LASER: EXPERIMENTAL DETERMINATION OF RETINAL DAMAGES THRESHOLDS INDUCED BY MULTIPLE PICOSECOND PULSES.

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INTRODUCTION

Most laser safety documents advise the laser user that caution must be used in the evaluation of exposure to repetitively pulsed radiation since they are only limited data on multiple pulse exposure criteria. The empirical multiple pulse formula is based on some data indicating that there is generally a cumulative effect in multiple-pulse exposures. This effect is a reduction in the threshold energy per pulse relative to the single pulse threshold. The best fit to experimental data predicts that the threshold energy per pulse decreases in proportion to the fourth root of the number of pulses *n*. This model has been reported to be in agreement for exposures ranging from picoseconds to seconds. However, the very limited data reported in the sub-nanosecond domain of time were obtained with a low pulse ratio frequency. No data exists in the litterature concerning the effect on the retina of ultrashort pulses delivered with a high repetition rate. Also, it has been hypothesized that one cannot extrapolate from longer pulses width because the ultrashort pulse (single or multiple) induce effects which may involve fundamentally different mechanisms of damage. Moreover, laser exposure limits for a single-pulse duration less than 1 ns cannot be specifically provided by ICNIRP Guidelines or other publications on laser radiation because of a lack of biological data (1). The limit values for these exposure times has been derived by maintening the irradiances applicable to nanosecond pulses. With the goal in mind to obtain adequate data base for single and multiple ultrashort pulses, an experimental study has been directed toward determining the retinal damage thresholds induced by picosecond pulses emited in the visible spectrum.

METHODS

The experimental system is a dye laser pumped by a mode locked pulse compressed, frequency doubled Spectra-Physic Nd:YAG laser. A cavity dumper produces single pulses with an adjustable pulse repetition rate between single pulse and 1 million pulses per second (1 MHz). The wavelength at the output of the dye laser is 590 nm. A sample of the laser beam at 590 nm was deflected onto an autocorrelator which measures the pulsewidth and onto a photodiode and a calorimeter. The output signal of the photodiode, calibrated by the calorimeter, was converted with 8 bits resolution by a 100 MHz analogic-digital converter and was transferred to a computer. The animal used in the experiments was the rabbit Fauve de Bourgogne. Rabbits were used primarily because the average ocular transmission and absorption characteristics are very similar to the human ocular media for the visible spectrum and secondly to compare with the other experiments (2). The theorically expected spot size formed on the rabbit retina with the dye laser optical system was about 30 µm. To aid alignment of the animal's eye, a 1 mW HeNe laser was mounted co-axially with the main laser

beam path. Rabbits were anesthetized with intramuscular injection of ketamine hydrochloride (10 mg kg^{-1}), placed on a stand permitting us to produce in most cases retinal lesions aligned on the visual streak. The pupils were dilated before to exposure, and the eyelids were held open with a wire speculum. The treatment and procedures used in this study conformed to the European Community Guidelines on Animal Experiments. Two investigative methods were used. A direct ophthalmoscopic examination was made 15 mn and 24 h after the exposure using a fundus camera. Any presence or suspicion of lesion was photographed. Immediately after the direct ophthalmoscopy, all animals were examined using fluorescein angiography. Photographic recordings were made for each observation of fluorescein leakage.

RESULTS

The results were obtained for repetitive pulse trains varying from 1 000 to 200 000 pulses. The pulse repetition frequency (PRF) used were 10 kHz, 100 kHz and 1 MHz. Because the low energy characterizing a single pulse in our experimental laser configuration, no retinal injury has been observed for 1 MHz pulse train including less than 100 pulses. Similarly, few damages have been detected with lower PRF than 1 MHz and the data were not enough to calculate a probit regression line. In our experimental conditions, the lowest fundoscopic threshold levels were obtained 24 h after exposure with the direct ophthalmoscopic method; whereas using fluorescein angiography, the best time to detect an injury was immediately after the exposure. The experimental results are in good agreement with earlier works (3-4). After employing both investigative methods, we founds that fluorescein angiography appeared to be the most sensitive technique. These observations show that a fluid leakage is involved in the damage process. For each experimental condition, data were scored and processed by a method of probit analysis (5). The median effective dose (ED_{50}^{MP}) which is the energy expected to produce a retinal damage in half of the exposures is given in Table 1.

Number of pulses *n*		**100**	**1 000**	**10 000**	**20 000**	**100 000**	**200 000**
Time period (interval) (s)		1.10^{-6}	1.10^{-6}	1.10^{-6}	1.10^{-6}	1.10^{-6}	1.10^{-6}
ED_{50}^{MP}	(μJ)	6 *	28	217	-	1 570	2 950
ED_{07}^{MP}	(μJ)	4 obs	21	133	-	677	1 483
Time period (interval) (s)		1.10^{-5}	1.10^{-5}	1.10^{-5}	1.10^{-5}	1.10^{-5}	1.10^{-5}
ED_{50}^{MP}	(μJ)				1100 *		
ED_{07}^{MP}	(μJ)	-	-	-	545 obs	-	-
Time period (interval) (s)		1.10^{-4}	1.10^{-4}	1.10^{-4}	1.10^{-4}	1.10^{-4}	1.10^{-4}
ED_{50}^{MP}	(μJ)		36 *				
ED_{04}^{MP}	(μJ)	-	25 obs	-	-	-	-

obs observed value
* extrapolated value

Table I. Median effective doses calculated from fluorescein angiographic data obtained on rabbit retinas with multiple pulses trains (pulsewidth 8.10^{-12} s, $\lambda = 590$ nm).

DISCUSSION-CONCLUSION

The results show a slight additivity for pulses separated by less than one microsecond. The injury threshold per pulse decreases when the number of pulse *n* included in the train increases. The best equation fitting the relation between the energy threshold of the pulsed train and the number of pulse *n* is given by:

$$ED_{50}^{MP} = 0.01n + ED_{50}^{SP}$$

where ED_{50}^{MP} is the threshold value for a multiple pulses train of n pulses corresponding to a probability of 50% and ED_{50}^{SP} the threshold value for a single pulse with the same probability. The ED_{50}^{SP} extrapolated from our results, in the range of 1 000 to 200 000 pulses, is 55 μJ and the correlation coefficient r^2 is 0.9995. This energy threshold is higher than other values reported in the litterature (6-7) but the threshold value of 6 μJ reported by Birngruber et al (6) for Chinchilla Grey rabbits give the same fitting equation with our results and the correlation coefficient is 0.9984.

The severity of the damage does not seem to increase with the number of pulses. The damage observed was very similar after an irradiation of 100 or 200 000 pulses. No hemorrhagic lesion has been induced by a train of 200 000 pulses. Such lesions have been histologically observed at different delay post exposure (8).

Number of pulses n	**100**	**1 000**	**10 000**	**100 000**	**200 000**
Limit value ($n^{-1/4}$ formula) (μJ)	$4.8\ 10^{-10}$	$2.7\ 10^{-10}$	$1.5\ 10^{-10}$	$8.5\ 10^{-11}$	$7.1\ 10^{-11}$
ED_{50} per pulse of the train (μJ)	$6\ 10^{-8}$	$2.8\ 10^{-8}$	$2.2\ 10^{-8}$	$1.6\ 10^{-8}$	$1.5\ 10^{-8}$

Table II. Comparison between the results (ED_{50}^{MP}/n) and the corresponding limit values.

The comparaison of the data with the limit values of the guidelines (Table II) show that the empirical multiple-pulse formula, predicting that the threshold energy per pulse decreases in proportion to the fourth root of the number of pulses n, seems acceptable for picosecond pulses delivered with a high repetition rate. The safety marge is reasonable but is also variable depending the number of pulse. The empirical $n^{-1/4}$ formula is acceptable but does not describe specifically the mechanim of the repetitive-pulse injury thresholds.

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