Original Article

The effect of aging on the antioxidative activity of astaxanthin in human aqueous humor

Hirotaka Hashimoto,[1,*] Kiyomi Arai,[2] Jiro Takahashi,[3] and Makoto Chikuda[2]

[1]Tsukuba Hashimoto Optical Clinic, 530 Furuku, Tsukuba-shi, Ibaraki 305-0021, Japan
[2]Department of Ophthalmology, Saitama Medical Center, Dokkyo Medical University, 2-1-50 Minamikoshigaya, Koshigaya, Saitama 343-8555, Japan
[3]Fuji Chemical Industry Co., Ltd., 55 Yokohoonji, Kamiichi-machi, Nakaniikawa-gun, Toyama 930-0397, Japan

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We previously evaluated the antioxidative effects of astaxanthin intake in the aqueous humor by measuring reactive oxygen species-related parameters, including $O_2^{\bullet-}$ scavenging activity, H_2O_2 level, and total hydroperoxides level. In this study, we analyzed the antioxidative effects of astaxanthin in relation to age in 16 males and 19 females (average age 71.3 and 70.6, respectively) who underwent bilateral cataract surgery on one side before and the other side after astaxanthin intake (6 mg/day for 2 weeks). None of the parameters correlated with age before astaxanthin intake, but only total hydroperoxides level was significantly correlated after the astaxanthin intake ($r = 0.4$, $p<0.05$). Total hydroperoxides levels were similar in younger and older patients (<70 vs ≥70 years) before astaxanthin, but decreased significantly more in younger patients (-0.21 ± 0.18 vs -0.05 ± 0.31, $p<0.05$) after the intake, resulting in significantly different levels ($p<0.05$). The previously observed decrease in mean total hydroperoxides levels following astaxanthin intake was therefore considered likely to be attributable to a greater response in younger subjects. Given that total hydroperoxides levels reflect general antioxidative status, astaxanthin intake may exert a greater antioxidative effect in younger patients. Further comparative studies involving younger subjects and different astaxanthin doses are needed.

Key Words: **astaxanthin, age, aqueous humor, superoxide, hydrogen peroxide, oxidation, antioxidant**

Oxidation has recently been shown to be involved in various pathologies, leading to increasing research into antioxidative substances.[1,2] In addition, numerous reports have described the involvement of oxidation reactions in various ophthalmologic pathologies, including cataract, glaucoma, diabetic retinopathy, uveitis, and age-related macular degeneration (AMD),[3,4] and have discussed the benefits of antioxidative agents in these conditions. For example, the carotenoid lutein is considered to be effective in AMD, and is recommended as a supplement in our daily practice.[5] We have focused on the antioxidant astaxanthin (AX) (Fig. 1), and explored its antioxidative effect from an ophthalmologic perspective.

We previously reported several findings,[6–12] including a beneficial anti-inflammatory effect of AX after cataract surgery.[6] AX intake also increased superoxide ($O_2^{\bullet-}$) scavenging activity in the aqueous humor in patients with diabetes,[7] and affected hydrogen peroxide (H_2O_2) levels in the aqueous humor.[8,9] AX intake lowered total hydroperoxides levels in the aqueous humor and generally suppressed oxidation.[10] AX intake was also suggested to have antioxidative effects on vascular endothelial growth factor (VEGF) through enhancement of $O_2^{\bullet-}$ scavenging activity and suppression of peroxide formation,[11] while VEGF levels were related to total hydroperoxides levels in the aqueous humor and AX intake affected $O_2^{\bullet-}$ scavenging activity in females.[12]

We reevaluated various reactive oxygen species (ROS)-related parameters and other factors using multiple linear regression analyses, which suggested that the antioxidative effect of AX in the aqueous humor may be strongly related to age. In the current study, we therefore analyzed the antioxidative effect of AX in relation to patient age.

Subjects and Methods

Subjects. Subjects who underwent bilateral cataract surgery (intraocular lens implantation) at Tsukuba Hashimoto Optical Clinic were included in the study. Patients with inflammatory diseases such as uveitis, with a high degree of refractive error (8.0 diopters or above), or who had been taking other supplements were excluded. The study was approved by the Bioethics Committee of Dokkyo Medical University Saitama Medical Center (approval number: 22025). All patients provided informed consent based on a detailed explanation of the purpose of the study.

Patients began AX (Astavita®; Fuji Chemical Industry, Toyama, Japan) 6 mg/day immediately after undergoing surgery on one eye, followed by surgery on the other eye 2 weeks later. Aqueous humor samples were taken from each eye during surgery for analysis of oxidation-related parameters ($O_2^{\bullet-}$ scavenging activity, and levels of H_2O_2, and total hydroperoxides).[13–16]

Materials. $O_2^{\bullet-}$ scavenging activity was measured by nitro-blue tetrazolium (NBT) reduction assay and H_2O_2 by titanium colorimetry.[13,14] The NBT assay was performed with a superoxide dismutase (SOD) test kit, (SOD Test Wako®; Wako Pure Chemical Industries Ltd., Osaka, Japan), which measures $O_2^{\bullet-}$ scavenging activities by various $O_2^{\bullet-}$ scavengers, including reduced glutathione and L-ascorbic acid, and is not limited to detecting SOD alone.

Total hydroperoxides was measured by microassay using the FREE d-ROMs reagent (Diacron Srl, Grosseto, Italy).[15] *N*,*N*-diethylparaphenylenediamine, the chromogen pigment in the FREE d-ROMs reagent, reacts with H_2O_2, lipid peroxides, peroxidized nucleic acids and nucleotides, as well as peroxides of proteins, peptides, and amino acids. The measured total hydroperoxides levels thus indicate the total amount of these peroxidized (-OOH modified) substances.[16]

Statistical analysis. The relationships between these parameters were analyzed before and after AX intake using Spearman's rank-correlation coefficient, and compared between younger (<70 years, $n = 15$) and older (≥70, $n = 20$) subjects using Mann–

*To whom correspondence should be addressed.
E-mail: hirotaka65@aol.com

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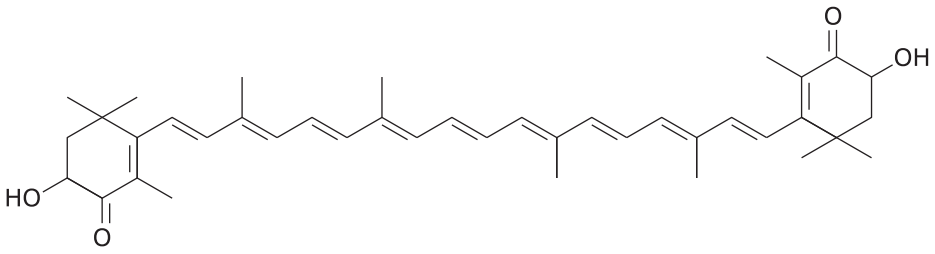

Fig. 1. Structural formula of astaxanthin

Table 1. The correlation between age and the levels of reactive oxygen species (ROS)-related parameters in human aqueous humor before and after the astaxanthin (AX) supplement intake

	Before the AX intake				
	$O_2^{\bullet-}$ scavenging activity	H_2O_2 level	Total hydroperoxides level	With or without DM	Gender
Age	r = –0.009 p = 0.960	r = 0.228 p = 0.188	r = 0.264 p = 0.126	r = –0.097 p = 0.580	r = 0.043 p = 0.807
	After the AX intake				
	$O_2^{\bullet-}$ scavenging activity	H_2O_2 level	Total hydroperoxides level	With or without DM	Gender
Age	r = 0.066 p = 0.707	r = –0.092 p = 0.599	r = 0.389* p = 0.021	r = –0.097 p = 0.580	r = 0.043 p = 0.807

Spearman rank-correlation coefficient, *p<0.05.

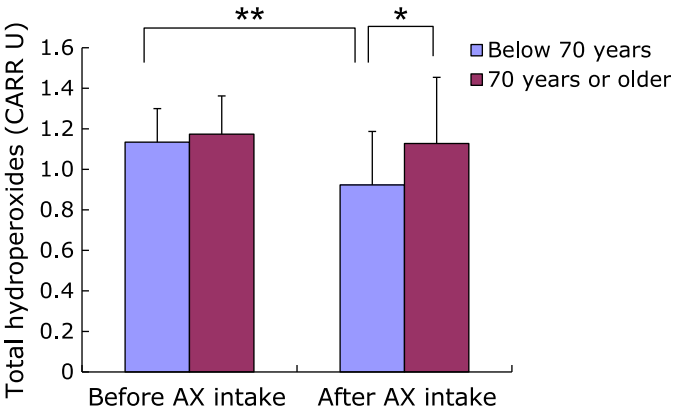

Fig. 2. Total hydroperoxides levels before and after AX intake of different age groups. *p<0.05, **p<0.01.

Whitney U tests. p<0.05 was considered significant. Changes between before and after AX intake were obtained by subtracting the respective levels.

Results

Thirty-five patients were included in the study (16 males, age 71.3 ± 6.4 years, and 19 females, age 70.6 ± 7.4 years). None of the measured ROS-related parameters showed any relationship with age before AX intake. However, total hydroperoxides level after AX intake was significantly related to age (r = 0.4, p<0.05) (Table 1). After AX intake, Total hydroperoxides levels decreased significantly more in younger (<70 years) group (–0.21 ± 0.18) compared with older (≥70 years) patients (–0.05 ± 0.31) (p<0.05), resulting in a significantly lower level in the younger group (p<0.05). (Fig. 2 and 3).

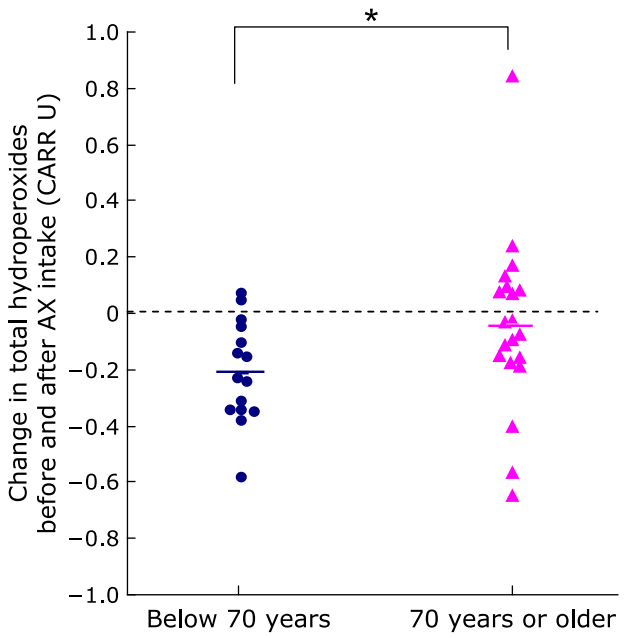

Fig. 3. Change in the level of total hydroperoxides before and after AX intake. Comparison between different age groups. *p<0.05.

Discussion

This study included only elderly patients (aged 58–85 years) who underwent cataract surgery, and no young or middle-aged patients were included. The results of the analysis revealed that the decrease in total hydroperoxides levels in the aqueous humor after the AX intake demonstrated in our previous study strongly reflected the responses of subjects aged younger than 70 years.[10]

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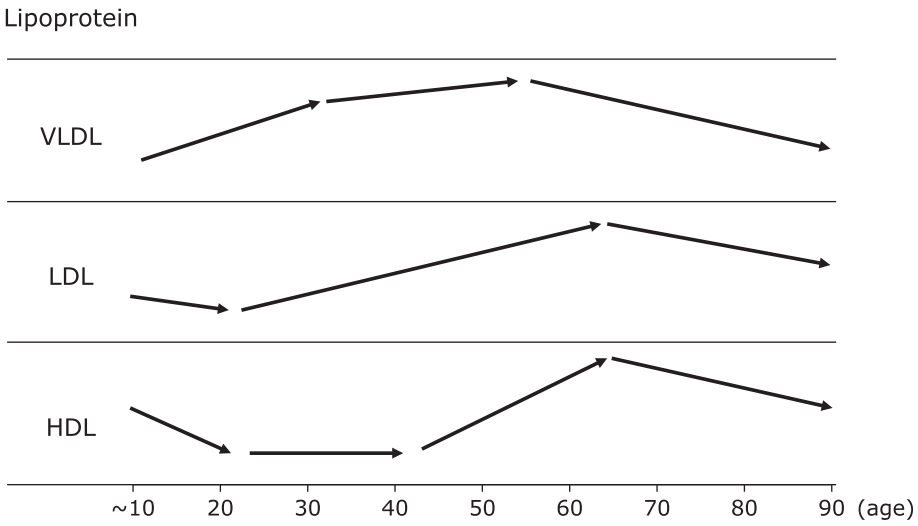

Fig. 4. Fluctuation patterns of lipoprotein levels with age (adapted from published literature).

Total hydroperoxides level is an indicator of general oxidation status, and the current results thus suggested that younger elderly subjects may benefited more from the antioxidative effect of AX than older elderly subjects.

We confirmed that there was no correlation between total hydroperoxides levels and age and no significant difference in total hydroperoxides levels between the younger and the older groups before AX intake, and no age-related differences in general oxidation status before AX intake. Based on this, we considered the results in light of the absorption, function, and metabolism of AX.

Regarding the pharmacokinetics of AX in the human body,[17,18] Østerlie *et al.*[17] reported that plasma AX after oral ingestion was mainly present in lipoproteins, with very-low-density (VLDL), low-density (LDL), and high-density lipoproteins (HDL) contained 36–64%, 29%, and 24% of total AX, respectively. Previous studies on age-related changes in lipoprotein levels showed that VLDL, LDL, and HDL levels increased with aging to a peak and then decreased.[19–21] The peak occurred at approximately 55 years for VLDL, 65–70 years for LDL, and 65 years for HDL in the Japanese population but varied among countries (Fig. 4). The results of the present study were in accord with these age-related changes in lipoproteins. Given that lipoproteins are involved in the absorption and transfer of AX in the body, this may provide a possible explanation for our results.

However, there are limitations to this study in interpreting our data. Firstly, the present study does not cover all age groups but enrolled only elderly patients who underwent cataract surgery. So, we haven't shown associations between total hydroperoxides levels and age for the younger ages when VLDL, LDL, and HDL levels are considered to increase with age. Secondly, we did not actually measure lipoprotein levels in the specimens taken from the subjects. Lipoprotein levels may vary by individuals or by races depending on the content of meals which can be affected by social backgrounds.

In this current study, we discussed the possibility that decreased lipoprotein levels due to aging might be one of the factors of lowering antioxidative effect of AX intake with age. For the future, we think further studies to elucidate the intestinal digestion and absorption, tissue functions, catabolism, and excretion of AX are needed to explain its effects, since there is currently no available information on age-related plasma concentrations of AX. Comparative clinical studies including younger or middle-aged populations and dose-ranging studies are also needed.

Abbreviations

AMD	age-related macular degeneration
AX	astaxanthin
HDL	high-density lipoproteins
H_2O_2	hydrogen peroxide
LDL	low-density lipoproteins
NBT	nitro blue tetrazolium
$O_2^{\bullet-}$	superoxide
-OOH	peroxidated substances
ROMs	reactive oxygen metabolites
ROS	reactive oxygen species
SOD	superoxide dismutase
VEGF	vascular endothelial growth factor
VLDL	very-low-density lipoproteins

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