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Regulation of the Hepatic Antioxidative System by Astaxanthin in the Rats

Ji-One Kang, Sung-Jin Kim* and Harriet Kim†

Department of Food and Nutrition, College of Human Ecology, Seoul National University, Seoul 151-742, Korea
**Department of Pharmacology, School of Dentistry, Kyung Hee University, Seoul 130-701, Korea*

Abstract

Astaxanthin is one of many carotenoids present in marine animals, vegetables and fruits. Since carotenoids are known to exert antioxidant actions, we explored to determine if astaxnathin could have such regulatory actions in normal- and CCl_4-treated rat liver. Astaxanthin treatment caused a slight increase in α-tocopherol levels in the control rat liver. Glucose-6-phosphatase activity was significantly increased by astaxanthin in a dose-dependent manner and its activity decreased in response to CCl_4 treatment tended to recover by astaxanthin. Production of conjugated dienes by CCl_4 treatment was slightly inhibited by astaxanthin. These results suggest that astaxanthin could protect liver damages induced by CCl_4 via inhibiting lipid peroxidation and it may have a potential to activate the anti-oxidant system of normal liver by stimulating α-tocopherol production.

Key words: astaxanthin, antioxidant

INTRODUCTION

Astaxanthin is a carotenoid which is widely distributed in marine animals. It is present in a large amount in the pigment of flesh and surface of salmon and trout as well as in vegetables and fruits (1). It has been reported that when astaxanthin is administered to the Wistar rats at the level of 300 mg/kg diet, the rate of absorption per day was approximately 34 mg/kg body weight, resulting in an accumulation of 0.853 μg per 1 g of liver (2). Carotenoids play a role in protecting marine animals against damages induced by free radicals and oxygen reactive species (1). Astaxanthin possesses an 80 times stronger quenching activity than α-tocopherol and a twice stronger quenching activity than β-carotene. The reactivity to other molecular oxygen decreases since singlet oxygen-associated carbon-centered radical of astaxanthin can form a more stable resonance structure by the carbonyl group and the hydroxyl group attached to the β-ionone ring of astaxanthin (3,4).

Astaxanthin can remove the chain-carrying lipid peroxyl radicals in the liposomal suspension more efficiently than β-carotene but less efficiently than α-tocopherol since the hydrogen bonds between the carbonyl group and the hydroxyl group in the β-ionone ring of astaxanthin. Also, the hydrophobic association by the polyene chain make astaxanthin fit in the membrane phospholipid structure well (5).

Carbon tetrachloride (CCl_4) is known to induce the fibrogenesis of hepatocytes and metabolizes to reactive trichloromethyl radicals by cytochrome P450 2E1. The trichloromethyl radicals can react with glutathione to form radicals containing glutathione that damage hepatocyte directly (6). The metabolic products of CCl_4 damage the plasma membrane through lipid peroxidation in endoplasmic reticulum, and calcium enters into the cell and accumulates. The increase of calcium in the cell induces activation of Kupffer cells and the activated Kupffer cells secrete cytokine which is toxic to hepatocytes (7).

The present study was performed to determine if astaxanthin has protective effects against rat liver damages induced by CCl_4 by measuring the levels of α-tocopherol and glucose-6-phosphatase activity, conjugated dienes and protein cabonylation.

MATERIALS AND METHODS

Animals and treatment

Sprague-Dawley male rats (120~180 g) were provided by the Animal Center of Seoul National Univeristy and accustomed to plastic cages for one week with common solid feed and water supply. Water and feed were freely supplied during experimental treatment. The temperature was kept at 20~25°C, the humidity at 55±1%, and the periodity of day and night at 12 hr interval (light 8:00~20:00). Experimental rats were randomly divided into three groups; control group (CON), 2 mg/kg b.w. astaxanthin-administered group (AX 1) and 100 mg/kg b.w. astaxanthin-administered group (AX 2). Each group was divided into CCl_4-untreated normal group and CCl_4-treated group. Astaxanthin was prepared daily by dissolving it in corn oil and administered by oral gavage for 15 days. At the sixteenth day, CCl_4-treated groups were treated by intraperitoneal injection of CCl_4 at dose of 1 ml/kg b.w. At the seventeenth day, experimental rats were weighed

†Corresponding author. E-mail: harriet@snu.ac kr
Phone: 82-2-880-6833, Fax: 82-2-884-0305
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after fasting for 16 hours and then decapitated.

Tissue preparation and fractionation

Blood was collected immediately after decapitation and centrifuged. The serum was frozen by liquid nitrogen and then used for the assay of glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) within 12 hours. Liver tissue was homogenized in 5 volumes of ice-cold homogenizing buffer (20 mM Tris-base, 250 mM Sucrose, 3 mM EDTA, 1 mM PMSF; pH 7.4) after chopping at 4°C and centrifuged at 1,000 g for 10 min. The supernatant was centrifuged at 10,000 g for 15 min and the resulting pellet was resuspended in storage buffer (20 mM Tris-base, 250 mM Sucrose, 3 mM EDTA, 1 mM PMSF, 1% (v/v) Triton X-100; pH 7.4) and frozen under liquid nitrogen as mitochondrial fraction. The resulting supernatant was further centrifuged at 105,000×g for 1 hour and the resulting supernatant was saved as a cytosolic fraction and the pellet as a microsomal fraction after resuspending in storage buffer (50 mM potassium phosphate, 1.15% KCl, 3 mM EDTA, 1 mM PMSF; pH 7.4) (8).

Measurement of α-tocopherol

α-Tocopherol contents were measured by HPLC which was originally described by Furr et al. (9). Briefly, rat liver (0.5 g) was homogenized in 2 ml of water, and 6 ml of chloroform : methanol (2 : 1) solution was added. The reaction mixture was vigorously shaked and subjected to centrifugation at 2,000 rpm for 15 min. The chloroform layer was removed and mixed with 2 ml of distilled water. The mixture was shaked and subjected to centrifugation at 2,000 rpm for 15 min. The resulting chloroform layer (3 ml) was collected and dried under the nitrogen gas. The final pellet was resuspended with methanol (130 μl) and filtered with a PTFE syringe filter. The filtrate was used for HPLC analysis. A Vydac C18 column was used and mobile phase was methanol : H_2O (95 : 5). Absorbance was measured at 290 nm.

Measurement of glucose-6-phosphatase activity

Glucose-6-phosphatase activity was measured in liver microsomal fraction as described by Baginski et al. (10). Microsomal homogenate was centrifuged at 100,000×g and the resulting pellet was resuspended in a homogenization buffer (20 mM Tris-base, 250 mM Sucrose, 3 mM EDTA, 1 mM PMSF, pH 7.4). Microsomal suspension (50 μl) was mixed with 50 μl of 0.25 M sucrose/1 mM EDTA solution, 50 μl of 0.1 M glucose-6-phosphate, and 50 μl of 0.1 m cacodylate buffer (pH 6.5) and subjected to incubation at 37°C for 7 min.

One ml of 2% ascorbic acid/10% trichloroacetic acid solution was added to the reaction mixture and subjected to centrifugation at 3,000×g for 3 min. To the supernatant, 1% ammonium molybdate, 2% arsenite/2% trichloroacetic acid were added and allowed to incubate for 15 min. Absorbance was measured at 700 nm.

Measurement of conjugated dienes

Conjugated diene was measured in liver microsome as essentially described by Recknagel and Glende (11).

Protein determination

The protein concentration in each fraction was measured by using the method of Lowry (12).

Statistical analysis

The data was estimated by using the SAS general linear model procedure, the ANOVA test and Duncan's multiple range test at $p<0.05$. The correlation between variables was calculated by Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Effect of astaxanthin on α-tocopherol levels

CCl_4 treatment considerably decreased α-tocopherol levels in liver homogenates of both the control and the astaxanthin-adminostered rats. The astaxanthin administration (100 mg/kg) increased the hepatic α-tocopherol levels as compared to those of the control group, whereas it has little effect on the CCl_4 treated group (Fig. 1). These results suggest that astaxanthin may play a role in increasing anti-oxidative potentials in the normal liver by elevating α-tocopherol levels.

Effect of glucose-6-phosphatase activity

Glucose-6-phosphatase activity is known to be negatively related to lipid peroxidation (13) and it could be used as an index of lipid peroxidation of membrane. During the metabolism of CCl_4, free radicals such as CCl_3 · or Cl · are generated and could play a role in the inactivation of glucose-6-phosphatase. Astaxanthin treatment caused a significant increase in the glucose-6-phosphatase activity in liver microsomal fractions dose dependently while CCl_4 treatment alone

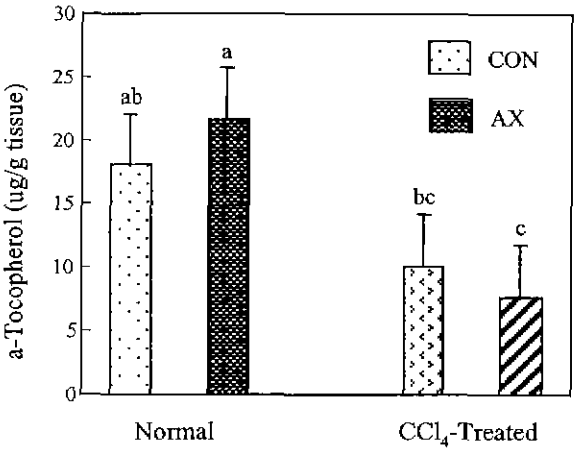

Fig. 1. The effect of astaxanthin on α-tocopherol levels of liver homogenate in rats. Astaxanthin was administered to rats daily at the dose of 2 mg/ kg body weight or 100 mg/kg body weight for 15 days. At the 16th day, CCl_4 was injected to rats in the CCl_4-treated group. Liver homogenates from the control and CCl_4-treated groups were prepared and α-tocopherol levels were measured as described in the Materials and Methods. Values are mean±S.D. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test. Con: control group; AX : astaxanthin administered group (100 mg/kg body weight).

reduced its activity as compared to the control group (Fig. 2). Strikingly, astaxanthin had some effect in recovering the glucose 6-phosphatase activity that is significantly reduced by the CCl_4 treatment.

These results suggest that astaxanthin has a potential to protect against lipid peroxidation by interfering with the inactivation of glucose 6-phosphatase activity by CCl_4.

The effect of astaxanthin on conjugated diene levels

Conjugated diene which is significantly influenced by diet patterns is an initial product of lipid peroxidation reaction. As production of conjugated diene in the liver increases, the liver is seriously damaged. Thus, the level of conjugated diene is regarded as a representative index of initial lipid peroxidation reactions (14). In this study, astaxanthin had little effect on the conjugated diene levels whereas it tended to cause a decrease in conjugated diene levels in the CCl_4 treated groups (Fig. 3). It is speculated that astaxanthin interferes with the generation of conjugated dienes in response to CCl_4, possibly by interacting with cell membranes efficiently and protecting the membrane from free radical attacks.

The effect of astaxanthin on protein carbonylation

As lipid peroxidation proceeds, cytotoxic products such as 4-hydroxynonenal and malondialdehyde are produced in addition to the breakdown of membrane lipids. These toxic materials react with thiol groups present in proteins, and carbonylated proteins are generated as a result (15). In this experiment, astaxanthin had little effect on protein carbonylation in normal as well as in CCl_4 treated groups (Fig. 4).

Taken together, it is concluded that astaxanthin could prevent liver damage induced by CCl_4 possibly via inhibition of lipid peroxidation. In addition, the increase of α-tocopherol production in the normal liver in response to astaxanthin may play a role in boosting the liver anti-oxidative system.

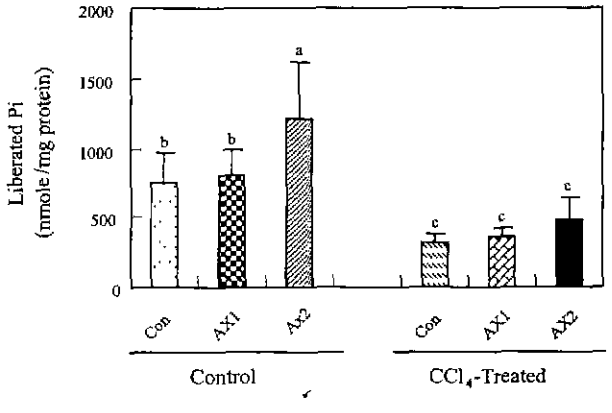

Fig. 2. The effect of astaxanthin on glucose-6-phosphatase activity of liver microsomal fraction in rats. Astaxanthin was administered to rats daily at the dose of 2 mg/kg body weight or 100 mg/kg body weight for 15 days. At the 16th day, CCl_4 was injected to rats in the CCl_4-treated group. Liver microsomal homogenates from the control and CCl_4-treated groups were prepared and glucose-6-phosphatse activities were measured as described in the Materials and Methods. Values are mean±S.D. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test. Con: control group; AX1: astaxanthin administered group (2 mg/kg body weight); AX2: astaxanthin administered group (100 mg/kg body weight).

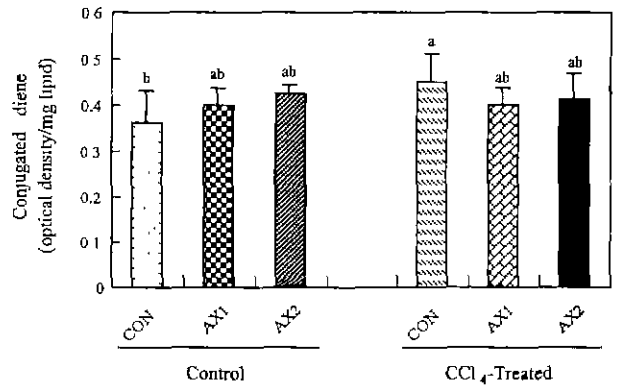

Fig. 3. The effects of astaxanthin on conjugated diene levels of liver microsomal fraction in rats. Astaxanthin was administered to rats daily at the dose of 2 mg/kg body weight or 100 mg/kg body weight for 15 days. At 16th day, CCl_4 was injected to rats in the CCl_4-treated group. Liver microsomal samples from the control and CCl_4-treated groups were prepared and conjugated diene levels were measured as described in the Materials and Methods. Values are mean±S.D. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test. Con: control group; AX1: astaxanthin administered group (2 mg/kg body weight); AX2: astaxanthin administered group (100 mg/kg body weight).

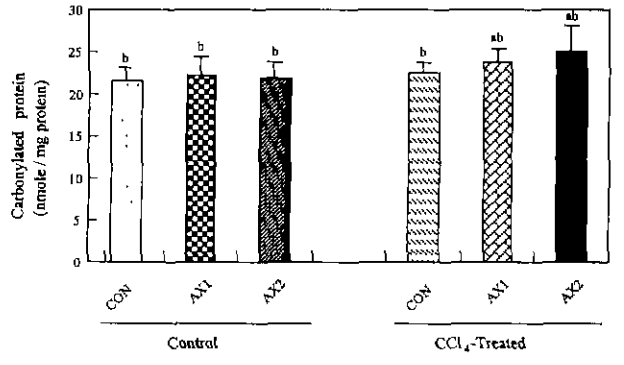

Fig. 4. The effects of astaxanthin on protein carbonylation levels of liver microsomal fraction in rats. Astaxanthin was administered to rats daily at the dose of 2 mg/kg body weight or 100 mg/kg body weight for 15 days. At the 16th day, CCl_4 was injected to rats in the CCl_4-treated group. Liver microsomal samples from the control and CCl_4-treated groups were prepared and protein carbonylation were measured as described in the Materials and Methods. Values are mean±S.D. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test. Con: control group; AX1: astaxanthin administered group (2 mg/kg body weight); AX2 : astaxanthin administered group (100 mg/kg body weight).

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