Investigation of Selective Retina Treatment (SRT) by Means of 8 ns Laser Pulses in a Rabbit Model

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Background: It has been shown that selective retina treatment (SRT) using a train of 1.7 microseconds laser pulses allows selective damage of the retinal pigment epithelium (RPE) while sparing the adjacent photoreceptors and thus avoiding laser scotoma. It was the purpose of this work to investigate SRT laser effects with Q-switched pulses of only 8 nanoseconds in duration by evaluating the angiographic and ophthalmoscopic damage thresholds and the damage range by histology in a rabbit model.
Materials and Methods: A flash lamp pumped frequency doubled (532 nm) Nd:YAG laser with 8 nanoseconds pulse duration was used. In total 210 laser lesions, each calculated to be 102 μm in diameter on retina, were applied through a slit lamp onto the fundus of six eyes of Chinchilla Bastard rabbits. The rabbits were irradiated with increasing energies with single pulses and a train of 10 laser pulses at 10 Hz. After treatment fundus photography and angiography were performed to determine the damage thresholds (ED_{50}-probability of RPE cell damage and neurosensory retinal damage) as well as the safety range between both thresholds (ratio of angiographic ED_{86} vs. ophthalmoscopic ED_{14}). Selected histology was taken for single and repetitive pulse lesions after treatment.
Results: Angiographic and ophthalmoscopic ED_{50}-thresholds decreased with increasing number of pulses. For single pulse application ophthalmoscopic and angiographic ED_{50} were determined to 365 and 144 mJ/cm^2, respectively. Regarding 10 pulses 266 and 72 mJ/cm^2 were found. No retinal hemorrhages or disruptions were observed for both sets of parameters. The therapeutic window between angiographic and ophthalmoscopic threshold revealed a factor of 3.1 for single pulses and 2.3 for repetitive pulse irradiation. The safety range respectively had a factor of 0.8 (single pulses) and 1.7 (10 pulses). Histologic examination of laser lesions with single and repetitive pulses at radiant exposures within the therapeutic window—292 and 213 mJ/cm^2 respectively—revealed damaged RPE, intact Bruch's membrane and choriocapillaries. Photoreceptors were partly spared but also damaged to various extents.
Conclusions: Short laser pulses of 8 nanoseconds pulse duration can damage the RPE without retinal hemorrhage or disruption. Selective damage of the RPE without affecting the photoreceptors can only rarely be achieved due to the small safety range. Thus, so far microsecond laser pulses for SRT seems favorable compared to nanosecond pulses in order to prevent unintentional photoreceptor damage. Lasers Surg. Med. 40:20–27, 2008. © 2008 Wiley-Liss, Inc.

Key words: RPE; laserphotocoagulation; SRT; threshold determination; therapeutic window; angiography

INTRODUCTION

Conventional retinal laser photocoagulation is usually performed using continuous wave (cw) argon-ion or frequency doubled Nd:YAG lasers at 514 and 532 nm, respectively. Generally the exposure times are longer than 50 milliseconds, typically 100–200 milliseconds. Using these parameters histologically an irreversible destruction of the outer and inner segments of the neuroretina due to strong and extended tissue denaturation occurs [1–4]. It was shown that a variety of retinal diseases such as diabetic macular edema, diabetic retinopathy, subforms of age-related macular degeneration, or central serous retinopathy can be treated successfully by laser irradiation; however, laser scotoma results. Many macular diseases are thought to be associated with a declined function of the retinal pigment epithelium (RPE). Therefore, a method for a selective destruction of the RPE cells in order to rejuvenate the RPE and widely improve the metabolism at the choriocapillary and retinal boundary, seems to be an appropriate treatment [5], especially when the neuroretina and the photoreceptors can be spared.

The selective effect on RPE cells, which absorb about 50% of the incident green light due to their high content of melanosomes [6], was first demonstrated by Roider using 5 microseconds Argon laser pulses at 514 nm with a repetition rate of 500 Hz [5]. By irradiating the fundus with a train of microsecond laser pulses it is possible to achieve high peak temperatures solely around the RPE-melanosomes leading to RPE damage probably without effects on photoreceptors due to the heat conduction [7].

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In several clinical studies using the selective RPE laser treatment, laser scotoma could be avoided while the course of the macular disease was improving [8–15]. Thus, significant drusen reduction was achieved in AMD [9,10]. In diabetic maculopathy a stabilizing effect on macular edema was observed [12,14] and in central serous chorioretinopathy in nearly all patients no leakage was seen 4 weeks after selective retina treatment (SRT) [13].

Ongoing experimental work on SRT aimed on optimizing treatment modalities as to consider different pulse duration, number of pulses and repetition rates [16,17], and a non-invasive online dosimetry control in order to avoid angiography to prove RPE leakage as a successful treatment criterion [18]. It could be shown, that the background temperature can be decreased and thus the treatment selectivity be increased when decreasing the pulse repetition rate from 500 to 100 Hz and also the number of pulses from 500 to actually 30 pulses in a train using 1.7 microseconds per pulse [11,19]. It has also been shown in rabbits that by reducing pulse duration to 200 nanoseconds the therapeutic window between angiographic visibility indicating RPE damage and ophthalmoscopic visibility indicating unintentional neurosensory tissue denaturation was increased [16,17].

In recent work, it was found that the origin of RPE damage can be attributed to micro-vaporization around the individual intracellular melanosomes when pulses shorter than 50 microseconds are applied [20]. The high peak temperatures occurring at the melanosomes during irradiation lead to short living microbubbles which mechanically disrupt the RPE cells due to a short but strong volume increase [21]. Moreover, it could be observed that the radiant exposure and thus the energy required for bubble formation decreases with pulse duration [22]. However, mechanical effects and bubble sizes become stronger when the pulse duration is shortened due to more and more explosive character of vaporization. The bubble diameter around individual RPE melanosomes in suspension increases proportionally with pulse energy. Owing to bubble oscillations the maximal diameter is restricted to about 4 μm when using microsecond pulses [23].

Based on the in vitro work, the aim of the experiments described here is to investigate the damage thresholds and the therapeutic window as well as the safety range for selective RPE laser exposure in vivo using short 8 nanoseconds pulses—in a train of 10 pulses and as single pulses—in a rabbit model. The benefit of less energy needed for SRT and thus less heat induction might be compromised by the stronger mechanical effects when using nanosecond laser exposure. Histologic evaluation was used to disclose the thermal and mechanical damage ranges associated with the explosive character of nanosecond pulses induced microbubble formation and dynamics.

MATERIALS AND METHODS

Laser

For irradiation a flash lamp pumped frequency-doubled Q-switched Nd:YAG laser (Spectron Ltd.) was used. The laser provided temporal and spatial Gaussian-shaped laser pulses with 8 nanoseconds pulse duration at a wavelength of 532 nm. Fluctuation of energy per pulse was smaller than 12%. The laser light was coupled through an optical fiber with 50 μm core diameter and a numerical aperture of NA 0.22. A fiber length of 50 m was used to minimize speckle formation.

It is known that due to transmission of laser light through multimode fibers interference is causing intensity modulation called speckles. This effect is based on the optical path length difference of the different fiber modes. If the path length difference is smaller than the coherence length of the laser, speckles are produced by interference. The speckles can be characterized by their contrast K and the speckle factor F, which were determined using a laser beam analyzer [16]. For the Nd:YAG-system used here with a 50 m slit-lamp fiber the speckle factor $F_{\mathrm{Nd:YAG}}$ was determined to be $F = 1.18 \pm 0.02$ (no speckle: $F = 1$) and the contrast $K_{\mathrm{Nd:YAG}}$ was 0.11 ± 0.01 (no speckle: $K = 0$). Thus, the speckle formation achieved with this system was neglectable, revealing an almost ideally homogeneous top hat profile. However, all radiant exposures H were speckle corrected, calculated with the pulse energy E_{p} and the retinal beam radius r according to the following equation:

$$H = F \frac{E_{\mathrm{p}}}{\pi r^2}$$

Settings

The laser beam was delivered to an ophthalmic laser slit lamp (30 SL/L, Zeiss, Oberkochen, Germany). The fiber tip was imaged onto the retinal surface of the central area in the rabbit eye using a Goldmann contact lens. Calculations showed that the use of a planoconcave contact lens in cycloplegic emmetropic rabbit eyes translates the aerial fiber image of 158 μm into a retinal spot size of $2r = 102$ μm [24]. A green helium–neon laser (543 nm) was used as an aiming beam.

Animals

The treatment of experimental animals in this study was in compliance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Chinchilla bastard rabbits were used because the density and location of light-absorbing pigments in the fundus are rather uniform and similar to that of the human eye [6]. The animals were anesthetized with ketamine hydrochloride (35 mg/kg of body weight) and xylazine hydrochloride (5 mg/kg of body weight). Pupillary dilatation was achieved with topical application of phenylephrine hydrochloride 2.5% and tropicamide 1%. The animals were placed into a special holder system which allowed movements into all directions. The contact lens was placed onto the mydriatic eye using methylcellulose as a contact gel. The lens was locked at the animal holder to prevent unfavorable movements. In summary, 210 laser lesions were placed in six eyes of six rabbits.

Treatment and Data Evaluation

Ophthalmoscopically visible suprathreshold marker lesions were placed into the central retinal zone of about 2 mm × 2 mm. The dominantly ophthalmoscopically invisible test lesions were placed in between these marker lesions using different energies. Irradiation took place with single 8 nanoseconds laser pulses or a train of 10 laser pulses (10 Hz). Laser lesions were documented by fundus photographs (Fundus camera, Carl Zeiss, Oberkochen, Germany). Afterwards standard fluorescein angiography with injection of 0.5 ml of 10% fluorescein sodium into the ear vein was performed. Each lesion was evaluated with respect to its angiographic and ophthalmoscopic visibility as already described in detail [16,17]. Probit analysis software programmed by CP Cain [25] was used to calculate the angiographic as well as the ophthalmoscopic ED_{50} damage threshold. Since in SRT, ophthalmoscopic visibility should be ideally 0% (no retinal side effects as coagulation) and angiographic visibility 100% (RPE damage) the therapeutic window is determined as the range between both of these thresholds and is conventionally calculated as the gap between the angiographic ED_{50} and ophthalmoscopic ED_{50} damage threshold. However, Sliney et al. stated that in laser injury studies a major point of discussion is always relates to the level of uncertainty of the threshold of injury, which relates to the slope of the transformed dose-response curve, or the "probit plot" and that the most cited point on the probit plot is the exposure that represents a 50% probability of injury. This value is frequently referred as the "threshold," even though a lot of experimental damage points exist below the "threshold." [26] Thus, a safety interval should be regarded as ratio of ophthalmoscopic ED_{14} to angiographic ED_{86} which takes the width of the logarithmic normal distribution fitted by probit into account.

Histology

Histology was obtained from single 8 nanoseconds pulses and trains of 10 pulses in a row irradiated at 0.8 times ED_{50}^{oph}, meaning treatment within the therapeutic window. Additional histologies were obtained from laser lesions irradiated at 1.2 times ED_{50}^{oph} meaning a suprathreshold irradiation. Eyes were enucleated 1 hour after irradiation (in one case 3 days after irradiation) under deep anesthesia in vivo to avoid choroidal swelling. The animals were sacrificed immediately after enucleation by a 5 ml bolus injection of T61 into the ear vein. Globes were incised anterior to the equator, and immersed with 2.5% Karnovsky's solution. The complete globe was put into this solution. Then the posterior eye cup was cut from the anterior segment 30 minutes later and the posterior globe again replaced in the fixative. After another 30 minutes the retinae were fixed in 2.5% glutaraldehyde, dehydrated in alcohol, and embedded in expoxy resin (Epon). Semi-thin sections (1.5 μm) were stained with toluidin-blue (0.1% solution of toluidin-blue in 2.5% sodiumcarbonate; staining for 2 minutes on a heating plate at 60°C).

RESULTS

With the Nd:YAG laser system in summary 210 laser lesions with a train of 10 8 nanoseconds pulses ($n = 120$) and also single pulses ($n = 90$) were applied to the fundus. Macroscopic disruptive effects or hemorrhage never occurred in the test lesions. For single pulses and trains of pulses irradiation was performed using energies from 3.5 up to maximally 40 μJ. With higher energies above ophthalmoscopic threshold, immediate blanching of the retina was noticed. This was rather a continuous, than an abrupt process. Bleeding was never observed up to the maximal energy used here. The ophthalmoscopic appearance of the whitening of the retina was similar to that after conventional cw photocoagulation. With increasing pulse energies much above ophthalmoscopic threshold bubble formation in the center of the laser spot was noticed and already described earlier [24]. Haemorrhage was observed at high energy levels above the bubble-threshold only in marker lesions. Bleedings never occurred without prior notice of long lasting macroscopic bubbles observable under white light at the slitlamp. Regarding the ED_{50} thresholds for RPE damage for single pulse irradiation the ophthalmoscopic threshold was 365 mJ/cm^2 and the angiographic one 144 mJ/cm^2. For the safety range according to Sliney [26] only a factor of 0.8 was calculated (ophthalmoscopic $ED_{14} = 231$ mJ/cm^2 vs. angiographic $ED_{86} = 305$ mJ/cm^2). The ophthalmoscopic ED_{50} threshold for 10 pulse irradiation was 266 mJ/cm^2 and the angiographic one 72 mJ/cm^2. For the safety range a factor of only 1.7 was calculated (ophthalmoscopic $ED_{14} = 186$ mJ/cm^2 vs. angiographic $ED_{86} = 111$ mJ/cm^2). All damage thresholds including therapeutic window and safety range are summarized in Table 1. Thus, damage thresholds for the 10 pulse regime are significantly lower than for single pulses, in accordance to previous results [22]. Comparison of the observed damage thresholds with those of former studies [16,17] are displayed in Figure 1 showing huge standard deviations for single pulse irradiation especially for the sensitive angiographic threshold in 8 nanoseconds pulse treatment. For 10 pulse irradiation using 8 nanoseconds pulses standard deviations are comparable to those of longer pulse durations; however, the safety range was smaller (Fig. 1).

Histology of single pulse laser lesions (Figs. 2 and 3) and also for 10 pulse laser lesions (Fig. 4) at a factor of 0.8 ED_{50}^{oph} within the safety range reveals open vessels within choroid and choriocapillaries, intact Bruch's membrane, a destructed flat and condensed RPE as well as partly intact but also significantly damaged photoreceptors in many of the lesions, despite using the same irradiation parameters (Fig. 3). This was seen in single pulse as well as 10 pulse irradiation; whereas in single pulse application the damage extent was regarded as being larger. However, in all sections the inner layers of the neurosensory retina are completely unaffected in terms of particular cellular damage. Some irregularities of the outer nuclear layers as in Figures 2a, 3c, and 4 seem to be attributed to the RPE/photoreceptor damage with

TABLE 1. Summary of All Calculated Damage Thresholds (mJ/cm^2) by Probit Analysis

Parameter	ED_{50}^{oph}	ED_{50}^{ang}	ED_{14}^{ph}	ED_{86}^{ang}	TW	SR
8 nanoseconds 1 pulse	365	144	231	305	2.5	0.8
8 nanoseconds 10 pulses	266	72	186	111	3.7	1.7

TW, therapeutical window; SR, safety range.

subsequent "downward contraction" of the following neurosensory layers.

In comparison, suprathreshold irradiation at 1.2 times ED_{50}^{oph} with ophthalmoscopically slightly visible lesions (as well as in cw macular treatment, e.g., for diabetic maculopathy) reveal complete coagulation of the photoreceptor layer and also damage of the outer nuclear layer to various extents (Fig. 5). In fact, the damage of the nuclear layer seems to be more enhanced in single pulse than in 10 pulse irradiation.

DISCUSSION

It was the aim of this study to evaluate 8 nanoseconds laser pulse irradiation as possible treatment modality for selective RPE effects in SRT. Laser pulses within the low nanosecond-regime were always regarded as too short for photocoagulation or selective RPE damage since the risk of retinal disruption and retinal hemorrhage increases strongly with shorter pulses [24]. The reason are the much higher RPE peak temperatures during shorter compared to longer pulses when the same pulse energy is used [24]. Recent studies on RPE-melanosomes in suspension showed that if the vaporization temperature is exceeded, microbubble formation becomes more explosive towards shorter pulses. Large bubbles with starting speeds up to 40 m/s at radiant exposure of 450 mJ/cm^2 with 12 nanoseconds pulse duration are observed [27] exhibiting a high mechanical damage potential. This probably is responsible for retinal disruption and bleeding due to retinal and choroidal disruptions.

However, on the other hand, nanoseconds pulses seem attractive since the amount of heat generated, which can flow into the adjacent tissue and cause collateral thermal damage, is significantly smaller for shorter pulses, since less energy is needed for the desired microbubble formation [22]. Moreover, Q-switched laser systems with nanoseconds pulse durations in the green spectral range are easier to build and commercially available compared to those emitting pulses in the microsecond-regime, which need special techniques to enlarge the pulse duration by

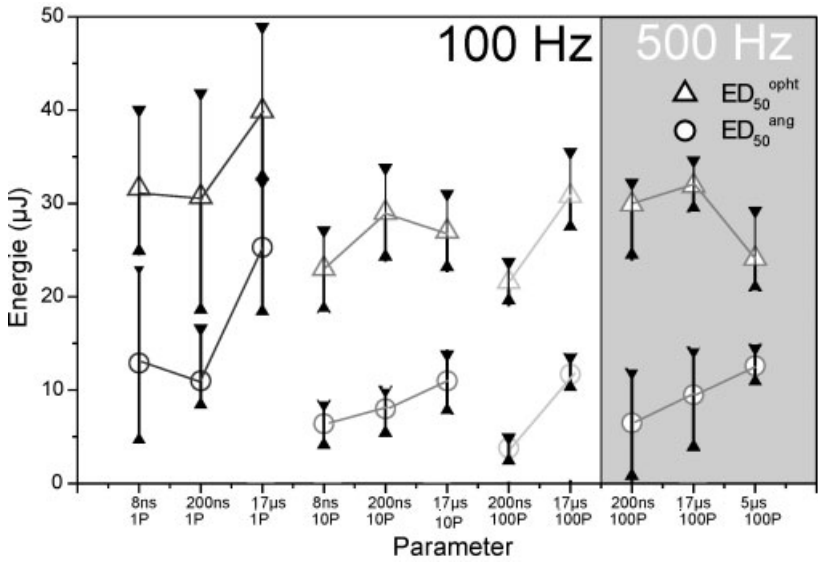

Fig. 1. Summarizes the threshold results of this study and two former studies from the authors using either 500 Hz repetition rate from 5 microseconds to 200 nanoseconds (100 pulses); ([16,17]), 100 Hz for 1.7 microseconds and 200 nanoseconds (single pulses up to 100 pulses) as well as 10 Hz for a train of 8 nanoseconds pulses or single pulses. As displayed the standard deviation for single pulse irradiation is always higher than for irradiation with 10 or 100 pulses making single pulse irradiation clinically not feasible. For comparison with the previous results of treatment with 200 nanoseconds and 1.7 microseconds at 100 Hz respectively 500 Hz also the dependence of the angiographic and ophthalmoscopic damage thresholds regarding number of pulses and pulse duration is shown, demonstrating lower thresholds for increasing number of pulses and smaller pulse duration having smallest standard deviation for 100 pulses rather than single pulses.

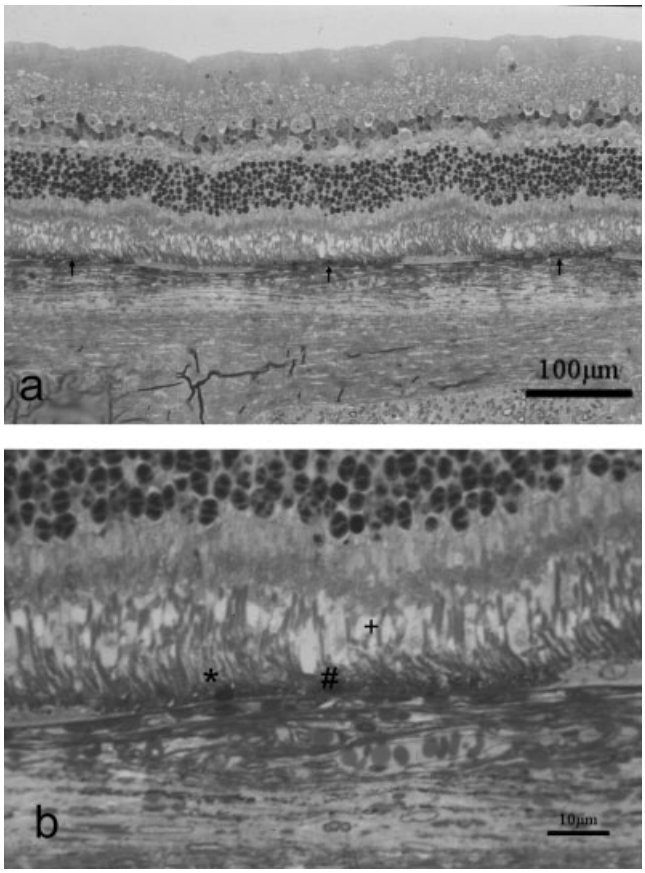

Fig. 2. **a**,**b**: Histology 40× (a) and 100× (b) 1 hour after irradiation with single pulses of 8 nanoseconds pulse duration at 0.8 ED_{50}^{oph}. In (a) three laser lesions (arrows) are visible showing condensed flat RPE and some rarefication of photoreceptors. b: Magnification of the central lesion reveals intact choriocapillaries, intact Bruch's membrane, coagulated melanin granules, and partly intact outer segments of the photoreceptors with preserved orientation (*) but also destructed outer segments (#). Within the whole lesion clear rarefication of the photoreceptor structure revealing a lot of empty spaces (+) is observable.

electronic pulse stretching [22] or overcoupled second harmonic generation [28].

As shown in this study, selective laser treatment with 8 nanoseconds laser pulses might be possible regarding to angiographic and ophthalmoscopic thresholds and regarding to histologic observations. However, due to the small safety range obtained for 8 nanoseconds treatment and the histologically observed variable damage extents within the lesions, clinical application of 8 nanoseconds laser treatment seems not to be feasible.

Regarding the damage thresholds in our study, it could be demonstrated—in association to previous studies ([16,17]; Fig. 1)—that using 10 pulses rather than single pulses lead to a decrease in the damage thresholds, especially the angiographic ones, and thus an enlargement of the safety range occurs. Generally larger therapeutic windows can be achieved with a higher number of repetitive pulses. According to Figure 1, 100 pulse irradiation with 200 nanoseconds or 1.7 microseconds pulse duration (both at 100 Hz repetition rate) results in a larger therapeutic window than for 10 pulse irradiation or even single pulses; however, treatment with less pulses as, for example, 10 pulses might be preferred to avoid unnecessary heat accumulation within the tissue. Thus, for 8 nanoseconds irradiation, treatment with a train of 10 pulses instead of just using single pulses would have been preferable.

For clinical application, it has to considered that fundus pigmentation in humans can inter- and intra-individually vary at least by a factor of 2 [6], comparable to those in rabbits. Since the safety range of single pulse irradiation using 8 nanoseconds is smaller than a factor of 1 (high standard deviation in angiographic and ophthalmoscopic threshold), clinical treatment using this parameter should not be recommended. However, also for 10 pulses of 8 nanoseconds the safety range revealed a factor of only 1.7, which is still very small to treat selectively. As shown in Figure 1 also for longer pulse duration as 200 nanoseconds or 1.7 µs, single pulse irradiation cannot be recommended since the safety range becomes very small and seems not to be suitable for clinical treatment.

Histology was performed from single pulsed laser lesions and 10 pulsed ones applied with a factor of 0.8 of the ophthalmoscopic threshold meaning irradiation within the safety range. Those specimens revealed RPE damage and even some restoration of the RPE layer due to proliferation 3 days after treatment. The photoreceptor layer in many of these lesions was grossly spared or partly unaffected and especially all other retinal layers were completely unaltered. However, we observed numerous lesions, which revealed significantly damaged photoreceptors despite using the same laser parameters. This seems to be attributed to the discussed very small safety range using 8 nanoseconds irradiation. For this, the extent of the damage also depends on the spatial pigmentation of the RPE. This pigmentation might vary highly even in a single eye leading to larger heat accumulation within more pigmented RPE and therefore, unselective thermal damage might occur. If irradiation would take place in lower levels of the safety range as, for example, at 1.2 times of angiographic threshold selectivity might be enhanced; however, the safety range is still very small. Since the impact of 10 pulse laser irradiation to the tissue is regarded as being lower than for single pulse irradiation (if the same energy is delivered to the tissue in a train of pulses rather than in a single pulse, peak temperature increase is lower) the extent of RPE and distal photoreceptor damage might be smaller using 10 pulses. In fact, histology for 10 pulse irradiation revealed selective RPE lesions, but due to the compromised safety range several lesions showed photoreceptor damage. If previous results of 200 nanoseconds and 1.7 microseconds irradiation ([17]; Fig. 1) are regarded, from the experimental point of view, 200 ns-irradiation should be recommended because the safety range was the largest. However, compared to 1.7 microseconds irradiation clinically no significant difference should be expected.

Histologic evaluation was additionally performed on laser lesions above ophthalmoscopic threshold at 1.2 ED_{50}^{oph}. In contrast to the previously described lesions, which were completely ophthalmoscopically invisible, these lesions were slightly visible comparable with weak cw laser coagulation, for example, diabetic maculopathy. Interestingly, complete damage of the photoreceptor layer was observed and also damage of the outer nuclear layer at various degrees, seemingly more enhanced for single pulse irradiation (438 mJ/cm^2) than for 10 pulses (10 times 319 mJ/cm^2).

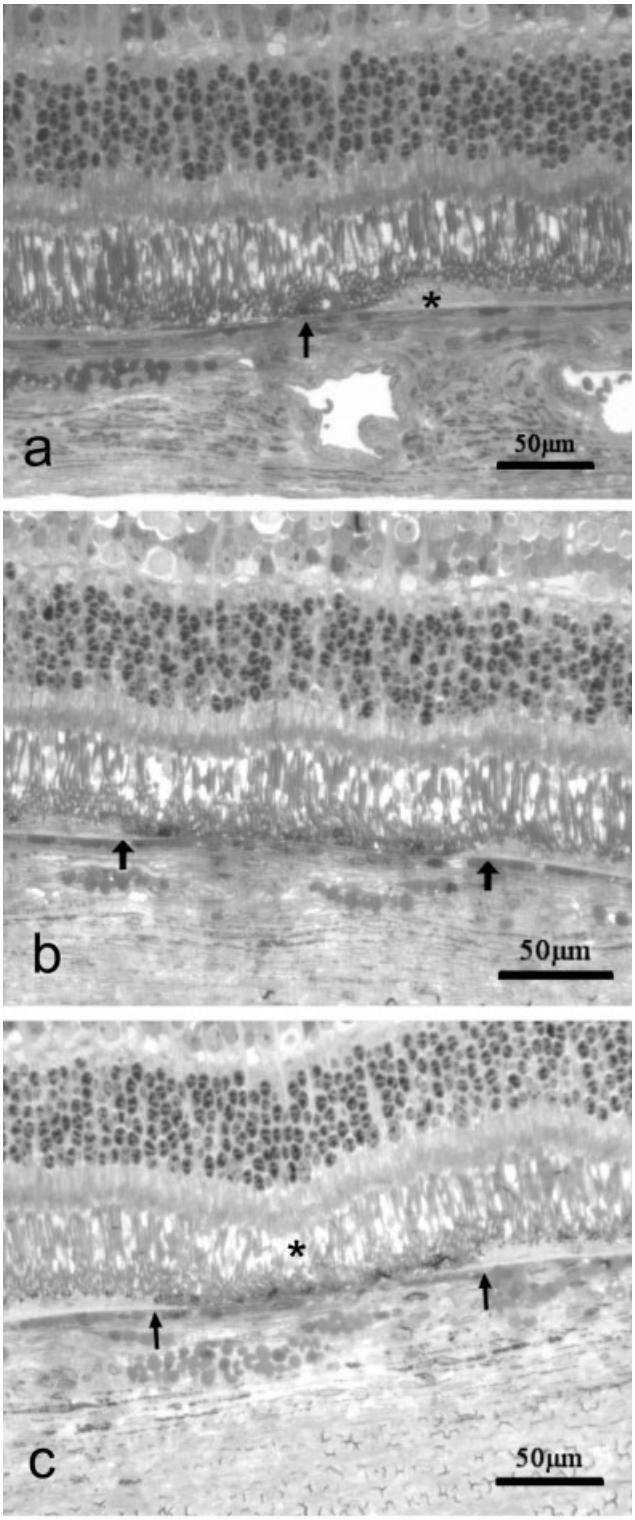

Fig. 3. **a–c**: Histologies (100×) for irradiation with single pulses of 8 nanoseconds pulse duration 3 days after treatment at 0.8 ED_{50}^{oph}. In (a) the RPE lesion is clearly visible and marked with an arrow. Photoreceptors seem to be grossly intact and also rarefication of the outer segments is only minor. Some RPE proliferation to cover the laser induced RPE defect seems to start (*). Some randomly seen big capillaries within the choroid—as in this image—are not associated with the SRT laser lesions. In (b) another lesion can be well demarcated from the healthy RPE (lesion borders marked by arrows). Rarefication of the outer segments is enhanced, coagulation of the outer tips could not be ruled out. Nuclei of the inner segments seem to be completely intact. Bruch's membrane can be followed as an intact line within the whole lesion. In another lesion (borders marked again with arrows) also applied with same laser energy (c) clearly damaged photoreceptors are observed (*). Orientation of cells is diffuse. The RPE is condensed and Bruch's membrane cannot be distinguished. Outer nuclear layer seems to be intact.

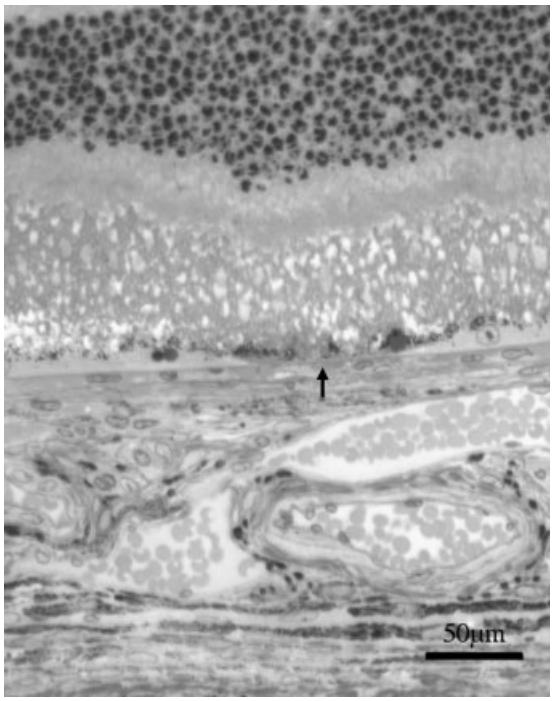

Fig. 4. Histology (100×) for irradiation with 10 pulses in a row of 8 nanoseconds pulse duration 1 hour after treatment at 0.8 ED_{50}^{oph}. The RPE damage is observable showing flat and condensed cells (arrow); Bruch's membrane seems to be intact. The photoreceptor layer is slightly compromised revealing some rarefication of cells within the lesion site. Extent of photoreceptor damage seems to be less enhanced than for single pulse irradiation.

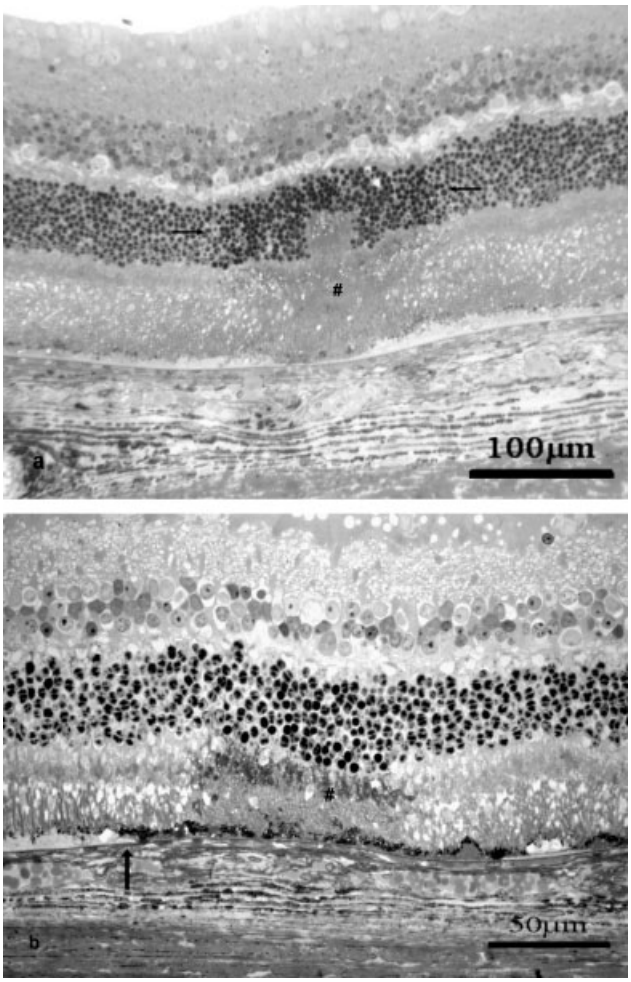

Fig. 5. **a**,**b**: Histologies (40×) 1 hour after application of suprathreshold laser lesions with single pulses (a) and a train of pulses (b) at a factor of 1.2 ED_{50}^{oph}. For both lesions, complete coagulation of the photoreceptor layer (#) was observed and also damage of the outer nuclear layer more enhanced with single pulses (arrows in (a)) rather than with a train of pulses (b, arrow demarcates the border to healthy tissue).

This might indicate that ophthalmoscopic visibility of laser lesions likely means irreversible tissue damage and not only reversible edema. In SRT suprathreshold single pulse irradiation rather than repetitive irradiation leads to a stronger energy impact on the tissue with extended damage to the neurosensory retina. Since this is observed at only 1.2 ED_{50}^{oph} above the threshold it becomes clear that a large safety range between angiographic and ophthalmoscopic threshold is necessary to avoid adverse retinal effects. The small safety range of nanosecond SRT is likely too small and thus is not recommended for clinical use.

In conclusion, 8 nanoseconds pulse irradiation for selective RPE damage seems to be possible; however, the safety range is very small using 10 pulses and even smaller using single pulse irradiation. As histologically seen laser lesions with 8 nanoseconds irradiation can be selective but this seems not to be repeatable since numerous lesions within the safety range also showed enhanced photoreceptor damage. The value of a large safety range is given by the histologic observations of slightly suprathreshold lesions showing distinct coagulation even of inner retinal layers leading to irreversible laser scotoma. Due to the various extent of pigmentation within the eye proper irradiation parameters should enforce a safety range of at least factor 2 to avoid unselective damage. Thus, a clinical application of 8 nanoseconds irradiation is not recommended.

REFERENCES

1. Birngruber R. Die Lichtbelastung unbehandelter Netzhautareale bei der Photokoagulation. Fortschr Ophthalmol 1984; 81:147–149.
2. Lorenz B, Birngruber R, Vogel A. Quantifizierung der Wellenlängenabhängigkeit laserinduzierter Aderhauteffekte. Fortschr Ophthalmol 1989;86:644–654.
3. Marshall J, Mellerio J. Pathological development of retinal laser photocoagulations. Exp Eye Res 1968;7:225–230.
4. Wallow IH, Birngruber R, Gabel VP, Hillenkamp F, Lund OE. Netzhautreaktion nach Intensivlichtbestrahlung. Adv Ophthalmol 1975;31:159–232.
5. Roider J, Michaud NA, Flotte TJ, Birngruber R. Response of the retinal pigment epithelium to selective photocoagulation. Arch Ophthalmol 1992;110:1786–1792.
6. Gabel VP, Birngruber R, Hillenkamp F. Visible and near infrared light absorption in pigment epithelium and choroid. In: Shimizu K, editor. International Congress Series No. 450, XXIII Concilium Ophthalmologicum, Kyoto. Princeton, NJ: Excerpta Medica; 1978. pp 658–662.
7. Roider J, Hillenkamp F, Flotte TJ, Birngruber R. Microphotocoagulation: Selective effects of repetitive short laser pulses. Proc Natl Acad Sci USA 1993;90:8643–8647.
8. Roider J, Wirbelauer C, Brinkmann R, Laqua H, Birngruber R. Control and detection of subthreshold effects in the first clinical trial of macular diseases. Invest Ophthalmol Vis Sci 1998;39:104.
9. Roider J, Brinkmann R, Wirbelauer C, Laqua H, Birngruber R. Subthreshold (retinal pigment epithelium) photocoagulation in macular diseases: A pilot study. Br J Ophthalmol 2000;84:40–407.
10. Framme C, Brinkmann R, Birngruber R, Roider J. Autofluorescence imaging after selective RPE laser treatment in macular diseases and clinical outcome: A pilot study. Br J Ophthalmol 2002;86(10):1099–1106.
11. Roider J, Brinkmann R, Birngruber R. Selective retinal pigment epithelium laser treatment—Theoretical and clinical aspects. In: Fankhauser F, Kwasniewska S, editors. Lasers in Ophthalmology—Basic, Diagnostic and surgical Aspects. The Hague: Kugler Publications; 2003. pp 119–129.
12. Liew M, Hussain B, Hamilton P, Elsner H, Klatt C, Shilling J, Theisen-Kunde D, Brinkmann R, Birngrubr R, Roider J, Marshall J. Selective retina treatment (SRT) of diabetic maculopathy: First multicenter study. in press, 2007.
13. Elsner H, Pörksen E, Klatt C, Bunse A, Theisen-Kunde D, Brinkmann R, Birngruber R, Laqua H, Roider J. Selective retina therapy (SRT) in patients with central serous chorioretinopathy (CSC). Graefes Arch Ophthalmol 2006; 244(12):1638–1645.
14. Elsner H, Klatt C, Liew SH, Porksen E, Bunse A, Rudolf M, Brinkmann R, Hamilton RP, Birngruber R, Laqua H, Roider J. Selektive retina therapiey (SRT) bei patienten mit diabetischer makulopathie. Ophthalmologe 2006;103(10): 856–860.
15. Brinkmann R, Roider J, Birngruber R. Selective retina therapy (SRT)—A review on methods, techniques, preclinical and first clinical results. Bull Soc Belge Ophtalmol 2006; 302:51–69.

16. Framme C, Schüle G, Roider J, Kracht D, Birngruber R, Brinkmann R. Threshold determinations for selective RPE damage with repetitive pulsed microsecond laser systems in rabbits. Ophthalmic Surg Lasers 2002;33(5):400–409.
17. Framme C, Schuele G, Roider J, Birngruber R, Brinkmann R. Influence of pulse duration and pulse number in selective RPE laser treatment. Lasers Surg Med 2004;34:206–215.
18. Schüle G, Elsner H, Framme C, Roider J, Birngruber R, Brinkmann R. Optoacoustic real-time dosimetry for selective retina treatment. J Biomed Opt 2005;10(6): 064022-1–064022-11.
19. Schüle G, Hüttmann G, Framme C, Roider J, Brinkmann R. Non-invasive optoacoustic temperature determination at the fundus of the eye during laser irradiation. J Biomed Opt 2004;9(1):173–179.
20. Schüle G, Rumohr M, Hüttmann G, Brinkmann R. RPE damage thresholds and mechanisms for laser exposure in the μs to ms time regimen. Invest Ophthalmol Vis Sci 2005; 46(2):714–719.
21. Neumann J, Brinkmann R. Nucleation and dynamics of bubbles forming around laser heated microabsorbers. Proc Soc Photo Opt Instrum Eng 2005;5863:19–27.
22. Brinkmann R, Hüttmann G, Rögener J, Roider J, Birngruber R, Lin CP. Origin of retinal pigment epithelium cell damage by pulsed laser irradiance in the nanosecond to microsecond time regimen. Lasers Surg Med 2000;27:451–464.
23. Neumann J, Brinkmann R. Microbubble dynamics around laser heated microparticles. Proc Soc Photo Opt Instrum Eng 2003;5142:82–87.
24. Birngruber R, Gabel VP, Hillenkamp F. Experimental studies of laser thermal retinal injury. Health Phys 1983; 44:519–531.
25. Finney DJ. Probit analysis, 3rd ed. London: Cambridge University Press; 1971.
26. Sliney DH, Mellerio J, Gabel VP, Schulmeister K. What is the meaning of threshold in laser injury experiments? Implications for human exposure limits. Health Phys 2002; 82(3): 335–347.
27. Neumann J, Brinkmann R. Nucleation dynamics around single microabsorbers in water heated by nanosecond irradiation. J Appl Phys 101(114701): 2007.
28. Kracht D, Brinkmann R. Green Q-switched microsecond laser pulses by overcoupled intracavity second harmonic generation. Opt Commun 2004;231:319–324.