2RT PAPER SUMMARY

Retinal Rejuvenation Therapy for Diabetic Macular Edema

Aim: Prospectively investigate the efficacy and safety of 2RT for the treatment of diabetic macular edmea.

Summary Outcome: Mean change in LogMAR visual acuity between baseline and 6 months for patients improved ≥ 3lines, improved ≥2lines and stable (within ± 1line) were 11%, 32% and 43% respectively. Central macular thickness decreased greater than 5% in 46% of treated eyes and stabilised (within ±5% change from baseline) in 39% treated eyes. Microperimetry examination confirmed preserved integrity of photoreceptors and improvement correlated with decreased macular thickness. Benefits were achieved without associated side effects of conventional thermal laser.

RETINA REJUVENATION THERAPY FOR DIABETIC MACULAR EDEMA

A Pilot Study

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Purpose: To prospectively investigate the safety and efficacy of a novel frequency-doubled nanosecond-pulsed laser with discontinuous beam energy distribution (2RT, Ellex) for the treatment of diabetic macular edema.

Methods: Twenty-three consecutive patients (38 eyes) with newly diagnosed diabetic macular edema were recruited and assessed with logarithm of the minimum angle of resolution best-corrected visual acuity, central macular thickness measured with optical coherence tomography (OCT/scanning laser ophthalmoscope, OPKO/OTI), microperimetry, fundus photography, and fundus fluorescein angiography. Macular grid treatments were performed with 2RT laser system by 1 operator. Patients were examined with logarithm of the minimum angle of resolution best-corrected visual acuity, central macular thickness, microperimetry, and fundus photography at 3 weeks and 6 weeks and 3 months and 6 months. Fundus fluorescein angiography was repeated at 3 months and 6 months.

Results: Six months postoperatively, 17 patients (28 eyes) completed the study. No complications were identified after 2RT therapy. Intraoperative retinal discoloration was observed in 2 cases, fully resolved at 3 months with no permanent anatomical or functional changes. Mean logarithm of the minimum angle of resolution visual acuity improved from 20/44 at baseline to 20/27 at 6 months. The change in best-corrected visual acuity was significant (P = 0.0190). Central macular thickness in the central 1-mm subfield, retinal exudates and vascular leakage decreased in the majority of patients at 6 months (46, 41, and 55%, respectively), although the change from baseline was not statistically significant. Microperimetry confirmed photoreceptor integrity and showed a trend of improvement that correlated with decreased central macular thickness.

Conclusion: For the first time, we achieved a beneficial effect on diabetic macular edema without the side effects of conventional laser therapy. The efficacy of this system in comparison with standard argon laser photocoagulation and in the treatment of other conditions affecting the retinal pigment epithelium needs further investigation.

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Diabetic macular edema (DMO) represents a significant cause of visual loss occurring in 27% of patients within 9 years of onset of diabetes mellitus.[1]

Abnormal fluid accumulation may result from either excessive leakage of retinal capillaries or loss of pumping capacity of the retinal pigment epithelium (RPE).[2,3] In DMO, the former mechanism is thought to be the causal agent. The fluid accumulates between the two plexiform layers and to a less extent between the outer plexiform layer and the outer limiting membrane.[4] Fluid accumulation within the retina causes displacement of retinal components and overall increase in retinal volume. The retina is a complex matrix within which the only connection between photoreceptors and ganglion cells is bipolar cells.[2,3] If the volume of the accumulating fluid is within elastic limits of the retina, then the connections between the plexiform layers can be maintained. However, if fluid volume overcomes the elasticity of the tissue, the bipolar axons may snap and the neuronal connections are lost.[3] This concept would explain the apparent relationship between central macular thickness (CMT) and visual acuity. In a previous optical coherence tomography (OCT) study, by measuring the residual connectivity between the plexiform layers in relation to eccentricity, we found a very strong relationship between bipolar survival and preservation

of visual function.[5] Therefore, retinal tissue integrity appears to be a reliable measure of preserved axonal connection and a stronger indicator of visual function compared with CMT.

There is an extensive literature investigating the association between CMT and visual acuity in macular edema.[6–8] At present, the measurement of CMT with OCT represents the accepted standard parameter at baseline and in longitudinal studies, despite its very poor correlation with visual function.[9] Similarly, the investigation of the relationship between patterns of macular edema and visual function has shown poor consistency across different studies.[10–12]

Even with the introduction of ranibizumab and bevacizumab, laser photocoagulation has remained the first-line treatment for DMO.[13,14] The Early Treatment Diabetic Retinopathy Study demonstrated that over a 3-year period, focal laser treatment reduced the risk of moderate visual loss by 50% compared with controls.[5] By contrast, diffuse edema involving the center of the macula was associated with higher risk of permanent visual loss and responded with limited functional improvement after grid laser.[16] Early treatment is associated with a lower prevalence of visual impairment, as demonstrated by a recent Wisconsin Epidemiologic Study of Diabetic Retinopathy report.[17]

In conventional macular laser therapy, laser impacts will destroy irradiated photoreceptors and thus induce microscotoma. Several authors reported additional side effects of grid macular laser such as choroidal neovascularization, enlargement of scars, subretinal fibrosis, and reduced color vision.[18–21]

The cascade of events triggered by macular laser photocoagulation and leading to the resolution of edema is not fully understood. Initially, it was thought that absorption of laser energy within the retinal capillaries had a direct effect on leaky microaneurysms. In early clinical studies, focal treatment of microaneurysms was common. More recently, it has been recognized that the therapeutic effects of laser result from irradiation of RPE, hence the idea of grid therapies. Laboratory studies have shown that subsequent to laser irradiation, migrating RPE cells release enzymes such as matrix metalloproteinases (MMPs), which facilitate removal of debris from Bruch membrane and increase transport processes.[22] Moreover, RPE division is associated with release of cytokines, which trigger endothelial cell divisions and changes in the neuroretinal capillaries, whereas MMPs may increase water outflow and cytokines may contribute to reduce water inflow into the retina.[23] Given the understanding of the importance of RPE-derived factors, clinicians have gradually applied lower energies producing lighter burns in an attempt to preserve photoreceptor cells. Even if these therapeutic regimens could produce RPE selective lesions, the extent of the area affected by RPE cell loss would result in secondary photoreceptor cell loss because of metabolic insufficiency. New treatment systems such as micropulse and selective retina treatment result in far less damage to the neuroretina but equal therapeutic effect to standard laser photocoagulation. However, they still result in microscotomas.[24–26]

The system investigated in this article, Retina Rejuvenation Therapy (2RT), includes 2 significant modifications. First, the time domain is reduced to 3 ns and results in damage from cavitation rather than the generation of thermal transients, thus only RPE cells are damaged by primary process. Second, the signal to noise ratio in the laser speckle has been manipulated such that only a few of the high points across the beam are sufficient to induce RPE cell death. The topography of the hot spots is such that each of the dead cells are surrounded by unaffected cells. In this case, sufficient cells should survive postoperatively, such that the overlying photoreceptors receive an ample supply of nutrient and therefore do not undergo secondary cell death.

Material and Methods

The study was a prospective investigation of safety and efficacy of a new laser device. The protocol of the study was approved by the institutional ethics committee of St. Thomas' Hospital, London, United Kingdom (Ref: 07/Q0702/29) and was compliant with the Declaration of Helsinki, and all participants' consent was obtained before enrollment.

Inclusion/Exclusion Criteria

Patients with age between 18 and 80 years, newly diagnosed focal or diffuse DMO in one or both eyes, according to the Early Treatment Diabetic Retinopathy Study definition of clinically significant macular edema, and willing to participate at all reviews were included in the study.

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J. Marshall is a nonexecutive director of Ellex; none of the authors have a proprietary interest.

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The presence of moderate or severe macular ischemia on fundus fluorescein angiography (FFA), proliferative diabetic retinopathy, previous laser, intraocular surgery, and other ocular pathologies were considered as exclusion criteria. Patients with uncontrolled diabetes, hypertension, hypersensitivity to fluorescein, or renal impairment were excluded from the study.

Subjects

During the first 3 months of the study, consecutive patients were recruited and received their first 2RT treatment. Each participant was involved in the study for a minimum of 12 months, and the total duration of the study was 18 months, including review of retreatments and data analysis.

Postoperative examinations occurred at 3 weeks and 6 weeks and 3 months and 6 months. Two early reviews, postoperatively, were carried out to monitor possible adverse effects on the RPE and Bruch membrane. Fundus color photography and FFA were performed at 3 months and 6 months.

Retreatment Criteria

After initial 2RT treatment, all patients were assessed regarding the need of retreatment at the 3-month visit. Criteria for retreatment included: increased OCT-measured CMT, decreased Log MAR BCVA by more than 1 line and increased angiographic leakage. If at least two out of three parameters had deteriorated from baseline, the patient was eligible for retreatment. If only one out of the three criteria had shown deterioration, the patient was observed. Retreatments were performed 4 months after the initial 2RT laser. All retreatments were performed using the same settings as for the original treatment.

Outcome Measures

In previous studies treating macular edema, there have been a number of outcome measures including visual acuity, CMT, and change in macular exudates and angiographic leakage.[27] The most important outcome measure for patients is represented by visual acuity change. Previous studies found some correlation between visual acuity change and CMT but little if any correlation between visual acuity and the other two parameters. For completeness, all have been included in this study.

To assess efficacy of 2RT, the following outcome measures were considered: improvement or stabilization of logarithm of the minimum angle of resolution best-corrected visual acuity (logMAR BCVA), change in CMT, and change in macular exudates and angiographic leakage. To assess the safety of 2RT laser, serial fundoscopy, FFA, and scanning laser ophthalmoscope microperimetry were performed to assess retinal anatomy and photoreceptor function.

Central macular thickness was defined as the average CMT in the central 1,000 μm of the fovea and measured from the topography map of a spectral OCT/SLO (OPKO/OTI, Miami, FL).

In previous studies, serious ocular adverse events were defined as evidence of retinal bleeding at the time of treatment, loss of visual acuity from the initial visit (two or more logMAR lines), development of RPE rips, RPE detachments, geographic atrophy, or cataract.[18–22] Given the short pulse duration of this laser, special attention was directed at the integrity of the Bruch membrane. Fundoscopy, OCT, and FFA were examined to identify possible adverse effects of laser such as choroidal neovascularization, hemorrhages, and pathologic RPE changes.

2RT Laser System

The laser device used in this clinical trial was a frequency-doubled Nd:YAG laser (emission wavelength of 532 nm, 3-ns pulse duration, 400-μm spot size) RPE. The most important modification embodied in the system was a discontinuous energy profile across the beam such that the threshold for cellular damage was only exceeded in discontinuous locations within the irradiated area. Thus, for any given exposure, only approximately 15% to 20% of the cells within the 400-μm spot size were raised above threshold for cellular damage. A useful analogy would be to consider a laser beam in which the bright points in the speckle are enhanced and the rest suppressed. The system was manufactured by Ellex, Adelaide, Australia, and named 2RT, standing for Retinal Rejuvenation Therapy.

All 2RT laser treatments were performed by the same clinician (A.M.P.H.) using an Area Centralis fundus contact lens Volk Optical Inc. (Mentor, OH). The laser was coupled to a slit lamp and used a digital interface to record the number of pulses, energy/pulse, and total treatment energy.

Before treating each patient, a series of laser exposures were made external to the inferior temporal arcade for calibration purposes. Extensive laboratory had established a lookup table to determine the boundary conditions for clinical exposures, and the initial exposure was carried out at the midpoint of this range.[28] If the initial pulse energy resulted in a visible lesion, the pulse energy was reduced until no lesion was visible. Typically, no more than three trial exposures were required. Our patients included blacks, Asians, and white. Having selected the energy, this

was used for the macula exposures and only readjusted if acute changes were seen at the sites of irradiation. The pulse energy ranged from 78 μJ to 131 μJ.

Laser treatments were guided by FFA images displayed on a computer screen adjacent to the laser console. Areas of edema were treated in a grid pattern sparing the foveal avascular zone. Although an attempt was made to ensure that lesions were separated by 400 μm, empirically, this was extremely difficult as none of the impacts could be seen. The total number of pulses and the geographic extent of the treated area varied between patients, depending on the extent of the edema and the leakage on FFA. No attempt was made to direct lesions specifically at microaneurysms.

Optical Coherence Tomography

Optical coherence tomography was carried out using a commercial model of spectral domain OCT/SLO (OPKO/OTI). The confocal fundus scanning laser ophthalmoscope image and the OCT images were generated through the same optics, displayed simultaneously on the computer screen with pixel-to-pixel correlation. The system used an infrared broadband super luminescent diode source with a wavelength between 790 nm and 950 nm and produced cross-sectional images of the retina along the *x*–*y* plane (B-scan) as well as along the *z* plane (coronal C-scans).

All patients were dilated using phenylephrine 2.5% and tropicamide 1%, before OCT/SLO imaging. Longitudinal B-scans and radial and topography scans of each eye were recorded by one operator.

Microperimetry

Reassessed before pupillary dilation (OPKO/OTI, 2007 version), a stimulus size equivalent to a Goldman I test spot (100 minutes of arc or 50 by 50 μm) and a stimulus duration of 100 ms, applying the sin all Light stimuli, were randomly presented during the examination, and results were reported in decibels, ranging from 4 dB to 20 dB, in 1.0-dB steps. A grid of 28 stimulus locations covering the central 8°, centered on the fovea, was used. The fixation target was represented by a 4° cross on a black background, and the test was performed in a dark room allowing 5 minutes for dark adaptation.

The program that presented a pattern of mean retinal sensitivities at increasing eccentricities was determined in 4 locations in the inner ring (2°), 12 locations in the middle ring (4°), and 12 locations in the outer ring (8°). Mean sensitivity was recorded at baseline, 3 weeks, 6 weeks, 3 months, and 6 months.

The scanning laser ophthalmoscope camera provided a real-time fundus image with 512 by 512 pixel resolution over a field of view of 29°. The scanning laser ophthalmoscope perimetry software included an automatic tracking system for fundus movements that evaluated every acquired frame for shifts in the *x* and *y* directions about a reference frame obtained by the scanning laser ophthalmoscope camera at the beginning of the examination.

Statistical Analysis

All data organization was carried out in Microsoft Office Excel 2010, whereas statistical analysis was carried out with SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL). The logMAR was used for clinical assessment and statistical analysis. Logarithm of the minimum angle of resolution best-corrected visual acuity was converted to Snellen equivalent for descriptive purposes.

Correlation coefficients were calculated to evaluate the association between logMAR BCVA and CMT. A 2-tailed significance test was carried out on logMAR BCVA and CMT at baseline, 3 months, and 6 months to investigate a difference in the outcome measures and to calculate the *P* value. A *P* value of <0.05 was considered significant and <0.01 highly significant.

Results

Study Patients

Over a period of 3 months, 38 eyes of 23 patients underwent 2RT laser treatment. The baseline characteristics of the 23 patients enrolled in the study are summarized in Table 1. The 2RT treatment settings are illustrated in Table 2. All participants had been newly diagnosed with DMO, and none of them had received laser treatment previously.

At baseline examination, 3 patients had focal extrafoveal macular thickening and were treated with 2RT applying a modified macular grid, as described in the Early Treatment Diabetic Retinopathy Study guidelines. The remaining eyes had diffuse DMO and received a conventional macular grid photocoagulation with 2RT system.

Six patients (10 eyes, 4 bilateral and 2 unilateral treatments) were reviewed at the 3-month visit but were lost to follow-up at 6 months. Two patients who had received unilateral treatment did not attend on more than 2 occasions and were excluded from the study after the 3-month review. Four patients who had received bilateral treatments could not attend the

Table 1. Summary of Preoperative Patient Characteristics

Patients Characteristics	Values
Number of subjects (eyes)	23 (38)
Age in years (mean ± SD, range)	65 ± 11 (47–83)
Gender, n (%)	9 F (39), 14 M (61)
Bilateral DMO, n (%)	15 (65); 5 F, 10 M
Unilateral DMO, n (%)	8 (35); 4 F, 4 M
Diffuse DMO, n (%)	20 (87)
Focal DMO, n (%)	3 (13)
Ethnicity, n (%)	13 (56) African/Caribbean
	6 (26) white
	4 (17) Asians
Duration of DM in years (mean ± SD, range)	10.4 ± 5.4 (3–22)
Treatment of DM, n (%)	8 (34) combined insulin + oral therapy
	13 (56) oral therapy
	1 (4) insulin therapy
	1 (4) diet control

F, female; M, male.

6-month review for the following reasons: development of uncontrolled diabetes and renal failure during the study period (2 patients, 4 eyes), metastatic pancreatic cancer (1 patient, 2 eyes), and chronic obstructive pulmonary disease (1 patient, 2 eyes). The logMAR and CMT outcomes of this subgroup with 3-month follow-up are shown in Table 3. No adverse effect from 2RT treatment was observed.

Seventeen patients (28 eyes, 6 women and 11 men) completed the 6-month review and their results are reported in 4 with subgroup analysis of men and women. There was no difference in characteristics and outcomes between men and women ($P > 0.10$). Table 5 shows a breakdown of 6-month results based on improvement, stability, and deterioration of the outcome measures.

Laser Treatment Settings

The 2RT treatments were completed without intraoperative or postoperative complications, and none of the patients reported discomfort or any subjective adverse effects after the laser. Single pulse duration of 3 ns was kept constant for all treatments. The end point lesion was an invisible retinal burn, although during laser delivery, a shimmering reflex from the retina where the laser spot was applied was observed by the operator in all cases.

On the immediate postoperative examination, changes in retinal pigmentation were observed in the first two patients, one black Caribbean and one Asian. These two patients had highly pigmented fundi, were treated with 0.6 mJ, and the retinal reaction probably represented mild overtreatment. As a consequence of visible lesions, the first 2 patients were subjected to further postoperative examination at 1 week and 3 weeks. The laser impacts appeared faintly visible at cessation of treatment and became more obvious 1 hour after treatment. The impacts appeared as annular areas of blanching at 1 week and as areas of choroidal vascular change at 3 weeks on FFA. By 3 months, virtually, all blanching areas had resolved and no patterns of vascular change could be determined. As a consequence, the energy was lowered for the next 4 treatments to 0.5 mJ. Although no intraoperative side effects were observed treating patients with 0.5 mJ, this resulted in variable degrees of retinal reaction, depending on the race and level of pigmentation of patients. Subsequent to these initial treatments, all remaining patients were treated at 0.3 mJ.

Dilated fundoscopy at 3 weeks and 6 weeks 3 months and 6 months after 2RT did not show clinically visible lesions, except for 2 cases of retinal discoloration at the time of laser treatment. In all remaining patients, the end point of an invisible

Table 2. 2RT Laser

Energy/Single Pulse, mJ (μJ)	Treated Eyes, n (%)	Number of Pulses, Mean (Range)	Total Energy, Mean (Range), mJ
0.3 (78)	22 eyes (75.8)	94.5 (78–232)	29.09 (2–77)
0.5 (109)	4 eyes (13.8)	75.75 (31–135)	39.75 (17–70)
0.6 (131)	3 eyes (10.3)	72 (14–124)	46 (18–74)

Table 3. Results in the 3-Month Subgroup (Patients Who Were Lost at 6-Months Follow-up)

Characteristics of 3-Months Subgroup	Values
Patients	6
Eyes	10
Correlation CMT vs. logMAR BCVA at baseline	$r = 0.22$
Correlation CMT vs. logMAR BCVA at 3 months	$r = 0.19$
Two-tailed test: logMAR BCVA baseline vs. 3 months	$P < 0.01$*
Two-tailed test: CMT baseline vs. 3 months	$P < 0.01$*
Baseline logMAR (Snellen)	0.37 (20/46)
Mean	0.4 (20/50)
Median	0.194
Standard deviation	0.1–0.6
Range	20/25–20/79
3 months logMAR (Snellen)	0.24 (20/34)
Mean	0.25 (20/35)
Median	0.135
Standard deviation	0.1–0.4
Range	20/25–20/50
Baseline OCT CMT (μm)	
Mean	317
Median	310
Standard deviation	59
Range	230–450
3 months OCT CMT (μm)	
Mean	266
Median	251
Standard deviation	57
Range	205–372

*statistically significant.

irradiation was achieved. Fundus fluorescein angiography at 3 months and 6 months postoperatively did not show any abnormalities related to the laser treatment.

Logarithm of the Minimum Angle of Resolution Best-Corrected Visual Acuity, Central Macular Thickness, Exudates, and Angiographic Leakage

Table 3 shows the results of the subgroup that completed the 3-month review but was lost at the 6-month review after 2RT treatment. Logarithm of the minimum angle of resolution visual acuity change and CMT change in the 3-month subgroup was significant ($P < 0.01$). Table 4 shows the 6-month results and men and women subgroup analysis, including the logMAR BCVA versus CMT correlation values and the P values. The correlation values were weak to moderate and showed high variability. The difference between mean logMAR visual acuity at baseline and 6 months (Figure 1) was statistically significant ($P = 0.019$), whereas no statistically significant difference was found between baseline and 3 months ($P = 0.2722$) and between 3 months and 6 months ($P = 0.2016$). Central macular thickness change was not statistically significant in the group of patients followed up for 6 months (Figure 2, Table 4). Evaluation of the amount of hard exudates and vascular leakage was carried out comparing fundus photographs and fluorescein angiograms at baseline, 3 months, and 6 months, in keeping with previous studies.[27]

Two patients (Cases 7-8 and 28-29) showed a bilateral deterioration in visual function at 6 months. Patient 7-8 was 1 of the 2 patients in whom retinal discoloration was observed intraoperatively and the reduced vision was the result of a preexisting cataract development. Patient 28-29 developed poorly controlled insulin-dependent diabetes mellitus, hypertension, and acute renal failure and required panretinal photocoagulation for bilateral proliferative diabetic retinopathy between 3 months and 6 months after 2RT treatment, resulting in being excluded from the study after 6-month review.

Microperimetry

The retinal sensitivity and fixation stability change after treatment are shown in Figures 3 and 4. At 3-month review, a moderate improvement in sensitivity and fixation stability within the central 2° was observed; however, the difference was not statistically significant. No deficit of photoreceptor cell function was detected over areas of nonvisible irradiation. In the first 2 treatments with 2RT, some loss in retinal sensitivity associated with suprathreshold lesions was observed at 3 weeks with full recovery of baseline sensitivities at 3 months.

Retreatments

Four months after initial 2RT treatment, all patients were evaluated regarding the need of retreatment. Retreatment was performed in 14 eyes of 10 patients (4 bilateral and 6 unilateral treatments). All retreatments were carried out using 0.3-mJ energy. No adverse effects were observed, and patients were reviewed 3 months postoperatively.

Discussion

The effectiveness of conventional green laser (CGL) photocoagulation for DMO has been demonstrated by several studies over the last three decades, with successful results of early treatment of focal DMO and less successful results on diffuse DMO.[15,16]

Table 4. Results at 3 and 6 Months with Gender Breakdown (Patients Who Completed All Reviews up to 6 Months)

logMAR BCVA and CMT at Baseline, 3-Month, and 6-Month Review	Results	Male Subgroup Results	Female Subgroup Results
Subjects (eyes)	17 (28)	11 (19)	6 (9)
Correlation: CMT vs. logMAR at baseline	–0.14	0.09	–0.58
Correlation: CMT vs. logMAR at 3 months	0.07	0.3	–0.2
Correlation: CMT vs. logMAR at 6 months	0.7	0.7	0.8
Two-tailed test: baseline logMAR vs. 6 months	$P = 0.019$*	$P < 0.01$*	$P = 0.04$*
Two-tailed test: baseline CMT vs. 6 months	$P = 0.19$	$P = 0.09$	$P = 0.845$
Baseline logMAR VA (Snellen)	0.348 (20/44)	0.36 (20/45)	0.34 (20/44)
Mean	0.3 (20/39)	0.3 (20/39)	0.3 (20/39)
Median	0.16	0.17	0.15
Standard deviation	0.1–0.7	0.1–0.7	0.2–0.6
Range	20/25–20/100	20/25–20/100	20/31–20/79
3-Month logMAR VA (Snellen)	0.29 (20/38)	0.31 (20/40)	0.26 (20/36)
Mean	0.28 (20/38)	0.34 (20/39)	0.26 (20/36)
Median	0.16	0.16	0.18
Standard deviation	0.0–0.6	0.0–0.58	0.02–0.06
Range	20/20–20/79	20/20–20/76	20/20–20/22
6-Month logMAR VA (Snellen)	0.14 (20/27)	0.12 (20/26)	0.18 (20/30)
Mean	0.1 (20/25)	0.1 (20/25)	0.1 (20/25)
Median	0.12	0.09	0.11
Standard deviation	0.0–0.4	0.0–0.3	0.1–0.4
Range	20/20–20/50	20/20–20/39	20/25–20/50
Baseline OCT-CMT (μm)			
Mean	296	290	320
Median	294	291	312
Standard deviation	88	66	125
Range	147–486	192–450	147–486
3-Months OCT-CMT (μm)			
Mean	279	280	292
Median	272	294	245
Standard deviation	99	65	150
Range	146–558	187–428	146–558
6-Months OCT-CMT (μm)			
Mean	275	261	311
Median	256	256	256
Standard deviation	85	48	137
Range	189–567	195–375	203–567

VA, visual acuity.

Adverse effects of CGL have been reported and may lead to permanent central vision impairement.[18–21] In view of potential risks of CGL, alternative laser strategies using lower energy and less visible burns have been investigated. Diode lasers with millisecond exposures were introduced in ophthalmology in the 1980s.[27] Clinical studies of diode lasers for diffuse DMO demonstrated resolution of edema in the majority of patients at 6 months and stabilization of visual acuity but with more selectivity for the outer retina compared with CGL.[26,27,29–31] More recent studies have investigated the efficacy of the so-called micropulse diode laser for DMO.[32] The rationale of micropulse diode laser systems is that, by using microsecond pulses, thermal transients would be confined to the RPE cells. In theory, this would limit damage to overlying photoreceptor cells. However, in practice, the 100 μs to 300 μs pulse would result in thermal damage to the tips of the photoreceptors.

Figueira et al[32] carried out a randomized controlled trial comparing micropulse diode laser with CGL and reported no significant difference in visual acuity between the two groups and equal efficacy.

Laboratory studies have suggested that a series of repetitive short laser pulses can be used to selectively damage absorbing structures and that short pulse

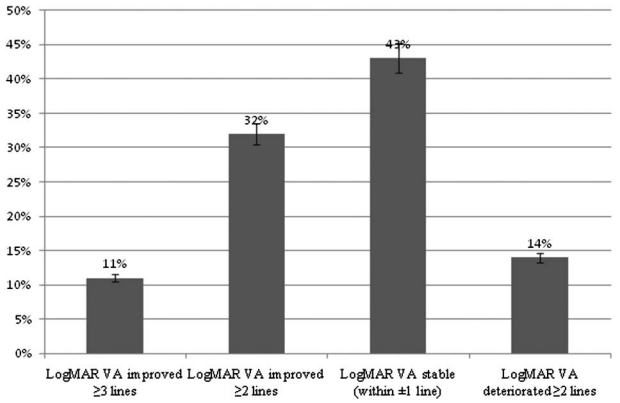

Fig. 1. Change in logMAR visual acuity between baseline and 6 months.

lengths and low duty cycle allow for maximum thermal localization.[33–35]

Conventional green laser is generally performed by laser pulses of a few hundred milliseconds producing a thermal interaction with tissues. Roider et al[36] showed that reducing the pulse duration produced non-thermal interaction mechanisms particularly for time domain in the nanosecond and microsecond range. According to a laser study on rabbit retina, a 200-ns laser pulse produces bubble formation or cavitation around single melanin granules.[36] Another laboratory study on rabbits investigated the influence of pulse duration and number of pulses on RPE damage thresholds and demonstrated a higher safety range with pulse duration of 200 ns rather than with 1.7 μs.[37] Selective retina treatment (8-ns pulse duration, 102 μm diameter) was tested in a rabbit model to evaluate the damage thresholds and range by histology.[38] The study demonstrated high RPE selectivity, intact Bruch membrane, and choriocapillaris but also occasional damage to photoreceptors.

A clinical pilot study with nonophthalmoscopically visible lesions caused by a train of repetitive short laser pulses (1.7 μs) with an Nd:YLF laser for various macular diseases showed resolution of leakage and

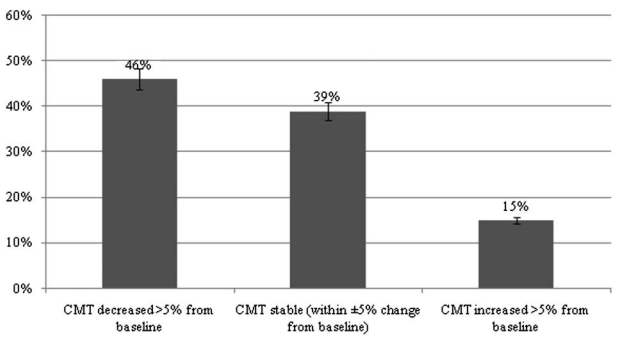

Fig. 2. Change in CMT between baseline and 6 months.

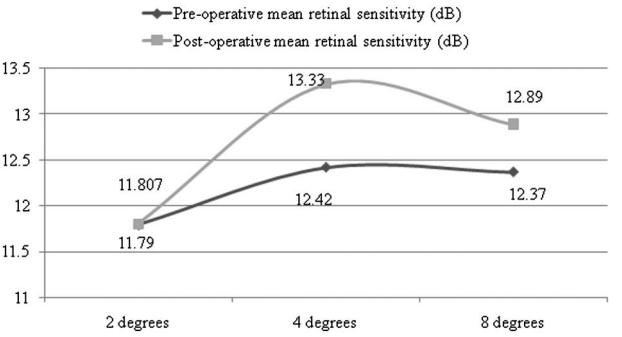

Fig. 3. Change in retinal sensitivity between baseline and 3 months post 2RT.

reduction of hard exudates in the majority of patients with DMO, with stabilization of visual acuity over a period of 6 months.[27]

To date, the problem with very short laser pulses has been the peak power generated, which may result in areas of cavitation that could induce rupture of Bruch membrane with a resultant subretinal hemorrhage. The novel laser described in this study, 2RT, uses a 3-ns pulse but deliberately avoids large areas of cavitation by only raising the energy distributed in the beam to levels at which cell damage will occur in regions widely separated. By using a technique that may be considered as enhanced speckled, bright spots distributed within the 400-μm spot size cause damage to only 15% to 20% of the cells. This energy distribution avoids problems of breaks in Bruch membrane, and the resultant cellular damage distribution means that each cell lost is surrounded by undamaged cells whose subsequent migration contributes to the therapeutic components of enzyme and cytokine release.

Previous laboratory studies have shown that enzymatic release is associated with RPE cell migration and that peak levels of MMP2 and MMP9 are seen within 7 days post irradiation. These enzymes have

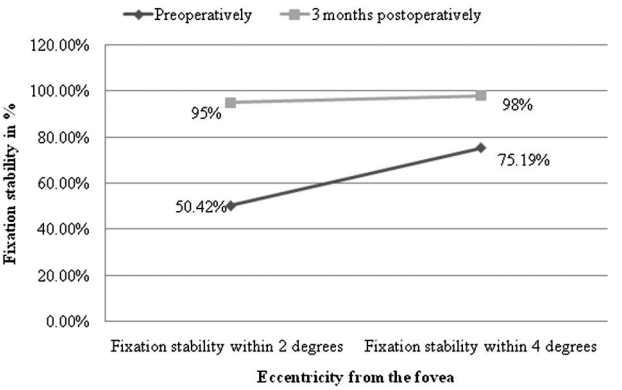

Fig. 4. Change in fixation stability between baseline and 3 months post 2RT.

been shown to clean Bruch membrane and enhance transport properties. By contrast, cell division occurs within 7 and 14 days and this is accompanied by the release of cytokines and changes on retinal capillaries. The energy degradation associated with absorption by the melanin granules is confined to the RPE with pulse duration of 3 ns or less and pulse energy ranging between 0.3 mJ and 0.6 mJ.

In conventional laser photocoagulation, suprathreshold energy is used and photoreceptors are destroyed. With shorter laser pulses, the heat flow may not inflict damage to photoreceptors, but because RPE cells are destroyed in the area corresponding to the laser burn, secondary damage of photoreceptors may develop while the RPE cells are reorganizing. Short pulses generate a nonthermal damage, and no heat is transmitted to photoreceptors. With discontinuous distribution of laser energy across the laser burn, photoreceptors have always access to nutrient supply. Therefore, there is no damage to photoreceptors especially over the areas of irradiation, as shown by microperimetry (MP).

This study describes the application of a novel laser for the treatment of DMO. Previous data were obtained from animal and laboratory studies. The results of this prospective pilot study indicate that 2RT is safe and effective in the treatment of DMO. The nature of this laser improves safety concerning collateral damage of the retina. Serial scanning laser ophthalmoscope microperimetry demonstrated stability of retinal sensitivity as a function of intact photoreceptors. The only features of the laser potentially responsible for collateral damage were the Q-switched pulses and potential problems induced by energy peak powers such as acute hemorrhages. However, by setting the laser energy within the limits that we applied, this risk was avoided and we did not see hemorrhages in any of the patients. Regarding the efficacy of this laser system, this was confirmed by the improved logMAR BCVA (P = 0.0190), a stable or decreased CMT, and FFA leakage in 85% of eyes 6 months postoperatively (Table 5).

The limitations of our study include the small size, short follow-up time, and the lack of controls and randomization. The study was intended to be a pilot safety study; therefore, a small size was planned as part of the original design. Six patients (10 eyes) were lost to follow-up as described in Results. This is the first report 6 months postoperatively, although each patient remained in the study for 12 months.

This study included patients with bilateral and unilateral macular edema. In cases of bilateral pathology, both eyes were treated simultaneously with 2RT.

Table 5. Results at 3 Months and 6 Months: Improvement, Stability, and Deterioration of Outcome Measures

Outcomes	3-Month Results, % (n)			6-Month Results, % (n)		
logMAR BCVA	Improved ≥ 2 lines	Stable ± 1 line	Decreased ≥ 2 lines	Improved ≥ 2 lines	Stable ± 1 line	Decreased ≥ 2 lines
	34 (13)	58 (22)	8 (3)	43 (12)	43 (12)	14 (4)
CMT	Decreased ≥ 5%	Stable ± 5%	Increased ≥ 5%	Decreased ≥ 5%	Stable ± 5%	Increased ≥ 5%
	55 (21)	24 (9)	20 (8)	46 (13)	39 (11)	15 (4)
Exudates	Decreased	Stable	Increased	Decreased	Stable	Increased
	66 (18)	27 (8)	7 (2)	41 (11)	43 (12)	16 (5)
Leakage	Decreased	Stable	Increased	Decreased	Stable	Increased
	83 (23)	7 (2)	10 (3)	55 (15)	31 (9)	14 (4)

We appreciate that systemic factors could affect the overall response to the laser and the resolution of macular edema, thereby introducing a bias in the results. We feel that any future efficacy study should consider the inclusion of controls and randomization to treatment in cases of bilateral edema.

There was a higher number of men than women in this study. This may represent a confounding factor affecting the results. However, analysis of results grouped by gender did not show a significant difference in the outcome measures at any time during the study (Table 4).

The proportion of patients with highly pigmented fundi was a crucial factor influencing the power needed to induce an effect and the incidence of intraoperative complications. Two cases presented intraoperative retinal discoloration, postoperative OCT changes at the level of the RPE, and decreased retinal sensitivity on MP 3 weeks postoperatively, with full resolution 3 months postoperatively. This clearly represented an overtreatment.

The disturbances affecting the RPE layer were not associated with changes in the outer nuclear or the inner retinal layers. Retinal pigment epithelial changes could be explained with temporary rearrangement of the RPE cells during the healing phase after laser treatment. The normalization of the OCT scan and the MP 3 months postoperatively suggested that the photoreceptor layer did not suffer any damage. This confirms the selective effect of 2RT laser. None of the patients presented permanent pigmentary changes or retinal scars after the 3-month review.

The inclusion of MP as an objective parameter to monitor the selectivity of 2RT is a strength of this study. No previous selective RPE laser studies have performed serial MP to monitor adverse effects of macular laser treatment. Microperimetry was repeated at baseline, 3 weeks, 6 weeks, and 3 months to monitor changes of retinal sensitivity overtime and detect unwanted neural retina damage after treatment. A mild overall decrease in retinal sensitivity was detected 3 weeks after treatment with a progressive improvement thereafter and full normalization 3 months postoperatively. We interpreted this finding as a result of the healing process involving RPE cell proliferation, migration, and reconnection with the photoreceptor layer. Full normalization of MP after 3 months is an indicator of photoreceptor integrity and confirms the RPE selectivity of 2RT.

Future development of this laser system will consider coupling this technology with real-time recording systems and Pascal-type predetermined patterns. In light of recent extensive laboratory studies showing the involvement of MMPs in the control of Bruch membrane thickness and transport processes, the efficacy of 2RT in treating other RPE conditions needs further investigation.[39] Furthermore, the value of retina regeneration therapy needs to be confirmed in a larger, controlled, clinical study in comparison with CGL.

Key words: diabetes mellitus, diabetic retinopathy, invisible laser, laser, macular edema, retina, subthreshold laser, visual acuity.

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