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Two-Year Randomized, Placebo-Controlled Study of Black Currant Anthocyanins on Visual Field in Glaucoma

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Key Words

Randomized placebo-controlled double-masked trial · Black currant anthocyanins · Glaucoma · Ocular blood circulation

Abstract

Aim: To examine the influence of the black currant anthocyanins (BCACs) on the disease progression of open-angle glaucoma (OAG), a randomized, placebo-controlled, double-masked trial was made in 38 patients with OAG treated by antiglaucoma drops. ***Methods:*** BCACs (50 mg/day, n = 19) or their placebos (n = 19) were orally administered once daily for a 24-month period. Systemic blood pressure, pulse rates, intraocular pressure (IOP), ocular blood circulation by laser-speckle flowgraphy, and Humphrey visual field mean deviation (MD) were measured during the 24-month period. ***Results:*** As a main outcome measurement, we evaluated the difference between the groups in MD deterioration in the eye with a better MD from the trial's baseline through 24 months. A statistically significant difference was observed between the treatment groups in mean change from baseline in MD 24 months after therapy (p = 0.039, unpaired t test). Upon administration of BCACs, the ocular blood flows during the 24-month observational period increased in comparison with placebo-treated patients. However, no significant changes were observed in systemic and ocular conditions including IOP during the 24-month period. ***Conclusions:*** Our results suggest that oral administration of BCACs may be a safe and promising supplement for patients with OAG in addition to antiglaucoma medication.

Introduction

Glaucomatous optic neuropathy is known as one of the major causes of irreversible blindness worldwide [1]. Elevated intraocular pressure (IOP) is widely known as the most important risk factor for glaucomatous optic neuropathy, and to this point, lowering the IOP by antiglaucoma medication and/or surgical intervention has been considered the most effective therapy [2–5]. However, in contrast, lowering the elevated IOP is also known to be insufficient to stop the progression in some patients with open-angle glaucoma (OAG) [2–4]. Therefore, although additional therapy or nutritional supplements are required in addition to the IOP control in OAG, no established evidence to support this belief has been available up to now.

In terms of the causative etiology for OAG, the retinal and optic disk blood supply is thought to be an important factor, based upon the following evidence: (1) disk hemorrhages are likely to exist in patients with OAG [6–8]; (2) retinal vascular diseases, such as retinal vein occlusion, are frequently associated with OAG [8, 9]; (3) there

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is decreased hemodynamics of ocular blood flow in patients with OAG [10, 11]; (4) there are abnormal levels of the concentration of plasma endothelin-1 (ET-1) in patients with OAG as compared with healthy control subjects [12]. In addition, our group recently found that the platelet aggregation ability is remarkably increased in patients with OAG compared with that in normal subjects [13]. 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 a type of polyphenols, rich in food and beverages such as red wine, cocoa and berries, and are known to have several kinds of beneficial effects on health [14]. Among these, the ACs in black currants (BC) in particular have been implicated in improvement of visual functions, such as dark adaptation and transient refractive alternation [15]. Matsumoto et al. [16] reported that in vitro, the black currant anthocyanins (BCACs) stimulated ET-dependent vessel dilatation in the bovine ciliary body. Since ET has been identified as one of the factors regulating ocular blood circulation [17], we speculated that BCACs may alter the metabolism of ET and may have beneficial effects in the hemodynamics of ocular blood flow, including that in the ONH. Therefore BCACs may rationally be effective for diseases with ocular blood circulation disturbance, such as OAG. Previously, in order to study the effect of BCACs on OAG, capsules containing 50 mg of BCACs, by which contrast sensitivity was improved in human volunteers [15], were orally administered to patients with OAG once daily for a 6-month period, and changes in blood flow at the ONH and concentrations of plasma ET-1 were evaluated. Upon oral administration of the BCAC capsules, the blood flows at the inferotemporal rim of the ONH significantly increased ($p < 0.05$), and serum ET-1 levels were changed to those obtained by normal subjects [18]. Thus, our preliminary pilot study suggested that oral administration of the BCACs could possibly be a promising supplement for patients with OAG by bringing about improvement in their ocular blood circulation.

In the current study, the purpose was to investigate whether the oral administration of BCACs could prevent the visual field deterioration in patients with OAG by improving their ocular blood circulation of the ONH. A randomized, placebo-controlled, double-masked, 24-month cohort study was performed, and differences in the patients' visual field deterioration were compared between groups.

Subjects and Methods

Subjects

A clinical diagnosis of the patients with OAG was assessed based upon the following diagnostic criteria: (1) glaucomatous visual field defects corresponded to the glaucomatous optic disk changes; (2) gonioscopically normal open angles; (3) no history or findings of pseudoexfoliation or secondary glaucoma; (4) no other ocular, neurological, otolaryngological or systemic diseases affecting optic disk damage. From among eligible 250 patients with OAG, a total of 40 patients meeting the following inclusion and exclusion criteria were enrolled in the study (fig. 1).

Inclusion Criteria

(1) More than 2 years of treatment by antiglaucoma drops and regularly receiving IOP measurements every 1–2 months and Humphrey visual field 30-2 test (Humphrey Instruments, San Leandro, Calif., USA) every 3–6 months in the glaucoma clinic of the Department of Ophthalmology, Sapporo Medical University Hospital. (2) Better than –12 dB of mean deviation (MD), early to moderate stages of glaucomatous optic neuropathy [19], at the trial baseline in at least one eye in the Humphrey visual field 30-2 test. (3) Best-corrected visual acuity of 0.6 or more in at least one eye at the trial baseline. (4) Reliable performance on the Humphrey visual field testing 30-2 program (fixation loss of less than 20%, a false-positive or false-negative response of less than 33%) and laser speckle flowgraphy with a pupil diameter of more than 3 mm.

Exclusion Criteria

(1) Ocular diseases other than glaucoma and an early-to-mild senile cataract that would not influence the Humphrey visual field testing; (2) history of cataract surgery until 2 years previously; history of glaucoma surgery; use of supplements, including ACs; and history of drug or food allergies.

The present study protocol was approved by the Ethics Committee of the Sapporo Medical University School of Medicine and conducted in accordance with the Declaration of Helsinki. After an explanation of the study's purpose and its protocol had been provided, written informed consent was obtained from all participants in our glaucoma clinic before inclusion.

Study Design

This was a randomized, double-masked, placebo-controlled single-center trial. The study enrollment and follow-up were conducted between November 1, 2006, and March 31, 2010, in our glaucoma clinic of the Department of Ophthalmology, Sapporo Medical University Hospital (fig. 1). Patients meeting the inclusion and exclusion criteria were randomly assigned to either 24-month administration of BCACs (n = 20) or placebo (n = 20). Randomization was based on a computer-generated list made by a biostatistician (Y.M.) who was not involved in any other aspect of the current study. Its list was disclosed after all patients had completed the 24-month administration. The effectiveness of the treatment was compared between groups.

In terms of our sample size, we estimated that more than 15 patients in each group would be required to detect a difference in the effectiveness between groups, based upon a recent randomized, placebo-controlled, double-masked study of a calcium blocker on visual field in patients with OAG [20].

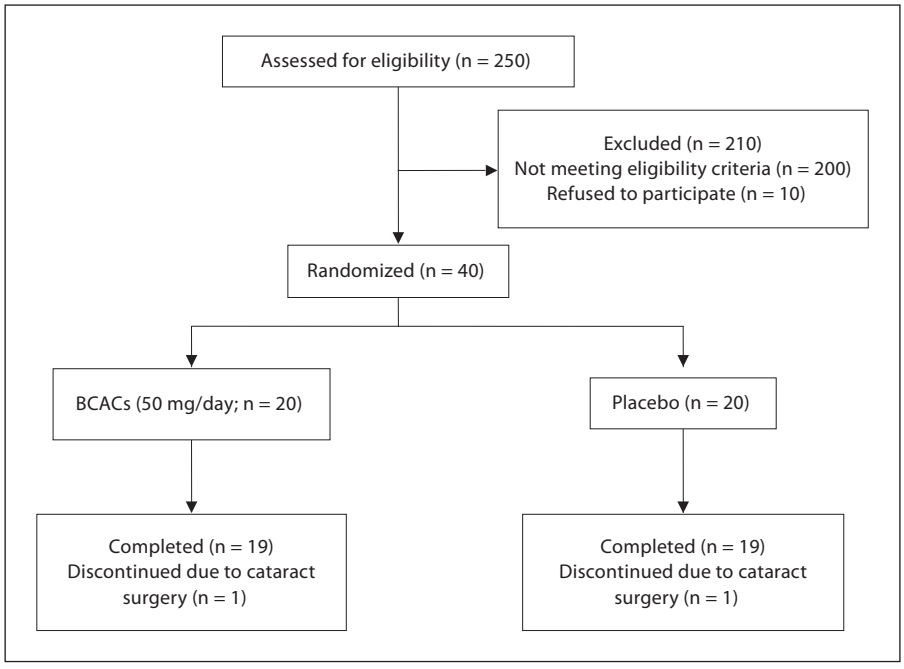

Fig. 1. Flow diagram of the present study.

Methods

ACs (25 mg) extracted from BCs were packed into the same capsules as placebos [18], making them indistinguishable to patients or physicians. Based on the assignment list numbers, subjects randomly received daily doses of BCACs (2 capsules, 50 mg/day, n = 20) or placebo capsules (n = 20) for 24 months (fig. 1). During the 24-month prospective observation period, compliance with the administration was confirmed by getting back used capsule packages, and slitlamp examinations, IOP measurements through a Goldmann applanation tonometer and ONH examinations were performed during each patient's monthly visits. Systemic blood pressure, ocular blood flow by laser speckle flowgraphy, visual acuity and a Humphrey visual field 30-2 test were measured at the trial baseline and every 6 months during the 24-month period. All examinations and measurements described above were carried out between approximately 9 and 11 in the morning by investigators or technicians unaware of the patients' group assignments. During the follow-up period, medications were not basically altered, and no additional medications or surgical interventions were added to lower the IOP levels. Patients were removed from the study when an investigator decided that it was impossible for the patient to continue the study because of adverse events, when the patient withdrew consent to participate in the study, when a patient's additional ocular disease influenced the visual field testing, when the patient required additional systemic medications such as antihypertensive drugs and cardiovascular drugs influenced the ocular blood flow measurements, when the patient needed surgical intervention for advancing cataract, or when an apparent progression in the visual field damage was found in the observational eye of the patient and required alteration of antiglaucoma drops or surgical intervention. For the evaluation of the visual field deterioration, the Advanced Glaucoma Intervention Study score calculated using the test points corresponding to those of the 24-2 program was used as described previously [21]. In cases in which scores worsened, visual field testing was repeated with 3-month intervals. Positive visual field deterioration was defined as when scores that had worsened in at least 4 units were observed in these consecutive 2 tests.

For evaluation of ONH and retinal circulation surrounding the optic disk, laser speckle flowgraphy was used as described previously [22]. Briefly, 30 min before measurement, the pupil was dilated with 1 drop of 0.4% tropicamide (Mydrin M; Santen, Osaka, Japan) and rest in a chair. Laser speckle flowgraphy (LSFG-NAVI, Softcare Co., Kyusyu, Japan; laser wave length: 830 nm, visual angle: 35°) was performed 3 consecutive times at 5 points including the ONH, the superior and inferior temporal disk rims, and the superior and inferior temporal peripapillary retinas in which chorioretinal atrophy and retinal vessels were excluded. The mean blur rate (MBR), a quantitative index indicating relative blood velocity calculated at each pixel point of these observational frames, was used. During the follow-up period, exactly the same areas were selected and the corresponding MBR was measured in each subject to compare at different time periods.

Statistical Analysis

The statistical difference of baseline characteristics between the BCAC and placebo groups was determined using an unpaired t test and Fisher test. During the 24-month follow-up periods, differences of changes of values at each time point from trial baseline in several observational parameters, including systolic, diastolic and mean blood pressure, IOP, visual acuity, MBR at the ONH, superior and inferior temporal disk rims, and su-

Table 1. Baseline characteristics of the patients

	BCACs	Placebo	p value
Gender (male/female), n	13/7	10/10	0.5231
Age, years	60.35 ± 7.22 (56.44 to 62.72)	63.20 ± 14.78 (55.45 to 69.92)	0.4333
Systolic blood pressure, mm Hg	128.4 ± 15.96 (121.14 to 136.76)	124.60 ± 17.33 (115.65 to 133.39)	0.4801
Diastolic blood pressure, mm Hg	74.70 ± 13.05 (69.42 to 81.74)	68.92 ± 10.62 (64.02 to 74.70)	0.1389
Mean blood pressure, mm Hg	92.60 ± 13.48 (86.91 to 99.82)	87.48 ± 11.63 (81.83 to 93.67)	0.2130
Pulse rate, beats/min	74.17 ± 14.28 (65.09 to 83.24)	72.21 ± 11.00 (67.17 to 78.77)	0.6793
IOP, mm Hg	13.33 ± 2.24 (12.37 to 14.52)	14.23 ± 3.46 (12.62 to 16.02)	0.3347
Glaucoma medications, n	1.26 ± 0.66 (0.96 to 1.56)	1.36 ± 0.76 (1.06 to 1.70)	0.3347
MD, dB	–4.59 ± 5.07 (–5.40 to –1.22)	–3.60 ± 3.99 (–2.89 to –1.47)	0.5043
Ocular blood flow (MBR) within			
Disk cup	2.36 ± 0.79 (2.02 to 2.98)	2.91 ± 1.30 (2.33 to 3.63)	0.1205
Superior/temporal disk rim	3.33 ± 1.04 (2.85 to 3.91)	3.75 ± 1.35 (3.14 to 4.49)	0.2891
Superior/temporal retina	2.48 ± 1.25 (1.86 to 3.20)	2.38 ± 0.72 (2.03 to 2.76)	0.7601
Inferior/temporal disk rim	4.03 ± 2.07 (2.87 to 4.46)	4.12 ± 2.07 (3.14 to 5.24)	0.9053
Inferior/temporal retina	2.29 ± 1.19 (1.76 to 3.15)	2.59 ± 1.16 (2.10 to 3.26)	0.4430

Patients received glaucoma medications including eyedrops of prostaglandin derivatives, β-blockers and carbonic anhydrase inhibitors. p values for differences between AC and placebo treatment groups were calculated by the Fisher test for gender and the unpaired t test for others; results are numbers or means ± SD; figures in parentheses are 95% confidence intervals.

perior and inferior temporal peripapillary retinas, and visual field MD, were compared by the unpaired t test between the BCAC and placebo groups. Additionally, differences in changes at each time point of the above observational data from baseline values within each group were compared by the paired t test. In terms of IOPs and visual field MD, these data obtained during the 24-month retrospective period from the baseline were also analyzed as above.

All statistical analyses were performed with SAS version 9.1 (SAS Institute Japan, Tokyo, Japan). The significance level was set at p = 0.05 for all statistical analyses.

Table 2. Visual acuity (decimal) of the patients at trial baseline and the 24-month time point

	BCACs	Placebo	p value
Baseline	1.06 ± 0.22 (0.96–1.16)	1.12 ± 0.26 (1.00–1.24)	0.281
24-month point	1.12 ± 0.27 (0.99–1.25)	1.16 ± 0.27 (1.04–1.28)	0.297

p values for differences between BCAC and placebo treatment groups were calculated by the unpaired t test; results are means ± SD; figures in parentheses are 95% confidence intervals.

Results

Initially, a total of 40 patients were assigned and involved in the present study (BCACs, n = 20; placebo, n = 20). During the observational 24-month period, 1 patient receiving BCACs and 1 patient receiving placebos were dropped from the study because they underwent cataract surgery due to the progression of cataracts in their observational eyes (fig. 1). Tables 1 and 2 summarize the baseline characteristics of the patients who completed the 24-month study (BCACs, n = 19; placebo, n = 19). Between the two groups, no significant difference was observed in age and gender, in addition to the several observational contents of the systemic conditions including systolic, diastolic and mean blood pressure, pulse rates or/and ocular conditions including visual acuity, IOP, number of glaucoma drop medications, MD in the Humphrey visual field testing 30-2 program, or blood flow at the ONH, its rims and its surrounding retinas. Next, each of the observational contents described above was compared between the BCAC and placebo groups every 6 months during the 24-month study.

Our previous preliminary pilot study demonstrated that oral administration of BCACs enhanced blood flow of the ONH and its surrounding retina [18]. Thus, we

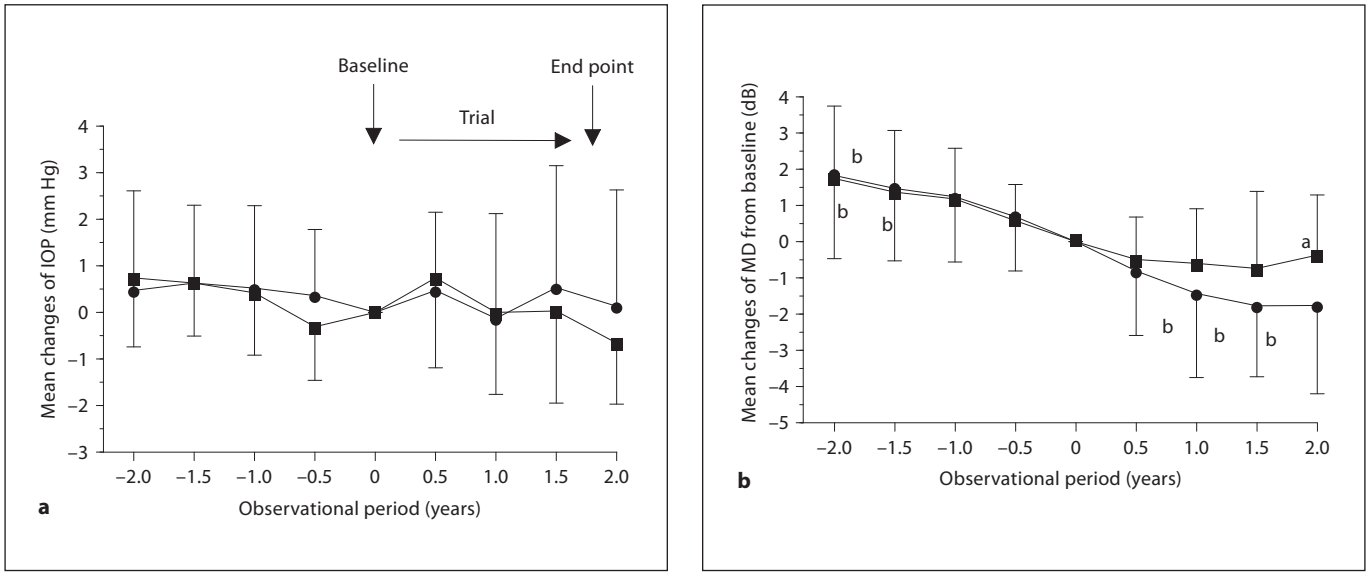

Fig. 2. The time course changes of IOPs and visual field MD. The differences between baseline and each time point of the retrospective period (–0.5, –1, –1.5 and –2 years) and trial period (0.5, 1, 1.5 and 2 years) of IOPs (**a**) and MD (**b**) in the BCAC intake group (■) and placebo intake group (●) were plotted. Data are expressed as means ± SD. [a] $p < 0.05$: significant difference between groups (unpaired t test); [b] $p < 0.05$: significant intergroup difference between each time point and baseline (paired t test).

speculated that BCACs could conceivably cause a slowing in the visual field progression in patients with OAG due to its efficacy toward ocular blood flow. To verify our hypothesis, the primary and secondary end points of the current study were chosen as comparison points of the deterioration of the visual field MD and changes of the ocular blood flow between the BCAC and placebo groups from the baseline through 24 months, respectively. As shown in figure 2, the mean deterioration of the MD at 24 months after the baseline was significantly less in the BCAC group than the placebo group (unpaired t test, p = 0.039). To explore when such effects of BCACs toward visual field MD were observed during the observational period until 24 months from baseline, within each BCAC or placebo group, intergroup changes occurring every 6 months from the baseline were examined. A statistically significant decrease in MD in the placebo group was observed after 12 months until the 24-month end point as compared with baseline (paired t test, 12 months: p = 0.03, 18 months: p = 0.003, 24 months: p = 0.007). In contrast, the BCAC group showed no significant changes of MD during the 24-month observational period. To exclude possibilities that MD deterioration and/or IOP control levels were already different between groups at the trial baseline, changes of MD and IOP were also examined during the 24-month retrospective period from the baseline, but no significant differences were found between groups (fig. 2). As another possibility to influence the MD changes between groups, it was speculated that cataract conditions in the BCAC group may be more progressive than those in the placebo group during the 24-month trial. Nevertheless, slitlamp examination revealed that none of the patients of both groups showed apparent progression in their cataract, and decimal visual acuity was not changed during the trial period and no statistical difference was observed between groups as shown in table 2.

In terms of ocular blood circulation, changes of mean MBR in the inferior temporal peripapillary retina at 18 months in the BCAC group were significantly higher than in the placebo group (unpaired t test, p = 0.01; fig. 3). In addition, mean changes of MBR in the BCAC group increased after 6 months from baseline levels at the ONH, its superior and inferior rims and inferior temporal peripapillary retina. Among these, the MBR increase within the inferior temporal retina at 6 months (paired t test, p = 0.03) and 18 months (paired t test, p = 0.01) and the ONH at 24 months (paired t test, p = 0.02) was statistically significant. However, the MBR of all tested areas in the placebo group was not significantly altered and was almost always less than those in the BCAC group during the study period (fig. 3).

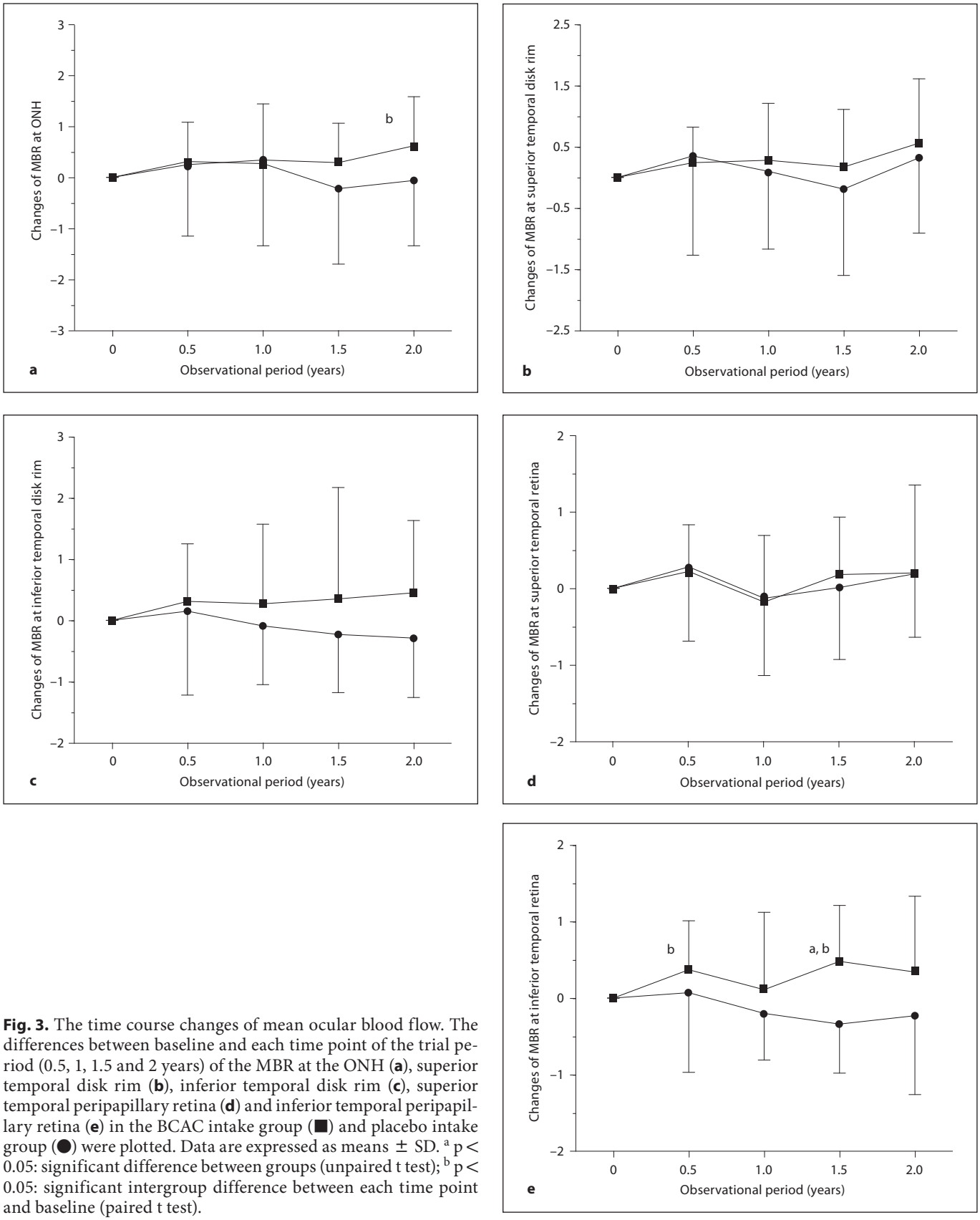

Fig. 3. The time course changes of mean ocular blood flow. The differences between baseline and each time point of the trial period (0.5, 1, 1.5 and 2 years) of the MBR at the ONH (**a**), superior temporal disk rim (**b**), inferior temporal disk rim (**c**), superior temporal peripapillary retina (**d**) and inferior temporal peripapillary retina (**e**) in the BCAC intake group (■) and placebo intake group (●) were plotted. Data are expressed as means ± SD. [a] $p < 0.05$: significant difference between groups (unpaired t test); [b] $p < 0.05$: significant intergroup difference between each time point and baseline (paired t test).

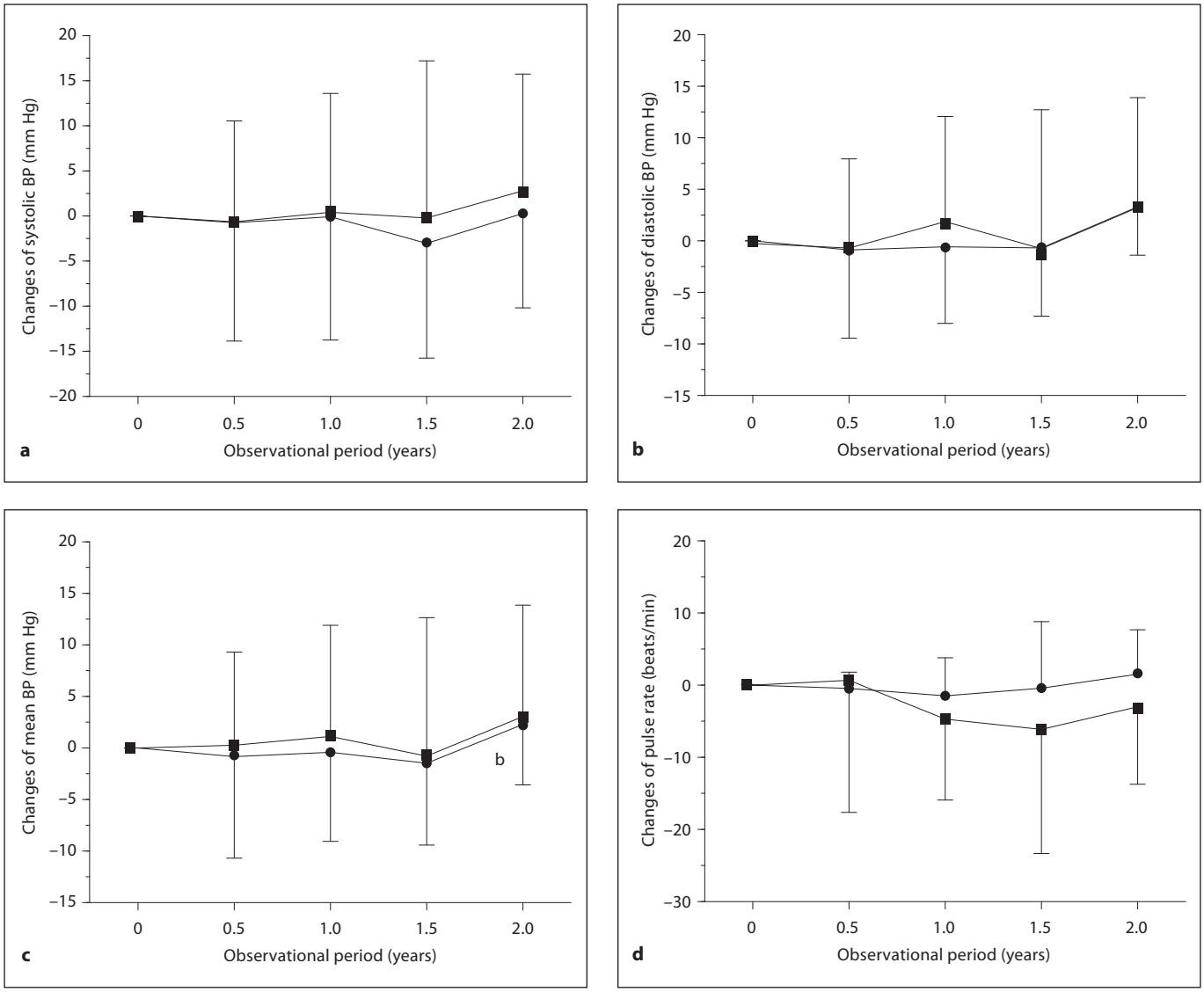

Fig. 4. The time course changes of mean systemic blood pressures and pulse rates. The differences between baseline and each time point of the trial period (0.5, 1, 1.5 and 2 years) of mean systemic systolic (**a**), diastolic (**b**) and mean blood pressures (**c**) and pulse rates (**d**) in the BCAC intake group (■) and placebo intake group (●) were plotted. Data are expressed as means ± SD. [b] $p < 0.05$: significant intergroup difference between each time point and baseline (paired t test).

In terms of the systemic conditions including blood pressure and pulse rates, no significant changes were observed in the analysis between groups and within groups, except for a significant intergroup change of mean diastolic blood pressure at 24 months in the placebo group ($p = 0.008$; fig. 4). In addition, no systemic and ocular side effects were detected in either trial group.

Discussion

Several clinical investigators [23–25] have revealed that both optic disk rim blood flow and peripapillary retinal blood flow are significantly lower in patients with OAG as compared with age-matched controls, and thus optic disk rim blood flow is significantly correlated with deterioration of existing visual field defects. Hafez et al. [26] evaluated and compared optic disk rim blood flow in patients with OAG and those with ocular hypertension

(OH) and found that OAG patients had significantly lower values than OH patients. They emphasized that optic disk rim blood flow levels were inversely correlated with an increased cup-to-disk ratio (C/D) in patients with OH; that is, rim blood flow levels were significantly lower in the disks with large C/D ratios as compared with those with small C/D ratios. They thereby suggested that optic disk rim perfusion may already be reduced in patients with OH with a large C/D ratio before their manifestation of visual field defects. Therefore, on the basis of this evidence, it was strongly suggested that the disturbance of the ocular blood flow around the ONH was indeed involved in the pathogenesis of OAG. Thus, improvement of the insufficient ONH blood circulation could reasonably be a promising therapy to prevent the deterioration of OAG.

Retinal nerve fiber layer thickness is well known to be different within part of the ONH in normal disks (the so-called ISN'T rule), that is the inferior one is thickest, followed by the superior, nasal and temporal parts [27]. Since the retinal nerve fiber layer is the thickest in the inferior portion of the ONH, the relative blood perfusion within this portion is the lowest among the ONH, and thus, this portion is known to be the most susceptible to glaucomatous optic nerve damage [28]. It has been shown that the upper visual field is more commonly involved than the lower in OAG [29]. If blood perfusion is the lowest in the inferior portion of the ONH as suggested, the beneficial effect of BCACs toward blood circulation should be most evident within the inferior portion of the ONH. In fact, in our previous [18] and current studies, systemic administration of BCACs consistently increased blood circulation within the inferotemporal portion of the ONH. Thus, these observations suggest that BCACs caused preventive effects toward the deterioration of OAG in addition to the antiglaucoma medications, presumably by improvement of the ocular blood circulation within the ONH and its surrounding retina.

It has been demonstrated that BC extract contains several kinds of flavonoids and ACs, and they possess several kinds of health benefit effects such as antioxidant [30, 31] or anti-inflammatory properties [32, 33]. However, the mechanisms underlying these effects are not understood well. There are actually 4 types of ACs that were purified and characterized: delphinidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-glucoside [34]. Upon oral administration, these 4 components were directly absorbed and presented as intact forms in plasma and then transferred beyond the blood-aqueous barrier and blood-retina barrier into ocular tissues including the retina, choroid and ciliary body [35]. In vitro evidence demonstrated that such BCACs transferred into ocular tissues caused several kinds of biological activities including stimulation of rhodopsin regeneration in frog retinas [36], suppression of ocular globe elongation in chick myopia models [37] in addition to the ET-dependent vasodilation in the bovine ciliary body [16]. In vivo experiments demonstrated that oral administration of the BCACs significantly improved dark adaptation and video display terminal work-induced transient refractive alteration in healthy human volunteers [15]. In terms of the relationship between ET-1 and the eye, some investigations have shown that ET-1 receptors are present in human uveal tissues [38], retina and ONH [39], suggesting that ET-1 may be implicated in ischemic vascular diseases such as diabetic retinopathy [40], retinal vein occlusion and retinal artery occlusion [41] and glaucoma [42–45]. Previous studies have shown that statistically significant differences of plasma ET-1 levels exist in glaucoma patients as compared to those in control subjects [12, 43, 46, 47]. In our recent study [18], we found that the oral administration of BCAC capsules (50 mg/day) normalized the abnormal plasma concentration levels of ET-1 in patients with normal-tension glaucoma and caused a significant increase in the ocular blood circulation around the ONH. From these observations, we speculated that orally administered BCACs possibly affect the ET-1 receptor functions such as its pharmacological reactivity and hypersensitivity, thereby resulting in beneficial effects toward the ocular blood circulation and visual field as demonstrated above. However, since BC extracts possess several kinds of biological effects as suggested by previous studies [30–33], identification of the molecular mechanisms causing beneficial effects of BCACs toward glaucomatous optic neuropathy is our next project.

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