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Beta-Carotene as a Modulator of Chromosomal Aberrations Induced in Mouse Bone Marrow Cells

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The inhibitory effects of β-carotene on cyclophosphamide (CPA)-induced chromosomal aberrations in mouse bone marrow cells were investigated. Male Balb C mice, 8–10 weeks old, were treated with β-carotene (0.5, 1.0, 2.0, 5.0, 10, 25, 50, 100, and 200 mg/kg) or with corn oil (0.05 ml/10 g b.w.) by gavage for 5 consecutive days. Four hours after the last treatment with or without β-carotene, the animals were intraperitoneally injected with CPA and killed 24 hr later for cytological preparations and analysis. The results obtained show that β-carotene provides significant protection against the clastogenicity of CPA. The maximum reduction in the frequency of aberrant metaphases (26.9%) and in total number of chromosomal aberrations were observed when β-carotene was used at 50 mg/kg. Nevertheless, no direct dose-response relationship was detected, suggesting that β-carotene might act through different mechanisms at differents doses. The results obtained in animals studies have served as part of the basis for the human intervention studies now underway to determine if β-carotene does indeed function as a chemopreventive agent in human nutrition.

Key words: cyclophosphamide, antimutagenic, clastogen, human nutrition

INTRODUCTION

Recently, β-carotene has received attention as a possible antimutagen, based on studies in bacteria, rodents, and human populations. Several papers have reported that β-carotene prevents chromosomal damage caused by mutagenic agents, including mutagenicity in a *Salmonella typhimurium* strain (Belisario et al., 1985), sister chromatid exchanges in mouse mammary cells (Manoharan and Banerjee, 1985), chromosome aberrations and micronuclei (Renner, 1985, 1990; Mukherjee et al., 1991), and micronuclei formation in the buccal mucosa of betel quid chewers (Stich et al., 1988).

The properties of β-carotene as an efficient antioxidant, its ability to trap organic free radical molecules, and its ability to be converted into vitamin A make it a desirable dietary prophylactic agent, particularly in chemical carcinogenesis. Nevertheless, there have been relatively few studies with β-carotene compared with the larger number of experiments dealing with vitamin A in carcinogenesis. Since β-carotene occurs naturally in fruits and vegetables, this provitamin appears important in accounting, in part, for the protective action of fruits and vegetables. Abraham et al. (1986) and Darroudi et al. (1988) have reported, for example, the antimutagenicity of carrot juice in both in vitro and in vivo tests. Carrot was chosen since it is one of the main source of β-carotene.

Because of its lack of toxicity, β-carotene is a very attractive agent for chemoprevention. In the present study we thus investigated the effects of some concentrations of pure β-carotene on cyclophosphamide (CPA)-induced chromosomal aberrations in mouse bone marrow cells.

MATERIAL AND METHODS

Eight- to 10-week-old male Balb C mice, obtained from our own colony, were used in this study. Animals were kept in temperature- (20–25°C) and humidity- (40–60%) controlled conditions with a 12-hr light-dark cycle and were maintained on mouse feed (Nuvital, Nuvilab) and water ad libitum until treatment.

β-carotene (beta-carotene 30%, Roche, Brazil) dissolved in corn oil was administered by gavage (0.05 ml/10 g body weight) for 5 consecutive days. Cyclophosphamide (Enduxan, Pravaz) was dissolved in distilled water and injected intraperitoneally (i.p) at 20 mg/kg b.w. 4 hr after the last treatment with β-carotene.

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The animals were divided into 13 groups. A negative control group received just corn oil (0.05 ml/10 g b.w.) by gavage and a positive control received just CPA (20 mg/kg), i.p. The concurrent control animals were treated with corn oil for 5 consecutive days and with CPA 4 hr after the last treatment with oil. Experimental groups were divided based on the concentration of β-carotene (0.5, 1.0, 2.0, 5.0, 10, 25, 50, 100, and 200 mg/kg b.w.), and each group consisted of 10 animals receiving β-carotene plus CPA. A group receiving only the highest concentration of β-carotene was also evaluated.

Two hours prior to sampling, colchicine was administered i.p. to each animal. The animals were killed by cervical dislocation 24 hr after the treatment with CPA, and one femur from each animal was dissected and stripped clean of muscles for cytological preparations (Hsu and Patton, 1969). The slides were stained in 10% aqueous Giemsa solution, and 100 bone marrow metaphases cells from each animal were scored under code. The types of chromosomal aberrations considered were chromatid and chromosome gaps, breaks and fragments, exchanges, and pulverization (severely damaged cells). Data were evaluated as the percentage of aberrant metaphase cells and as the total number of aberrations per 1000 cells. For the mitotic index, 1,000 cells per animal were scanned.

The reduction factor due to β-carotene treatment was calculated using the formula:

$$\% \text{ reduction} = \frac{\left(\begin{array}{c}\text{aberrant cells}\\ \text{in control}\end{array}\right) - \left(\begin{array}{c}\text{aberrant cells in}\\ \beta\text{-carotene}\\ + \text{CPA}\end{array}\right)}{\left(\begin{array}{c}\text{aberrant cells}\\ \text{in control}\end{array}\right) - \left(\begin{array}{c}\text{aberrant cells in}\\ \text{negative}\\ \text{controls}\end{array}\right)} \times 100.$$

For analysis of significant differences in aberration frequencies between control (corn oil plus CPA) and the groups treated with β-carotene plus CPA, the X^2 (chi-square) test was applied (Pereira, 1991).

RESULTS

The frequencies of aberrant cells, as well as the types of aberrations and the frequency of chromosomal aberrations per 1,000 cells analysed, observed in the experiments are presented in Tables I and II, respectively. Because a statistically significant difference was not observed between the analysis including or excluding gaps, the results are presented considering all the chromosomal alterations observed in the cell.

Data in Table I clearly show that β-carotene is capable of causing a significant decrease of cells with chromosomal aberrations induced by CPA. This observation, however, shows no direct dose-response relationship for the concentrations of β-carotene tested. Although no statistically significant differences were observed among the effective con-

TABLE I. Effect of β-Carotene on the Frequency of Cells With Chromosome Aberrations Induced by Cyclophosphamide (20 mg/kg)

Treatment	β-carotene (mg/kg)	Number of pulverized cells	Types of chromatid aberrations				% Aberrant cells (mean ± SEM)	Reduction (%)	Mitotic index (mean ± SEM)
			Gaps	Breaks	Fragments	Exchanges			
Oil[a]	0	0	1	0	11	0	1.1 ± 0.32	—	1.50 ± 0.12
CPA[b]	0	9	36	25	141	2	12.7 ± 1.05	—	1.45 ± 0.12
CPA + oil[c]	0	7	36	30	161	5	13.0 ± 1.06	—	1.35 ± 0.11
β-carotene	200	0	0	1	6	0	0.7 ± 0.26	—	1.53 ± 0.12
CPA + β-carotene	0.5	3	32	12	97	0	9.9 ± 0.94[d]	26.0	1.51 ± 0.12
	1.0	4	42	13	102	1	11.2 ± 0.99[d]	15.1	1.67 ± 0.13
	2.0	5	37	14	97	0	10.0 ± 0.94[d]	25.2	1.41 ± 0.11
	5.0	8	43	20	140	2	11.7 ± 1.01[d]	10.9	1.36 ± 0.11
	10	8	35	18	139	2	11.2 ± 0.99[d]	15.1	1.56 ± 0.12
	25	7	36	15	102	1	9.9 ± 0.94[d]	26.0	1.53 ± 0.12
	50	3	21	20	90	4	9.8 ± 0.94[d]	26.9	1.57 ± 0.12
	100	6	24	24	156	5	13.5 ± 1.08	—	1.37 ± 0.11
	200	8	37	13	170	1	13.5 ± 1.08	—	1.51 ± 0.12

1,000 cells from 10 animals were analysed for each point.
[a]Negative control.
[b]Positive control—cyclophosphamide.
[c]Control.
[d]Significant at the 5% level ($P < 0.05$).

TABLE II. Effect of β-Carotene on the Distribution and Frequency of Chromosome Aberrations Induced by Cyclophosphamide (20 mg/kg)

Treatment	β-carotene (mg/kg)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6–9	
Oil[a]	0	989	10	1	0	0	0	0	12
CPA[b]	0	873	73	26	8	5	3	3	204
CPA + oil[c]	0	870	72	25	9	9	3	5	233
β-carotene	200	993	7	0	0	0	0	0	7
CPA + β-carotene	0.5	901	72	14	4	2	3	1	141[d]
	1.0	888	77	18	9	3	0	1	158[d]
	2.0	900	64	21	4	4	0	2	148[d]
	5.0	883	62	22	13	4	4	4	205
	10	888	61	21	10	6	3	3	194
	25	901	62	16	5	4	2	3	154[d]
	50	902	69	16	6	4	0	0	135[d]
	100	865	82	28	10	7	1	1	209
	200	865	80	23	12	3	7	2	221

1,000 cells from 10 animals were analysed for each point.
[a]Negative control.
[b]Positive control—cyclophosphamide.
[c]Control.
[d]Significant at the 5% level ($P < 0.05$).

centrations of β-carotene (0.5, 1.0, 2.0, 5.0, 10, 25, and 50 mg/kg), the greatest reduction (26.9%) in the frequency of aberrant metaphases was observed with 50 mg/kg of β-carotene. On the other hand, no reduction was detected for the two higher concentrations (100 and 200 mg/kg) tested.

Regarding the frequencies of chromosomal aberrations (Table II), a statistically significant decrease was observed only for 5 concentrations of β-carotene (0.5, 1.0, 2.0, 25, and 50 mg/kg). Thus, the two higher concentrations used (100 and 200 mg/kg), together with the two intermediates (5 and 10 mg/kg), did not induce a significant decrease in the frequency of chromosomal aberrations.

The data did not show any significant difference in clastogenicity when the positive control (treated just with CPA) was compared with the control group (oil plus CPA). The same result can also be observed between the negative control (corn oil) and the group exposed to the highest concentration of β-carotene used.

No statistically significant difference was observed in the distribution of damage among cells in the control group when compared with those groups treated with CPA plus β-carotene, i.e., a preferential reduction of aberrations was not observed in cells with more or less damage (Table II).

The mitotic index did not show any statistically significant difference between all treated groups and controls.

DISCUSSION

Several investigators have attempted to assess the hypothesis that β-carotene might function to reduce cancer rates. Although not yet proven, this hypothesis has generated a great deal of work with respect to the possible role of β-carotene as either an anticarcinogenic or an antimutagenic compound.

It is clear from the data presented here that β-carotene reduced the clastogenicity of CPA. Nevertheless, its modulatory action was not dose dependent; in other words, no direct dose-response relationship was observed.

According to De Flora and Ramel (1988), prevention of mutagenesis may be achieved at the metabolic stage, either by inhibiting those biochemical mechanisms that are responsible for the activation of promutagens to electrophilic metabolites, by stimulating the enzymatic detoxification of chemicals, or, since the mechanisms are quite often competitively or sequentially involved, by shifting their balance in favor of detoxification. Thus, as CPA is an indirect-acting mutagen, alterations affecting the process of enzymatic activation might modify the formation of its ultimate mutagen metabolite.

Masuda et al. (1990) reported that the CPA-induced SCEs were substantially lower in rats predisposed to hereditary hepatitis and liver cancer, which exhibited significantly reduced cytochrome P-450 contents and monooxygenase activities.

The effects of dietary supplements of β-carotene on hepatic microsomal drug metabolizing enzyme activities were studied in mice by Basu et al. (1987). Supplementation for 14 days resulted in a marked reduction in the concentration of biphenyl 4-hydroxylase and cytochrome P-450, the terminal oxygenase for the mixed-function oxidase system.

Darroudi et al. (1988) observed that carrot juice, and possibly β-carotene, may have an inhibitory effect on the genotoxic activity of CPA, possibly by interfering with the enzymatic process of its activation. In addition to the ability of β-carotene to affect metabolic activation, the anticlastogenic activity of this carotenoid may also be due to its antioxidative property, which eliminates free radicals by formation of complexes with one of its double bonds (Burton and Ingold, 1984). Many authors have attributed the antimutagenicity/anticarcinogenicity of β-carotene to its capacity to inactivate electronically excited molecules (Ames, 1983; Malone, 1991; Singh and Gaby, 1991).

Mukherjee et al. (1991), using 4 mice per concentration and 2 concentrations (2.7 and 27 mg/kg) of β-carotene for 7 days, reported a significant dose-related efficiency of β-carotene in reducing chromosomal aberrations induced by CPA in mice bone marrow cells in vivo. Nevertheless, they observed that β-carotene alone (27 mg/kg) showed significantly higher clastogenicity than the negative control. In our experiments no statistically significant difference was observed between the negative control group and that exposed just to β-carotene (200 mg/kg).

Although Renner (1985) did not observe an inhibitory effect of β-carotene on CPA-induced chromosome aberrations, this result is in agreement with our result, since we also did not find an anticlastogenic effect when doses above 100 mg/kg of β-carotene were used.

In a previous study conducted in our laboratory (Salvadori et al., 1992) using the same methodology as that described here, but another β-carotene formulation (water soluble, WS) at concentrations of 5, 10, 25, 50, 100, and 200 mg/kg, we also observed that β-carotene was effective in reducing chromosomal damage induced by CPA. However, in agreement with the present results, no dose-response relationship was observed. On the other hand, 7 concentrations of β-carotene under the formulation used in the present study (soluble in oil) were active in reducing the frequency of cells with chromosomal aberrations, while only 2 concentrations of β-carotene WS tested (10 and 25 mg/kg) were effective. This difference could be attributed to a better absorption of β-carotene when dissolved in oil.

The absence of a protective effect at the 2 highest concentrations of β-carotene observed in the present study might be due to the fact that at low levels of β-carotene intake the efficiency of absorption and utilization is fairly good and the conversion efficiency of β-carotene to vitamin A decreases tremendously at higher levels (Erdman, 1988).

In conclusion, before the potential benefits of β-carotene in humans can be established more experimental studies must be conduced to elucidate its antimutagenic and anticarcinogenic mechanisms, as well as the best strategies (doses and formulations) for its use as a chemopreventive agent.

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