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Comparison of Subthreshold 577 and 810 nm Micropulse Laser Effects on Heat-Shock Protein Activation Kinetics: Implications for Treatment Efficacy and Safety

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Received: September 20, 2019
Accepted: January 10, 2020
Published: April 28, 2020

Keywords: micropulse laser; subthreshold; retinal pigment epithelium; melanin; heat shock proteins; activation threshold; therapeutic range; retina; Arrhenius integral

Citation: Chang DB, Luttrull JK. Comparison of subthreshold 577 and 810 nm micropulse laser effects on heat-shock protein activation kinetics: implications for treatment efficacy and safety. Trans Vis Sci Tech. 2020;9(5):23, https://doi.org/10.1167/tvst.9.5.23

Purpose: To compare the safety and efficacy of 810 versus 577 nm laser wavelengths for micropulse subthreshold (sublethal) laser treatment by mathematical analysis.

Methods: Two different representative laser parameter sets for micropulsed subthreshold diode laser treatment, one employing 810 nm and the other 577 nm, are compared with regard to efficacy by analysis of the kinetics of laser-induced heat-shock protein (HSP) activation; and for safety, by scaling law analysis.

Results: Kinetics analysis of laser-induced HSP activation shows that the primary therapeutic effect of laser is thermal incitement of a long-term wavelength-independent increase in the rate of HSP-mediated protein repair specific to sick and dysfunctional cells, rather than from short-term increases in free intracellular HSP concentrations. Scaling law analysis of the same 810 and 577 nm laser parameters, however, finds treatment safety highly wavelength-sensitive, favoring 810 over 577 nm.

Conclusions: Mathematical analyses of the effects retinal laser-induced HSP activation provide important insights into the mechanism of action and the importance of wavelength selection in modern retinal laser therapy. Our analyses find 810 and 577 nm to be equally effective, but 810 nm having a significantly wider therapeutic range/safety margin, and thus less likely to cause inadvertent, and thus unpredictable, laser-induced retinal damage, than 577 nm.

Translational Relevance: Mathematical analysis of enzyme reaction kinetics provides important insights into the mechanism of action and clinical implications of wavelength selection in modern retinal laser therapy.

Introduction

Despite the recent rise of drug therapy, retinal laser treatment continues to be important in the management of the complications of diabetes mellitus and other chronic progressive retinopathies.[1–4] Laser-induced retinal damage (LIRD), such as photocoagulation, is responsible for the many well-known risks, adverse treatment effects, and limitations of traditional retinal laser treatment. Despite these well-recognized and long-accepted drawbacks, LIRD is not known to have any intrinsic or direct therapeutic effects; these arising instead from cells affected, but not killed, by laser application.[5–15]

Defining photocoagulation, and indeed any degree of LIRD, as complications rather than goals of treatment, modern retinal laser therapy seeks to maximize both treatment safety and efficacy and broaden treatment indications by precluding LIRD.[2,7,16–36] The cornerstones of modern retinal laser therapy were established and defined by low-intensity/high-density subthreshold diode (810 nm) micropulse laser (SDM).[23,27] These include treatments (1) selective

to the retinal pigment epithelium (RPE) and sparing the neurosensory retina; (2) reliably and predictably sublethal to the RPE; and (3) clinically optimized by preservation and normalization of RPE function at the cellular level, with amplification of the cellular response by en masse recruitment of large areas of dysfunctional retina in confluent treatment to maximize therapeutic effects, reverse the disease process, and thereby reduce the risks of visual loss.[2,17–19,22,23,27] Implementation of the principles of modern retinal laser therapy allows effective treatment for conventional retinal laser indications, including diabetic macular edema, proliferative diabetic retinopathy, central serous chorioretinopathy, and retinal vein occlusions with improved safety allowing transfoveal treatment and early treatment, and thus improved visual results.[21,23–36] Moreover, modern retinal laser therapy allows effective therapeutic and preventive treatment of other important sight-threatening disorders, all neurodegenerations, precluded to conventional photocoagulation. The improved understanding of the mechanisms and neuroprotective effects of laser action introduced by SDM predicted these new laser applications. These effects include improved visual acuity, visual fields, mesopic visual function and retinal electrophysiology in age-related macular degeneration (AMD), inherited retinopathies, and open angle glaucoma (OAG); reversal of drug tolerance in neovascular AMD; reduced neovascular conversion in high-risk dry AMD; and improved ganglion cell and optic nerve function in OAG.[2,17–22] Other applications reported include pseudophakic and uveitic cystoid macular edema, retinal vasculitis, macular telangiectasia, dome-shaped maculae, macular macroaneurysms, and macular edema associated with epiretinal membranes.[33–36]

Our current understanding of the therapeutic mechanism of SDM is that it acts as a physiologic "reset" stimulus, improving, and thus normalizing, RPE and hence retinal function by activating RPE heat-shock proteins (HSPs).[8–15,17,18,27,37] This effect is agnostic to the cause of retinal dysfunction, accounting for the wide range of clinical applications afforded to modern retinal laser therapy.[17,18,27,37] Thermally mediated, therapeutically effective retinal laser treatment is a form of bioactivation, dependent on reaching an intra-RPE cell temperature above the threshold for HSP activation. Modern retinal laser therapy does this below the thermal threshold for cell death to also preclude RPE damage.[17,18,21,27,37] Any laser wavelength can achieve the former (ideally avoiding wavelengths below 550 nm, which also carry photochemical effects cytotoxic to the neurosensory retina), whereas low-frequency pulsed lasers, such as micropulsed lasers, are better suited to the latter.[7,24–27,37–42]

In the era of photocoagulation, the therapeutic characteristics of different wavelengths were said to have been summarized by W. Richard Green as "a burn is a burn is a burn."[44] But what of modern retinal laser therapy, in which there is no immediate visible indicator of "effective" treatment, and an explicit goal of treatment is avoidance, rather than causation, of LIRD? How does wavelength selection affect treatment efficacy and safety at the physiologic and functional level?

Although the first micropulsed lasers for retinal use employed the near-infrared wavelength of 810 nm, such as is employed in SDM, in recent years 577 nm has largely replaced 810 nm in the marketplace.[43] The reasons for this shift are not entirely clear.[43] In a prospective randomized clinical trial designed to compare these wavelengths, Vujosevic et al.[45] reported in 2015 that in the treatment of diabetic macular edema, micropulsed 577 nm seemed to work a bit better than 810 nm, although this difference was not significant. In light of the marked commercial shift to 577 nm and away from 810 nm micropulsed lasers since that time, the question remains as to whether one wavelength might be better than the other for subthreshold micropulsed laser treatment intended to be both effective, and also reliably sublethal to the RPE, in accordance with the goals of modern retinal laser therapy. In the current study, we examine the reaction kinetics of laser-induced HSP activation for the 577 and 810 nm laser parameters compared clinically by Vujosevic et al.[45] Then, applying a scaling law analysis, we examine the relative safety (likelihood of retinal damage avoidance) of those same sets of treatment parameters.[21,38,42,45–48]

Methods

The kinetics of HSP activation by subthreshold micropulsed laser treatment sublethal to the RPE were examined for two different published sets of laser parameters: one employing 810 nm and the other 577 nm radiation (Table 1).[45] To determine if exceeding the threshold for HSP activation (Ω HSP) improved the treatment response, the 10 equations developed by Rybinski et al.[46] were employed to depict the chain of reactions initiated by laser-induced HSP activation. The coupled simultaneous mass conservation equations are:

$$1\quad \Omega = \int dt\ A \exp[-E/k_B T(t)]$$

$2a_1$ $[HSP]_{initial} = [HSP.HSF]_{before\ irradiation}(1- \exp[-\Omega]) + [HSP]_{before\ irradiation}$

$2a_2$. $[HSP.HSF]_{initial} = [HSP.HSF]_{before\ irradiation} \exp[-\Omega]$

$2a_3$. $[HSF]_{initial} = [HSP.HSF]_{before\ irradiation}(1- \exp[-\Omega]) + [HSF]_{before\ irradiation}$

$2b_1$. $d[HSP]/dt = (l_1+k_{10})[HSPS] + l_2[HSPHSF] + k_4[mRNA] - k_1[S][HSP] - k_2[HSP][HSF] - l_3[HSP][HSF_3] - k_9[HSP]$

$2b_2$. $d\{HSF]/dt = l_2[HSPHSF] + 2l_3[HSP][HSF_3] + k_6[HSPHSF][S] - k_2[HSP][HSF] - 3k_3[HSF]3 - l_6[HSPS][HSF]$

$2b_3$. $d[S]/dt = k_{11}\{[P] + l_1[HSPS] + l_6[SPS][HSF] - k_1[S][HSP] - k_6[HSPHSF][S]$

$2b_4$. $d[HSPHSF]/dt = k_2[HSP][HSF] + l_6[HSPS][HSF] + l_3[HSP][HSF_3] - l_2[HSPHSF] - k_6[HSPHSF][S]$

$2b_5$. $d[HSPS]/dt = k_1[S][HSP] + k_6[HSPHSF][S] - (l_1+k_{10})[HSPS] - l_6[HSPS][HSF]$

$2b_6$. $d[HSF_3]/dt = k_3[HSF]^3 + l_7[HSF_3][HSE] - l_3[HSP][HSF_3] - k_7[HSF_3][HSE]$

$2b_7$. $d[HSE]/dt = l_7[HSF_3][HSE] - k_7[HSF_3][HSE]$

$2b_8$. $d[HSF_3HSE]/dt = k_7[HSF_3][HSE] - l_7[HSF_3][HSE]$

$2b_9$. $d[mRNA]/dt = k_8[HSF_3HSE] - k_5[mRNA]$

$2b_{10}$. $d[P]/dt = k_{10}[HSPS] - k_{11}[P]$

In these expressions, k = rate constant; [] = cellular concentration of the quantity inside the bracket; A = Arrhenius rate constant for HSP activation; E = activation energy; T(t) = temperature of the thin RPE layer, including the laser-induced temperature rise; HSP = free/active HPS molecule; HSF = heat shock (transcription) factor; HSP.HSF = sequestered HSP/HSP complex with inactive HSP; HSE = heat shock element, a DNA site that initiates transcription of HSP when bound to HSP_3; HSP_3 = HSP trimer capable of binding to DNA; mRNA = messenger RNA; S = substrate for binding (damaged/misfolded protein); P = properly folded protein; HSF_3.HSE = complex that induces transcription of new HSP mRNA molecule; HSP.S = complex of HSP attached to and actively repairing misfolded protein.

In addition, it is expected that the kinetic rate constants in the Rybinski et al.[46] equations will be affected by SDM-induced changes in the conformations of the reactants. This effect would be expected to depend on the magnitude of the HSP activation Arrhenius integral Ω, being very small for small Ω and exhibiting saturation at large Ω when maximum conformational changes or optimization have been achieved. To describe this threshold/saturation behavior, we shall approximate the rate constant dependence of all constants k_i like $k_{10,}$ that are expected to increase with the SDM Arrhenius integral Ω by the simple expression:

$$2a_5.\ k_i = k_{io}\ (1+\tanh[2\Omega])$$

where k_{1o} denotes the value of the rate constant before SDM irradiation.

Similarly, for rate constants k_j that decrease with Ω (those for inverse reactions, for instance), we shall describe the dependence on Ω by the simple expression:

$$2a_6.\ k_j = k_{jo}\ (1+\tanh[2\Omega])^{-1}$$

where k_{jo} denotes the value of the rate constant before SDM irradiation. These two expressions give kinetic constants that depend exponentially on the Arrhenius integrals when $\Omega<<1$, but exhibit saturation when $\Omega>>1$.

Results

Please refer to the Supplementary Data for the detailed analyses.

The subthreshold micropulse laser parameters and Arrhenius integrals for HSP activation (Ω HSP) compared in the current study are listed in Table 1.

Solving the coupled kinetic equations for laser-mediated HPS effects shows that the long-term effects of SDM laser result from laser-induced conformational changes in activated HSPs that increase the kinetic reaction rate for protein repair in dysfunctional cells (those with high concentrations of misfolded proteins and short-lived normal proteins), rather than immediate/short-term increases in the concentration of

Table 1. The 577 and 810 Subthreshold (Sublethal) Diode Micropulsed Laser Parameters for Comparison, from Vujosevic et al.[45]

Wavelength (nm)	Duty Cycle	Retinal Spot Size (um)	Duration	Power (watts)
577	5%	105	0.2 sec	0.25
810	5%	131	0.2 sec	0.75

See Supplementary Tables S2–S5.

Table 2. Comparison of Subthreshold (Sublethal) Diode Micropulse Laser Arrhenius Integrals, Therapeutic and Cell Death Thresholds for the Vujosevic et al.[45] 577 and 810 nm SDM Parameter Sets

Wavelength[45]	P (Treatment) Laser Power Watts	Arrhenius Integral for HSP activation	P (Reset) Watts	P (Death) Watts	TR Watts	SM Watts
577	0.25	2.59	0.16	0.45	0.29	0.20
810	0.75	0.84	0.85	2.67	1.82	1.92

P (Reset) = power required for the HSP activation Arrhenius integral to reach a value of unity; P (Death) = power required for the Arrhenius integral for damage to reach a value of unity, marking the upper limit of the therapeutic range, avoidance of RPE damage being a desirable end of treatment. TR = therapeutic range = P (Death) – P (Reset), or the width (expressed in laser power) of the nominal effective interval for SDM treatment sublethal to the RPE. The laser powers delineating the P (Reset) and P (Death) vary based on the particular combinations of each of the other laser parameters, including wavelength, duty cycle, pulse duration, and spot size. SM = safety margin = P (Death) – P (Treatment). The powers for the P (Reset) and P (Death) assume an Arrhenius integral for HSP activation of 1.0.

activated HSPs (illustrated in Supplementary Figs. S1–S9). Kinetic analysis shows no difference between the therapeutic HSP effects of the Vujosevic et al.[45] 577 and 810 nm parameters. This is despite the Arrhenius integral for HSP activation (Ω HSP) for the given parameters is substantially higher for the 577 nm parameters than the 810 nm parameters (Ω HSP 2.59 vs. 0.84). Thus there is no therapeutic advantage from exceeding an Arrhenius integral (Ω HSP) of 1.0, and the 810 nm Ω HSP of 0.84 does not reduce effectiveness. Our findings also suggest that the power of the Vujosevic et al.[45] 577 nm parameters could be reduced to 0.16 watts or less to increase treatment safety without sacrificing effectiveness (Table 2; Supplementary Figs. S1–S9).

Scaling law analysis shows that the Vujosevic et al.[45] 810 nm parameters have a markedly larger therapeutic range and greater margin of treatment safety (lower likelihood of inadvertent retinal damage) than the 577 nm parameters.

Discussion

Our analysis shows that, as a threshold phenomenon, there is no difference in the HSP activation efficacy between the compared 577 and 810 nm micropulsed laser parameters, despite markedly different Ω HSPs.[23,45] Laser-induced increases in free intracellular HSPs are modest for both, consistent with the findings of in vivo and in vitro studies, which demonstrate low levels of HSP activation at exposure levels sublethal to the RPE.[9,11–13] This finding is also consistent with long clinical experience and prior studies that find no notable differences in therapeutic effectiveness in retinal treatment efficacy based on laser wavelength.[7,39]

Kinetic analysis shows that the principal laser-triggered HSP effect is not to increase the level of free and activated intracellular HSPs in the short-term (immediate or subsecond time frame); however, rather in the longer term (minutes to hours), to induce a conformational change in free HSPs that substantially increases the rate of protein repair (via rate constant k_{10}) in only sick cells. This finding accounts for the reset phenomenon, manifest clinically by the normalization of retinal function observed following SDM in various clinical settings independent of the cause and proportional to the degree of retinal dysfunction. Our findings also support the property of "pathoselectivity," wherein HSP activation exposure improves and normalizes the function of dysfunctional cells without any notable effect on healthy cells.[2,16–36,49,50]

Kinetic analysis confirms laser-induced HSP activation to be a form of bioactivation, showing no difference in the therapeutic response to laser parameters significantly exceeding the Ω HSP = 1.0 (2.59 for the 577 nm parameters) to those below (0.84 for the 810 nm parameters). This is because of the catalytic nature of the response to HSP activation (Table 2; Supplementary Figs. S1–S9). By analogy, HSP activation can be thought of as a fuse attached to an explosive device. It is the size of the device (in this case the catalytic reaction and cascade of effects initiated by HSP activation) and not the fuse that matters.[8–15,37,38,42,47–50] Because exceeding the threshold for HSP activation does not increase HSP activation but increases the risk of thermal cell death and retinal damage, laser parameters exceeding this threshold (Ω HSP = 1) should be avoided. Because of the catalytic response to laser HSP activation, treatment parameters with an Ω HSP < 1.0 can also be used to increase treatment safety, without compromising efficacy (Supplementary Figs. S1–S9).

Although our calculations find no difference in therapeutic efficacy, defined by HSP activation,

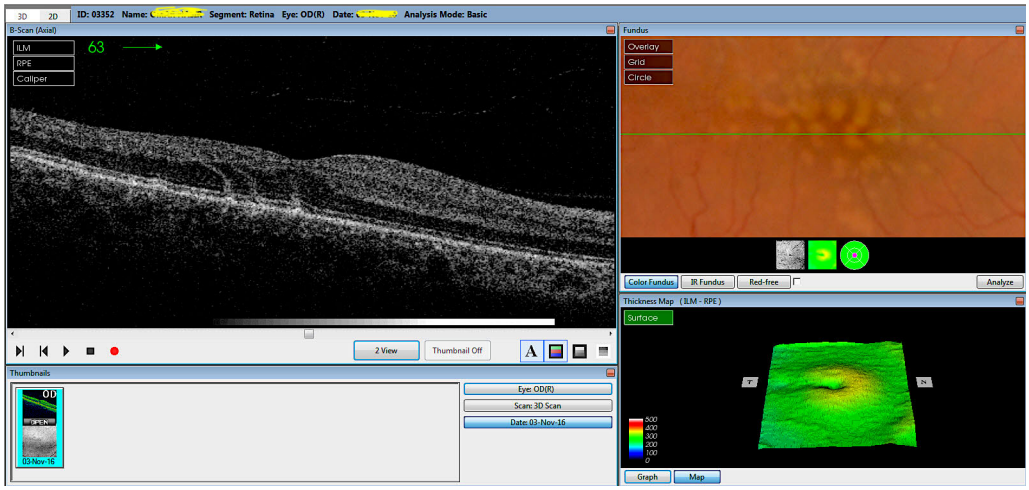

Figure. Fundus photograph and optical coherence tomography (OCT) of eye after 577 nm micropulse laser treatment. The patient was treated for the indication of macular edema owing to a branch retinal vein occlusion. A titration algorithm was used to determine the subthreshold laser treatment parameters. A low-density conventional grid pattern of treatment application was employed, which traversed the fovea using a 5% duty cycle. The day following treatment the patient noted visual loss and multiple spots in his vision. One week postoperatively the fundus photograph demonstrates a grid of threshold macular photocoagulation lesions, including the fovea, and near-full thickness retinal damage at the foci of laser spot applications by OCT.

between the Vujosevic et al.[45] 577 and 810 nm parameters, we find a substantial difference in safety. Modern retinal laser therapy defines treatment safety as the likelihood of causing inadvertent LIRD. In practical terms, the key determinant of treatment safety for any intervention is the therapeutic range (TR). The TR of modern retinal laser therapy, extending from biologic efficacy to the 50% risk of RPE death, can be thought of as the "target size" of treatment.[21,24,27] Within this range, treatment is sublethal to the RPE, and thus maximized with respect to safety and efficacy, preserving and normalizing the RPE and permitting amplification of the cellular effects via high-density laser application to achieve en masse recruitment of the RPE to maximize clinically therapeutic benefits.[23] As a rule, the TR for nanosecond lasers is zero, as they are photodisruptive to the RPE (thus destroying the cell before achieving HSP activation); narrow for continuous wave (CW) lasers; and wide for micropulsed lasers, with the width or target size of the TR increasing exponentially with decreasing pulse frequency and longer wavelengths.[21,24,27,38–40] For CW lasers, such as the Pattern Scanning Laser (PASCAL; Topcon, Tokyo, Japan), the TR is 0.010 watts, making it theoretically possible, but clinically unlikely, to consistently "hit" the very small treatment target; treating below the TR being ineffectual, and above the TR resulting in retinal damage.[9,21,23,24,27,32,37–40] Thus inherent unpredictability renders CW lasers unsuitable for modern retinal laser therapy, for which reliable safety is a prerequisite.[7,22,24,25,51] The ability create, with appropriate treatment parameters, TRs wide enough to achieve predictable and reliable avoidance of retinal damage in all clinical settings appears unique to micropulsed lasers.[24,27,40]

Scaling law analysis of the 577 and 810 nm parameters compared by Vujosevic et al.[45] finds the TR of 810 nm 6X wider than for 577 nm (1.82 vs. 0.29 watts) (Table 2).[45] This reflects the higher energy and RPE melanin absorption of 577 nm compared with 810 nm.[39,51] By treating at Ω HSP < 1.0, the safety margin of the 810 nm settings examined becomes larger than the TR (1.92 watts), increasing treatment safety. In contrast, exceeding an Ω HSP of 1.0 by 2.59X, the safety margin of the 577 nm parameters becomes less than the TR, falling to just 0.20 watts, making the safety margin of the 810 nm parameters employed by Vujosevic et al.[45] 9.6X larger (and thus safer) than the 577 nm parameters (Tables 1 and 2). Thus compared with 810 nm, 577 nm is more likely to cause inadvertent retinal damage; such as might result from titration with a faulty titration algorithm or misestimation of test-burn intensity, incorrect laser settings, individual patient or local retinal variations in RPE melanin density or heterogeneity, or media absorption or scatter.[40] Clinically, this is illustrated by the fact that it is difficult if not impossible to burn the retina with current 810 nm lasers pulsed at a 5% DC, whereas it is easily done with 577 nm (Fig.).[21,30,40] Illustrating these principles is a recent multicenter clinical trial

comparing low-duty cycle (5%) micropulsed 810 nm laser to half-dose photodynamic therapy for central serous chorioretinopathy (CSR).[30–32] Despite inadequate, and thus ineffective micropulse laser treatment in that study, the 810 nm laser power used (1.80 watts) was markedly higher than that used by Vujosevic et al.[45] (0.75 watts).[23,30–32,45] As a clinical demonstration of the very wide TR/safety margin of low frequency pulsed 810 nm, no LIRD was reported by investigators in the CSR trial.[30] Our calculations suggest that increasing the Vujosevic et al.[45] 577 nm power a similar degree (2.4X) would have resulted in retinal burns, and possibly visual loss.

A further implication of our calculations regards selection of clinical treatment parameters. Visual titration of retinal burn intensity was standard practice in the photocoagulation era. However, there are no scientifically based titration algorithms known to be reliably safe and effective for titration of micropulsed laser treatment sublethal to the RPE. Thus titration, a per-eye experiment with set size of 1, should be avoided. Instead, we favor use of published fixed (the same in all eyes of all patients) laser parameter sets found to be safe and effective in extensive clinical use, such as those compared in this study.[2,17–21,23,24,27,29] This appears especially important with 577 nm as the narrow safety range significantly increases the likelihood of miscalculation and inadvertent retinal damage (Fig.).

Conclusions

As there appears to be no difference in efficacy, but significantly different margins of safety, we find 810 nm better suited to modern retinal laser therapy than 577 nm toward the goals of achieving therapeutically effective retinal laser treatment reliably sublethal to the RPE. Offering no therapeutic advantage but reducing treatment safety, we suggest reconsideration of the current commercial emphasis on 577 nm (and shorter wavelengths) for micropulsed lasers intended for subthreshold retinal laser treatment; and increased caution and avoidance of the fovea by those using 577 nm and shorter wavelengths, particularly if using a titration algorithm.[21,43]

Acknowledgments

Disclosure: **D.B. Chang**, Ojai Retinal Technologies, LLC (C, I); **J.K. Luttrull**, Ojai Retinal Technologies, LLC (I), Retinal Protection Sciences, LLC (I), Replenish, Inc. (I)

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