Antihypertensive Potential and Mechanism of Action of Astaxanthin: III. Antioxidant and Histopathological Effects in Spontaneously Hypertensive Rats

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We investigated the effects of a dietary astaxanthin (ASX-O) on oxidative parameters in spontaneously hypertensive rats (SHR), by determination of the level of nitric oxide (NO) end products nitrite/nitrate (NO_2^-/NO_3^-) and lipid peroxidation in ASX-O-treated SHR. Oral administration of the ASX-O significantly reduced the plasma level of NO_2^-/NO_3^- compared to the control vehicle ($p<0.05$). The lipid peroxidation level, however, was reduced in both ASX-O- and olive oil-treated groups. We also analyzed the post-treatment effects of ASX-O on the vascular tissues by examining the changes in the aorta and coronary arteries and arterioles. The dietary ASX-O showed significant reduction in the elastin bands in the rat aorta ($p<0.05$). It also significantly decreased the [wall : lumen] aerial ratio of the coronary arteries. These results suggest that ASX-O can modulate the oxidative condition and may improve vascular elastin and arterial wall thickness in hypertension.

Key words astaxanthin; hypertension; antioxidant; elastin

The oxidative status and the physiological profile of the cardiovascular system represent crucial elements in evaluating the development and progression of a number of cardiovascular diseases (CVD), including hypertension. On one hand, hypertension is associated with structural changes in the resistance vasculature.[1—3] These alterations, known as 'remodeling', had been considered to be a complex process that might involve an increase (hypertrophy), a decrease (hypotrophy), or a rearrangement (eutrophy) of the vascular wall material.[3,4] In the majority of hypertension animal models, the arterial internal diameter was generally reduced and the wall : lumen ratio was increased in the small arteries if they are compared under equivalent biophysical conditions.[1—3]

On the other hand, free radicals and oxidative stress have been reported to play an important role in the pathogenesis of a variety of heart disease conditions,[5] ischemia-reperfusion injury,[6] congestive heart failure,[7] coronary artery disease,[8] and hypertension.[9,10]

Some antioxidant compounds and diets have been shown to be effective in reducing oxidative damage in rats, especially in heart disease.[11,12] Antioxidants, including a number of carotenoids, have been hypothesized to inhibit lipid peroxidation[13] and play a protective role against chronic diseases such as CVD.[14] The antioxidant astaxanthin (ASX), a natural oxygenated carotenoid with no pro-vitamin A activity, has been reported in a number of studies to have a superior antioxidant activity.[15,16]

We previously investigated the blood pressure (BP) lowering effect of a dietary astaxanthin (ASX-O) in spontaneously hypertensive rats (SHR)[17] and its suggested action mechanisms.[18] In the present study, the effects of ASX-O on the oxidative status and the vascular physiology in SHR were examined. We measured the levels of nitrite/nitrate (NO_2^-/NO_3^-) anions and lipid peroxidation in ASX-O-treated SHR. The histological effects were assessed by light microscopy of slides from specimens of the heart, aorta, and coronary arteries.

MATERIALS AND METHODS

General Procedures Spontaneously hypertensive rats (SHR) (♂, 7 weeks) obtained from colonies of specific pathogen-free rats maintained by Japan SLC (SLC, Shizuoka, Japan), were used. Housing conditions were thermostatically maintained at 24±1 °C with constant humidity (60%) and lighting (12 h light/dark cycle, lights on: 07:30—19:30). The animals were acclimatized for at least 1 week before the experiments and fed a normal diet (Lab MR, NOSAN, Yokohama, Japan) and given water *ad libitum*. Body weight was measured daily during the experimental period. ASX-O, composed of 5.5% astaxanthin in an edible oil base, was obtained from Fuji Chemical (Fuji Chemical Industry Co. Ltd, Toyama, Japan) and dissolved and diluted in olive oil (Wako Pure Chemicals, Osaka, Japan). Doses were calculated as ASX in the dietary ASX-O. The animals were divided into two groups (8 rats/group) and treated daily for 7 weeks. One group was administered ASX-O (5 mg/kg/d, *p.o.*) and the other one was similarly treated with olive oil (1 ml/kg/d).

All experimental procedures were performed in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals of University of Toyama.

Measurement of Blood Pressure and Heart Rate in Conscious Rats Arterial blood pressure (BP) and heart rate (HR) were determined by a tail cuff system. The rats were lightly supported in a mesh holder made of cloth and maintained at 37±1 °C (Model THC-1 Digital Thermo, Softron, Tokyo, Japan). BP from the tail artery was indirectly measured using a tail-cuff apparatus (BP-98, Softron) which was controlled with a personal computer. Values are presented as the average of three separate measurements.

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Measurement of Plasma Nitric Oxide (NO) and Lipid Peroxide Levels Blood from the heart of each sacrificed SHR was separately collected into heparinized syringes containing 5% heparin and 2% sodium citrate, and was instantly and gently mixed. Plasma was immediately separated from the blood by centrifugation at 3000 *g* for 15 min (Kubota 8700, Kubota, Tokyo). Nitrite (NO_2^-) and nitrate (NO_3^-) anions, designated as NOx, were used as indices of *in vivo* NO generation. A portion of the plasma was filtered through a syringe microfilter (4 mm Millex-HV, 0.45 mm, Millipore Japan Ltd., Tokyo) and the NOx level was measured with an automated high-performance liquid chromatography-Griess system, ENO-10 (Eicom Co., Kyoto, Japan). Briefly, the system consisted of a separation column, a reduction column (to reduce NO_3^- to NO_2^-), a flow reactor (with Griess reagent), and a detector at 540 nm. The sensitivity of the setup was 0.1 μM for both NO_2^- and NO_3^- with a loading volume of 10 μl. To minimize NOx contamination, all laboratory glassware was washed several times with pure water (almost NOx-free by filtration through a Milli-Q [Millipore, Bedford, MA, U.S.A.]).

The lipid peroxide level was determined by measuring the thiobarbituric acid reactive substance (TBARS) concentration as an index of lipid peroxidation, according to the Yagi method.[19] The measurement of TBARS was conducted by Special Reference Laboratories (SRL) (SRL Tokyo Medical Inc., Tokyo).

Histological Studies After the 7 week treatment, the rats were anesthetized (50 mg/kg i.p. pentobarbital) and sacrificed by cutting the abdominal aorta. Instantly, the heart, aorta and coronary arteries were excised, trimmed of adhering tissues, placed in buffered formalin (10%), and fixed. Specimens from each of the rats were embedded in paraffin and sections were stained with hematoxylin–eosin for the light microscopy study. Verhoff's stain was used to evaluate the expression and distribution of vascular elastin. The specimens and slides were prepared by SRL (Tokyo). Sections of the heart, coronary arteries and aortae were examined and photographed using a light microscopy system (Olympus Provis AX80, Olympus Optical Co. Ltd., Tokyo).

Elastin Evaluation The microstructural changes in the vascular elastin were assessed by determining the number of elastin bands in several sections of each aorta.

Wall-Thickness The change in vascular wall-thickness was assessed by the [wall : lumen] aerial ratio of the coronary arteries and arterioles. The aerial calibration was carried out using a standard area of (1/20×1/20 mm) (Burker-Turk haemocytometer, JIS No. E3871, Kayagaki Irika Kogyo Co., Ltd., Tokyo). The measurement and analysis of the slides was conducted using UTHSCSA Image Tool for Windows, version 3.00 (Texas, U.S.A.). The slides were further examined and evaluated blindly by two investigators.

Statistical Analysis Statistical significance was determined using Student's *t*-test for unpaired observations and the Mann–Whitney Rank Sum Test. One-way analysis of variance (ANOVA) was performed for multiple comparisons between the groups. Differences with $p<0.05$ were considered statistically significant.

Drugs and Chemicals Analytical grades of the following reagents were purchased: pentobarbital sodium from TCI (Tokyo Kasei, Tokyo), heparin from Mochida (Novo-Heparin, Mochida, Tokyo) and sodium citrate from Fuso (Osaka, Japan).

RESULTS

Blood Pressure (BP) Oral administration of ASX-O (5 mg/kg/d, for 7 weeks) had significant BP-lowering effects on arterial BP ($p<0.05$) (Table 1), consistent with our previous results.[17] The effect at this dose was significant from day 21, *i.e.* the 3rd week, of the treatment period.

NOx and Lipid Peroxidation The effects of ASX-O on *in vivo* NO generation in the animals were evaluated by the NO_2^-/NO_3^- indices (referred to as NOx). ASX-O significantly reduced NOx in the SHR (Fig. 1). However, the lipid peroxidation levels were very similar in the ASX- and olive oil-treated groups, with no significant difference being observed (Fig. 2).

Table 1. Characteristics of ASX-O-Treated SHR

	0 weeks		7 weeks	
	Control	ASX-O	Control	ASX-O
BW (g)	166±1.6	166±1.3	266±3.3	260±4.0
SBP (mmHg)	173±1.6	171±1.8	197±3.2	173±1.1‡
MBP (mmHg)	152±1.9	152±1.8	174±2.4	148±0.9*
Plasma NO_2^-/NO_3^- (μM)			62.3±8.7	9.8±1.9***
Lipid peroxides (μM)			0.7±0.1	0.6±0.1
Aortic elastin bands (number)			10.8±0.4	6.9±0.4***
Arterial [wall : lumen] ratio			2.8±0.7	1.5±0.1**

BW=body weight, SBP=systolic blood pressure, MBP=mean blood pressure. Each value represents the mean±S.E.M. (n=5—8). The elastin bands were counted from 3 different sections/specimens of 4 rats/group. *$p<0.05$; **$p<0.01$; ***$p<0.001$ *vs.* control (olive oil) group (*t*-test). ‡$p<0.05$ *vs.* the control (Mann-Whitney Rank Sum Test).

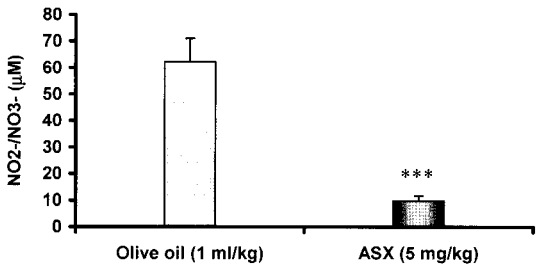

Fig. 1. Effects of Oral Administration of ASX-O on the Level of Plasma NO_2^-/NO_3^- in SHR

The animals were treated with ASX-O and olive oil (control) for 7 weeks. Data represent the mean±S.E.M. (n=5—7). ***$p<0.001$ *vs.* the control (*t*-test).

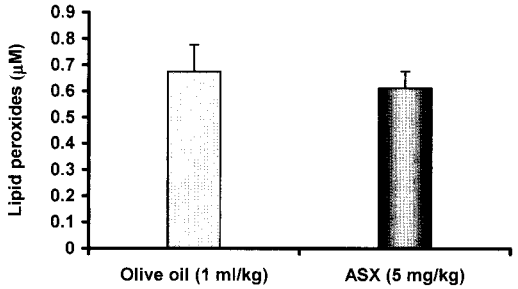

Fig. 2. Effects of Administration of ASX-O on Plasma Lipid Peroxide Level

Data are represented as the mean±S.E.M. (n=6—7).