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Damage Threshold of *In Vivo* Rabbit Cornea by 2 μm Laser Irradiation

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ABSTRACT

To support refinement of the Maximum Permissible Exposure (MPE) safety limits, a series of experiments were conducted *in vivo* on Dutch Belted rabbit corneas to determine corneal minimum visible lesion thresholds for 2.0 μm continuous-wave laser irradiation. Single pulse radiant exposures were made at specified pulse durations of 0.1 sec, 0.25 sec, 0.5 sec, 1.0 sec, 2.0 sec and 4.0 seconds for spot $1/e^2$ diameters of 1.17 mm and 4.02 mm. Lesions were placed in rows without overlap on rabbit cornea. The effect of each irradiation was evaluated within one minute post exposure and the final determination of lesion formation was made using a slit lamp one hour post exposure. Threshold lesions were defined as the presence of a superficial surface whitening one hour after irradiation. Probit analysis was conducted to estimate the dose for 50% probability (ED50) of laser-induced damage. Approximately 20 different radiant exposures were made for each exposure duration-spot size combination. At the threshold level, the diameters of barely visible opaque white lesions were smaller than the Gaussian $1/e^2$ beam diameter. In selected survival animals, most of the threshold lesions were still visible 24 hours after exposure. The average lesion radius was approximately 0.4 ± 0.12 mm diameter for the 1.17 mm spot size and 1.0 ± 0.20 mm diameter for the 4.02 mm spot size. The exposure duration dependence of threshold average radiant exposure was described by an empirical power law equation: Threshold radiant exposure[J/cm2] = $a \times$ exposure duration[s] ^{b}, experimentally derived coefficient a was 9.79 and b was 0.669 for the 1.17 mm spot diameter; values of a and b were 4.57 and 0.456 respectively for the 4.02 spot diameter. Based on the experimental data and the empirical power law, the safety factors which were defined as threshold radiant exposure divided by MPE values were predicted for the 2.0 μm wavelength at various exposure durations and spot diameters. The minimum limit of the safety factor was approximately a factor of four for both 4.02 mm and 1.17 mm spot diameters. Due to the very sharp boundary and small uncertainties of damage threshold determination, it is suggesting that a factor of 4 "padding" is adequate and safety standard may not need to be changed.

Keywords: Cornea damage; ED50 threshold; Gaussian laser irradiation; laser injury; laser safety; Maximum Permissible Exposure (MPE)

1. INTRODUCTION

The eye and skin are the most susceptible parts of the body to accidental laser irradiation and due to the importance of vision to the quality of life, eye hazards are by far the more important consideration for safety. Wavelengths greater than 1.4 μm are primarily absorbed in the cornea and aqueous humor with insignificant energy reaching the retina. Since the absorption coefficient of cornea at 2.0 μm has been reported to be 45.9 cm^{-1}[1], approximately 90% of the irradiation delivered to the anterior surface of the human cornea is absorbed within average central thickness of 520 μm.

Early safety studies for wavelengths beyond 1.4 μm investigated CO_2 laser radiation of the cornea at 10.6 μm [2-6], where the 1/e penetration depth was approximately 10 μm [1]. Bargeron et al. found that most of the CO_2 radiation was absorbed with in the 50 μm thick human corneal epithelium [5]. In 1992, McCally et al. reported corneal damage thresholds for Tm:YAG laser radiation (2.02 μm) on New Zealand white rabbits [7]. The laser spot diameter was approximately 1 mm and exposure durations were 0.082 sec, 0.235 sec and 4.28 seconds. Based on the very little experimental data and mainly on the extrapolation of CO_2 threshold data, the American National Standard for Safe Use

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of Lasers (ANSI Z136.1-2000 [8]) defined the Maximum Permissible Exposure (MPE) for the eye at wavelengths between 1.8 μm and 2.6 μm and laser exposures from 1.0 ms to 10.0 s (listed in Table 1):

Wavelength(μm)	Exposure Duration, t (s)	MPE (J cm^{-2})	Limit Aperture Diameter (mm)
1.800 to 2.600	10^{-3} to 0.3	$0.56\ t^{0.25}$	1.0
	0.3 to 10	$0.56\ t^{0.25}$	$1.5\ t^{0.375}$

TABLE 1. Maximum Permissible Exposure (MPE) for Corneal Exposure to a Laser Beam (From ANSI Z136.1-2000).

A recent threshold damage study of skin to 2.0 μm laser irradiation suggested that the current laser safety standard may need to be adjusted for spot diameters larger than 3.5 mm [9]. Therefore, this study was conducted to provide large spot size thresholds for the cornea to 2.0 μm laser irradiation.

Safety standards are specified in terms of the MPE which is typically defined as a factor of ten below the ED50 damage threshold [10]. Since irradiation of the skin or eye to levels at the published MPE values may be "uncomfortable", it is good practice to maintain exposure levels sufficiently below the MPE [8]. In this paper, we report the corneal minimal visible damage thresholds for two spot diameters and exposure duration from 0.1 sec to 4 seconds. These results may help refine current ANSI MPE values.

2. MATERIAL AND METHODS

2.1. Experimental Setup

A rack mountable thulium fiber optic CW laser (IPG Photonics Corporation; Oxford, MA; Model: TLR-20-2000-LP) with a maximum 20 W output at a wavelength 2.0 μm provided the source of CW irradiation. A small fraction of the incident laser power was reflected onto a powermeter (Molectron Detector, Inc.; Portland, OR; Model: EPM2000 with an air-cooled powermeter probe PM30) using a beam splitter. Telescopes were employed to generate collimated laser beams with desired spot diameters. A low power alignment beam (fiber optic stable source 600nm-700nm; OZ optics, Ltd; Canada) was injected into the 2.0 μm beam path using a beam combining cube. Co-alignment of the two laser beams was accomplished at the cornea plane and at an intermediate aperture by adjustment of the position of the alignment laser fiber mount with respect to the combining cube. An iris shutter system (Uniblitz, Inc.; Rochester, NY; Model VMM-T1) was used to control exposure duration. Laser power was controlled by adjusting the current setting on the control panel of the laser. After energizing the laser, a settling time prior to corneal exposure was allowed until a stable power meter reading was obstained. A pulse generator (Stanford Research Systems. Inc; Sunnyvale, CA; Model DG535) was used to trigger the iris shutter system as well as a function generator (Hewlett-Packard, Ltd; Model HP 33120A), which controlled the imaging rate of an IR array detector thermal camera (Phoenix™ DAS camera system, Indigo, CA). The IR camera employed a 320 x 256 InSb array which detected wavelengths between 3 μm and 5 μm. The IR camera began capturing infrared images 0.1 second before laser irradiation, and continued recording for about 7 seconds after the laser was turned off. The imaging rate of IR camera was set at 100 Hz. The measurement system was arranged as depicted in Figure 1. The telescope, IR mirror and the IR camera were mounted together to ensure all burn sites were located at a fixed distance from the laser for the same spot size. Temperature calibration for the IR camera was done by using a blackbody radiator before laser irradiations. Power calibration of the reference was accomplished prior to and following each day in which laser exposures were delivered. The power meter used for calibration (Molectron Detector, Inc.; Portland, OR; Model: PM-3) was placed in the plane of the cornea and at least fifteen measurements, spanning the operational range used in testing for that day, were collected prior to corneal exposure. Normal incidence to the cornea for the 2.0 μm laser was assured by incorporating a video camera, co-imaged with the IR camera at the corneal plane. First (anterior cornea) and second surface (posterior cornea) specular reflections from the visible alignment laser were imaged on a video monitor using the video camera. When first and second surface reflections overlapped at the detector plane of the video camera, constructive interference was observed on the monitor and the incident beam was deemed normal to the corneal surface. To facilitate this alignment, fine positioning of the cornea was accomplished with the use of a goniometric animal stage which had 5-1/2 axes of adjustment.

Two different laser spot sizes were employed. The laser beam profiles were measured using a beam profiler (Pyrocam I; Spiricon, Inc; Logan, UT) and were confirmed by a measurement of temperature distribution on a plastic plate prior to heat conduction [9]. Moreover, beam diameters were checked with the knife-edge method [11]. All measurements indicated that the laser beam profiles were nominally Gaussian with $1/e^2$ diameters of 1.17 mm and 4.02 mm for the two telescope settings.

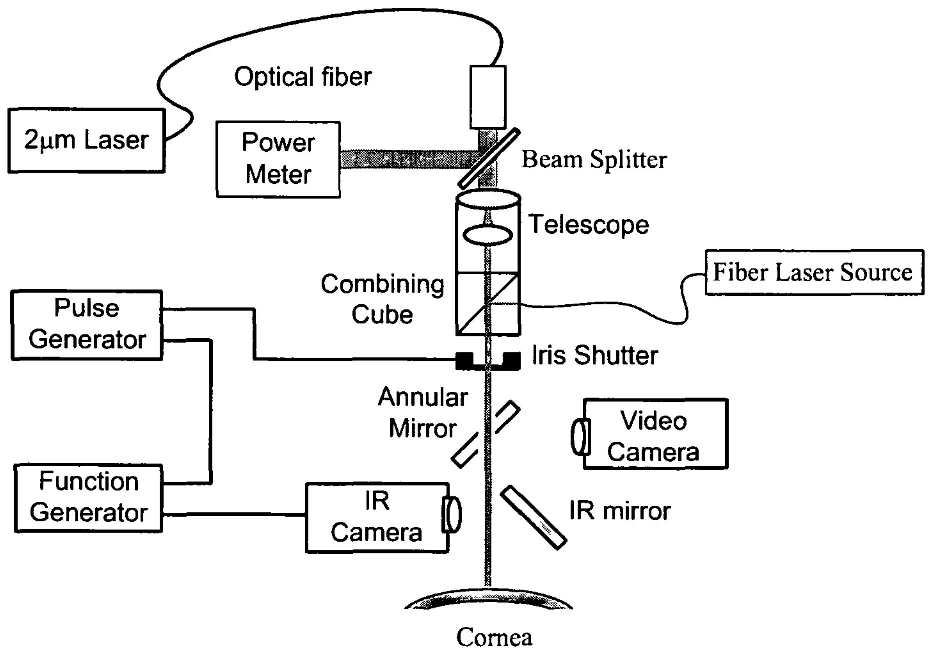

Figure 1. Experimental configuration for corneal damage study.

2.2. Animal

Rabbit cornea is an established animal model for determining laser damage thresholds with infrared radiation. Seventeen Dutch Belted rabbits of either sex weighting 2.14-2.36 kg were used in this study. The animal use protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin. The rabbits were chemically restrained using 20-40 mg/kg ketamine and 3-5 mg/kg xylazine , intramuscularly (IM). Two drops each of 0.5% proparacaine hydrochloride (Bausch and Lomb) and 5% homatropine hydrobromide (Alcon), were administered to both eyes for analgesia, cycloplegia and pupillary dilation. Anesthesia was maintained with isoflurane (1.5-3%) inhalant anesthesia. Isoflurane was supplemented by acepromazine (0.5-1.0 mg/kg) administered intramuscularly as needed for individual rabbits demonstrating a high intolerance to isoflurane. Prior to corneal laser exposures and once again after the completion of corneal irradiations, the animal was transported to a surgical table for slit lamp (Topcon; Paramus, New Jersey; Model: SL6E) examination. Once inhalant anesthesia was induced, the animal was physically secured in the sternal position on a specially designed adjustable animal stage to facilitate precise laser exposure. One hour prior to termination of isoflurane, buprenorphine 0.01-0.05 mg/kg was administered IM. Oxygen saturation and pulse, heart and respiration rate were continuously monitored throughout the study.

Eyelids of the rabbits were held open with a wire-lid speculum, and moisture of the cornea was maintained by frequent irrigation with 0.9% buffered saline solution at room temperature. In order to create a reproducible tear film, the irrigation was stopped 1 minute prior to laser exposure and the excess fluid was blotted at the limbus. Thermal measurement showed that the corneal surface returned to its normal temperature before the time of exposure.

On several animals, fluorescein sodium drops (0.25%) and benoxinate HCl (0.4%) ophthalmic solution (Alcon Laboratories, Ft. Worth, TX) were applied topically to the cornea to enhance lesion visibility during the slit lamp examination. Excess dye was flushed using buffered saline solution. A blue-wavelength pass filter was used with the slit lamp to enhance visibility of epithelial damage sites which were stained by the fluorescent dye.

After completion of the final examination, the subject was euthanized and the eyes were enucleated. Sutures were placed in the nasal and superior aspects of the enucleated eye to mark orientation for histological sectioning. Enucleated eyes were stored in formalin (10% buffered) and preserved for histology.

2.3. Damage Determination

At lease two examiners evaluated all exposure locations acutely and approximately one-hour post irradiation. In addition, twenty-four hour examinations were conducted for selected laser conditions. A grade of "yes" or "no" was recorded acutely and a numerical grade as described below was assigned at the one and twenty-four hour examinations.
Grade 0, no visible damage;
Grade 1, superficial damage minimally visible without magnification;
Grade 2, readily apparent lesion on surface with some circular symmetry;
Grade 3, severe lesion, circular symmetric opacity with shrinking of epithelium at the center.

Digitized video and still frame photography was used to document the exposure of the cornea and post exposure slit-lamp examination respectively. Probit analysis [12] was conducted to estimate ED50 damage thresholds. Data points (damage/no damage for each condition) were entered into the probit statistical analysis package (Lund, B., Probit Fit Dose-Response Data Analysis Program, Version 1.02, U.S. Army Medical Research and Material Command, Hazards Research Branch) to calculate the ED50 values.

Average radiant exposure [J/cm^2] reported in this paper was calculated as the applied laser energy divided by the $1/e^2$ spot area rather than $1/e$ spot area used in some laser safety classifications. The peak radiant exposure for our near Gaussian profile was twice the average value.

2.4. Experimental Procedures

After anesthetized rabbits were secured upon the animal stage, reflections from the cornea surface were observed on the video camera and the rabbit's position was adjusted within the FOV of the video camera until the angle of incidence of the co-aligned laser beams at the cornea was determined to be zero (i.e., normal approach). Constructive interference observed at the video monitor between first surface and second surface corneal reflections was used as an indication of "good alignment". Once alignment was established, the eye was irrigated with saline solution and one minute passed before laser irradiation. Power readings from the reference power meter were obtained at the time of corneal exposure. Thermal imagery was automatically recorded for each exposure. The thermal video was observed after each exposure to verify stability of the eye. Radiant exposures were made at specified exposure durations of 0.1 sec, 0.25 sec, 0.5 sec, 1 sec, 2 sec and 4 seconds for spot diameters of 1.17 mm and 4.02 mm. The number of irradiations for each of the 12 spot size-exposure duration conditions was 8 - 33 with an average of 20 per condition. The variation in laser power provided sufficient data points for probit analysis of damage/no damage response as a function of power. After each laser exposure the test location was examined to evaluate the presence or absence of corneal lesions with the aid of an overhead surgical light. A lesion was recorded as a "yes" if both readers identified it as positive. The consensus "yes" or "no" response of the observers was recorded on the data sheet. If consensus of the two observers could not be reached on damage a note of "questionable" was recorded on the data sheet and a third reader was used to give final decision based on slit lamp observation 1 hour later. This align-expose-examine procedure was repeated until all accessible corneal locations using the adjustable animal platform were exhausted. Nominally nine exposure locations were tested on each cornea with the 1.17 mm diameter beam and five were tested with the 4.02 mm beam. Practically the number of accessible corneal locations was limited by eye movement during testing which led to a disorganized grid pattern thus limiting the available corneal area for testing.

3. RESULTS

The effect of each irradiation was evaluated acutely by visual observation of the eye and the final determination of lesion formation was made using slit lamp at 1 hour post exposure. For some selected laser conditions, twenty-four hour evaluations were conducted to exam the damage at post exposure interval longer than 1 hour. The grossly apparent minimal visible lesion was defined as the presence of a superficial surface whitening at 1 hour after irradiation (see figure 2). At the threshold level, the barely visible opaque lesions were smaller than the Gaussian beam diameter. The threshold lesion sizes were measured roughly using slit lamp images. These diameters ranged from 0.3 mm - 0.6 mm (0.4 ± 0.12 mm) and 0.8 mm - 1.1 mm (1.0 ± 0.12 mm) for the 1.17 mm and 4.02 mm spot sizes respectively.

The ED50 threshold powers and standard deviations for 1-hour post exposure are listed in Table 2 along with their fiducial limits and slopes of the probit curves. The "standard deviation" (σ) was derived from the probit fit curve using the definition:

$$\sigma = (ED_{84} - ED_{16})/2, \qquad (1)$$

where ED_{84} represented the dose for 84% probability of laser-induced damage, and similarly for ED_{16}. Fiducial limits were calculated at the 95% confidence level. Slope of the probit curve was defined as:

$$\text{Slope} = \frac{\delta p}{\delta d}, \qquad (2)$$

where δp was the probability change and δd was the dose change.[12]

D(mm) \ T (s)	4	2	1	0.5	0.25	0.1
1.17	73.2 ± 0.8* (73.2–73.2)** slope 217.7 #	87.6 ± 1.9 (87.6–87.6) slope 211.2	114.9 ± 5.1 (114.9-114.9) slope 52.4	116.5 ± 1.1 (116.5-116.5) slope 250.1	154.1 ± 3.6 (154.1-154.1) Slope 200.8	236.1 ± 2.7 (236.1-236.1) Slope 200.3
4.02	303.6 ± 3.5 (303.6-303.6) slope 207.6	402.4 ± 48.6 (402.4-402.4) slope 19.0	538.2 ± 60.8 (446.0-585.9) slope 20.3	788.1 ± 5.5 (788.1-788.1) slope 328.7	1209 ± 23.1 (1153.3-1251.0) slope 120.3	2272.8 ± 45.2 (2272.8-2272.8) slope 115.1

Table 2. The ED50 Threshold Power [mw] and Standard Deviation associated with Their Fiducial Limits and Probit Curve Slopes. *: ED50 threshold power [mw] ± standard deviation. **: fiducial limits at the 95% confidence level. #: the slope of probit curve

4. DISCUSSION

Most of the one hour threshold lesions were still visible 24 hours after exposure. However, some barely visible superficial lesions disappeared. Nevertheless, all one hour threshold lesions were included in our evaluation of threshold. Damage threshold evaluated at one hour may give slightly more conservative threshold values than at 24 hours. As laser power was increased beyond threshold, readily apparent surface lesions with somewhat circular symmetry were seen. More severe lesions with circular symmetric opacity and shrinking of epithelium at the center were observed at powers around 1.5 times threshold. In this case, dehydration and coagulation of corneal epithelium as well as denaturization of corneal stroma occurred. In 1992, McCally *et al.* studied the corneal damage thresholds for Tm:YAG laser radiation (2.02 μm) on new Zealand white rabbits [7]. Based on their histopathlogic study of corneal threshold lesions, McCally *et al* claimed that the threshold damage was confined to the epithelium and no obvious stromal abnormalities were present [13]. Our observation of 2.0 μm threshold lesions using a slit lamp also suggested that the corneal opacity was present in a very thin layer, most probably only in the epithelium. Qualitative and quantitative histopathologic study of corneal damage will be performed to determine the mechanisms of laser induced damage and map the extent and severity of the lesions to confirm this conclusion.

Generally, the small standard deviations of threshold powers, closeness of lower and upper fiducial limits and sharp slopes of the probit curves indicated that the damage thresholds were well defined with a little overlap between exposures that produce damage and those that do not. The larger spot size (4.02 mm) had more uncertainty in damage thresholds than 1.17 mm spot at most of the exposure durations. The observational uncertainties of barely visible whitening on the corneal surface was more notable for the larger spot size (4.02 mm diameter). These threshold lesions appeared much more superficial and fainter than threshold lesions associated with the 1.17 mm spot size.

Typically, non uniform beam profiles are specified as 1/e diameter rather than $1/e^2$ to give a more conservative radiant exposures to compare to published MPE values in the laser safety classification. The radiant exposures based on 1/e diameter are twice as large as the $1/e^2$ diameter radiant exposure for a Gaussian beam. The 1/e radiant exposure is equal to the peak radiant exposure for a Gaussian shape laser beam. Although 1/e diameter is a conservative estimation of the laser hazard classification, $1/e^2$ diameters must be used to truly evaluate laser damage thresholds which require average irradiance. All calculations in this paper are based on the $1/e^2$ definition of spot size. The average radiant exposure (*H*) is calculated as:

$$H = \frac{J}{\pi r^2}, \quad [\mathrm{J/cm^2}] \tag{3}$$

where *J* is the threshold energy and *r* is the $1/e^2$ radius of the laser spot.

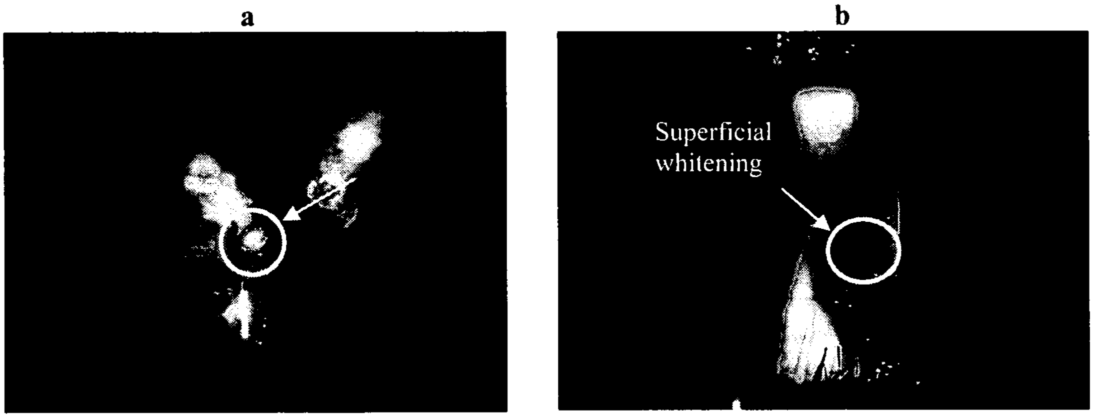

Figure 2. Slit lamp images of threshold damage. a) spot diameter 1.17 mm, exposure duration 0.25 s, power 181 mw (approximately 1.2 times threshold power). Grade 2 was assigned as the severity of the lesion. b) spot diameter 4.02 mm, exposure duration 0.5 s, power 789.7 mw (approximately equals threshold power). Grade 1 was assigned.

In Fig 3, the 2.0 μm threshold radiant exposures are compared with the single-pulse damage thresholds that were determined for Tm:YAG laser (2.02 μm wavelength) radiation by McCally *et al* [7]. McCally *et al.* studied the corneal damage thresholds for Tm:YAG laser radiation (2.02 μm) on new Zealand white rabbits. Threshold average radiant exposures were determined as 4.23 J/cm^2 and 29.6 J/cm^2 for 1.33 mm $1/e^2$ spot diameter and exposure duration of 0.235 sec and 4.28 seconds respectively [7,13]. The slightly higher threshold values at 2.02 μm wavelength than our experimental results for 2.0 μm laser at 1.17 mm spot size are mainly due to the smaller absorption coefficient of cornea at 2.02 μm. Water is the primary absorber around 2 μm wavelength, the absorption coefficient of cornea can be estimated by the product of absorption coefficient of water and the water content of cornea:

$$\mu_a = \mu_{water} \times w, \quad [1/\mathrm{cm}] \tag{4}$$

where μ_{water} is the absorption coefficient of water, which is 69.12 cm^{-1} at 2.0 μm and 55.99 cm^{-1} at 2.02 μm wavelength [14], and *w* is the water content of the cornea, approximately equals 0.78 for the rabbit [15]. Therefore, the corneal absorption coefficients are estimated to be 53.91 cm^{-1} and 43.67 cm^{-1} at 2.0 μm and 2.02 μm wavelengths respectively. Consequently, for 2.02 μm laser radiation, more input energy is needed to achieve the same temperature rise resulting from radiant exposure at 2.0 μm. Comparing McCally's threshold results (1.33 mm spot size) at exposure duration 0.235 sec and 4.28 seconds with our corresponding results (1.17 mm spot size) at 0.25 s and 4 seconds respectively, the relative differences of threshold radiant exposures are close to 20 %, which corresponds to the percent difference in corneal absorption coefficients at these two wavelengths. A more precise analysis should consider the transient temperature profiles and the temperature dependent changes in the absorption coefficients at the water absorption peak.

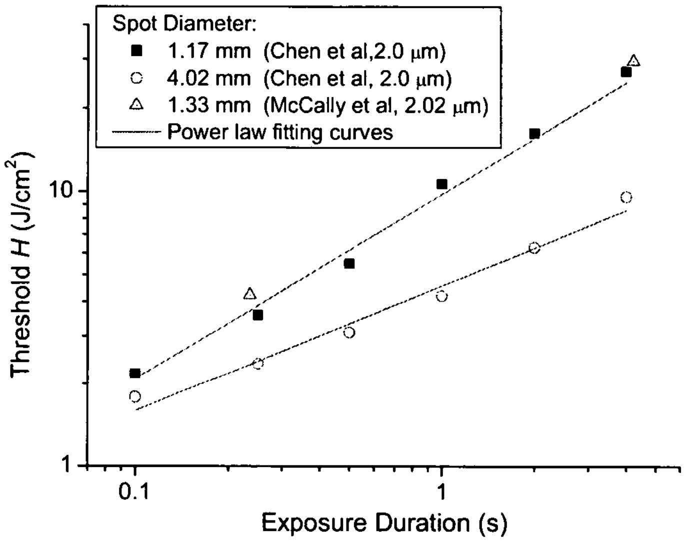

Figure 3. Comparison of threshold average radiant exposures along with the power law fitting curves for 1.17 mm and 4.02 mm spot sizes.

An empirical power law relation of threshold radiant exposure and exposure duration was proposed by McCally *et al.* [13]. They investigated single-pulse threshold damage on cornea at three different wavelengths (10.6 μm CO_2 laser, 2.02 μm Tm:YAG laser and 1.54 μm Er fiber laser), and suggested that the threshold radiant exposure followed an empirical power law relationship:

$$H = at^b \quad , \qquad [\mathrm{J/cm^2}] \tag{5}$$

where H is the threshold radiant exposure, t is the exposure duration, a and b are two positive coefficients selected to fit the experimental data. Although the absorption coefficients for these wavelengths span nearly 2 orders of magnitude, McCally found that the coefficient b's were nearly identical at all three wavelengths. Our results confirmed the general hypothesis of equation (5). However when spot size was considered (see Figure 3) different coefficients for a and b were determined. Our power law fitting results are listed in Table 3.

The light 1/e penetration depth ($\delta = 1/\mu_a$) in the cornea at 2.0 μm is approximately 180 μm and the associated characteristic thermal diffusion time for spot diameters much larger than δ is about 250 ms [16]. The power law relation is not valid when the exposure duration is much less than characteristic diffusion time such that heat conduction during the laser pulse is negligible and the impulse response is a function of pulse energy and independent of pulse duration. Threshold peak temperature predicted by the damage rate process integral is only a function of the temperature decay transient at the end of the short laser pulse [17].

In the IR wavelength range of 1.800 to 2.600 μm, the ANSI Z136.1-2000 defined MPE as follows:

$$H_{\max} = 0.56\, t^{\,0.25} \, , \qquad [\mathrm{J/cm^2}] \tag{6}$$

where $H_{\max}$ is the maximum allowed radiant exposure and t is exposure duration. The ANSI standard of MPE for eye exposure to 2.0 μm laser and the experimental results of threshold average radiant exposure at various durations and spot sizes are compared in Table 4. A safety factor is defined as the threshold radiant exposure divided by MPE value.

The safety factors calculated from experimental radiant exposures are listed in Table 6 as well. Dividing the empirical threshold power law equation (5) by MPE definition (equation (6)), the safety factor is predicted by following equation:

$$k = \frac{a}{0.56} t^{(b-0.25)} \quad , \qquad (7)$$

where k is the safety factor and a and b are the power law coefficients in equation (5). The safety factor monotonically decreases as exposure duration decreases, and reaches 7.1 for 1.17 mm and 5.8 for 4.02 mm spot sizes at 100 ms. If equation (7) is extrapolated to 30 ms, the predicted safety factor is about 4 for both spot sizes. Although the safety factor is smaller than the typical choice of 10 for MPE definition, more careful consideration should be given due to the very sharp boundary and small uncertainties of damage threshold determination. The concept of a safety factor must consider the overall level of uncertainty in the threshold data, experimental detail, sources of potential error, differences between animal and humans, and knowledge of the injury mechanisms and the biological sequelae. Although the typical safety factor is set as 10 in most cases for skin and retina thresholds, a smaller safety factor may be adequate for corneal MPE determination due to the small uncertainty of cornea damage [10]. For exposure durations shorter than 30 ms at 2 μm, absorbed energy is confined and for any spot size there is a constant threshold radiant exposure until non-thermal damage mechanisms begin to play a role.

Spot size [mm]	a [J/cm^2]	b	R (correlation coefficient)
1.17	9.79	0.669	0.98
4.02	4.57	0.456	0.97
MPE*	0.56	0.25	N/A

Table 3. The Results of Power-Law Fitting of Threshold Radiant Exposure. ($H = at^b$). *: ANSI MPE definition for corneal exposure at 2.0 μm. See Table 1.

T (s) / D (mm)	4	2	1	0.5	0.25	0.1
1.17	27.2	16.3	10.7	5.5	3.58	2.19
4.02	9.6	6.3	4.2	3.1	2.38	1.79
MPE	0.79	0.67	0.56	0.47	0.40	0.31
Safety factor, k	34.4(12.2)*	24.3(9.4)	19.1(7.5)	11.7(6.6)	9.0(6.0)	7.1(5.8)

Table 4. Experimental Threshold Average Radiant Exposures [J/cm^2] and ANSI MPE Values along with Their Safety Factors. *: safety factor calculated based on: 1.17 mm data (4.02 mm data).

Besides observational uncertainties, other experimental uncertainties were spot diameter and power measurements as well as exposure duration. Laser beam spatial profiles measured by a beam profiler were elliptical rather than circular, with the major axis about 10 % longer than the minor axis. To simplify calculations, spot diameters were estimated by arithmetically averaging the major and minor diameters. Uncertainties in calculated radiant exposure was magnified by uncertainties in spot diameter. The air-cooled power meter probe PM30 had 3% uncertainty, and the power meter EPM2000 had 1% read-out error. The iris shutter system with shutter LS6Z (Uniblitz, Inc.; Rochester, NY) used to control exposure duration at 1.17 mm spot size radiation has a 0.7 ms opening time, while VS25 shutter (Uniblitz, Inc.; Rochester, NY) used for 4.02 mm spot size setting had a 3 ms opening time. In conclusion, there was about 4% uncertainty in power measurements and 14% uncertainty in calculated radiant exposure.

5. CONCLUSIONS

We have defined and determined the *in-vivo* minimal visible threshold lesions on Dutch Belted rabbit corneas to 2.0 μm continuous-wave laser irradiation at six specified exposure durations (0.1 s - 4.0 s) and two spot sizes (1.17 mm and 4.02 mm in diameter). Threshold lesions were defined as the presence of superficial surface whitening one hour post irradiation. A power law relation between threshold radiant exposure and exposure duration was evaluated for different spot diameters: *Threshold radiant exposure*[J/cm^2] = $a \times$ *exposure duration*[s] ^{b}. Coefficient a was 9.79 and b was 0.669 for the 1.17 mm spot diameter; values of a and b were 4.57 and 0.456 respectively for the 4.02 spot diameter. Based on the empirical power laws, safety factors at 2.0 μm were calculated and predicted to have a minimum limit of four for both 4.02 mm and 1.17 mm spot diameters. Due to the very sharp boundary and small uncertainties of damage threshold determination, it is suggesting that a factor of 4 "padding" is adequate and safety standard may not need to be changed.

6. ACKNOWLEDGMENTS

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the University of Texas, the United States Air Force or the Department of Defense.

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