Beta-carotene prevents x-ray induction of micronuclei in human lymphocytes\textsuperscript{1,2}

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ABSTRACT The effects of \( \beta \)-carotene and ascorbic acid on spontaneous and x-ray-induced appearance of micronuclei (MNs) in human lymphocytes were studied. For 12 d, three groups of healthy volunteers were given \( \beta \)-carotene-deficient meals containing 100 mg ascorbic acid. There was no supplementation in the first 6 d but, in the last 6 d, the respective groups were given \( \beta \)-carotene (30 mg), ascorbic acid (300 mg), or placebo. Blood samples were drawn on days 7 and 13 before breakfast, exposed either to x-ray irradiation or left unexposed and were cultured. Lymphocytes containing MNs were then counted. On day 7 the three groups showed comparable MN frequencies. On day 13 lymphocytes containing x-ray-induced MNs became less frequent in the \( \beta \)-carotene but not the ascorbic acid group. Both before and after the supplementation, the MN frequency of irradiated lymphocytes showed a significant inverse correlation with plasma \( \beta \)-carotene. These results strongly suggest that \( \beta \)-carotene protects human lymphocytes from x-ray-induced genetic damage. \textit{Am J Clin Nutr} 1994;59:409–12.

KEY WORDS \( \beta \)-Carotene, vitamin C, micronuclei, x-ray irradiation, lymphocytes

Introduction

A large body of epidemiologic studies suggests that intake of antioxidant nutrients, especially \( \beta \)-carotene, has a protective effect against the development of cancer at various sites (1–4). On the other hand, accumulating evidence has demonstrated that oxygen radicals are involved in mutation, tumor promotion, and cancer (5–9). The oxygen radicals cause damage to protein, lipids, and DNA. DNA damage is directly associated with mutation and cancer. In fact, oxidative DNA damage after the formation of oxygen radicals was demonstrated when cells or animals were treated with radiation or carcinogenic chemicals (7–10). Antioxidant nutrients such as vitamin C, vitamin E, and \( \beta \)-carotene are thought to give protection against such oxidative damage.

To further the understanding of the relationship between antioxidants and the incidence of cancer, many experiments have been performed. These studies suggest that antioxidant nutrients inhibit chromosomal damage, tumor promotion, cell transformation, and cancer induced by chemical carcinogens (11–13) or radiation (14, 15). Most of these studies have used rodents as experimental animals. Because rodents do not require dietary ascorbic acid and do not accumulate \( \beta \)-carotene in their tissues (16), it seems difficult to evaluate the effect of \( \beta \)-carotene and ascorbic acid in such experiments. Little is known about whether the supplementation of \( \beta \)-carotene or ascorbic acid gives protection against chromosomal damage in humans.

In this study we examined the effect of \( \beta \)-carotene or ascorbic acid supplementation on micronucleus (MN) frequencies in the lymphocytes of healthy volunteers, whose blood samples were cultured after being either irradiated with a low dose of x-rays in vitro or left unexposed. MNs represent chromosome fragments or whole chromosomes that have not been incorporated into the main nuclei during mitosis and are indicative of chromosomal damage. The MNs were examined by the cytochalasin B blocked method, which has been shown to be a simple, reliable, and sensitive method for the detection of radiation-induced chromosomal damages in lymphocytes (17–19).

Subjects and methods

Materials

Beta-carotene and ascorbic acid were obtained from Nippon Roche KK (Tokyo) and Iwaki Pharmaceutical Co (Tokyo), respectively. Fetal bovine serum, RPMI 1640 medium, antibiotics (penicillin 10 000 MU/L, streptomycin 10 000 \( \mu \)g/L), and phytohemagglutinin were purchased from GIBCO (Grand Island, NY). Giemsa’s staining solution was from Merck (Darmstadt, Germany), and cytochalasin B was from Sigma (St Louis).

Human subjects and blood sampling

The volunteers were 17 nonsmoking, healthy female students aged 20–21 y. They lived in a dormitory and were served meals that were \( \beta \)-carotene deficient and contained \( \approx 100 \) mg ascorbic acid throughout the 12 d of the experimental period. The meals contained 33% fat and the calculated energy was \( \approx 7.95 \) MJ (1900 kcal). According to their heights and weights, 5 to 6 women were placed into three groups according to the supplementation they would receive during the last 6 d: placebo, \( \beta \)-carotene, or ascorbic acid. The average weight was 52 kg and


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 Analyses of α-tocopherol, ascorbic acid, β-carotene, and retinol in plasma

Heparinized whole blood was centrifuged at 1500 X g for 15 min at 4 °C and the plasma was isolated. For the analysis of ascorbic acid, the aliquot of plasma (0.5 mL) was immediately mixed with an equal volume of 30% metaphosphoric acid. These samples were stored at −80 °C until analyzed. Alpha-tocopherol and retinol in the plasma were analyzed by reversed-phase HPLC according to Vuilleumier et al (20). Beta-carotene in the plasma was assayed by the HPLC method of Nells and Leenheer (21). Ascorbic acid was analyzed by the hydrazine method (22).

Statistical analysis

Data were analyzed with a repeated-measures analysis of variance and post hoc test. The Pearson correlation coefficient was used to assess the relationship between MN frequencies in lymphocytes and plasma vitamin concentration(s). These statistical analyses were done by using the SYSTAT computer program (Intelligent Software, Evanston, IL).

Results

Plasma concentrations of β-carotene, ascorbic acid, retinol, and α-tocopherol are shown in Fig 1. Before the supplementation of β-carotene or ascorbic acid, the plasma concentrations of these vitamins were not different among the groups. After the supplementation of ascorbic acid or β-carotene for 6 d, the plasma concentration of each vitamin was significantly elevated only in the respectively supplemented groups. Beta-carotene is a precursor of retinol; however, the supplementation did not influence the plasma retinol concentration, which was well maintained.

The appearance of MNs in lymphocytes with or without irradiation was examined before and after the supplementation (Fig 2). The spontaneous MN frequencies of lymphocytes were ≈2%, and increased to ≈7% by a low dose (0.6 Gy) of x irradiation. Before the supplementation of β-carotene or ascorbic acid, both spontaneous and x-irradiated MN frequencies of the cells did not differ among the three groups. In contrast, the supplementation

Lymphocyte culture, slide preparation, and scoring

Heparinized peripheral blood from each volunteer was divided into two parts (0.4 mL each) in culture tubes; one sample (non-irradiated) was analyzed for spontaneous MN formation and the other (x-ray irradiated) for MN formation after irradiation. X-ray irradiation was performed by using a soft x-ray unit (model OM-150RS; Ohmic Inc, Tokyo) for 2 min at the dose rate of 0.3 Gy/min. The x-ray beam was filtered through 0.1 mm Cu and 0.2 mm Al filter. After the irradiation the blood was cultured immediately as follows.

To the whole blood (0.4 mL), 4.5 mL RPMI 1640 culture medium supplemented with 15% fetal calf serum and 1% antibiotics was added, and then the cultures were initiated by adding 0.1 mL phytohemagglutinin. Cytochasin B (final concentration 3 μg/L) was added at 44 h to induce binucleated cells according to the method of Fenech and Morley (17). The cells were harvested at 72 h, treated with hypotonik solution (0.1 mol KCl/L) by the method of Balasem and Ali (19) for 3 min, fixed in two changes of methanol-acetic acid (3:1), and finally suspended in methanol containing 1% acetic acid. The cells were spread onto glass slides and stained with 4% Giemsa for 30 min. The slides were coded and scored blind at 1000× magnification. At least 500 binucleated cells with preserved cytoplasm were scored for each culture. Percentages of lymphocytes with MNs in the binucleated cells were calculated from each culture.

FIG 1. Concentrations of various vitamins in plasma before and after supplementation with β-carotene (30 mg/d) or ascorbic acid (300 mg/d). P, placebo group (n = 6); β, β-carotene-supplemented group (n = 6); C, ascorbic acid-supplemented group (n = 5). Vertical bars indicate x ± SEM. **P < 0.01, *P < 0.05.
of β-carotene tended to decrease the x-irradiated MN frequencies.

The relationship between MN frequencies in lymphocytes and the plasma concentration of β-carotene, retinol, ascorbic acid, or α-tocopherol was examined by using pooled data (Table 1). None of the vitamins in plasma correlated with the spontaneous MN frequencies in lymphocytes either before or after the supplementation. In contrast, a significant ($P < 0.01$) negative correlation between the MN frequencies of the irradiated samples and β-carotene in plasma was found both before and after the supplementation. A significant ($P < 0.01$) negative correlation was also observed between MN frequencies of the irradiated samples and α-tocopherol in plasma before the supplementation. Correlation-coefficient analysis was also performed on the data pooled from before and after the supplementation. As shown in Fig 3, a significant negative correlation ($P < 0.001$) was noted between the MN frequencies in the irradiated lymphocytes and the β-carotene concentration in plasma, whereas no such relationship was observed between the other vitamins in plasma and MN frequencies in lymphocytes with and without irradiation.

**Discussion**

Epidemiological evidence suggests that intake of antioxidant nutrients, especially β-carotene, has a preventive effect on the development of cancer at various sites (1–4). On the other hand, accumulated evidence indicates that oxygen radicals are involved in many biological events, such as tumor promotion, cancer, and aging (5–9). Therefore, it would be interesting to know how the supplementation of antioxidant nutrients influences MN frequencies, which are indicative of chromosomal damage in human cells exposed to radiation, which damages DNA by the formation of oxygen radicals (9, 10).

In this study, supplementation of β-carotene significantly increased the plasma β-carotene concentration and tended to inhibit MN frequencies in irradiated lymphocytes (Figs 1 and 2). In addition, a significant negative correlation was noted between MN frequencies of irradiated lymphocytes and plasma concentration of β-carotene when pooled data were analyzed both before and after supplementation (Table 1 and Fig 3). Interestingly, such a correlation was not observed between plasma retinol and MN frequencies in lymphocytes. These results are consistent with the epidemiologic finding that plasma β-carotene, but not retinol, is negatively correlated with the incidence of cancer (3). The present data strongly suggest that supplementation with β-carotene protects against x-ray–irradiated chromosomal damage in humans.

Epidemiologic studies indicate that vitamins C and E also have some cancer preventive effect (1, 2). Vitamins C and E are known to be important antioxidants in the hydrophilic and the lipophilic compartment, respectively. The effect of these two antioxidants on MN frequencies in lymphocytes was not clear in the present experiment. Supplementation with ascorbic acid increased its plasma concentration but did not attenuate both spontaneous MN frequencies in lymphocytes and those in irradiated samples (Fig 2). The correlation-coefficient analyses performed on pooled data demonstrated the lack of correlation between plasma concentration of ascorbic acid and MN frequencies in lymphocytes, regardless of irradiation (Table 1). Plasma concentration of α-tocopherol was negatively correlated with MN frequencies in irradiated lymphocytes only before the supplementation. Further study will be needed to confirm the cancer preventive effect of these two antioxidant nutrients on the MN frequencies in lymphocytes.

The preventive effect of β-carotene on chromosomal damage induced by chemicals has also been reported in rodent bone marrow cells (12, 13) and in human buccal mucosa (23). In these studies it was hard to distinguish whether β-carotene inhibited carcinogen activation or protected against chromosome damage by the activated carcinogen. In contrast, our present experimental system using x-ray irradiation to induce chromosomal damage may simply indicate the preventive effect of β-carotene against chromosomal damage. It has been demonstrated that radiation-induced oxidative damage in DNA results in the formation of oxidatively modified base such as 8-hydroxy-deoxyguanosine and thymidine glycol (9, 10). On the other hand, it has been reported that β-carotene is an excellent antioxidant and free radical scavenger (24, 25) whereas retinol is a relatively poor antioxidant (24). Therefore, it is reasonable to speculate from the present data that the preventive effect of β-carotene on MN formation in irradiated samples is due to the antioxidant action of β-carotene. In cultured mammalian cells, Weitberg et al (26) demonstrated that β-carotene gave protection against oxygen radical–generated chromosome injuries, which were measured by sister chromatid exchanges. Their results are similar to those of our present study.

**TABLE 1**

Coefficient ($r$) analysis between micronucleus (MN) frequencies in lymphocytes and plasma concentrations of vitamins*

<table>
<thead>
<tr>
<th>MN frequencies in lymphocytes</th>
<th>β-Carotene</th>
<th>Retinol</th>
<th>α-Tocopherol</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment ($n = 16$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>-0.192</td>
<td>-0.429</td>
<td>-0.001</td>
<td>-0.348</td>
</tr>
<tr>
<td>X-ray irradiated</td>
<td>-0.707†</td>
<td>-0.322</td>
<td>-0.740†</td>
<td>-0.106</td>
</tr>
<tr>
<td>After treatment ($n = 17$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>-0.408</td>
<td>0.312</td>
<td>-0.181</td>
<td>-0.048</td>
</tr>
<tr>
<td>X-ray irradiated</td>
<td>-0.616†</td>
<td>0.370</td>
<td>-0.006</td>
<td>0.205</td>
</tr>
</tbody>
</table>

*Correlation-coefficient analysis was performed on the pooled data. †$P < 0.01$. 

![FIG 3. Correlation between β-carotene in plasma and micronuclei in irradiated lymphocytes. Correlation–coefficient analysis was performed on the pooled data.](https://academic.oup.com/aajn/article-abstract/59/2/409/4731986/)
It has been reported that β-carotene enhanced immunological function in experimental animals and humans. Schwartz et al (27) reported that β-carotene increased the concentration of tumor necrosis factor in macrophages. Alexander et al (28) reported that oral supplementation with β-carotene increased the number of OKT4+ cells (helper-inducer T cells) in human subjects. Seifter et al (14) reported that β-carotene reduced the morbidity and mortality of mice who received total-body γ irradiation, and the β-carotene effect was observed even though the supplementation was started 2 d after irradiation. Such results may indicate that the anticarcinogenic effect of β-carotene against radiation operates both during and after the exposure to radiation. Because lymphocytes are important cells of the immune system, the chromosomal damage of lymphocytes through radiation may disturb normal functions of the cells and have an influence on many immunological defense systems. Therefore, the protective effect of β-carotene against chromosomal damage in lymphocytes as observed in this study may indicate not only the prevention of chromosomal damage but also the maintenance of normal function of lymphocytes in the immune systems.

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References