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Protective effects of β-carotene against the genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*

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ABSTRACT. β-carotene (BC), pro-vitamin A, is an efficient antioxidant, effective in the neutralization of oxygen reactive species, which cause serious damage to DNA. Various studies have been conducted on the effectiveness of BC for chemoprevention of cancer and heart disease. Doxorubicin is a chemotherapeutic agent used for cancer treatment that generates free radicals. We examined the effects of BC (1, 2 and 4 mg/mL) on the genotoxicity of doxorubicin (0.125 mg/mL), using the wing spot test in *Drosophila melanogaster* (somatic mutation and recombination test). The BC alone had no significant effect on the frequency of mutant spots. However, it significantly reduced the number of spots caused by doxorubicin. We concluded that BC is not genotoxic and that it exerts protective effects against the genotoxic action of the chemotherapeutic free-radical generator doxorubicin.

Key words: Antioxidants; Antigenotoxicity; β-carotene; SMART; *Drosophila melanogaster*; Doxorubicin

INTRODUCTION

β-carotene (BC), which is the principal source of vitamin A for most of the world's population (Peto et al., 1981), is effective in the neutralization of singlet oxygen ($^1O_2$) and for the inhibition of oxidation by peroxide compounds (Krinsky, 1991). Its effects, however, are modified under certain conditions and concentrations. Zhang and Omaye (2001) demonstrated that the antioxidant and prooxidant effects of BC are dependent on the level of oxygen and on the concentration of BC. As for most biological tissues, when the oxygen level is low, BC, as other carotenoids, becomes important as an antioxidant (Burri, 1997).

This characteristic of BC as an efficient antioxidant plays an important role in decreasing the incidence of cancer (Gandini et al., 2000; Riboli and Norat, 2003), cardiovascular diseases (Bast and Haenen, 2002; Oliveira et al., 2007), and aging (Ames et al., 1993), in addition to acting as an immunological regulator (Burri, 1997).

The chemopreventive action of BC is effective mainly in the beginning of the carcinogenic process or in the initial stages of its promotion, inhibiting the formation of preneoplastic lesions in experimental models *in vitro* and *in vivo* (Moreno et al., 1995; He et al., 1997; Gradelet et al., 1997). Apoptosis can be stimulated by BC in B16F cells (melanoma) (Guruvayoorappan and Kuttan, 2007) and in the cell line MCF-7 (Cui et al., 2007).

The somatic mutation and recombination test (SMART), developed with *Drosophila melanogaster*, is an assay that permits the detection of a range of genetic alterations, such as DNA point mutations, nucleotide deletions in DNA and mitotic DNA recombination (Graf et al., 1984). Flies with gene markers, such as *multiple wing hairs (mwh*) and *flare*^{3} *(flr*^{3}*)*, are used to detect the loss of heterozygosis in these genes. During the embryonic development of *D. melanogaster*, the cells of the imaginal disk proliferate mitotically to form the body of the adult fly. Genetic alterations in some of these cells of the imaginal disk result in the formation of descendent cells with alterations, forming clones of mutant cells. Such alterations are easily detected by phenotypic modifications in the hairs of the wings of the adult fly (Guzmán-Rincón and Graf, 1995).

The antineoplastic drug doxorubicin (DXR) is a potent and effective chemotherapeutic agent in the treatment of several forms of cancer, but its use is limited by the development of heart toxicity (Tallaj et al., 2005). Cellular enzymes are capable of converting DXR into free-radical metabolites (Benchekroun et al., 1993; Menegola et al., 2001). The free radicals generated act directly in the cell nucleus making the process of cell division unfeasible. For this reason, the cytotoxic mechanism seems to constitute the principal antitumor effect of DXR (Keizer et al., 1990). Doxorubicin is also a genotoxic agent that inhibits the activity of the enzyme topoisomerase II, resulting in the accumulation of DNA strand breaks that, if not repaired by the cell, can cause mutations and chromosomal aberrations (Islaih et al., 2005). In *D. melanogaster* SMART, DXR induces recombinations and is therefore used as a positive control (Lehmann et al., 2003; Costa and Nepomuceno, 2006; Fragiorge et al., 2007).

Burri (1997) suggests that daily supplements of BC should increase serum BC concentration in most individuals to the concentrations that have been associated with reduced risks of cancer and heart disease in epidemiological studies. Nutritional therapy with antioxidants administered concomitantly with antineoplasic drugs offers several benefits in the treatment of cancer patients. The use of antioxidant vitamins mitigates the side effects associated with antiblastic drugs and has a beneficial effect on the course of treatment since the toxicity of antineoplastic drugs is a limiting factor in this type of therapy. Antioxidant-based nutritional therapy can there-

fore be a valuable adjuvant in oncological therapy by improving the control of cancer (Santos and Cruz, 2001). In this context, the intake of antioxidant molecules such as carotenoids, ascorbic acid, vitamin E, and phenolic compounds, particularly flavonoids, is considered to be a nutritional indicator of good eating habits and a healthy life style (George et al., 2004). Therefore, the objective of the present study was to evaluate the protective effects of BC against the genotoxic action of DXR, using SMART for the detection of wing spots in *D. melanogaster*.

MATERIAL AND METHODS

Chemical compounds

β-carotene, Lot No. 04020136, manufactured by the Pharma Nostra Comercial Ltda. Laboratory in São Paulo, Brazil, and doxorubicin hydrochloride (DXR), known commercially as Doxolem®, Lot No. 80344, manufactured by Eurofarma Laboratórios Ltda. and distributed by Zodiac Pharmaceutical Products S/A, in São Paulo, Brazil, were used in this research.

Somatic mutation and recombination test

Drosophila stock

The tests were conducted using two mutant strains of *D. melanogaster*, bearers of genetic markers - *mwh* (*3-0.3*) and *flr*^{3} (*3-38.8*): 1) *mwh*, with genetic constitution: *mwh jv* and 2) *flr*^{3}, with genetic constitution *flr*^{3}*/ In(3LR)TM3, ri p*^{p} *sep I*(3)*89Aa bx*^{34e} and *Bd*^{s}.

Crosses between the mutant strains, collection of larvae and treatment

For the standard (ST) cross, virgin *flr*^{3}*/ In(3LR)TM3, ri p*^{p} *sep I(3)89Aa bx*^{34e} and *Bd*^{s} females were crossed with *mwh/mwh* males (Guzmán-Rincón and Graf, 1995). Oviposition occurred 48 h after the beginning of the crosses during a period of 8 h, in flasks containing a solid base of agar (4% agar in water) and a layer of yeast (*Saccharomyces cerevisiae*) supplemented with sugar. Three-day-old larvae, in the third embryonic stage of development, were washed in reverse osmosis water, and groups of approximately 100 larvae were transferred to glass vials containing 1.5 g instant mashed potatoes (HIKARI®, São Paulo, Brazil) rehydrated with 5 mL BC (1, 2 or 4 mg/mL) or not, added to DXR (0.125 mg/mL). For positive control, DXR (0.125 mg/mL) was used, and reverse osmosis water for negative control. Since the possible compounds were photosensitive, all vials were wrapped in aluminum foil.

Preparation and microscopic analysis of the fly wings

After hatching, the individual adults were collected and preserved in a container containing 70% ethanol. The wings of the flies were removed under a stereomicroscope using a pair of entomological tweezers, and were mounted in Faure's solution (30 g gum arabic; 20 mL glycerol, 1.5 g chloral hydrate and 50 mL of distilled water). Both the dorsal and ventral surfaces of the wings were analyzed under a compound microscope at 400X magnification. During

the analysis, the positions of spots were noted according to wing sections (Graf et al., 1984).

Statistical analysis

Statistical analysis of the experiments, for the verification of possible BC genotoxic action, was carried out using the test described by Frei and Würgler (1988). For the analysis of antigenotoxicity, the frequencies of each type of spot per fly were compared in pairs (negative control versus BC; genotoxic agent separately versus BC + genotoxic agent), using the Mann-Whitney U-test and the Wilcoxon test (Frei and Würgler, 1995). The percentage of BC inhibition was calculated using the frequencies of cloned 10^5 cells, corrected by the control (DXR - DXR combined with BC / DXR) x 100 (Abraham, 1994).

RESULTS AND DISCUSSION

The present study investigated the protective effects of BC against the genotoxic action of DXR in somatic cells of *D. melanogaster*. The concentrations of 1, 2, and 4 mg/mL BC were used as described in the literature (Burri, 1997; Silva and Naves, 2001).

Doxorubicin was selected in this study because it is an effective clastogenic and potent carcinogenic agent (Dhawan et al., 2003). The successful use of this antitumor agent is restricted by the risk of developing cardiotoxicity. Possible clinical options for reducing DXR-induced cardiotoxicity include agents such as antioxidants that prevent oxygen free radical generation (Fragiorge et al., 2007; Dutra et al., 2009).

Table 1 presents the results obtained from analysis of the marked-trans-heterozygous (MH) descendants of the ST cross, treated with different concentrations of BC (1, 2, or 4 mg/mL) using the positive (DXR - 0.125 mg/mL) and negative (reverse osmosis water) controls. The data showed that there was no significant increase ($P \geq 0.05$) in the frequencies of spots induced by BC when compared with the negative control, for all classes of spots. These results are in agreement with Konopacka and Rzeszowska-Wolny (2001) who showed the absence of BC genotoxicity when added (at 1-5 μg/mL) before, or immediately after, the exposure of human lymphocytes to γ rays. Similar results were obtained by Salvadori et al. (1992) who reported the absence of genotoxicity when male BALB/c mice were treated with BC (0.5, 1.0, 2.0, 5.0, 10, 25, 50, 100, and 200 mg/kg) by gavage for 5 consecutive days. In addition, cotreatments with DXR and BC produced responses that were significantly lower than the responses produced by DXR alone.

It was noted that DXR alone produced a positive response in both the MH and balancer heterozygous (BH) descendants of the ST cross, indicating that DXR was genotoxic in the assay. The appearance of twin spots indicated the recombinogenic activity of the chemotherapeutic agent DXR. The relation between an increase in the frequency of twin spots and an increase of the recombinogenic activity was confirmed by the analysis of the BH descendants. In the BH descendants, only mutation and deletion are detected, since every recombination event leads to the nonviability of the flies due to the presence of multiple inversions in the balance chromosome *TM3* (Graf et al., 1984). Results from DXR recombinogenic action in somatic cells of *D. melanogaster* have been reported by Rodriguez-Arnaiz et al. (2004), Costa and Nepomuceno (2006), Fragiorge et al. (2007), and Dutra et al. (2009).

Figure 1 graphically shows the effects of chronic treatment with different concentrations of BC and DXR on the distribution of the spot sizes in the MH descendants from the ST cross. The data indicate that the frequency of all sizes of mutant spots was reduced by cotreatment.

Table 1. Summary of results obtained in the *Drosophila* wing spot test after combined treatments (co-treatment) with doxorubicin (DXR) and β-carotene (BC).

Series			Spots per fly (No. of spots); stat. diagnoses[a]				Spots with *mwh* clone[c]	Mean clone size class	Frequency of clone formation per 10^5 cells[d]		Inhibition[e] (%)
DXR (mg/mL)	BC (mg/mL)	No. of flies	Small single spots (1-2 cells)[b]	Large single spots (>2 cells)[b]	Twin	Total spots			Observed	Control corrected	
mwh/flr³ (MH)											
0	0	40	0.60 (24)	0.03 (01)	0.03 (01)	0.65 (26)	26	1.69	1.33	{0.47}	
0	1	50	0.78 (39) ns	0.10 (05) ns	0.00 (00) ns	0.88 (44) ns	44	1.55 {1.13}	1.80	-{0.06}	
0	2	50	0.50 (25) ns	0.08 (04) ns	0.04 (02) ns	0.62 (31) ns	31	1.94 -{3.33}	1.27	-{0.18}	
0	4	50	0.50 (25) ns	0.02 (01) ns	0.04 (02) ns	0.56 (28) ns	28	1.54 {2.67}	1.15	{0.47}	
0.125	0	20	4.20 (84)+	5.70 (114)+	8.30 (166)+	18.20 (364)+	350	3.51 {3.58}	35.86	{34.53}	
0.125	1	40	1.70 (68)*	1.13 (45)*	1.53 (61)*	4.35 (174)*	171	3.02 {3.26}	8.76	{7.43}	78.5%
0.125	2	40	1.23 (49)*	0.63 (25)*	0.65 (26)*	2.50 (100)*	99	2.81 {3.21}	5.07	{3.74}	89.2%
0.125	4	40	1.03 (41)*	0.23 (9)*	0.23 (9)*	1.48 (59)*	58	2.05 {2.34}	2.97	{1.64}	95.3%
mwh/TM3 (BH)											
0	0	40	0.50 (20)	0.00 (0)		0.50 (20)	20	1.45	1.02		
0.125	0	40	0.65 (26) ns	0.38 (15)+		1.03 (41)+	42	2.40 {3.27}	2.15	{1.13}	
0.125	1	40	0.45 (18) ns	0.10 (4)*		0.55 (22)*	22	1.86 {6.00}	1.13	{0.10}	91.0%
0.125	2	40	0.53 (21) ns	0.00 (0)*		0.53 (21)*	21	1.29 -{2.00}	1.08	{0.05}	95.6%
0.125	4	40	0.40 (16) ns	0.03 (1)*		0.43 (17)*	17	1.29 {2.33}	0.87	-{0.15}	113.3%

Larvae from standard cross. Marker-trans-heterozygous (*mwh/flr³* - MH) and balancer heterozygous (*mwh/TM3* - BH) flies. [a]Statistical diagnoses according to Frei and Würgler (1995): U-test, two-sided, probability levels: $^{+}P \leq 0.05$ *vs* untreated control; $*P \leq 0.05$ *vs* DXR only. ns = not significant. [b]Including rare *flr³* single spots. [c]Considering *mwh* clones from *mwh* single and twin spots. [d]Frequency of clone formation: clones/flies/48,800 cells (without size correction). [e]Calculated according to Abraham (1994): [(genotoxin alone - genotoxin + BC) / genotoxin alone] x 100.

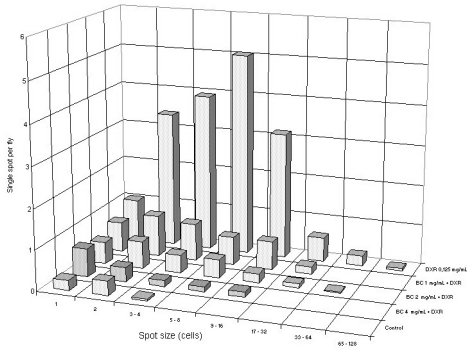

Figure 1. Size distributions for single spots after chronic treatment with different concentrations of β-carotene (BC) and doxorubicin (DXR). Larvae from the ST cross.

The combinations of different concentrations of BC with DXR, yielded a significant reduction, in relation to the DXR control, in all classes of spots of MH individuals, in the frequency of large single spots and in the total spots of BH individuals. There was no significant reduction of small single spots, however, among the three tested concentrations of BC with DXR, among the BH descendants. For the MH descendants, the reduction in the frequency of BC spots, when used with DXR, was 78.5% for 1 mg/mL, 89.2% for 2 mg/mL and 95.3% for 4 mg/mL. For the BH descendants, the reduction was 91.0% for 1 mg/mL, 95.6% for 2 mg/mL and 113.3% for 4 mg/mL.

The reduction of spots in BH descendants, in relation to MH, permitted the calculation of the mutation and recombination rates. For calculation of the recombination frequency the following formula was used: mutation frequency = a/b [a = frequency of *mwh* clones (x 10^5 cells) in the BH individuals, b = frequency of *mwh* clones (x 10^5 cells) in the MH individuals]; recombination frequency = 1 - mutation frequency.

The mutation and recombination percentages, observed in MH and BH individuals treated with DXR (0.125 mg/mL), demonstrated for the DXR control, 6% mutation, and 94% recombination. For the analysis of BC at the concentrations of 1, 2 and 4 mg/mL with DXR, there were significant reductions in BH descendants, indicating a significant reduction in mutagenic activity.

It is known that DXR induces peroxide production in various tissues. Cellular enzymes are capable of converting DXR into free radical metabolites. DXR cytotoxicity may be mediated by free radicals derived from this drug (Quiles et al., 2002). The mechanisms by which BC inhibited the genotoxicity of DXR were not directly analyzed. However, since DXR generates free radicals and produces DNA damage (Keizer et al., 1990; Quiles et al., 2002), it is suggested that there are mechanisms by which BC may protect against the genotoxicity of DXR. Costa and Nepomuceno (2006) and Fragiorge et al. (2007) suggested a similar mechanism to account for a reduction in mutant spot frequencies in the larvae of *D. melanogaster* that were treated with DXR plus vitamin antioxidants.

Costa and Nepomuceno (2006) evaluated the antigenotoxic effects of a mixture of vitamins (vitamins C and E and β-carotene) and minerals (copper, selenium and zinc), known commercially as Vitergan® Zinc Plus. The authors used doses of 12.5, 25, and 50 mg/mL of the mul-

tivitamins/minerals capsule. These authors showed that the mutation frequency induced by DXR decreased to 63.0% (with 25 mg/mL multivitamins) and 75.0% (with 50 mg/mL multivitamins). However, in the present study, we found a significant reduction in mutagenic activity induced by DXR by 91% (for 1 mg/mL), 95.6% (for 2 mg/mL), and 113.27% (for 4 mg/mL). Similar to the study with Vitergan® Zinc Plus, our results also showed a reduction in all types of spots relative to the frequencies of spots produced by DXR alone. Therefore, these results suggest that supplements of multivitamins with much higher doses were not essential in reducing the genotoxicity of DXR when compared with low doses (1, 2, and 4 mg/mL) of BC alone. BC alone was sufficient to inhibit the genotoxicity of DXR. We suggest that the supplement of BC alone mitigates the side effects associated with antiblastic drugs and has a beneficial effect on the course of treatment.

As the cotreatment of BC was carried out with DXR, a desmutagenic action of BC can be proposed. This desmutagenic action is characterized by the chemical or enzymatic inactivation of the genotoxic agent, inhibiting metabolic activation of promutagens or by sequestration of reactive molecules (Kada et al., 1982). Thus, it is possible that BC inactivated DXR, or its metabolic products, the free radicals generated in the transformation of this genotoxic agent. Trekli et al. (2003) found similar results of desmutagenic action in studies with human cells. In that study, BC, under appropriate conditions, acted as a powerful antioxidant. Picada et al. (2003) suggest that carotenoids have a stabilizing effect on simple oxygen molecules by transferring their energy to the antioxidant molecule. The possibility of a desmutagenic action mechanism, by a compound of antioxidant vitamins (E, C, and β-carotene), against the genotoxicity of DXR in *D. melanogaster* has also been examined by Costa and Nepomuceno (2006). The direct interactions between BC and DXR or their metabolites are possible, and a desmutagenic mode of action for BC is a plausible mechanism. However, Konopacka et al. (1998) showed that the radio-protective effects of vitamins C and E, as well as BC, depend on the treatment concentration, and are not only due to the capturing of free radicals but also to an increase in DNA repair. The results of this study showed that oral administration of vitamins C and E and BC protected against micronucleus induction caused by exposure of mice to gamma rays.

Another explanation for the reduction of mutant spots in the treatments combining DXR with BC could be that the cytochrome P-450 enzymes are inhibited by BC causing a reduction in the bioactivity of DXR. Although DXR is considered to be a direct-acting mutagen, it requires metabolic reduction of its quinine ring to a semiquinone radical to induce toxicity (Ramji et al., 2003). De Flora et al. (1999), in a review, noted that most of the data were consistent with the conclusion that the major mechanism involved in the antigenoxicity of carotenoids and vitamin A is interference with the metabolism of xenobiotics, by inhibition of the cytochrome P-450 system. Gülkaç et al. (2004) suggested a similar mechanism to account for a reduction in chromosomal aberrations in bone marrow cells of rats that were treated with DXR and vitamin A.

Finally, it can be concluded, based on the results and on the experimental conditions mentioned in this study, that BC in the concentrations used is not mutagenic, and that it actually exerts a protective effect against the genotoxic action of the chemotherapeutic agent DXR (0.125 mg/mL), as tested using *Drosophila melanogaster*.

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REFERENCES

Abraham SK (1994). Antigenotoxicity of coffee in the *Drosophila* assay for somatic mutation and recombination. *Mutagenesis* 9: 383-386.

Ames BN, Shigenaga MK and Hagen TM (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U. S. A.* 90: 7915-7922.

Bast A and Haenen GRMM (2002). The toxicity of antioxidants and their metabolites. *Environ. Toxicol. Pharmacol.* 11: 251-258.

Benchekroun MN, Sinha BK and Robert J (1993). Doxorubicin-induced oxygen free radical formation in sensitive and doxorubicin-resistant variants of rat glioblastoma cell lines [corrected and republished erratum originally printed in FEBS *Lett*. 1993 May 17;322 (3): 295-8]. *FEBS Lett.* 326: 302-305.

Burri BJ (1997). β-carotene and human health: a review of current research. *Nutr. Res.* 17: 547-580.

Costa WF and Nepomuceno JC (2006). Protective effects of a mixture of antioxidant vitamins and minerals on the genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. *Environ. Mol. Mutagen.* 47: 18-24.

Cui Y, Lu Z, Bai L, Shi Z, et al. (2007). β-carotene induces apoptosis and up-regulates peroxisome proliferator-activated receptor gamma expression and reactive oxygen species production in MCF-7 cancer cells. *Eur. J. Cancer* 43: 2590-2601.

De Flora S, Bagnasco M and Vainio H (1999). Modulation of genotoxic and related effects by carotenoids and vitamin A in experimental models: mechanistic issues. *Mutagenesis* 14: 153-172.

Dhawan A, Kayani MA, Parry JM, Parry E, et al. (2003). Aneugenic and clastogenic effects of doxorubicin in human lymphocytes. *Mutagenesis* 18: 487-490.

Dutra ES, Dias CD, Araújo BC, Castro AJS, et al. (2009). Effect of organic tomato (*Lycopersicon esculentum*) extract on the genotoxicity of doxorubicin in the *Drosophila* wing spot test. *Genet. Mol. Biol.* 32: 133-137.

Fragiorge EJ, Spanó MA and Antunes LMG (2007). Modulatory effects of the antioxidant ascorbic acid on the direct genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. *Genet. Mol. Biol.* 30: 449-455.

Frei H and Würgler FE (1988). Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative, or inconclusive result. *Mutat. Res.* 203: 297-308.

Frei H and Würgler FE (1995). Optimal experimental design and sample size for the statistical evaluation of data from somatic mutation and recombination tests (SMART) in *Drosophila*. *Mutat. Res.* 334: 247-258.

Gandini S, Merzenich H, Robertson C and Boyle P (2000). Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. *Eur. J. Cancer* 36: 636-646.

George B, Kaur C, Khurdiya DS and Kapoor HC (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Food Chem.* 84: 45-51.

Gradelet S, Astorg P, Le Bon AM, Berges R, et al. (1997). Modulation of aflatoxin B1 carcinogenicity, genotoxicity and metabolism in rat liver by dietary carotenoids: evidence for a protective effect of CYP1A inducers. *Cancer Lett.* 114: 221-223.

Graf U, Wurgler FE, Katz AJ, Frei H, et al. (1984). Somatic mutation and recombination test in *Drosophila melanogaster*. *Environ. Mutagen.* 6: 153-188.

Gülkaç MD, Akpinar G, Ustun H and Ozon KA (2004). Effects of vitamin A on doxorubicin-induced chromosomal aberrations in bone marrow cells of rats. *Mutagenesis* 19: 231-236.

Guruvayoorappan C and Kuttan G (2007). β-carotene down-regulates inducible nitric oxide synthase gene expression and induces apoptosis by suppressing bcl-2 expression and activating caspase-3 and p53 genes in B16F-10 melanoma cells. *Nutr. Res.* 27: 336-342.

Guzmán-Rincón J and Graf U (1995). *Drosophila melanogaster* Somatic Mutation and Recombination Test as a Biomonitor. In: Biomonitors and Biomarkers as Indicators of Enviromental Change (Butterworth FM, Corkun LD and Guzmán-Rincón J, eds.). Plenum Press, New York, 169-181.

He Y, Root MM, Parker RS and Campbell TC (1997). Effects of carotenoid-rich food extracts on the development of preneoplastic lesions in rat liver and on *in vivo* and *in vitro* antioxidant status. *Nutr. Cancer* 27: 238-244.

Islaih M, Halstead BW, Kadura IA, Li B, et al. (2005). Relationships between genomic, cell cycle, and mutagenic responses of TK6 cells exposed to DNA damaging chemicals. *Mutat. Res.* 578: 100-116.

Kada T, Inoue T and Namiki M (1982). Environmental Desmutagens and Antimutagens. In: Environmental Mutagenesis, Carcinogenesis and Plant Biology (Klekowski EJ Jr, ed.). Praeger, New York, 133-152.

Keizer HG, Pinedo HM, Schuurhuis GJ and Joenje H (1990). Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. *Pharmacol. Ther.* 47: 219-231.

Konopacka M and Rzeszowska-Wolny J (2001). Antioxidant vitamins C, E and β-carotene reduce DNA damage before as well as after gamma-ray irradiation of human lymphocytes *in vitro*. *Mutat. Res.* 491: 1-7.

Konopacka M, Widel M and Rzeszowska-Wolny J (1998). Modifying effect of vitamins C, E and β-carotene against

gamma-ray-induced DNA damage in mouse cells. *Mutat. Res.* 417: 85-94.
Krinsky NI (1991). Effects of carotenoids in cellular and animal systems. *Am. J. Clin. Nutr.* 53: 238S-246S.
Lehmann M, Franco A, de Souza Prudente V, Lukza RM, et al. (2003). Doxorubicin and two of its analogues are preferential inducers of homologous recombination compared with mutational events in somatic cells of *Drosophila melanogaster*. *Mutat. Res.* 539: 167-175.
Menegola E, Broccia ML and Renzo FD (2001). Teratogenic effects of doxorubicin in rats at midgestation and at term. *Teratog. Carcinog. Mutagen.* 21: 283-293.
Moreno FS, Wu TS, Penteado MV, Rizzi MB, et al. (1995). A comparison of β-carotene and vitamin A effects on a hepatocarcinogenesis model. *Int. J. Vitam. Nutr. Res.* 65: 87-94.
Oliveira GS, Figueiredo ASP, Santos RS and Vianna LM (2007). Effect of β-carotene supplementation on the blood pressure of rats. [Efeito da suplementação de beta-caroteno na pressão arterial de ratos]. *Rev. Nutr.* 20: 39-45.
Peto R, Doll R, Buckley JD and Sporn MB (1981). Can dietary β-carotene materially reduce human cancer rates? *Nature* 290: 201-208.
Picada JN, Kern AL, Ramos LLP and Saffi J (2003). O Extresse Oxidativo e as Defesas Antioxidantes. In: Genética Toxicológica (Silva J, Erdtmann B and Henriques JAP, eds.). Alcance, Porto Alegre, 251-268.
Quiles JL, Huertas JR, Battino M, Mataix J, et al. (2002). Antioxidant nutrients and adriamycin toxicity. *Toxicology* 180: 79-95.
Ramji S, Lee C, Inaba T, Patterson AV, et al. (2003). Human NADPH-cytochrome p450 reductase over expression does not enhance the aerobic cytotoxicity of doxorubicin in human breast cancer cell lines. *Cancer Res.* 63: 6914-6919.
Riboli E and Norat T (2003). Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am. J. Clin. Nutr.* 78: 559S-569S.
Rodriguez-Arnaiz R, Sortibran AC and Tellez GO (2004). Detection of mitotic recombination and sex chromosome loss induced by adriamycin, chlorambucil, demecolcine, paclitaxel and vinblastine in somatic cells of *Drosophila melanogaster in vivo*. *Mutagenesis* 19: 121-127.
Salvadori DM, Ribeiro LR, Oliveira MD, Pereira CA, et al. (1992). β-carotene as a modulator of chromosomal aberrations induced in mouse bone marrow cells. *Environ. Mol. Mutagen.* 20: 206-210.
Santos HS and Cruz WMS (2001). The antioxidant vitamin nutritional therapy and the chemotherapy treatment in oncology. [A terapia nutricional com vitaminas antioxidantes e o tratamento quimioterápico oncológico]. *Rev. Bras. Cancerol.* 47: 303-308.
Silva CRM and Naves MMV (2001). Vitamin supplementation in cancer chemoprevention. [Suplementação de vitaminas na prevenção de câncer]. *Rev. Nutr.* 14: 135-143.
Tallaj JA, Franco V, Rayburn BK, Pinderski L, et al. (2005). Response of doxorubicin-induced cardiomyopathy to the current management strategy of heart failure. *J. Heart Lung Transplant.* 24: 2196-2201.
Trekli MC, Riss G, Goralczyk R and Tyrrell RM (2003). β-carotene suppresses UVA-induced HO-1 gene expression in cultured FEK4. *Free Radic. Biol. Med.* 34: 456-464.
Zhang P and Omaye ST (2001). Antioxidant and prooxidant roles for β-carotene, α-tocopherol and ascorbic acid in human lung cells. *Toxicol. In Vitro* 15: 13-24.