraperitoneally with 4 mg kg^{-1} b.w. of colchicine (Sigma Chemical Co., St. Louis, MO, USA) 90 min. before euthanasia, which occurred 24 h after cDDP or saline administration.

For the analysis of chromosomal damage in metaphase cells, bone marrow preparations were prepared according to Preston *et al.* (1987) and the slides stained with Giemsa (Sigma Chemical Co., St. Louis, MO, USA). One hundred metaphase cells were scored per rat to determine total chromosomal aberrations and the mitotic index was obtained by counting the number of mitotic cells in 1000 cells per rat. The endpoints analyzed were mitotic index, total chromosome aberrations and percentage of cells with chromosomal aberrations. Only well-spread metaphases with 42 ± 1 chromosomes were randomly analyzed by light microscopy at 100x magnification. Chromosomal damage reduction percentages were calculated by comparing the treated groups with the group that received only the antitumor drug cisplatin.

Statistical analysis

The results were tabulated and experimental values expressed with \pm standard deviations (SD). One-way ANOVA was carried out and Student's t-test was used to detect significant differences amongst different treatment groups. The level of significance was $p < 0.05$. Gaps were not included in the statistical analysis.

Results

The cytogenetic analysis of bone-marrow cells from Wistar rats treated with dietary oils and cDDP revealed various types of chromosomal aberrations, which consisted of chromatid gaps, chromatid and chromosome breaks and exchanges. Chromatid and chromosome breaks were the most frequent type of aberrations. Chromatid gaps were not included in the total chromosomal aberrations or in the percentage of cells with aberrations. The results of the chromosomal aberrations test after multiple doses of CAO, OLO, VOO, and COO, alone or in combination with cDDP, are summarized in Tables 1 and 2. The frequencies of cells with chromosomal aberrations increased in the rats that received i.p. cDDP, and a statistically significant difference was observed for the negative control ($p < 0.05$). The percentages of metaphases with aberrations in the groups treated with cDDP were 23.16, 21.66, 22.83, and 22.16 %. There was no significant difference between the group treated with dietary oils and the negative control regarding the induction of chromosomal damage ($p > 0.05$).

Multiple doses of dietary oils produced a statistically significant decrease in cDDP-induced chromosomal aberrations ($p < 0.05$). Results shown in Table 1 indicate a 48.9% and 57.6% decrease of the cDDP-induced cells with aberrations when the animals were pre-treated with multiple doses of CAO or OLO, respectively. VOO was also efficient as a pre-treatment, resulting in a decrease of 54.7% of cells with aberrations, when compared with the group that received cDDP i.p. (Table 2). A reduction in chromosomal aberrations was registered in the group pre-treated with corn oil when compared with the group treated with cDDP, but the difference was not statistically significant ($p > 0.05$).

The mitotic index evaluated as the percentage of dividing cells did not show any significant variation between treatments with the dietary oils and cDDP when compared with the negative control ($p > 0.05$). Mitotic indices of

4.3% (CAO), 3.8% (OLO), 4.1% (VOO) and 4.2% (COO) were recorded (Tables 1 and 2). This study implies that the administration of dietary oils does not have the potential to inhibit the division of bone marrow cells.

Discussion

Toxicological data on dietary oils are scarce in the literature, and the potential involvement of corn oil gavage test-compound administration in unexpected carcinogenesis-toxicity testing has been reported (Landers *et al.*, 1986). The guideline to conduct a scientifically valid *in vivo* chromosomal aberration bioassay recommended that the test substance, if insoluble in water or saline, should be dissolved or homogeneously suspended in vegetable oil (Tice *et al.*, 1994). However, few studies have been published on the biological activity of vegetable oils including their possible mutagenic/antimutagenic effects, despite the

Table 1 - Mitotic index, and distribution of different types of chromosome aberrations, in bone marrow cells of Wistar rats pretreated with canola oil (CAO), olive oil (OLO), cisplatin (cDDP) and their respective controls.

Treatments groups	Mitotic index %	Chromatid gaps	Chromatid breaks	Chromosome breaks	Chromosome exchange	Total chromosomal aberrations	Cells with chromosomal aberrations %	Reduction cells with aberrations %
Control	3.8	2	6	0	0	6	1.00 ± 0.83	
CAO	4.3	2	7	1	0	8	1.33 ± 1.14	
cDDP	3.5	4	131	14	3	148	23.16 ^a ± 6.22	
CAO + cDDP	2.8	0	72	3	2	77	11.83 ^{a,b} ± 3.96	48.9
Control	4.1	4	5	0	0	5	0.83 ± 0.70	
OLO	3.8	3	11	0	0	11	1.66 ± 1.22	
cDDP	3.7	15	124	10	3	137	21.66 ^a ± 10.21	
OLO + cDDP	3.8	5	56	3	2	61	9.16 ^{a,b} ± 3.76	57.6

Six-hundred cells were analyzed per treatment. Gaps were not included in the total and in the cells with chromosomal aberrations.

^aSignificantly different from negative control ($p < 0.05$).

^bSignificantly different from the cDDP group ($p < 0.05$).

Table 2 - Mitotic index, and distribution of different types of chromosome aberrations, in bone marrow cells of Wistar rats pretreated with virgin olive oil (VOO) or corn oil (COO), Cisplatin (cDDP) and respective controls.

Treatment groups	Mitotic index %	Chromatid gaps	Chromatid breaks	Chromosome breaks	Exchange	Total chromosomal aberrations	Cells with chromosomal aberrations %	Reduction cells with aberrations %
Control	4.0	1	6	0	0	6	1.00 ± 0.63	
VOO	4.1	2	5	0	0	5	0.83 ± 0.40	
cDDP	3.2	4	126	9	10	145	22.83 ^a ± 9.10	
VOO + cDDP	3.5	9	62	0	1	63	10.33 ^{a,b} ± 2.16	54.7
Control	4.4	2	6	1	1	8	1.33 ± 1.03	
COO	4.2	0	6	0	0	6	1.00 ± 0.63	
cDDP	3.7	5	147	13	2	162	22.16 ^a ± 4.87	
COO+cDDP	3.9	2	104	6	9	119	15.66 ^a ± 6.50	29.3

Gaps were not included in the total and in the cells with chromosomal aberrations.

^aSignificantly different from negative control ($p < 0.05$).

^bSignificantly different from the cDDP group ($p < 0.05$).

beneficial properties of CAO, OLO and VOO on human health.

DNA damage is often measured as single-strand breaks, double-strand breaks or chromosomal aberrations, and increase in their frequencies is frequently associated with mutagenesis and carcinogenesis (Surh and Ferguson, 2003). In the present study, we investigated the cytogenetic effects of multiple doses of four dietary oils, and their association with the antitumor drug cDDP in rats using the chromosomal aberrations assay. The percentages of cells with chromosomal aberrations in the animals exposed to multiple doses of dietary oils were not statistically greater than those of the negative controls. A significant increase in the induction of chromosomal aberrations was observed in cDDP groups when compared with the oil groups and negative controls. Chromatid and chromosome breaks were the predominant types of damage induced by cDDP, and in general one aberration per cell was found. The cytogenetic effects caused by cDDP can be ascribed by the generation of reactive oxygen species.

cDDP is one of the most widely antineoplastic drugs used in the treatment of patients with a variety of malignancies. The generation of free radicals is believed to be an important mechanism in cDDP-induced mutagenicity, clastogenicity and toxicity (Wozniak *et al.*, 2004). This antitumor drug is usually selected as a positive control for short-term antimutagenicity tests. Our data support the literature reports that show an increased frequency of chromosomal damage in rodent bone marrow cells after administrations of cDDP (Antunes *et al.* 2000; Mora *et al.*, 2002).

The results demonstrated that the animals that received CAO, OLO, and VOO presented a marked statistically significant decrease in total chromosomal damage and in the percentage of cells with aberrations when compared with the cDDP group. The reduction in the percentage of cells with aberrations was 48.9% for CAO, 57.6% for OLO and 54.7% for VOO. The reduction produced by COO was 29.3%, but it was not statistically significant. The exact mechanisms of the antimutagenic effects of vegetable oils are not well understood. In this study, the oils may have acted as an antioxidant by intercepting the free oxygen radicals generated by cDDP, since it is known that OLO and VOO are remarkably rich in effective phenolic antioxidants which could provide protection by inhibiting oxidative damage (Owen *et al.*, 2000).

The antioxidant effects of OLO are probably ascribable to a combination of its high oleic acid content and its content of a variety of plant antioxidants, particularly oleuropein, hydroxytyrosol, tyrosol, and some minor components such as rutin, leuteolin and squalene (Visioli and Galli, 1998; Wahle *et al.*, 2004). Manna *et al.* (2002) reported that the phenolic fraction extracted from VOO had a protective effect against the cytotoxic effects of reactive oxygen species in human erythrocytes and intestinal

Caco-2 cells. Hydroxytyrosol is a potent hydrogen peroxide scavenger and could be used as a possible chemoprotective agent in the metabolic pathways related to oxidative stress (O'Dowd *et al.*, 2004). Antioxidant and antimutagenic activities of canolol, isolated from CAO, have been reported in the suppression of peroxynitrite-induced mutation in *Salmonella typhimurium* TA102 (Kuwahara *et al.*, 2004).

Several polyphenols have been demonstrated to have clear antioxidant properties attributable to their free radical scavenging and metal chelating properties. It should be noted that polyphenols might exert other biological activities, such as effects on cell signaling pathways and on gene expression (Soobrattee *et al.*, 2005). The COO, OLO, sesame, or soybean oils, commonly used as drug vehicles, may have different effects on specific hepatic CYP isoforms and may add to the variability in metabolism when xenobiotics are administered using dietary oils as drug vehicles (Brunner and Bai, 2000). Recent studies suggested that dietary polyphenols can stimulate the transcription of antioxidant and detoxification defense systems through antioxidant responsive elements, which are found in the promoters of many genes that may be induced by oxidative or chemical stress (Masella *et al.*, 2005).

In our present study, the data obtained with multiple doses of CAO, OLO and VOO have shown that the dietary oils were neither cytotoxic nor mutagenic but had protective effects against cDDP-induced chromosomal aberrations in bone marrow cells. The OLO and VOO were the most effective in the inhibition of cytogenetic damage induced by the antitumor drug. Although COO was not mutagenic it failed to inhibit cDDP-induced chromosomal damage. These results suggested that phenolic compounds in corn oil may not have antioxidant effects. This could have benefits, since COO is the main vehicle usually used in mutagenicity assays.

Research on pharmacological intervention or diet is viable when based on risk/benefit analysis evaluating the efficacy and security of protecting agents in a variety of test systems, followed by clinical and epidemiological studies. Our results suggest that dietary oils might be useful for eventual therapeutic or dietary interventions.

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