The Effect of Astaxanthin on Retinal Capillary Blood Flow in Normal Volunteers

Nagaki Yasunori　Mihara Miharu　Takahashi Jiro　Kitamura Akitoshi
Horita Yoshiharu　Sugiura Yuri　Tsukahara Hiroki

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The Effect of Astaxanthin on Retinal Capillary Blood Flow in Normal Volunteers

Yasunori Nagaki [1)] Miharu Mihara [1)] Jiro Takahashi [2)] Akitoshi Kitamura [2)]
Yoshiharu Horita [2)] Yuri Sugiura [2)] Hiroki Tsukahara [2)]

Objective: We evaluated the effect of astaxanthin on retinal circulation in healthy volunteers.

Design: A double blind randomized placebo controlled study.

Methods : Thirty-six volunteers were randomized into two groups: An astaxanthin group that consisted of 18 subjects who received oral astaxanthin, 6mg/day, for 4 weeks and a placebo group that consisted of 18 subjects who received an identical looking oral placebo for 4 weeks. Retinal capillary blood flow was measured using a Heidelberg Retina Flowmeter. Changes in blood pressure, blood cell counts, fasting plasma glucose level, fasting plasma astaxanthin level, retinal capillary blood flow and intraocular pressure were examined and a survey about eye strain taken before and after supplementation in both groups.

Results : The fasting plasma astaxanthin level in the astaxanthin group was significantly ($p<0.001$) higher than before supplementation. The fasting plasma astaxanthin level in the placebo group after placebo treatment remained unchanged. After 4 weeks supplementation, retinal capillary blood flow in the astaxanthin group was significantly ($p<0.01$) higher than before supplementation in both eyes, while retinal capillary blood flow in the placebo group after placebo treatment was unchanged. Intraocular pressures in both groups remained unchanged during the supplementation period.

Conclusion: Our results suggest that astaxanthin supplementation may increase retinal capillary blood flow.

Key words: Astaxanthin; Retinal capillary blood flow; Plasma astaxanthin level

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[1)] Yasunori Nagaki, Miharu Mihara Toyama Medical and Pharmaceutical University, Department of Ophthalmology

[2)] Jiro Takahashi, Akitoshi Kitamura, Yoshiharu Horita, Yuri Sugiura, Hiroki Tsukahara Fuji Chemical Industry Co., Ltd., Life Science Division

Introduction

Astaxanthin (AX) is one of a number of carotenoids similar to β-carotene. AX is a red pigment found mainly in crustaceans, fish, algae and yeast. In recent years, AX has been reported to have a strong antioxidant effect[2]. Effects also have been reported in many other fields, including an anti-inflammatory effect[3], anti-atherogenic action[4], a protective effect against brain damage due to cerebral ischemia[5], and improvement in visual function and amelioration of muscle fatigue in athletes[6]. In the field of ophthalmology, such results as anti-inflammatory effects in endotoxin-induced uveitis (EIU) in rats[7], prevention of age-related macular degeneration in humans[8], improvement of amplitude of accommodation in visual display terminal (VDT) workers[9] and improvement of ocular accommodation in humans[10] have been reported. In such reports, however, what pharmacologic action AX exerts on the eyes, particularly concerning improvement in amplitude of accommodation, has not been clarified. In our research, we studied the effect AX has on blood rheology dynamics in the human retina.

I Materials and Methods

1. Medical institution implementing the study

The study was implemented under the control of physicians at the Department of Ophthalmology at Toyama Medical and Pharmaceutical University. Conduct of the study was implemented in accordance with the Declaration of Helsinki, after having obtained the approval of the clinical trial review board and having explained the details of the study sufficiently to each subject and obtained each subject's written consent.

2. Study materials

The AX derived from *Haematococcus* algae used for the study (*AstaReal Oil 50F* containing approximately 5.5% AX (as free form), Fuji Chemical Industry Co., Ltd.) was AX enriched material extracted from *Haematococcus pluvialis* algae. This substance was placed in soft capsules, each containing AX 3mg, and used as the sample for the AX group. Soft capsules containing medium chain triglyceride substituted for *AstaReal Oil 50F*, which were indistinguishable by external appearance, were used as capsules for the placebo group.

3. Subjects

Individuals taking medication, and individuals with a serious ophthalmic or systemic illness including diabetes, were excluded from the study. The study was conducted as a double-blind controlled trial. Thirty-six subjects were divided randomly into two groups. There were no differences between the two groups in terms of gender, age and physical characteristics (**Table 1**).

4. Study design

The subjects orally ingested two placebo or AX capsules (0 or 6mg) daily, 30 minutes before dinner, for four weeks. On the day before peroral administration began (8-11 AM that day) and on the day after final peroral ingestion over the four-week period (8-11 AM), examination interviews, ophthalmologic examinations including intraocular pressure measurement and retinal perfusion measurement, physical examinations including height, weight and blood pressure, and blood chemistry and blood biochemistry examinations using blood samples, were

conducted by physicians. Blood samples were taken before peroral ingestion began, after two weeks and after four weeks, and the samples supplied for measurement of blood AX levels. In addition, during the study period, the subjects kept an ingestion diary in which they answered a questionnaire concerning subjective symptoms before they began ingestion and after two weeks and four weeks.

Table 1 Subject profile

	Male (number)	Female (number)	Age (Years)	Weight (kg)
Placebo group	15	3	40.8±10.4	67.4±10.5
AX group	14	4	40.6±11.0	64.2±9.1

Values for age and weight are mean ± standard deviation

The details of the study are shown below.

1) Retinal perfusion measurement

A Scanning Laser Doppler Retina Flowmeter (Heidelberg Retina Flowmeter; Heidelberg Engineering, Inc., Germany) was used. Measurements were taken in a comparatively dark room by the same physician, before and after ingestion. The measurements were made without pupil dilation. Perfusion was measured above the retina in the macular region within one disk diameter from the optic papilla, in the area without cortical layer blood vessels. The right and left eye were each measured three times. Using the mean value obtained from the measurements of each image as a measurement value, Flow (retinal capillary perfusion), one of the Heidelberg Retina Flowmeter indicators, was calculated. In addition, detailed observation of the optic papilla was performed to check for the presence of glaucomatous change.

2) Ophthalmotonometry of both eyes

After ocular perfusion had been measured, intraocular pressure was measured using a tonometer (non-contact tonometer, Kowa).

3) Physical examination

Height, weight and blood pressure (mercury sphygmomanometer) were measured.

4) Blood tests

The following items were measured using vein blood samples collected after fasting.

Total protein, albumin, A/G ratio, GOT, GPT, ALP, LDH, γ -GTP, CPK, total bilirubin, total cholesterol, HDL cholesterol, TG, arterial stiffness index, BUN, CRE, uric acid, Na, K, Cl, Mg, glucose, leukocyte count, erythrocyte count, blood platelet count, hemoglobin content, hemoglobin content %, hematocrit value, MCV, MCH, MCHC

5) Measurement of blood AX concentration

Venous blood was collected using heparin-treated plasma separation tubes. The blood plasma samples obtained by centrifuging at 4°C and 3,000rpm for 10 minutes were preserved by freezing at -20°C and supplied for measurement after allocation tables for maintenance of the double blind study were affixed. Blood plasma AX concentrations were measured by the LC/MS

Method. An HP1100 LC/MS system (Hewlett Packard/Yokogawa Analytical Systems) was used to make the measurements. Although all-trans, 9-cis and 13-cis were detected as geometric isomers, in order to look at the change in concentration after ingestion only all-trans, which accounted for the majority of isomers, was used as an indicator. The limit of quantitation was established as 2.0ng/ml.

6) Subjective symptom questionnaire

The subjects entered scores to indicate changes in the following items after two weeks and four weeks compared with prior to ingestion.

■Items

a) My eyes tire easily, b) My eyes hurt, c) My eyesight is dim, d) My eyes tear easily, e) My eyes redden easily, f) I see objects that flit about, g) I see things double, h) My shoulders and waist get stiff, i) I become irritated easily, j) My head becomes heavy easily, k) I get headaches easily, l) My eyesight has become weaker, m) My field of vision has narrowed, n) My eyelids feel heavy, o) The insides of my eyes are painful, p) My eyes get red easily, q) My eyes get bleary, r) My eyes become dry easily, s) My eyes can't focus properly, t) I have mucus discharge from my eyes, u) My eyes feel hot, v) Light is too bright, w) My eyes itch, x) My eyelids twitch, y) It hurts to open my eyes, z) It feels like I have something in my eyes.

■Scoring

Compared to prior to ingestion: Has become much better: :3; Has become somewhat better: 2; Has become a little better: 1; No change: 0; Has become a little worse: -1; Has become somewhat worse: -2; Has become much worse: -3

5. Statistical analysis

For retinal perfusion, a paired t-test was used for comparison within a group, and the Wilcoxon signed-rank test was used for comparisons between groups, before and after ingestion. The Mann-Whitney test was used for the subjective symptom questionnaire. Results with a p value of less than 0.05 were assumed to have a statistically "significant difference," while results with a p value of less than 0.1 were assumed to show a "tendency."

II Results

1. Retinal perfusion measurement

The retinal capillary perfusion (Flow) results are shown in **Table 2**. No significant difference was detected in Flow between the AX group and placebo group before ingestion. Flow (mean ± standard deviation) for the AX group before ingestion was 221±32 (right eye) and 215±28 (left eye). After ingestion, Flow was 241±35 (right eye) and 238±34 (left eye), and compared with before ingestion, a significant ($p<0.01$) increase was seen after ingestion for both eyes. The percentage increase in Flow before and after ingestion for the AX group was 9.0% for the right eye and 10.7% for the left eye. For the placebo group, the figures were 2.6% for the right eye and 2.8% for the left eye. Compared with the placebo group, for the AX group a significant ($p<0.01$) percentage increase was noted for both eyes. For the placebo group, there was no significant difference before and after ingestion.

Table 2 Retinal capillary perfusion (Flow)

		AX group	Placebo group
Before ingestion	Right eye	221±32	230±42
	Left eye	215±28	218±38
After ingestion	Right eye	241±35[a] (9.0%[b])	236±33 (2.6%)
	Left eye	238±34[a] (10.7%[b])	224±30 (2.8%)

a: $p<0.01$ (comparison before and after ingestion), b: $p<0.01$ (AX group vs. placebo group)

(%) Percent of increase in value after ingestion

2. Ophthalmotonometry of both eyes

For the AX group, there was almost no change in mean intraocular pressure (mean±standard deviation), which was 13.0±2.9mmHg before ingestion and 12.8±3.1mmHg after ingestion. For the placebo group, mean intraocular pressure was 12.6±2.6mmHg before ingestion and 12.7±3.0mmHg after ingestion, with no change before and after administration.

3. Physical examination

In the physiological examination of body weight, height and blood pressure (diastolic phase, systolic phase), there were no significant physiological differences before and after ingestion for either the AX group or the placebo group.

4. Blood tests

In the results of the blood chemistry and blood biochemistry tests, there were no significant differences before and after ingestion for either the AX group or the placebo group.

5. Measurement of blood AX concentration

The results for blood AX concentration (mean±standard deviation) are shown in **Table 3**. For the AX group, blood AX concentration increased gradually with each passing day, from 0.2±0.78ng/mL before ingestion to 27.9±10.25ng/mL after two weeks and 35.6±12.64ng/mL after four weeks. On the other hand, for the placebo group, blood AX concentration remained at about 2.0ng/mL throughout the study period. For the AX group, compared with the placebo group blood AX concentration rose significantly ($p<0.001$).

Table 3 Blood AX concentration

	Before ingestion	After 2 weeks	After 4 weeks
Placebo group	2.3±3.72	trace (1.2±2.25)	2.0±2.07
AX group (6mg/day)	trace	27.9±10.25[a]	35.6±12.64[a]

	(0. 2±0.78)		

Values: Mean value (ng/mL) ± standard deviation, limit of quantitation (trace): less than 2.0ng/mL calculated as 0ng/mL

a: $p<0.001$ (comparison with before ingestion)

6. Subjective symptom questionnaire

When changes in the subjective symptom scores before and after ingestion between both groups were compared, an improvement from AX ingestion was found for three of the 26 items after two weeks. These results were an improvement tendency in "My eyes tire easily," a significant improvement in "My eyes get red easily" and an improvement tendency in "My eyes can't focus properly."

III Discussion

Retinal capillary perfusion has been reported to decrease when individuals have glaucoma[11)-13)]. Furthermore, retinal perfusion has been reported to increase in diabetes patients without retinopathy[14),15)]. Because of these reported results, cases with a history of glaucoma or diabetes were excluded from this study. Moreover, in the blood chemistry and blood biochemistry tests, blood sugar levels after fasting etc. were all within normal range. Intraocular pressure also was within normal range for all subjects, and there were no cases for which glaucomatous change was seen in the optic papilla.

Measurement of retinal capillary perfusion using the Heidelberg Retina Flowmeter is simple, and reproducibility is high[16)-19)]. Therefore this device was used to perform the measurements of retinal capillary perfusion for this study.

Retinal perfusion has been reported to increase as a result of instillation of therapeutic agents for glaucoma such as timolol and betaxolol[16),17)]. Because glaucoma instillation agents decrease intraocular pressure, however, the relationship between percentage of intraocular pressure decrease and increase in perfusion is not yet understood completely.

In this study, there was no change in intraocular pressure before and after ingestion of AX. That is, AX increased retinal capillary perfusion without lowering intraocular pressure. Currently, the involvement of circulatory failure in the optic papilla area is conjectured to be a factor in glaucoma optic nerve disorders. Thus an increase in the quantity of blood flow near the papilla has been reported to be important for glaucoma therapeutic agents[13),20)]. Based on our study, AX was shown to enhance blood perfusion in the area surrounding the papilla, and the possibility of AX being effective in the treatment of glaucoma was suggested.

Moreover, we reported that earlier research showed the decline in amplitude of accommodation because of VDT work and subjective symptoms of tired eyes were ameliorated by AX ingestion[9)]. The agent at the tip of the eye's accommodation mechanism is contraction and relaxation of the ciliary muscle, and it is the long posterior ciliary artery and short posterior ciliary artery branching from the ophthalmic artery that nourish this muscle. For this study, the retinal capillary perfusion measured is believed to be the blood flow volume at the

division of the central retinal artery branching from the same ophthalmic artery. Because it is a division from the same ophthalmic artery, the probability that perfusion to the ciliary body increases when retinal blood flow increases is thought to be high. Although the precise reason for improvement in the amplitude of accommodation as a result of AX ingestion is unclear, there is a possibility the retinal perfusion improvement action of AX shown in this study is involved in the improvement in amplitude of accommodation. On the subjective symptom questionnaire covering VDT workers in our previous study, we found a marked improvement effect in "tired eyes," and in the results of this latest study there was an improvement tendency in "tired eyes." The reason for this difference is believed to be that while all of the subjects in the prior study were VDT workers who complained of tired eyes, not all of the subjects in this latest study were individuals who complained of tired eyes in particular.

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