Oral Administration of β-Cryptoxanthin Prevents Bone Loss in Streptozotocin-Diabetic Rats in Vivo

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The effects of β-cryptoxanthin, a carotenoid, on bone components in the femoral-diaphyseal and -metaphysseal tissues of streptozotocin (STZ)-diabetic rats was investigated. Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight), and then the animal were orally administered β-cryptoxanthin (5 or 10 µg/100 g body weight) once daily for 7 or 14 d. The administration of STZ caused a significant decrease in body weight and a significant increase in serum glucose, triglyceride, and calcium levels, indicating a diabetic state. These alterations were significantly prevented by the administration of β-cryptoxanthin (5 or 10 µg/100 g) for 14 d. The administration of β-cryptoxanthin (5 or 10 µg/100 g) to normal rats for 14 d did not have a significant effect on body weight or on serum glucose, triglyceride, and calcium levels. Calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and -metaphysseal tissues were significantly decreased in STZ-diabetic rats. These decreases were significantly prevented by the administration of β-cryptoxanthin (5 or 10 µg/100 g) for 14 d. The administration of β-cryptoxanthin to normal rats for 14 d caused a significant increase in calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and -metaphysseal tissues. This study demonstrates that the intake of β-cryptoxanthin has a preventive effect on bone loss in STZ-diabetic rats.

Key words β-cryptoxanthin; bone; osteoporosis; diabetes

Aging induces a decrease in bone mass, and osteoporosis with its accompanying decrease in bone mass is widely recognized as a major public health problem.1,2) The most dramatic expression of the disease is represented by fractures of the proximal femurs.3) Bone loss with increasing age may be due to decreased bone formation and increased bone resorption. Pharmacologic and nutritional factors may prevent bone loss with aging.4,5) Chemical compounds in food that act on bone metabolism, however, are poorly understood.

Micronutrients and phytochemicals found in vegetables and fruit, such as carotenoids, are potential chemopreventive agents. Carotenoids have been shown to play a possible role in cancer prevention.6,7) This preventive effect on osteoporosis, however, has not been fully clarified. Vitamin A is known to have a detrimental effect on bone at high doses. High levels of vitamin A lead to accelerated bone resorption, bone fractures, and osteoporotic bone lesions in animals.8–10)

β-Cryptoxanthin is a carotenoid abundant in Satsuma mandarin (Citrus unshiu Marc.), and it is enzymatically converted from β-carotene (provitamin A) in plants. Of the various carotenoids (including β-cryptoxanthin, lutein, lycopene, and β-carotene) and rutin (queretin-3-rutinoside), β-cryptoxanthin has been found to have a unique anabolic effect on bone calcification in vitro.11) β-Cryptoxanthin has a stimulatory effect on bone formation and an inhibitory effect on bone resorption in rat femoral tissue culture in vitro.11) β-Cryptoxanthin can stimulate cell proliferation and mineralization in osteoblastic cells in vitro.12,13) and it can inhibit osteoclast-like cell formation induced by bone-resorbing factors in mouse marrow cultures in vitro.14) Thus β-cryptoxanthin has been demonstrated to have a stimulatory effect on osteoblastic bone formation and an inhibitory effect on osteoclastic bone resorption in vitro.

Oral administration of β-cryptoxanthin has been shown to have an anabolic effect on bone components in young and aged rats in vivo.15,16) This study was undertaken to determine whether β-cryptoxanthin has a preventive effect on bone loss in the pathophysiologic state. We found that oral administration of the agent induces this effect on femoral tissues in streptozotocin (STZ)-diabetic rats in vivo.

MATERIALS AND METHODS

Chemicals STZ was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), β-Cryptoxanthin was obtained from Extrasynthese (Lyon-Nord, France). Other chemicals were of reagent grade from Wako Pure Chemical Industries (Osaka, Japan).

Animals Male Wistar rats (conventional) weighing 90–95 g (4 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 1.1% calcium and 1.1% phosphorus at room temperature of 25 °C, with free access to distilled water.

Administration Procedures STZ was dissolved in sodium citrate 50 mM (pH 4.5) solution containing NaCl 150 mM.17) The solution (6.0 mg/0.5 ml/100 g body weight) was subcutaneously administered to rats, and 7 or 14 d later the animals were killed by exsanguination. In another experiment, β-cryptoxanthin was dissolved in corn oil at a concentration of 10 or 20 µg/ml and then 5 or 10 µg/0.5 ml/100 g body weight was orally administered to rats through a stomach tube once daily for 7 or 14 d. Control rats received corn oil (0.5 ml/100 g body weight) orally. β-Cryptoxanthin was orally administered 3 h after the administration of STZ (6.0 mg/100 g). The animals were killed 24 h after the last administration by cardiac puncture under light ether anesthesia, and the blood and femur were removed immediately.

Analytical Procedures Blood samples obtained by cardiac puncture were centrifuged 30 min after collection, and the serum was separated. Serum was frozen at −80 °C until
Table 1. Effects of β-Cryptoxanthin (CRP) Administration on the Change in Body Weight and Serum Glucose and Triglyceride Levels in STZ-Diabetic Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Serum level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>7 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>118.8±3.4</td>
<td>134.8±5.0</td>
</tr>
<tr>
<td>STZ</td>
<td>87.6±2.2*</td>
<td>590.1±15.1*</td>
</tr>
<tr>
<td>STZ + CRP (5 µg/100 g)</td>
<td>101.8±5.0*</td>
<td>527.0±34.5*</td>
</tr>
<tr>
<td>STZ + CRP (10 µg/100 g)</td>
<td>96.9±3.4*</td>
<td>519.3±34.8*</td>
</tr>
<tr>
<td>14 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>143.9±4.5</td>
<td>138.5±5.7</td>
</tr>
<tr>
<td>STZ</td>
<td>81.8±3.0*</td>
<td>801.1±24.3*</td>
</tr>
<tr>
<td>STZ + CRP (5 µg/100 g)</td>
<td>121.2±1.6*</td>
<td>562.9±26.4*</td>
</tr>
<tr>
<td>STZ + CRP (10 µg/100 g)</td>
<td>115.8±4.8*</td>
<td>587.3±25.7*</td>
</tr>
</tbody>
</table>

Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight), and 3 h later the animals were orally administered β-cryptoxanthin (5 or 10 µg/100 g) once daily for 7 or 14 d. The animals were killed 24 h after the last administration. Each value is the mean±S.E.M. for six rats. *p<0.01, compared with the control (none) value.

Table 2. Effects of β-Cryptoxanthin (CRP) Administration on the Change in Serum Calcium and Inorganic Phosphorus Levels in STZ-Diabetic Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcium</td>
</tr>
<tr>
<td>7 d</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.50±0.14</td>
</tr>
<tr>
<td>STZ</td>
<td>11.03±0.15</td>
</tr>
<tr>
<td>STZ + CRP (5 µg/100 g)</td>
<td>10.88±0.21</td>
</tr>
<tr>
<td>STZ + CRP (10 µg/100 g)</td>
<td>10.89±0.30</td>
</tr>
<tr>
<td>14 d</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.82±0.10</td>
</tr>
<tr>
<td>STZ</td>
<td>12.59±0.15</td>
</tr>
<tr>
<td>STZ + CRP (5 µg/100 g)</td>
<td>10.17±0.30</td>
</tr>
<tr>
<td>STZ + CRP (10 µg/100 g)</td>
<td>10.69±0.21*</td>
</tr>
</tbody>
</table>

The procedure of administration is described in the legend to Table 1. Each value is the mean±S.E.M. for six rats. *p<0.01, compared with the control (none) value.

Serum glucose and triglyceride levels were markedly elevated in rats administered STZ, indicating that the administration induces a diabetic state. These increases were significantly prevented by the administration of β-cryptoxanthin (5 or 10 µg/100 g) for 14 d (Table 1). The administration of β-cryptoxanthin (5 or 10 µg/100 g) for 14 d did not have any effect on serum glucose and triglyceride levels in normal rats (data not shown).

The serum calcium levels were significantly elevated 14 d after the the administration of STZ (Table 2). This increase was significantly prevented by administration of β-cryptoxanthin (5 or 10 µg/100 g) for 14 d. Serum inorganic phosphorus levels were significantly decreased 7 or 14 d after STZ administration (Table 2). This decrease was significantly prevented by the administration of β-cryptoxanthin (5 or 10 µg/100 g) for 7 or 14 d. The administration of β-cryptoxanthin (5 or 10 µg/100 g) to normal rats for 7 or 14 d had no effect on serum calcium and inorganic phosphorus levels (data not shown).

Effects of β-Cryptoxanthin Administration on Bone Components in STZ-Diabetic Rats The effects of β-cryptoxanthin administration on bone components in the femoral-diaphyseal and -metaphyseal tissues of STZ-diabetic rats was examined. Calcium content (Fig. 1) and alkaline phosphatase assay. Serum glucose, triglyceride, calcium, and inorganic phosphorus concentrations were determined using an assay Kit (Wako Pure Chemical Industries).

The diaphyseal or metaphyseal tissues were dried for 16 h at 110°C. Calcium was determined using atomic absorption spectrophotometry. (9) Calcium content in bone tissues was expressed as milligrams per gram of dry bone.

To assay alkaline phosphatase activity, the diaphyseal or metaphyseal tissues were immersed in 3.0 ml of ice-cold barbital buffer 6.6 mM (pH 7.4), cut into small pieces, and disrupted for 60 s with an ultrasonic device. The supernatant centrifuged at 600 x g for 5 min was used to measure enzyme activity. The enzyme assay was carried out under optimal conditions. Alkaline phosphatase activity was determined using the method of Walter and Schutt. (19) Enzyme activity was expressed as micromole of p-nitrophenol liberated per minute per milligram of protein. The protein concentration was determined using the method of Lowry et al. (20)

To measure bone DNA content, the diaphyseal or metaphyseal tissues were shaken with 4.0 ml of ice-cold 0.1 N NaOH solution for 24 h after the homogenization of the bone tissues. (21) After alkaline extraction, the samples were centrifuged at 1000 x g for 5 min, and the supernatant was determined using the method of Ceriotti (22) and expressed as the amount of DNA (mg) per g wet weight of bone tissue.

Statistical Analysis The significance of differences between values was estimated using Student's t-test. We also used multiple ANOVA to compare the treatment groups. A p value of less 0.05 was considered to indicate a statistically significant difference.

RESULTS

Effects of β-Cryptoxanthin Administration on Serum Components in STZ-Diabetic Rats Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight), and the animals were orally administered β-cryptoxanthin (5 or 10 µg/100 g body weight) once daily for 7 or 14 d. The body weight of animals was significantly decreased 7 or 14 d after the administration of STZ. This reduction was significantly prevented by the administration of β-cryptoxanthin (5 or 10 µg/100 g) for 14 d (Table 1).
activity (Fig. 2) in the femoral-diaphyseal and -metaphyseal tissues were significantly decreased 7 or 14 d after the administration of STZ (6.0 mg/100 g), respectively. These decreases were significantly prevented by the administration of β-cryptoxanthin for 7 or 14 d. DNA content in the femoral-diaphyseal and -metaphyseal tissues was significantly decreased 7 or 14 d after the administration of STZ (6.0 mg/100 g) (Fig. 3). The decrease was not caused by the administration of β-
cryptoxanthin (5 or 10 μg/100 g) for 7 or 14 d. Metaphyseal DNA content was significantly decreased 14 d after STZ administration. This decrease was significantly prevented by the administration of β-cryptoxanthin (5 or 10 μg/100 g) for 14 d. The administration of β-cryptoxanthin (5 or 10 μg/100 g) to normal rats for 14 d caused a significant increase in calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and -metaphyseal tissues (data not shown).

**DISCUSSION**

The administration of STZ to rats caused a significant increase in serum glucose and triglyceride levels, indicating a diabetic state. STZ administration also induced a significant increase in serum calcium and a significant decrease in diabetic state. STZ administration also induced a significant increase in serum glucose and triglyceride levels, indicating a diabetic state. STZ administration in diabetic rats and that the administration has a partial preventive effect on the increase in glucose and triglyceride levels with diabetes in vivo.

Bone loss may be induced in the diabetic state. Calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal (cortical bone) and -metaphyseal (trabecular bone) tissues was significantly decreased in STZ-diabetic rats. Alkaline phosphatase is an enzyme marker of osteoblasts and the enzyme participates in bone mineralization. DNA content in bone tissues is an index of the number of bone cells. Presumably, STZ administration induces bone loss by inhibiting osteoblastic bone formation and stimulating osteoclastic bone resorption.

Oral administration of β-cryptoxanthin was found to have a preventive effect on the decrease in calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and -metaphyseal tissues of STZ-diabetic rats. β-Cryptoxanthin has been shown to have a stimulatory effect on osteoblastic bone formation and an inhibitory effect on osteoclastic bone resorption. As a result, oral administration of β-cryptoxanthin may restore the hypercalcemia and hypophosphatemia induced in the diabetic state.

Oral administration of β-cryptoxanthin was found to have a significant preventive effect on the increase in glucose and triglyceride levels in the serum of STZ-administered rats. The mechanism by which β-cryptoxanthin administration has a partial preventive effect on the serum components in STZ-diabetic rats is unknown. It is speculated that β-cryptoxanthin administration stimulates the tissue transport of serum glucose and triglycerides. This remains to be elucidated. It has been reported that the serum concentrations of β-cryptoxanthin increases due to the consumption of vegetable juice in women in the range of 1.34×10⁻⁴ to 5.3×10⁻⁴ M. The anabolic effect of β-cryptoxanthin on bone formation and bone resorption was observed to 10⁻⁴ and 10⁻⁷ M in vivo. Presumably, the supplemental intake of β-cryptoxanthin has a preventive effect on bone loss induced in the diabetic state in vivo.

In conclusion, it has been demonstrated that the oral administration of β-cryptoxanthin can prevent bone loss in STZ-diabetic rats and that the administration has a partial preventive effect on the increase in serum glucose and triglyceride levels with diabetes in vivo.

**REFERENCES**