Clinical Trials

Effectiveness of Palmitoylethanolamide on Endothelial Dysfunction in Ocular Hypertensive Patients: A Randomized, Placebo-Controlled Cross-Over Study

Ernesto Strobbe, Mauro Cellini, and Emilio C. Campos

Purpose. We assessed the effect of palmitoylethanolamide (PEA) on systemic endothelial function in ocular hypertensive patients (OH).

Methods. We enrolled in this randomized, double-blind, placebo-controlled, crossover single-center study 40 never-treated OH patients and 40 healthy age-matched controls. At baseline, each participant underwent endothelium-dependent flow-mediated vasodilation (FMD) measurement using a noninvasive high-resolution 2-dimensional ultrasonographic imaging of the brachial artery. OH patients were assigned randomly to receive either 300 mg PEA (Group A) or a matching placebo (Group B), twice a day for three months (T1). The first medication period was followed by a two-month washout period (T2), and then patients switched to PEA or placebo (depending on the first drug received) for another three months (T3). FMD evaluations were repeated at T1, T2, and T3.

Results. At baseline FMD values in OH patients and controls were 6.06 ± 0.60% vs. 10.85 ± 1.80%, respectively ($P < 0.001$). At T1, FMD and IOP of Group A were, respectively, 8.46 ± 1.09% vs. 6.08 ± 0.62% ($P < 0.001$, $r = 0.96$) and 22.18 ± 1.26 vs. 23.03 ± 0.88 mm Hg ($P < 0.001$). At T2, Group A had better FMD values than at baseline (6.59 ± 0.33% vs. 6.08 ± 0.62%, $P < 0.05$). At T3, subjects in Group B showed better FMD and IOP than at T2 (8.52 ± 1.07% vs. 6.05 ± 0.68%, $P < 0.001$, $r = 0.97$; and 22.43 ± 1.17 vs. 23.03 ± 0.83 mm Hg, $P < 0.01$, respectively). No side effects were observed.

Conclusions. Three-month PEA intake reduced IOP and led to significantly improved FMD values in OH patients compared to placebo, by ameliorating peripheral endothelial function, and its positive effect lasted longer than the period of PEA consumption. No adverse events were recorded. (Controlled-trials.com number, ISRCTN72647928.) (*Invest Ophthalmol Vis Sci.* 2013;54:968–973) DOI:10.1167/iovs.12-10899

From the Department of Surgery Science and Anesthesiology, Ophthalmology Unit, University of Bologna, Bologna, Italy.

Supported in part by the University of Bologna (ECC-MIUR ex-60%), in part through a grant from the "Fondazione Banca del Monte di Bologna e Ravenna," and in part through a gift from the "Fondazione Cassa di Risparmio di Bologna." The authors alone are responsible for the content and writing of this paper.

Submitted for publication September 4, 2012; revised November 26 and December 17, 2012, and January 3, 2013; accepted January 4, 2013.

Disclosure: **E. Strobbe**, None; **M. Cellini**, None; **E.C. Campos**, None

Corresponding author: Ernesto Strobbe, University of Bologna, Pelagi 9, 40126, Italy; strobern@libero.it.

Although glaucoma is a multifactorial optic neuropathy of unknown etiology, in which increased IOP is the most important risk factor because it can be measured easily, and currently is the only modifiable and treatable factor,[1] ischemia, vascular dysregulation,[2,3] and vasospasm[4] also have been implicated in the causative mechanism of glaucoma. Indeed, the vascular hypothesis of glaucomatous optic neuropathy development implies that ganglion cell damage is caused at least partly by a chronic impairment of the blood supply in the optic nerve head,[5,6] which possibly is due to an increase in plasma and aqueous humor endothelin 1 (ET-1) levels.[7,8]

Moreover, to confirm and strengthen the key role of the vascular system in the pathogenesis of glaucoma, a generalized peripheral endothelial dysfunction has been demonstrated in ocular hypertension (OH),[9] normal tension glaucoma (NTG),[10] primary open angle glaucoma (POAG),[11] and patients with pseudoexfoliation syndrome.[12]

The endocannabinoid (eCB) system, which was discovered in the late 1990s, is a complex and pleiotropic endogenous signaling system that is activated "on demand" following a perturbation of cell homeostasis to aid in the re-establishment of this homeostasis,[13] seems to exert an important role in endothelial protection,[14] and is composed of several substances, including 2-arachidonoylglycerol (2-AG), anandamide (AEA), and palmitoylethanolamide (PEA), which have been found in different human systems, including vascular system and ocular tissues,[15] where they also may provide IOP reduction.[16]

We assessed the effect of PEA therapy on peripheral vascular endothelial function and IOP in OH patients using noninvasive brachial artery ultrasound assessment of flow-mediated vasodilation (FMD).

Methods

Study Design

This randomized, double-blind, placebo-controlled, crossover, single-center study was conducted between September 2010 and March 2012, at the Ophthalmology Unit of University of Bologna, in which 40 untreated OH patients and 40 healthy age-matched controls were enrolled. None of the healthy controls in this study was treated.

OH subjects were assigned randomly to one of the two parallel treatment arms at baseline: 300 mg PEA or a matching placebo, per os, twice a day, for a period of 90 days (T1) under medical supervision to assess possible side effects. Then, the first medication period was followed by a 2-month washout period (T2), and each patient subsequently was switched to the other treatment for a further 3 months (T3).

FMD and IOP values were measured at baseline, and at T1, T2, and T3, and the safety profile of both drugs (PEA and placebo) was assessed by interviewing study patients about the occurrence of adverse events,

Investigative Ophthalmology & Visual Science, February 2013, Vol. 54, No. 2

Table 1. Demographic and Ocular Characteristics of OH Patients and Healthy Controls

	Controls	OHT Patients	*P* Value
Male	18	24	
Female	22	16	
Age, y	56.2 ± 10.4	56.8 ± 8.1	0.431
IOP, mm Hg	16.7 ± 1.5	23.11 ± 0.93	0.001*
Mean spherical equivalent refractive error, diopters	−0.2 ± 1.5	−0.4 ± 1.6	0.320
Visual acuity	1.0 ± 0.0	1.0 ± 0.1	1.00
Corneal thickness, μm	552.3 ± 3.08	558.4 ± 4.15	0.274
C/D ratio	0.33 ± 0.11	0.37 ± 0.12	0.068
Visual field MD, dB	−1.66 ± 0.59	−1.80 ± 0.68	0.131
Visual field PSD, dB	2.00 ± 0.31	2.17 ± 0.27	0.527
FMD, %	10.85 ± 1.80	6.06 ± 0.60	<0.002*

MD, mean deviation; PSD, pattern standard deviation.
* Statistically significant.

including serious adverse events and discontinuation of the drug before the end of the treatment period.

The randomization schedule was generated by using a computer algorithm. The generator of the random allocation was not involved in the study, the executors did not participate in generating the schedule, and investigators and patients were blinded to the treatment allocation. Medications were prepared by the hospital pharmacy and packaged identically.

The study was performed in accordance with the tenets of the Declaration of Helsinki, the protocol was approved by the institutional review board and ethical committee of University of Bologna, and a written informed consent was obtained from all participants. This clinical trial is registered in the Current Controlled Trials Web site (available online at www.controlled-trials.com, number ISRCTN72647928).

Subject Recruitment

Each subject enrolled in this study was aged <65 years and, to eliminate possible confounders that could affect endothelial function from the study sample, had no cardiovascular disease or known cardiovascular risk factors, such as hypertension, diabetes mellitus, hypercholesterolemia, dyslipidemia, and smoking, and was not taking vasoactive medications. OH patients were required to meet the following inclusion criteria: baseline IOP ≥22 mm Hg for at least two measurements, an open anterior chamber angle at gonioscopy, a cup-to-disc (C/D) ratio <0.4, normal visual field parameters (MD <3 dB and PSD <2.5 dB), normal Glaucoma Hemifield Test, and a normal corneal central thickness (530–560 μm).

Baseline Measurements

Each patient underwent an ophthalmologic examination, including visual acuity and applanation IOP assessment, corneal thickness evaluation with a Tomey SP3000 pachymeter (Tomey Corp., Nagoya, Japan), biomicroscopy of the anterior and posterior segment with automatic measurement of the C/D area ratio of the optic nerve head by using a Stratus OCT III (Zeiss-Humphrey, Dublin, CA), and standard achromatic perimetry (SAP) with a Humphrey Field Analyzer-SITA program (Zeiss-Humphrey, Table 1).

The criteria for the reliability of the visual fields were ≤33% false positive, ≤33% false negative, and ≤20% fixation losses. For each patient, body mass index was calculated, and blood samples were drawn from an antecubital vein of a sitting subject after an overnight fast to assess lipid profile, fasting serum glucose, and ET-1 plasma levels, before beginning the ultrasound measurement to exclude OH patients who had an evident peripheral endothelial dysfunction compared to normal subjects (Table 2).

ET-1 Plasma Level Assessment

For each patient, a plasma sample was collected in a container with EDTA, cooled, and stored on ice, and then centrifuged at 4°C and frozen at −25°C. After centrifugation, extraction was performed by using a Sep-column containing C-18 (Peninsula Laboratories, Belmont, CA) and ET-1 concentration was determined by using a commercial radioimmunoassay (RIA) kit (Peninsula Laboratories). For the RIA, the samples and standards first were incubated with rabbit anti-ET-1 serum for 24 hours at 4°C, and then, after the addition of an iodinated tracer [^{125}I]-ET-1, for a further 24 hours. Free and bound radioligands were separated by centrifugation, and the radioactivity in the precipitate was counted with an automatic gamma counter.

FMD Evaluation

Before the examination, every patient was asked to fast for at least 12 hours and avoid caffeinated beverages. Then FMD evaluation was performed by using high-resolution 2-dimensional ultrasonographic

Table 2. Vascular and Biochemical Parameters of OH Patients and Healthy Controls

	Controls	OHT Patients	*P* Value
Body mass index, kg/m^2	25.77 ± 2.73	23.85 ± 3.47	0.103
Systolic blood pressure, mm Hg	125.13 ± 15.03	123.00 ± 15.22	0.702
Diastolic blood pressure, mm Hg	76.80 ± 7.45	78.67 ± 7.66	0.504
Total cholesterol, mg/dL	194.42 ± 29.21	188.73 ± 24.41	0.567
HDL cholesterol, mg/dL	53.37 ± 13.16	48.66 ± 12.38	0.321
LDL cholesterol, mg/dL	116.22 ± 26.81	115.70 ± 24.85	0.956
Triglycerides, mg/dL	107.85 ± 62.77	103.54 ± 47.75	0.834
Plasma ET-1, pg/mL	1.89 ± 0.33	1.75 ± 0.25	0.104
Fasting serum glucose, mg/dL	92.30 ± 7.97	93.29 ± 9.20	0.754

HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 3. Main Clinical Characteristics of OH Patients Randomized to Group A and Group B

	Group A	Group B	*P* Value
IOP, mm Hg	23.03 ± 0.88	23.25 ± 0.76	0.201
Visual field MD, dB	−1.86 ± 0.64	−1.76 ± 0.54	0.308
Plasma ET-1, pg/mL	1.73 ± 0.24	1.77 ± 0.20	0.291
FMD, %	6.08 ± 0.62	6.04 ± 0.58	0.401

imaging of the brachial artery by a Philips ENVISOR echographic machine (Philips Medical Systems, Best, The Netherlands) and a 4 to 7 Hz linear probe, according to a previously described and validated methodology.[17]

The examination was performed in a quiet room at a temperature of 23°C to 26°C, in which each patient rested in a supine position for 10 minutes before the first imaging scan and held that position until the final measurements were recorded. During this time, baseline 2-D images and pulsed Doppler blood flow velocity of the brachial artery were acquired, whereas baseline blood pressure was measured in the left arm and a continuous electrocardiographic recording was obtained. To induce hyperemia, a 14 cm sphygmomanometer cuff was placed on the right arm and inflated to 250 mm Hg for 5 minutes, with the transducer positioned carefully approximately 5 cm above the antecubital fossa by an expert examiner who was blinded to the condition of the subjects. Then, the cuff was deflated rapidly and a pulsed Doppler velocity signal was recorded 5 to 10 seconds after deflation.

Bidimensional images of the brachial artery were recorded 60 seconds after cuff deflation and continuously for 5 minutes, whereas FMD was calculated as the percentage change in the brachial artery diameter in response to reactive hyperemia:

$$FMD = ([VDHyperemia - VDBaseline]/VDBaseline) \times 100\%$$

$$VD = vessel\ diameter.$$

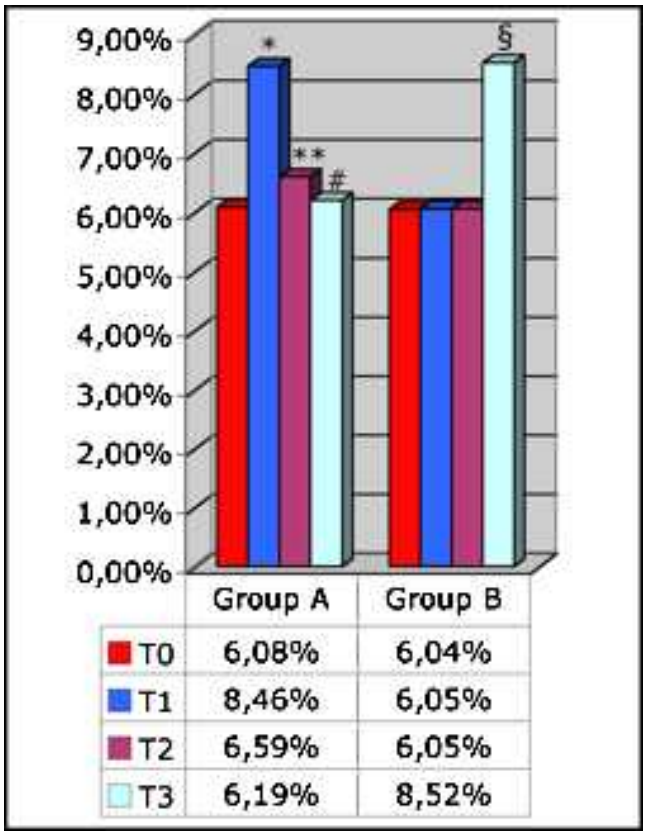

Figure. FMD in OH patients at baseline (T0), after 3 months of PEA or placebo treatment (T1), after 2 months of wash-out (T2), and after a further 3 months of placebo or PEA therapy (T3). *Group A T1 versus baseline, $P < 0.001$. ** Group A T2 versus baseline, $P < 0.05$. #Group A T3 versus baseline, $P < 0.001$. §Group B T3 versus baseline, $P < 0.001$.

Statistical Analysis

All data were expressed as the mean ± SD. A statistical analysis was done to assess FMD in OH patients and controls using Student's *t*-test for unpaired data. For both OH groups, the change in IOP values before and after PEA or placebo intake, and the differences in FMD values between baseline and 3-month therapies, between T0 and the end of the wash-out period, and between T0 and T3 were analyzed by repeated measures ANOVA model followed by the Bonferroni post-hoc test.

A Pearson's correlation test was used to evaluate the relationships between endothelial function and PEA intake, and between IOP and FMD before and after PEA consumption. *P* values less than 0.05 were regarded as being statistically significant.

Results

All of the OH patients who were included at the onset of the study completed the study. At baseline, FMD values of the OH patients were significantly lower than FMD values of the controls (6.06 ± 0.60% vs. 10.85 ± 1.80%, $P < 0.001$, Table 1). No significant differences were evaluated between demographic and clinical parameters of OH subjects randomized to PEA and those randomized to placebo (Table 3).

At T1, patients who were undergoing PEA therapy (Group A) showed a significant improvement in FMD values (8.46 ± 1.09% vs. 6.08 ± 0.62%, $P < 0.001$, $r = 0.96$) and a significant IOP reduction 22.18 ± 1.26 vs. 23.03 ± 0.88 mm Hg, $P < 0.001$), whereas subjects who were administered a placebo (Group B) had stable FMD and IOP values (6.05 ± 0.68% vs. 6.04 ± 0.58%, $P > 0.05$ and 22.95 ± 0.90 vs. 23.25 ± 0.76 mm Hg, $P > 0.05$, respectively, see Figure).

After 2 months of washout, Group A maintained better FMD values than at baseline (6.59 ± 0.33% vs. 6.08 ± 0.62%, $P < 0.05$) and IOP moved from 22.18 ± 1.26 to 22.88 ± 0.85 mm Hg ($P < 0.001$), whereas FMD and IOP of Group B did not show any significant change. Then, each patient switched to the other treatment, depending on the first drug received, and at T3, subjects who started PEA intake showed a significant improvement in their FMD and a significant IOP reduction (8.52 ± 1.07% vs. 6.05 ± 0.68%, $P < 0.001$, $r = 0.97$ and 22.43 ± 1.17 vs. 23.03 ± 0.83 mm Hg, $P < 0.01$, respectively), whereas in patients who started the placebo, FMD and IOP moved from 6.59 ± 0.33% to 6.19 ± 0.41% ($P < 0.001$) and from 22.88 ± 0.85 to 22.78 ± 1 mm Hg ($P > 0.05$), respectively (see Figure). As regards the relationship between FMD and IOP values, there was a very good correlation in both OH groups at baseline ($r = 0.605$, $P < 0.004$ for Group A and $r = 0.707$, $P < 0.001$ for Group B) and after PEA intake ($r = 0.887$, $P < 0.0001$ for Group A and $r = 0.944$, $P < 0.0001$ for Group B). None of the study patients discontinued therapy before the end of the treatment period because they did not experience either systemic/local adverse events or intolerance to PEA.

Discussion

IOP is the most targeted risk factor in the diagnostic and therapeutic routines of patients who are suffering from glaucoma or ocular hypertension. However, increasing attention has been focused on gaining a better understanding of the other factors that are involved in their causative mechanism, such as the role of the endothelial dysfunction. Indeed, in accordance with other studies,[9–12] as regards FMD values in OH patients, we found a reduction of 44% compared to controls, and after 3 months of PEA intake, patients had approximately 40% improvement of FMD values and an IOP reduction of 3.7%.

Furthermore, we demonstrated that the effect of PEA on FMD is maintained partially over its intake period. Indeed, after 2 months from the end of PEA consumption, the residual FMD improvement of 8.4% was observed, whereas the residual hypotensive effect was reduced until the value of 0.15 mm Hg.

The vascular endothelium is a complex organ that maintains vascular homeostasis in every part of the body[18]; has a key role in angiogenesis, inflammatory response, and hemostasis; controls vascular tone and blood flow[19]; regulates microcirculation; and, although it is a single layer in vessel walls, it has autocrine, paracrine, and endocrine properties, and produces several vasoactive substances,[20] including prostacyclins, acetylcholine, bradykinin, and histamine,[2,21] in addition to the vasodilator nitric oxide and the vasoconstrictor ET-1. All these molecules function together to maintain perfect homeostasis of the vascular system in response to several stresses and stimuli.

Endothelial dysfunction is a common condition in many systemic diseases, such as coronary artery disease, chronic renal failure, diabetic vasculopathy, and atherosclerosis,[22] is a negative prognostic factor in their progression, and can be assessed by several invasive and noninvasive methods. At present, indeed, brachial artery ultrasound assessment of FMD is the method that is used most extensively in the cardiovascular field to determine cardiovascular disease risk factors,[23] because it is noninvasive and provides information on the NO production by endothelia of blood vessels,[24] but this technique also has been adopted recently in ophthalmologic research to assess peripheral vascular functionality in patients with POAG, OH, NTG, and pseudoexfoliation syndrome with and without glaucoma.[9–12]

Strategies to ameliorate endothelial function include changes in lifestyle, treatment with statins,[25] inhibitors of angiotensin-converting enzyme, angiotensin-1 receptor blockers, an increase in high-density lipoprotein cholesterol, and the inhibition of cholesterol acyltransferase.[26] An endogenous fatty acid amide, PEA, which was discovered in 1957 and belongs to the eCB system together with 2-AG and AEA, a complex network of receptors and enzymes responsible for their degradation, as fatty acid amide hydrolase (FAAH) and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA),[27] is a congener of AEA, seems to increase AEA levels or prolong its effects by inhibiting the activity of FAAH, acts by inhibiting mast cell degranulation[28] and peripheral inflammation,[29] interacting with cyclo-oxygenase–2,[30] exerting neuroprotective effects in rats and mice,[31,32] modifying nitric oxide production by macrophages,[33] and having a role in endothelial protection in ischemic rat hearts.[14]

In our study, we demonstrated that 600 mg PEA administered daily over a period of three months may improve systemic endothelial function in patients with ocular hypertension with no local side effects or systemic adverse events, and we showed that its protective action may last longer than its intake period as the effect on IOP.

The dosage and route of administration of PEA were decided taking into account studies in which PEA was used to contrast chronic pelvic pain,[34,35] whereas a washout period of 2 months was defined to minimize any possible residual action on IOP and FMD.

In accordance with other studies,[16,36,37] we showed that oral PEA also may provide reduction of IOP, even if the exact mechanism is not understood completely. Indeed, it has been shown that PEA may lower IOP by modulating AEA levels by inhibiting its degradation[38] to restore proper eCBs levels, which are, for example, altered in glaucomatous eyes.[15]

AEA is able to activate CB_1 receptors in the pigmented epithelium of the ciliary body, trabecular meshwork, Schlemm's canal, and ciliary muscle influencing the production and drainage of aqueous humor,[39] but it also may act on vascular beds via an endothelial anandamide receptor,[40] leading to vasodilation and vasorelaxion, although the precise vascular endothelial functions are complex and controversial.[41]

The hypotensive effect of PEA we found was lower than that shown by Gagliano et al. (0.85 vs. 3.5 mm Hg),[16] but this finding may be explained by different eligibility criteria and different treatment regimens of our patients. Indeed, in our study we evaluated the hypotensive effect of PEA alone in OH patients, whereas Gagliano et al. enrolled OH and POAG patients who were treated previously with timolol 0.5% twice a day.[16] Thus, a combination of two hypotensive drugs, PEA and timolol, might justify the enhancement of the magnitude of IOP reduction as shown also by Oltmanns MH, et al. (*IOVS* 2007;48:ARVO E-Abstract 4807).

The correlation between IOP and FMD values ($r = 0.944$), we found, could open a new intriguing scenario in which the endothelial dysfunction in the anterior chamber due to an endothelial shear-stress, as it happens in the peripheral endothelial cells, might cause a poor production of nitric oxide by endothelial nitric oxide synthase, a relative local improvement of ET-1, and a remodeling of F-actin architecture of endothelial cells,[42] with IOP improvement due to outflow resistance either directly at the level of the trabecular meshwork, Schlemm's canal, and collecting channels, or indirectly through alteration in the tone of the longitudinal ciliary muscle.[43]

A systemic endothelial dysfunction might prevent the physiologic regulation of ocular blood flow, leading to modifications in the optic nerve head supply, and contributing to ganglion cell damage, and a trabecular and Schlemm's endothelial dysfunction might contrast the normal aqueous outflow.

However, to confirm this hypothesis, long-term prospective studies and further investigations are necessary, because at present the extent to which this systemic dysfunction may contribute to the glaucomatous process remains unclear. We presume that PEA capacity to modify eCBs levels may reduce IOP, restore normal endothelial function, ameliorate the regulation of microcirculation, improve the optic nerve head blood supply, and guarantee the necessary ocular blood flow demand in OH patients.

Our study has some limitations. First, the sample was chosen from a selected cohort of patients with very strict eligibility criteria, but it is likely that results might be more heterogeneous considering a general glaucomatous population. We expect that, in the future, larger samples could be analyzed. Second, despite very well trained examiners who perform the examination and the widespread use of brachial artery ultrasound technique to evaluate endothelial function, this technique remains vulnerable to criticisms of reproducibility, and presents technical and interpretative limitations. Third, we know that our hypothesis of a correlation between intraocular and peripheral endothelial dysfunction is the result of a speculation, but it could be corroborated by the molecular analysis of the aqueous humor in search of local key molecules related to intraocular endothelial dysfunction, for example in experimental studies.

Acknowledgments

Caterina Villani performed ultrasonographic evaluations, and Luigi Cellini generated the random allocation of ocular hypertensive patients.

References

1. Quigley HA. Glaucoma: macrocosm to microcosm. The Friedenwald Lecture. *Invest Ophthalmol Vis Sci*. 2005;46: 2662–2670.
2. Flammer J, Orgül S. Optic nerve blood-flow abnormalities in glaucoma. *Prog Retin Eye Res*. 1998;17:267–289.
3. Haefliger IO, Dettmann E, Liu R, et al. Potential role of nitric oxide and endothelin in the pathogenesis of glaucoma. *Surv Ophthalmol*. 1999;43:51–58.
4. Nicolela MT, Ferrier SN, Morrison CA, et al. Effects of cold-induced vasospasm in glaucoma: the role of endothelin-1. *Invest Ophthalmol Vis Sci*. 2003;44:2565–2572.
5. Flammer J. The vascular concept of glaucoma. *Surv Ophthalmol*. 1994;38(suppl):3–6.
6. Hayreh SS. Factors influencing blood flow in the optic nerve head. *J Glaucoma*. 1997;6:412–425.
7. Emre M, Orgül S, Haufschild T, et al. Increased plasma endothelin-1 levels in patients with progressive open angle glaucoma. *Br J Ophthalmol*. 2005;89:60–63.
8. Iwabe S, Lamas M, Vásquez Pélaez CG, Carrasco, FG. Aqueous humor endothelin-1 (Et-1), vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) levels in Mexican glaucomatous patients. *Curr Eye Res*. 2010;35:287–294.
9. Fadini GP, Pagano C, Baesso I, et al. Reduced endothelial progenitor cells and brachial artery flow-mediated dilation as evidence of endothelial dysfunction in ocular hypertension and primary open-angle glaucoma. *Acta Ophthalmol*. 2010;88: 135–141.
10. Su WW, Cheng ST, Hsu TS, Ho WJ. Abnormal flow-mediated vasodilation in normal-tension glaucoma using a noninvasive determination for peripheral endothelial dysfunction. *Invest Ophthalmol Vis Sci*. 2006;47:3390–3394.
11. Su WW, Cheng ST, Ho WJ, et al. Glaucoma is associated with peripheral vascular endothelial dysfunction. *Ophthalmology*. 2008;115:1173–1178.
12. Atalar PT, Atalar E, Kilic H, et al. Impaired systemic endothelial function in patients with pseudoexfoliation syndrome. *Int Heart J*. 2006;47:77–84.
13. De Petrocellis L, Di Marzo V. An introduction to the endocannabinoid system: from the early to the latest concepts. *Best Pract Res Clin Endocrinol Metab*. 2009;23:1–15.
14. Bouchard JF, Lépicier P, Lamontagne D. Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sci*. 2003;72:1859–1870.
15. Chen J, Matias I, Dinh T, et al. Finding of endocannabinoids in human eye tissues: implications for glaucoma. *Biochem Biophys Res Comm*. 2005;330:1062–1067.
16. Gagliano C, Ortisi E, Pulvirenti L, et al. Ocular hypotensive effect of oral palmitoyl-ethanolamide: a clinical trial. *Invest Ophthalmol Vis Sci*. 2011;52:6096–6100.
17. Corretti MC, Anderson TJ, Benjamin EJ. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *J Am Coll Cardiol*. 2002; 77:257–265.
18. Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leucocyte emigration. *Annu Rev Physiol*. 1995;57:827–872.
19. Resch H, Garhofer G, Fuchsjäger-Mayrl G, et al. Endothelial dysfunction in glaucoma. *Acta Ophthalmol*. 2009;87:4–12.
20. Behrendt D, Ganz P. Endothelial function: from vascular biology to clinical applications. *Am J Cardiol*. 2002;90:40L–48L.
21. Adams JA. Endothelium and cardiopulmonary resuscitation. *Crit Care Med*. 2006;34(suppl 12):458–465.
22. Fadini GP, Agostini C, Sartore S, Avogaro A. Endothelial progenitor cells in the natural history of atherosclerosis. *Atherosclerosis*. 2007;194:46–54.
23. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). *J Am Coll Cardiol*. 2000;35:60–66.
24. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol*. 2005;568:357–369.
25. Dimmeler S, Aicher A, Vasa M, et al. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase / Akt pathway. *J Clin Invest*. 2001;108:391–397.
26. Landmesser U, Drexler H. The clinical significance of endothelial dysfunction. *Curr Opin Cardiol*. 2005;20:547–551.
27. Järvinen T, Pate DW, Laine K. Cannabinoids in the treatment of glaucoma. *Pharmacol Ther*. 2002;95:203–220.
28. Aloe L, Leon A, Levi-Montalcini R. A proposed autacoid mechanism controlling mastocyte behaviour. *Agents Actions*. 1993;39:C145–C147.
29. Mazzari S, Canella R, Petrelli L, et al. N-(2-hydroxyethyl)hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. *Eur J Pharmacol*. 1996;300:227–236.
30. Costa B, Conti S, Giagnoni G, Colleoni M. Therapeutic effect of the endogenous fatty acid amide, palmitoylethanolamide, in rat acute inflammation: inhibition of nitric oxide and cyclooxygenase systems. *Br J Pharmacol*. 2002;137:413–420.
31. Franklin A, Parmentier-Batteur S, Walter L, et al. Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. *J Neurosci*. 2003;23: 7767–7775.
32. Skaper SD, Buriani A, Dal Toso R, et al. The aliamide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proc Natl Acad Sci U S A*. 1996;93:3984–3989.
33. Ross RA, Brockie HC, Pertwee RG. Inhibition of nitric oxide production in RAW264.7 macrophages by cannabinoids and palmitoylethanolamide. *Eur J Pharmacol*. 2000;401:121–130.
34. Calabrò RS, Gervasi G, Marino S, Mondo PN, Bramanti P. Misdiagnosed chronic pelvic pain: pudendal neuralgia responding to a novel use of palmitoylethanolamide. *Pain Med*. 2010;11:781–784.
35. Indraccolo U, Barbieri F. Effect of palmitoylethanolamide-polydatin combination on chronic pelvic pain associated with endometriosis: preliminary observations. *Eur J Obst Gynec Reprod Biol*. 2010;150:76–79.
36. Laine K, Järvinen T, Savinainen J, et al. Effects of topical anandamide-transport inhibitors, AM404 and olvanil, on intraocular pressure in normotensive rabbits. *Pharm Res*. 2001;18:494–499.
37. Buchwald A, Browne CE, Wu WM, Ji F, Bodor N. Soft cannabinoid analogues as potential anti-glaucoma agents. *Pharmazie*. 2000;55:196–201.
38. Di Marzo V, Melck D, Orlando P, et al. Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and

enhances the anti-proliferative effect of anandamide in human breast cancer cells. *Biochem J.* 2001;358:249–255.

39. Pate DW, Jarvinen K, Urtti A, et al. Effects of topical alpha-substituted anandamides on intraocular pressure in normotensive rabbits. *Pharm Res*. 1997;14:1738–1743.
40. Jarai Z, Wagner JA, Varga K, et al. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB_1 or CB_2 receptors. *Proc Natl Acad Sci U S A*. 1999;96:14136–14141.
41. Randall MD, Harris D, Kendall DA, Ralevic V. Cardiovascular effects of cannabinoids. *Pharmacol Ther*. 2002;95:191–202.
42. Ross Ethier C, Read TA, Chan D. Biomechanics of Schlemm's canal endothelial cells: influence on F-actin architecture. *Biophys J*. 2004;87:2828–2837.
43. Nathanson JA, McKee M. Identification of an extensive system of nitric oxide-producing cells in the ciliary muscle and outflow pathway of the human eye. *Invest Ophthalmol Vis Sci*. 1995;36:1765–1773.