Therapeutic Window of Retinal Photocoagulation With Green (532-nm) and Yellow (577-nm) Lasers

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■ **BACKGROUND AND OBJECTIVE:** The 577-nm (yellow) laser provides an alternative to the 532-nm (green) laser in retinal photocoagulation, with potential benefits in macular treatment and through ocular opacities. To assess relative risk of thermomechanical rupture of Bruch's membrane with yellow laser in photocoagulation, the therapeutic window, the ratio of threshold powers for mild coagulation and rupture, was measured.

■ **MATERIALS AND METHODS:** Retinal coagulation and rupture thresholds, visualized ophthalmoscopically, were measured with 577- and 532-nm lasers using 10- to 100-ms pulses in 34 rabbit eyes. Lesions at 1 and 7 days were assessed histologically.

■ **RESULTS:** Coagulation threshold with yellow laser was 26% lower than with green laser. The therapeutic window increased linearly with log-duration for both wavelengths with a difference in parallel-slope intercept of 0.36 ± 0.20, corresponding to 8% to 15% wider therapeutic window for yellow wavelength.

■ **CONCLUSION:** The therapeutic window of retinal photocoagulation in rabbits at 577 nm is slightly wider than at 532 nm, whereas histologically the lesions are similar.

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INTRODUCTION

Retinal laser photocoagulation is a standard therapy for several retinal vascular conditions. Laser energy is absorbed by the retinal pigment epithelium (RPE) and choroid and converted into heat, diffusing into the inner retina. Typically, 100-ms exposures with argon ion (514-nm) or frequency-doubled Nd:YAG (532-

nm) lasers are used due to the high absorption by melanin and hemoglobin at green wavelengths, but 577-nm (yellow) wavelength has been suggested as a preferable alternative.[1]

Not only has 577-nm wavelength been shown to be efficacious in the treatment of various retinal vascular diseases,[2-6] but it also has several potential advantages over 532-nm wavelength. It is less affected by small-angle scattering in the transparent ocular media[7] and provides greater transmittance through some corneal or lenticular opacities. Reduced scattering theoretically enables better focusing of the treatment beam onto the retina, whereas better transmittance through opacities should make laser delivery more consistent. In addition, the 577-nm wavelength occurs outside the absorption spectrum of retinal xanthophylls,[8] potentially allowing for treatment close to the fovea with lower light absorption in the inner retina than at green wavelengths. Finally, this wavelength occurs at an absorption maximum of oxyhemoglobin,[1] hypothetically allowing for better selective treatment of vasculature and compensating for the reduction in absorption by RPE melanin compared to 532 nm. High choriocapillaris absorption should also help to provide more uniform effects in patients with light or irregular fundus pigmentation. Thus, 577-nm wavelength has been described as optimal for macular photocoagulation[1,9] and treatment of vascular lesions and subretinal vascular proliferations.[10]

Traditionally, yellow wavelengths for retinal photocoagulation have been generated by krypton (568 nm) or dye (577 nm) lasers, but the advent of optically pumped semiconductor lasers based on NIR-pumped**AQ1** quantum well structures has allowed for much more compact and cost-effective high power yellow lasers to become available. Several companies have recently released laser systems including yellow wavelengths for ophthalmic applications (Integre Yellow, Ellex Medical Lasers Ltd.; IQ 577, Iridex Corp.; Supra 577, Quantel Medical**AQ2**). A clinical study with one of these systems (the YELL-1 study) comparing efficacy of micropulse 577-nm laser with 532-nm laser for diabetic macular edema demonstrated similar improvement in best-corrected visual acuity and macular volume.[11]

Laser power in photocoagulation is typically titrated to a visible clinical effect (graying or whitening of the retina), which corresponds to damage to the photoreceptors and, with longer exposures, to the inner retina.[12] The recent innovation of patterned scanning retinal photocoagulation has led to the use of shorter pulses (< 50 ms), which require higher retinal temperatures to coagulate, and corresponding higher laser powers.[12] This leads to an increased risk of thermomechanical rupture of Bruch's membrane ("rupture"). Consequently, early argon laser studies showed that threshold energies for coagulation and hemorrhage in rabbits decreased with decreasing pulse duration.[13,14] The therapeutic window, defined as the ratio of the threshold powers for mild coagulation and rupture, was found to approach unity at 1 ms for 200-µm beam diameter, 532-nm wavelength exposures in rabbits.[12]

Despite the potential clinical advantages, the increasingly more common clinical use of yellow wavelengths, and the theoretical differences in retinal absorption, no comparable study of the therapeutic window with 577-nm wavelength has been performed. This is of particular concern for sub-50 ms duration pulses, where rupture risk is expected to be higher. In this study, we measured the therapeutic window of photocoagulation for 10- to 100-ms pulse durations in live rabbit eyes with both 532- and 577-nm wavelengths. Because previously published histology of yellow wavelength lesions has been restricted to exposures of 50 ms or longer,[15-18] we also examine the histologic character of mild coagulation lesions produced with 20-ms pulses.

MATERIALS AND METHODS

Photocoagulation Systems

Two laser systems were used in this study. The 532-nm laser system (PASCAL; Topcon Medical Laser Systems, Inc., Santa Clara, CA) provided optical radiation from a diode-pumped solid-state continuous wave laser coupled into a scanning system integrated with a slit lamp. In this system, the laser beam (flat-top profile) is projected onto the retina and a graphic user interface is used to control laser parameters including the spot size, laser power, and pulse duration. Once the treatment parameters are selected, a foot pedal is used to activate the laser. The 577-nm system was a PASCAL Streamline 577 photocoagulator (Topcon Medical Laser Systems, Inc.), operated in the same manner as the 532-nm system. Both systems provided 10- to 100-ms pulses with up to 2 W of power and an aerial spot size of 200 µm.

Laser Application

Twenty-one Dutch Belted rabbits (weight: 1.5 to 2.5 kg) were used in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Ophthalmic and Vision Research, with approval from the Stanford University Animal Institutional Review Board. Ketamine (35 mg/kg), xylazine (5 mg/kg), and glycopyrrolate (0.01 mg/kg) were used intramuscularly for anesthesia. Pupil dilation was achieved by one drop each of 1% tropicamide and 2.5% phenylephrine, and topical tetracaine 0.5% was used for local anesthesia. Laser exposures of 10 to 100 ms were placed in both eyes of 11 rabbits with the 532-nm system; similarly, 6 additional rabbits were treated in both eyes with the 577-nm system. Four additional rabbits were treated with each system in both eyes for histologic analysis.

The threshold powers of mild coagulation and rupture at each pulse duration were measured for the two wavelengths. Between 12 and 22 eyes were used with each pulse duration, and 14 to 48 separate exposures were administered per eye for each tested duration. Power was titrated to yield clinical appearance varying from invisible outcome (no lesion) to rupture. A standard retinal laser contact lens (OMRA-S; Ocular Instruments, Bellevue, WA) was placed onto the mydriatic eye using hydroxypropyl methylcellulose as a contact gel. Taking into account the combined magnifications of the contact lens and rabbit eye of 0.6619, the aerial spot size of 200 μm corresponded to a retinal spot size of 132 μm. Thresholds for mild coagulation were measured prior to rupture to limit the view obscuration due to vitreous hemorrhage with rupture burns. All lesions were placed in the central fundus in areas with similar pigmentation. Animals involved in experiments with retinal rupture were killed immediately following placement of all lesions with a lethal dose of intravenous pentobarbital in the marginal ear vein.

The clinical appearance of the laser lesions was graded by one observer (L-SBL), who was blinded to the laser power and duration, within 3 seconds of delivering the laser pulse. Lesions were graded by means of the following scale: invisible, barely visible, mild, intense, and rupture. A barely visible or minimally visible lesion was one that just crossed the limit of detection and produced no retinal whitening. A mild lesion produced some blanching but no whitening. An intense lesion had an area of central whitening, with or without a ring of translucent edema. Thermomechanical rupture of Bruch's membrane was assumed when a vapor bubble or discontinuity (hole or rip) in the retinal architecture was visualized, with or without hemorrhage.

Statistical Analysis

The ED_{50} is the median effective dose, or the laser power required to produce the specific effect (light coagulation or rupture) in 50% of the measurements. ED_{50} threshold powers for mild coagulation and rupture at each pulse duration were calculated by Probit analysis[20] in MATLAB software (v7.4; Mathworks, Inc., Natick, MA) separately for each eye. Therapeutic window was calculated as the ratio of these thresholds, and Lilliefors and Levene's tests were performed to check for normality and homogeneity of variance.[21] One-way analysis of covariance (ANCOVA)[21] was used to evaluate the covariation of therapeutic window with log-transformed pulse duration for the two treatment wavelengths. Regression intercepts, P values, and confidence intervals were determined. Statistical significance was determined at a P value of less than .05.

Lesion Histology

To examine the general character of photocoagulation lesions, mild coagulation lesions were produced in both eyes of 4 rabbits with both wavelengths at 20-ms pulse duration. Columns of lesions with each laser system were placed roughly three beam diameters apart to facilitate side-by-side comparison between the two wavelengths in similar pigmentation conditions. In this set of animals, 10 lesions were placed per eye 1 and 7 days prior to death and enucleation.

The enucleated eyes were fixed in 1.25% glutaraldehyde/1% paraformaldehyde in cacodylate buffer at pH 7.4. They were then post-fixed in osmium tetroxide, dehydrated with a graded series of ethanol, and embedded in epoxy resin. Sections of 1-μm thickness were stained with toluidine blue and examined by light microscopy. Lesion width, indicated by disruption of the photoreceptor–RPE junction or RPE cell collapse, was measured using ImageJ software.[22] Lesions were evaluated by an observer blinded to treatment wavelength.

RESULTS

Mean ED_{50} threshold powers decreased with pulse duration, and thresholds for mild coagulation and rup-
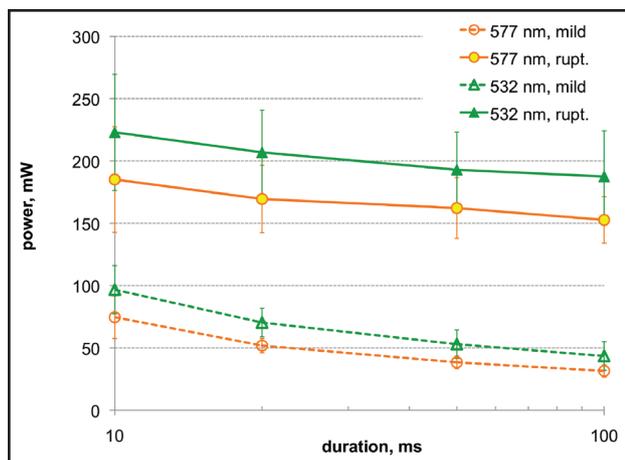
Figure 1. Mean ED$_{50}$ mild coagulation and rupture threshold powers as a function of pulse duration for 532-nm (square) and 577-nm (triangle) wavelength treatments. Power for 577 nm was 26% and 17% lower for coagulation and rupture, respectively. Error bars indicate one standard deviation in thresholds measured for each eye.

Figure 2. Mean therapeutic window (TW), the ratio of rupture and mild coagulation powers, for 532- and 577-nm treatment as a function of pulse duration. Error bars indicate the standard deviation in TW measured for each eye. Shared-slope linear regressions for TW to log-duration are shown, with 95% simultaneous confidence bounds (shaded). A difference in regression intercept ΔTW = 0.36 ± 0.20 was found to be statistically significant (*P* < .001).

ture with 577 nm were 26% and 17% lower than with 532 nm, respectively (Fig. 1). Mean therapeutic window increased logarithmically with pulse duration for both wavelengths, with 577-nm therapeutic window being approximately 10% larger at all pulse durations (Fig. 2). Due to the substantial variation of therapeutic window with pulse duration, ANCOVA was used to test for a significant difference across all data. The logarithmic dependence of therapeutic window on pulse duration suggested the use of log-transformed durations in this analysis. Physically, this dependence can be understood as a consequence of Arrhenius-law denaturation of intercellular proteins during photocoagulation, a process occurring roughly exponentially with temperature and linearly with duration.[23]

Therapeutic window values at each pulse duration for both wavelengths were normally distributed (Lilliefors test, *P* > .05), and variance between wavelengths (based on separate-slope linear regression residuals) was homogeneous (Levene's test, *P* > .05). The 532-nm therapeutic window demonstrated heteroscedasticity across pulse duration (Levene's test, *P* < .05), but ANCOVA has been shown to be robust to this condition.[24] Equal regression slopes could not be rejected (F-test, *P* = .24), dictating the use of parallel-slope ANCOVA. A difference in intercept of 0.36 ± 0.20 (F-test, *P* < .001) was found between the 577- and 532-nm therapeutic window regressions. This corresponded to an improvement in therapeutic window of 8% to 15% with 577-nm wavelength, de-
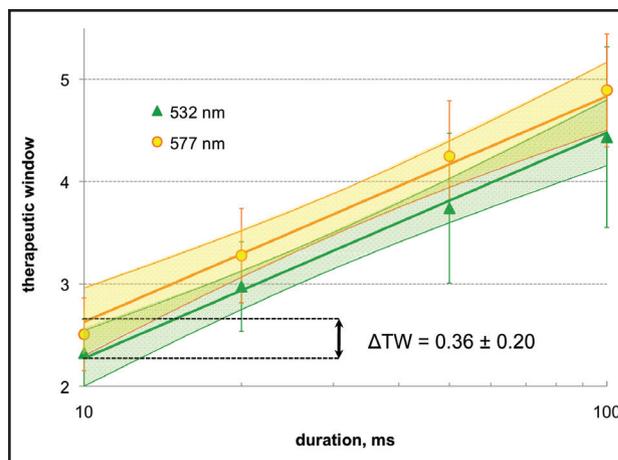
pending on pulse duration. Figure 2 shows the parallel-slope ANCOVA regressions with 95% confidence bands.

Histology demonstrated little difference between mild coagulation lesions produced with 20-ms pulses at 532- and 577-nm wavelengths. Figure 3 shows representative histologic sections with both wavelengths at 1 and 7 day time-points. No statistically significant difference in lesion width between the treatments was observed at any of the time points (paired two-tailed *t* tests, *P* > .05, n = 5 pairs per time-point). At 1 day with both treatments, edema was present in the inner and outer nuclear layers, and vacuolization was occasionally observed. Inner and outer segments of photoreceptors were shortened, and nuclei in the outer nuclear layer were pyknotic within the lesion. RPE continuity appeared disrupted and RPE cells were collapsed. At 7 days, RPE continuity was restored, with some regions of hypertrophy. The defect at the RPE–photoreceptor junction had shrunk to 34% of the initial lesion size for both lesion types: from 160 ± 14 μm at 1 day to 106 ± 22 μm at 7 days.

DISCUSSION

We compared the therapeutic window of retinal photocoagulation and histologic character of lesions produced by 532- and 577-nm wavelengths. The modest statistically significant improvement in therapeutic
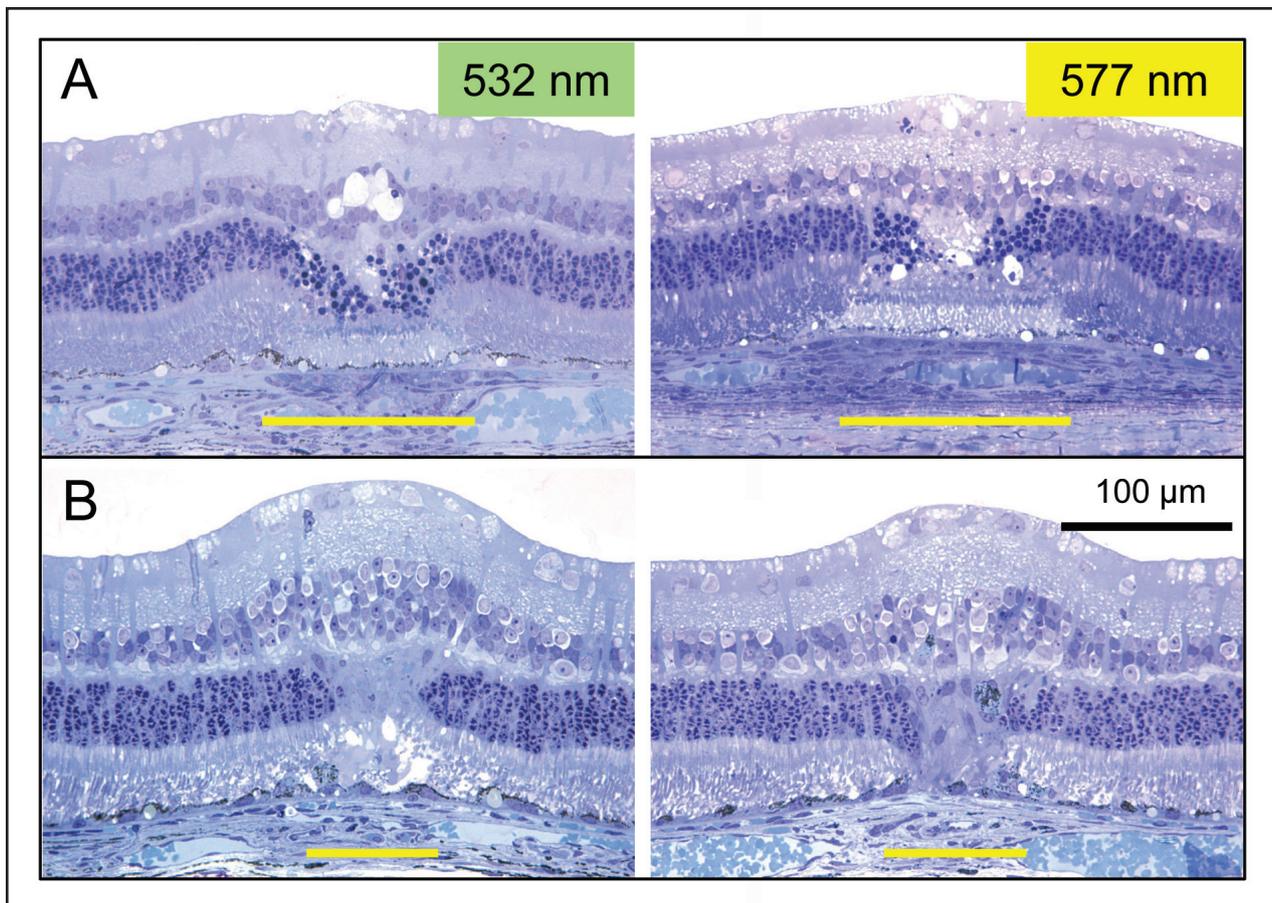
Figure 3. Representative histologic sections (toluidine blue staining **AQ3**) of 577- and 532-nm mild coagulation lesions (20-ms pulse duration) at (A) 1 day and (B) 7 day post-laser time-points. Lesion width at the retinal pigment epithelium does not appear to vary between 577- and 532-nm laser lesions, and similar shrinkage of the lesion (34% reduction in lesion width) occurred over 1 week for both types of lesions. No difference in inner retinal or choroidal damage is observed. Yellow bar indicates the width of the lesion at the retinal pigment epithelium–photoreceptor junction.

window with 577-nm wavelength inferred from the ANCOVA with pulse duration suggests that use of this wavelength in retinal photocoagulation does not pose any additional risk of Bruch's membrane rupture over conventional 532-nm wavelength, if the results of this animal study are confirmed clinically. The observed logarithmic dependence of therapeutic window on pulse duration is consistent with previous measurements with frequency-doubled Nd:YAG,[12] argon,[13] and diode (810-nm)[14] lasers. The slightly lower thresholds of coagulation with yellow wavelength than with green wavelength has been previously described with dye laser[2,5,25] and could be attributed to decreased intraocular scattering. Lesions were placed in the visual streak, which functions similarly to the human macula.[26] However, unlike in humans, the rabbit retina is xanthophyll free, and the lack of visible damage to

the inner retina observed histologically with 532-nm wavelength supports the idea that inner retinal chromophores play at most a small role in determining relative thresholds in rabbits. Absorption at both wavelengths thus predominantly occurs in the RPE and choroid, and no substantial difference in choroidal or RPE histologic appearance was observed between the two treatments. Although melanin absorption coefficient is estimated to decrease by 25% from 532 to 577 nm, oxyhemoglobin absorption coefficient increases by roughly the same amount.[1] Decreased absorption in the RPE at 577 nm may be balanced by increased absorption in the adjacent choriocapillaris, with diffusion during the pulse creating a similar thermal profile as the 532-nm exposure.

In contrast to the previous evaluation of therapeutic window and lesion character by Jain et al.,[12]

histopathologic examination in this study was limited to 8 eyes of 4 rabbits with a single-pulse duration (20 ms) and lesion grade. These laser parameters were chosen based on the common use of 20-ms pulse duration in patterned scanning photocoagulation, the lack of yellow wavelength lesion histology with sub-50 ms pulse durations, and the recent interest in clinical use of lighter lesion grades than the moderate lesions with central whitening prescribed by the Early Treatment of Diabetic Retinopathy Study.[27-29] Replication of a comprehensive histologic analysis of different lesion grades and pulse durations was thought to be excessive in light of (1) the similarities observed in lesion appearance and healing dynamics at the evaluated grade and pulse duration and (2) the similar histopathologic findings at longer pulse durations with green and yellow wavelengths in other studies.[16]

Several factors influence the translation of these animal findings to a clinical context. Although the architectural discontinuity or hemorrhage occurring with rupture is principally observer-independent, mild coagulation thresholds are inherently a more subjective assessment. Although the minimally visible lesion threshold is more objective, because it depends solely on visibility rather than judgment of the lesion character, the mild coagulation threshold is more clinically relevant and more familiar as a photocoagulation endpoint. We attempted to control for subjectivity by using a single observer to perform lesion grading, blinding the observer to the laser power and duration, and using the same lesion judgment criteria for all treatment types. Paired threshold measurements (placing lesions with both wavelengths in the same eye) would have been preferable, but 532- and 577-nm thresholds were measured with separate laser systems at different times. However, pigmentation was fairly consistent between individual animals; standard deviation in threshold power relative to the mean was 18%, averaged over all thresholds.

The retinal coagulation lesions applied in this study were confined to an area of similar pigmentation adjacent to the medullary ray in rabbits, a well-established animal model in retinal damage studies.[12,30,31] Although RPE pigmentation is similar in rabbits and humans, limitations exist with regard to the extrapolation of these data to humans. Lesions with 532- and 577-nm wavelengths were indistinguishable histologically after 1 week (Fig. 3), suggesting similar retinal ef-

fect. However, the ongoing trial assessing relative clinical efficacy of these particular wavelengths is welcomed to supplement past observations of wavelength-independence in efficacy.[5,32-35] Additional oxyhemoglobin-mediated light absorption occurs in the holangiotic, vascularized human inner retina compared to the merangiotic, avascular inner rabbit retina. Equivalence in clinically observed rupture complication rate should be scrutinized as use of 577-nm wavelength becomes more common.

Yellow laser provides low intraocular light scattering in opacified media, high transmittance through yellow, cataracts, negligible xanthophyll absorption, and high choriocapillaris absorption relative to green laser. The benefits of these attributes of 577-nm wavelength in retinal photocoagulation, in addition to a potentially increased therapeutic window, await clinical validation.

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AUTHOR QUERIES

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