Effect of Dietary \( \beta \)-Carotene on Hepatic Drug-Metabolizing Enzymes in Mice

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Summary The effects of dietary supplements of \( \beta \)-carotene (20–500 mg per kg diet) on hepatic microsomal drug-metabolizing enzyme activities were studied in mice. Supplementation for 14 days resulted in marked reductions in the concentrations of cytochrome P-450 and biphenyl 4-hydroxylase. The antipyrine N-demethylase and \( p \)-nitroanisol O-demethylase activities, however, were unchanged. Also apparently unchanged were the hepatic concentrations of microsomal protein, lipid peroxides, and superoxide dismutase. Supplemental \( \beta \)-carotene was weakly protective against the acute toxic effects of an injection of 1,2-dimethylhydrazine (DMH), as indicated by a lowered mortality. This anti-carcinogenic action of \( \beta \)-carotene, including a protection against DMH-induced colon carcinogenesis, suggests an alteration by this carotenoid in the metabolism of carcinogens by the liver.

Key Words: cytochrome P-450, \( \beta \)-carotene, demethylase, superoxide dismutase, 1,2-dimethylhydrazine toxicity

Several epidemiological studies have indicated that an increased intake of foods rich in carotenoids is related to a reduced incidence of various types of cancer [1, 2]. This circumstantial evidence has been supported by experimental studies where dietary supplementation of \( \beta \)-carotene has been shown to have a protective effect against chemically-induced tumor formation at several sites in rats, mice, and hamsters [3–5]. In our recent study, \( \beta \)-carotene appeared to have a strong inhibitory action against dimethylhydrazine (DMH)-induced colon tumors in mice, as manifested by a markedly reduced incidence and multiplicity of tumors as well as by a decreased mortality rate [6].

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The mode of action of \( \beta \)-carotene is not yet understood. However, it has been suggested that \( \beta \)-carotene may behave as an antioxidant, especially at the relatively low oxygen partial pressures found in most tissues under physiological conditions [7]. The trapping of free radicals is the presumed mechanism of action. For that reason we have tested whether \( \beta \)-carotene affects two liver indices of tissue oxidation, namely superoxide dismutase (SOD) and lipid peroxides (thiobarbituric acid-reactive substances). The former removes potentially toxic, oxygen-derived free radicals, while the latter, a measure of lipid peroxidation, indicates the extent of free radical damage to cells.

Another possibility is that \( \beta \)-carotene alters the metabolism of carcinogens so that they are preferentially detoxified. We have therefore examined the dose-response effects of \( \beta \)-carotene on hepatic microsomal enzymes in mice, particularly cytochrome P-450, the terminal oxygenase for the mixed-function oxidase system [8]. As a test of how this might be related to DMH-induced tumors, we tested whether \( \beta \)-carotene protects against the lethality of DMH.

**MATERIALS AND METHODS**

*Reagents.* Specialized reagents were mostly purchased from Sigma Chemical Co., St. Louis, MO, except \( p \)-nitroanisole (Aldrich Chemical Co., Milwaukee, WI) and thiobarbituric acid (J.J. Baker Chemical Co., Phillipsburg, NJ).

*Mice and treatment.* Male Swiss (ICR) mice (aged 6–10 wk; mean wt, 31.6 g; range, 25–40 g) were used from a colony maintained in the University animal facilities.

Experiment 1. The mice were divided into 4 groups. The control group received no supplementation (\( \beta \)-carotene level: 2 mg/kg diet). The other groups received dietary supplements of \( \beta \)-carotene (type III, Sigma) at a dose level of 20, 100 or 500 mg/kg diet for 14 days. The diets consisted of 98.1% Wayne Rodent Blox meal (Continental Grain Co., IL) and 1.9% corn oil and were prepared as described previously [6]. Wayne meal consists mainly of cereal and vegetable foods and contains 24% protein, 3.6% crude fibre, 4.1% fat, 15,000 I.U. vitamin A per kg, and an adequate concentration of all nutrients. Animals were given food and water *ad libitum*.

Experiment 2. Treatment was as in Experiment 1 except that only 2 diets were fed (unsupplemented and that supplemented with 500 mg \( \beta \)-carotene per kg). On the fifteenth day the mice received a subcutaneous injection of DMH. The DMH was dissolved in 1 mM EDTA and neutralized with saturated sodium bicarbonate. The mice were then observed for several days and deaths recorded.

Experiment 3. Mice received only the unsupplemented diet. They received 4 daily intraperitoneal injections of \( \beta \)-carotene (2.53 mg in 0.1 g corn oil). Control mice received vehicle only. These dose levels are equivalent to the daily \( \beta \)-carotene intake of mice eating the diets of Experiment 2. Eight hours after the fourth injection the mice were injected with DMH as above.

Preparation of liver samples. On the fifteenth day the mice from Experiment 1 were killed by cervical fracture. The livers were quickly removed, chilled, weighed and homogenized. Part of each liver was homogenized in 0.06 M potassium phosphate (pH 7.8; about 50 mg/ml) using a Potter-Elvehjem homogenizer and analyzed for thiobarbituric acid-reactive substances. A portion of this homogenate was centrifuged at 17,000 × g for 60 min, and the supernatant was stored at −30°C until analyzed for SOD activity. Another piece of liver was homogenized in 1.15% KCl in 20 mM Tris-HCl (pH 7.6; 250 mg/ml) and centrifuged at 10,000 × g for 20 min. The resulting supernatant was analyzed for N-demethylase and O-demethylase activities. Some of this supernatant was centrifuged at 100,000 × g for 60 min and the pellet resuspended in the original volume of buffer (microsomes).

Analyses. The cytochrome P-450 content of microsomes was determined from the carbon monoxide difference spectra of dithionite-reduced samples (400 to 550 nm). The absorbance at 490 nm was deducted from that at 450 nm and the cytochrome P-450 content was calculated using the molar extinction of 91 cm⁻¹·mm⁻¹ [9]. The cytochrome P-450-dependent hydroxylase activity in microsomes was measured using biphenyl as a substrate, by the method of Creaven et al. [10]. N-Demethylase [11] and O-demethylase [12] were measured using anti-pyrine and p-nitroanisole as substrates, respectively. However, these enzymes were determined only in the controls and the animals fed 500 mg β-carotene per kg diet.

Lipid peroxides were measured within 1 h of sample preparation by heating with thiobarbituric acid [13] and expressed in terms of malondialdehyde nmol/g. The absorbance at 600 nm was deducted from that at 535 nm. 1,1,3,3-Tetramethoxypropane was used as standard. Total SOD (essentially Cu, Zn enzyme) was assayed by measurement of the reduction of nitro blue tetrazolium after generation of superoxide ions with xanthine and xanthine oxidase [14]. Triton X-100 was not used since it was strongly inhibitive. One unit of SOD is defined as the quantity of SOD which causes a 50% inhibition of the reaction rate in the absence of SOD. Protein was determined by the method of Lowry et al. [15].

Statistics. Statistical analysis was performed using either one way ANOVA followed by the Scheffe procedure or else by Student's t-test.

RESULTS

Supplementing the mouse diet with β-carotene (20–500 mg/kg diet) for 14 days had no apparent effect on food intake, liver weight, or body weight. Mean food intake was 5.3 g per mouse per day. The dose-response relationships of β-carotene with respect to various microsomal enzymes are shown in Table 1. The hepatic concentrations of both cytochrome P-450 and biphenyl 4-hydroxylase activities were reduced in all supplemented groups compared with those of the control animals. Furthermore, the concentrations tended to decline with increasing intake of β-carotene. The correlation between the levels of cytochrome P-450
Table 1. Effect of dietary supplementation with β-carotene on the activity of hepatic enzymes and on lipid peroxides in mice.

<table>
<thead>
<tr>
<th>Supplemental β-carotene (mg/kg)</th>
<th>Biphenyl 4-hydroxylase (μmol/g)</th>
<th>Cytochrome P-450 (nmol/g)</th>
<th>Antipyrine N-demethylase (μmol/g/h)</th>
<th>p-Nitroanisole O-demethylase (μmol/g/h)</th>
<th>SOD (units/mg)</th>
<th>Thioarbituric acid-reactive substances (nmol/g)</th>
<th>Microsomal protein (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>2.84 ± 0.17*</td>
<td>28.4 ± 5.4*</td>
<td>6.04 ± 0.59</td>
<td>12.7 ± 3.7</td>
<td>15.8 ± 2.1</td>
<td>289 ± 55</td>
<td>19.9 ± 6.6</td>
</tr>
<tr>
<td>20</td>
<td>2.46 ± 0.17**</td>
<td>22.5 ± 5.2</td>
<td>15.8 ± 2.9</td>
<td>289 ± 62</td>
<td>17.3 ± 3.1</td>
<td>15.3 ± 2.0</td>
<td>288 ± 48</td>
</tr>
<tr>
<td>100</td>
<td>2.16 ± 0.15**</td>
<td>19.3 ± 3.5**</td>
<td>15.3 ± 2.0</td>
<td>288 ± 48</td>
<td>18.0 ± 5.8</td>
<td>14.0 ± 2.3</td>
<td>271 ± 64</td>
</tr>
<tr>
<td>500</td>
<td>1.97 ± 0.17**</td>
<td>15.4 ± 4.9**</td>
<td>5.57 ± 1.63</td>
<td>11.9 ± 2.4</td>
<td>18.6 ± 7.8</td>
<td>10.9 ± 2.1</td>
<td>264 ± 62</td>
</tr>
</tbody>
</table>

Experiment 1. Each value is the mean of 6-8 animals ± SD. Activities refer to liver weight. *p<0.05 for trend (one way ANOVA), **p<0.05 vs. the control diet (Scheffe procedure, multiple comparison).

Fig. 1. Correlation between the content of cytochrome P-450 and biphenyl 4-hydroxylase activity.

and biphenyl 4-hydroxylase was found to be highly significant (Fig. 1; r = 0.994). In contrast to these two enzyme activities the hepatic microsomal levels of antipyrine N-demethylase and p-nitroanisole O-demethylase were unaffected in mice given 500 mg β-carotene per kg diet (Table 1).

Hepatic tissues were analyzed for lipid peroxides and SOD activity. Neither appeared to be affected by β-carotene (Table 1). We also observed that 500 mg β-carotene/kg did not affect lipid peroxide levels in the mucosa of the distal colon (data not shown).
Table 2. Effect of β-carotene treatment on mortality of mice injected with DMH.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>β-Carotene</th>
<th>Dose DMH</th>
<th>Mortality (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-</td>
<td>36</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>36</td>
<td>79</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>33</td>
<td>56</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>33</td>
<td>48</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>33</td>
<td>71</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>33</td>
<td>54</td>
<td>24</td>
</tr>
</tbody>
</table>

*β-Carotene supplementation was given in the diet (500 mg/kg for 14 days; Experiment 2) or by ip injection (2.63 mg/mouse/day for 4 days; Experiment 3). *The dose is mg DMH dihydrochloride/kg body wt.

In another experiment female Swiss Webster mice were given a dietary supplement of β-carotene (20 mg/kg diet) for 17 weeks (i.e., low-level supplementation for an extended period). Compared with mice fed the control diet supplemental β-carotene did not affect hepatic lipid peroxides or SOD activity (data not shown).

In Experiments 2 and 3 mice were given an injection of DMH at a high dosage. Deaths, which all occurred 31–80 h post-DMH, were then recorded. There was a trend, albeit not significant, for β-carotene treatment (both dietary and by intra-peritoneal injection) to reduced mortality (Table 2).

**DISCUSSION**

It has been suggested that altered metabolism of carcinogens is one of the factors that influence the activation of a carcinogen [16, 17]. Many studies have shown that the hepatic microsomal enzyme system, which is involved in the metabolism of potential carcinogens, is depressed by retinol deficiency [18–20], an effect which is reversed by retinol supplementation. The protective effect of retinol against chemical carcinogenesis has been extensively investigated [21]. The present study examined the effects of dietary β-carotene, the precursor of retinol, on the hepatic metabolism in mice of prototype drug substrates, namely, biphenyl, antipyrine, and p-nitroanisole. The unsupplemented (control) diet contained 2 mg β-carotene/kg and 15,000 I.U. vitamin A/kg. The experimental diets were supplemented with β-carotene ranging from 20–500 mg/kg (i.e., from a nutritional intake to an unphysiological one).

The hepatic microsomal biphenyl 4-hydroxylase enzyme responded to dietary β-carotene differently from antipyrine N- and p-nitroanisol O-demethylases. Thus, whilst the hepatic concentration of the former enzyme was reduced by feeding a diet containing 20–500 mg/kg for 14 days, the activity of the latter enzymes remained unaffected. Cytochrome P-450 was reduced in parallel with the loss in activity of the aromatic hydroxylase. This agrees with the established relationship between these two enzymes [22]. It therefore seems that the effect of β-carotene on drug-metabolizing enzymes depends on the substrate used.

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Like β-carotene in our study, an excess intake of retinol in rats has been shown to cause no change in the levels of hepatic microsomal demethylase activity [23]. Furthermore, an in vitro study has shown that the addition of retinol (12–600 μM) to an incubation mixture containing rabbit liver microsomes results in a gradual inhibition of benzpyrene hydroxylase activity with increasing retinol concentration [24]. It is therefore possible that the inhibitory effect of β-carotene on the hepatic microsomal hydroxylase enzyme is a reflection of its retinol activity. However, it seems unlikely that enough β-carotene would be converted to vitamin A since the basal diet was already adequate in preformed retinol [25].

As another test of whether β-carotene protects against carcinogens by altering their metabolism, we studied the effect of supplemental β-carotene on DMH induced mortality. Deaths occurred 31–80 h following the DMH injection and were probably caused by liver damage [26]. A trend was observed suggesting that β-carotene treatment (dietary or by injection) lowers the mortality to a limited extent. Since DMH is metabolized in the liver [27], these observations possibly reflect the altered levels of drug-metabolizing enzymes and may be related to the protective effect of β-carotene against DMH-induced colon carcinogenesis [6]. Further study should be made on the influence of β-carotene on the metabolism and toxicity of foreign chemicals including carcinogens.

Evidence to date indicates that β-carotene may have an antioxidant action, particularly at the relatively low oxygen partial pressures found in most tissues under physiological conditions [7]. Liver samples were therefore analyzed for SOD in post-mitochondrial supernatant (essentially the Cu,Zn-enzyme) and for thiobarbituric acid-reactive substances, an index of lipid peroxidation. Neither parameter was apparently affected by β-carotene-supplemented diets. This suggests that the depressed hydroxylase enzyme activity was not caused by the antioxidant property of β-carotene.

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REFERENCES

