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Ginkgo biloba Extract Increases Ocular Blood Flow Velocity

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ABSTRACT

We evaluated a possible therapeutic effect of *Ginkgo biloba* extract (GBE) on glaucoma patients that may benefit from improvements in ocular blood flow. A Phase I cross-over trial of GBE with placebo control in 11 healthy volunteers (8 women, 3 men: Age; 34 ± 3 years, mean ± SE) was performed. Patients were treated with either GBE 40 mg or placebo three times daily orally, for 2 days. Color Doppler imaging (Siemens Quantum 2000) was used to measure ocular blood flow before and after treatment. There was a two week washout period between GBE and placebo treatment.

Ginkgo biloba extract significantly increased end diastolic velocity (EDV) in the ophthalmic artery (OA) (baseline vs GBE-treatment; 6.5 ± 0.5 vs 7.7 ± 0.5 cm/sec, 23 % change, p=0.023), with no change seen in placebo (baseline vs GBE-treatment; 7.2 ± 0.6 vs 7.1 ± 0.5 cm/sec, 3 % change, p=0.892). No side effects related to GBE were found. *Ginkgo biloba* extract did not alter arterial blood pressure, heart rate, or IOP.

Ginkgo biloba extract significantly increased EDV in the OA and deserves further investigation in ocular blood flow and neuroprotection for possible application to the treatment of glaucomatous optic neuropathy as well as other ischemic ocular diseases.

INTRODUCTION

Leaf extracts of *Ginkgo biloba* (*Ginkgo biloba* extract, GBE) have been used in Chinese traditional medicine since 3000 BC for treating asthma and bronchitis (1). This "fossil" tree is the single surviving member of the Family and Order of the earliest known trees, having originated in the Permian period approximately 250 millions years ago. At the present time, GBE is widely used in Europe and is the most commonly prescribed drug in Germany, where it has been approved for a variety of symptoms associated with aging. In the United States, it is freely available as a nutritional supplement. GBE has been claimed effective in numerous disorders, including cerebrovascular disease, peripheral vascular disease, intermittent claudication, dementia, tinnitus, bronchoconstriction, and sexual dysfunction. Many studies have been uncontrolled or poorly controlled. In the past few years, studies using improved methodology

suggest that GBE may actually be beneficial in these disorders. Most recently, it has been shown to be effective in treating Alzheimer's disease (2-4).

Treatment of glaucoma has long been centered on lowering IOP by one means or another. Intraocular pressure, however, is not the disease itself, but the most important risk factor for it. In the past decade, investigation into other risk factors for glaucomatous damage has been steadily increasing. Much of this research has centered on the adequacy of the circulation to the eye. Patients with either idiopathic (primary) open-angle glaucoma or normal-tension glaucoma exhibit numerous ocular blood flow deficits (5,6). Fluorescein angiography reveals reduced total retinal blood flow, and dye leakage from optic nerve head capillaries, suggesting pre-capillary ischemia (7,8). Choroidal circulation in glaucoma patients fails to vasodilate appropriately, and delays in choroidal filling may be associated with the thinning of the entire choroid (9-11). These vascular abnormalities may be among the earliest manifestations of the illness. Nevertheless, despite accumulating evidence that glaucoma patients suffer from inadequate ocular blood flow, current clinical treatment of the illness involves neither documentation of nor treatment for these deficits.

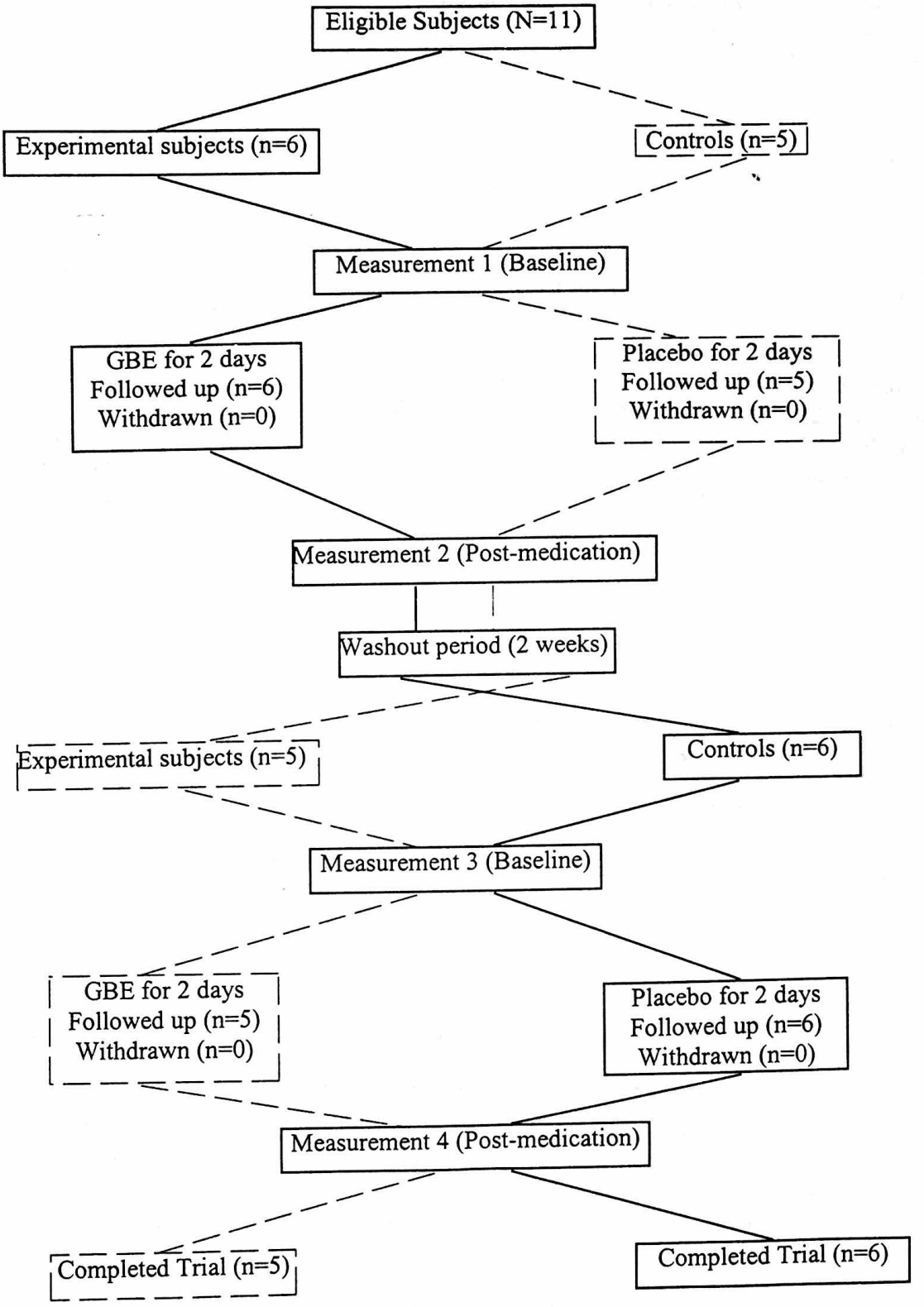
As a major aspect of the pathophysiology of the illness, blood flow deficiencies represent crucial opportunity for development of new treatment strategies. The authors hypothesized that, extrapolating from its known effect in other systems, GBE could be of benefit in treating non-pressure-dependent aspects of glaucoma. In this study, we evaluated the effect of GBE on ocular blood flow in normal volunteers.

MATERIALS AND METHODS

We performed a randomized, prospective, double-masked, cross-over Phase I clinical trial of GBE with a placebo control in eleven healthy volunteers (8 women, 3 men; mean age ± S.E., 34 ± 3 years, range from 20 to 61 years). All volunteers were recruited at Indiana University Medical Center. No subject had a history of ocular or systemic disease or was taking topical or systemic medication at the time of the study. Measurements were taken in the left eye for all subjects. Informed consent was obtained from all subjects, and the protocol was approved by an institutional review board. All procedures conformed to the tenets of the Declaration of Helsinki. After baseline pretreatment measurements (measurement 1) were made, subjects were treated in random order with either *Ginkgo biloba* extract (Ginkoba®, Pharmaton, Ridgefield CT) 40 mg (24% flavonoid glycosides, 6% terpenes) or placebo taken orally three times a day, for 2 days. Fructose was used as placebo. Identical capsules (Empty gelatin capsules No.00, Eli Lilly and Company, Indianapolis) were filled with either 1 tablet containing 40 mg of GBE or placebo. At the completion of post-treatment measurements (measurement 2), the second baseline control measurements (measurement 3) were repeated after 2 weeks of drug washout. Subsequent to this second control, the other drug regimen was carried out over 2 days, culminating in a final set of measurements (measurement 4). All measurements were obtained at the same time of day for each of the four visits. Subjects and investigators were masked to the phase of the study.

We measured IOP by Goldmann applanation tonometry, heart rate, and arterial blood pressure. Color Doppler imaging was performed on the ophthalmic artery (OA), central retinal artery (CRA), and short posterior ciliary artery (SPCA) with subjects comfortably reclining at a 60-degree angle. A Siemens Quantum 2000 (Issaquah, Washington) with a 7.5-MHz linear phase transducer was used for imaging. From each vessel, peak systolic velocity (PSV) and end diastolic velocity (EDV) were recorded. Resistance index (RI) [(PSV-EDV)/PSV] was calculated for each vessel. To examine the OA, the sample volume is oriented nasally and superior to the optic nerve, just lateral to and abutting the visible hyporeflective stripe representing the nerve. The CRA is anterior to the optic nerve: the sample volume is placed about 3 mm behind the surface of the optic disk. The SPCA are temporal or nasal to the optic nerve shadow. Because of their small size (less than 200 μm) relative to the sample volume, it is not always possible to resolve individual vessels (12). Nevertheless, the presence of colored pixels in this

Flow Diagram of a Randomized Controlled Trial with Crossover



region and the characteristic Doppler spectrum obtained from them confirm the presence of posterior ciliary artery flow.

Statistical comparisons were made with two tailed paired t-tests. A p value of less than 0.05 was considered statistically significant. The sample size was determined to provide 90% power to detect a difference of 15% change in flow velocity or RI in the OA; 80% power for a 15% change in the CRA and SPCA.

RESULTS

GBE did not alter systemic arterial blood pressure, heart rate or IOP (Table 1). GBE significantly increased EDV in the OA (baseline vs GBE-treatment; 6.5 ± 0.5 vs 7.7 ± 0.5 cm/sec, p=0.023), whereas placebo produced no change (Table 2, Fig. 1). Changes of EDV in the OA were 23.0 ± 8.8% for GBE and 3.0 ± 8.1% for placebo. GBE also showed a tendency to increase PSV (baseline vs GBE; 29.6 ± 2.4 vs 33.4 ± 2.8 cm/sec, p=0.075) (Table 2). Changes of PSV in the OA was 15.0 ± 8.5% for GBE and -2.4 ± 4.8% for placebo. GBE did not alter PSV and EDV in the CRA or SPCA (Table 2). There were no significant changes in RI in any of the three vessels studied. When results from the 3 vessels in a single subject were averaged (using percentage of changes from baseline so that each vessel was equally weighted), GBE showed a tendency to increase EDV (p=0.070)(Fig. 2). No side effects or discomfort related to GBE were found in this study.

TABLE 1.

Blood Pressure (BP), Intraocular Pressure (IOP), and Heart Rate (HR) Responses to Placebo and GBE

Parameters	Baseline	Placebo	p	Baseline	GBE-treated	p
Systolic BP (mm Hg)	117±3	115±4	0.277	117±2	117±3	0.847
Diastolic BP (mm Hg)	76±3	74±4	0.211	76±3	74±4	0.230
IOP (mm Hg)	14.6±0.8	14.0±1.0	0.290	13.5±0.9	14.0±1.0	0.716
HR (bpm)	75±4	71±4	0.209	74±2	70±2	0.107

Values represent mean± SEM.

TABLE 2.

Hemodynamic Responses to Placebo and GBE Measured by Color Doppler Imaging.

Vessels	Parameters	Baseline	Placebo	p	Baseline	GBE-treated	p
OA	PSV (cm/sec)	30.6±2.2	29.6±2.2	0.504	29.6±2.4	33.4±2.8	0.075
	EDV (cm/sec)	7.2±0.6	7.1±0.5	0.892	6.5±0.5	7.7±0.5	0.023*
	RI	0.76±0.02	0.76±0.01	0.642	0.78±0.02	0.76±0.01	0.392
CRA	PSV (cm/sec)	7.6±0.7	7.5±0.7	0.851	7.8±1.0	8.0±1.1	0.416
	EDV (cm/sec)	1.7±0.3	1.6±0.3	0.638	1.7±0.4	1.7±0.4	0.548
	RI	0.79±0.02	0.80±0.03	0.704	0.81±0.02	0.80±0.03	0.522
SPCA	PSV (cm/sec)	7.2±0.8	7.7±0.8	0.295	7.4±0.7	8.0±1.6	0.575
	EDV (cm/sec)	1.9±0.5	1.9±0.5	0.840	1.8±0.5	2.2±0.7	0.292
	RI	0.76±0.02	0.77±0.09	0.111	0.76±0.03	0.75±0.02	0.332

Values represent mean± SEM.

*Statistically significantly different from baseline

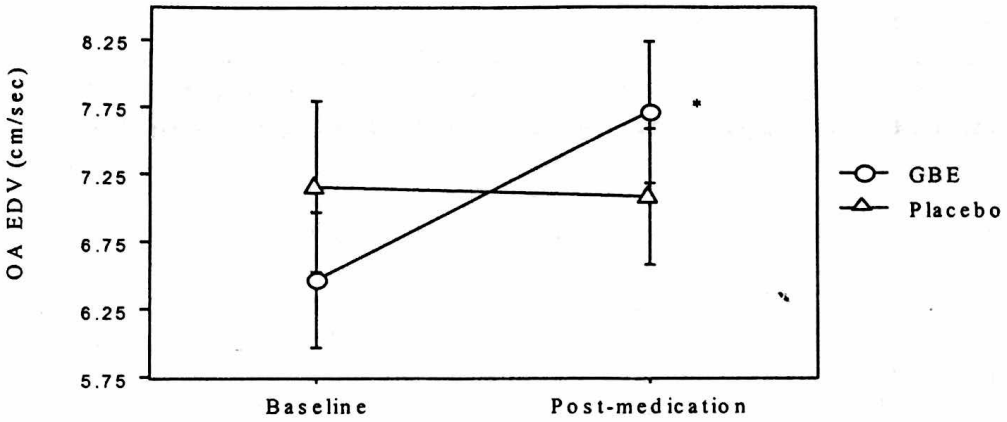


FIGURE 1. *Ginkgo biloba* Extract (GBE) Significantly Increased End Diastolic Velocity (EDV) in Ophthalmic Artery (OA) Measured by Color Doppler Imaging (N=11). Values represent mean ± SEM. *Statistically significant difference from baseline (p=0.023).

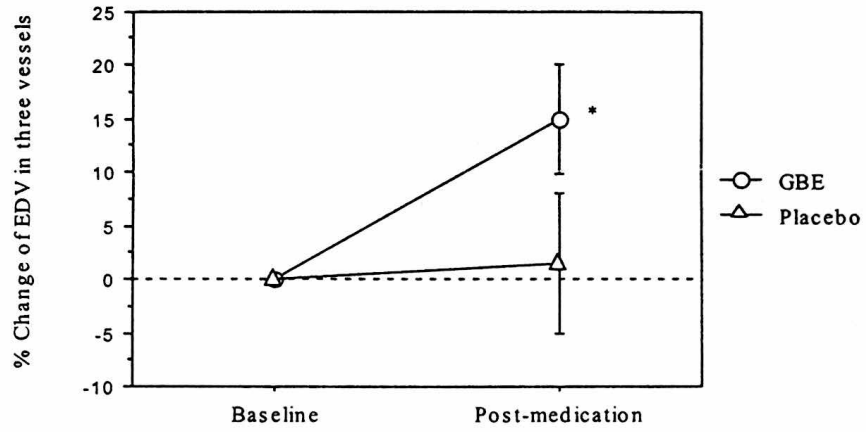


FIGURE 2. *Ginkgo biloba* Extract (GBE) Showed a Tendency to Increase Three-vessel Mean End Diastolic Velocity (EDV) [Percentage Change of 15.0 ± 5.1% for GBE-treated; 1.6 ± 6.6% for Placebo (N=11)] Measured by Color Doppler Imaging. Three vessels; Ophthalmic, central retinal, and short posterior ciliary arteries. Values represent mean ± SEM. *difference between placebo and GBE (p=0.070).

DISCUSSION

Ginkgo biloba extract contains over 60 known bioactive compounds. Most available brands contain approximately 24% flavone glycosides (flavonoids). Some, but not all, brands contain approximately 6% terpene lactones (ginkgolides and bilobalide), approximately 7% proanthocyanidines, and other, uncharacterized compounds (13). The natural ginkgolides inhibit platelet activating factor (PAF) by binding to its membrane receptor. The most powerful antagonist of PAF is ginkgolide B (14). Other, minor components include shikimic acid, 3,4-hydroxybenzoic acid, 3-methoxy-4-hydroxybenzoic acid, 6-hydroxykynurenic acid, kynurenic acid, and ascorbic acid (13).

The ginkgo flavonoids are active primarily as free radical scavengers with activity comparable to vitamins A and C (15-17). GBE inhibits red cell membrane lipid peroxidation in a time and concentration dependent manner (18). GBE significantly reduced lipid peroxidation in experimental spinal cord injury in rats (19). *Ginkgo biloba* has the ability to scavenge nitric oxide (20).

Neuroprotection is the postinjury protection of neurons that initially were undamaged or only marginally damaged, but are at risk from toxic stimuli released by damaged cells which cause secondary degeneration. PAF enhances glutamatergic excitatory synaptic transmission and may amplify excitotoxicity produced by excessive glutamate release during neuronal injury (21). PAF levels are known to increase in the CNS during brain trauma causing an increase in free intracellular calcium ion concentrations (22). Ginkgolide B is an extremely potent PAF antagonist.

The flavonoids and terpene lactones contribute to the ability of GBE to improve both peripheral and cerebral blood flow (23). GBE has been reported to increase skin perfusion and decrease blood viscosity (24), and increase microcirculation in a dose-dependent fashion (25). It has been reported to improve electroencephalographic findings in patients with cerebrovascular insufficiency (26), and improve both patient and physician assessment of such symptoms as fatigue, depression, and memory loss (27). It has most recently been shown to be of value in the treatment of Alzheimer's disease. Improvement of symptoms related to intermittent claudication has been assumed to result from an increase in oxygen supply to the muscles (24).

This study demonstrated that GBE significantly increased EDV of the OA, and there was a trend of increasing PSV in the OA. The average changes from baseline were 15% for PSV and 23% for EDV, which are substantial when it is considered that the coefficient of variation for the OA measurement are 12% for PSV and 6% for EDV (12). Measurements of CDI are indirect, and interpretation of them must proceed cautiously. It is, however, clear from *in vitro* studies that CDI indices are validated as good indicators of volumetric flow. In larger cerebral vessels, change in PSV provides a direct indication of change in bulk blood flow. In smaller vessels, such as in the eye, measurement of both PSV and EDV are needed. *In vitro* analysis confirmed that simultaneous increases in the PSV and EDV imply increased bulk flow (28). Therefore, our data showing parallel increases of PSV and EDV in the OA indicates an increase of volumetric blood flow in the OA. GBE did not alter measurements of CRA and SPCA in normal subjects. The response of CRA and SPCA, however, may be different in glaucoma patients. Because of vascular dysfunction and insufficient autoregulation, GBE could presumably increase blood flow of CRA and SPCA in addition to the OA in glaucoma patients or in other patients with ocular vascular deficiencies. Improvement of ocular blood flow and neuroprotective effect of GBE may provide for an ideal medical treatment modality combined with current hypotensive glaucoma medications.

In conclusion, *Ginkgo biloba* extract significantly increased OA blood flow and deserves further investigation in ocular blood flow and neuroprotection for possible application to the treatment of glaucomatous optic neuropathy as well as other ischemic ocular diseases.

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