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 dedicated solution of two-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 < 0.01). 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

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, ischemia, vascular dysregulation, and vasospasm also have been implicated in the causative mechanism of glaucoma. Indeed, the vascular hypothesis of glucomatous 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, which possibly is due to an increase in plasma and aqueous humor endothelin 1 (ET-1) levels.

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), normal tension glaucoma (NTG), primary open angle glaucoma (POAG), and patients with pseudoexfoliation syndrome.

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, seems to exert an important role in endothelial protection, 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, where they also may provide IOP reduction.

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.
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 <5 dB and PSD <2.5 dB), normal Glaucoma Hemifield Test, and a normal corneal central thickness (550–560 μm).

### Baseline Measurements

Each patient underwent an ophthalmologic examination, including visual acuity and applanation IOP assessment, corneal thickness evaluation with a Tomey SP30000 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 ≤3% false positive, ≤35% 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 (C. After centrifugation, extraction was performed by an automatic gamma counter. The criteria for the reliability of the visual fields were ≤3% false positive, ≤35% 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).

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

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>OHT Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>18</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>56.2 ± 10.4</td>
<td>56.8 ± 8.1</td>
<td>0.431</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>16.7 ± 1.5</td>
<td>23.11 ± 0.93</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean spherical equivalent refractive error, diopters</td>
<td>-0.2 ± 1.5</td>
<td>-0.4 ± 1.6</td>
<td>0.520</td>
</tr>
<tr>
<td>Visual acuity</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.1</td>
<td>1.00</td>
</tr>
<tr>
<td>Corneal thickness, μm</td>
<td>552.3 ± 3.08</td>
<td>558.4 ± 4.15</td>
<td>0.274</td>
</tr>
<tr>
<td>C/D ratio</td>
<td>0.35 ± 0.11</td>
<td>0.37 ± 0.12</td>
<td>0.068</td>
</tr>
<tr>
<td>Visual field MD, dB</td>
<td>-1.66 ± 0.59</td>
<td>-1.80 ± 0.68</td>
<td>0.131</td>
</tr>
<tr>
<td>Visual field PSD, dB</td>
<td>2.00 ± 0.31</td>
<td>2.17 ± 0.27</td>
<td>0.527</td>
</tr>
<tr>
<td>FMD, %</td>
<td>10.85 ± 1.80</td>
<td>6.06 ± 0.60</td>
<td>&lt;0.002*</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>OHT Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.77 ± 2.73</td>
<td>23.85 ± 3.47</td>
<td>0.104</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>125.15 ± 15.03</td>
<td>123.00 ± 15.22</td>
<td>0.702</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76.80 ± 7.45</td>
<td>78.67 ± 7.66</td>
<td>0.504</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>194.42 ± 29.21</td>
<td>188.73 ± 24.41</td>
<td>0.567</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>53.37 ± 13.16</td>
<td>48.66 ± 12.38</td>
<td>0.321</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>116.22 ± 26.81</td>
<td>115.70 ± 24.85</td>
<td>0.956</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>107.85 ± 62.77</td>
<td>103.54 ± 47.75</td>
<td>0.834</td>
</tr>
<tr>
<td>Plasma ET-1, pg/mL</td>
<td>1.89 ± 0.33</td>
<td>1.75 ± 0.25</td>
<td>0.104</td>
</tr>
<tr>
<td>Fasting serum glucose, mg/dL</td>
<td>92.30 ± 7.97</td>
<td>93.29 ± 9.20</td>
<td>0.754</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; LDL, low density lipoprotein.
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 2D 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:

\[
FMD = \left( \frac{VD_{\text{Hyperemia}} - VD_{\text{Baseline}}}{VD_{\text{Baseline}}} \right) \times 100\%
\]

\[VD = \text{vessel diameter}.\]

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 ± 0.58% 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, we observed, whereas the residual hypotensive effect was reduced until the value of 0.15 mm Hg. The endothelial dysfunction is a common condition in many systemic diseases, such as coronary artery disease, chronic renal failure, diabetic vasculopathy, and atherosclerosis.

Endothelial dysfunction might cause a poor production of nitric oxide by endothelial nitric oxide synthase, a relative local improvement of EF-1, and a remodeling of F-actin architecture of endothelial cells, 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.

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.

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, whereas a washout period of 2 months was defined to minimize any possible residual action on IOP and FMD.

In accordance with other studies, 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 to restore proper eCBs levels, which are, for example, altered in glaucomatous eyes.

AEA is able to activate CB1 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, but it also may act on vascular beds via an endothelial anandamide receptor, leading to vasodilation and vasorelaxation, although the precise vascular endothelial functions are complex and controversial. The hypotensive effect of PEA we found was lower than that shown by Gagliano et al. (0.85 vs. 3.5 mm Hg), 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. 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 = 0.94), 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 EF-1, and a remodeling of F-actin architecture of endothelial cells, 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.

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

38. Di Marzo V, Melck D, Orlando P, et al. Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and...


