Notice: This material may be protected by copyright law [Title 17 US Code]. Provided by the Pacific Regional Aquaculture Information Service.
Effects of Astaxanthin and Vitamin C on the Prevention of Gastric Ulcerations in Stressed Rats

Yoshiyuki NISHIKAWA¹, Yoshiharu MINENAKA¹, Mika ICHIMURA¹, Kaori TATSUMI¹, Tomonori NADAMOTO² and Kimiko URABE²

¹Laboratory of Food Science and Nutrition, College of Nutrition, Koshien University, 10-1 Momijigaoka, Takarazuka, Hyogo, 665-0006, Japan
²Laboratory of Food Science and Nutrition, School of Human Culture, The University of Shiga Prefecture, 2500 Hassaka-cho, Hikone, Shiga, 522-8533, Japan

(Received November 5, 2004)

Summary  Astaxanthin (Asx), one of the carotenoids, is a red pigment in fish and Crustaceans, and possesses stronger reduction properties than conventional carotenoids, like β-carotene. However, little is known about the biochemical properties and physiological functions of astaxanthin. The effects of astaxanthin and vitamin C on stressed rats were studied physiologically and biochemically. β-Carotene and three kinds of astaxanthins, which were extracted from Haematococcus and Phaffia, and synthesized chemically, were used in these experiments. These rats given astaxanthins or β-carotene had stress induced on the 12th day by immersing the rats in chest-level water at 20°C for 24 h after fasting for 24 h. Rats given astaxanthins or β-carotene prior to stressing were appreciably protected against the evolution of gastric ulcerations in relation to control rats. Ulcer indexes in particular were smaller with the rat group fed astaxanthin extracted from Haematococcus than the other groups. Next, the effects of Asx and/or vitamin C on the protection of evolution of gastric ulcer in stressed rats were pursued by the same methods as described above. The results showed that rats given Asx or vitamin C were appreciably protected against the evolution of gastric ulcerations in relation to control rats. The effects were more intense, especially in rats simultaneously supplied Asx and vitamin C than in rats taking either Asx or vitamin C. It was suggested that the simultaneous supplementation of food substances with astaxanthin and vitamin C would supply enough antioxidants to offset stress-related injuries.

Key Words  ascorbic acid, vitamin C, carotenoids, β-carotene astaxanthin, gastric ulcers, stress

Triggers for adult diseases such as diabetes mellitus, hypertension and cancer are assumed to be related to the overproduction and over accumulation of cellular peroxides like lipid peroxides, free radicals and active reactive oxygen species in the body (1–3). Accordingly, effective methods for protection against adult diseases rely on the intake of substances which contain reducing agents like vitamin C, vitamin E and β-carotene (4–7). Generally, with regard to diabetes, nutritional supplementation with antioxidants such as α-tocopherol, α-tocopherol and vitamin C were shown to be beneficial (8). Further, cement workers in Turkey are exposed to more oxidative stress when compared to control subjects, and to overcome the oxidative stress, dietary supplementation with antioxidant vitamins such as α-tocopherol and ascorbic acid, were found to be effective (9). The authors have reported that the blood pressure of SHR (spontaneously hypertensive rats) significantly increased upon the administration of salt, but when given concomitantly with vitamin C, the hypertension was considerably restrained (10) by the antioxidative effect (11). Astaxanthin, one of the carotenoids, has been regarded as a more powerful antioxidant in relation to ascorbic acid. α-tocopherol and other carotenoids like β-carotene. Haematococcus pluvialis algae (12. 13) and Xanthophyllomyces dendrorhous (Phaffia rhodozyma) (14) produce high levels of astaxanthin, and extracts from these algae are used as aquaculture colorants, egg yolks and crustaceans. Astaxanthin was expected to confer numerous health benefits and desirability in food and pharmaceutical industries due to its potent antioxidant properties. Studies which examined whether astaxanthin could elicit beneficial effects were carried out (15–17). Oxidative stress under hyperglycemic conditions caused dysfunctions in pancreatic β-cells and various forms of tissue damage. Astaxanthin could exert beneficial effects in diabetes, with preservation in β-cell function, and was potentially useful for reducing glucose toxicity (15). Vitamin C and carotenoids have numerous benefits in human health. Glavin et al. (18) showed that the rats loaded with cold restraint-stress suffered from gastric ulcers, and the rats given vitamin C in advance exhibited less gastric damage than control rats.
Vitamin C was guessed to have protective effects against injury from the stress. So, in this paper, the authors tried to obtain reliable evidence of the effects of Vitamin C and astaxanthin. Rats were stressed by chest level immersion in water for 24 h. Vitamin C and astaxanthin, which show strong antioxidant activities, were evaluated for the prevention of the evolution of gastric ulcers and liver dysfunctions and for the improvement of other physiological and biological functions.

**MATERIALS AND METHODS**

1. **Physiological experiments designed to feed carotenoids (astaxanthin, β-carotene) and vitamin C (VC) to rats**

   1) Physiological experiments, by giving carotenoids (Haematococcus astaxanthin (Haem-asx), Phaffia astaxanthin (Pha-asx)), astaxanthin synthesized organochemically (Syn-asx) and β-carotene to rats: Six-week-old Wistar male rats (50 rats: mean body weight of about 100 g) were divided into 10 groups (5 rats per group). Groups were labeled A to J and housed in individual wire screen bottomed cages.

   Haem-asx was added at 40 mg/100 g (High dose: HD) into the diet of group A and at 8 mg/100 g (Normal dose: ND) (19) into the diet of group B. Pha-asx, Syn-asx and β-carotene were added at HD and ND dose level into the diet of C and D, E and F, and G and H, respectively. The rats of I group were the stressed control without carotenoids, and the rats of J group were the non-fasted and non-stressed control. The animals were housed at 25°C with a 12 h light cycle (7:00–19:00) in a SPF environment. Rats were allowed free access to water and a stock chow diet, which contained hardly any Asx. (Clea Japan; CE2).

   The body weight of rats and their dietary and water intakes were measured every day. Rats in groups A to I were given their respective diets for 11 d, fasted for 24 h from 10:00 a.m. to the next 10:00 a.m., and introduced to stress by immersing the rats in chest-level water at 20°C for 24 h from 10:00 a.m. to the next 10:00 a.m. All of the rats (group A to J) were dissected from 11:00 a.m. (20, 21).

   2) Physiological experiments designed to feed Haem-asx, and vitamin C to rats: Nine-week-old Wistar male rats (25 rats: mean body weight was about 200 g) were divided into 5 groups (5 rats per group) labeled A to E. The rats were fed the following: A group (Haem-asx: 24 mg/100 g), B group (Haem-asx+VC), C group (VC), D group (Control; stressed) and E group (Control; non-stressed). The A to E groups were fed diets with test compounds for 6 d, fasted for 24 h, and introduced to stress by immersing the rats in chest-level water at 20°C for 24 h, as described above (20, 21). Astaxanthin was given to rats in the form of 24 mg in 100 g diet, and vitamin C (500 mg) was dissolved in drinking water (100 mL) (10, 22).

2. **Biochemical experiments designed to feed carotenoids and vitamin C to rats.** After stressing, rats were dissected and their hearts, livers, spleens, kidneys, adrenals and stomach were removed. The organs were weighed and observed morphologically. The evolution of gastric ulcers in stressed rats was observed by a method described in the following paragraph. Several blood biochemical tests were carried out on rat sera. Enzyme activities for ALT, AST and γ-GT and the amount of TGs in sera were assayed to confirm biological rat functions. Blood drawn from cardiac aortas was collected in vessels, and centrifuged for 1 min at 3,000 rpm (K. Daiichi, Japan CF-9510). Sera, and supernatants obtained after centrifugation were measured using an autoanalyzer (K. Daiichi, Japan SP-4410).

3. **Assays and gastric ulcer indices.** Lumina from removed stomachs were washed by passing physiological saline solutions from pylori to cardios using syringes. Sections of pylori and cardios taken from washed stomachs were tied with threads, and stomachs were filled with 1% formalin and immersed in 10% formalin for 10 min to fix gastric ulcers. Stomachs were incised along greater curvatures, and examined for ulcer disease histopathologically using a dissecting microscope 10 mm scale eyepiece adapter (Olympus, Japan: SZN). The cumulative area in square millimeters of the ulcers was quantified (18, 20).

4. **Ethical approval.** This study was carried out according to the guidelines for animal experiments of the Journal of Nutritional Science and Vitaminology, and also that of the College of Nutrition, Koshien University.

5. **Data analyses.** Data are presented as means±SD (n=5). The statistical significance of the differences between values was determined using an analysis of variance (one-way ANOVA) and Duncan’s multiple-range test (23).

---

**Fig. 1.** Changes of body weight gains in rats fed astaxanthin and β-carotene. The marks, • (A), ■ (B), ▲ (C), □ (D), — (E), ● (F), △ (G), □ (H), ○ (I) and ◆ (J), denote the experimental groups described in Materials and Methods. That is, A: Haem-asx (ASX extracted from Haematococcus) (High dose: HD), B: Haem-asx (Normal dose: ND), C: Syn-asx (ASX synthesized chemically: HD). D: Syn-asx ND, E: Pha-asx (ASX extracted from Phaffia: HD), F: Pha-asx ND, G: β-carotene HD, H: β-carotene ND, I: stressed Control and J: non-stressed Control. The rat groups from A to I group were stress-loaded. HD and ND mean high dose (40 mg/100 g diet) and normal dose (8 mg/100 g diet) for these test substances. All of the rats except for group J were fasted on the 11th day for 24 h, followed by loaded stressing for 24 h.
Fig. 2. Effects of astaxanthin and β-carotene on liver and spleen weights of stressed rats. Organ weights (g) for livers and spleens per 100 g of body weight of stressed rats are shown. The capital letters A to J denote the experimental groups described in the legend of Fig. 1. Columns show the mean±SD for 5 rats per group. When F values were significant, differences among the groups were inspected by Duncan’s multiple range test (p<0.05). Means within the same column not sharing a common superscript letter denote significant differences (p<0.05).

Fig. 3. Effects of astaxanthin and β-carotene on serum triacylglyceride concentration, ALT and γ-GT activities and gastric ulceration of stressed rats. The ALT, γ-GT and TGs were determined using an autoanalyzer for clinical examinations (Kyoto Daiichi, Japan). Ulcer index was determined by quantifying the area of ulcer disease. The capital letters A to J denote the experimental groups described in the legend of Fig. 1. Columns show the mean±SD for 5 rats. When F values were significant, differences among the groups were inspected by Duncan’s multiple range test (p<0.05). Means within the same column not sharing a common superscript letter denote significant differences (p<0.05).

RESULTS

1. Physiological experiments designed to feed carotenoids Haem-asx, Pha-asx, Syn-asx and β-carotene to rats

The effects of several astaxanthins (Haem-asx, Pha-asx and Syn-asx) and β-carotene on the physiological conditions of stressed rats were investigated in this experiment. As shown in Fig. 1, body weight gains in rats increased linearly for 11 d, and did not differ significantly (p<0.05) for the 10 experimental groups (A to J). Amongst these groups, some were fed carotenoids like astaxanthin and β-carotene, while others were not given antioxidants. However, increments in body weights from group A to F rats given astaxanthin were liable to be greater than those of rats given β-carotene (groups G and H). On the 11th day, rats from group A to I were fasted for 24 h after the bleeding of test compounds, followed by induction of stress by immersing animals in chest-level water for 24 h. Body weights decreased almost identically with rats from group A to I, except for group J, which consisted of non-fasted and

Fig. 4. Changes of body weight in rats fed astaxanthin and vitamin C. The marks, • (A), ■ (B), ▲ (C), ● (D) and ○ (E) denote experimental groups described in Materials and Methods. That is, A: Astaxanthin, B: Astaxanthin+vitamin C, C: vitamin C, D: stressed Control and E: non-stressed Control. All of the rats except for group E were fasted on the 6th day for 24 h, followed by loaded stressing for 24 h.
non-stressed control rats. Food and water intakes for rats given carotenoids were almost the same as those of control rats. On day 13 all the rats were dissected and their livers, spleens and stomachs were removed. Compared to the organ weights (g/100 g body weight) of livers and spleens of unstressed control rats (group J), those of stressed rats (group A to I) significantly ($p<0.01$) decreased. However, values for groups A to I, which were stressed rats, were almost similar compared to the non-stressed rats as shown in Fig. 2. Blood biochemical tests for enzymatic activities of ALT and γ-GT, and triacylglyceride concentrations were carried out to detect primary liver functions (Fig. 3). ALT activities in stressed control rats (group I) were much greater than in unstressed control rats (group J). There was not a great difference among activities from group A to I. It is compelling to say, ALT activities were almost identical in 9 rat groups (A to I). Mean values of ALT activities were significantly smallest in the rats given Haem-aax (HD) (group A) among groups A to H. γ-GT activities were higher in stressed control rats (group I) than in rats given carotenoids (groups A to H), though there were large individual differences among these groups. TG contents significantly decreased in groups A to I for non-stressed control rats (group J). Decrements were assumed to mainly be the result of fasting and stressing rats. The ulcer index of non-stressed control rats (group J) was 0 mm², but in stressed control rats (group I), the index remarkably increased. Ulcer indices in rats taking carotenoids (groups A to H) decreased significantly and were lower than 50% of those obtained from stressed control rats (group I). Mean values were especially
smaller in the rats which were given Haem-asx (HD) (group A).

2. Physiological experiments designed to feed Haem-asx and vitamin C to rats

Experiments with the simultaneous supply of astaxanthin and vitamin C were carried out using 25 renewed rats. As shown in Fig. 4, body weights from 5 rat groups increased gradually over 6 d. The rats given vitamin C took about 25 mL of water containing vitamin C per day. Accordingly, the intake of vitamin C was estimated to be 125 mg/d. On the 6th day, rats from groups A to D were fasted for 24 h after bleeding the test compound, followed by stress loading by immersing rats in chest-level water at 20°C for 24 h. Body weights decreased for 2 d from day 6 to day 8 in direct consequence of fasting and stressing animals. The results were almost identical for rats from group A to D (loaded stress), but not for non-fasted and non-stressed control rats (group E). There were no significant differences in body weight gains for rats among groups A to D. Rat organ weights (g/100 g body weight) are shown in Fig. 5. The heart and kidney weights from groups A to D were slightly higher than those of non-stressed control rats (group E). There were no differences in adrenal weights between rat groups. Liver and spleen weights of stressed rats were significantly decreased when compared to non-stressed control rats (group E) in a similar manner as in the previous experiment, and were not altered among groups A to D.

Blood biochemical test results and ulcer indices are shown in Fig. 6. ALT and AST enzymatic activities for non-stressed group E rats were normal (14 ± 3 IU). On the other hand, those of stressed rats in groups A to D were 241 ± 59, 213 ± 25, 197 ± 41 and 282 ± 73 IU, respectively. Among groups A to D, ALT activities of the rats given vitamin C (groups B and C) were more or less decreased in comparison to those of stressed control rats (group D). γ-GT enzyme activities were significantly increased in stressed rats from groups A to D similar to ALT and AST activities. Only intake of vitamin C significantly decreased γ-GT activities in stressed control rats. The concentration of blood triacylglycerides in stressed rats (group A to D) was considerably decreased for non-stressed rats (group E), but individual differences from stressed rats were practically unchanged in this experiment. Ulcer indices from non-stressed rats (group E) were 0 mm², in short, and non-stressed control rats did not develop gastric ulcers. However, stressed control rats (group D) showed remarkably large ulcer indices (17.3 ± 9.6 mm²). Groups A to C given astaxanthin and/or vitamin C showed considerably and significantly (p<0.05) smaller ulcer indices of 10.2 ± 4.2, 6.4 ± 4.4 and 7.1 ± 5.6 mm², respectively. In this experiment, the group B rats simultaneously given astaxanthin and vitamin C were found to be most protected against the evolution of gastric ulcers.

**DISCUSSION**

In the present paper, the evolution of gastric ulcers was observed by stress loading rats: rats were fixed in chest level water at 20°C for 24 h in small cages. Gastric ulcerations are thought to evolve when stress causes necrosis of stomach mucosal epithelial and basement membrane cells, and necrotized cells are left outside of gastric mucosa. The authors presumed the mechanisms for the evolution of gastric ulcers by stress as follows: The secretion of gastric acid in the stomach increases according to increments in the secretion of gastrin, acetylcholine and histamine. The concentration of active oxygen in the blood vessels of stomachs increases with ischemia-reperfusion, and reactive oxygen species break vessel cells. The secretion of mucin to protect mucous cells from attacks by pepsin and gastric acid is reduced by stress. The gastric mucosal epithelial cells are extinguished through the steps described above (1–3).

Medicines to suppress the secretion of gastric acid, like antagonists of histamine, muscarin, and gastrin receptors, along with inhibitors for proton pumps have been used in the therapeutic treatment of ulcers. In the beginning of this experiment, the authors thought that dietary nutrients might prevent gastric ulcers and restore organ injuries generated by stress.

At first, effects from high and normal doses (19) were studied for three kinds of astaxanthins and β-carotene. Body weight gains for the tested rats groups were almost identical to experimental and negative control rats. After fasting and stressing the rats for 24 h, livers and spleens shrank remarkably against non-fasted and non-stressed rats. ALT enzyme activities from non-stressed rats were normal, but stressed rats from groups A to I experienced an increase in ALT activities. There was no great difference among values from rats in groups A to I. In the prevention of gastric ulcers, all the carotenoids were effective in decreasing the incidence of gastric ulcers. Haem-asx (groups A and B) had more protective effects against the evolution of gastric ulcers and liver dysfunctions.

When studying the effects of astaxanthin on physiological functions under stress, Kurihara et al. (24) reported using mice in which the total number of spleen cells, and levels of NK (natural killer) cell activities per spleen were reduced to nadir and the lipid peroxidation of liver tissue increased significantly. Further astaxanthin (100 mg/kg/d) improved the immunological dysfunction induced by restraint stress, and daily oral administration of astaxanthin (1 mg/kg/d) markedly attenuated the promotion of hepatic metastasis induced by restraint stress. Astaxanthin was suggested to improve antitumor immune responses by inhibiting lipid peroxidation induced by stress.

Next, the simultaneous supply of Haem-asx and vitamin C was carried out. The amount of vitamin C given to rats was about 20 times that given to normal human beings. It was determined by converting the mean body weight of the rats to that of an average human and by the metabolic turnover (22). There were no differences in body weight gains for rats stress-loaded in groups A to D. Compared to heart and kidney hypertrophies in stressed rats (group A to D), livers and spleens con-
versely atrophied. ALT and AST enzyme activities from stressed rats were remarkably higher than in non-stressed control rats. The subsequent intake of vitamin C decreased γ-GT activities raised in stressed control rats. The concentration of blood triacylglycerides from stressed rats considerably decreased in comparison to non-fasted and non-stressed rats (group E). The main cause of the decrements was surmised to be fasting for 48 h. Gastric ulcers evolved strongly in stressed control rats against the non-evolution of gastric ulcers in non-stressed rats. Three rat groups (A to C) were given astaxanthin and/or vitamin C, and these rats showed significantly small ulcer indices against stressed control rats (group D).

Cheney and Rudrud (25) reported that when rats were given vitamin C in their drinking water prior to and during three consecutive 47 h food deprivation periods, they did not develop ulcers. Rats given water or deactivated vitamin C developed severe gastric pathologies. Glavin et al. (18) designed a study to replicate Cheney’s findings. It was shown that vitamin C did not afford total protection against ulcer development in rats subjected to various forms of stress. On the contrary, the intake of vitamin C exacerbated ulcer formation in the stomach of rats. Most of the previous reported studies had used only single doses of vitamin C. Glavin et al. examined the effects of pretreatment with three doses of vitamin C on cold restraint-induced gastric ulcers in rats. The relationship between average daily vitamin C intake and ulcer severity identified in the high dose (50 g/L) group replicated results previously reported. On the other hand, rats given vitamin C at the low dose (10 g/L) were found to exhibit less gastric damage than control rats. Accordingly, a lower dose vitamin C was surmised to exert a protective effect on the gut.

In the experiment of Brzozowski et al. (26), aspirin significantly delayed ulcer healing and this effect was accompanied by a marked increase in lipid peroxidation and a fall in the gastric blood flow (GBF) at ulcer margin. Vitamin C significantly attenuated both the aspirin-induced gastric damage and the accompanying fall in the GBF at ulcer margin and reversed the aspirin-induced lipid peroxidation. Patients with normal endoscopic findings had significantly higher intragastric concentrations of vitamin C than those with gastric cancer, pernicious anemia, gastric ulcers, duodenal ulcers, or relative baseline values determined after gastric surgery. There was an especially strong trend towards lower intragastric concentrations of vitamin C in patients with chronic atrophic gastritis. Gastric levels secretly studied in five volunteers showed that vitamin C concentrations increased significantly after intramuscular pentagastrin and that vitamin C was secreted by the human stomach (27). In these results, it was suggested that a deficiency in vitamin C might easily invite gastric ulcer formation, and vitamin C secreted into the stomach from blood might be useful to protect against gastric ulcer evolution.

On the other hand, astaxanthin was expected to confer numerous health benefits due to its potent antioxidan properties. In our rat experiments, astaxanthin might be surmised to reduce the evolution of gastric ulcers because of its antioxidant effects. Furthermore, simultaneous intakes of astaxanthin and vitamin C might work to inhibit the secretion of gastric acid and prevent the evolution of gastric ulcers. The reperfusion of blood may occur after the depression of blood flow by vasosclerosis in livers by stress and active reactive oxygen species, which are thought to break into organ cells, are extensively generated in liver cells; astaxanthin may work as a protector of blood vessel cells and gastric mucosal epithelial cells in combination with vitamin C. In this paper, antioxidants like astaxanthin and vitamin C were shown to be useful in preventing gastric ulcer evolution caused by stress.

**REFERENCES**

767–771.