The Comparative Histologic Effects of Subthreshold 532- and 810-nm Diode Micropulse Laser on the Retina

Alfred K. Yu, Kevin D. Merrill, Steven N. Truong, Krisztina M. Forward, Lawrence S. Morse, and David G. Telander

PURPOSE. Therapeutic retinal laser photocoagulation can damage the neurosensory retina and cause iatrogenic visual impairment. Subthreshold micropulse photocoagulation may decrease this risk by selective tissue treatment. The aim of this study was to compare subthreshold 810-nm diode micropulse laser and subthreshold 532-nm micropulse laser on the retina by histologic examination and differential protein expression.

METHODS. Fourteen Dutch-belted rabbits received subthreshold 810-nm diode micropulse laser photocoagulation in their right eye and subthreshold 532-nm micropulse laser photocoagulation in their left eye. Histology and immunohistochemical detection of stromal cell-derived factor-1 (SDF-1), β-actin, vascular endothelial growth factor (VEGF), glial fibrillary acidic protein (GFAP), and insulin-like growth factor 1 (IGF-1) were analyzed 12 hours, 3 days, 14 days, and 28 days post–laser photocoagulation.

RESULTS. Histologically, all time points produced a similar degree of retinal disruption in both wavelengths. Immunohistochemically, SDF-1 expression was greatest at the 12-hour time point and decreased thereafter. SDF-1, VEGF, and β-actin up-regulation was detected at early time points in both the 810- and 532-nm micropulse laser-treated animals.

CONCLUSIONS. Subthreshold micropulse retinal laser photocoagulation caused equivalent histologic changes from both 532- and 810-nm diode lasers. Differential protein expression was not evident between the different laser conditions. (Invest Ophthalmol Vis Sci. 2013;54:2216–2224) DOI:10.1167/iovs.12-11382

Micropulse laser photocoagulation has been demonstrated to be a clinically effective therapeutic treatment of diabetic macular edema and has been suggested to be less destructive to the retina compared with conventional laser photocoagulation.1,2 Conventional laser photocoagulation of the retina delivers a continuous wave (CW) laser, which normally results in a full-thickness visible lesion due to thermal damage of the retinal pigmented epithelium (RPE), neurosensory retina, and the choroid. This treatment has been successfully used in the treatment of diabetic retinal edema and proliferative diabetic retinopathy.3,4 Retinal laser does cause photoreceptor loss, which leads to scotoma formation, though it is often asymptomatic. In addition, laser therapy can cause choroidal neovascularization, retinal or subretinal hemorrhage, foveal distortion, and subretinal fibrosis. Ideally, therapeutic retinal laser would treat only specific histologic retinal layers, such as altering the RPE but sparing the neurosensory retina.

Conventional retinal photocoagulation is a photothermal process, in which laser energy is mainly absorbed by melanin in the RPE. Properties of the laser such as wavelength, duration, and power play a major role in the effective treatment of different ocular diseases. Thermal destruction of a significant fraction of the photoreceptor layer has been suggested to improve retinal oxygenation, reduce metabolic activity, inhibit angiogenic stimulators, and increase production of angioinhibitory factors.5 During the laser exposure, the temperature rises from baseline (36°C) and spreads by conduction toward the adjacent choroid and neurosensory retina, which, if damaged (±20°C to ±30°C), starts scattering the light and showing the familiar intraoperative grayish endpoint, or laser lesion. With conventional laser exposure, the initial damage is followed by further thermal spread and decay. The zone of latent lethal damage, which is invisible during the laser treatment, manifests at various later times.6 Therefore, clinically, the lesion visible at the time of the original procedure will eventually expand and manifest as a larger lesion and an area of expanding atrophy.6

Recent studies have suggested that it may be possible to deliver a subthreshold lesion that is above the threshold of biochemical effect but below the threshold of a visible, destructive lesion.7 Micropulse laser has been proposed as one effective way to deliver the laser energy without causing retinal and photoreceptor damage.7 The goal of micropulse lasers is to treat the retinopathy while potentially limiting progressive enlargement of laser scars, which can lead to scotoma and loss of color vision. Continuous conventional laser (of any wavelength) can also be delivered in a subthreshold fashion using lower energy, but this is not the same as dividing the energy into micropulses. Micropulse-divided laser energy will heat the tissue less than a continuous pulse laser even if the total amount of energy is the same.

Micropulse laser delivers a pulse train of interrupted laser energy, which can limit thermal damage to the RPE and overlying neurosensory retina.7 To confine the thermal gradient to the RPE and spare the neurosensory retina, the laser exposure time should not exceed 10–4 seconds (100 μs), which can be achieved with a micropulse laser.7 While CW laser applies an uninterrupted stream of energy, a micropulse laser applies small micropulses of energy over a given period of time. One can set the duty cycle (percent time) that the laser is actually active during an envelope period of time (cycle).
Micropulse lasers have been shown in the past to give a clinical effect, but only the 810-nm (infrared) wavelength has been well studied. One study showed that micropulse laser treatment of diabetic macular edema patients was equally effective compared with conventional argon laser treatment. Another study showed that subthreshold diode micropulse laser minimized chorioretinal damage in that no laser scarring was observed while successfully treating clinically significant macular edema. Most recently, subthreshold micropulse laser has been used for panretinal photocoagulation in treatment of diabetic retinopathy. Nakamura et al. demonstrated that subthreshold micropulse diode photocoagulation is effective in the reduction of macular edema. The functional improvement was limited to visual acuity, and there was no improvement to the reduction of macular edema. Ohkoshi and Yamaguchi showed that subthreshold micropulse laser photocoagulation might be useful for maintaining visual acuity in patients with early macular edema, while causing minimal damage.

Laser photocoagulation has been found to regulate the expression of many molecules important in capillary leakage and angiogenesis. Laser photocoagulation has also been shown induce migration of bone marrow-derived stem cells to laser lesions. In addition, stromal cell-derived factor-1 (SDF-1) has been shown to be up-regulated after laser treatment. SDF-1 is a chemokine involved in the recruitment of hematopoietic stem cells in ocular diseases, such as diabetic retinopathy, and it has been found to play an important role in neuroprotection of the retina. SDF-1 is normally produced in RPE cells and is instrumental in attracting bone marrow cells after injury.

Prior studies have indicated that applying 810-nm diode laser pulses of short duration almost solely affects the RPE, with little histopathologic effect on the outer retina and choriocapillaris. However, the histopathologic effects of a 532-nm wavelength micropulse laser have not been well studied. Therefore, the purpose of this study was to compare the histologic effects on the retina of the 532-nm micropulse laser with those of the 810-nm micropulse laser at multiple time points. In addition, we assessed the effects of subthreshold 532- and 810-nm diode micropulse lasers on gene regulation in the retina, including SDF-1.

**Methods**

**Animals**

Pigmented Dutch-belted rabbits (Covance, Princeton, NJ) with a body mass of 1.21 to 1.68 kg were acclimated under controlled conditions for a week. Fourteen rabbits were used in total. All animals were given 5 oz. of Purina rabbit chow (solid feed) once a day; water was supplied ad libitum. All animal procedures adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and were approved and monitored by the Institutional Animal Care and Use Committee of the University of California, Davis.

**Laser Photocoagulation**

Rabbits were anesthetized using ketamine hydrochloride (50 mg/kg), xylazine (5 mg/kg), and acepromazine (0.5 mg/kg) administered subcutaneously 15 minutes before the procedure. One drop each of 1% tropicamide and 2.5% phenylephrine hydrochloride was applied topically 30 minutes prior to laser micropulse procedure to achieve pupillary dilation. One drop of proparacaine 0.5% was instilled in each eye before treatment to act as an anesthetic. A Mainster standard retinal laser contact lens (0.96 image magnification) was used to focus the laser on the rabbit fundus. The threshold power for each eye was determined with CW laser using the standard Mainster lens for 200 ms.

Two separate experiments were conducted in which the laser spot diameter varied. Aerial spot sizes for the two experiments were 500 and 200 μm, creating retinal spot diameters of 195 and 130 μm, respectively. In both experiments, the right eye was treated with the 810-nm diode micropulse laser (Iridex, Mountain View, CA) and the left eye with the 532-nm micropulse laser (Iridex) using duty cycles of 5%, 10%, 20%, and 40%.

Grid laser patterns were made in the fundus of rabbits in two separate experiments. The pattern was slightly different for each group of rabbits (Figs. 1a, 1b). For each experiment, once the visible threshold for a laser lesion for each eye was determined, a grid of subthreshold spots was made by using various duty cycles. Spots were made one lesion width apart. Visible 100% threshold lesions served as visible markers so as to identify the location of nonvisible subthreshold lesions during histologic analysis. A triangular pattern of marker lesions was created at one edge of the grid to determine orientation. For the first group of eight rabbits, four were sacrificed at 2 weeks posttreatment and four at 4 weeks posttreatment.

In the second experiment, rabbits were treated with the same two micropulse wavelengths using a modified grid pattern. Six rabbits, totaling six eyes for each wavelength were studied. Six study lesions were created for each duty cycle, with each lesion separated by one laser spot. Grid laser patterns were made in the fundus of rabbits in two separate experiments. The pattern was slightly different for each group of rabbits (Figs. 1a, 1b). For each experiment, once the visible threshold for a laser lesion for each eye was determined, a grid of subthreshold spots was made by using various duty cycles. Spots were made one lesion width apart. Visible 100% threshold lesions served as visible markers so as to identify the location of nonvisible subthreshold lesions during histologic analysis. A triangular pattern of marker lesions was created at one edge of the grid to determine orientation. For the first group of eight rabbits, four were sacrificed at 2 weeks posttreatment and four at 4 weeks posttreatment.

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**Figure 1.** (a) Laser micropulse grid of first experiment. (b) Laser micropulse grid of second experiment.
Immunohistochemistry

After the rabbits were euthanized with an intracardiac injection of Beuthanasia-D (Merck Animal Health, Summit, New Jersey) under anesthesia, the eyes were injected with 0.2 mL of 10% formalin. After 5 minutes, the eyes were enucleated and placed in 10% formalin. The eyes were processed, embedded, and sectioned in paraffin for histologic and immunohistochemical analysis. Tissue sections were cut at 4-μm thickness.

Sections were stained with hematoxylin and eosin to analyze histologic changes caused by laser photoocoagulation. Immunohistochemical analysis included detection of SDF-1, β-actin, vascular endothelial growth factor (VEGF), glial fibrillary acidic protein (GFAP), and insulin-like growth factor 1 (IGF-1). All tissue sections were rehydrated and treated with H2O2 blocking solution.

For SDF-1 detection, tissue sections were permeabilized with 5% normal goat serum (Sigma-Aldrich) in TBS-T (tris-buffered saline with tween) and a rabbit-to-rabbit blocking reagent (ScyTek Laboratories Inc., West Logan, UT). Rabbit anti-SDF-1 (200 μg/mL; Santa Cruz Biotechnology Inc., Santa Cruz, CA) primary antibody was applied with a 1:1000 dilution in blocking buffer. Sections were washed with TBS-T before the addition of donkey antimouse secondary antibody (0.8 mg/mL; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) with a 1:500 dilution in blocking buffer. The protein of interest was detected using Vector VIP (Vector Laboratories) with a dilution of 1:400 in tris-buffered saline. The protein of interest was detected using Vector VIP (Vector Laboratories) with a dilution of 1:400 in tris-buffered saline. The protein of interest was detected using Vector VIP (Vector Laboratories) with a dilution of 1:400 in tris-buffered saline.

For β-actin detection, tissue sections were permeabilized with 5% normal donkey serum (Sigma-Aldrich) in TBS-T. Mouse anti-β-actin (Novus Biologicals, Littleton, CO) primary antibody was applied with a 1:5000 dilution in blocking buffer. Sections were washed with TBS-T before the addition of donkey antirabbit secondary antibody (0.8 mg/mL; Jackson Immunoresearch Laboratories Inc., West Grove, PA) with a 1:5000 dilution in blocking buffer. The protein of interest was detected using Vector VIP (Vector Laboratories), and a methyl green (Vector Laboratories) counterstain was applied. Staining of a control untreated eye was used to compare SDF-1 up-regulation.

For VEGF detection, tissue sections were permeabilized with 5% normal donkey serum (Sigma-Aldrich) in TBS-T. Mouse anti-VEGF (200 μg/mL; Santa Cruz Biotechnology, Inc.) primary antibody was applied with a 1:500 dilution in blocking buffer. Sections were washed with TBS-T before the addition of goat antimouse secondary antibody (0.8 mg/mL; Jackson Immunoresearch) with a 1:2000 dilution in blocking buffer. The protein of interest was detected using Vector VIP (Vector Laboratories), and a methyl green (Vector Laboratories) counterstain was applied.

For GFAP detection, tissue sections were permeabilized with 5% normal donkey serum (Sigma-Aldrich). Mouse anti-GFAP (Thermo Fisher Scientific Inc., Waltham, MA) primary antibody was applied with a 1:250 dilution. Sections were washed with TBS-T before the addition of goat antimouse secondary antibody (0.6 mg/mL; Jackson Immunoresearch) with a 1:1000 dilution in blocking buffer. The protein of interest was detected using Vector Blue (Vector Laboratories).

For IGF-1 detection, tissue sections were permeabilized with 5% normal donkey serum (Sigma-Aldrich). Mouse anti-IGF-1 (0.10 mg/mL; Abcam, Cambridge, MA) primary antibody was applied with a 1:100 dilution in blocking buffer. Sections were washed with TBS-T before the addition of goat antimouse secondary antibody (0.6 mg/mL; Jackson Immunoresearch) with a 1:1100 dilution in blocking buffer. The protein of interest was detected using Vector Blue (Vector Laboratories).

RESULTS

Ophthalmoscopy

Laser photoocoagulation was applied to the retina of rabbits using the slit lamp and contact lens. All rabbits (n = 14) were treated based on ophthalmoscopic determination of threshold as the laser energy needed to cause minimal RPE and retinal whitening. Micropulse laser was then applied based on this visual determination of threshold in each rabbit. Because rabbits were pigmented, the threshold energy was low varying from 30 to 42 mJ. The ophthalmoscopic appearance of the threshold lesions was also found to be heterogeneous, and slightly different intensities of whitening occurred despite using the same laser settings. Micropulse lesions were not initially observed by ophthalmoscopic exam. Of note, after 3 to 5 minutes 40% duty cycle lesions could occasionally be visualized with slight whitening of the tissue.

Histology

The rabbits’ retinal tissues were examined at 12 hours, 3 days, 2 weeks, and 4 weeks following laser treatment. Gross examination clearly showed the threshold lesions at all time points (Fig. 2). Forty percent duty cycle lesions were seen on gross examination in approximately 50% of the lesions at the 2- and 4-week time points. No significant difference was found between the 532- and 810-nm laser-treated eyes.

At the 12-hour time point, morphologic changes were noted in the photoreceptor layers at the threshold lesions, with disorganization of the inner segments. Morphological changes in the 40% duty cycle lesions at both wavelengths appeared in 50% of the lesions. All other duty cycles showed no retinal changes with either laser wavelength at the 12-hour time point.

At the 3-day time point, morphologically detectable lesions appeared in the same proportion, with more developed lesions for both wavelengths.

At the 2- and 4-week time points, 100% marker lesions at 810 and 532 nm showed full thickness retinal changes, including retinal disruption, edema, retinal compaction, and pigment migration in all retinal layers. At the 2- and 4-week time points.

Micropulse lesions created with the 40% duty cycle micropulse laser at both wavelengths showed neurosensory retinal disruption, edema, retinal compaction, and pigment migration at all lesions, but to a lesser degree than the visible control lesion (Figs. 3c, 3d). Tissue disruption was not usually through all layers of the retina (unlike threshold lesions), and lateral spread of the lesion was less. Tissue changes were the same in the rabbits treated with 532- and 810-nm micropulse lasers at both the 2- and 4-week time points.

FIGURE 2. Post-laser photoocoagulation gross dissection.
Figure 3. Images of hematoxylin and eosin stained retinal section (×10) following various laser treatments: (a) 810-nm micropulse laser at threshold power stained at 4 weeks; (b) 532-nm micropulse laser at threshold power stained at 4 weeks; (c) 810-nm micropulse laser with 40% duty cycle at 4 weeks; (d) 532-nm micropulse laser with 40% duty cycle at 4 weeks; (e) 810-nm micropulse laser with 20% duty cycle at 4 weeks; (f) 532-nm micropulse laser with 20% duty cycle at 4 weeks; (g) 810-nm micropulse laser with 10% duty cycle at 4 weeks; (h) 532-nm micropulse laser with 10% duty cycle at 4 weeks; (i) 810-nm micropulse laser with 5% duty cycle at 4 weeks; and (j) 532-nm micropulse laser with 5% duty cycle at 4 weeks.
The 532- and 810-nm diode laser lesions from the 20% duty cycle showed some pigment migration and limited changes to the neurosensory retina and morphological changes limited to the outer retina at the subthreshold lesion site. Overall, there was less retinal disruption from this duty cycle as compared with the 40% subthreshold lesions, and the amount of histologic change was similar between the 810- and 532-nm laser lesions (Figs. 3e, 3f) at both the 2- and 4-week time points.

The 10% subthreshold lesions at both 810 and 532 nm showed histologic changes mainly in the RPE layer, with limited, if any, neurosensory retinal change (Figs. 3g, 3h). Again, no differences were evident between the 810- and 532-nm laser lesions at either the 2- or 4-week time point.

Using the 5% duty cycle, it was difficult to distinguish any retinal disruption or RPE damage at the injury site from either the 810- or 532-nm micropulse laser at the 2- or 4-week time point (Figs. 3i, 3j).

During the clinical application of the laser, all lesions were subthreshold, and therefore were not visibly detectable; however, most but not all of the lesions had histologic sequelae. Specifically, of all the laser treatments with a 40% duty cycle, 94% resulted in histologically detectable changes (75 detectable lesions/80 total lesions). With the 20% duty cycle, histologic changes were evident in 60% of the examined lesions (45/75). For the laser lesions from the 10% duty cycle, changes were evident in 36% (27/75). With the 5% duty cycle, only 24% (18/75) were even detectable by histological examination. The 20%, 10%, and 5% duty cycle totals were out of 75 instead of 80 because the retina was missing on some slides.

No significant difference was noted between the 2- and 4-week time points. Analysis of the percentage of detectable change in the 12-hour and 3-day time points was not possible due to lack of lesion formation.

**Immunohistochemistry**

There was no detectable expression of GFAP or IGF-1 in eyes treated with either the 532- or 810-nm diode micropulse laser at any of the four time points (data not shown). Staining for SDF-1 expression was easily detected at the 12-hour time point for all duty cycles at both wavelengths. SDF-1 expression was up-regulated to a similar degree by both wavelengths in the 100% lesions (Fig. 4a) and appeared to be up-regulated to a similar degree in the treatment lesions. Moreover, the degree of up-regulation of SDF-1 appeared to not
FIGURE 4. Representative ×20 images of SDF-1 expression (*violet stain*) in retinal tissue treated with the following (a) 532-nm micropulse laser with threshold power at 12 hours post-laser photocoagulation; (b) 532-nm micropulse laser 10% duty cycle lesion at 12 hours post-laser photocoagulation; (c) 532-nm micropulse laser threshold power at 3 days post-laser photocoagulation; (d) 810-nm micropulse laser with 40% duty cycle lesion at 3 days post-laser photocoagulation; (e) SDF-1 positive control (rabbit kidney); and (f) SDF-1 negative control (rabbit retina not treated with laser photocoagulation).
be significantly different between the various duty cycles and the CW lesion created by energy comparable to the 10% duty cycle. SDF-1 up-regulation was not limited to the area of the laser treatment but rather extended beyond the area of histologic change caused by the laser. At the 3-day time point, SDF-1 staining was still intense in the 100% visible lesions (Fig. 4c); however, staining in the treatment areas (40%, 20%, 10%, and 5% duty cycle) was only weakly detectable (Fig. 4d). Staining appeared throughout each section of the retina and RPE. The staining that varied between normal control eyes (not treated with laser) and laser-treated eyes occurred mainly in the outer segments of the retina (data not shown). SDF-1 staining at the 2-week (Fig. 5a) and 4-week (Fig. 5b) time points did not reveal any SDF-1 expression.

VEGF expression was similar to SDF-1 expression. Staining was observed predominantly in the outer segments of the retina at 12 hours post–laser photocoagulation (Fig. 6). Minimal staining was detected at 3 days post–laser photocoagulation except at the threshold marker lesions.

β-Actin expression was confined to the area of laser application and was clearly discernible in the 40%, 20%, and CW laser treatments predominately in the outer nuclear layer (Fig. 7). Both wavelengths displayed this up-regulation of β-actin to the same degree.

**DISCUSSION**

Conventional laser photocoagulation has been proven to be an effective means to treat ocular diseases. However, it results in full-thickness damage to the RPE, choroid, and neural retina, potentially leading to photoreceptor loss, scotoma formation, choroidal neovascularization, foveal distortion, and subretinal fibrosis. Subthreshold laser delivery by micropulse technology aims to limit the permanent damage to the retina and thus minimize the scotoma that can develop, while still allowing therapeutic effect. A study with the 810-nm diode micropulse laser done by Stanga et al.8 showed that the micropulse diode laser is an effective way of treating diabetic macular edema.

We decided to compare the 810-nm micropulse laser with a 532-nm micropulse laser. While both wavelengths are used clinically, the 532-nm wavelength is the most common for the treatment of retinal edema or retinal neovascularization; however, most micropulse studies have been conducted with the 810-nm laser. We hypothesized that equivalent subthreshold micropulse duty cycles at both wavelengths would result in equivalent histologic changes in the retina. This study is the first to verify that the histologic changes induced by 532-nm micropulse laser are indistinguishable from those of the 810-
nm micropulse diode laser. Moreover, protein regulation by the lasers appears to be the similar as well.

In this study, full-thickness changes were not seen with subthreshold laser powers with the 40%, 20%, 10%, and 5% duty cycles. Micropulse lesions from both the 532- and 810-nm lasers showed limited tissue damage to the RPE layer, largely sparing the choriocapillaris and overlying neurosensory retina. By decreasing the duty cycle, the duration of pulse delivery can be reduced to limit heat conduction to layers adjacent to the RPE. The heat production time is therefore shorter than the thermal relaxation time for the space between the RPE and the neural retina, which results in axial confinement of the increase in heat at the RPE.7

Variable tissue effects were seen after laser photocoagulation with both the 532- and 810-nm micropulse lasers. The absorption of laser energy by melanin in the RPE is what causes disruption of the RPE and subsequent retinal distortion. The RPE has a normal variation in cell pigmentation, size, and shape, which causes a variation in energy uptake at different locations in the RPE.7 As a result, there is a heterogeneous appearance of laser spots at both wavelengths and with both the continuous and micropulse lasers. Equivalent amounts of laser energy will damage some RPE cells while leaving adjacent cells unaffected. Clinically, this presents a problem in defining the continuous and micropulse lasers. Equivalent amounts of laser energy will damage some RPE cells while leaving adjacent layers. The selectivity of tissue damage appeared best with duty cycles of 10% and 20%. Control laser (100% duty cycles) showed limited tissue damage to the RPE layer, largely sparing the choriocapillaris and overlying neurosensory retina. By decreasing the duty cycle, the duration of pulse delivery can be reduced to limit heat conduction to layers adjacent to the RPE. The heat production time is therefore shorter than the thermal relaxation time for the space between the RPE and the neural retina, which results in axial confinement of the increase in heat at the RPE.7

As compared with 100% threshold (visible) lesions, the subthreshold lesions showed decreased lateralization or spread of retinal histologic change over time. Most likely this is due to the fact that the laser-induced heat in the RPE diffuses much more rapidly with subthreshold micropulse lesions compared with 100% threshold lesions.5 Therefore, based on hematoxylin and eosin staining, the damage does not extend laterally or to adjacent layers. The selectivity of tissue damage appeared greatest with duty cycles of 10% and 20%. Control laser (100% threshold power) showed full-thickness tissue disruption, the effects of which were consistent among the subjects. Other investigators have found similar results. Paulus et al.18 demonstrated in two separate experiments that the 532 nm Nd:YAG (PASCAL) micropulse laser was able to selectively target the RPE and at higher power of the photoreceptors without permanent scarring or inner retinal damage. Restoration of retinal continuity and absence of scarring might reduce microscotomas typical of conventional pan retinal photoocoagulation (PRP) and may allow for retreatment.5

An interesting finding was that at the 4-week time point, the 40% subthreshold lesions became visible on gross pathologic sections for both wavelength treatments. These lesions were not visible at the 2-week time point. This indicates that lesions from the 40% duty cycle are more like lesions from the visible threshold CW laser than from a subthreshold micropulse laser.

We studied expression of proteins that we thought might be up-regulated, such as SDF-1, which has been shown to be up-regulated in laser-induced choroidal neovascular membrane (CNVM).19 Immunohistochemically, SDF-1 staining was strongest at 12 hours post-laser photocoagulation, and expression began to decrease thereafter. The close proximity of the 100% marker laser lesions to the treatment lesions and the diffuse expression of SDF-1 made distinguishing the area of up-regulation difficult. Therefore, in this current model we were unable to differentiate which laser lesions were most closely associated with the increased SDF-1 expression. It has been shown that the SDF-1 gradient is associated with increased bone marrow cell trafficking to the site of injury.16 SDF-1 intensity did not differ between the 532- and 810-nm micropulse lasers.

SDF-1 was not detected at the 2- and 4-week time points after laser photocoagulation, which confirms that SDF-1 is only up-regulated for a short period of time postinjury. A previous ischemia–reperfusion experiment done by Lai et al.20 showed that there was prominent staining of SDF-1 in the inner retina 6 hours after ischemia–reperfusion injury, and that SDF-1 protein diffused in the inner retinal layer at 12 hours. Only faint staining remained at 24 hours in the whole retina, specifically along the outer nuclear layer.

Similar to SDF-1, VEGF protein expression was also briefly up-regulated 12 hours post-laser application and declined within 3 days. Silva et al.21 also found increased SDF-1 and CXCR4, the receptor for SDF-1, and VEGF following laser treatment in their mouse model of laser-induced ocular neovascularization. However, the presence of SDF-1 availability does not confirm that SDF-1 is related to ocular neovascularization, only that it may be present in damaged retina. In addition, Chen et al.22 demonstrated that CXCR4 is selectively expressed in vascular endothelial cells and is up-regulated by the angiogenesis factors VEGF and basic fibroblast growth factor (bFGF). SDF-1 enhanced production of VEGF and bFGF; therefore, SDF-1 and VEGF may act synergistically in diseased or damaged retina.

We also looked for the expression of additional protein markers that we thought might be up-regulated by micropulse laser application. We chose to probe retinal tissue treated with 810-nm diode micropulse laser and 532-nm micropulse laser for SDF-1, VEGF, and IGF-1 because it has been shown that VEGF and IGF-1 stimulate SDF-1-induced angiogenesis.14 Of all the proteins tested, β-actin was the only protein marker that showed up-regulation confined to the area of micropulse laser application. This increased expression of β-actin suggests that 532- and 810-nm micropulse lasers were able to induce increased protein expression when applied with the 40% duty cycle, 20% duty cycle, and subthreshold CW.

Modifiable properties of the laser include duration, power, and wavelength. In this experiment, by modifying the duty cycle of micropulse laser application, we changed the duration and in effect the total power applied. The ideal wavelength is characterized by good penetration through ocular media and maximal absorption in the target tissue. Shorter wavelengths scatter more easily; therefore, red light (620–750 nm) has better penetration than blue light (450–495 nm). Laser absorption is also dependent upon pigment composition of the target tissue. The three major ocular pigments are melanin,
xanthophylls, and hemoglobin. Melanin absorbs most of the visible to near-infrared portion of the light spectrum. Since melanin is the most effective light absorber, the major sites of laser absorption are the RPE and choroid. Xanthophyll has maximal absorption of blue light and is found predominantly in the macula. Green laser has become the most popular due to its minimal absorption by xanthophylls coupled with its strong affinity for melanin and hemoglobin. Therefore, it can be used in the macular region as well as in the periphery and can target abnormal vessels. Selective treatment of a micropulse laser coupled with the low energy requirement of the green laser may be ideal in treating ocular diseases.

In summary, this study finds that the 532-nm micropulse laser, like the 810-nm micropulse laser, can be used to selectively photocoagulate the RPE and, with higher duty cycles, the RPE and outer retina. There does not appear to be a difference between the 532- and 810-nm micropulse lasers in regard to effecting retinal histologic changes at particular duty cycles. At both the 532- and 810-nm wavelengths, lower duty cycles gave less retinal photocoagulation and more selectivity in regard to specific histologic retinal layers. Since the 532-nm laser is more commonly used for macular laser photocoagulation, a micropulse laser at this wavelength may be more convenient for the clinician. SDF-1 is up-regulated in the same manner in both wavelengths. Further experiments must be conducted to determine if micropulse laser lesions induce SDF-1 expression in the same manner as 100% laser lesions. The 532-nm laser provides the same histologic effects as the 810-nm micropulse laser, which may be more available in clinical settings.

References