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BASIC SCIENCE

# Astaxanthin increases choroidal blood flow velocity

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**Abstract**
*Purpose* Previous studies have reported that astaxanthin (AXT) has antioxidative and anti-inflammatory effects in addition to its ability to shorten blood transit times. As laser speckle flowgraphy (LSFG) can noninvasively visualize the hemodynamics of the choroidal circulation, we used the technique to evaluate whether continuous ingestion of 12 mg of AXT per day could increase quantitative blood flow velocity.
*Methods* In this randomized, double-blind, placebo-controlled study, we examined 20 healthy volunteers who ingested 12 mg AXT or placebo capsules over a 4-week period. LSFG was measured in the right eyes of all subjects at pre-ingestion, and at 2 and 4 weeks after the treatment of AXT. LSFG values were used to calculate the square blur rate (SBR), which is a quantitative index of relative blood flow velocity.
*Results* A significant increase of the macular SBR was seen 4 weeks after AXT ingestion when compared to the pre-ingestion values (Wilcoxon signed-rank test, $P$=0.018). In contrast, no statistical difference in the macular SBR was detected in the placebo group (Friedman test, $P$=0.598). No subjective or objective adverse events were found after the 12-mg AXT ingestion.
*Conclusions* Results suggest that administration of AXT over a 4-week period can elevate the choroidal blood flow velocity without any adverse effects.

**Keywords** Astaxanthin · Laser speckle flowgraphy · Choroidal blood flow velocity

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## Introduction

Astaxanthin (AXT) is one of the most common carotenoids, and is found in the red pigment of crustacean shells (e.g., crabs and shrimp), salmon, salmon roe, and members of Asteroidea (e.g., starfish). The chemical structure of this carotenoid has been shown to be modified, with the long-chain conjugate double bond and the b-ionone ring at both ends replaced by a keto- and hydroxyl-group respectively. Discovery of AXT's strong antioxidant effect has led to a large number of studies focusing on the compound, with these studies demonstrating a wide variety of biological activities that have included antioxidant [1], anti-tumor [2], anti-Helicobacter pylori effects [3], anti-arteriosclerosis [4], anti-inflammatory [5], or even perhaps control diabetic nephropathy (kidney disease) [6, 7].

Additionally, it has been reported that AXT ingestion has the effect of improving the microcirculation [8, 9]. A

microchannel array flow analyzer apparatus has shown, in vivo, that there is improvement of the blood rheology after ingestion of AXT 6 mg/day for 10 days in normal human subjects [8]. Moreover, Hussein G et al. reported a modulatory effect on nitric oxide (NO)-induced vasorelaxation by the NO-donor sodium nitroprusside ($P<0.05$) and hemorheological effect by decreasing the microchannel transit time of whole blood in spontaneously hypertensive rats with 7–9 weeks AXT administration [9].

Laser speckle flowgraphy (LSFG), which uses no contrast agents, has recently been used to investigate ocular blood flow distribution. LSFG is able to target moving red blood cells in the eye by using a diode laser (wavelength 830 nm) to illuminate the ocular fundus. The light that is reflected from the ocular tissue produces a speckled pattern on the plane where the area sensor is focused. The reflected lights from the moving erythrocytes then induce blurring within the speckle pattern. Square blur rate (SBR) is a quantitative index of the relative blood flow velocity that is calculated from variations in the blurring. It has been reported that after induction of branch retinal artery occlusion in monkey eyes, there is little change in the post-induction panoramic map when compared to vascular patterns prior to the occlusion. Therefore, as the blood flow velocities calculated by LSFG are believed to be choroidal in origin, they may be useful in evaluating the relative choroidal hemodynamics. We have previously reported that LSFG showed an increase in the foveolar choroidal blood velocity during the treatment of Vogt–Koyanagi–Harada (VKH) disease [10].

Recently, it has been elucidated that disturbance of choroidal circulation is involved in the pathogenesis of ocular diseases such as age-related macular degeneration (AMD) [11] and VKH [10]. Taken together, these reports suggest that treatment regimens designed to elevate the choroidal blood flow velocity should be of benefit in patients with these diseases. However, no investigations of AXT's effect on the choroidal circulation have ever been reported.

In the present study, we investigated the impact of oral administration of AXT on the effect to the choroidal blood circulation.

## Materials and methods

### Subjects

A total of 20 healthy volunteers (15 women, five men) were enrolled in the study.

This clinical trial was performed from January 2008 to February 2008 at Hokkaido University Hospital. Subjects were excluded from the study if any systemic or ocular abnormalities, such as diabetes or drug allergies, were found. In the case of female subjects, we also excluded pregnant subjects. Some of the volunteers did have mild or moderate refractive errors. This study followed the rules and guidelines of the Declaration of Helsinki, with all subjects signing an informed consent form after the nature and possible consequences of the study had been explained.

### Study design

To collect the data, we used a randomized, double-blind, placebo-controlled design without crossover. Subjects were divided into two groups. The AXT group contained two men and eight women, and the placebo group three men and seven women. Over a duration of 4 weeks, the intervention group received two capsules of 6 mg AXT (total 12 mg) capsules once daily at 7 A.M., while the control group received identical-looking placebo capsules.

### AXT

In this study, a soft gel capsule containing Haematococcus pluvialis natural AX of 6 mg was used (AX 5% by weight, Astarile® oil 50 F,Fuji Chemical Industry). In the previous report, the half-life of 9 mg ingestion was determined as 26.0±4.8 h [12]. For control groups, an identical-looking similar soft gel capsule containing no AXT was used.

### Medical examinations

Prior to administration of the capsules, all subjects passed a screening examination, serologic test, and physical or medical examination. On the first day, 2 and 4 weeks after starting capsule ingestion, study physicians also examined each of the subjects for the presence of adverse events during subjects' visits. The information about adverse events was recorded daily, and collected at the time of subjects' visits. In addition, the serologic tests were performed at pre-ingestion and at 4 weeks after starting capsule ingestion.

### LSFG

The LSFG were performed at pre-ingestion and then at 2 and 4 weeks after initiation of the ingestion. The measurements were performed in the morning about 4 hours after the capsule ingestion each time. The test was performed as reported elsewhere [13, 14]. Tropicamide 0.5% and phenylephrine hydrochloride 0.5% were used for mydoriatics. It has been shown that LSFG has little effect on the blood flow decrease at 30 minutes after the instillation of them [15].

Using a previously reported method, we were able to track the movement of the subject's eye in all directions during the measurement period [16]. To obtain these measurements, the patients had to maintain a good fixation for three cardiac cycles, which was approximately 7 seconds. The average of

five results was used as the SBR value. To evaluate changes in the relative blood flow velocity, a square was set at the macula (Fig. 1). An area of 600×280 pixels was created, so that the viewing angle was about 20 deg and included the macula. The average SBR was calculated by using the macular squares in the right eye.

To calculate the coefficient of the reproducibility of measurements, in our preliminary study, SBR was measured in 34 eyes of 34 healthy volunteers twice a day, with 1-minute intervals between each of the measurements. The equation used for the coefficient of the reproducibility of measurements was as follows:

$$|\mathrm{SBR1} - \mathrm{SBR2}|/(1/2)(\mathrm{SBR1} + \mathrm{SBR2})(\%).$$

The coefficient of reproducibility for the SBR measurements for the 1-minute intervals was 2.1±0.4% (mean ± SEM, $n$=34).

### Hemodynamics, IOP and OPP

Previous studies have demonstrated that there is a linear relationship between the choroidal blood flow and the ocular perfusion pressure (OPP) within a certain range [17]. After the LSFG measurements, the patient's blood pressure, pulse rate (PR) and intraocular pressure (IOP) were measured. Mean arterial pressure (MAP) was calculated from the systolic blood pressure (SBP) and the diastolic blood pressure (DBP), which was based on the following equation:

$$\mathrm{MAP} = \mathrm{DBP} + 1/3(\mathrm{SBP} - \mathrm{DBP}).$$

OPP was calculated using the following equation:

$$\mathrm{OPP} = 2/3\mathrm{MAP} - \mathrm{IOP}.$$

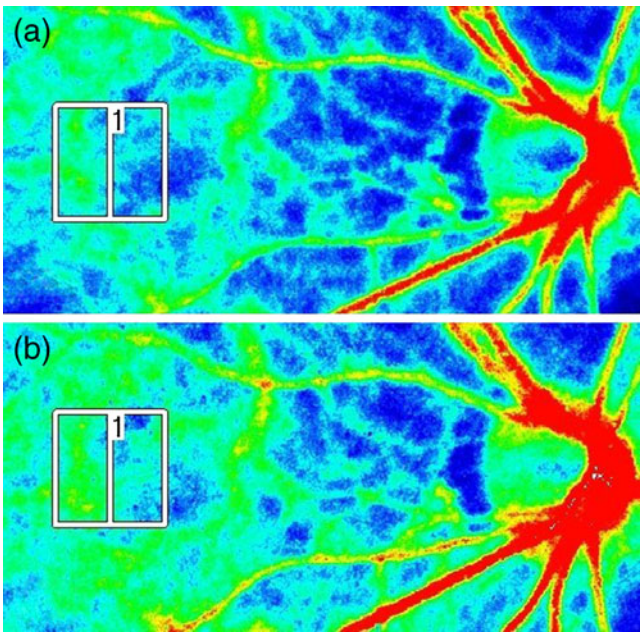


**Fig. 1** Composite color map using the square blur rate (SBR) as measured by laser speckle flowgraphy (LSFG) at (**a**) the pretreatment and (**b**) 4 weeks after systemic 12 mg AXT treatment in the right eye. The *red color* indicates high SBR and the *blue color* indicates low SBR. The SBR increased at the macula during the 4-week treatment (square 1)

### Statistics

Comparisons between the changing rates for SBR and those seen at pretreatment were performed using a previously described statistical method [18]. Nonparametric tests such as the Friedman test or the Wilcoxon signed-rank test were used to analyze SBR values. Wilcoxon tests were performed to determine differences between periods for 3 times (pre-treatment vs 2 weeks, pre-treatment vs 4 weeks, 2 weeks vs 4 weeks) when time was a significant factor in the Friedman test. Wilcoxon tests were adjusted for multiplicity with the Bonferroni method.

The IOP, MAP, OPP and PR before, and 2 and 4 weeks after the treatments in the right eyes were compared using a repeated analysis of variance (ANOVA) test and a paired $t$-test. Since all of their values were found to have approximately normal distributions, we used the ANOVA and paired $t$-tests to analyze all of our data [19]. Differences were considered statistically significant when $P$<0.05.

## Results

### Systemic data

Table 1 presents the subjects' backgrounds. At the start of the ingestion period, the mean age of the subjects was 38.2±11.7 and 38.8±6.8 (mean ± SD) years in the AXT and placebo

**Table 1** Subjects' backgrounds. The mean age of the subjects at the start of the ingestion was 38.2±11.7 and 38.8±6.8 years in the AXT and placebo ingestion groups respectively. Body weight, body fat percentage and pulse rate were all within normal ranges, and did not differ significantly between the two groups ($t$-test, $P$>0.1, for all). All of the subjects in the AXT and the placebo ingestion groups had nearly identical backgrounds

| | Placebo group | AXT group |
|---|---|---|
| Number of subjects | 10 | 10 |
| Age (years) | 38.8±6.8 | 38.2±11.7 |
| Weight (kg) | 53.4±6.6 | 55.3±7.2 |
| Body fat percentage (%) | 25.5±3.6 | 24.3±5.4 |
| Pulse rate (/min) | 84.7±9.5 | 81.5±24.6 |

Mean value ± standard deviation

groups respectively. Body weight, body fat percentage, pulse rate, and all other measures were within normal ranges. There were no significant differences noted for the physical parameters between these groups ($P>0.05$, for all). The members of the AXT and placebo ingestion groups had nearly identical backgrounds.

## Serologic test data

Serologic test data is summarized in Table 2. No significant differences were noted for the standard laboratory parameters between these two groups prior to capsule ingestion.

Furthermore, at 4 weeks after starting capsule ingestion, there were no significant differences between the two groups for the following parameters: white blood cells, red blood cells, hemoglobin, hematocrit, platelets, total protein, albumin, albumin–globulin ratio, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, total bilirubin, creatinine, blood urea nitrogen, uric acid, creatine phosphokinase, total cholesterol, high-density lipoprotein cholesterol, triglyceride, sodium, potassium, chlorine, calcium, blood glucose levels, and hemoglobin A1c ($P>0.05$, for all).

**Table 2** Serologic test data. The serologic tests were performed at pre-ingestion and at 4 weeks after starting capsule ingestion in both groups. At 4 weeks after starting capsule ingestion, there were no significant differences between the two groups for parameters listed in this table ($P>0.05$, for all). Moreover there were no significant changes for 4 weeks in either group ($P>0.05$, for all)

| | | Placebo group | | AXT group | |
|---|---|---|---|---|---|
| Item | Unit | Before ingestion | 4-week ingestion | Before ingestion | 4-week ingestion |
| WBC | /μl | 5998±2162.2 | 5931±1778.2 | 5089±803.3 | 5261±1857.4 |
| RBC | $\times10^4$/μml | 446.3±44.2 | 454.6±36 | 456.9±57.1 | 462.5±63.4 |
| Hb | g/dl | 14.1±1.3 | 13.9±1.2 | 14±1.3 | 13.7±1.6 |
| Ht | % | 41.6±3.6 | 43.2±3.3 | 42.4±4.3 | 43.5±4.7 |
| MCV | fl | 93.6±3.3 | 95.1±3.3 | 93.1±3 | 94.4±2.9 |
| Plt | $\times10^4$/μml | 25.3±6 | 24.5±6.6 | 21.8±3.6 | 22.6±3.1 |
| TP | g/dl | 7.3±0.2 | 7.3±0.3 | 7.2±0.3 | 7.2±0.6 |
| Alb | g/dl | 4.5±0.2 | 4.5±0.2 | 4.4±0.2 | 4.3±0.3 |
| A/G | – | 1.6±0.2 | 1.6±0.1 | 1.6±0.1 | 1.5±0.2 |
| GOT | U/L | 18.4±2.7 | 17.8±3 | 18.9±4 | 22±11.2 |
| GPT | U/L | 14.5±3 | 14±2.7 | 14.8±5.6 | 17.8±9.3 |
| LDH | U/L | 170.9±22.4 | 173.1±26.1 | 175.9±37.4 | 181.8±30.9 |
| ALP | IU/l | 161.9±35.3 | 153.8±34.7 | 165.6±46 | 161.9±52 |
| γ-GTP | IU/l | 19±5.1 | 18.2±5.2 | 24.2±19.9 | 22.2±12.2 |
| T-Bil | mg/dl | 0.7±0.1 | 0.8±0.1 | 0.7±0.2 | 0.8±0.3 |
| Cre | mg/dl | 0.6±0 | 0.6±0 | 0.6±0.1 | 0.6±0.1 |
| BUN | mg/dl | 12±2.8 | 12.1±2.3 | 15.4±3.3 | 14.3±4 |
| UA | mg/dl | 4.7±1.1 | 4.5±0.9 | 4.7±1.6 | 4.7±1.6 |
| CPK | IU/l | 85.5±33.3 | 90.6±36.6 | 88.7±27.6 | 97.3±36.8 |
| TC | mg/dl | 194.7±24.1 | 192.3±24.5 | 190.1±32.9 | 181.8±30.7 |
| HDL | mg/dl | 74.7±17.7 | 76.8±16.8 | 72±17.4 | 72.1±13.5 |
| TG | mg/dl | 81.1±48.9 | 63.3±30.3 | 56±21.6 | 47.5±17 |
| Na | mEq/L | 141.2±1.5 | 141.5±1.1 | 140.8±1.5 | 141.1±2.2 |
| K | mEq/L | 4.2±0.5 | 4.2±0.7 | 4.5±0.6 | 4.4±1.1 |
| Cl | mEq/L | 102.8±1.9 | 103.4±0.6 | 103.2±2 | 103.3±2.6 |
| Ca | mEq/L | 9±0.3 | 9.2±0.3 | 8.9±0.3 | 9.1±0.4 |
| FBS | mg/dl | 83.8±6.4 | 87.3±4.9 | 88±6.6 | 85.8±4.6 |
| A1c | % | 4.5±0.4 | 4.5±0.3 | 4.7±0.2 | 4.8±0.2 |

WBC = white blood cells; RBC = red blood cells; Hb = hemoglobin; Ht = hematocrit; MCV = mean cell hemoglobin concentration; Pt = platelets; TP = total protein; Alb = albumin; A/G = albumin–globulin ratio; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase; γ-GTP = gamma–glutamyl transpeptidase; T-Bil = total bilirubin; creatinine; Cre = creatine phosphokinase; BUN = blood urea nitrogen; UA = uric acid; CPK = creatine phosphokinase; TC = total cholesterol; HDL = high-density lipoprotein cholesterol; TG = triglyceride; Na = sodium; K = potassium; Cl = chlorine; Ca = calcium; FBS = fasting blood sugar levels; A1c = hemoglobin A1c ; mean ± standard deviation

## Refractive errors and BCVA

Pre-treatment average spherical equivalent of the refractive errors in the right eye were −1.15 (ranging from 2.63 to −4.25) diopters in the AXT group and −1.28 (from 2.75 to −4.0) diopters in the placebo group. There was no significant difference in the refractive errors noted between the groups ($P$=0.89, $t$-test).

The mean log MAR best-corrected visual acuity (BCVA) values for the right eyes that were obtained prior to and at 4 weeks after beginning of capsule ingestion were 0.106 and 0.144 in the placebo group and 0.018 and 0.104 in the AXT group respectively. There were no significant differences in either group (placebo group $P$=0.863, AXT group $P$=0.571, paired $t$-test).

## Hemodynamics, OPP and IOP

As seen in Table 3, no significant differences were noted for the MAP ($t$-test, $P$=0.99), IOP ($t$-test, $P$=0.16), OPP ($t$-test, $P$=0.52), and PR ($t$-test, $P$=0.71) between the groups prior to capsule ingestion. In the AXT group, there were also no significant differences for the IOP of the right eyes (repeated ANOVA, $P$=0.14), MAP (repeated ANOVA, $P$=0.59), and OPP (repeated ANOVA, $P$=0.28) for 4 weeks. The pulse rate was also measured at 2 and 4 weeks after start of treatment. There were no significant changes both in AXT and placebo groups (repeated ANOVA, $P$=0.45 and $P$=0.09 respectively).

**Table 3** Variations in hemodynamic parameters observed during administration of AXT or the placebo. No significant differences were noted for the MAP ($t$-test, $P$=0.99), IOP ($t$-test, $P$=0.16), or OPP ($t$-test, $P$=0.52) between the groups prior to capsule ingestion. The placebo and AXT groups exhibited no significant differences for the MAP, IOP, and OPP between the pre-ingestion results and the results at 2 or 4 weeks after capsule ingestion (repeated ANOVA, $P$>0.1).The pulse rate was also measured at 2 and 4 weeks after start of treatment. There were no significant changes in either the AXT or the placebo group (repeated ANOVA, $P$=0.45 and $P$=0.09 respectively)

| | Before ingestion | 2-week ingestion | 4-week ingestion |
|---|---|---|---|
| Placebo group | | | |
| MAP (mmHg) | 89±2 | 87±2 | 85±2 |
| IOP (mmHg) | 15±0.8 | 15±0.6 | 14±0.7 |
| OPP (mmHg) | 45±1 | 43±1 | 42±2 |
| PR (/min) | 85±3 | 81±3 | 77±3 |
| AXT group | | | |
| MAP (mmHg) | 89±2 | 89±2 | 88±3 |
| IOP (mmHg) | 14±0.6 | 14±0.7 | 14±0.9 |
| OPP (mmHg) | 46±2 | 46±1 | 44±2 |
| PR (/min) | 82±8 | 74±3 | 74±3 |

MAP = mean arterial pressure; IOP = intraocular pressure; OPP = ocular perfusion pressure; PR = pulse rate; mean ± standard error ($n$=10)

## LSFG data

With regard to the choroidal blood flow velocity, a summary of the measurement results for both groups' SBR at the macular area during capsule administration is shown in Fig. 2. Because SBR is a quantitative index of the "relative" blood flow velocity, the SBR at 2 and 4 weeks after AXT ingestion was indicated as ratio to the pre-ingestion values. Therefore, no error bars are shown in the pre-treatment levels of SBR as in the manuscripts previously reported [19, 20].

Significant changes in the SBR values at the macular area were noted after AXT ingestion (Friedman test, $P$=0.016). In addition, there were significant increases in the SBR at the macula at 4 weeks after AXT ingestion when compared to the

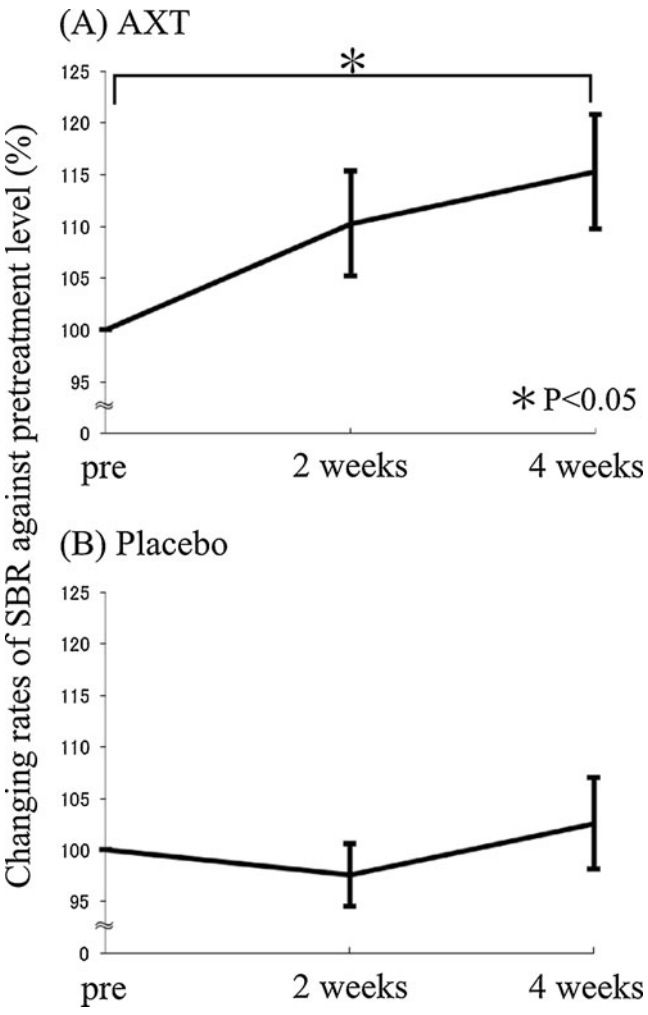


**Fig. 2** Changing rates of macular flow compared to the pretreatment level ($n$=10; mean ± standard error) after the ingestion of astaxanthin (**a**) and placebo (**b**). Significant changes in the SBR values at the macular area were noted after AXT ingestion (Friedman test, $P$=0.016). The SBR at the macula significantly increased at 4 weeks after AXT ingestion as compared to pre-ingestion values (Wilcoxon signed-rank test, $P$=0.018 ). Conversely, there were no significant changes in the SBR in the placebo group (Friedman test, $P$=0.598)

pre-ingestion values (Wilcoxon signed-rank test, $P$=0.018 ). When the changing rates of the macular flow were compared to the pretreatment levels, a 10.3% increase was noted at 2 weeks, while a 15.3% increase was noted at 4 weeks after the AXT administration.

In contrast, no statistical differences in the macular SBR were detected among pre-, 2-week and 4-week ingestion values in the placebo group (Friedman test, $P$=0.598).

Adverse effects

There were no subjects in either group that exhibited adverse effects at any time during the study.

## Discussion

In the present study, we used LSFG to evaluate macular area blood flow velocity prior to and after ingestion of AXT. It has been reported that the choroidal blood flow exceeds the retinal flow by approximately 96% in areas outside of the fovea [21]. In addition, there are no large retinal vessels found near the fovea [22]. Therefore, the SBR calculations we performed at the square of the macula can be considered to reflect the choroidal blood flow velocity.

The significant increase in the SBR was noted in volunteers who continuously ingested 12 mg of AXT per day over a 4-week period. In contrast, no statistical differences in the macular SBR were detected among pre-, 2-week and 4-week in the placebo group. Upon continuous ingestion of 12 mg of AXT, there were no abnormal findings or adverse events noted either subjectively or objectively, which included changes in the arterial blood pressure and heart rate response (Table 3).These results suggest that a 4-week AXT administration can elevate the choroidal blood flow velocity without adverse effects.

Our results showed that AXT increases the choroidal blood flow velocity. Since no significant differences were noted for the OPP prior to, and at 2 and 4 weeks after ingestion, the choroidal blood flow velocity increase was not due to systemic hemodynamic changes, but rather was due to either vasodilation in the choroid or changes in blood rheology. In this study, we were not discriminative about which mechanism was attributed to the increase of SBR. The vasodilation due to a modulatory effect on NO-induced vasorelaxation might also be responsible for even normal human chroidal vessels. On the other hand, it has been previously reported that AXT ingestion shortens the blood transit time [8, 9], suggesting that AXT improves the blood rheology. A similar hemorheological mechanism might also be responsible for the elevated choroidal blood flow velocities.

In the previous reports, the mechanism responsible for the improvement in blood rheology by AXT was not determined. However, it has been reported that AXT prevents over-oxidation of lipids via its strong antioxidant effect, and controls oxidation of low density lipoprotein [23]. AXT is believed to be able to effectively work against oxidation in lipid metabolism, and also has been shown to be widely distributed in the lipoprotein areas of the body [24, 25]. The actions of AXT are thought to work by preventing oxidation of the biomembranes, which includes the cell membranes. Hence, it was hypothesized that there was an antioxidant effect for both within and outside of the lipid double membrane of erythrocytes, which renders the form flexible, thereby making it possible to longitudinally obtain such suitable membranes [8].

This study is intended to be an exploratory proof of concept study, requiring confirmatory studies in the future. While this study has shown that AXT significantly increases the choroidal blood flow in the absence of any side-effects, it is still unknown as to whether this effect is temporary or permanent. With regard to the design of this study, the statistic was conducted with small sample size. However, in this study, when the two-sided significance level was 0.05, the powers of analysis of the Friedman test in the AXT group and the placebo group were 0.91 and 0.78 respectively. These values are almost sufficient, because most researchers assess the power of their tests using 0.80 as a standard for adequacy [26]. Additionally, the change of SBR was examined using a randomized, double-blind, placebo-controlled without cross-over design. The intra-group comparison for the changing rates of SBR by the Wilcoxon test clearly indicated the effect of AXT on the SBR. However, studies that examine these long-term effects on wider cohort are desirable for the future evaluation of the AXT effects on choroidal circulation in humans.

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**Conflict of interest** Hiroki Tsukahara is an employee of Fuji Chemical Industry Co., Ltd. The AXT capsules were provided by the company.

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