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Topical Mecamylamine for Diabetic Macular Edema

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Introduction

Nicotinic acetylcholine (nACh) receptors play an important role in neurotransmission. They are ligand-gated ion channels made up of 5 subunits that form a central pore (for review see ¹). Seventeen subunits have been identified, $\alpha 1$ - $\alpha 10$, $\beta 1$ - $\beta 4$, γ , δ , and ϵ . Homomeric and heteromeric pentamers can form so that there are numerous nACh receptor subtypes. Binding of acetylcholine, the endogenous ligand, induces a conformational change that opens the ion pore causing influx of cations. The heteromeric nAChRs are differentially permeable to sodium, whereas the homomeric nAChRs are differentially permeable to

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Potential Conflicts of Interest

QDN, DVD, and PAC receive research support and have an institutional consulting agreement through which JHU receives compensation with Genentech, Inc. PAC has an institutional consulting agreement with GlaxoSmithKline. QDN is a consultant for Bausch and Lomb and has research support from Genentech, Inc. and Regeneron, Inc. PAC serves on the data and safety monitoring committee for a phase III trial sponsored by Regeneron, Inc. and has research support from Alimera and CoMentis for diabetic macular edema trials. These activities are being managed by the Conflict of Interest Committee of the Johns Hopkins University School of Medicine. JPC is a scientific advisor and holds equity in CoMentis, and receives royalties as an inventor on Stanford patents licensed to CoMentis. JSH is a consultant for Genentech, Alcon, Allergan, Bausch and Lomb, Eyemaginations, Fovea, Genzyme, Heidelberg, IScience, ISTA, Jerini, LPath, NeoVista, Nodal Vision, Novagali, Novartis, Ophtherion, Oxigene, Paloma, Pfizer, Regeneron, Resolvix, Schering Plough, Scyfix, VisionCare and has received honoraria from Genentech, Heidelberg, Jerini, NeoVista, Optimedica, and Regeneron.

Contributions of authors

Peter A. Campochiaro: conception and design, analysis and interpretation, writing the article, critical revision of the article, final approval of the article, data collection, provision of materials patients and resources, literature search
Syed Mahmood Shah: analysis and interpretation, writing the article, critical revision of the article, final approval of the article, data collection, statistical expertise
Gulnar Hafiz: analysis and interpretation, final approval of the article, data collection, administrative and technical support
Jeffery Heier: critical revision of the article, final approval of the article, data collection, provision of patients
Eugene Lit: critical revision of the article, final approval of the article, data collection, provision of patients
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Jim Monk: final approval of the article, data collection, and logistical support
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Ken Kengatharan: helped in study design, provided materials and resources, and approved the manuscript
Henry Hsu: helped in study design, provided materials and resources, and approved the manuscript

calcium^{2, 3}. Sustained exposure to acetylcholine may result in desensitization, a progressive loss of response.⁴ Receptor subtypes differ with respect to activation and desensitization depending upon their subunit makeup and extent of phosphorylation resulting in different magnitudes of effect or even opposing effects mediated by different subtypes on the same cell.⁵

In addition to binding acetylcholine, nACh receptors bind nicotine; the activation of heteromeric nAChRs by nicotine is responsible for addictive effects of cigarette smoke.⁶ Binding of nicotine distinguishes nACh receptors from muscarinic acetylcholine receptors, which are activated by acetylcholine, but not nicotine. The muscarinic receptors are G protein-coupled 7-transmembrane spanning receptors that are very different in structure. Nicotine provides a tool for identification of nACh receptors and has been used to determine that they are present on some non-neuronal as well as neuronal cells. Vascular endothelial cells contain nACh receptors which may be activated by exogenous nicotine, or endogenous acetylcholine. Endothelial nAChR activation increases proliferation, migration, and tube formation of endothelial cells *in vitro*, and neovascularization in atherosclerotic plaques and tumors *in vivo*^{7, 8}. One source of endogenous acetylcholine is the endothelium itself, as endothelial cells synthesize, store and release acetylcholine resulting in autocrine loops⁹. In the eye, nicotine stimulates choroidal neovascularization, which may contribute to the increased progression to neovascular age-related macular degeneration (AMD) seen in smokers with non-neovascular AMD.^{10, 11} Nicotine also stimulates increased vascular permeability in brain.¹² Endothelial $\alpha 7$ -nAChRs have been implicated as the major subtype mediating nicotine-induced vascular permeability and angiogenesis.^{12, 13}

There are several nACh receptor antagonists that bind to nACh receptors and reduce activation by agonists. Mecamylamine is a nonspecific nACh receptor antagonist that was approved as an oral antihypertensive agent in the 1950s.¹⁴ It reduces blood pressure by blocking neuronal nAChR-mediated neurotransmission at sympathetic ganglia permitting blood vessels to dilate. Mecamylamine has a good safety profile at antihypertensive doses of 30–90 mg/day but may cause orthostatic hypotension, as well as anti-cholinergic side effects including constipation, urinary retention, and dry mouth.¹⁵ Mecamylamine is able to cross the blood-brain barrier and has been used as an antidepressant¹⁶ and to facilitate cessation of smoking.¹⁷

The previous use of mecamylamine in humans made it a good choice to test whether blocking nAChRs can reduce abnormal retinal vascular permeability in patients with diabetic macular edema (DME). To avoid orthostatic hypotension, constipation, urinary retention and dry mouth, a formulation for local topical ocular delivery was developed. Application of this formulation containing 0.1 or 1% mecamylamine three times a day in rabbits resulted in detectable levels of mecamylamine in the retina and suppressed choroidal neovascularization in mice.¹¹ During 6 hours of sampling after a single application of 1% mecamylamine to the cornea of rabbits, high levels of mecamylamine were detected by high performance liquid chromatography in retina/choroid with mean peak levels of 5000–11300 ng/mL (Henry Hsu, personal communication, November 17, 2009). Area-under-the-curve was ~7500 ng/mL•hr using a trapezoidal rule on a population pharmacokinetic basis and comparison of levels in different parts of the eye suggested that topically delivered mecamylamine gains access to the choroid and retina by penetration through the conjunctiva and sclera. Topical application of 1% mecamylamine twice a day in normal volunteers was well-tolerated with no adverse effects. In this study, we tested the effect of topical mecamylamine on foveal thickness and visual acuity in patients with DME.

Materials and Methods

This is a phase I/II, open-label clinical trial conducted at 3 sites in the U.S. through an IND granted by the Food and Drug Administration. The study adhered to the guidelines of the Declaration of Helsinki and the protocol and consent form were approved by a local investigational review board (IRB) or by Western IRB. Each subject provided written informed consent. The study is registered at www.clinicaltrials.gov under the identifier NCT00536692.

Patient Eligibility and Exclusion Criteria

Patients (18 or older) with type 1 or type 2 diabetes and DME were eligible if they had reduction in visual acuity between 20/40 and 20/400 and met the following criteria: (1) center subfield thickness measured by optical coherence tomography (OCT) ≥ 250 μm , (2) no potential contributing causes to reduced visual acuity other than DME, (3) no indication of permanent vision loss such as atrophy and pigmentary change in the fovea, (4) no intraocular surgery, laser photocoagulation, or treatment with an antagonist of vascular endothelial growth factor (VEGF) within 3 months, (5) no media problems that would preclude retinal evaluation, (6) no treatment with any investigational agent within 3 months, (7) $\text{HbA1C} \leq 12\%$.

Study Drug

Mecamylamine HCl ophthalmic solution (CoMentis, Inc., South San Francisco, CA) is a clear colorless buffered aqueous solution containing 0.01% benzalkonium chloride and ethylenediaminetetraacetic acid as a chelating agent. It was supplied as a sterile 1% solution in clear low density polyethylene bottles with controlled dropper tips for multi-use.

Study Protocol

Consenting patients were screened for the study with a medical history, physical examination, measurement of best-corrected visual acuity (BCVA) by an experienced examiner using the ETDRS protocol,¹⁸ a complete eye examination, an OCT, a fluorescein angiogram, and laboratory tests on blood and urine. If both eyes met eligibility criteria one was selected by the enrolling investigator to be the study eye. Eligible patients returned for a baseline visit and had measurement of BCVA, complete eye examination, and OCT. Patients were given 1% mecamylamine eye drops and instructed in their use. It was requested that the drops be administered twice a day. Follow up study visits were at 1, 4, 8, 12, and 16 weeks. At each visit, patients had safety assessments, measurement of BCVA, complete eye examination, and OCT. At 12 weeks, the primary endpoint, patients also had a repeat physical examination and hematology and blood chemistry lab tests. At one site, patients had an anterior chamber tap at baseline, 1 week and 12 weeks for measurement of aqueous VEGF levels.

OCTs

OCT scans were performed by an experienced investigator with a StratusOCT3™ (Carl Zeiss Meditec, Dublin, CA) using the Fast Macular Scan protocol. This protocol consists of 6 line scans that are 6.0 mm long centered on fixation and spaced 30° apart around the circumference of a circle. Each line consists of 128 A-scan measurements. With each A-scan, the OCT software measures the distance between the inner surface of the retina and the anterior border of retinal pigmented epithelium (RPE)-choriocapillaris complex based on changes in reflectivity. The center point thickness, also known as the foveolar thickness, is a mean value generated by the StratusOCT software from the 6 central A-scan thickness values of each of the radial lines comprising the fast macular thickness map. We did not use

this value generated from only 6 data points for our primary measure of central retinal thickness, but instead used the foveal or central 1mm thickness, which is an average interpolated value based on central 21 scans of each of the six lines passing through the patient's fixation. The number of data points used to compute this value is $21 \times 6 = 126$, which provides a better representation of the thickness of the central retina than a value generated from only 6 points around fixation. Readers at the Wilmer Retinal Imaging Research and Reading Center examined the images for each OCT file to be sure that there were no artifacts such as misidentification of inner or outer surface of the retina. When artifacts were present, corrected measurements were obtained using RetinaTOMOGRAPHER software (version 1.1, Retinal Imaging Research and Reading Center, Baltimore, MD). Excess foveal thickness (EFT) was calculated by subtracting the measured foveal thickness value from $212 \mu\text{m}$, the upper limit of the normal range of center subfield thickness determined from measurements on a large population of subjects¹⁹.

Measurement of VEGF levels in aqueous

Anterior chamber taps were performed under topical anesthesia by biomicroscopic observation of the eye with the patient seated at a slit lamp. A 30-gauge needle was inserted into the anterior chamber and 0.1 ml of aqueous was removed and stored frozen at -80°C until assayed. Aqueous VEGF levels were measured with the Human VEGF Quantikine ELISA kit (R&D Systems, Minneapolis, MN) using the manufacturer's instructions. Three separate assays were performed to ensure results were reproducible.

Results

The primary objective of this study was to evaluate the safety of topical mecamlamine in patients with DME. A few patients reported transient stinging after application, but in general the drops were well-tolerated. Twenty-three patients were enrolled in the study and 19 completed all study visits. Two patients withdrew consent before the week 1 visit and no data are available for those patients; one patient decided that the time commitment was too great and the other decided that an eye drop would be unlikely to be strong enough to help their condition. One patient was withdrawn from the study for alternative treatment due to worsening of DME after the month 2 visit and this patient's observations at month 2 were carried forward to months 3 and 4. One patient made all study visits through the primary endpoint and missed the month 4 visit for which the data at month 3 were carried forward. Thus there are 21 patients for whom data are evaluated. There were no serious adverse events and no drug-related adverse events.

The secondary objective of the study was to assess for potential bioactivity of topical mecamlamine. In early phase studies, it is useful to examine the course of each patient in great detail and therefore BCVA and OCT scans are presented for each patient at each study visit.

Subgroup of eight patients showing improvement

Figure 1 shows the course for eight patients who showed signs of improvement on topical mecamlamine. Patients 01–008, 03–007, 02–003, and 01–003 showed improvements in BCVA of 9, 6, 14, and 15 letters at the week 12 primary endpoint along with a substantial reduction in EFT at some point during the course. Red free photographs of patient 01–008 show a reduction in exudates between baseline and 3 months and fluorescein angiography illustrated a corresponding reduction in leakage and pooling of dye in the macula during the late phase of the angiogram (Figure 2). The concordance of anatomical and functional improvement increases confidence that these patients truly had benefit. Patient 03–005 showed improvement in BCVA of 22 letters despite a modest change in EFT. Patient 01–

011 did not show a substantial change in EFT at any time, but showed an improvement in BCVA of more than 2 lines at 1 week (generally a 2 line change is our threshold for taking a BCVA change as clinically significant) and although it fluctuated somewhat thereafter, an improvement was substantiated. In both of these patients, the improvement in vision was substantial, occurred soon after starting the drug, and was reproduced in multiple measurements for the remainder of the study. Both of these patients had modest EFT at baseline and therefore even modest changes might be expected to have a substantial functional impact. Patients 01–002 and 03–009 had mild macular edema at BL that was essentially completely eliminated by week 12 with normal appearing scans, but this was not accompanied by substantial improvement in BCVA. Patients may have permanent visual loss from chronic macular edema so that BCVA cannot improve despite elimination of the edema. In retrospect, this situation might have been predicted for these two patients, because despite the mild edema at BL, the BCVA was quite poor.

Subgroup of nine patients showing no change

Patients 01–010, 02–005, and 03–013 showed a similar pattern in that they showed some evidence of improvement through week 8, but during the last 4 weeks of mecamlamine treatment there was a substantial drop in BCVA that recovered one month after mecamlamine was stopped (Figure 3).

Patients 01–009 and 03–012 showed improvements in BCVA of 8 and 7 letters, respectively, but this was not accompanied by a substantial change in EFT (Figure 4). Patient 03–012 had a reduction in EFT of 41 and 38 μm at 4 and 8 weeks and since the reproducibility limit of time domain OCT is in the range of 30 μm , this could represent a real change that might explain the improvement of 7 letters. However, this patient never had an improvement of 2 or more lines in BCVA at any time point and is therefore listed as equivocal. Similarly, patients 01–009 and 03–012 had severe edema at baseline, but did not have a substantial change in EFT. Patients 01–006 and 01–007 showed minimal change in BCVA and no change (01–006) or modest worsening (01–007) in EFT. Patients 03–004 and 02–004 showed essentially no change in BCVA (+3 and +1) and a modest improvement (03–004) or worsening (02–004) in EFT.

Subgroup of four patients showing mecamlamine-induced worsening

Patient 03–002 had a 15 letter reduction in BCVA and a 248 μm increase in excess central subfield thickening over the 3 months on mecamlamine. Notably, 1 month after stopping mecamlamine, there was resolution of the macular edema with a reduction of 516 μm in EFT accompanied by a gain of 14 letters in BCVA (Figure 5). The magnitude of the worsening as well as the rapidity and magnitude of the reversal after mecamlamine was discontinued implicate mecamlamine, rather than a spontaneous change in DME status. Patient 01–005 had a 20 letter reduction in BCVA and a 238 μm increase in EFT over the 3 months on mecamlamine; within 1 month of cessation of mecamlamine the BCVA improved by 10 letters and the EFT decreased by 20 μm . Patient 03–010 had severe edema with EFT at BL of 492 μm that was reduced by 128 μm at 8 weeks. However, at 12 weeks, EFT increased by 78 μm , and BCVA fell by 9 letters compared to baseline. After stopping mecamlamine the edema continued to worsen and the BCVA remained the same. Patient 03–011 was withdrawn from the study for alternative treatment at M2 because of a reduction in BCVA of 14 letters and an increase in EFT thickness of 288 μm .

Mean and median changes in best-corrected visual acuity and excess foveal thickness

There was no change in mean EFT throughout the study (Figure 6A). Median EFT was slightly above BL at the primary endpoint and below BL at month 4 (Figure 6B). There was an increase in mean BCVA of 2.8 letters at 1 week after starting mecamlamine, but there

was a substantial dip to 0.8 letters at week 12, the primary endpoint with rebound to 3.1 letters 4 weeks after stopping mecamlamine. Median improvement in BCVA peaked at 2 letters at month 2 and was 1 letter at the primary endpoint.

Aqueous VEGF levels

To determine if mecamlamine influenced VEGF levels in patients with DME, aqueous VEGF levels were measured in 9 patients enrolled at one site at baseline, day 7, and day 84, the primary endpoint. There were small reductions in mean and median VEGF levels between baseline and day 84 that were not statistically significant (Figure 7A). Two of 4 patients in category 1 who showed improvement on mecamlamine had reductions in aqueous VEGF of 105 and 168 pmol/ml during the 12 weeks of treatment, but the other two showed increases of 52 and 62 (Figure 7B). Patient 1-005 who worsened while on mecamlamine had a reduction in aqueous VEGF of 93 pmol/ml. Thus, although the numbers are very small which would preclude any firm conclusions in any case, there were no consistent correlations of outcome with alterations of VEGF.

Glycemic control

Sometimes when patients enter clinical trials, their compliance improves resulting in improvement in glycemic control which can have an impact on DME. The mean ($\pm$ standard error of the mean) HbA1C at baseline and week 12 was 7.88 ± 0.29 and 8.33 ± 0.28 , respectively. None of the patients in this trial had a substantial improvement in HbA1C and therefore changes in glycemic control did not play any role in the changes in DME that were observed.

Discussion

This is the first study investigating the use of topical mecamlamine for an ocular disease and the formulation was found to be safe and well-tolerated. Although the study was not powered to assess the efficacy of topical mecamlamine in patients with DME, there were several findings that suggest that mecamlamine gained access to the retina and had biological effects. Eight of the 21 patients for whom data were available showed evidence of improvement. While patients with DME may occasionally show small spontaneous improvements, one would not expect 1/3 of the patients to show spontaneous improvement. Furthermore, the magnitude of changes in several of the patients was substantial and not likely part of the natural history of DME. Four patients showed substantial worsening of DME while receiving topical mecamlamine. While worsening is part of the natural history of DME, it usually occurs gradually. In patient 01-005, the increase in EFT of 134 μ m within 1 week of starting mecamlamine and the increase of 238 μ m over 12 weeks accompanied by a 20 letter drop in BCVA is unusual. Patient 03-002 had a very similar course in which there was an increase in EFT of 140 μ m with a week of starting mecamlamine and an increase in EFT of 248 μ m over 12 weeks accompanied by a 15 letter drop in BCVA. In addition, both patients had a sudden reversal with improvements in BCVA of 10 and 14 letters 1 month after mecamlamine was stopped. The accompanying sudden reduction in EFT from 527 μ m to 11 μ m with resolution of large cysts in patient 03-002 is particularly dramatic and strongly implicates mecamlamine in the worsening and its withdrawal in the sudden reversal. This interpretation is strengthened by observations in patients 03-010 and 03-011. Patient 03-010 had an initial reduction in EFT, but between months 2 and 3, there was an increase of 68 μ m accompanied by reduction in BCVA of 6 letters. Patient 03-011 had an increase of 288 μ m in EFT and reduction of 14 letters in BCVA over 2 months on mecamlamine and as a result the patient was withdrawn from the study to receive alternative treatment. Patients 01-010, 02-005, and 03-013 provide additional evidence for a mecamlamine-induced paradoxical response, because they

initially showed some evidence of benefit or stability, but had a substantial worsening in BCVA between weeks 8 and 12 followed by a rebound improvement after mecamlamine was stopped. Thus, there were a total of 4 patients who clearly got worse on mecamlamine, 2 of whom improved when mecamlamine was stopped, and 3 additional patients that had a sudden reduction in BCVA during the third month of mecamlamine treatment that reversed when mecamlamine was stopped. These patients suggest that mecamlamine may cause a reversible exacerbation of DME in some patients, but more follow up would have been useful to determine if those patients who improved after mecamlamine was stopped showed continued improvement over time.

Viewed in aggregate, the study population did not show substantial changes in mean and median BCVA and EFT. If these data were obtained without a detailed look at individual patients, it could easily be concluded that mecamlamine has no effect in patients with DME. Instead, it seems likely that a single topical dose of 1% of mecamlamine given twice a day has different effects in different patients, causing benefit in some, little effect in others, and worsening in others.

One possible explanation for this paradoxical result has its basis in the multiple receptor subtypes that exist for nAChRs. Since receptor subtypes differ with regard to activation and desensitization depending upon their subunit makeup, these different subtypes may mediate different magnitudes of effect or even opposing effects. If patients differ with regard to the population of nAChR subtypes they express on retinal vascular endothelial cells, this would explain the differing responses to mecamlamine. Much of nicotine's proangiogenic and pro-permeability effects appear to be mediated through $\alpha 7$ homomeric nAChRs, but cultured retinal vascular endothelial cells express mRNA for a diverse set of subunits.¹¹ Notably, one of these other subunits, $\alpha 9$, forms $\alpha 9$ homomeric or $\alpha 9\alpha 10$ heteromeric receptors that oppose the effects of the $\alpha 7$ homomeric receptor in human microvascular endothelial cells.²⁰ Using siRNA methodology, it was found that the $\alpha 7$ nAChR plays a dominant role in nicotine-induced endothelial cell signaling (assessed by intracellular calcium imaging and studies of protein expression and phosphorylation), as well as nicotine-activated endothelial cell functions (proliferation, survival, migration and tube formation). Notably, selectively knocking down the expression of the $\alpha 9$ or the $\alpha 7$ nAChRs revealed that these receptors have opposing effects on nicotine-induced endothelial cell proliferation and survival.

The simplest explanation for the divergent effects of mecamlamine in our DME patients is that one or more nAChR subtypes have opposing effects on endothelial permeability. Furthermore, these nAChRs may be expressed to a variable degree in patients with DME. Patients with a high percentage of pro-permeability nAChR subtypes on their retinal endothelial cells would have a substantial contribution to leakage from acetylcholine and/or nicotine and would benefit from mecamlamine. Those patients with a low percentage of pro-permeability nACh receptor subtypes would not benefit, and may even worsen if anti-permeability nAChRs were antagonized by the non-specific nAChR mecamlamine.

It is unclear how nAChR signaling in endothelial cells interacts with VEGF signaling. There was a slight decrease in mean and median aqueous VEGF levels during 3 months of treatment with mecamlamine in 9 patients at one site. This difference was not statistically significant and the number of patients tested is too small to draw any conclusions, but there was not a good correlation between outcome and alteration in VEGF levels. Measurements in a much larger cohort of DME patients treated with mecamlamine would be needed to determine if mecamlamine alters VEGF levels and impacts edema in that way.

Further work is needed to determine which nAChR subtypes contribute to acetylcholine- and nicotine-induced retinal vascular permeability and which, if any, oppose it. Such

knowledge could lead to the development of more specific nAChR antagonists, directed against the pro-permeability nAChRs (eg. the α_7 nAChR). Such specific antagonists would be expected to be more consistently efficacious in patients with DME. On the other hand, if it were possible to predict which patients are likely to respond favorably to topical mecamlamine, it still might have a role in the treatment of DME. Identification of the nAChR subtypes that contribute to excessive permeability in DME, and technology to determine endothelial nAChR expression in patients, could provide therapeutic direction in the use of topical mecamlamine. Even without that information, it may be reasonable to consider additional trials with different treatment regimens. Most patients who worsened on mecamlamine did so only after 2 months and most patients who improved maintained benefit for at least a month after treatment was stopped. It might be reasonable to test intermittent treatment periods of 2 months separated by one month treatment holidays.

Intraocular injections of ranibizumab provide benefit in patients with DME, but repeated injections are needed to sustain benefit.^{21, 22} There is great need for treatments that can be given by routes other than intraocular injection such as topical mecamlamine, if they will allow reduction in the frequency of intraocular injections of ranibizumab in a substantial number of patients. Before abandoning mecamlamine, all strategies that may definitively prove or disprove its worth should be considered.

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Figure 1. Eight patients who showed evidence of improvement on topical mecamlamine
 Each column containing nine rows is shown for 8 patients who demonstrated some evidence of improvement. The top row shows the patient identifier with the first two digits indicating the site and the next 3 digits indicating the enrollment number. Rows 2–7 show a horizontal cross-section time domain optical coherence tomography scan through the fovea at each time point of the study, baseline (BL), day 7 (D7), month 1 (M1), month 2 (M2), month 3 (M3), and month 4 (M4). The seventh row in each column shows a bar chart depicting excess center subfield thickening at each time point and the eighth row show a line graph depicting the change from BL in best-corrected visual acuity (BCVA) shown as number of letters read at 4 meters on an Early Treatment Diabetic Retinopathy (ETDRS) chart. Mecamlamine drops were stopped at M3, the primary endpoint of the study, and thus the data for M4 was obtained 1 month after stopping mecamlamine. Patients 01–008, 03–007, and 02–003 showed improvements in BCVA of 9, 6, and 14 letters at the primary endpoint along with substantial reduction in excess center subfield thickening. Patient 01–003 showed improvement in BCVA of 15 letters along with a modest reduction in excess center subfield thickening. Patient 03–005 showed improvement in BCVA of 22 letters despite a modest change in EFT and Patient 01–011 showed an improvement in BCVA of 9 letters with little change in EFT. Patients 01–002 and 03–009 had mild macular edema at BL that was essentially completely eliminated by M3 with normal appearing scans, but this was not accompanied by substantial improvement in BCVA.

Figure 2. Red free fundus photographs and fluorescein angiogram patient 01-009 at baseline (BL) and month 3 (M3) after starting mecamlamine

The red free photograph at M3 shows a substantial reduction in exudates compared to the photograph at BL. The fluorescein angiogram at BL shows substantial leakage during the early and mid phases resulting in pooling of dye in a petaloid pattern during the late phase of the angiogram. At M3 there is less leakage and less pooling of dye in the macula during the late phase of the angiogram.

Figure 3. Three patients who showed some evidence of improvement during their course, but had a sudden drop in best-corrected visual acuity (BCVA) at the primary endpoint and rebound improvement after stopping mecamlamine

The top row shows the patient identifier with the first two digits indicating the site and the next 3 digits indicating the enrollment number. Rows 2–7 show a horizontal cross-section time domain optical coherence tomography scan through the fovea at each time point of the study, baseline (BL), day 7 (D7), month 1 (M1), month 2 (M2), month 3 (M3), and month 4 (M4). The seventh row in each column shows a bar chart depicting excess center subfield thickening at each time point and the eighth row show a line graph depicting the change from BL in BCVA shown as number of letters read at 4 meters on an Early Treatment Diabetic Retinopathy (ETDRS) chart. Mecamlamine drops were stopped at M3, the primary endpoint of the study, and thus the data for M4 was obtained 1 month after stopping mecamlamine. Patients 01–010, 02–005, and 03–013 all had a similar pattern in that they showed some evidence of improvement through week 8, but during the last 4 weeks of mecamlamine treatment there was a substantial drop in BCVA that recovered one month after mecamlamine was stopped.

Figure 4. Six patients who showed equivocal change or no substantial change on topical mecamylamine

The top row shows the patient identifier with the first two digits indicating the site and the next 3 digits indicating the enrollment number. Rows 2–7 show a horizontal cross-section time domain optical coherence tomography scan through the fovea at each time point of the study, baseline (BL), day 7 (D7), month 1 (M1), month 2 (M2), month 3 (M3), and month 4 (M4). The seventh row in each column shows a bar chart depicting excess center subfield thickening at each time point and the eighth row show a line graph depicting the change from BL in best-corrected visual acuity (BCVA) shown as number of letters read at 4 meters on an Early Treatment Diabetic Retinopathy (ETDRS) chart. Mecamylamine drops were stopped at M3, the primary endpoint of the study, and thus the data for M4 was obtained 1 month after stopping mecamylamine. Patients 01–009 and 03–012 showed improvements in BCVA of 8 and 7 letters, respectively, but this was not accompanied by a substantial change in excess center subfield thickness. Patients 01–007 and 01–006 showed minimal change in BCVA and no change (01–006) or modest worsening (01–007) in excess center subfield thickening. Patients 03–004 and 02–004 showed essentially no change in BCVA (+3 and +1) and a modest improvement (03–004) or worsening (02–004) in excess center subfield thickness.

Figure 5. Four patients who showed evidence of worsening on mecamylamine

The top row shows the patient identifier with the first two digits indicating the site and the next 3 digits indicating the enrollment number. Rows 2–7 show a horizontal cross-section time domain optical coherence tomography scan through the fovea at each time point of the study, baseline (BL), day 7 (D7), month 1 (M1), month 2 (M2), month 3 (M3), and month 4 (M4). The seventh row in each column shows a bar chart depicting excess center subfield thickening at each time point and the eighth row show a line graph depicting the change from BL in best-corrected visual acuity (BCVA) shown as number of letters read at 4 meters on an Early Treatment Diabetic Retinopathy (ETDRS) chart. Mecamylamine drops were stopped at M3, the primary endpoint of the study, and thus the data for M4 was obtained 1 month after stopping mecamylamine. Patient 03–002 had a 15 letter reduction in BCVA and a 248 μm increase in excess central subfield thickening over the 3 months on mecamylamine and 1 month after stopping mecamylamine, there was resolution of the macular edema by a reduction of 516 μm in excess center subfield thickness accompanied by a gain of 14 letters in BCVA. Patient 01–005 had a 20 letter reduction in BCVA and a 238 μm increase in excess central subfield thickening over the 3 months on mecamylamine and 1 month after stopping mecamylamine the BCVA had improved by 10 letters and the excess center subfield thickening had a small reduction of 20 μm . Patient 03–010 had severe edema with excess center subfield thickness at BL of 492 μm that was reduced by 128 μm at M2, but increased by 78 μm at M3 when BCVA was reduced by 9 letter compared to baseline. After stopping mecamylamine the edema continued to worsen and the BCVA remained the same. Patient 03–011 was withdrawn from the study for alternative treatment at M2 because of a reduction in BCVA of 14 letters and an increase in excess center subfield thickness of 288 μm .

Figure 6. Change from baseline in best-corrected visual acuity (BCVA) and excess foveal thickness (FTH) during 12 weeks of treatment with topical mecamlamine and 4 weeks after discontinuation

The mean (upper panel) and median (lower panel) change from baseline in BCVA (Y axis on left) in letters read at 4 meters on an ETDRS visual acuity chart is shown by the black lines. The mean (upper panel) and median (lower panel) change from baseline in excess FTH (Y axis on right) is shown at each study visit by the bars.

Figure 7. Aqueous VEGF levels at baseline and after 7 or 84 days of treatment with topical mecamlamine

All of the nine patients entered at one site had aqueous taps at baseline, day 7, and day 84 and VEGF levels were measured by ELISA. Each value represents a mean of 3 separate assays. There were small reductions in mean and median VEGF levels between baseline and day 84 that were not statistically significant (upper panel). The VEGF levels for each patient at the 3 time points are shown in the lower panel along with their outcome category. Category 1 consists of patient shown in Figure 1 who showed evidence of improvement. Category 2 consists of patients shown in Figure 3 who showed some evidence of improvement during their course, but had a sudden drop in best-corrected visual acuity at the primary endpoint and rebound improvement after stopping mecamlamine. Category 3 consists of patients shown in Figure 4 who showed equivocal change or no substantial change. Category 4 consists of patients shown in Figure 5 who showed evidence of worsening on mecamlamine. There was no consistent correlation between alteration of VEGF level and outcome category.

TABLE

Correlation or Diabetic Macular Edema Patient Characteristics with Outcome

ID	Duration (yrs)		HbA1C		BP		Creatinine		Cholesterol		TG	
	DM	DME	BL	WK 12	BL	WK 12	BL	WK 12	BL	WK 12	BL	WK 12
Patients who improved (Figure 1)												
01-002	4	3	10.7	9.9	160/92	120/80	0.8	1.3	210	149	100	106
01-003	42	27	7.4	7.4	120/78	130/64	1	0.9	196	180	125	246
01-008 ^a	14	0.1	8.9	8.1	104/72	140/90	0.7	0.7	229	215	134	101
01-011	9	0.1	6.4	9.1	130/68	130/75	0.7	0.7	158	190	88	87
02-003	9	0.002	6.1	6.3	118/58	118/64	1.1	1.1	125	145	120	113
03-005	9	7	10.4	10	153/94	168/99	0.87	1.76	257	236	100	111
03-007	9	8	8.2	9	122/64	128/75	0.65	0.64	172	182	340	324
03-009	9	3	7	7.7	140/87	168/79	0.69	0.75	171	150	101	99
Patients who improved then worsened then improved (Figure 3)												
01-010	18	7	8.9	7.6	100/68	100/70	2.5	2.4	170	200	222	138
02-005	9	2	8.7	9.6	120/74	132/68	0.7	0.8	181	171	73	117
03-013	22	0.3	7.6	7.7	124/70	97/46	1.65	1.71	156	196	91	138
Patients who showed equivocal change or no change (Figure 4)												
01-006	9	4	7.2	7.4	115/72	110/76	0.8	0.9	161	144	113	116
01-007	15	15	6.9	7.4	140/72	134/58	0.9	0.8	268	219	249	233
01-009	2	1	7.9	10.3	138/72	120/74	0.9	0.8	175	169	215	261
02-004	15	5	7.7	8.6	122/62	118/62	1	0.7	136	162	175	224
03-004	13	4	8.9	8.4	115/62	182/93	1.53	1.29	156	194	132	138
03-012	16	1	7.6	8	132/80	123/80	0.87	0.85	104	111	102	115
Patients who worsened (Figure 5)												
01/005	19	3	9.5	9	120/76	138/78	0.6	0.6	199	169	324	284
03-002	8	2	6.8	6.1	112/68	109/63	0.58	0.63	211	228	190	201
03-010	27	5	9.2	10.7	152/78	134/70	1.20	1.31	148	156	246	186
03-011	2	1	6.8	6.7	130/65	136/78	0.54	0.55	176	187	164	193

BL = baseline; DM = diabetes mellitus; DME = diabetic macular edema; HbA1C = hemoglobin A1C; BP = blood pressure; TG = triglycerides; WK 12 = week 12 (primary end point).

Units for HbA1c, cholesterol, creatinine, and TG are in mg/dL.

^a Only current smoker in the study

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