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ORIGINAL CONTRIBUTION

Astaxanthin suppresses scavenger receptor expression and matrix metalloproteinase activity in macrophages

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Abstract
Background Astaxanthin is a red carotenoid pigment which has significant potential for antioxidant activity. The macrophages in atherosclerotic lesions, known as activated macrophages, express scavenger receptors responsible for the clearance of pathogenic lipoproteins. In addition, the expression and secretion of proteolytic enzymes, matrix metalloproteinases (MMPs), and pro-inflammatory cytokines are remarkably promoted in activated macrophages.
Aim of the study In this study, we investigated the effects of astaxanthin on the expression of scavenger receptors, MMPs, and pro-inflammatory cytokines in macrophages.
Methods THP-1 macrophages were incubated with 5–10 μM astaxanthin for 24 h. The expression levels of scavenger receptors, MMPs, and pro-inflammatory cytokines were determined by Western blot analysis or real-time RT-PCR. The MMP-9 and -2 activities were examined by gelatin zymography and total MMP activity was measured by fluorometry.
Results We found that astaxanthin remarkably decreased the class A scavenger receptor and CD36 expression in the protein and mRNA levels. Astaxanthin also reduced MMP-1, -2, -3, -9, -12, and -14 activity and expression. The mRNA expression of tumor necrosis factor-α, interleukin-1β, interleukin-6, inducible nitric oxide synthase, and cyclooxygenase-2 were significantly suppressed by astaxanthin. Furthermore, astaxanthin inhibited the phosphorylation of nuclear factor-κB.
Conclusions These results indicate that astaxanthin has inhibitory effects on macrophage activation, such as scavenger receptors up-regulation, MMPs activation, and pro-inflammatory cytokines secretion.

Keywords Astaxanthin · Macrophage · Scavenger receptor · Matrix metalloproteinase · Atherosclerosis

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Introduction

Astaxanthin is a red carotenoid pigment contained in some seafood, such as salmons, crabs, and shrimps. The astaxanthin content of salmon is 1.7–2.6 mg/100 g. Astaxanthin belongs to the xanthophyll class of carotenoids and is closely related to β-carotene, lutein, and zeaxanthin, sharing with them many of the general metabolic and physiological functions attributed to carotenoids. Several previous studies have demonstrated that astaxanthin exhibits a wide variety of biological activities, including antioxidant [19, 31] and anti-tumor effects [5, 21]. The antioxidant activity of astaxanthin is greater than that of β-carotene or α-tocopherol [11, 31]. Astaxanthin has unique chemical

properties based on its molecular structure. The presence of the hydroxyl and keto moieties on each ionone ring explains its higher antioxidant activity. We previously reported that astaxanthin inhibited low density lipoprotein (LDL) oxidation in vitro and ex vivo [18]. However, little information is available about the effects of astaxanthin on atherosclerosis progression beyond its antioxidant ability.

One of the earliest events in atherosclerosis is the accumulation of oxidized LDL (ox-LDL) in the intima. Scavenger receptors are integral membrane proteins that bind to a wide variety of ligands, including modified and ox-LDL, lipoproteins, apoptotic cells and pathogens. Scavenger receptor-mediated recognition of ox-LDL by macrophages leads to the formation of foam cells and plaque. In vitro experiments have shown that the class A scavenger receptor (SR-A) and CD36 are responsible for the major part of ox-LDL uptake by macrophages, suggesting pro-atherogenic roles for SR-A and CD36 [24, 29].

Additionally, macrophages play particularly important roles in inflammation via the production of matrix-degrading enzymes, pro-inflammatory cytokines/chemokines, cell adhesion molecules, nitric oxide (NO), and cyclooxygenase-2 (COX-2) [35]. Matrix metalloproteinases (MMPs), a family of Zn^{2+}-dependent endopeptidases, are responsible for the degradation of most extracellular matrix proteins. MMPs also mediate tissue remodeling in various pathologic conditions, including several inflammatory diseases [2]. Expression of MMPs is increased in atherosclerotic lesions and is linked to weakening of the vascular wall due to degradation of the extracellular matrix [4, 39, 41]. Furthermore, several biomarker studies show associations between increased plasma MMPs levels and acute coronary syndromes [13, 17]. Inducible nitric oxide synthase (iNOS) has been detected in macrophages and T-lymphocytes of advanced atherosclerotic plaques of human coronary arteries. The local release of large amount of NO metabolites has been linked to the production of harmful oxidative products such as peroxynitrite. Prostaglandin which is synthesized by cyclooxygenase is another important mediator of inflammation. COX-2 is induced by several pro-inflammatory stimuli, such as, growth factors, cytokines, and endotoxin. Many macrophage-derived factors have been hypothesized to play an important role in generating inflammatory responses during atherosclerosis.

Thus, macrophages are central to the initiation and progression of atherosclerosis and can be very appropriate targets for therapy. The aim of this study was to elucidate the potential of astaxanthin on macrophage atherogenesis-related functions. We investigated whether astaxanthin inhibited scavenger receptors up-regulation, MMPs activation, and pro-inflammatory cytokines expression in THP-1 macrophages.

Materials and methods

Materials and reagents

Astaxanthin was kindly provided by Fuji Chemical Industries (Toyama, Japan). RPMI 1640 medium, fetal bovine serum (FBS), and penicillin/streptomycin were purchased from GIBCO (Paisley, UK). Oligonucleotide primers were synthesized by Sigma-Genosys (Hokkaido, Japan). RNeasy Mini Kit was purchased from Qiagen (Hilden, Germany), and cDNA Reverse Transcription Kit and SYBR green PCR mix from Applied Biosystems (CA, USA). Polyclonal antibody against SR-A, CD36, LOX-1, MMP-9, MMP-2, nuclear factor-κB (NF-κB) (p65), and phospho NF-κB (p65) were purchased from Santa Cruz Biotechnology (CA, USA). Polyclonal antibody against actin was obtained from Sigma-Aldrich (Tokyo, Japan). All other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan), unless otherwise specified.

Cell culture

The human monocytic cell line THP-1 was obtained from the RIKEN CELL BANK (Tsukuba, Japan) and cultured in PRMI-1640 supplemented with 50 units/mL penicillin and streptomycin, and 10% FBS, at 37 °C and 5% CO_2. THP-1 cells were stimulated with 320 nM Phorbol-12-myristate-13-acetate (PMA) for 72 h to induce differentiation into macrophages. After differentiation, cells were treated with 5–10 μM astaxanthin dissolved in dimethyl sulfoxide for 24 h.

MMPs activity

Gelatinolytic activities of MMP-9 and -2 were examined by gelatin zymography assay. Electrophoresis was performed on culture supernatants at 4 °C on 10% polyacrylamide gels containing 1% SDS and 0.1% gelatin without boiling at a constant voltage of 80 V. The gels were incubated overnight at 37 °C in incubation buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 10 mM $CaCl_2$, and 1 nM $ZnCl_2$. The gels were then stained with a solution of coomassie brilliant blue R-250. Gelatinolytic activity was quantified by scanning densitometry with LAS-4000 (Fujifilm, Tokyo, Japan). Total MMP activity in culture supernatants was measured by using a fluorogenic peptide substrate (R&D systems, MN, USA). The MMP substrate was added to the culture supernatants and incubated at 37 °C for 60 min. Total MMP activity was determined by fluorometer (POWERSCAN-HT, DS Pharma Biomedical, Osaka, Japan) at excitation at 320 nm and emission at 405 nm. The tern 'total MMP activity' is used throughout the paper synonymously with the activity of MMP-1, -2, -7, -8, -9, -12, -13, -14, -15, and -16.

Real-time RT-PCR analysis

Total cellular RNA was extracted using RNeasy Mini kit and the total RNA (2 μg) was then reverse-transcribed into cDNA using a thermal cycler (MJ Research, MA, USA). Real-time PCR was performed on the ABI 7300 cycler (Applied Biosystems, CA, USA) using SYBR green PCR mix. The results were expressed as a copy number ratio of the target mRNA to β-actin mRNA. Primer sequences used in the study were shown in Table 1.

Western blot analysis

After treatment, cells were washed with ice-cold PBS and lysed with protein extraction reagent (Pierce Biotechnology, IL, USA) containing 10 μg/mL leupeptin, 10 μg/mL aprotinin, and 1 mM phenylmethylsulfonyl fluoride. Aliquots of cellular proteins were electrophoresed on 10% SDS-polyacrylamide gel and transferred to an Immobilon-P-membrane (Millipore, MA, USA). The membrane was allowed to react with a specific antibody, and detection of specific proteins was carried out by enhanced chemiluminescence. Loading differences were normalized using polyclonal actin antibodies. All signals were detected by LAS-4000 (Fujifilm, Tokyo, Japan). Densitometric analysis was performed using Multi Gauge version 3.0 (Fujifilm, Tokyo, Japan) to scan the signals.

Table 1 List of primer sequence

Gene/primer	Sequence (5′–3′)
SR-A	Forward: AGGGCCCTCTTAAGATCAGG
	Reverse: ACAACACGGGAACCAAAGTC
CD36	Forward: CAATTAAAAAGCAAGTTGTCCTCGA
	Reverse: ATCACTTCCTGTGGATTTTGCA
MMP-9	Forward: GTGCGTCTTCCCCTTCACTTTCCT
	Reverse: GGAATGATCTAAGCCCAGCG
MMP-2	Forward: GGGACAAGAACCAGATCACATAC
	Reverse: CTTCTCAAAGTTGTAGGTGGTGG
MMP-1	Forward: GACAGAAAGAGACAGGAGAC
	Reverse: GAGTTATCCCTTGCCTATCC
MMP-3	Forward: GGCACAATATGGGCACTTTA
	Reverse: CCGGCAAGATACAGATTCAC
MMP-12	Forward: ACACCTGACATGAACCGTGA
	Reverse: CTAGGATTCCACCTTTGCCA
MMP-14	Forward: GAAGCCTGGCTACAGCAATATG
	Reverse: TGCAAGCCGTAAAACTTCTGC
TNF-α	Forward: TGGAGAAGGGTGACCGACTC
	Reverse: TCCTCACAGGGCAATGATCC
IL-1β	Forward: CTGTACGATCACTGAACTGC
	Reverse: CACCACTTGTTGCTCCATACT
IL-6	Forward: GGTACATCCTCGACGGCATC
	Reverse: GCCTCTTTGCTGCTTTCACAC
iNOS	Forward: CCAAGAGAAGAGAGATTCCATTGAA
	Reverse: TGATTTTCCTGTCTCTGTCGCA
COX-2	Forward: CCAGCACTTCACGCATCAGT
	Reverse: ACGCTGTCTAGCCAGAGTTTCAC
β-Actin	Forward: CTTCTACAATGAGCTGCGTG
	Reverse: CATGAGGTAGTCAGTCAGG

Statistical analysis

Statistical analyses were performed with GraphPad Prism for Windows version 5.0 (GraphPad Software, CA, USA). All results were expressed as mean ± SEM, and differences between groups were analyzed by one-way ANOVA followed by Dunnett's test. The minimum significance level was set at a P value of 0.05 for all analysis. All experiments were performed at least three times.

Results

Effects of astaxanthin on scavenger receptors expression

We first checked the cytotoxic effects of astaxanthin in THP-1 macrophages by MTT assay. Astaxanthin did not affect cell viability under our experimental conditions (data not shown).

We next examined the effect of astaxanthin on scavenger receptors (SR-A and CD36) expression. SR-A and CD36 expressions were remarkably increased during differentiation into macrophages. Astaxanthin gradually decreased SR-A and CD36 protein levels at 5–10 μM in THP-1 macrophages (Fig. 1a).We performed the real-time RT-PCR analyses to determine whether the inhibition of scavenger receptors expression by astaxanthin was due to decreased levels of transcription. Astaxanthin decreased the mRNA expression levels of SR-A and CD36 at 10 μM (48 and 58%, respectively) (Fig. 1b). Furthermore, astaxanthin also suppressed SR-A and CD36 expression in the protein and mRNA levels within human primary macrophages (data not shown).

Effects of astaxanthin on MMPs activity and expression

We analyzed the MMPs activity by using gelatin zymography assay and fluorometry. Quiescent THP-1 cells secreted MMPs at very low levels, and differentiation into macrophages markedly augmented MMPs activity in the culture supernatants. The MMP-9 activity was slightly inhibited in the presence of 10 μM astaxanthin (Fig. 2a). The medium from THP-1 macrophages contained very

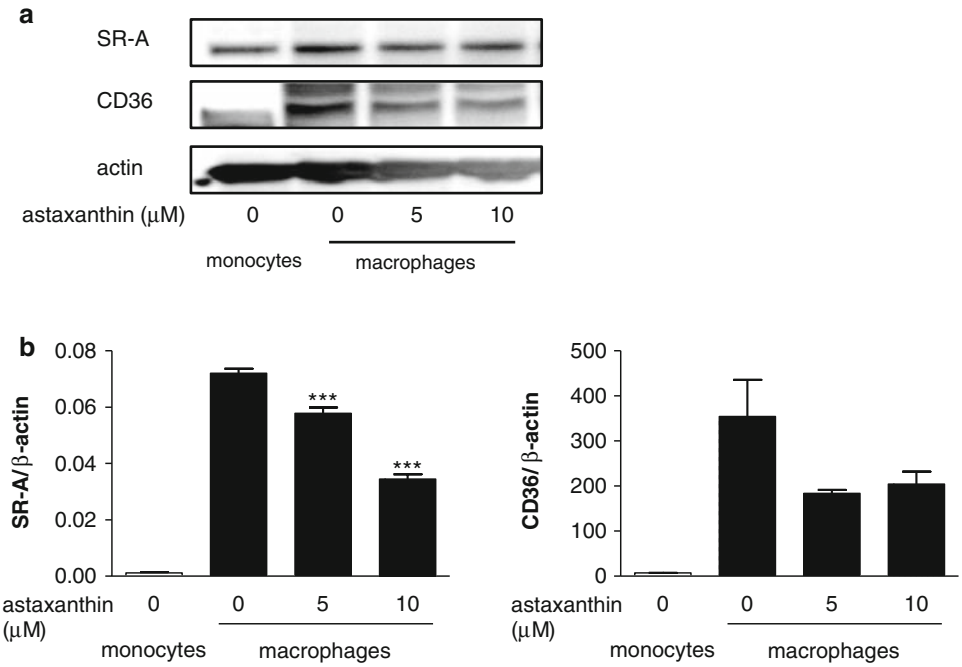

Fig. 1 Effects of astaxanthin on scavenger receptor expression in THP-1 macrophages. Macrophages were treated with 5–10 μM astaxanthin for 24 h. **a** SR-A and CD36 protein expression were determined by Western blot. Equal loading of proteins was confirmed with total actin antibody. Representative blots from three independent experiments are shown. **b** SR-A and CD36 mRNA expression were determined by real-time RT-PCR analysis. Each mRNA levels were normalized to the levels of β-actin mRNA. ***$P < 0.001$ compared with untreated macrophage

weak proteolytic activity at 72 kDa, corresponding to MMP-2. Total MMP activity was measured by digestion of fluorogenic peptide substrate. As shown in Fig. 2b, astaxanthin significantly reduced total MMP activity at 10 μM (73%, $P < 0.05$). The protein expression levels of MMP-9 and -2 were determined by Western blot analysis. The expression levels of MMP-9 protein decreased (Fig. 2c), indicating that reduced MMP-9 enzyme activity was the result of decreased amounts of MMP-9 protein. MMP-2 protein expression was also decreased by astaxanthin. Figure 2d showed the effects of astaxanthin on MMPs expression in the mRNA levels. MMP-9, -2 and the other MMPs expressed in macrophages, MMP-1, -3, -12, and -14 expressions were decreased by 10 μM astaxanthin (29, 66, 74, 15, 10 and 37%, respectively). Furthermore, astaxanthin showed the inhibitory effects on MMP-9 and -2 expressions in mRNA and protein level in human primary macrophages (data not shown).

Effects of astaxanthin on pro-inflammatory cytokines, iNOS and COX-2 expression

As shown in Fig. 3, the differentiation of monocytes into macrophages-induced up-regulation of pro-inflammatory cytokines, iNOS and COX-2. Astaxanthin remarkably suppressed the mRNA expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), iNOS, and COX-2 at 10 μM (39, 31, 28, 32, and 19%, respectively). We found that astaxanthin also inhibited the pro-inflammatory cytokines expression in human primary macrophages (data not shown).

Effects of astaxanthin on nuclear factor-κB activation

To evaluate the mechanism by which astaxanthin down-regulates the expression of scavenger receptors, MMPs, and pro-inflammatory cytokines, we examined its inhibitory effect on NF-κB activation. Protein kinase C activated by PMA is known to stimulate the activities of NF-κB. We found that astaxanthin inhibited the phosphorylation of NF-κB in THP-1 macrophages (Fig. 4).

Discussion

Macrophages play central roles in the initiation, progression, development, and rupture of atherosclerotic plaque. In the present study, we evaluated the inhibitory effects of astaxanthin on macrophage activation. Astaxanthin effectively suppressed the expression of scavenger receptors, MMPs, and pro-inflammatory cytokines in THP-1 macrophages. Moreover, we found that astaxanthin inhibited the activation of NF-κB.

Atherosclerosis is known as an inflammatory disease. Recently, it has been proposed that oxidative stress and inflammation are evoked thought the similar pathway. Therefore, the antioxidants, such as carotenoids, are expected to inhibit inflammation and improve atherosclerosis progression. Several epidemiologic studies have proposed an inverse relationship between serum carotenoids and cardiovascular disease [6, 9]. Astaxanthin is considered to have stronger antioxidant ability than other carotenoids due to the presence of the hydroxyl and keto

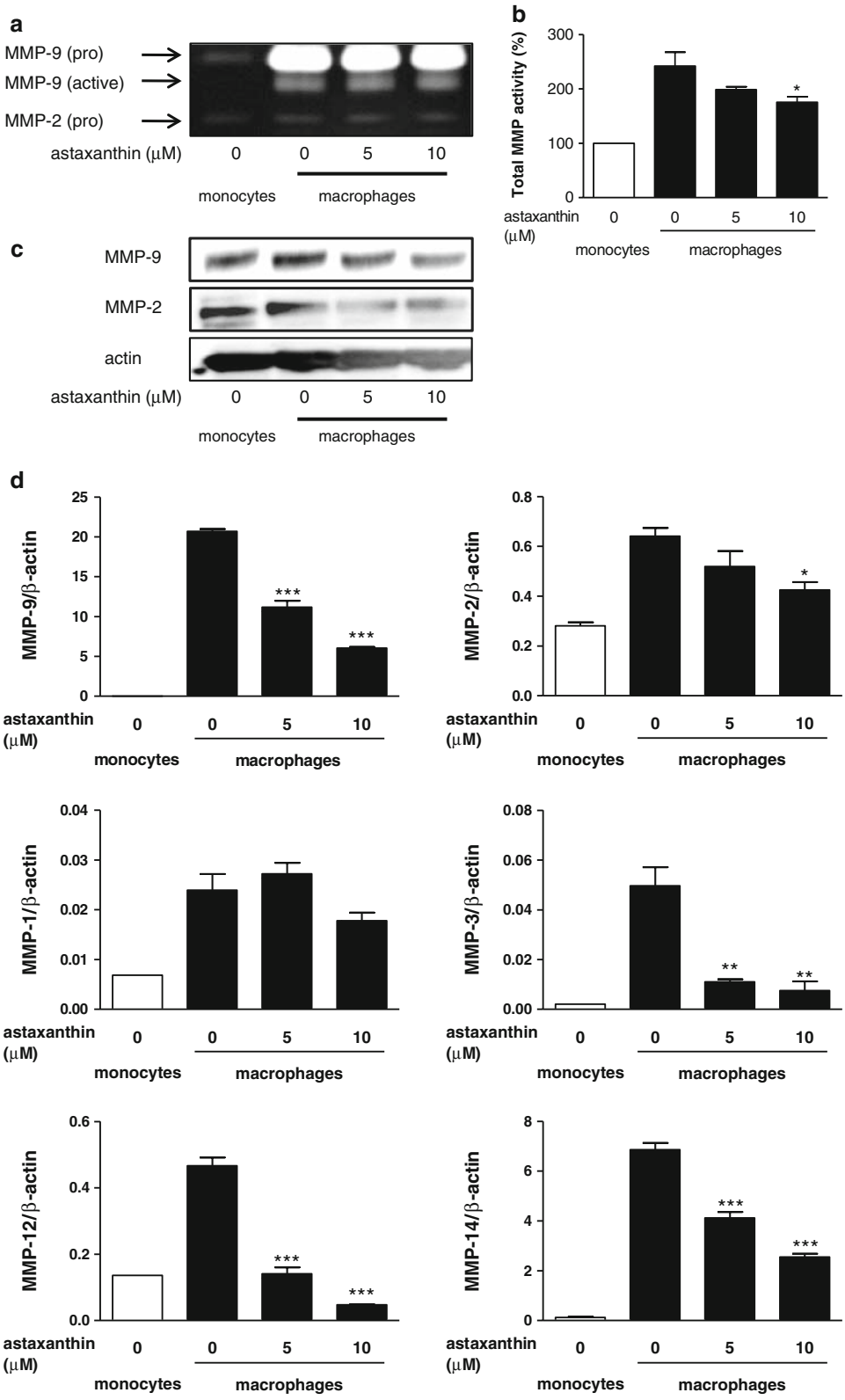

Fig. 2 Effects of astaxanthin on MMP activity and expression in THP-1 macrophages. Macrophages were treated with 5–10 μM astaxanthin for 24 h. **a** MMP-9 and -2 activities in culture supernatants were analyzed by gelatinolytic zymography. **b** Total MMP activity was measured by digestion of fluorogenic peptide substrate. **c** MMP-9 and -2 protein expressions were determined by Western blot. Equal loading of proteins was confirmed with total actin antibody. Representative blots from three independent experiments are shown. **d** MMP-9, -2, -1, -3, -12, and -14 mRNA expression were determined by real-time RT-PCR analysis. Each mRNA levels were normalized to the levels of β-actin mRNA. Data are presented as mean ± SEM of three separate experiments. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared with untreated macrophage

moieties on each terminal ring. In addition, astaxanthin is likely to be located in the membrane in such a way that its polar terminal ring is oriented at or near the membrane surface, with the polyene chain in the interior of the membrane [14]. This structure could be effective for the terminal ring of astaxanthin to scavenge reactive oxygen

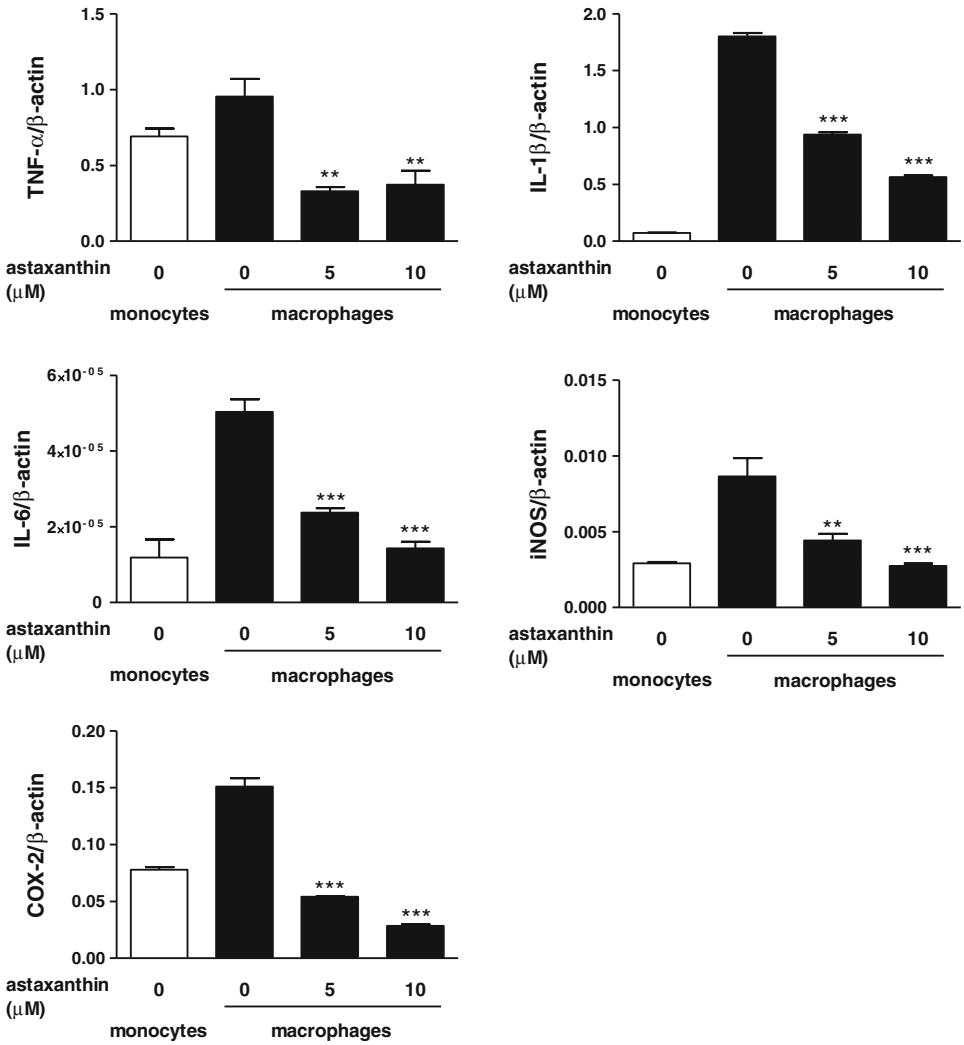

Fig. 3 Effects of astaxanthin on pro-inflammatory cytokines, iNOS and COX-2 expression in THP-1 macrophages. Macrophages were treated with 5–10 μM astaxanthin for 24 h. TNF-α, IL-1β, IL-6, COX-2, and iNOS mRNA expression were determined by real-time RT-PCR analysis. Each mRNA levels were normalized to the levels of β-actin mRNA. $^{***}P < 0.001$, $^{**}P < 0.01$ compared with untreated macrophage

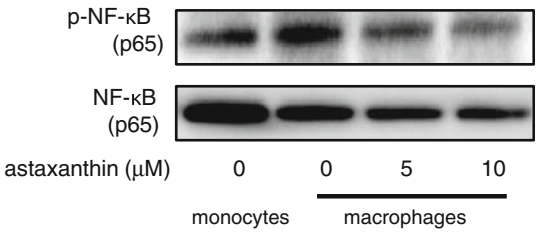

Fig. 4 Effects of astaxanthin on NF-κB activation in THP-1 macrophages. Macrophages were treated with 5–10 μM astaxanthin for 24 h. NF-κB activation were determined by Western blot with phospho-specific antibodies. Equal loading was confirmed with the total antibody. Representative blots from three independent experiments are shown

species (ROS) at/near the membrane surface, and for its polyene chain to inhibit the radical chain reaction in the membrane.

We showed that astaxanthin decreased SR-A and CD36 up-regulations in THP-1 macrophages. Unregulated uptake of ox-LDL via macrophage scavenger receptors is a key event in atherosclerosis progression. Mamputu et al. [28] showed that gliclazide, a sulfonylurea with antioxidant properties, but not glyburide, without antioxidant properties, decreased CD36 expression in human macrophages. These results suggest that oxidative stress is involved in the regulation of CD36 expression. Recent studies demonstrated that some antioxidants, such as flavonoids [8, 22] and α-tocopherol [7, 33], suppressed CD36 expression in macrophages. Though the precise mechanisms for developmental regulation of SR-A have not been clear, in vitro and animal studies probably suggest that ROS generated from a protein kinase C-dependent NAD(P)H oxidase pathway plays a role in the high glucose-induced up-regulation of SR-A [10]. Hence, we think that the antioxidant property of astaxanthin may account for its inhibitory effect on SR-A and CD36 expression. We previously reported that astaxanthin protected LDL oxidation in healthy subjects [18]. These data, as well as our current results, suggest that astaxanthin can inhibit the uptake of ox-LDL and foam cells formation.

Macrophage-derived foam cells associate clinically with unstable plaques [26]. Elevated levels of MMPs, such as MMP-1, -2, -3, -9, -11, -12, -13, -14, and -16 are all found in macrophage-rich regions of human atherosclerotic plaque [32]. In the current study, we found that astaxanthin reduced the gelatinolytic activity (MMP-9 and -2) and total peptidase activity (total MMP) in THP-1 macrophages. Astaxanthin decreased MMP-9 and -2 in both the mRNA and protein levels, and MMP-1, -3, -12, and -14 in mRNA levels. It has been reported that the gelatinolytic activity in plaques is ROS-dependent and thus inhibitable by ROS scavengers such as *N*-acetyl-L-cysteine [12]. Our results indicated that astaxanthin might prove useful for inhibiting matrix degradation and improving vascular stability.

The pro-inflammatory cytokines, prostaglandins, and NO produced by activated macrophages play critical roles in atherosclerosis. The inhibition of pro-inflammatory cytokines secretion from macrophages might be one of the mechanisms mediating the beneficial effects of antioxidants on atherosclerosis development. This idea is based primarily on studies with vitamin E, which has been shown to reduce the secretion of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, by monocytes and macrophages [20, 36]. We found that astaxanthin inhibited TNF-α, IL-1β, IL-6, COX-2, and iNOS mRNA expressions in THP-1 macrophage. The pro-inflammatory mediators, such as TNF-α, IL-1β, or IFNγ is known to further increase expression of MMPs [37, 42].

Nuclear factor-κB is one of most important transcription factors in terms of inflammatory responses, and controls the expressions of numerous genes, such as TNF-α, IL-1β, IL-6, COX-2, and iNOS [1]. Moreover, it is known that MMPs expressions are also regulated with NF-κB [3]. Previous studies showed that the inhibitor of the NF-κB signaling pathway blocked the MMP-9 activation [27, 40]. We found that astaxanthin inhibited the phosphorylation of NF-κB in THP-1 macrophages, suggesting that the inhibitory effects of astaxanthin on the MMPs activation and pro-inflammatory cytokines expression were associated with the suppression of NF-κB activation. Lycopene and β-carotene have been shown to regulate NF-κB pathways [23, 34].

A few animal studies have reported the beneficial effects of oral astaxanthin supplementation on prevention of cardiovascular disease. The astaxanthin derivative, astaxanthin disodium salt (ADD), protected ischemia-reperfusion injury in rat and dog [15, 16]. ADD has also demonstrated efficacy of carotid artery rethrombosis and platelet aggregation [25]. Though the anti-inflammatory effects of astaxanthin in human subject is still unclear, lycopene or β-carotene supplementation suppressed ROS generation in neutrophils [38]. Serum lycopene levels inversely correlated with neopterin, a sensitive indicator for macrophage immune activation [30]. Results from previous reports and our study support the antioxidant/anti-inflammatory properties of astaxanthin, suggesting it as a candidate for a therapeutic agent for cardiovascular oxidative stress and inflammation. However, it remains to be seen if the results of these in vitro and animal studies can be reproduced in human subjects.

In conclusion, astaxanthin suppressed the scavenger receptors up-regulation, MMPs activation, and pro-inflammatory cytokines expression in macrophages. Our findings suggest that astaxanthin can be effective to regulate the macrophage atherogenesis-related functions.

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Conflict of interest statement We have no conflict of interest.

References

1. Baeuerle PA, Baltimore D (1996) NF-kappa B: ten years after. Cell 87:13–20
2. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T (2003) Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 253:269–285
3. Chase AJ, Bond M, Crook MF, Newby AC (2002) Role of nuclear factor-kappa B activation in metalloproteinase-1, -3, and -9 secretion by human macrophages in vitro and rabbit foam cells produced in vivo. Arterioscler Thromb Vasc Biol 22:765–771
4. Chen F, Eriksson P, Hansson GK, Herzfeld I, Klein M, Hansson LO, Valen G (2005) Expression of matrix metalloproteinase 9 and its regulators in the unstable coronary atherosclerotic plaque. Int J Mol Med 15:57–65
5. Chew BP, Park JS, Wong MW, Wong TS (1999) A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice in vivo. Anticancer Res 19:1849–1853
6. De Waart FG, Schouten EG, Stalenhoef AF, Kok FJ (2001) Serum carotenoids, alpha-tocopherol and mortality risk in a prospective study among Dutch elderly. Int J Epidemiol 30:136–143
7. Devaraj S, Hugou I, Jialal I (2001) Alpha-tocopherol decreases CD36 expression in human monocyte-derived macrophages. J Lipid Res 42:521–527
8. Eguchi A, Murakami A, Ohigashi H (2006) Nobiletin, a citrus flavonoid, suppresses phorbol ester-induced expression of multiple scavenger receptor genes in THP-1 human monocytic cells. FEBS Lett 580:3321–3328
9. Evans RW, Shaten BJ, Day BW, Kuller LH (1998) Prospective association between lipid soluble antioxidants and coronary heart disease in men. The multiple risk factor intervention trial. Am J Epidemiol 147:180–186
10. Fukuhara-Takaki K, Sakai M, Sakamoto Y, Takeya M, Horiuchi S (2005) Expression of class A scavenger receptor is enhanced by high glucose in vitro and under diabetic conditions in vivo: one mechanism for an increased rate of atherosclerosis in diabetes. J Biol Chem 280:3355–3364

11. Fukuzawa K, Inokami Y, Tokumura A, Terao J, Suzuki A (1998) Rate constants for quenching singlet oxygen and activities for inhibiting lipid peroxidation of carotenoids and alpha-tocopherol in liposomes. Lipids 33:751–756
12. Galis ZS, Asanuma K, Godin D, Meng X (1998) *N*-acetyl-cysteine decreases the matrix-degrading capacity of macrophage-derived foam cells: new target for antioxidant therapy? Circulation 97:2445–2453
13. Giansante C, Fiotti N, Di Chiara A, Altamura N, Wasserman S, Fioretti P, Guarnieri G (2007) In-hospital outcome of patients with acute coronary syndrome: relationship with inflammation and remodeling markers. J Cardiovasc Med (Hagerstown) 8:602–607
14. Goto S, Kogure K, Abe K, Kimata Y, Kitahama K, Yamashita E, Terada H (2001) Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. Biochim Biophys Acta 1512:251–258
15. Gross GJ, Lockwood SF (2005) Acute and chronic administration of disodium disuccinate astaxanthin (Cardax) produces marked cardioprotection in dog hearts. Mol Cell Biochem 272:221–227
16. Gross GJ, Lockwood SF (2004) Cardioprotection and myocardial salvage by a disodium disuccinate astaxanthin derivative (Cardax). Life Sci 75:215–224
17. Inokubo Y, Hanada H, Ishizaka H, Fukushi T, Kamada T, Okumura K (2001) Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. Am Heart J 141:211–217
18. Iwamoto T, Hosoda K, Hirano R, Kurata H, Matsumoto A, Miki W, Kamiyama M, Itakura H, Yamamoto S, Kondo K (2000) Inhibition of low-density lipoprotein oxidation by astaxanthin. J Atheroscler Thromb 7:216–222
19. Jacobsson LS, Yuan XM, Zieden B, Olsson AG (2004) Effects of alpha-tocopherol and astaxanthin on LDL oxidation and atherosclerosis in WHHL rabbits. Atherosclerosis 173:231–237
20. Jung WJ, Sung MK (2004) Effects of major dietary antioxidants on inflammatory markers of RAW 264.7 macrophages. Biofactors (Oxford) 21:113–117
21. Jyonouchi H, Sun S, Iijima K, Gross MD (2000) Antitumor activity of astaxanthin and its mode of action. Nutr Cancer 36:59–65
22. Kawai Y, Nishikawa T, Shiba Y, Saito S, Murota K, Shibata N, Kobayashi M, Kanayama M, Uchida K, Terao J (2008) Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries: implication in the anti-atherosclerotic mechanism of dietary flavonoids. J Biol Chem 283:9424–9434
23. Kim GY, Kim JH, Ahn SC, Lee HJ, Moon DO, Lee CM, Park YM (2004) Lycopene suppresses the lipopolysaccharide-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and nuclear factor-kappaB. Immunology 113:203–211
24. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW (2002) Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. J Biol Chem 277:49982–49988
25. Lauver DA, Driscoll EM, Lucchesi BR (2008) Disodium disuccinate astaxanthin prevents carotid artery rethrombosis and ex vivo platelet activation. Pharmacology 82:67–73
26. Lendon CL, Davies MJ, Born GV, Richardson PD (1991) Atherosclerotic plaque caps are locally weakened when macrophages density is increased. Atherosclerosis 87:87–90
27. Liao YF, Hung HC, Hsu PC, Kao MC, Hour TC, Tsay GJ, Liu GY (2008) Ornithine decarboxylase interferes with macrophage-like differentiation and matrix metalloproteinase-9 expression by tumor necrosis factor alpha via NF-kappaB. Leuk Res 32:1124–1140
28. Mamputu JC, Li L, Renier G (2006) Gliclazide inhibits differentiation-associated biologic events in human monocyte-derived macrophages. Metab Clin Exp 55:778–785
29. Miller YI, Chang MK, Binder CJ, Shaw PX, Witztum JL (2003) Oxidized low density lipoprotein and innate immune receptors. Curr Opin Lipidol 14:437–445
30. Murr C, Winklhofer-Roob BM, Schroecksnadel K, Maritschnegg M, Mangge H, Bohm BO, Winkelmann BR, Marz W, Fuchs D (2009) Inverse association between serum concentrations of neopterin and antioxidants in patients with and without angiographic coronary artery disease. Atherosclerosis 202:543–549
31. Naguib YM (2000) Antioxidant activities of astaxanthin and related carotenoids. J Agric Food Chem 48:1150–1154
32. Newby AC (2008) Metalloproteinase expression in monocytes and macrophages and its relationship to atherosclerotic plaque instability. Arterioscler Thromb Vasc Biol 28:2108–2114
33. Ozer NK, Negis Y, Aytan N, Villacorta L, Ricciarelli R, Zingg JM, Azzi A (2006) Vitamin E inhibits CD36 scavenger receptor expression in hypercholesterolemic rabbits. Atherosclerosis 184:15–20
34. Palozza P, Serini S, Torsello A, Di Nicuolo F, Piccioni E, Ubaldi V, Pioli C, Wolf FI, Calviello G (2003) Beta-carotene regulates NF-kappaB DNA-binding activity by a redox mechanism in human leukemia and colon adenocarcinoma cells. J Nutr 133:381–388
35. Ross R (1999) Atherosclerosis is an inflammatory disease. Am Heart J 138:S419–S420
36. Singh U, Devaraj S, Jialal I (2005) Vitamin E, oxidative stress, and inflammation. Annu Rev Nutr 25:151–174
37. Vaday GG, Franitza S, Schor H, Hecht I, Brill A, Cahalon L, Hershkoviz R, Lider O (2001) Combinatorial signals by inflammatory cytokines and chemokines mediate leukocyte interactions with extracellular matrix. J Leukoc Biol 69:885–892
38. Walrand S, Farges MC, Dehaese O, Cardinault N, Minet-Quinard R, Grolier P, Bouteloup-Demange C, Ribalta J, Winklhofer-Roob BM, Rock E, Vasson MP (2005) In vivo and in vitro evidences that carotenoids could modulate the neutrophil respiratory burst during dietary manipulation. Eur J Nutr 44:114–120
39. Watanabe N, Ikeda U (2004) Matrix metalloproteinases and atherosclerosis. Curr Atheroscler Rep 6:112–120
40. Woo JH, Park JW, Lee SH, Kim YH, Lee IK, Gabrielson E, Lee SH, Lee HJ, Kho YH, Kwon TK (2003) Dykellic acid inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting nuclear factor kappa B transcriptional activity. Cancer Res 63:3430–3434
41. Zeng B, Prasan A, Fung KC, Solanki V, Bruce D, Freedman SB, Brieger D (2005) Elevated circulating levels of matrix metalloproteinase-9 and -2 in patients with symptomatic coronary artery disease. Intern Med J 35:331–335
42. Zhou M, Zhang Y, Ardans JA, Wahl LM (2003) Interferon-gamma differentially regulates monocyte matrix metalloproteinase-1 and -9 through tumor necrosis factor-alpha and caspase 8. J Biol Chem 278:45406–45413