Contributed Reviews

Astaxanthin, a Carotenoid with Potential in Human Health and Nutrition[⊥]

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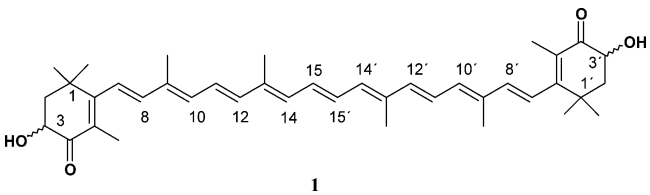
Astaxanthin (**1**), a red-orange carotenoid pigment, is a powerful biological antioxidant that occurs naturally in a wide variety of living organisms. The potent antioxidant property of **1** has been implicated in its various biological activities demonstrated in both experimental animals and clinical studies. Compound **1** has considerable potential and promising applications in human health and nutrition. In this review, the recent scientific literature (from 2002 to 2005) is covered on the most significant activities of **1**, including its antioxidative and anti-inflammatory properties, its effects on cancer, diabetes, the immune system, and ocular health, and other related aspects. We also discuss the green microalga *Haematococcus pluvialis*, the richest source of natural **1**, and its utilization in the promotion of human health, including the antihypertensive and neuroprotective potentials of **1**, emphasizing our experimental data on the effects of dietary astaxanthin on blood pressure, stroke, and vascular dementia in animal models, is described.

Introduction

To date, a number of review articles have been published on the carotenoid astaxanthin (**1**), including its sources and chemical properties,[1−6] genetic and biochemical nature,[7,8] and its applications in human health and nutrition.[9] In the current review, we update the scientific data and reports on **1** during the period of 2002−2005. Studies on **1**, with an emphasis on those related to human health and nutrition, have been summarized. Additionally, we discuss the results of our investigation on the potential of **1** against life-style diseases, in particular its antihypertensive and neuroprotective effects in experimental animals.

The Carotenoid Astaxanthin (1) in Nature. Astaxanthin (**1**) is the main carotenoid pigment found in aquatic animals.[10] It is also found in some birds, such as flamingoes, quails, and other species.[11,12] This carotenoid is included in many well-known seafoods such as salmon, trout, red seabream, shrimp, lobster, and fish eggs.[13] Compound **1**, similar to other carotenoids, cannot be synthesized by animals and must be provided in the diet. Previous reports have described that mammals, including humans, lack the ability to synthesize **1** or to convert dietary **1** into vitamin A.[14]



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Commercially, photobioreactor (PBR) technology has been utilized largely in the production of microalgal biomass of two Chlorophyte algae, *Chlorella zofingiensis* Dönz and *Haematococcus pluvialis* Flotow, in the form of open ponds, enclosed PBRs, or fermentation reactors.[15] *H. pluvialis* is believed to have the highest capacity to accumulate **1** in nature.

Molecular Structure and Chemical Forms of Astaxanthin (1). Compound **1** [3*R*,3′*R*, 3*R*,3′*S*, 3*S*,3′*S* (25:50:25)] belongs to the xanthophyll class of carotenoids and has the semisystematic name 3,3′-dihydroxy-β,β-carotene-4,4′-dione. It is closely related to β-carotene, lutein, and zeaxanthin, sharing with them many of the general metabolic and physiological functions attributed to carotenoids. In addition, **1** has unique chemical properties based on its molecular structure. The presence of the hydroxyl (OH) and keto (C═O) moieties on each ionone ring explains some of its unique features, namely, the ability to be esterified and a higher antioxidant activity and a more polar nature than other carotenoids. In its free form, **1** is considerably unstable and particularly susceptible to oxidation. Hence it is found in nature either conjugated with proteins (e.g., salmon muscle or lobster exoskeleton) or esterified with one or two fatty acids (monoester and diester forms), which stabilize the molecule. In *H. pluvialis*, the esterified form predominates, mostly as **1** monoester.[3] Various astaxanthin isomers have been characterized on the basis of the configuration of the two hydroxyl groups on the molecule. The 3*S*,3′*S* stereoisomer is the main form found in *H. pluvialis*, while synthetic **1** contains primarily the 3*R*,3′*S* isomer.

Bioavailability and Pharmacokinetics of Astaxanthin (1). A few reports have described the uptake and distribution of **1** in fish[16] and some animals, including chickens and rats.[17,18] These results show that the geometrical and optical isomers of **1** are distributed selectively in different tissues and that levels of free **1** in the liver are greater than the corresponding concentration in the plasma, suggesting concentrative uptake by the liver. Of the chicken tissues examined, the highest concentration of **1** was detected in the intestine, followed by subcutaneous fat, abdominal fat, spleen, liver, heart, kidney, and skin. The lowest concentration was in the muscle. Of these tissues, the small intestine, subcutaneous fat, abdominal fat, liver, and skin showed a proportional increase of the level of **1** with dietary content. In the liver, higher concentrations were found in the microsomal fraction than in the mitochondrial one.

[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

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In humans, a recent small-scale clinical study by Coral-Hinostroza et al.[19] described the appearance of unesterified astaxanthin geometrical *E*/*Z* and optical *R*/*S* isomers in human plasma after a single dose of a mixture containing optical 3- and 3′*R*/*S* isomers of fatty acyl diesters of **1**. They showed that the *Z* isomers of **1** were absorbed selectively into plasma and accounted for approximately 32% of total **1** 6−7.5 h post-prandially. The proportion of all-*E*-**1** was significantly higher in the very low-density lipoprotein (VLDL) and chylomicron plasma lipoprotein fraction than in the high-density lipoprotein (HDL) and the LDL fractions. These results suggest that a selective process may be involved in an increase of the relative proportion of the *Z* isomers of **1** compared to the all-*E*-**1** before uptake in blood and that the esters of **1** are hydrolyzed selectively during absorption.

Astaxanthin (**1**), similar to other carotenoids, is a very lipophilic compound and has a low oral bioavailability. This criterion has limited the ability to test this compound in well-defined rodent models of human disease. Several attempts, with variable success, to solve this difficulty have been reported. In a recent study, Mercke Odeberg et al.[20] showed that incorporation of lipid-based formulations can enhance oral bioavailability in humans. This approach was previously successful in mesenteric lymph duct-cannulated rats, where different types of oils (such as corn oil and olive oil) influenced the absorption accordingly.[21] Some preclinical studies have cited improvement of the solubility of **1** by employing emulsifiers such as polyoxyethylene sorbitan monopalmitate (Tween 40), the monooleate form (Tween 80),[22] and a sulfobutyl ether β-cyclodextrin.[23]

The increasing interest in **1** and its applications in human health and nutrition have triggered a concern about its safety. A randomized, double-blind, placebo-controlled clinical study conducted by Spiller and Dewell[24] demonstrated that 6 mg of **1** per day from a *H. pluvialis* algal extract for 8 weeks can be safely consumed by healthy adults.

Metabolism of Astaxanthin (1). A number of studies have addressed the metabolic fate of many carotenoids, including β-carotene[25−30] and lycopene.[28,29,31] However, there is little information on **1**. Previous studies in rat hepatocytes[32] showed the conversion of **1** into two metabolites: 3-hydroxy-4-oxo-β-ionone and its reduced form, 3-hydroxy-4-oxo-7,8-dihydro-β-ionone, both in conjugated forms. Astaxanthin has also been reported as a potent inducer of cytochrome P450 (CYP) enzymes in the rat liver.[33,34]

A recent report by Kistler et al.[35] described four metabolites, the above-mentioned ionones and their corresponding ionols. They also showed that **1** is a significant inducer of the major CYP3A4, as well as of CYP2B6, but not of other CYPs, in cultured hepatocytes. Although these data showed some contradictory metabolic differences, it has been suggested that **1** may stimulate the liver expression of CYP genes in a species-specific manner and that its metabolism, as well as its CYP-inducing capacity in humans and in rats, is different.

Astaxanthin (1) and Cellular Health. The energy needed by the cell is generated in the mitochondria via multiple oxidative chain reactions, which are accompanied by the production of a large amount of reactive oxygen species (ROS) such as superoxide anion radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and peroxinitrite anion ($ONOO^-$). These ROS need to be neutralized in order to maintain the proper functions of this cellular component and to protect the cell from degradation and aging. There are few recent reports on the cellular effects of **1**. Kim et al.[36,37] described a protective effect of **1** on naproxen-induced and ethanol-induced gastric antral ulcerations in rats in a dose-dependent manner, accompanied by a significant increase in the activities of radical-scavenging enzymes.

Anticancer Properties of Astaxanthin (1). Several studies have highlighted the contribution of the antioxidative property of **1** to its anticancer effect. This compound has markedly attenuated the promotion of hepatic metastasis induced by restraint stress in mice. This effect was suggested to be through inhibition of the stress-induced lipid peroxidation.[38] Recent studies on human and animal cells have demonstrated that connexin 43 (Cx43) protein, the most widely expressed connexin in tissues, is up-regulated at the message and protein level by chemopreventive retinoids and carotenoids, leading to a decreased proliferation and decreased indices of neoplasia. A recently published study showed that combinations of **1** and retinoids were capable of superadditive up-regulation of the tumor suppressor gene Cx43.[39] Another report also described that derivatives of **1** delivered in several aqueous formulations induced expression of Cx43 and significantly increased gap junctional intercellular communication, suggesting that these derivatives retain the potent chemopreventive activity of the parent compound (**1**).[8]

Astaxanthin (1) and Inflammatory Responses and the Immune System. Previous reports have described enhanced cell-mediated immune responses by carotenoids.[40,41] However, the exact mechanism of action is still unclear. Interestingly, some recent studies have presented new insights on how the immune function can be regulated by carotenoids, in particular the highly active non-provitamin-A carotenoids, including **1**. In vitro and ex vivo studies by Lee et al.[42] have revealed that **1** can prevent inflammatory processes by blocking the expression of pro-inflammatory genes as a consequence of suppressing the nuclear factor kappaB (NF-κB) activation. It also inhibited the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) and the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β). Similar effects were further reported by Ohgami et al.[43] using lipopolysaccharide-induced uveitis in rats in vivo and the endotoxin-induced production of inflammatory mediators in a mouse macrophage cell line in vitro. They showed that **1** can exhibit a dose-dependent ocular anti-inflammatory effect by suppression of NO, PGE_2, and TNF-α production via direct blockage of nitric oxide synthase (NOS) enzyme activity. The effect of 100 mg/kg **1** appeared to be comparable to that of 10 mg/kg prednisolone.

In the past few years, a study on peripheral blood mononuclear cells from asthmatic patients has shown that **1** alone, or in a combination with ginkgolide B, can suppress T-cell activation comparably to two commonly used antihistamines, cetirizine dihydrochloride and azelastine.[44] This finding suggests that **1** may be a candidate in antiasthmatic combination formulations.

On the other hand, randomized double-blind placebo-controlled studies by Kupcinskas et al.[45,46] showed different responses of *Helicobacter pylori* negative and positive patients to the treatment of nonulcer dyspepsia with an algal meal rich in **1**. In the *H. pylori* infection, the active inflammatory response is induced by infiltration of neutrophils, which together with macrophages and/or monocytes produce ROS that can cause DNA damage to the adjacent cells.

Astaxanthin (1) and Diabetes. Oxidative stress induced by hyperglycemia may possibly cause the dysfunction of pancreatic beta-cells and various forms of tissue damage in patients with diabetes mellitus. It is also implicated as an important mechanism by which diabetes may cause nephropathy. A few years ago, a report by Naito et al.[47] indicated that **1** ameliorated the progression and acceleration of diabetic nephropathy in diabetic db/db mice, a rodent model of type 2 diabetes. It also preserved the beta-cell function of insulin secretion and decreased the higher level of blood glucose in the diabetic animal.[48]

Astaxanthin (1) and Ocular Health. A recent in vitro study has reported that pretreatment of human lens epithelial cells with a **1**-containing xanthophyll complex significantly decreased ultraviolet B (UVB) light-induced lipid peroxidation and stress signaling and showed that the xanthophylls are more potent than α-tocopherol in protecting human lens epithelial cells against UVB insult.[49] A double-blind study has also revealed that astaxanthin (**1**) has improved the accommodation amplitude in visual display terminal workers.[50]

Astaxanthin (1) and Skeletal Muscle Health. Free radicals play an important role as mediators of skeletal muscle damage and inflammation after strenuous exercise, as well as in the presence of a muscle disease. It has been postulated that the generation of ROS is increased during exercise as a result of increase in mitochondrial oxygen consumption and electron transport flux, leading to lipid peroxidation.[51,52] The literature suggests that dietary antioxidants may prevent muscle damage by scavenging ROS produced during exercise.[53] Compound **1** has also been found to attenuate exercise-induced damage in mouse skeletal muscle and heart, including an associated neutrophil infiltration that induces further damage.[54] A further study showed that supplementation with **1** may preferentially attenuate perceptions of delayed onset muscular soreness in currently trained individuals.[55]

Astaxanthin (1) and Skin Health. The skin is exposed to both endogenous and environmental pro-oxidant agents, leading to ROS generation and possibly oxidative stress, which is suggested to be involved in the damage of cellular constituents, such as DNA, cell membrane lipids, and proteins. When the oxidative stress overwhelms the skin antioxidant chemical and enzymatic network, subsequent modification of cellular redox apparatus leads to an alteration of cell homeostasis and the generation of degenerative processes. Compound **1** was reported recently to have protective effects on the skin. An in vitro study, using human skin cell line cultures, has shown that preincubation with either natural algal or synthetic **1** prevents ultraviolet A (UVA)-induced alterations in cellular superoxide dismutase (SOD) activity and cellular glutathione content and displayed protection against UVA-induced DNA damage.[56]

Astaxanthin (1) and Reproduction. Mammalian spermatocytes contain a highly specific lipoid composition and high content of polyunsaturated fatty acids, plasmalogenes, and sphingomyelins, all of which afford the flexibility and the functional ability of these cells. Such lipids represent the main substrates for peroxidation, whereas immature spermatozoa and leukocytes are considered to be the main sources of ROS in the semen. Excessive ROS generation can overwhelm the protective mechanisms and initiate changes in lipid and/or protein layers of sperm plasma membranes, and may further induce changes in DNA. Some recent reports have reviewed pro-oxidative and antioxidative imbalance in human semen and its relation with male fertility.[57,58] A recent study, based on a self-administered food and supplements frequency questionnaire, has revealed that higher healthy antioxidant intake, including carotenoids, is associated with greater sperm numbers and motility in healthy humans.[59]

A clinical study has reported that supplementation with **1** improves sperm quality and function in male patients with deficient semen. It also described double-blind trials in which **1** increased spontaneous or intrauterine insemination-assisted conception rates.[60]

Applications of Astaxanthin (1). It may be argued that microalgal technology has not yet attracted the attention of large pharmaceutical companies, possibly due to the unavailability of the source organisms or to the lack of successful track record. Nevertheless, the potential of this technology in astaxanthin (**1**) production has had some success that may lead to the fulfillment of future expectations.[15] Recently, several successful applications in the development of **1** have been described. The most significant is a water-dispersible derivative of **1** (disodium disuccinate **1**; Cardax), which has been reported as a myocardial salvage agent in a rat infarction model[61] and as a cardioprotective in canines.[62]

Astaxanthin (1) and Cardiovascular Health. In many industrialized and developing countries, cardiovascular diseases stand at the forefront among other life-threatening diseases and have attracted growing attention by a number of institutions and research groups. Nevertheless, the last few years have witnessed few successful studies on natural products as targets or candidates in cardiovascular health. Some recent reports have demonstrated a linkage between oxidative stress and the cardiovascular diseases and their consequent implications.[63−68] In accordance with these reports and other similar ones, it has been suggested that the powerful antioxidant **1** can be one of these future candidates. Recent studies have reported a blood pressure (BP)-lowering effect of **1** in hypertensive animal models.[69−71] These effects have been further augmented by the report mentioned above on the myocardial salvage potential of a naturally oriented **1** disuccinate derivative in a rat model of infarction.[61] Another study has suggested that **1** may improve plaque stability in atherosclerosis by decreasing macrophage infiltration, apoptosis, and vulnerability in atheroma of hyperlipidemic rabbits.[72]

Antihypertensive and Neuroprotective Potentials of Astaxanthin (1)

In this section of the review, we describe and discuss studies in our laboratory on the cardiovascular and neurological effects of astaxanthin (**1**), particularly the BP-lowering effect in spontaneously hypertensive rats (SHR) and their suggested mechanisms of action.

Some diseases, such as hypertension, atherosclerosis, hyperlipidemia, and diabetes, are associated with vascular, functional, and structural changes, including endothelial dysfunction, altered contractility, and vascular remodeling. The role of the vascular endothelium in modulating vascular tone and maintaining cardiovascular homeostasis in blood vessels, through the production of an array of both relaxant (e.g., NO, prostacyclin) and constrictor factors (such as thromboxane, endothelin), has been widely reported.[73−76] In general, the development of hypertension is accompanied by changes in the rheological properties of blood, particularly by an increased red blood cell (RBC) aggregation, leading to further pathological complications. Plasma viscosity is one of the parameters that contribute to cardiovascular risks. It may be of special importance in cases of reduced blood flow, as commonly occurs in patients with advanced atherosclerosis,[77] and is also related to the extension of coronary heart disease.[78,79]

It is widely accepted that 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, including hypertension. On one hand, hypertension is associated with structural changes in the vasculature.[80−82] These alterations, known as "remodeling", have 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.[82,83] In most hypertension animal models, it has been demonstrated that the arterial internal diameter is generally reduced and the wall-to-lumen ratio is increased in the small arteries if they are compared under equivalent biophysical conditions.[80−82] 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,[84] ischemia-reperfusion injury,[85] congestive heart failure,[86] and coronary artery disease,[87] as well as in hypertension.[88,89]

Some antioxidant compounds and health diets have been shown to be effective in reducing oxidative damage in experimental rats, especially in heart disease.[90,91] Antioxidants, including a number of carotenoids, can inhibit lipid peroxidation[92] and have protective effects against chronic diseases such as cardiovascular diseases.[93]

Cerebrovascular dementia and ischemic-stroke are closely related to some cardiovascular diseases, particularly hypertension, all of which share the criteria of vascular remodeling and dysfunction. Stroke occurs due to hemorrhage or occlusive injury and results in ischemia and reperfusion injury. A variety of destructive mechanisms have been suggested to be involved. These include oxygen radical generation, calcium overload, cytotoxicity, and apoptosis as well as the generation of inflammatory mediators.[94]

One of the crucial factors involved in triggering and maintaining the post-ischemic insult to brain tissues is the oxidative stress that

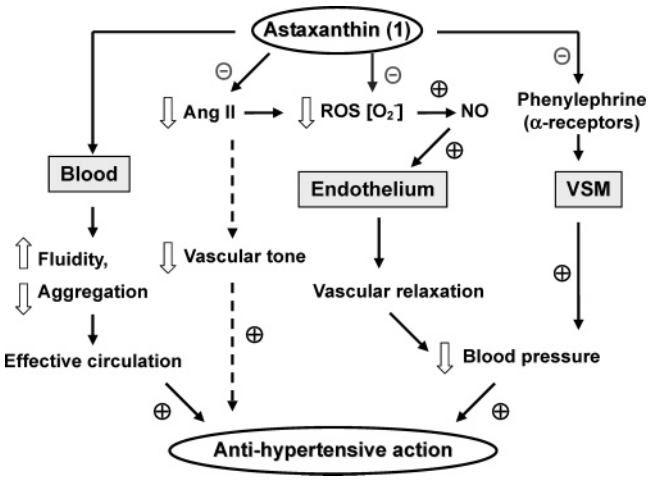

Figure 1. Action mechanisms of the antihypertensive effects of ASX. VSM, vascular smooth muscle; Ang II, angiotensin II; ROS, reactive oxygen species; NO, nitric oxide; Phe, phenylephrine. ↑, ⊕: induce or increase; ↓, ⊖: inhibit or decrease.

occurs in the reperfusion phase of the stroke. In the ischemic brain, this reperfusion together with the excessive amount of excitatory amino acids (e.g., glutamate) and infiltration by neutrophils have been considered as the major sources of ROS generation, which in turn amplify the imbalance in the neurons and astroglial cells of the ischemic core and penumbra.[95] The changes in the antioxidant status of brain tissues before, during, and after ischemic-reperfusion greatly affect post-ischemic brain damage and have been widely studied in the past few years. Moreover, the chronically reduced cerebral blood flow in vascular dementia contributes to behavioral and cognitive deficits.[96−98] The studies have largely agreed that the significance of an additional supply of antioxidant moieties, over and above the natural SOD enzyme levels, is crucial for a selective protection of brain tissue against ischemic injury.[99−101]

In our ongoing studies on astaxanthin, we investigated, for the first time, the antihypertensive effect and action mechanisms of dietary natural **1** (designated as **1**-O) in SHR and neuroprotective effects in mice that received transient brain ischemia.[69−71]

Antihypertensive Effects of Astaxanthin (1). In preliminary investigations, oral administration of **1** (50 mg/kg/day) for 2 weeks induced a significant reduction in BP in SHR, but not in the normotensive Wistar Kyoto (WKY) strain. A separate study showed that **1**-O can further delay the incidence of stroke in the stroke-prone SHR-SP upon a 5-week treatment.[69] In another more detailed study, a 7-week long-term administration of **1** (5 mg/kg/day) showed the BP-lowering effect in SHR, accompanied with (1) improved blood fluidity, (2) decreased aortic elastin band number and a smaller wall-to-lumen ratio of the coronary arteries and arterioles, and (3) reduced plasma nitric oxide (NOx) level, compared to the vehicle control (olive oil, 1 mL/kg/day).[70,71] However, throughout the studies, **1**-O did not produce a consistent change in the heart rate nor did it show a modifying effect on body weight or blood cell count.

Action Mechanisms of the Antihypertensive Effects of Astaxanthin (1). We evaluated the contractions induced by phenylephrine (Phe), angiotensin II (Ang II), and the xanthine/xanthine oxidase (Xan/XOD) system and the relaxations induced by sodium nitroprusside, as well as endothelium-dependent relaxations mediated by acetylcholine in thoracic aortae of the SHR, with and without **1**-O intervention in the long-term study. The results of the study suggest that the antihypertensive effects of **1**-O are exerted through some mechanisms, including normalization of the sensitivity of the adrenoceptor sympathetic pathway, particularly [alpha]-adrenoceptors, and by restoration of the vascular tone through attenuation of the Ang II- and the reactive oxygen species-induced vasoconstriction (Figure 1). The data also suggest that **1**-O can act in modulating blood fluidity, ameliorating NOx, and improving vascular elastin and coronary wall thickness in hypertension.[70,71]

Astaxanthin (1) and Superoxide Anion. The effects of **1** on the contractile and relaxant responses of the vascular system are important factors. It is generally accepted that under physiological conditions vascular production of ROS and the consequent activation of redox-dependent signaling pathways and expression of redox-sensitive genes are tightly controlled by regulatory systems. However, in pathological conditions, such as in hypertension and atherosclerosis, where generation of ROS is increased and the renin angiotensin system may be up-regulated, these redox-sensitive events may contribute to cellular processes involved in vascular dysfunction and structural remodeling.[102−104]

Increased levels of vascular $^{\bullet}O_2^-$ and H_2O_2 impair the endothelium-dependent relaxation and increase the contractile reactivity, and further alter the vascular tone. These effects may be mediated directly by elevating the cytosolic Ca^{2+} concentration or indirectly by reducing the concentration of the vasodilator NO.[105] Increased generation of $^{\bullet}O_2^-$ has been suggested to scavenge the endothelium-derived NO, resulting in increased vascular tone and BP.[106,107] The in vivo interaction between $^{\bullet}O_2^-$ and NO produces peroxynitrite anion ($ONOO^-$),[108] a highly reactive moiety that can alter vascular reactivity and favor vascular injury. Moreover, an overproduction of $^{\bullet}O_2^-$ and a concomitant decrease of antioxidant levels have also been reported to occur in human essential hypertension,[109] as well as in SHR.[110]

In our experiment, the vascular contraction induced by the Xan/XOD system was inhibited by the **1**-O treatment. This contraction appears to be induced by the oxygen-derived free radicals that were produced by the system. Therefore, it is likely that **1**, by scavenging the free radicals in the circulation, namely, $^{\bullet}O_2^-$, preserves the amount of NO that is required to maintain the endothelial and vascular functions.

Astaxanthin (1) and Angiotensin II. The vasoconstrictor Ang II plays a critical role in regulating the BP and fluid homeostasis in physiological conditions, as well as in the vascular damage in pathological conditions.[102−104] The role of Ang II in the production of ROS in vascular cells through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase has been reported.[102,103] It has been described that the addition of Ang II to cultured vascular smooth muscle cells in vitro leads to an increase in NADPH oxidase activity and $^{\bullet}O_2^-$ production.[111] Those findings were consistent with the observation that increased vascular $^{\bullet}O_2^-$ concentrations were detected in Ang II-induced hypertensive rats.[112] Interestingly, a down-regulation of the expression of the NADPH oxidase subunits and a lowering of the $^{\bullet}O_2^-$ level in endothelial cells appear to be induced by some polyphenols.[113] Astaxanthin (**1**) has potential antioxidant activity, and our present findings may urge us to consider the link between the vascular effects of **1** and Ang II-induced ROS production. In our study, **1**-O significantly reduced the contractile response to Ang II in the SHR aortic preparations.[70]

Astaxanthin (1) and Nitric Oxide. Another factor is the direct antioxidative effect of **1** on the blood circulation that may play an important role in the antihypertensive effect of this compound. It is well known that NO is synthesized from the amino acid L-arginine by a family of enzymes, the nitric oxide synthases (NOS), through a metabolic route, namely, the L-arginine−nitric oxide pathway.[114] The synthesis of NO by the vascular endothelium is responsible for the vasodilator tone that is essential for the regulation of the BP[115] through the activation of the NO receptor, the soluble guanylate cyclase (sGC), thereby increasing cyclic guanylate monophosphate (cGMP) and causing smooth muscle relaxation.[116] Once NO is released from the cells, it rapidly autoxidizes to yield the nitrite anion (NO_2^-), which, in turn, interacts with hemoglobin to yield nitrate (NO_3^-) species.[117] Because NO_2^- plus NO_3^- (termed NOx) are relatively stable in the blood, the concentration of NOx in blood has been used as an indicator of the endogenous formation

of NO.[118] In our study,[71] **1**-O significantly reduced the plasma NOx level in the SHR and showed a significant scavenging effect of systemically administered **1** on the NO metabolite radicals (i.e., NOx) in the blood circulation, indicating its potential as an antioxidant.

Astaxanthin (1) and the Sympathetic Nervous System. In a current study with **1**-O,[70] we focused on determining the response to the [alpha]$_1$-selective agonist Phe. The Phe-evoked exaggerated vasoconstrictor responses in the SHR strain have been attributed to endothelial dysfunction as a result of a decreased basal NO production.[119] Such a reduced basal NO concentration could be attributed to increased $^{\bullet}O_2^-$ production in these animal systems.[119,120] The response to Phe has also been reported to be enhanced by Ang II.[121] **1**-O significantly ameliorated the Phe-induced contraction of the aortic rings. Hence, **1**-O can be postulated to improve the cardiovascular functions in the SHR by normalization of the sensitivity of the adrenoceptor sympathetic pathway, particularly α_1-adrenoceptors.

Astaxanthin (1) and the Microcirculation. Hemorheological alterations are important factors that have been implicated in the pathogenesis of different cardiovascular diseases.[122] BP is positively associated with plasma viscosity, for which fibrinogen is considered as the main determinant factor.[123] Some substances included in natural medicines have been reported to affect erythrocyte deformability, blood viscosity, fibrinogen concentration, and blood rheology in rats.[124,125] In our studies,[70] we used the microchannel MC-FAN, which is rationally applied in the analysis of blood rheology in pathophysiological conditions.[126−128] **1** (5 mg/kg) significantly decreased the microchannel transit time of whole blood compared to the vehicle control (1 mL/kg), without affecting the blood cell counts, suggesting that **1**-O acts in modulating blood fluidity in hypertension. It is unlikely that this improvement of the hemorheology by **1**-O is related to the plasma fibrinogen level, since the level of plasma fibrinogen was not significantly affected by **1**-O. The improvement is suggested to be due to modulatory effects on the deformability or aggregation of the blood cells; however the mechanism underlying this rheological effect is unclear.

Astaxanthin (1) and the Cardiovascular Tissues. In addition to the above-mentioned factors, **1**-O has shown interesting histological changes in SHR. Vascular elastin is considered an important determinant of the arterial wall mechanical properties and essential for vascular structural integrity and function.[129,130] The well-known features of the inward eutrophic remodeling—the reduced lumen, the increased wall thickness, and the increased wall-to-lumen ratio—had been abolished by elastin degradation.[131] In our findings,[71] **1**-O has demonstrated an outward eutrophic remodeling in the SHR vascular system. **1**-O also decreased the vascular wall thickness in the coronary arteries and the aortae of treated rats, as reflected by a significant decrease in the (wall-to-lumen) ratio. Increased wall thickness is a common structural feature of hypertensive vessels[132] and conduit arteries such as the aorta.[133] Previous reports have shown that pharmacological treatment in SHR attenuates the hypertensive structural changes in large arteries and that this effect is associated with a reduction in the BP.[134,135] Similarly, the **1**-O-induced alterations in the aorta may decrease arterial stiffness and pulse pressure.

An alternative explanation of this finding is that the diminished wall thickness and the lower number of elastin fibers could be a consequence of the reduction in the BP. The vessel wall thickness is related to the pressure, according to the principles of Laplace's law, and in hypertension as much as the BP increases, the arterial walls exhibit hypertrophy and may become thicker.

Neuroprotective Effects of Astaxanthin (1). The effects of **1**-O on the transient ischemia-induced impairment of Morris water maze performance were studied in ICR mice. Transient cerebral ischemia was induced by occlusion of carotid arteries (2 vessel occlusion, 2VO). **1**-O was given orally to the animals 1 h before 2VO. Two days after the ischemia, the mice received training trials of 4 trials per day for 5 days, and 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 **1** (55 and 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.[69] This effect is suggested to be due to the significant antioxidant property of **1** on ischemia-induced free radicals and their consequent pathological cerebral and neural effects. Our findings indicate that, although a relatively high dose of **1** is needed, it may have beneficial effects in preventing the memory deficit in vascular dementia.

Concluding Remarks

Several recent reports and reviews have described and discussed the role of antioxidants and vitamin supplements, including carotenoids, in health and nutrition and their successes and failures in clinical trials and applications in cardiovascular diseases.[68,136−139] These reports have described several factors that may explain the lack of agreement between the predicted positive benefits and the results of the clinical trials conducted to date. They largely agree that there is a lack of knowledge about the oxidative mechanisms in vivo and the biochemical markers with which to evaluate candidate antioxidant compounds and that antioxidant treatment may need to begin early in life in order to be effective.

In conclusion, astaxanthin (**1**) is suggested as a promising natural product in health promotion, and a number of health products containing **1** are under development. Nevertheless, further prospective clinical and research studies addressing the protective effects of **1** are crucial and strongly recommended.

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