Ocular Hypotensive Effect of Oral Palmitoyl-ethanolamide: A Clinical Trial

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Purpose. To investigate the effect of oral palmitoyl-ethanolamide (PEA) on intraocular pressure (IOP) in primary open angle glaucoma (POAG) and ocular hypertension (OH).

Methods. In a prospective, randomized, double-blind, crossover clinical trial, 42 patients with POAG or OH who were treated with timolol 0.5% and whose IOP was between 19 and 24 mm Hg received oral PEA (300-mg tablets twice a day) or placebo (PEA vehicle tablets twice a day) for 2 months (period 1), and, after a 2-month washout, received the other treatment for 1 month (period 2). IOP, best-corrected visual acuity, and visual field parameters were considered.

Results. After PEA treatment (mean baseline IOP, 21.6 ± 1.7 mm Hg), IOP was reduced by 3.2 ± 1.3 mm Hg at 1 month and by 3.5 ± 1.2 mm Hg (15.9% ± 5.1%) at 2 months (ANOVA, $P < 0.001$; both Tukey-Kramer, $P < 0.01$ vs. baseline); after placebo (mean baseline IOP, 21.5 ± 1.5 mm Hg), IOP was reduced by 0.4 ± 1.2 mm Hg at 1 month and by 0.3 ± 1.3 mm Hg at 2 months (*t*-test at both time points, $P < 0.001$ vs. PEA). No statistically significant vital signs, visual field, visual acuity changes, or adverse events were detected in either group.

Conclusions. Systemic administration of PEA reduces IOP in patients with glaucoma and ocular hypertension. PEA could be a valuable tool for the treatment of glaucoma (http://www.umin.ac.jp/ctr/index/htm number, UMIN000002833). (*Invest Ophthalmol Vis Sci.* 2011;52:6096–6100) DOI:10.1167/iovs.10-7057

Glaucoma is the second most prevalent cause of blindness worldwide.[1] Although several factors are involved in the pathogenesis of glaucoma, the main therapeutic strategy is actually based on intraocular pressure (IOP) reduction.

In 1971, Helper and Frank[2] reported that smoking marijuana reduced IOP in human volunteers, with an apparent dose-response relationship.[2] Marijuana is a complex pharmacologic mixture containing 420 natural products; some of them, known as cannabinoids,[2] have several pharmacologic actions that affect the cardiovascular and central nervous systems (with psychotropic effects) and cause IOP reduction.[2–5] Systemic and topical administration of several cannabinoids and cannabinoid derivatives lowered IOP in normal and glaucomatous eyes.[2,6–11]

A cannabinoid system composed of several receptors and of endogenous cannabinoids, including anandamide (AEA) and 2-arachidonylglycerol (2-AG),[12,13] has been identified in the brain, the peripheral tissue, and the eye.[14–18] Two principal cannabinoid receptors have been described (CB1, which is predominant in neurons, and CB2, which is localized in immune cells and peripheral tissue cells).[19] AEA, the most investigated endocannabinoid, acts as a partial CB1 agonist and a weak CB2 agonist and activates vanilloid type 1 receptor.[15,20–22] In vivo, AEA has a short duration of action: it is transported to cells by a carrier-mediated uptake mechanism, and it is hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH).[19]

Palmitoyl-ethanolamide (PEA) is an endogenous congener of AEA that is cosynthesized with AEA by most cell types; PEA does not bind to CB1 or CB2 receptors,[21,23] but it is a competing substrate with AEA for the FAAH active site, and it has been hypothesized to increase or prolong the effect of AEA[23–26] (entourage effect)[27] without the systemic side effects of cannabinoids.

PEA is synthesized during inflammation and tissue damage, and it shares with AEA anti-inflammatory, analgesic, and antioxidant properties. PEA is a component of the tissue-protective mechanisms acting through the downregulation of mediator release from mast cells. It prevents mast cell degranulation, through an autocoid local injury antagonism mechanism[28]; the anti-inflammatory effect is mediated by the action on mast cells and by its binding to peroxisome proliferator-activated receptor alpha.[29] It is also a ligand for the orphan GPR55 receptor.[30] The relief of neuropathic pain could be mediated through action on receptors located on the nociceptive pathway and on mast cells.[31]

In the human eye, CB1 receptors were found in trabecular meshwork (TM) and Schlemm canal cells.[18,32] Recently, CB2 receptors were demonstrated in porcine TM cells in culture.[33]

AEA and the CB1- and CB2-selective agonists enhance aqueous humor outflow through the conventional pathway and significantly decrease IOP in rabbits, primates, and humans after topical application.[34–39] Conversely, the CB1 antagonist elevated IOP.[8] FAAH was found in the TM tissues; inhibitors of FAAH, by prolonging the effect of AEA, reduce IOP.[38]

In rabbits, instillation of 31.25 μg AEA caused an immediate reduction in IOP; AEA doses of 62.5 μg caused initial increases and subsequent decreases in IOP in the treated eyes.[40] The maximum IOP reduction occurred at 2 hours, and IOP returned to baseline values by 7 hours after administration.[41] Activation of CB1 and CB2 receptors was found to affect TM cell migration, morphology, contractility, actin cytoskeletal architecture, and focal adhesion formation.[42] However, topical administration of PEA had no effect on IOP.[41]

AEA and PEA were found in many human ocular tissues; decreased 2-AG and PEA levels were detected in the ciliary body of glaucomatous eyes,[43] but levels of PEA were not examined in the trabecular meshwork.

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PEA has been used at a dosage of 600 to 1200 mg/d in patients with chronic pain (entrapment neuropathy of the median in the wrist,[44] chronic pelvic pain of pudendal neuralgia)[45] or associated with other compounds in endometriosis.[46]

In the present study, we investigated the effect of systemic administration of PEA (Visimast, 300 mg; Medivis s.r.l., Catania, Italy) on IOP in patients with ocular hypertension and primary open glaucoma (POAG). Secondary outcomes of the study included visual acuity, visual field, vital sign, and psychotropic effects.

Patients, Materials, and Methods

Patients

Forty-two patients were enrolled in a prospective, randomized, double-blind, crossover clinical trial held between November 2008 and August 2009 at the Institute of Ophthalmology of the University of Catania, Italy. This phase IV trial is registered at http://www.umin.ac.jp/ctr/.

The protocol and the consent forms were approved by the Institutional Review Board. The study was performed in accordance with good clinical practice guidelines and adhered to the tenets of the Declaration of Helsinki. All participants provided written informed consent after having received an explanation of the nature and possible consequences of the study.

All study patients (18 years and older) were affected by POAG or ocular hypertension and were treated in both eyes with timolol 0.5% eyedrops twice daily from at least 3 months; all had IOP ranging between 19 and 24 mm Hg. Other treatments were not allowed. All patients were able to perform a reliable visual field (a minimum of 3 tests); OH patients had normal visual fields, and POAG patients had nonadvanced glaucomatous visual field defects (MD, 6–12 dB) nonprogressive for at least 1 year (with at least two visual field examinations during this year); the C/D ratio was lower than 0.6.

Exclusion criteria were need for glaucoma surgical or laser therapy, ocular surgery in the previous year, no tolerability to product under evaluation, visual acuity <8/10 with refractive error >3 diopters, pupillary diameter <2.5 mm, concomitant systemic or ocular pathologies, pregnancy or lactation, vasoactive systemic therapies (Ca- antagonists, oral β-blocker, others), and current tobacco smoker.

Study Design

In this study, 42 patients were randomly assigned to two different groups, (21 each); the first group (group A) received PEA at a dose of 300 mg orally twice daily, and the second group (group B) received placebo tablets (PEA vehicle) for 2 months (period 1).

After a 1-month washout, in the second period of the study, the patients in group A received the placebo treatment and those in group B received PEA treatment, both for 2 months (period 2). Patients and investigators were masked to the treatment assigned. Placebo tables were identical in appearance to the PEA tablets. Study parameters were evaluated at the first, second, third, fourth, and fifth months of treatment.

PEA was administered after the morning and evening meals; timolol 0.5% eyedrops had to be instilled at 7 AM and 7 PM in both eyes; IOP was measured at the defined time (±10 minutes) in both eyes; one eye randomly chosen was considered for the analysis.

Interventions

At baseline and at the following examinations, these parameters were evaluated: best-corrected visual acuity, anterior and posterior segment findings (by biomicroscopy and binocular indirect ophthalmoscopy in mydriasis), evaluation of vertical and horizontal cup-disc ratio, and IOP.

Central corneal thickness measurement and visual field tests were performed at the beginning and at the end of the two periods of the study. IOP was measured in both eyes by Goldmann applanation tonometry (the means of three consecutive readings) with the patient in a sitting position at the slit lamp four times a day (8 am, 12 pm, 4 pm, and 7 pm) (±10 minutes). The mean circadian IOP was the mean of the four measurements.

Visual field was tested by automatic computerized perimetry (24-2 SITA standard test, Humphrey Visual Field Analyzer II; Carl Zeiss Meditec, Inc., Dublin, CA); mean defect (MD) and pattern SD (PSD) were considered. During the study, both eyes were examined; for statistical analysis, one eye was chosen randomly at the beginning of the study.

Patients were asked to complete an adverse events and mood disorder questionnaire (MDQ)[47] for each evaluation period to monitor adverse events, including irritability, anxiety, sleeping disorders, and mood disorders. It is a brief, self-report screening instrument that can be used to identify patients most likely to have bipolar disorder. This form was collected at each visit, and additional comments regarding treatment and adverse events were encouraged from each subject.

Data were collected by two experienced ophthalmologists (CG, EO) between November 2008 and August 2009 and were reported at each visit on an investigator's treatment evaluation form from each patient.

Statistical Analysis

Baseline characteristics of the patients in the two groups were compared by *t*-test. In a nonrandomized open pilot study with PEA in eight glaucomatous patients, with a mean follow-up of 2 weeks, we found an IOP decrease of 12%. The sample size (at least 20 eyes for each group) was determined from the results of our preliminary data to detect, with an alpha of 0.05 and a 90% power (two-tailed), a 15% reduction in IOP.

In each phase, IOP values detected in each group were compared by repeated-measures ANOVA; if significant, multiple comparisons were performed by Tukey-Kramer test. IOP values of two groups at each time point were compared by *t*-test. $P < 0.05$ was considered as statistically significant. Statistical analysis was performed (SPSS, version 13.0; SSPS Inc., Chicago, IL) from Neurovisual Science Technology.

Results

Of the 42 patients enrolled in this trial, 21 received first PEA and 21 received placebo. All patients concluded the study. At baseline, no significant difference was seen between the two groups in age, sex, best-corrected visual acuity, CCT, vertical C/D ratio, MD, PSD, or mean circadian IOP value. (Table 1).

PEA treatment reduced IOP significantly (ANOVA, $P < 0.001$). At baseline, the mean IOP ± SD was 21.6 ± 1.7 mm Hg; at 1 and 2 months, respectively, the mean IOP was 18.4 ± 1.8 mm Hg and 18.1 ± 1.4 mm Hg (both Tukey-Kramer, $P < 0.01$ vs. baseline), and the mean IOP reduction was 3.2 ± 1.3 mm Hg (14.7% ± 6.1%) and 3.5 ± 1.2 mm Hg (15.9% ± 5.1%) (Table 2).

Treatment with placebo did not significantly change the IOP (ANOVA, ns) (mean IOP ± SD at baseline, 21.5 ± 1.5 mm Hg). At 1 and 2 months, respectively, the mean IOP was

Table 1. Baseline Characteristics of Both Groups

	Group A (n = 21)	Group B (n = 21)	P (t-test)
Age, y	65 ± 12	63 ± 11	0.638
Sex, male/female	4:17	7:14	—
Best-corrected visual acuity	0.9 ± 0.1	0.9 ± 0.1	0.795
Central corneal thickness, μm	537 ± 26	542 ± 26	0.550
Vertical C/D ratio	0.4 ± 0.2	0.4 ± 0.2	0.433
Mean defect, dB	−5.83 ± 7.08	−6.42 ± 6.87	0.790
Pattern standard deviation	5.36 ± 3.66	5.54 ± 3.75	0.879
Mean circadian IOP, mm Hg	21.7 ± 0.9	21.6 ± 1.1	0.665

Values are mean ± SD.

TABLE 2. IOP in POAG and OH Patients Who Received PEA or Placebo

Treatment	8 AM	12 AM	4 PM	8 PM	Mean
PEA					
Baseline	21.7 ± 1.9	21.7 ± 1.6	21.1 ± 1.9	21.8 ± 1.5	21.6 ± 1.7
1 mo	18.1 ± 1.9	18.5 ± 1.8	18.5 ± 1.8	18.5 ± 1.7	18.4 ± 1.8
2 mo	17.9 ± 1.5	18.2 ± 1.3	18.5 ± 1.4	18.0 ± 1.5	18.1 ± 1.4
Placebo					
Baseline	21.9 ± 1.8	21.5 ± 1.3	21.3 ± 1.6	21.3 ± 1.2	21.5 ± 1.5
1 mo	21.5 ± 1.2	21.1 ± 1.6	21.0 ± 1.8	20.8 ± 1.7	21.1 ± 1.6
2 mo	21.2 ± 1.6	21.1 ± 1.7	21.4 ± 1.4	21.2 ± 2.2	21.2 ± 1.7

Values are mean ± SD.

21.1 ± 1.6 mm Hg and 21.2 ± 1.7 mm Hg, and the mean IOP reduction was 0.4 ± 1.2 mm Hg (1.8% ± 5.5%) and 0.3 ± 1.3 mm Hg (1.2% ± 6.2%).

IOP was significantly lower in PEA-treated subjects than in placebo-treated subjects at all time points (*t*-test $P < 0.001$). No statistically significant changes were seen in best-corrected visual acuity, CCT, vertical C/D ratio, MD, or PSD. No severe adverse events were recorded; adverse events included influenza (two subjects). One subject had dyspepsia that regressed with regular assumption of the tablets after meals; no changes were found in answers on the MDQ questionnaire.

DISCUSSION

The aim of this study was to investigate the effect of oral administration of PEA on IOP. We found an IOP decrease of 3 mm Hg (~16%) at the 1- and 2-month follow-up visits, which we considered clinically significant. This value was comparable to that of several other antiglaucomatous drugs, as topical carbonic anhydrase inhibitors, (dorzolamide, brinzolamide 17%–20%), α-agonists (brimonidine 18%–25%), and betaxolol (20%–23%).[48]

PEA is an endogenous congener of AEA, and it is cosynthesized with AEA by most cell types. PEA is present in several foods as peanut oil, egg yolk, and soybean lecithin,[49] and it is physiologically present in the mammalian blood at concentrations ranging from 9.4 to 16.7 pmol/mL.[50]

In humans after oral administration of one 300 mg tablet, the plasma concentration doubled after 2 hours and returned to baseline after 6 hours (data on file; submitted to the Ministry of Health, Italy). In rats, higher PEA concentrations were found in the adrenal gland after oral administration, but 0.95% of administered PEA was found in the brain, suggesting that PEA can penetrate the blood-brain barrier.[51] In brain, it was dosed in particular in the hypothalamus and the pituitary gland.[52]

Like anandamide, PEA has analgesic[53] and anti-inflammatory[54] activities. Marketed as a medical food in several European countries, PEA has been used at a dosage of 600/1200 mg/d in chronic pain of entrapment neuropathy of the median in the wrist,[44] in chronic pelvic pain of pudendal neuralgia,[45] or associated with other compounds in endometriosis.[46] It was also used as a topical application in atopic eczema[55] and atopic dermatitis.[56]

Several mechanisms have been ascribed to PEA for analgesic and anti-inflammatory effect, many of them including the effect on mast cells.[29–31] Some of the activities of PEA could result from an increase of cannabinoid tone by an "entourage effect," increasing the cellular levels of AEA through an interference of AEA enzymatic degradation by FAAH.[27]

The effect of PEA on IOP is unclear. AEA and PEA have been found in many human ocular tissues. In glaucomatous patients, decreased 2-AG and PEA levels were detected in the ciliary body, suggesting that both compounds may have a role in this disease, particularly with regard to the regulation of IOP.[43] PEA levels were not examined in the trabecular meshwork.

In rabbits, topical applications of AEA reduced IOP by enhancing the aqueous humor outflow through the conventional pathway.[34–37,39] In the human eye, CB1 receptors were found in trabecular meshwork (TM) and Schlemm canal cells.[18,32] Recently, CB2 receptors were demonstrated in porcine TM cells in culture.[33] Activation of CB1 and CB2 receptors was found to affect TM cell migration, morphology, contractility, actin cytoskeletal architecture, and focal adhesion formation.[42]

Because PEA has no effect on CB1 or CB2 receptors, the effect of PEA could be mediated by an entourage effect, leading to an increase of the cannabinoid tone. Topical administration on PEA did not affect IOP in rabbits,[41] possibly because of the very low solubility of oily dissolved PEA in the tears. However, other factors could be involved; topical delta 9-tetrahydrocannabinol at 0.05% and 0.1% reduced IOP in laboratory animals but not in subjects with POAG.[57]

Several aspects must be clarified. The action of PEA may involve the AEA, but the role of AEA is not completely known. It has been hypothesized that in the eye, AEA could be synthesized and released "on demand" in certain physiological and pathologic conditions. In perfused anterior segment organ culture, URB597, a selective inhibitor of the enzyme FAAH, induced an increase of outflow facility under higher pressure (15 mm Hg) but had no effect under normal perfusion pressure (7.35 mm Hg).[39] There are no data about PEA levels in ocular tissue after systemic administration.

This study was performed after the observation of IOP reduction in patients who had been treated with PEA for a possible neuroprotective effect: in a group of glaucomatous patients, with a mean follow-up of 1 month, we found an IOP decrease of 12%. All patients included were treated with timolol 0.5% eyedrops twice a day to exclude any possible source of variation. The dosage and route of administration of PEA are the same used as those in other studies. A washout period of 1 month was defined to exclude any possible residual action on IOP.

No specific side effects have been reported with PEA, and no contraindications have been defined. We excluded patients with concomitant systemic or ocular pathologies, pregnancy or lactation, or vasoactive systemic therapies (Ca- antagonists, oral β-blocker, others) and those who were current tobacco smokers. We detected few and nonspecific adverse events; however, the safety assessment of a drug requires a large series and a long follow-up.

We did not found significant changes in systemic blood pressure; cannabinoids reduce blood pressure, with an effect particularly involving CB1 receptors.[58] The lack of effect of PEA on CB1 and CB2 receptors could explain our findings and could also explain the absence of significant psychotropic effects after 2 months of treatment. We used MDQ for evalu-

ating this aspect, but further studies with more sensitive tests should evaluate PEA effects, also after long-term treatment. In addition, longer studies with a wider series are required to fully elucidate the effect of PEA on IOP. This study has the limitation of a small number of patients treated and a short period of treatment.

In conclusion, systemic administration of PEA reduces IOP in patients with glaucoma and ocular hypertension; mild adverse events were recorded. The decrease in IOP was 16% of baseline IOP, not much different from that of some ocular hypotensive drugs currently used (topical CAI, alpha agonists). This suggests that PEA, a drug of a class that has been proposed for the treatment of glaucoma but that is not used because of concerns about side effects,[59,60] could be a valuable tool in the treatment of such disease.

References

1. Quigley HA. Number of people with glaucoma worldwide. *Br J Ophthalmol.* 1996;80:389–393.
2. Hepler RS, Frank IR. Marihuana smoking and intraocular pressure. *JAMA.* 1971;217:1392.
3. Drew WG, Miller LL. Cannabis: neural mechanisms and behavior—a theoretical review. *Pharmacology.* 1974;11:12–32.
4. Benowitz NL, Rosenberg J, Rogers W, Bachman J, Jones RT. Cardiovascular effects of intravenous delta-9-tetrahydrocannabinol: autonomic nervous mechanisms. *Clin Pharmacol Ther.* 1979;25: 440–446.
5. Grotenhermen F. Cannabinoids in pain management: cannabinoid receptor agonists will soon find their place in modern medicine. *BMJ.* 2001;323:1250–1251.
6. Lockhart AB, West ME, Lowe HI. The potential use of *Cannabis sativa* in ophthalmology. *West Indian Med J.* 1977;26:66–70.
7. Colasanti BK. Ocular hypotensive effect of marihuana cannabinoids: correlate of central action or separate phenomenon? *J Ocul Pharmacol.* 1986;2:295–304.
8. Pate DW, Jarvinen K, Urtti A, Mahadevan V, Jarvinen T. Effect of the CB1 receptor antagonist. SR141716A on cannabinoid-induced ocular hypotension in normotensive rabbits. *Life Sci.* 1998;63: 2181–2188.
9. Buchwald A, Browne CE, Wu WM, Ji F, Bodor N. Soft cannabinoid analogues as potential anti-glaucoma agents. *Pharmazie.* 2000;55: 196–201.
10. Beilin M, Neumann R, Belkin M, Green K, Bar-Ilan A. Pharmacology of the intraocular pressure (IOP) lowering effect of systemic dexanabinol (HU-211), a non-psychotropic cannabinoid. *J Ocul Pharmacol Ther.* 2000;16:217–230.
11. Laine K, Jarvinen K, Pate DW, Urtti A, Jarvinen T. Effect of the enzyme inhibitor phenylmethylsulfonyl fluoride on the IOP profiles of topical anandamides. *Invest Ophthalmol Vis Sci.* 2002;43: 393–397.
12. Hanus L, Gopher A, Almog S, Mechoulam R. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem.* 1993;36:3032–3034.
13. Mechoulam R, Ben-Shabat S, Hanus L, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol.* 1995;50:83–90.
14. Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol.* 1988;34:605–613.
15. Devane WA, Hanus L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992;258:1946–1949.
16. Matsuda LA, Bonner TI, Lolait SJ. Cannabinoid receptors: which cells, where, how, and why? *NIDA Res Monogr.* 1992;126:48–56.
17. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993;365:61–65.
18. Straiker AJ, Maguire G, Mackie K, Lindsey J. Localization of cannabinoid CB1 receptors in the human anterior eye and retina. *Invest Ophthalmol Vis Sci.* 1999;40:2442–2448.
19. Wan Z, Woodward DF, Stamer WD. Endogenous bioactive lipids and the regulation of conventional outflow facility. *Expert Rev Ophthalmol.* 2008;3:457–470.
20. Di Marzo V, De Petrocellis L, Fezza F, Ligresti A, Bisogno T. Anandamide receptors. *Prostaglandins Leukot Essent Fatty Acids.* 2002;66:377–391.
21. Howlett AC, Barth F, Bonner TI, et al. International union of pharmacology, XXVII: classification of cannabinoid receptors. *Pharmacol Rev.* 2002;54:161–202.
22. Ross RA. Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol.* 2003;140:790–801.
23. Lambert DM, Di Marzo V. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? *Curr Med Chem.* 1999;6:757–773.
24. Lambert DM, Vandevoorde S, Jonsson K-O, Fowler CJ. The palmitoylethanolamide family: a new class of anti-inflammatory agents? *Curr Med Chem.* 2002;9:663–674.
25. De Petrocellis L, Davis JB, Di Marzo V. Palmitoylethanolamide enhances anandamide stimulation of human vanilloid VR1 receptors. *FEBS Lett.* 2001;506:253–256.
26. 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.
27. Ben-Shabat S, Fride E, Sheskin T, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol.* 1998;353:23–31.
28. Aloe L, Leon A, Levi-Montalcini R. A proposed autacoid mechanism controlling mastocyte behaviour. *Agents Actions.* 1993;39(spec no):C145–C147.
29. LoVerme J, La Rana G, Russo R, Calignano A, Piomelli D. The search for the palmitoylethanolamide receptor. *Life Sci.* 2005;77: 1685–1698.
30. Ryberg E, Larsson N, Sjögren S. et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol.* 2007;152:1092–101.
31. Re G, Barbero R, Miolo A, Di Marzo V. Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in protection against tissue inflammation and pain: potential use in companion animals. *Vet J.* 2007;2007 173:21–30.
32. Stamer WD, Golightly SF, Hosohata Y, et al. Cannabinoid CB(1) receptor expression, activation and detection of endogenous ligand in trabecular meshwork and ciliary process tissues. *Eur J Pharmacol.* 2001;431:277–286.
33. Zhong L, Geng L, Njie Y, Feng W, Song ZH. CB2 cannabinoid receptors in trabecular meshwork cells mediate JWH015-induced enhancement of aqueous humor outflow facility. *Invest Ophthalmol Vis Sci.* 2005;46:1988–1992.
34. Chien FY, Wang RF, Mittag TW, Podos SM. Effect of WIN 55212–2, a cannabinoid receptor agonist, on aqueous humor dynamics in monkeys. *Arch Ophthalmol.* 2003;121:87–90.
35. Song ZH, Slowey CA. Involvement of cannabinoid receptors in the intraocular pressure-lowering effects of WIN55212–2. *J Pharmacol Exp Ther.* 2000;292:136–139.
36. Pate DW, Jarvinen K, Urtti A, et al. Effects of topical anandamides on intraocular pressure in normotensive rabbits. *Life Sci.* 1996;58: 1849–1860.
37. Porcella A, Maxia C, Gessa GL, Pani L. The synthetic cannabinoid WIN55212–2 decreases the intraocular pressure in human glaucoma resistant to conventional therapies. *Eur J Neurosci.* 2001; 13:409–412.
38. Njie YF, Kumar A, Qiao Z, Zhong L, Song ZH. Noladin ether acts on trabecular meshwork cannabinoid (CB1) receptors to enhance aqueous humor outflow facility. *Invest Ophthalmol Vis Sci.* 2006; 47:1999–2005.
39. Njie YF, Qiao Z, Xiao Z, Wang W, Song ZH. N-arachidonylethanolamide-induced increase in aqueous humor outflow facility. *Invest Ophthalmol Vis Sci.* 2008;49:4528–4534.
40. Pate DW, Jarvinen K, Urtti A, Jarho P, Jarvinen T. Ophthalmic arachidonylethanolamide decreases intraocular pressure in normotensive rabbits. *Curr Eye Res.* 1995;14:791–797.

41. Mikawa Y, Matsuda S, Kanagawa T, Tajika T, Ueda N, Mimura Y. Ocular activity of topically administered anandamide in the rabbit. *Jpn J Ophthalmol.* 1997;41:217–220.
42. Kumar A, Song ZH. CB1 cannabinoid receptor-mediated changes of trabecular meshwork cellular properties. *Mol Vis.* 2006;12:290–297.
43. Chen J, Matias I, Dinh T, et al. Finding of endocannabinoids in human eye tissues: implications for glaucoma. *Biochem Biophys Res Commun.* 2005;330:1062–1067.
44. Conigliaro R, Drago V, Foster PS, Schievano C, Di Marzo V. Use of palmitoylethanolamide in the entrapment neuropathy of the median in the wrist. *Minerva Med.* 2011;102:141–147.
45. 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.
46. Indraccolo U, Barbieri F. Effect of palmitoylethanolamide-polydatin combination on chronic pelvic pain associated with endometriosis: preliminary observations. *Eur J Obstet Gynecol Reprod Biol.* 2010;150:76–79.
47. Hirschfeld RM, Williams JB, Spitzer RL, et al. Development and validation of a screening instrument for bipolar spectrum disorder: the Mood Disorder Questionnaire. *Am J Psychiatry.* 2000;157:1873–1875.
48. European Glaucoma Society. *Terminology and Guidelines for Glaucoma*. 3rd ed. Savona, Italy: Dogma Publishers; 2008.
49. Kuehl FA, Jacob TA, Ganley OH, Ormond RE, Meisinger MAP. The identification of N-(2-hydroxyethyl)-palmitamide as a naturally occurring anti-inflammatory agent. *J Am Chem Soc.* 1957;79:5577–5578.
50. Zolese G, Bacchetti T, Ambrosini A, Wozniak M, Bertoli E, Ferretti G. Increased plasma concentrations of palmitoylethanolamide, an endogenous fatty acid amide, affect oxidative damage of human low-density lipoproteins: an in vitro study. *Atherosclerosis.* 2005;182:47–55.
51. Zhukov OD. [Distribution of N-([1–14C]-palmitoyl)ethanolamine in rat tissues]. *Ukr Biokhim Zh.* 1999;71:124–125.
52. Artamonov M, Zhukov O, Shuba I, et al. Incorporation of labelled N-acylethanolamine (NAE) into rat brain regions in vivo and adaptive properties of saturated NAE under x-ray irradiation. *Ukr Biokhim Zh.* 2005;77:51–62.
53. Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. *Nature.* 1998;394:277–281.
54. Berdyshev E, Boichot E, Corbel M, Germain N, Lagente V. Effect of cannabinoid receptor ligands on LPS-induced pulmonary inflammation in mice. *Life Sci.* 1998;63:PL125–PL129.
55. Eberlein B, Eicke C, Reinhardt HW, Ring J. Adjuvant treatment of atopic eczema: assessment of an emollient containing N-palmitoylethanolamine (ATOPA study). *J Eur Acad Dermatol Venereol.* 2008;22:73–82.
56. Kircik L. A nonsteroidal lamellar matrix cream containing palmitoylethanolamide for the treatment of atopic dermatitis. *J Drugs Dermatol.* 2010;9:334–338.
57. Merritt JC, Perry DD, Russell DN, Jones BF. Topical delta 9-tetrahydrocannabinol and aqueous dynamics in glaucoma. *J Clin Pharmacol.* 1981;21:467S–471S.
58. Pacher P, Bátkai S, Kunos G. Cardiovascular pharmacology of cannabinoids. *Handb Exp Pharmacol.* 2005;168:599–625.
59. Green K. Marijuana smoking vs cannabinoids for glaucoma therapy. *Arch Ophthalmol.* 1998;116:1433–1437.
60. Jarvinen T, Pate DW, Laine K. Cannabinoids in the treatment of glaucoma. *Pharmacol Ther.* 2002;95:203–220.