Antihypertensive and Neuroprotective Effects of Astaxanthin in Experimental Animals

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Astaxanthin is a natural antioxidant carotenoid that occurs in a wide variety of living organisms. We investigated, for the first time, antihypertensive effects of astaxanthin (ASX-O) in spontaneously hypertensive rats (SHR). Oral administration of ASX-O for 14 d induced a significant reduction in the arterial blood pressure (BP) in SHR but not in normotensive Wistar Kyoto (WKY) strain. The long-term administration of ASX-O (50 mg/kg) for 5 weeks in stroke prone SHR (SHR-SP) induced a significant reduction in the BP. It also delayed the incidence of stroke in the SHR-SP. To investigate the action mechanism of ASX-O, the effects on $PGF_{2\alpha}$-induced contractions of rat aorta treated with N^G-nitro-l-arginine methyl ester (L-NAME) were studied *in vitro*. ASX-O (1 to 10 μM) induced vasorelaxation mediated by nitric oxide (NO). The results suggest that the antihypertensive effect of ASX-O may be due to a NO-related mechanism. ASX-O also showed significant neuroprotective effects in ischemic mice, presumably due to its antioxidant potential. Pretreatment of the mice with ASX-O significantly shortened the latency of escaping onto the platform in the Morris water maze learning performance test. In conclusion, these results indicate that astaxanthin can exert beneficial effects in protection against hypertension and stroke and in improving memory in vascular dementia.

Key words astaxanthin; hypertension; spontaneously hypertensive rat (SHR); carotenoid; nitric oxide (NO)

Astaxanthin (ASX), a red carotenoid pigment, is a biological antioxidant that occurs naturally in a wide variety of living organisms. ASX has shown some pharmacological activities, including anti-oxidative,[1—4] anti-tumor and anti-cancer,[5,6] anti-inflammatory,[7,8] anti-diabetic[9] and immunomodulatory activities.[10,11] It was reported to exhibit strong free radical scavenging activity and to protect cells against lipid peroxidation and the oxidative damage of low-density lipoproteins (LDL)-cholesterol and of the cell membrane.[12] The role of the oxidation of unsaturated lipids in LDL and its complex sequelae in the pathogenesis of a number of cardiovascular diseases (CVD) has been widely investigated.[13,14] Epidemiological and clinical data have indicated that dietary antioxidants, such as carotenoids, might protect against CVD[15,16] and help to prevent several human diseases such as prostate cancer and inflammatory disorders.[12]

In this study, we first investigated the effects of ASX on hypertension in rats *in vivo*. A dietary product of astaxanthin (ASX-O) was used in this experiment. The present study was also designed to investigate mechanisms of action and effects of ASX-O on vascular reactivity in rat aortic preparations. We evaluated the effects of ASX-O on the aorta in relation to the endothelium and nitric oxide (NO). Moreover, the effects of ASX-O administration on behavior were studied in mice.

To the best of our knowledge, this is the first report on the antihypertensive and neuroprotective effects of ASX in experimental animals; however a mixture composition for lowering BP consisting of a group of carotenoids has previously been cited.[17]

MATERIALS AND METHODS

General Procedures Male rats of Wistar Kyoto (WKY) (7 weeks), Wistar standard breed (20—40 weeks), spontaneously hypertensive rat (SHR) (7 weeks) and stroke-prone SHR-SP strains (10 weeks) were used in this study. The neuroprotective effects were studied in a strain of Institute of Cancer Research (ICR) mice (8 weeks). The animals were obtained from colonies of specific pathogen-free rats and mice maintained by Japan SLC (SLC, Shizuoka, Japan). Housing conditions were thermostatically maintained at 24±1 °C with constant humidity (60%) and lighting (12 h light/dark cycle, light on: 07:30—19:30). The animals were housed for at least 1 week before the experiments and fed a normal diet (Lab MR, NOSAN, Yokohama, Japan) and water given *ad libitum*. Body weights were 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). Administered doses were calculated as ASX in the dietary ASX-O.

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

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, Tokyo) which was controlled with a personal computer. Values are presented as the average of three separate measurements.

Relaxation Experiments Using Rat Aorta The experiments were conducted as by Goto *et al.*[18] Briefly, Wistar rats (♂, 20—40 weeks) weighing 350—450 g were anesthetized

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(50 mg/kg *i.p.* pentobarbital) and sacrificed by cutting the abdominal aorta. Aortic rings (3 mm) were prepared from the thoracic aorta, mounted on steel hooks in a Magnus chamber (Kishimoto UC-5TD, Kyoto, Japan) and attached to a force-displacement transducer (Kishimoto UM-203, Kyoto) and a recorder (Recti-Horiz 8K, NEC, Tokyo). Baths were filled with Krebs solution maintained at 37 °C and bubbled continuously with 5% CO_2 in O_2 at a pH of 7.4. The rings were equilibrated for 40 min at an initial resting tension of 1 g, and then contracted with 60 mM KCl to determine the optimal resting tension. When the contraction reached a steady maximal response, 10^{-6} M acetylcholine (ACh) was added to confirm the status of the endothelium. ACh induced relaxation in rings with intact endothelium, whereas the relaxation disappeared in the denuded ones. The Krebs solution was then replaced and the experiments were carried out.

In a series of experiments, each of the rings was contracted by treatment with 3×10^{-6} M PGF2α then ASX-O was added at concentrations ranging from 1×10^{-6} to 1×10^{-4} M cumulatively. Relaxation was expressed as the percentage of the decrease in maximal tension obtained by PGF2α-induced contraction.

The effects of ASX-O on the responses induced by the xanthine/xanthine oxidase (XOD) system were studied by the addition of xanthine and XOD to ASX-O-pretreated aortic rings.

To investigate the effects of nitric oxide (NO), the preparations with the endothelium were exposed to 10^{-4} M N^G-nitro-1-arginine methyl ester (L-NAME) for 60 min prior to the addition of ASX-O. In some experiments, the endothelial lining of the rings was removed by pressing the ring slightly and rolling it gently onto a filter paper few times. Removal of the endothelial lining was functionally confirmed by ACh, as described above.

Transient Cerebral Ischemia ICR mice (♂, 8 weeks) were subjected to transient cerebral ischemia induced by bilateral common carotid artery occlusion, 2 vessel occlusion (2VO), 1 h after administration of the substance as described by Watanabe *et al.*[19] In brief, the mice were anesthetized with pentobarbital sodium (50 mg/kg *i.p.*), and the arteries were exposed and carefully separated from the adjacent veins and sympathetic nerves, then occluded by artery clips (Roboz Surgical Instruments, MD, U.S.A.) for 20 min. While the arteries were clamped, 0.3 ml of blood was withdrawn from the tail vein. Then, the clips were removed and cerebral blood flow was restored. The skin incision was closed and the mice were kept in an air-conditioned room at 25±1 °C. Sham-operated mice were subjected to the same procedure without carotid clamping and withdrawal of the blood.

Morris Water Maze Learning Performance One day before the start of learning, mice were given a pre-training session in which they were allowed to swim freely in a pool (70 cm diameter, with a depth of 13 cm of water maintained at 25±1 °C) for 60 s without an escape platform. The pool was placed in a dimly lit large test room and surrounded by visual cues. In the learning block, the pool was filled to the 13 cm depth with water, and a platform (5 cm diameter) was situated 1 cm below the surface of the water. The pool was divided into four quadrants, with the platform in a fixed position in one quadrant. Daily learning consisted of four trials in which the mouse was placed in the water from four different starting points, and the latency of escaping onto the platform was recorded. This was conducted for 5 consecutive days. A maximum of 60 s was allowed for the mouse to find the platform and climb onto it. On day 7, each mouse was subjected to a probe trial in which there was no platform present. The time of crossing the former platform quadrant and the total time of crossing all quadrants were recorded for 1 min.

Statistical Analysis Statistical significance was determined by Student's *t*-test for unpaired observations and by the Mann–Whitney Rank Sum Test. One-way analysis of variance (ANOVA) was performed for multiple comparisons between the groups. The significance between different groups in the Morris water maze test was analyzed by two-way repeated measures ANOVA. Differences with $p<0.05$ were considered statistically significant.

Chemicals Prostaglandin F2α (PGF2α), xanthine, xanthine oxidase (XOD), N^G-nitro-1-arginine methyl ester (L-NAME), dimethyl sulphoxide (DMSO) and acetylcholine chloride (ACh) were obtained from Wako (Wako Pure Chemicals, Tokyo, Japan), and pentobarbital sodium was from TCI, Tokyo Kasei, Tokyo.

RESULTS

Antihypertensive Effects of ASX-O In the first series of experiments, the effects of oral administration of ASX-O on BP in WKY rats (♂, 7 weeks) and SHR (♂, 7 weeks) were investigated. A single administration of ASX-O at doses of 5, 50 and 500 mg/kg *p.o.* did not affect the BP or HR in both WKY rats and SHR. Then, ASX-O was administered at 50 mg/kg *p.o.* to WKY rats and SHR once daily for 2 weeks. ASX-O significantly lowered the BP in SHR, while it did not produce any changes in WKY rats (Fig. 1).

Thereafter, the effect of long-term administration of ASX-O at the doses of 5 and 50 mg/kg, *p.o.*, was examined in SHR-SP (♂, 10 weeks), which showed BP over 200 mmHg. ASX-O at the dose of 5 mg/kg produced no significant change in BP after one week of administration, although it induced a progressive reduction in BP from the second week. In contrast, at the dose of 50 mg/kg, ASX-O showed the BP lowering effect from the first week. After 5 weeks of administration, ASX-O produced a significant reduction in SBP (−6%, −4%), DBP (each −10%) and MBP (−9%, −8%) at doses of 5 mg and 50 mg/kg, respectively. On the other hand, the BP of the control group increased nearly 18 mmHg (Fig. 2). There was no significant change in the HR after ASX-O treatment. Moreover, ASX-O did not modify or show any effect on body weight in either the SHR-SP or WKY rats (data not shown).

Time Course Effects of ASX-O on BP The effects on BP were measured 40 min before administration, at 0 min, and every 40 min after the administration for a 4-h period.

Compared to the control, ASX-O (50 mg/kg) administration produced significant BP lowering effects. The effects on MBP were significantly different from the control after 80 min (Fig. 3). An initial effect was shown shortly after administration, but within the first 40 min, as indicated by 0 min time in Fig. 2, suggesting an acute phase of effect by ASX-O. The HR did not show significant change during the time course ($p>0.1$) (data not shown).

Effects of ASX-O on Stroke Incidence In this study,

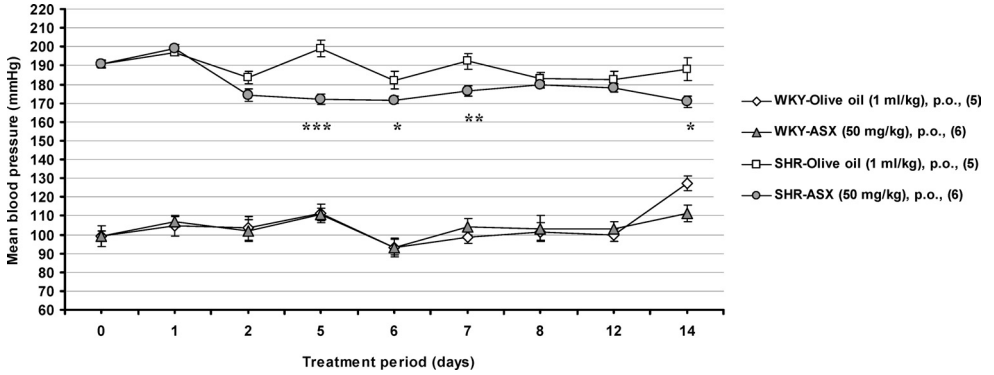

Fig. 1. Effects of Short-Term Oral Administration of ASX-O on MBP in SHR and WKY Rats

Each data point represents the mean±S.E.M. of 5—6 rats per group. Doses are calculated as ASX in the dietary ASX-O. $*p<0.05$: $**p<0.01$: $***p<0.001$ *vs.* the vehicle control group (*t*-test).

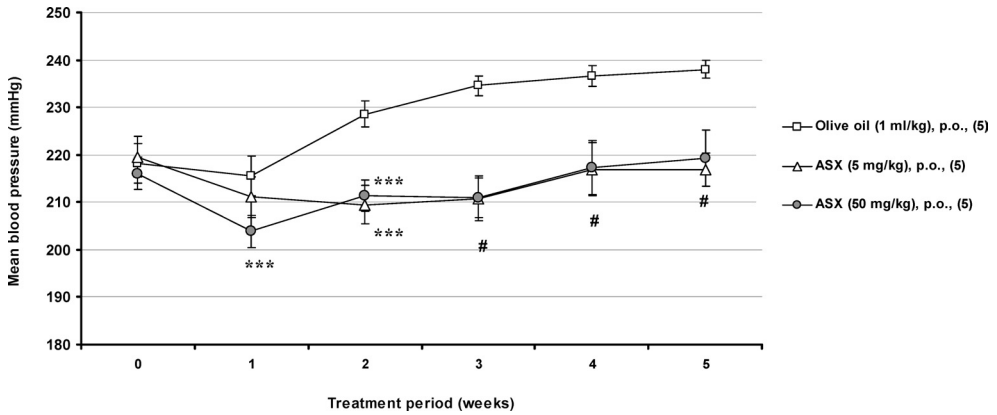

Fig. 2. Effects of Long-Term Oral Administration of ASX-O on MBP in SHR-SP

Each data point represents the mean±S.E.M. ($n=5$ rats per group). $***p<0.001$ *vs.* the vehicle control group (*t*-test). $\#p<0.001$ significant difference between groups (Mann–Whitney test).

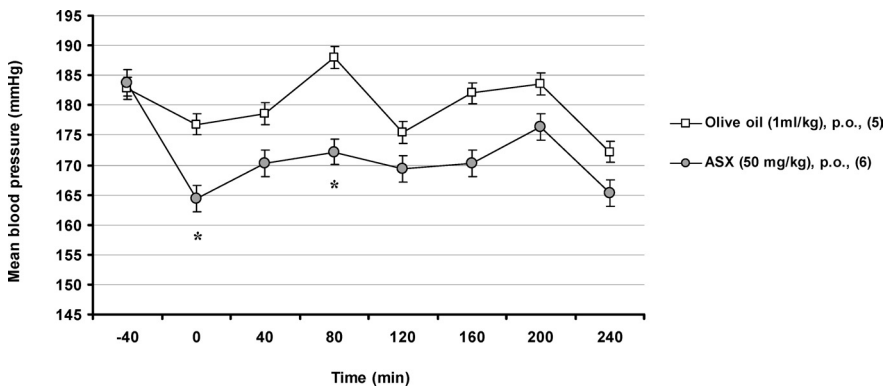

Fig. 3. Time Course Effects of Single Oral Administration of ASX-O on MBP in SHR

Data are represented as mean±S.E.M. ($n=5$—6 rats for each group). $*p<0.05$ *vs.* the vehicle control (*t*-test).

ASX-O (50 mg/kg) significantly delayed the incidence of stroke in SHR-SP. By day 4 of the post-treatment period, the stroke rate was 60% in the control and lower dose (5 mg/kg) groups, whereas the third group (50 mg/kg) did not show any sign of stroke. On day 7, the ASX-O (50 mg/kg) group showed a small incidence of stroke (20%) compared to the control that in which the incidence increased to 80% on day 14 (Fig. 4).

Vascular Reactivity of ASX-O on Rat Aorta, *in Vitro*. Effects on Contraction Induced by the Xanthine/Xanthine Oxidase (XOD) System In the first relaxation experiment, the 1×10^{-4} M L-NAME-pretreated preparations were treated with ASX-O (1×10^{-7} M) and (1×10^{-5} M), separately. After 10 min of treatment, xanthine (1×10^{-4} M) was added, followed by XOD (10 mU/ml) 5 min later. A transient contraction was thereby induced. The xanthine–XOD system produced 10.8% contraction, whereas ASX-O (1×10^{-7} M) produced 8% and ASX-O (1×10^{-5} M) 6.9% in the system-treated aorta (Fig. 5).

Effects of ASX-O and Nitric Oxide (NO) on Contraction Induced by Prostaglandin PGF2α After washing the preparations and equilibrating them with Krebs solution,

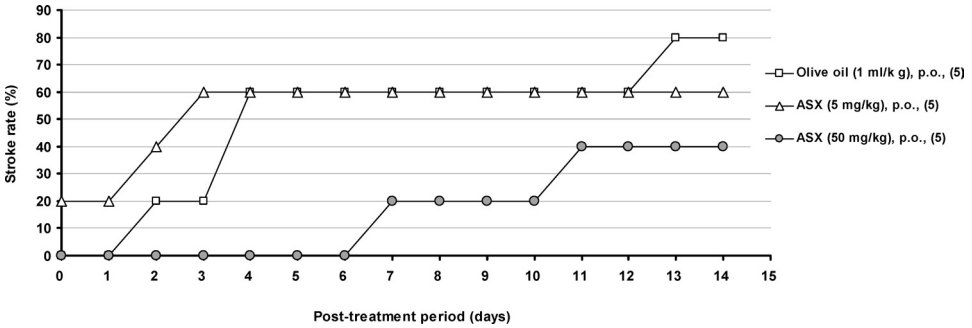

Fig. 4. Effects of Chronic Oral Administration of ASX-O on the Incidence of Stroke in SHR-SP after Cessation of the Treatment

Data points are represented as mean±S.E.M. ($n=5$ rats per group).

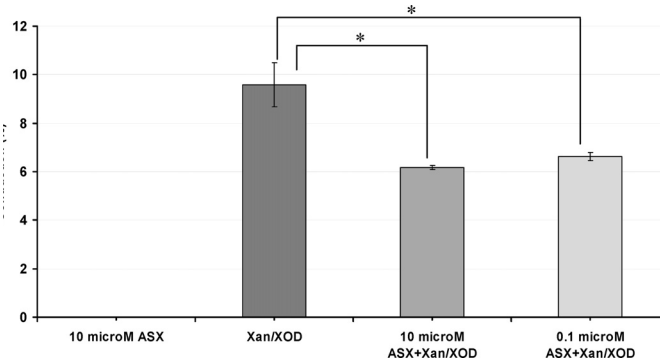

Fig. 5. Effects of ASX-O on Xan/XOD-Induced Transient Contractions on Aortic Rings of Wistar Rat

All the rings had intact endothelium and the preparations were pretreated with 1×10^{-4} M L-NAME. Values are expressed as a percentage of the maximal tension contracted with 60 mM KCl. Data represent mean±S.E.M. ($n=5$). $*p<0.05$ significant difference between groups (one way ANOVA).

some preparations were contracted by treatment with PGF2α (1×10^{-5} M). When the PG-induced contraction reached a plateau, ASX-O was added separately at concentrations ranging from 1×10^{-7} to 1×10^{-4} M.

A similar experiment was carried out simultaneously, in which the preparations were pretreated with L-NAME (1×10^{-4} M) for 60 min before the addition of PG. L-NAME is known to inhibit NO synthesis. In this experiment, ASX-O showed relaxation effects on the preparations with (+) or without (−) L-NAME-pretreatment. Relaxation was greater [percentage: 64.2, (−); 45, (+), and 63.4, (−); 14.6, (+)] at the concentrations of 1×10^{-4} M (100 μM) and 3×10^{-5} M (30 μM), respectively, while the lesser concentrations, *i.e.* 1×10^{-6}—10^{-5} M (1—10 μM), showed smaller effects ($\leq$11.2%) (Fig. 6A). Dimethyl sulphoxide (DMSO), used to dissolve ASX-O, showed no significant effect on the preparations at concentrations $\leq$1%.

Endothelium-Dependent and Endothelium-Independent Relaxation Endothelium-dependent (ED) and endothelium-independent (EIND) relaxation experiments were carried separately on aortic preparations with intact and denuded endothelia, respectively. ASX-O produced both ED and EIND relaxations when added at cumulative concentrations of ASX-O (1×10^{-8} to 1×10^{-5} M). The intensity of the ED relaxation produced by ASX-O was greater than the EIND relaxation, 95.4% and 70.9%, respectively (Fig. 6B). Moreover, the onset and cumulative effects of the relaxation were faster and more intense in the ED relaxation than in the EIND one.

Neuroprotective Effects of ASX-O in Mice, *in Vivo* The effects of ASX-O on the transient ischemia-induced impairment of Morris water maze performance in mice were studied in ICR mice. Transient cerebral ischemia was induced by bilateral common carotid occlusion, 2 vessels occlusion (2VO). ASX-O was given orally to the animals 1 h before the ischemia. Two days after the ischemia, the trial was performed at a rate of four trials/block/d for 5 d. The time course of change in the latency of escaping to the pool platform was recorded. The swimming time in the platform quadrant was recorded at the probe trial for 1 min after the platform was removed on day 7 of the test. These experiments showed that pretreatment of mice with ASX-O, (55, 550 mg/kg) significantly shortened the latency of escaping onto the platform, and increased the time of crossing the former platform quadrant in the probe trial (Fig. 7).

DISCUSSION

The role of ASX, as well as of many other antioxidant carotenoids (*e.g.* β-carotene, lycopene), in reducing atherogenesis and lowering the risk of some coronary heart diseases has been previously studied.[20—23] However there is no previous report on the antihypertensive action of ASX. In the present study, dietary ASX-O showed a significant antihypertensive effect by lowering the BP in SHR compared to the control. It produced this effect in SHR but not in WKY. It also delayed the incidence of stroke in SHR-SP. The administration of ASX for 5 weeks produced a significant reduction in the MBP (−9, −8%), as well as in the SBP (−6, −4%) and DBP (each −10%) (data not shown) at the doses of 5 mg and 50 mg/kg, respectively, in SHR-SP. The time course effect of ASX-O suggests that ASX-O may have an initial acute effect on BP, probably due to a sympatholytic effect; however, further studies are needed to confirm this. The present results suggest that ASX-O can attenuate the development of hypertension and may help to protect the brain against stroke and ischemic insults.

There is a number of reports indicating that ASX is a more powerful antioxidant than other carotenoids and vitamin E, and that it may confer numerous health benefits.[7,24—26] The antioxidative properties of ASX had been demonstrated in a number of different biological membranes.[7,27—29] In the present study, when ASX-O was incorporated with the xanthine/XOD system, it produced relaxation of the transient contraction of the aortic rings. This transient contraction was produced by oxygen-derived free radicals that were gener-

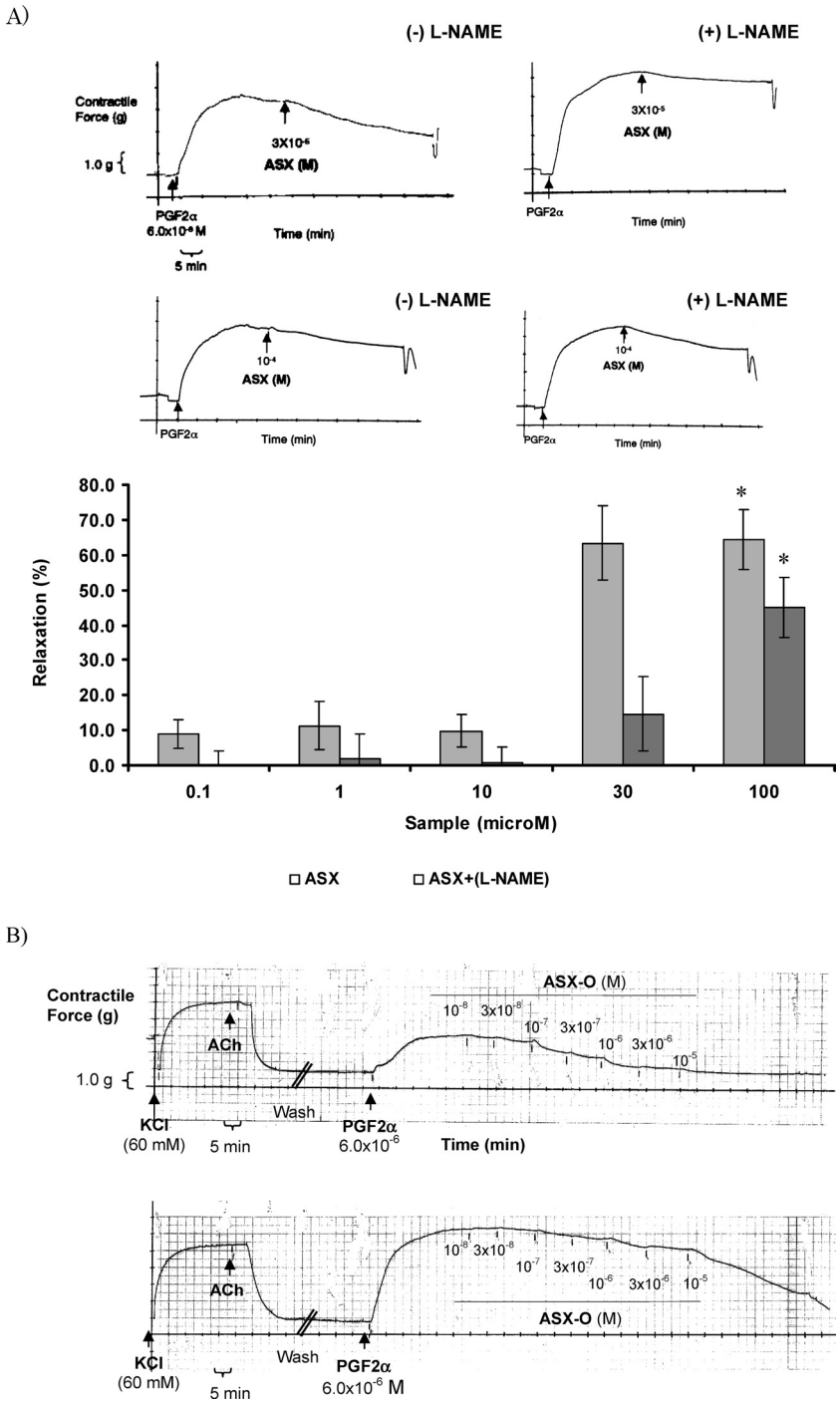

Fig. 6. Vascular Reactivity of ASX-O on Rat Aorta

(A) Nitric oxide (NO)-related vasorelaxation induced by ASX-O on the aortic rings of Wistar rat, pretreated with 1×10^{-4} M L-NAME (+) and without pretreatment (−). All the rings had intact endothelium. ASX-O at 3×10^{-5} M (30 μM) produced higher relaxation (−) without L-NAME, indicating NO-dependent relaxation (6A-upper charts). At 1×10^{-4} M (100 μM), ASX-O showed greater relaxation (+) with L-NAME, suggesting NO-independent relaxation (6A-lower charts). $*p<0.05$ *vs.* the corresponding L-NAME (+) group (*t*-test). (B) Endothelium-dependent (ED) and endothelium-independent (EIND) relaxation induced by ASX-O. ASX-O at cumulative concentrations (1×10^{-8}—1×10^{-5} M) showed ED relaxation (6B-upper chart) and EIND ones (6B-lower chart). Values are expressed as a percentage of decrease in the maximal tension contracted with 1×10^{-5} M $PGF_{2\alpha}$. Data represent the mean±S.E.M. (n=4).

ated by the system.

In this study, ASX-O also showed vascular effects on the aortic preparations by inducing ED and EID vasorelaxation effects. The ED effect might result from antioxidative properties, presumably the superoxide scavenging effects of ASX in preventing superoxide-induced NO degradation, which would thus prolong its half-life and consequently its vasorelaxation effect. Therefore, the restoration of NO-induced vasorelaxation may explain, partially, the antihypertensive effects of ASX-O. In the current experiments, the effective vasorelaxant concentrations of ASX-O (30—100 μM) produced relaxation on aortic responses. This relaxation is NO-dependent at the lower dose of ASX-O (30 μM), and NO-independent at the higher dose (100 μM), as depicted in the charts and graph of Fig. 6A. However, more intensive investigations are necessary to verify the mechanisms of these effects.

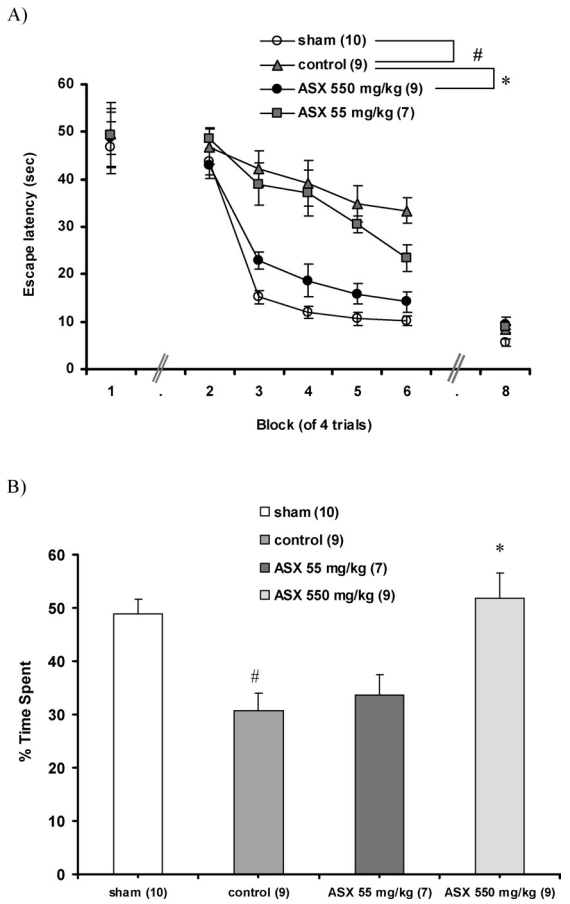

Fig. 7. Neuroprotective Effects of ASX-O on Transient Ischemia (2VO)-Induced Impairment of Morris Water Maze Performance in Mice

ASX-O was orally given to animals 1 h before the ischemia. Two days after the ischemia, the trial test was performed (four trials/block/d for 5 d). (A) Time course of the change in latency of escaping to the platform in the pool. (B) The swimming time in the platform quadrant was recorded at the probe trial for 1 min after removal of the platform, on day 7 of the test. Data represent the mean±S.E.M. of 7—10 mice per group. $^{\#}p<0.001$ *vs.* sham group. $*p<0.05$ *vs.* ischemic control (two way RM-ANOVA).

In addition, ASX-O showed significant neuroprotective effects at relatively high doses by preventing the ischemia-induced impairment of spatial memory in mice. This effect is suggested to be due to the significant antioxidant property of ASX on ischemia-induced free radicals and their consequent pathological cerebral and neural effects. The current result indicates that ASX-O may have beneficial effects in improving memory in vascular dementia.

Some clinical studies have revealed that healthy adults can safely consume 6 mg of ASX per day from a *Haematococcus pluvialis* algal extract.[30] In human blood, ASX is carried by the lipoproteins VLDL, LDL and HDL. ASX was reported to reduce LDL oxidizability *in vitro* and *ex vivo*, and a daily dosage as low as 3.6 mg of ASX for two consecutive weeks demonstrated that ASX could protect LDL-cholesterol against induced-oxidation in an *in vitro* test.[2] A number of ASX-containing health products are under development based on these findings.[31] Nevertheless, prospective clinical studies addressing the protective effects of ASX in essential hypertensive patients are crucial and recommended.

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REFERENCES

1) O'Connor I., O'Brien N., *J. Dermatol.*, **16**, 226—230 (1998).
2) Iwamoto T., Hosoda K., Hirano R., Kurata H., Matsumoto A., Miki W., Kamiyama M., Itakura H., Yamamoto S., Kondo K. J., *Atheroscler. Thromb.*, **7**, 216—222 (2000).
3) Kang J. O., Kim S. J., Kim H., *Methods Find. Exp. Clin. Pharmacol.*, **23**, 79—84 (2001).
4) Aoi W., Naito Y., Sakuma K., Kuchide M., Tokuda H., Maoka T., Toyokuni S., Oka S., Yasuhara M., Yoshikawa T., *Antioxid. Redox. Signal.*, **5**, 139—144 (2003).
5) Chew B. P., Park J. S., Wong M. W., Wong T. S., *Anticancer Res.*, **19**, 1849—1853 (1999).
6) Jyonouchi H., Sun S., Iijima K., Gross M. D., *Nutr. Cancer*, **36**, 59—65 (2000).
7) Kurashige M., Okimasu E., Inoue M., Utsumi K., *Physiol. Chem. Phys. Med. NMR.*, **22**, 27—38 (1990).
8) Ohgami K., Shiratori K., Kotake S., Nishida T., Mizuki N., Yazawa K., Ohno S., *Invest. Ophthalmol. Vis. Sci.*, **44**, 2694—2701 (2003).
9) Uchiyama K., Naito Y., Hasegawa G., Nakamura N., Takahashi J., Yoshikawa T., *Redox. Rep.*, **7**, 290—293 (2002).
10) Okai Y., Higashi-Okai K., *Int. J. Immunopharmacol.*, **18**, 753—758 (1996).
11) Chew B. P., Wong M. W., Park J. S., Wong T. S., *Anticancer Res.*, **19**, 5223—5227 (1999).
12) Guerin M., Huntley M. E., Olaizola M., *Trends in Biotech.*, **21**, 210—216 (2003).
13) Pryor W. A., *Free Rad. Biol. Med.*, **28**, 141—164 (2000).
14) Liu K., Cuddy T. E., Pierce G. N., *Am. Heart J.*, **123**, 285—290 (1992).
15) Kritchevsky S. B., *J. Nutr.*, **129**, 5—8 (1999).
16) Stahl W., Ale-Agha N., Polidori M. C., *Biol. Chem.*, **383**, 553—558 (2002).
17) Harris A., Zelkha M., Paran E. PCT Int. Appl. WO 02/058683 A2 (Cl. A61K31/00), 1 Aug 2002, IL Appl. 141,038, 23 Jan 2001; 14 pp.
18) Goto H., Sakakibara I., Shimada Y., Kasahara Y., Terasawa K., *Am. J. Chin. Med.*, **28**, 197—203 (2000).
19) Watanabe H., Zhao Q., Matsumoto K., Tohda M., Murakami Y., Zhang S. H., Kang T. H., Mahakunakorn P., Maruyama Y., Sakakibara I., Aimi N., Takayama H., *Pharmacol. Biochem. Behav.*, **75**, 635—643 (2003).
20) Berset C., *Med. Nutr.*, **35**, 215—223 (1999).
21) Rimm E. B., Stampfer M. J., *Med. Clin. North Am.*, **84**, 239—249 (2000).
22) Giugliano D., *Nutr. Metab. Cardiovasc. Dis.*, **10**, 38—44 (2000).
23) Agrawal S., Rao A. V., *Drug Metab. Drug Interact.*, **17**, 189—210 (2000).
24) Di Mascio P., Devasagayam T. P. A., Kaiser S., Sies H., *Biochem. Soc. Trans.*, **18**, 1054—1056 (1990).
25) Miki W., *Pure Appl. Chem.*, **63**, 141—146 (1991).
26) Shimidzu N., Goto M., Miki W., *Fish. Sci.*, **62**, 134—137 (1996).
27) Palozza P., Krinsky N. I., *Arch. Biochem. Biophys.*, **297**, 291—295 (1992).
28) Oshima S., Ojima F., Sakamoto H., Ishiguro Y., Terao J., *J. Nutr. Sci. Vitaminol.*, **39**, 607—615 (1993).
29) Nakagawa K., Kang S. D., Park D. K., Handelman G. J., Miyazawa T., *J. Nutr. Sci. Vitaminol.*, **43**, 345—355 (1997).
30) Spiller G. A., Dewell A., *J. Med. Food*, **6**, 51—56 (2003).
31) Miki W., Hosoda K., Kondo K., Itakura H., Patent application number 10155459. Japanese Patent Office. Publication date 16 June 1998.