Comparative studies on genotoxicity and antigenotoxicity of natural and synthetic β-carotene stereoisomers

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Abstract

To evaluate the practical value of natural β-carotene (NβC) and to elucidate the apparent discrepancy between epidemiological observations and intervention trials on the role of β-carotene (βC) in tumor prevention, the genotoxicity and the antigenotoxicity of NβC and synthetic βC crystal (SβCC) stereoisomers were studied comparatively using chromosome aberration analysis and the micronucleus test in human lymphocytes in vitro. NβC was extracted from the halotolerant algae Dunaliella salina. The NβC crystal (NβCC) preparation is about 70% all-trans (TβC) and 8% 9-cis (CβC). The NβC oil (NβCO) preparation is about 40% all-trans and 38% 9-cis. SβCC is more than 97% all-trans, and the 9-cis can not be detected. The mixture of βC (βCM) preparation is 74% SβCC and 26% NβC. Our results show no genotoxicity of 1–30 μg/ml NβCC, but this concentration of NβCO inhibited significantly γ-ray-induced micronucleus formation in human lymphocytes in vitro. One to thirty μg/ml NβCO was most effective against both γ-ray-induced and spontaneous micronucleus formation. However, no influence of NβCO on spontaneous chromosome aberrations in human lymphocytes in vitro was observed. NβCO suppressed significantly mitomycin C (MMC)-induced chromosome aberrations. One to thirty μg/ml SβCC induced a dose-dependent increase in micronucleus frequency, and also inhibited γ-ray-induced micronucleus formation. No effect of βCM on spontaneous chromosome aberrations was found. One to thirty μg/ml βCM is more effective against MMC-induced chromosome aberrations than NβCO. These results suggest that CβC might play a critical role in the genotoxicity and antigenotoxicity of SβCC and NβC. The genotoxic activity of SβCC might be involved in carcinogenesis. NβC or βCM could be of practical value in tumor prevention and supplementary treatment. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Genotoxicity; Antigenotoxicity; β-carotene; Stereoisomer

1. Introduction

β-Carotene is a plant pigment and major precursor of vitamin A and is widely distributed in dark-green leafy vegetables and some violet to yellow fruits. Several epidemiological studies have indicated that an increased intake of vegetables and fruits rich in βC, and resulting higher serum βC levels can be related to a reduced incidence of lung, stomach and other cancers [1–5]. Experimental investigations in vivo have shown that administration of NβC or carrot juice could suppress genotoxic actions induced...
by chemotherapeutic drugs in humans and animals [6–8]. Even high doses of NBBC did not produce significant genotoxicity [9].

Recently, two large-scale prospective studies have reported that supplementation with βC or βC combined with retinol increased significantly the incidence of lung cancer in current smokers and asbestos-exposed workers in the United States and Finland; but the exact mechanism by which βC increased the risk of lung cancer is not yet clear [1,2]. The βC used in the above-mentioned intervention trials is synthetic [2]. Synthetic βC is greater than 99% all-trans with only traces of 9-cis, or 9Cis not detected. However, NBBC extracted from plants contains different amount of 9-cis; Algal sources of βC have approximately equal proportions of all-trans and 9-cis [10,11]. Although some fresh vegetables do not contain 9-cis, the total βC can contain up to 50% 9-cis after processing [12]. Therefore, the composition of NBBC stereoisomers is very different from CβCC. Comparative studies on their genotoxicity and antigenotoxicity have not been reported so far. This study not only could develop new drug sources, but also might help elucidate the apparent discrepancy between the epidemiological observations and the intervention trials on the role of βC in tumor prevention.

2. Materials and methods

2.1. βC preparations

Various βC preparations were provided by the Department of Biology Nanjing Normal university NBBC was extracted from the halotolerant algae Dunaliella salina. NBCC is 70% all-trans and 8% 9-cis. NBCC is 40% all-trans and 38% 9-cis. βCM is 74% SβCC and 26% NBCC. BCM contains about 82% all-trans and 10% 9-cis. βC was dissolved in 15% DMSO prepared with medium RPMI 1640, and was diluted again with the medium until the desired concentrations (1.6 and 30 μg/ml) were obtained.

2.2. Micronucleus test using human lymphocytes in vitro

Blood samples were taken from 8 healthy donors, 4 males, 4 females, their ages were 18 to 22 years. Each blood example was divided into 8 groups. In the negative control group only 15% DMSO was added to whole blood. The positive control group was irradiated with 3 Grays of γ-rays at a dose rate of 2.2 Grays/min; 3 groups were treated with various βC preparations (1, 6, 30 μg/ml); the remaining 3 groups were irradiated with 3 Grays of γ-rays and then were immediately treated with the βC preparations.

The procedures for micronucleus test have been described in detail elsewhere [13]. Our previous experiments have demonstrated that the increase of micronucleus frequencies detected by the micronucleus test is mutagen dose-dependent, and the relationship among these micronucleus frequencies and chromosome aberration rates as well as conventional, cytokinesis-block micronucleus frequencies detected simultaneously is apparently linear within certain dose range [14,15]. The main points of the procedures are as follows, either βC solution or 15% DMSO was added to the irradiated or non-irradiated whole blood in a ratio of 1:4. The final DMSO concentration is 3%. The blood samples were incubated for 16–18 h at 37°C. The smears of isolated lymphocytes were stained with Giemsa. Micronuclei were counted in 2000 lymphocytes.

2.3. Chromosome aberration analysis

The donors, experimental groupings and βC concentrations were the same as in the micronucleus test. The short culture of micro-whole blood was performed as described by Xue et al. [16]. After 24 h of blood culture, NBCC or βCM was added to the experimental groups and to the positive control and antigenotoxicity groups, 0.12 μg/ml MMC was added singly or simultaneously. The experimental and control groups were cultured for 46 h and colchicine was added. After 2 h, the metaphase cells were collected. The chromosome preparation and analysis was carried out using conventional techniques [16].

2.4. Data analysis

The U-test was used to evaluate the differences in micronucleus frequencies (MNFs) between 2 groups.
Table 1
Influence of SβCC, NβCC and NβCO on micronucleus formation induced by γ-rays in human lymphocytes in vitro

<table>
<thead>
<tr>
<th></th>
<th>MNF (%)</th>
<th>MNF (%)</th>
<th>MNF (%)</th>
<th>MNF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>γ-rays</td>
<td>βC/μg ml⁻¹</td>
<td>γ-rays + βC/μg ml⁻¹</td>
</tr>
<tr>
<td>SβCC</td>
<td>0.5 ± 0.7</td>
<td>4.0 ± 1.0</td>
<td>1.0 ± 0.5*</td>
<td>1.8 ± 1.5*</td>
</tr>
<tr>
<td>NβCC</td>
<td>0.5 ± 0.7</td>
<td>4.0 ± 1.0</td>
<td>0.5 ± 0.8*</td>
<td>0.2 ± 4.4*</td>
</tr>
<tr>
<td>NβCO</td>
<td>0.5 ± 0.7</td>
<td>4.0 ± 1.0</td>
<td>0.2 ± 0.4*</td>
<td>0.1 ± 0.3*</td>
</tr>
</tbody>
</table>

No. of observation groups = 15. ± s.
*P > 0.05; *P < 0.05; *P < 0.01 vs. control group; *P < 0.01 vs. γ-ray group.

The χ² test was used to evaluate the differences in the aberrative cell rates (ACRs) between 2 groups.

3. Results

3.1. Influence of SβCC, NβCC and NβCO on spontaneous and γ-ray-induced micronucleus formation

One to thirty μg/ml SβCC induced significant dose-dependent increase in MNF in human lymphocytes in vitro, and also inhibited significantly γ-ray-induced micronucleus formation. An obvious influence of 1–30 μg/ml NβCC on spontaneous MNF in lymphocytes has not been found, however 1–6 μg/ml NβCO suppressed spontaneous micronucleus formation. Compared with the negative control group, the decrease in MNF in lymphocytes treated with 6 μg/ml βC was statistically significant, but in the groups treated with 30 μg/ml NβCO this effect has not been observed (Table 1).

Among SβCC, NβCC and NβCO, NβCO was the most effective in reducing γ-ray-induced micronucleus formation. The MNFs in the groups treated with NβCO were nearly the same as those of the negative control group (Table 1).

Table 2
Effects of NβCC and βCM on chromosome aberrations induced by MMC in human cultured lymphocytes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose/βC μg ml⁻¹</th>
<th>Donors</th>
<th>Scored cells</th>
<th>Aberration * cells</th>
<th>ACR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>5</td>
<td>500</td>
<td>14</td>
<td>2.8</td>
</tr>
<tr>
<td>MMC</td>
<td>0.12</td>
<td>5</td>
<td>375</td>
<td>115</td>
<td>32.2</td>
</tr>
<tr>
<td>NβCO</td>
<td>1</td>
<td>5</td>
<td>500</td>
<td>13</td>
<td>2.6*</td>
</tr>
<tr>
<td>NβCO</td>
<td>6</td>
<td>5</td>
<td>500</td>
<td>13</td>
<td>2.6*</td>
</tr>
<tr>
<td>NβCO</td>
<td>30</td>
<td>5</td>
<td>500</td>
<td>17</td>
<td>3.4*</td>
</tr>
<tr>
<td>βCM</td>
<td>1</td>
<td>3</td>
<td>300</td>
<td>7</td>
<td>2.3*</td>
</tr>
<tr>
<td>βCM</td>
<td>6</td>
<td>3</td>
<td>300</td>
<td>8</td>
<td>2.7*</td>
</tr>
<tr>
<td>βCM</td>
<td>30</td>
<td>3</td>
<td>300</td>
<td>9</td>
<td>3.0*</td>
</tr>
<tr>
<td>MMC + NβCO</td>
<td>1</td>
<td>5</td>
<td>415</td>
<td>90</td>
<td>21.7f</td>
</tr>
<tr>
<td>MMC + NβCO</td>
<td>6</td>
<td>5</td>
<td>425</td>
<td>85</td>
<td>15.3f</td>
</tr>
<tr>
<td>MMC + NβCO</td>
<td>30</td>
<td>5</td>
<td>414</td>
<td>36</td>
<td>8.7f</td>
</tr>
<tr>
<td>MMC + βCM</td>
<td>1</td>
<td>3</td>
<td>300</td>
<td>33</td>
<td>11.0f</td>
</tr>
<tr>
<td>MMC + βCM</td>
<td>6</td>
<td>3</td>
<td>300</td>
<td>25</td>
<td>8.3f</td>
</tr>
<tr>
<td>MMC + βCM</td>
<td>30</td>
<td>3</td>
<td>300</td>
<td>17</td>
<td>5.7f</td>
</tr>
</tbody>
</table>

*P > 0.05 vs. control; *P < 0.01 vs. MMC; *P < 0.01 vs. MMC + NβCO 1 μg ml⁻¹ group; *P < 0.01 vs. MMC + NβCO 6 μg ml⁻¹ group.
*Aberration types: breakage/fragment, gap, triradial, quadriradial, ring.
3.2. Influence of NβCO and βCM on spontaneous and MMC-induced chromosome aberrations

A remarkable effect of both NβCO and βCM (1, 6, 30 µg/ml) on spontaneous ACR in human lymphocytes in vitro was not found. The inhibitory action of both NβCO and βCM on chromosome aberrations induced by MMC was proportional to the βC concentrations. βCM was more effective in reducing ACR than NβCO. A statistically significant difference in reducing ACR was observed between the βCM and NβCO groups when 1–6 µg/ml βC was added (Table 2).

4. Discussion

To our knowledge, this paper is the first attempt to study comparatively the genotoxicity and the antigenotoxicity of NβC and SβC. The experimental results (Table 1) show that the influence of 1–30 µg/ml NβCC on spontaneous micronucleus formation in human lymphocytes in vitro has not been observed. MNF in lymphocytes treated with 1 to 6 µg/ml NβCO display a trend toward a reduction when compared with negative group. Six µg/ml NβCO inhibited significantly spontaneous micronucleus formation. The above-mentioned results demonstrated that the inhibitory effect of NβC on spontaneous micronucleus formation occurred with the increase in 9-cis βC content. One to thirty µg/ml SβCC not containing 9-cis induced a dose-dependent increase of MNF in lymphocytes in vitro. The genotoxic action of high doses of SβC has been observed in animal experiments in vivo. SβC was administered by gavage, 27 mg/kg b.wt SβC induced significant increases in MNF and ACR in bone marrow cells in mice. However, these effects were not found in mice treated with 3 mg/kg b.wt SβC [24]. Two more recent experiments suggested that high doses of supplemental SβC could accelerate tumorigenesis induced by chemical carcinogens [18,19]. Therefore, the mutagenic and tumor promotional effects of high doses of SβC should arouse general concern.

Many physical and chemical agents used in cancer therapy, for example, MMC and γ-rays are also mutagens and carcinogens, and could induce the development of secondary tumors and suppress bone marrow cell production. Therefore, it is valuable for the prevention of secondary and harmful adverse effects to detect compounds which could suppress cellular mutagenesis and carcinogenesis. Our results (Table 1) indicated that all of SβC, NβCC and NβCO inhibited micronucleus formation induced by γ-rays. SβCC and some flavonoids are similar in possessing both genotoxicity and antigenotoxicity [20].

Because we observed by the micronucleus test that SβCC both genotoxic and antigenotoxic effects, and NβCO has the highest inhibitory effect on spontaneous and γ-ray-induced micronucleus formation, we compared further the genotoxicity and the antigenotoxicity of NβCO with that of a mixture of SβCC and NβCO using the chromosomal aberration analysis in human lymphocytes in vitro. The experimental results show that no major influence of βCM or NβCO on ACRs in human lymphocytes in vitro was observed, and βCM has a more inhibitory action against MMC-induced chromosomal aberrations than NβCO (Table 2). This suggests that SβC mixed with NβCO in a the ratio of about three to one might lose its genotoxicity which was observed in the micronucleus test and could increase significantly its antigenotoxicity. This finding could result in to providing a new approach to chemical tumor prevention and treatment of the harmful adverse effects of cancer therapy.

The exact mechanisms of the antigenotoxicity, genotoxic and promotional effects of βC are not well understood [21–23]. Recently, many authors considered that the βC antigenotoxicity is due to its antioxidant properties and inhibition of the metabolic activation of promutagens; the βC mutagenic and promotional effects might result from its pro-oxidant activity under conditions of high oxidative stress and high concentration [2,17,23]. Our investigations further indicated that βC stereoisomers play a very important role in its genotoxicity and the antigenotoxicity. NβCO containing the most CβC (38%) had the highest effectiveness against γ-ray-induced and spontaneous micronucleus formation in human lymphocytes in vitro; NβCC containing more CβC (8%) had only moderate effectiveness against the γ-ray-induced micronucleus formation, but was not effective against spontaneous micronucleus formation. SβCC
without CβC induced the dose-dependent increase of MNF, and a very high dose of SβCC also reduced the inhibitory effects on the γ-ray-induced micronucleus formation (Table 1). Other authors have reported that high doses of SβCC also had genotoxic and promotional activities [18,19,24]. Multiple mutations are of critical events in carcinogenesis [21,25], so the mutagenic action of SβCC could be involved in the increased incidence of lung cancer induced by supplemental SβCC in smokers. The NβC from dietary intake of fruits and vegetables, contains a certain amount of CβC [10–12]. The base-line serum βC concentration reflects fruit and vegetable intake. Plant foods also contain numerous compounds with antioxidant, antimutagenic and cancer-inhibiting properties for example, indoles, isothiocyanates and flavonoids, etc. [26–28]. They could protect βC from oxidation, and inhibit the harmful effects of tobacco etc. Therefore, an increased intake of foods rich in βC and higher serum βC levels could reduce the risk of some cancers, especially lung cancer.

Lower doses of SβCC did not exhibit obvious genotoxicity or have only weaker genotoxicity, but have more remarkable antigenotoxic effects than high doses (Table 1, [24]). The beneficial effect of lower dose of SβCC supplementation (15 μg daily) on tumor prevention in a micronutrient-deficient population consuming less fruits and vegetables also was observed [29]. Our experimental results indicate that higher doses of SβCC had significant genotoxicity, and the auto-oxidation of βC in vitro is dose-dependent [23,30]. Some βC intervention studies used higher doses of βC (20 mg or 30 mg daily) for long periods in population with good nutrition so that the median serum βC levels were 12 times and 17 times higher than the base line level in separate CARET and ATBC studies [1,2]. On the other hand the chemicals in cigarette smoke can produce free radicals-rich atmosphere, so βC in the lungs of current smokers, especially heavy smokers, might undergo oxidant attack [2,23]. Under such conditions of high βC concentrations and high oxidative stress, SβCC can have genotoxicity and pro-oxidant activities [2,17,23], therefore, high doses of βC supplement increased the risk of lung cancer in current smokers in ATBC and CARET studies. In the physician’s health study, the serum βC levels of βC-supplemented smokers were considerably lower than those in the ATBC and CARET studies, so lung cancer risk was not elevated among βC supplemented smokers [23].

In summary, our preliminary observations demonstrated that CβC is critical in the genotoxicity and the antigenotoxicity of βC stereoisomers. High doses of SβCC not containing CβC have genotoxic activity. Under high βC doses and high oxidative stress which was produced by cigarette smoking SβCC could have pro-oxidant activity, it also might enhance βC mutagenicity. As a result SβCC could increase the lung cancer risk of smokers. High doses of SβC supplementation should be avoided by such smokers. On the other hand our results also show that NBC and βCM in the same doses as SβCC did not exhibit obvious genotoxicity and could inhibit significantly mutagen-induced clastogenesis. NBC has the highest effectiveness against γ-ray-induced and spontaneous micronucleus formation. Other authors reported that lower doses of βC supplement reduced the incidence of certain cancers in a micronutrient-deficient population. Therefore, it is a possible approach to use low doses of NBC or βCM in tumor prevention, especially in populations consuming less vegetables and fruits.

References


