Effects of black currants anthocyanins on hemodynamics of aqueous humor and peripheral blood circulation.

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ABSTRACT

Purpose: In our previous studies, we demonstrated the beneficial effects that black currant anthocyanins (BCACs) have on glaucomatous optic neuropathy (GON) through their possible mechanisms toward reactivity to endothelin-1 (ET-1). The purpose of the current study is to examine the influence of BCACs on peripheral blood circulation as well as aqueous humor (AH) circulation.

Methods: In our examination of peripheral blood circulation, responses toward finger cooling stimulation were monitored by a thermography in 8 healthy subjects receiving one time administration of BCACs (130 mg) and 5 separate subjects receiving BCACs daily (50 mg/day) for one week. To examine aqueous humor (AH) circulation, AH outflow resistance or AH production was evaluated by means of a Weight on-off test at baseline and again at week 1, 2 or 3 of BCACs administration.

Results: Compared to pre-administration, recovery of the surface temperature of the middle finger was achieved more rapidly after both the one time BCAC intake and when they were administered over 7 days. Upon administration of BCACs for three weeks, the IOP drop rate and IOP recovery time increased and shortened, respectively.

Conclusions: Our present results indicate that BCACs intake effectively suppresses cold-induced vasospasms and modulates AH circulation by a possible ET-1 mediated mechanism.

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INTRODUCTION

Glaucomatous optic neuropathy (GON) affects approximately 90 million people worldwide, and is the major cause of irreversible blindness[1)]. Clinically, GON is characterized by a glaucomatous excavation of the optic nerve head (ONH) with concomitant visual field defects[2)]. As possible causative factors, elevated intraocular pressure (IOP) and other mechanisms independent to IOP such as ocular blood circulation are known to be involved in the GON etiology[3-7)].

GON may be involved in the insufficiency of ocular blood circulation based upon the following evidence: 1) Disc hemorrhages frequently exist in patients with open angle glaucoma (OAG) [8)]; 2) Retinal vascular diseases, such as retinal vein occlusion, are frequently associated with OAG[9)]; 3) A decreased hemodynamic of ocular blood flow is found in patients with OAG [10,11)]; 4) Abnormal levels of the concentration of plasma endothelin-1 (ET-1) are observed in patients with OAG as compared with healthy control subjects[12-15)]; 5) Platelet aggregation ability is remarkably increased in patients with OAG compared with that in normal subjects[16)].

In addition to ocular blood circulation, systemic peripheral blood circulation may also be affected by GON. In fact, abnormal recovery from cold stimulation has been noticed in patients with GON[17)]. Thus, taken together, blood circulatory disturbances at the optic nerve head (ONH) are indeed involved in the etiology of OAG and are therefore an additional therapeutic target against OAG.

Anthocyanins (ACs) are types of polyphenols, rich in food and beverages such as red wine, cocoa and berries, and are known to have several beneficial effects on human health[18,19)]. Among these, the ACs in black currants (BC) in particular has been implicated in improvement of visual functions, including dark adaptation, and transient refractive alternation[20)]. A previous *in vitro* study demonstrated that the BCACs stimulated ET-dependent vessel dilatation in the bovine ciliary body [21)]. Since ET has been identified as one of the factors regulating ocular blood circulation[22)], it was speculated that BCACs possibly had some effects in the hemodynamics of ocular blood flow. To test this hypothesis, we administered BCACs (50 mg/day) to OAG patients (n=30) for six months, and their ocular blood circulation and visual fields were monitored. Results indicated that BCACs caused a significant increase in the blood flow at the ONH ($P<0.05$), but none of the subjects showed progression in their visual field defects[23)]. In the subsequent randomized, placebo-controlled, double-masked, 24-month trial, we demonstrated that OAG patients administered with BCACs

showed significantly less deterioration in the visual field test index, mean deviation (MD) and pattern standard deviation (PSD), and increased ocular blood flow in comparison with placebo-treated patients[24]. In this trial, a total of 21 glaucoma patients (BCACs, n=12; placebo, n=9) treated with a single dose of anti-glaucoma medication were selected and analyzed. Inter-group and between-group analyses revealed statistically significant decreases in mean IOP in the glaucoma patients taking BCACs (*P*=0.027, paired t-test; *P*=0.024, unpaired t-test) at 24 months after the baseline[25]. Furthermore, an additional placebo-controlled, double-masked, crossover study of healthy subjects (n=12) demonstrated that a statistically significant decrease in mean IOP was observed at 2 weeks (*P*=0.002, paired t-test) and 4 weeks (*P*=0.039, paired t-test) from the baseline of BCAC administration[25]. Taken together, our results suggested that oral administration of BCACs may induce favorable effects against visual field deterioration in patients with GON as well as decrease IOP levels in both patients with GON and healthy subjects.

Although the underlying mechanism of these beneficial effects of BCACs toward ocular blood circulation and IOPs has yet to be elucidated, following three evidences have lead us to speculate that the effects may be due to the ET-1 mediated mechanism. First, in contrast to the control subjects, GON patients have an abnormal hyperactivity of ET-1 in response to vasospastic stimuli such as cold[17]. Next, an imbalance between vasoconstrictor substances such as ET-1 and vasodilators such as nitric oxide are the cause of vasospasm in glaucoma[26]. Finally, GON patients who have vasospasm have a higher susceptibility to glaucomatous damage, which could be a consequence of a decreased dilation of blood vessels that properly autoregulated blood flow[13].

To test our hypothesis about the possible influence of the ET-1 mediated mechanism, we assayed serum ET-1 concentrations of BCAC-treated and placebo-treated OAG patients. Results showed that (1) serum ET-1 concentration was significantly lower in OAG patients than in the control subjects[15] and (2) continuous supplementation of BCACs caused normalization of decreased levels of serum ET-1 concentrations in patients with OAG[27]. Taken together with the fact that BCACs react with ET-1 receptors in the ciliary body as described above, we speculated that the response of normalized ET-1 and its receptors to BCACs may cause the suppression of ET-1 dependent vasospasms. This in turn leads to an increase of ocular blood flow as well as a decrease of IOP by modulating the aqueous humor (AH) dynamics.

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In the present study, to study the effect of BCACs to prevent vasospasms, we conducted finger cooling tests before and after administration of BCACs. In addition, the effect of BCACs on AH dynamics was also investigated using a recently developed Weight on-off test to elucidate the possible mechanisms of the BCAC dependent IOP decrease.

1. SUBJECTS AND METHODS

This study was approval by the local Ethics Committee and according to the tenets of the Declaration of Helsinki and national laws for the protection of personal data. Informed consent was obtained from all subjects in the study.

1.1 Subjects

Two groups, consisting of 8 healthy volunteers (34.88±4.85 years) and 5 healthy volunteers (27.2±3.49 years) were included for peripheral blood circulation analysis. Nine healthy volunteers (28.67±4.09 years) were included for analysis of AH dynamics by the Weight on-off test. None of the subjects showed ocular pathologic conditions other than minor refractive errors within a spherical equivalent of less than 5 diopters, had any history of drug or food allergies, or used any medications or supplements.

1.2 Effects of the BCACs on peripheral blood circulation

Effects of the BCACs on peripheral blood circulation were evaluated in healthy volunteers administrated one time with 130 mg of the BCACs or 50 mg of the BCACs daily for a week using a finger cooling test described previously[28]. Amounts of the BCACs used in the present study were followed by a previous *in vivo* study using human healthy volunteers or our clinical trials as described above[20,23-25].

1.2.1 Effects of single administration of the BCACs on peripheral blood circulation.

The 8 healthy subjects receiving 130 mg of the BCACs assumed a sitting position 30 min before the test in a room with the temperature set at 25°C. Before the finger cooling, the skin temperature of the subjects' fingers was measured by thermography composed of the infrared camera InfReC H2640 and InfReC Analyzer NS9500 Standard (Nippon Avionics, Tokyo, Japan). Subjects' fingers were then kept at 4°C in ice-cold water for 20 sec and subsequently dried with a paper towel. Immediately after the finger cooling and every 1 min for the following 15 min, finger skin temperatures were again measured as above. This set of measurements was repeated one hour after the administration of the 130 mg of BCACs. Temperatures

were measured at 3 points, the tip and second and third joints of the subjects' middle fingers.

1.2.2 Effects of one week-administration of the BCACs on peripheral blood circulation.

To evaluate the long-term effects of the BCACs on peripheral blood circulation, BCACs (50 mg daily) were administrated to 5 healthy subjects for 1 week. The finger cooling test as described above was performed before and after the administration of BCACs.

For this test, recovery rate of skin temperature after finger cooling (%,R_x) was calculated as follows:

$$R_x\ (\%)=(T_x-T_0)/(T_B-T_0) \times 100$$

T_x: Temperature at x min after cooling; T_0: Temperature at 20 sec after cooling

T_B: Temperature at baseline

1.3 Effects of the BCACs on AH dynamics by the Weight on-off test

Effects of the BCACs on AH dynamics were estimated by the Weight on-off test as described previously[29]. The Weight on-off test was performed at baseline and once a week during the three-week administration of BCACs (50 mg daily). Experimental protocol and basic principles of the Weight on-off test were followed by the method described previously[29]. Briefly, 9 healthy subjects assumed a supine position 10 min before the test and maintained this position throughout its duration. A salt wrapped weight (50 g) was put on one eye for 5 min. During the 5 min, subjects gazed at a fixed target in front of their other eye to keep the primary eye position. IOP was measured with a rebound tonometer (ICare®, Finland) prior to placement of the weight and immediately after its removal, and thereafter once a minute until IOP returned to the pre-weight placement levels. At the time of measurements, subjects turned their necks to a lateral position so that IOP could be suitably measured by the rebound tonometer. All measurements were conducted by trained ophthalmologists. Values were the mean results of three to six consecutive measurements until the inter-measurement variability was less than 5%. The AH resistance factor (IOP drop rate (IOP_{drop}) (%)) and AH production factor (IOP recovery time (IOP_{rec}) (min)) were defined as follows:

(1) IOP drop rate (IOP_{drop}) (%)

$$IOP_{drop}\ (\%) = (P_0-P_e) - (P_5-P_e) / (P_0-P_e) \times 100$$

P_0: IOP at baseline; P_e: assumed episcleral venous pressure of 8 mm Hg; P_5: IOP at 5 min after weight on an eye.

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(2) IOP recovery time (IOP_{rec}) (min)

IOP_{rec} was defined as the amount of time (min) necessary for IOP to reach the base line level after removal of the weight.

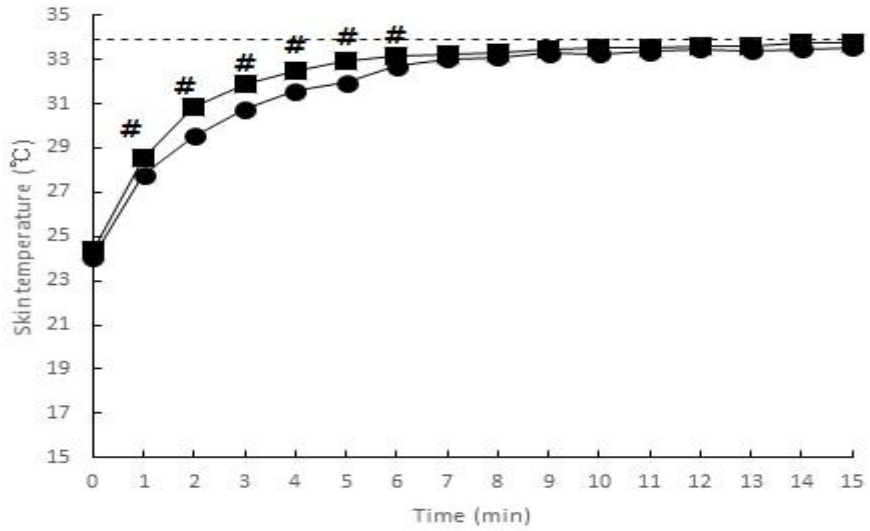
1.4 Other analytical methods

All statistical analyses were performed with MS-Excel. The significance level was set at $P<0.05$ for all statistical analysis.

2. RESULTS

In the present study, to investigate the effect of BCACs have toward preventing vasospasms, we conducted finger cooling stimulation tests before and after oral intake of one-time administrations of BCACs (130 mg) or daily administrations for 7 days (50 mg per day). As shown in Fig. 1, compared to pre-administration of BCACs, the surface temperature of middle fingers in healthy volunteers (n=8) more rapidly rewarmed to the base levels (dotted line) after the one-time BCAC intake.

Fig 1



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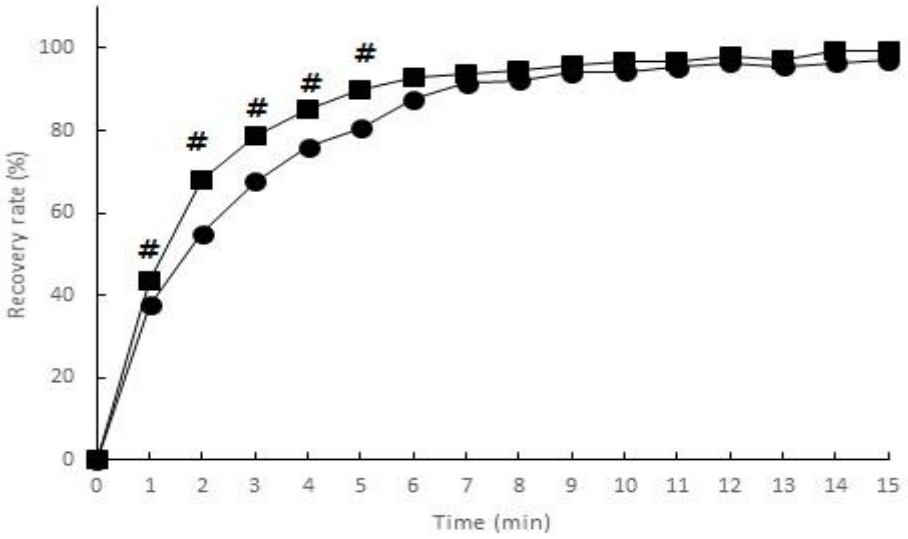


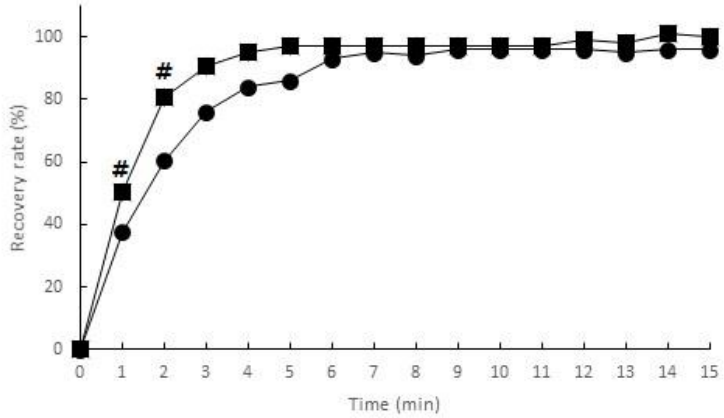
Figure 1. Effects of single administration of BCACs on finger cooling test.

Mean finger temperatures of 8 healthy subjects were measured by thermography immediately after cooling and then again every minute for 15 minutes this set of measurements was performed before (filled circles) and one hour after (filled squares) single administration of BCACs (130 mg) (upper panel). Mean skin temperature at the baseline point is indicated as a dotted line. Rates of recovery to basal skin temperature at each time point are shown in the lower panel. Significant difference ($^{\#}$: $P<0.05$, unpaired t-test).

This effect by BCACs was more evident in the tip of the finger (Fig. 2).

Fig 2

Distal interphalangeal joint



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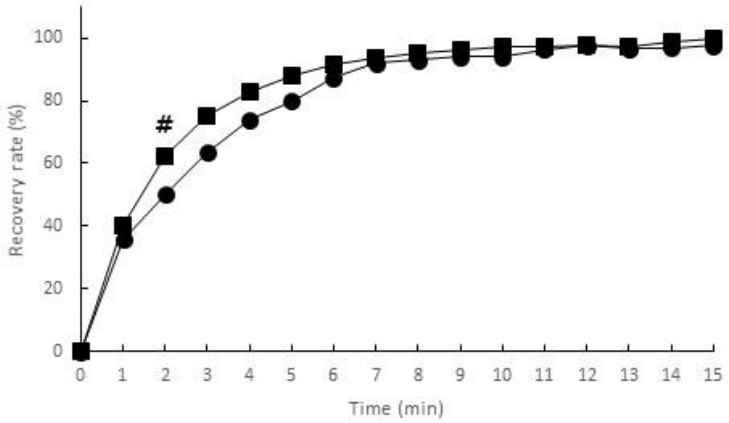
Proximal interphalangeal joint



Metacarpophalangeal joint

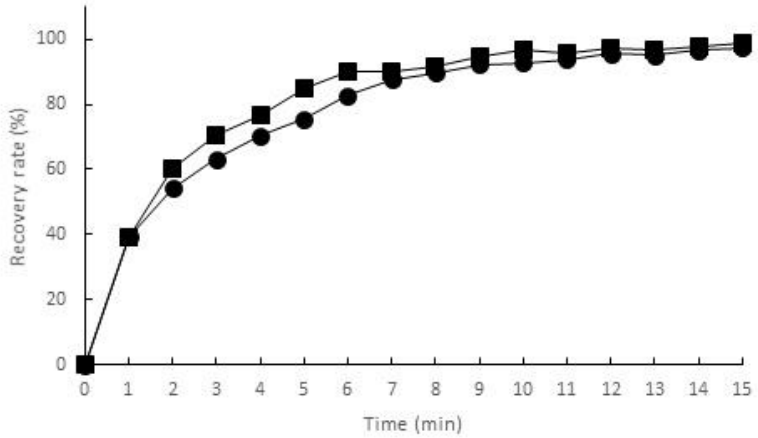


Figure 2. Time course changes of mean recovery rates of skin temperatures at distal interphalangeal, proximal interphalangeal or metacarpophalangeal joints of the middle finger in finger cooling test before and after single administration of BCACs.

Skin temperatures at distal interphalangeal, proximal interphalangeal or metacarpophalangeal joints of the middle finger observed during the finger cooling tests conducted before (filled circles) and after (filled squares) a single administration of BCACs were measured by thermography as described in the legend of Fig. 1. Rates of recovery to basal skin temperature of each joint at each time point were plotted. Significant difference ($^{\#}$: $P<0.05$, unpaired t-test).

To a lesser degree, administration of BCACs over 7 days also increased the speed in which middle fingers rewarmed to base levels (Figs. 3 and 4). These

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results indicated that BCAC intake effectively suppresses cold-induced vasospasms through a possible ET-1 mediated mechanism as described above.

Fig 3

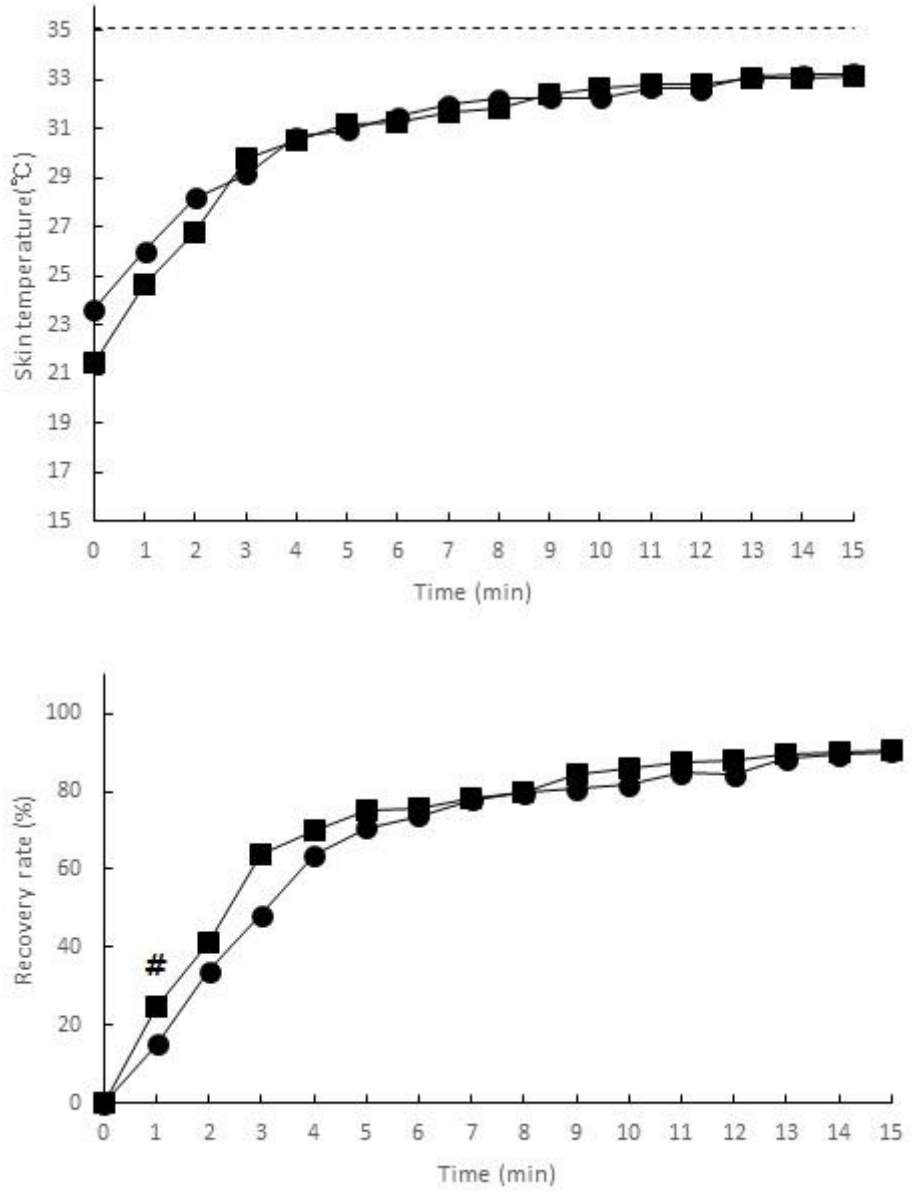

Figure 3. Effects of daily administration of BCACs for one week on finger cooling test.

Mean finger temperatures were measured by thermography at baseline and after the cooling finger test in 8 healthy subjects as described in the legend of Fig. 1. This set of measurements was performed before (filled circles) and after (filled squares) administration of BCACs for a week (upper panel). Mean skin temperature at the baseline point is indicated as a dotted line. Rates of recovery to basal skin temperature at each time point are shown in the lower panel. Significant difference ($^{\#}$: $P<0.05$, unpaired t-test).

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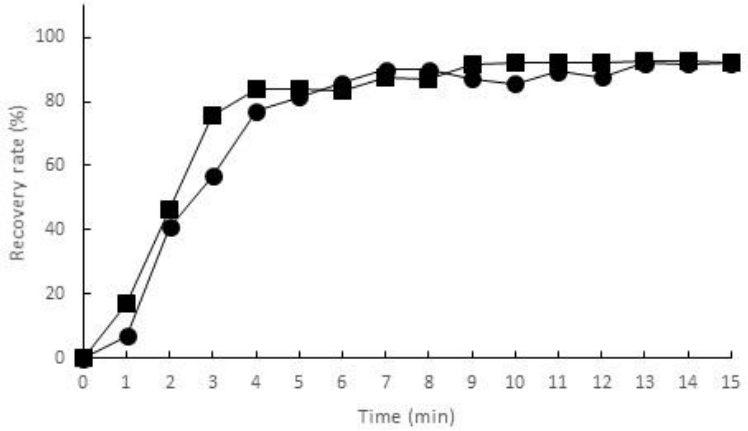
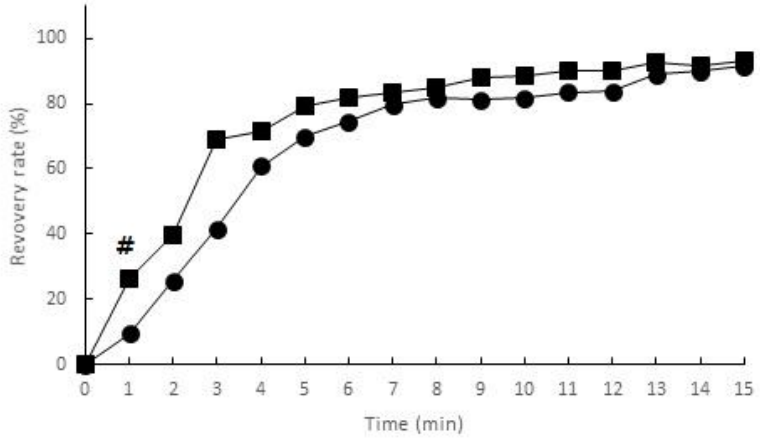
Fig 4

Distal interphalangeal joint



Proximal interphalangeal joint



Metacarpophalangeal joint

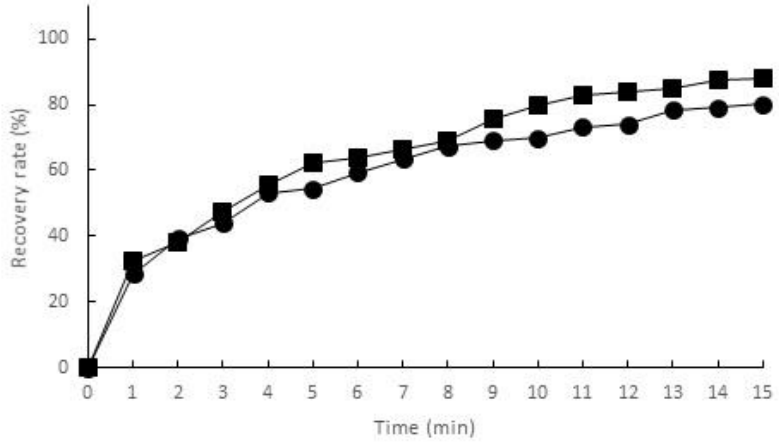


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Figure 4. Time course changes of mean recovery rates of skin temperatures at distal interphalangeal, proximal interphalangeal or metacarpophalangeal joints of the middle finger in finger cooling test before and after administration of BCACs for a week.

Skin temperatures at distal interphalangeal, proximal interphalangeal or metacarpophalangeal joints of the middle finger in the finger cooling test before (filled circles) and after (filled squares) administration of BCACs for a week were measured by thermography as described in the legend of Fig. 1. Rates of recovery to basal skin temperature of each joint at each time point were plotted. Significant difference ($^{\#}$: $P<0.05$, unpaired t-test).

To further investigate the effects of BCACs toward ET-1 dependent blood circulation in the ciliary body, AH dynamics was evaluated using a recently developed Weight on-off test in healthy subjects (n=9). This methodology enabled us to estimate AH outflow resistance by decreased levels of IOPs (IOP drop rate) after a weight on for 5 min, as well as AH production by the period of recovery of the decreased levels of IOPs to the basal (IOP recovery time) after the weight was removed. As shown in Fig. 5, both the IOP drop rate and IOP recovery time were increased and shortened during the three weeks of the BCAC supplementation (50 mg per day). This suggested BCACs supplementation may lead to a decrease in AH outflow resistance and an increase in AH production.

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Fig 5

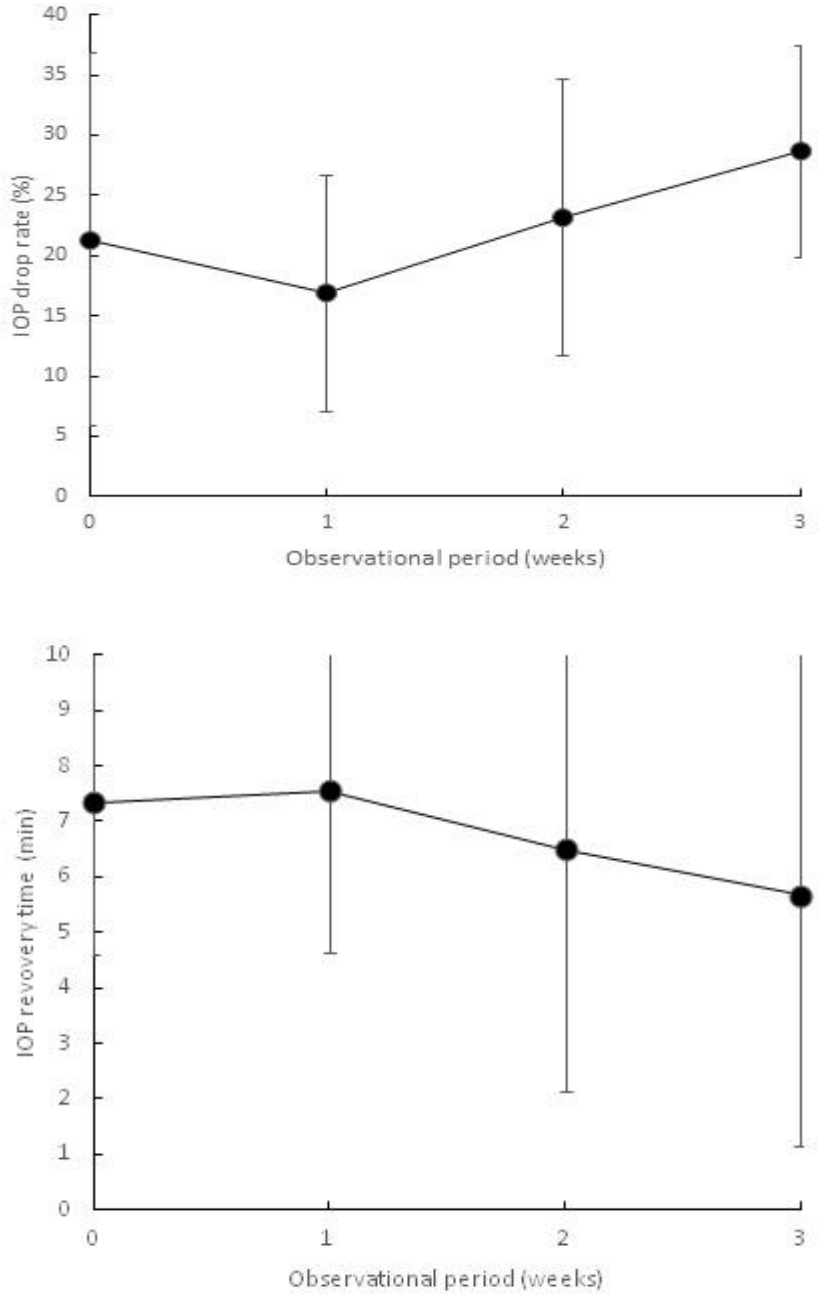

Figure 5. Effects of BCACs on aqueous humor dynamics.

Effects of the BCACs on aqueous humor dynamics were estimated by the "Weight on-off test" as described in the "Subjects and Methods" section. BCACs (50 mg) were administrated daily to 9 healthy subjects for three weeks. At baseline and at 1, 2 and 3 weeks after administration, the Weight on-off test was conducted. IOP drop rate (IOP_{drop}) (%) (upper panel) and IOP recovery time (IOP_{rec}) (min) (lower panel) at each time point were plotted. Data is expressed as mean±SD.

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DISCUSSION

A previous study described how four forms of BCACs, delphinidin-3-rutinoside (D3R), delphinidin-3-glucoside (D3G), cyaniding-3-rutinoside (C3R) and cyaniding-3-glucoside (C3G), could be isolated and characterized[30]. Another study showed that after systemic administration these BCACs were directly absorbed into plasma without degradation and transferred beyond both the blood-aqueous barrier and blood-retina barrier into ocular tissues[31]. Additional in vitro studies described how BCAC administration resulted in several benefits toward ocular biological activities including stimulation of rhodopsin regeneration in frog retinas[32], suppression of ocular globe elongation in chick myopia models[33] and ET-dependent vasodilation in the bovine ciliary body[21]. Finally, an in vivo study showed how oral intake of BCACs resulted in significant improvement in the dark adaptation and video display terminal work-induced transient refractive alteration in healthy human volunteers[20].

Several studies have shown that ET-1 may also be implicated in GON[12-15] as well as other ocular ischemic vascular diseases including diabetic retinopathy[34], retinal vein occlusion and retinal artery occlusion[35]. It has been noted that there are statistically significant differences of plasma ET-1 levels in glaucoma patients as compared to those in control subjects[12-15]. Thus, ET metabolism is believed to be involved in the glaucoma etiology and modulation of ET metabolism through its specific receptors is believed to be a possible and promising therapeutic target for the above ocular diseases[35]. The ET-1 receptors belong to the family of G-protein coupled receptors, and three types, ETA, ETB (subtypes 1 and 2) and ETC, have been characterized in humans[36]. Within ocular tissues, ETA and ETB are constitutively expressed in human uveal tissues[37], the retina and ONH[38]. ET-1 induces vasoconstriction by its interaction with ETA receptors on vascular smooth muscle cells, but can also stimulate NO-mediated vasodilation by activation of ETB receptors on the endothelium[39]. Therefore, blocking of ETA receptor action or stimulation of ETB receptor action is theoretically required for the purpose of increasing ocular blood circulation and AH dynamics, resulting in decreased IOP. In fact, human and animal studies demonstrated that ETA receptor blockers[40,41] and a ETB receptor agonist[42,43] indeed caused IOP reduction and increased ocular blood flow. Previous knowledge that BCACs could stimulate the ETB receptor in a ciliary body[20] suggests that systemic administration of BCACs may also affect ET-1 dependent

blood circulation. In the present study, we could confirm that BCACs were able to effectively suppress ET-1 induced vasospasms based upon our present data of the finger cooling stimulation test.

Recently, we developed a simple method to evaluate AH dynamics, AH production and AH outflow resistance. We called this the "Weight on-off test" based upon external ocular compression[29]. When conducting this test, we observed that AH outflow resistance factors, IOPdrop and Raq out, were significantly lower and higher, respectively, in POAG, PACG or PEX subjects than in control subjects, but these factors were not significantly affected by anti-glaucoma medications. Additionally, AH production, IOPrec and Paq, in POAG, PACG or PEX subjects was slightly decreased and increased, respectively, when compared to production in control subjects. In POAG patients, PG caused a significant delay of IOPrec and a slight decrease in Paq. Our recent data therefore suggested that the Weight on-off test may be simple and useful in estimating AH dynamics in patients with GON and could help provide a better understanding of GON etiology as well as pharmacology of anti-glaucoma medications. By using this method, we evaluated the effects of BCACs on AH dynamics, and found that BCACs increase AH production as well as decrease AH outflow resistance. Since it was revealed that BCACs can stimulate the ETB receptor in a ciliary body[20], we speculated that those effects of BCACs on AH circulation may also be related to the ETB receptor functions in the ciliary body. If this speculation is correct, it confirms our previous observation that oral administration of BCACs caused a decrease in IOP levels in healthy subjects as well as patients with GON.

In conclusion, oral administration of BCACs may be a safe and promising supplement that affects ocular hemodynamics as well as systemic peripheral blood circulation through a ET-1 related mechanism.

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