Lasers in Surgery and Medicine 33:102–107 (2003)

Transpupillary Thermotherapy (TTT) With Short Duration Laser Exposures Induce Heat Shock Protein (HSP) Hyperexpression on Choroidoretinal Layers

Thomas Desmettre, MD, PhD,[1,2,3] Claude-Alain Maurage, MD,[4] and Serge Mordon, PhD[1]*
[1]*UPRES-EA2689 INSERM IFR 114, University Hospital, 59037 Lille Cedex, France*
[2]*Imaging, Laser, and Low Vision Rehabilitation Center, 59130 Lambersart, France*
[3]*Ophthalmology Department, Lariboisière University Hospital, 75010 Paris, France*
[4]*Pathology Department, Lille University Hospital, 59037 Lille, France*

Background and Objectives: To assess a choroidal heat shock protein hyperexpression after transpupillary thermotherapy (TTT) performed with exposures shorter than 60 seconds.

Study Design/Materials and Methods: Nine male pigmented rabbits were anesthetized and TTT was performed on their right eye with a 810 nm diode laser (Iridis, Quantel-Medical (France)) (spot size: 1.3 mm). Three exposure durations (60, 30, or 15 seconds) were used with three ranges of power for each duration ("high," "mild," or "low"). A series of laser impacts was delivered to the posterior pole of the retina. Left eyes were used as controls. Twenty-four hours after laser irradiation, the animals were killed and histological study was performed on chorioretinal layers. Tissue samples were fixed in formalin and embedded in paraffin. A monoclonal antibody was used to detect Hsp70 immunoreactivity (mouse IgGl, SPA-810, Stress Gen, Victoria, BC, Canada), followed by a biotinylated goat anti-mouse antibody (Dako, Glostrup, Denmark), revealed by the avidin–biotin complex (Vectastain kit, Vector Laboratries, Burlingame, CA, USA) and the AEC chromogen. Retinal structures were further identified by HES coloration.

Results: During the experiments, the laser spots were not visible except for the strongest "high" powers for each exposure duration, where a whitening was discernable at the end of the laser exposures. A strong HSP70 immunoreactivity was detected in choroidal, non-pigmented cells for laser exposures lasting 60, 30, or 15 seconds with "mild" laser powers. On the contrary, rare HSP hyperexpression was detected with "high" or "low" laser powers lasting 60, 30, or 15 seconds. No HSP-70 immunoreactivity was detected on control eyes nor outside of the irradiated zones of treated eyes.

Conclusions: Transpupillary laser irradiation lasting 15, 30, or 60 seconds induces an hyperexpression of HSP on choroidal layers. This could be a basis for the use of TTT with "short" laser exposures. Lasers Surg. Med. 33:102–107, 2003. © 2003 Wiley-Liss, Inc.

Key words: apoptosis; heat shock protein; laser; macular degeneration; neovascularization

INTRODUCTION

Transpupillary thermotherapy (TTT) is currently being evaluated for the treatment of subfoveal occult choroidal neovessels of age related macular degeneration (AMD) in the TTT4CNV study. The results of pilot studies have shown promising results for the treatment of occult or classic choroidal neovessels of AMD with laser parameters thoughtfully established by E. Reichel [1–8]. Angiographies performed early after TTT have shown vascular changes giving a hint about the target of the laser procedure [9–11].

According to these parameters, the retinochoroidal temperature elevation is induced with an infrared laser (diode 810 nm), a specific spot diameter (1.2, 2, or 3 mm), and a long duration pulse (60 seconds). Some rare cases of overdosage have been reported, leading to a drop in visual acuity related to a central scotoma [8,12]. These overdosages illustrate that the window of efficiency of the temperature elevation corresponding to TTT is closely below the photocoagulation threshold.

A particularity of TTT is the lack of biomicroscopically visible modification, i.e., absence or faint whitening of the area of laser irradiation at the end of the laser pulse [1]. This hampers a control of the tissue effect by the ophthalmologist during the laser pulse. In addition, for long exposures, such as 60 seconds, the control of the temperature rise throughout the duration of the laser pulse remains poorly documented [13–15].

The cellular mechanism of action of the moderate thermal elevation on chorioretinal tissues has been recently highlighted [15]. An experimental study has shown an hyperexpression of heat shock protein (HSP70) following TTT [16]. In this perspective, TTT would be a non-lethal suprahysiologic hyperthermia inducing an hyperexpression of

*Correspondence to: Serge Mordon, PhD, UPRES EA 2689, INSERM (French National Institute of Health and Medical Research) IFR114, Pavillon Vancostenobel, Lille University Hospital, 59037 Lille Cedex, France.
E-mail: mordon@lille.inserm.fr
Accepted 17 April 2003
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.10193

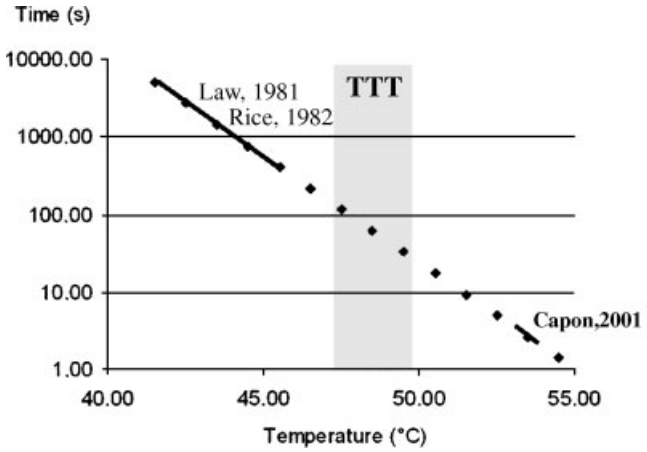

Fig. 1. Data from the literature illustrating a logarithmic relationship between duration and temperature for an equivalent thermotolerance in various tissues [18–20]. TTT with the use of standard parameters established by E. Reichel or with adapted parameters for pigmented, pseudophakes can take place within this figure (gray area).

HSP70 modulating apoptosis in choroidoretinal layers. Such a thermotolerance could decrease the amount of necrosis and the release of inflammatory cytokines in the choroidal tissue, leading to a decrease of neovascularization and the associated increase in vascular permeability [15,16]. Conversely, an overdosage of the laser light would result in a lethal hyperthermia and an underdosage would result in a physiologic hyperthermia. These two dosages would be inappropriate to enhance a strong HSP70 production and a thermotolerance.

A notion of "heat dose" is often used to emphasize that both the magnitude of the hyperthermia and the duration of exposure determine the final cellular effect [17]. According to this notion, with low temperatures, the duration of exposure has to be increased in order to reach the same degree of HSP hyperexpression and thermotolerance [18–20]. In Figure 1 data collected from the literature about thermotolerance of different tissues have been plotted, illustrating a logarithmic relationship between duration and temperature for an equivalent thermotolerance (Fig. 1). With a duration of 60 seconds and a temperature elevation that can be estimated between 45 and 50°C, TTT can logically take place in this representation.

Interestingly, on this figure, the use of a 30 or 15 second exposure instead of 60 seconds would still be under the photocoagulation threshold. In this perspective, the present study aimed to determine on an animal model of TTT if such "short" 30 or 15 seconds laser exposure durations under the photocoagulation threshold could induce a HSP hyperexpression on choroidal layers.

MATERIALS AND METHODS

Animals

The animal experimental procedure was approved by the Lille University Animal Ethics Committee (protocol 2001-102) and carried out in accordance with the ARVO statement for the use of animals in ophthalmic and vision research. Rabbit eyes were used in this study as a healthy eye model for TTT without any opacities or heterogeneity of the lens. The laser exposures were performed on the posterior pole limiting focusing errors and near the optic disk to be easily retrieved during histology study. The homogeneous pigmentation of the fundus reduced the variation of laser light absorption. Nine male pigmented rabbits weighing between 3.0 and 3.5 kg were lightly anesthetized with an intra muscular injection of a combination of Ketamine (150 mg/kg) and Chlorpromazine (0.50 mg/kg). Ketamine and Chlorpromazine were then injected into the marginal ear vein to ensure the anesthesia of the animal during the whole experimentation. Pupil dilatation was achieved with Tropicamide 0.5% and Phenylnephrine (10%) eye drops. After completing the experiments, the animals were sacrificed with an overdose of Ketamine and Chlorpromazine.

Laser

An 810 nm diode laser, IRIDIS, Quantel-Medical (Clermont-Ferrand, France) with a TTT adaptator was installed on a slit lamp (Haag Streit, Bern, Swiss). This diode laser system was used in a continuous mode with a Quadraspheric® contact lens (Veatch Opthalmic Instruments, Tempe, AZ, USA) in order to magnify the laser spot and counter balance the minifying effect of the rabbit's eye. The power was verified with a Laserstar power meter (Ophir Optronics, Israel) before series of irradiation. The laser spot size and profile were assessed using a laser beam scanner (BeamScan®, Photon Inc., San Jose, CA). The size was controlled on the retina (diameter: 1.3 ± 0.1 mm, actual size on the retina). In that case, a 10 milliseconds pulse and a high power was used in order to reduce the effect of lateral heat diffusion usually observed when using a duration longer than 1 second.

Methods

Nine male pigmented rabbits were anesthetized and TTT was performed on their right eyes. A series of six to nine adjacent laser impacts was delivered to the posterior pole region of the retina. The spot diameter was kept constant. Three different pulse durations were used: 15, 30, or 60 seconds. The laser power was tuned as presented in Table 1, according to a previous study [16]. The "high" value of the laser power aimed to reach the photocoagulation threshold. The "mild" value aimed to stay under the photocoagulation threshold but to produce a sublethal supraphysiologic hyperthermia corresponding to TTT. The "low" value aimed to stay under the stressful temperatures thereby avoiding the expression of HSP (Table 1).

Histological Study

Routine preparations. The animals were sacrificed 24 hours after irradiation. The whole eyes were immediately immersed after choroidal incision in 10% formalin for 48 hours. Eyes were then totally paraffin-embedded. Sections corresponding to the area of laser irradiation were

TABLE 1. Power of the Laser Used for 15, 30, and 60 Seconds Exposures and 1.3 mm Spot

	15 seconds	30 seconds	60 seconds
High	300 mW	250 mW	150 mW
Mild	250 mW	210 mW	130 mW
Low	180 mW	150 mW	90 mW

The "high" value of the laser power aimed to reach the photocoagulation threshold (lethal hyperthermia). The "mild" value aimed to stay under the photocoagulation threshold but to produce a sublethal supraphysiologic (stressful) hyperthermia corresponding to TTT. The "low" value aimed to stay under the stressful temperatures (physiologic hyperthermia) and avoid HSP hyperexpression.

stained with haematoxylin erythrosin saffron (HES) to identify retinal structures. Further, 5 μm thick adjacent sections from selected areas were mounted on silane prepared microscope slides.

Immunodetection of HSP-70 reactivity. Slides were deparaffinized in xylene, followed by absolute ethanol, 95% ethanol, and distilled water. Before immuno-staining procedures, sections were incubated for 5 minutes in pH 6.0 citrate buffer in a pressure cooker, at 112°C, to enhance the immunoreactivity of the samples. A monoclonal antibody was used to detect Hsp70 immunoreactivity (mouse IgGl, SPA-810, Stress Gen, Victoria, Canada), followed by a biotinylated goat anti-mouse antibody (Dako, Denmark), revealed by the avidin–biotin complex (Vectastain kit, Vector, CA) and the AEC chromogen.

Controls. Human lymphocytes were used for immunocytochemistry. Lymphocytes were separated by a single-density gradient method (Ficoll; Pharmacia Upjohn, Uppsala, Sweden) [21], then kept in RPMI. The half part was heated for 30 minutes at 40°C. Cytospins of heated and non-heated lymphocytes were then quickly fixed in acetone/alcohol, and immunostained. On cytospins of heated lymphocytes, 40% of cells were immunoreactive for HSP70. Conversely, no immunostaining was detected among the non-heated lymphocytes.

To control the antibody specificity, the primary anti-HSP-70 antibody was replaced by non-immune horse serum applied on paraffin sections or cytospins. These slides did not display any immunoreactivity (data not shown).

RESULTS

For the 60 second exposures, impacts corresponding to three laser powers have been studied: at 150 mW (one eye: six spots), the photocoagulation threshold was obtained for most laser exposures.

With the use of a laser power of 130 mW (one eye, six spots) no visible change was observed during the experiments, and no modification of the irradiated areas was detected on biomicroscopy nor on gross examination. On light microscopy, low magnification disclosed a mild congestion on choroid: the lumen of capillaries was dilated by erythrocytes and there was neither blood extravasation nor thrombosis. No hemorrhage, necrosis, atrophy, nor detachment was observed after HES staining and no tissue architecture modification could be observed. Endothelial cells and vascular smooth muscle cells were not vacuolized. The nuclei appeared similar to the controls; chromatin was not abnormally clumped without pyknotic nuclei. Similarly, the neuroretina appeared undamaged, without any morphologic alteration of the photoreceptors. For these six laser spots, the HSP70 immunoreactivity was found to be strong. It was detected in spindle shaped choroidal non-pigmented cells, and in some choroidal capillary endothelial cells. No HSP-70 immunoreactivity was observed on the retina.

For the laser power 90 mW (one eye, eight spots), as for the sections from non-irradiated eyes, no HSP-70 immunoreactivity was detected either on the choroid or on the retina. This 90 mW power corresponded to a laser dose not powerful enough to induce a thermal stress detectable by either microscopy or HSP70 hyperexpression.

For the 30 seconds exposures, impacts corresponding to three laser powers have been studied: at 250 mW (one eye: seven spots), the photocoagulation threshold was reached for most laser exposures with a whitening within the laser spot occurring at the end of the pulse. At 210 mW (one eye, six spots), no visible change was observed during the experiments, no modification of the irradiated areas was detected on biomicroscopy nor on gross examination. On light microscopy, low magnification showed a discrete congestion on choroid without either blood extravasation or thrombosis. No hemorrhage, necrosis, atrophy, nor detachment was observed after HES staining and no tissue architecture modification was observed. Endothelial cells and vascular smooth muscle cells were not vacuolized.

In contrast, the neuroretina appeared undamaged, without any morphologic alteration of the photoreceptors. For these seven laser spots, the HSP70 immunoreactivity was strong. It was detected in spindle shaped choroidal non-pigmented cells, and in some choroidal capillary endothelial cells. No HSP-70 immunoreactivity was observed in the retina (Fig. 2).

For the laser power 150 mW (one eye, nine spots), as for the sections from non-irradiated eyes, no HSP-70 immunoreactivity was detected either in the choroid or in the retina. This power corresponded to a laser dose not powerful enough to induce a thermal stress detectable by neither microscopy nor HSP70 hyperexpression.

For the 15 seconds exposures, impacts corresponding to three laser powers have been studied: at 300 mW (one eye, six spots), the photocoagulation threshold was obtained for most laser exposures with a whitening occurring at the end of the pulse.

With the use of a laser power of 250 mW (one eye, nine spots), no modification of the irradiated areas was detected during the laser pulse nor on biomicroscopy or on gross examination. With the light microscopy and with low magnification, only a mild congestion was observed.

After HES staining, no bleeding nor necrosis, atrophy, or detachment was observed. A vacuolization of endothelial cells and vascular smooth muscle cells was observed,

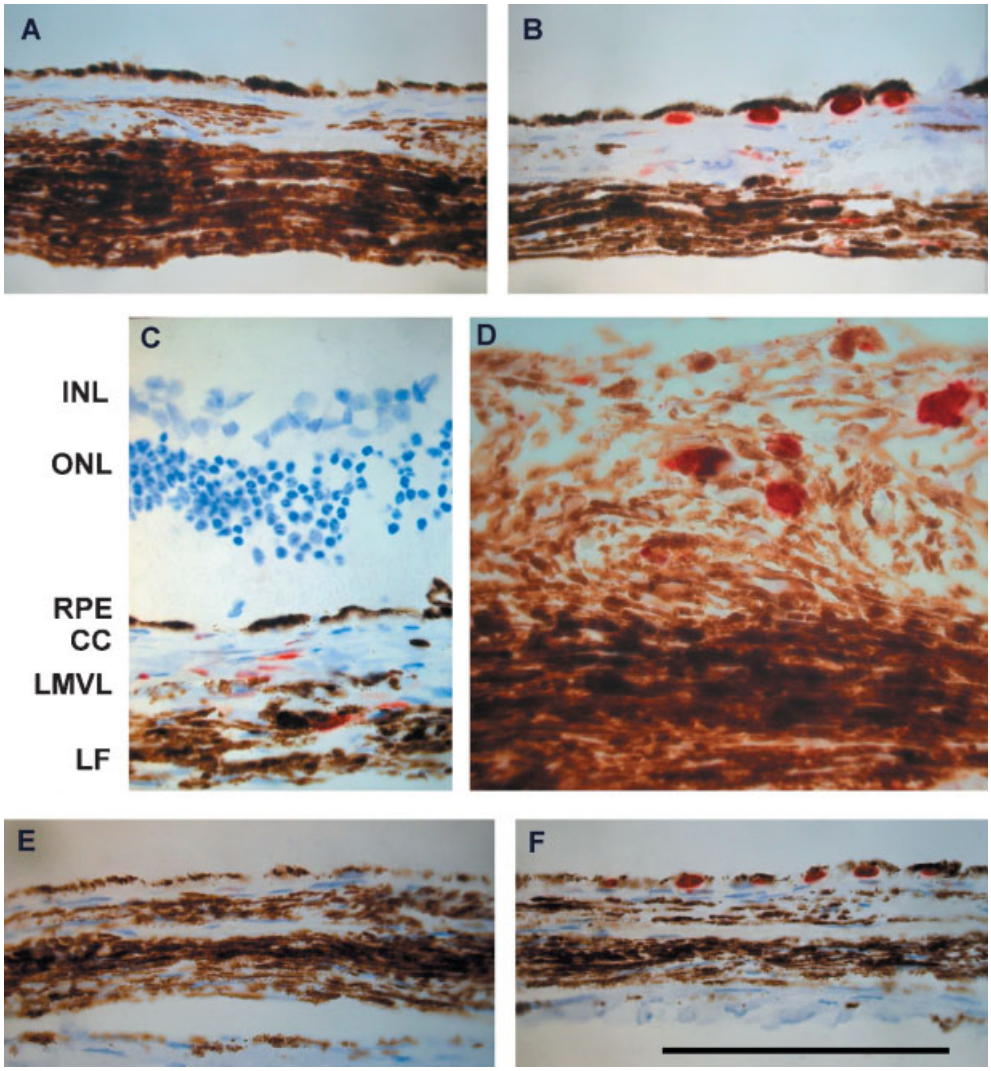

Fig. 2. Chorioretinal samples after transpupillary laser irradiation. The HSP-70 labeling appears as red deposits, contrasting from the brown color of melanin. **A** and **B** are from the same eye sample irradiated with 15 seconds laser exposure (P = 180 mW). A shows the lack of HSP immunostaining on choroid outside of the irradiation zone, whereas (B) (P = 250 mW) shows focalized HSP labeling within the choriocapillaris predominant under the RPE. A delicate staining is disclosed deeper on the lamina fusca. **C** and **D** correspond to eye samples irradiated with 30 seconds laser exposure (P = 210 mW), showing on C the absence of HSP immunostaining on the retina contrasting with a HSP staining on the choriocapillaris and extended to the large and medium size vessels layer. D shows at a higher magnification the HSP staining of non-vascular cells on the pigmented choroid. Most of these immunostained cells are non-pigmented, **E** and **F** are on the same eye sample irradiated with a 60 seconds laser exposure. E (P = 90 mW) shows the lack of HSP immunostaining on choroid aside the irradiation zone. F (P = 130 mW) shows HSP labeling predominantly located in the choriocapillaris. Scale bar: 100 μm for A, B, C, E, F, and 40 μm for D, INL. inner nuclear layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium; CC, choriocapillaris; LMVL, large and medium vessels layer; LF, lamina fusca.

restricted to the areas of laser irradiation. The nuclei appeared similar to the controls.

For these nine laser spots, HSP70 immunoreactivity was observed and considered strong. It was detected in choroidal, non-pigmented cells but also in some choroidal capillary endothelial cells. The labeled vessels lay beneath the Bruch's membrane, within the choriocapillary layer. The overlying Bruch membrane was mildly folded in opposition to undamaged retina. Spindle-shaped labeled cells were observed deeper within the large vessel layer

choroid (Fig. 2). No HSP-70 immunoreactivity was observed on the retina.

For the laser power 180 mW (one eye, six spots), no HSP-70 immunoreactivity was detected either on the choroid or on the retina. We then assumed that this power corresponded to a laser dose not powerful enough to induce a thermal stress detectable by either microscopy or HSP70 hyperexpression.

DISCUSSION

In these experiments, the use of a sublethal supraphysiologic hyperthermia with 15, 30, or 60 seconds duration laser exposures induced an HSP hyperexpression in choroidal layers within the irradiation zones. Conversely, no HSP hyperexpression could be detected in non-irradiated eyes or outside of the irradiation zone in the irradiated eyes. Since the HSP hyperexpression has been advocated to be at least a part of the mechanism of the therapeutic action of TTT [15,16], it can be hypothesized that TTT could be performed with laser exposures shorter than 60 seconds and that a therapeutic action could be expected with such laser exposures.

In these experiments, the magnification factor of the rabbit eye led us to adapt the laser procedure in order to obtain a spot size comparable to the spot used for TTT in the clinical studies [22,23]. We have already used this model in a previous study, as well as other recent authors [24].

In this study, the results obtained with 60 seconds exposures confirmed those obtained in a previous study [16]. The use of three decreasing laser powers producing: (i) slight photocoagulation, i.e., lethal hyperthermia to, (ii) supraphysiologic sublethal hyperthermia and further down to, (iii) physiologic hyperthermia was the best way to establish a model of TTT. This gradation of hyperthermia has been used in several clinical studies [17].

The presence of HSP70 hyperexpression for the 30 and the 15 seconds laser exposures was consistent with our hypothesis based on the notion of "heat dose." As illustrated in Figure 1, an equivalent HSP hyperexpression has been found, following long duration of heating such as 130 minutes or short duration such as 7.5 minutes [20]. More recently it has been shown with a laser technique used for skin closure using a 810 nm diode laser with a low dose, under the photocoagulation threshold, that an HSP hyperexpression could be induced with laser exposures as short as 3 seconds [25,26]. These data are consistent with the concept of heat dose that could be applied to TTT.

Heat shock 70 protein family probably plays an important role in ocular protection from various biologic and environmental stresses. Some authors have proposed that decreased levels of heat shock constitutive proteins of this family (HSC70) in the retina during aging may contribute to the apparent increased susceptibility of the retina to age-acquired retinal diseases [27]. In different models, some experimental studies have shown an increase in the synthesis of heat shock proteins that is induced in cells in vitro by hyperthermia or other types of metabolic stress. This increase in HSP synthesis correlates with enhanced cell survival upon further stress [28–30]. In another therapeutic perspective, it has been shown that intravitreally injected HSC/HSP70 can be taken up by retinal cells and are associated with an increase of the number of surviving photoreceptors after an acute injury such as light damage [31].

In our experiments, the localization of the HSP immunostaining close to the RPE after most exposures (Fig. 2) could be related with studies demonstrating a modulation of cytokines production by the RPE induced by laser irradiation. TGF-beta produced by photocoagulated RPE cells, associated with a down-regulation of angiogenic factors in repaired RPE cells, could possibly play a role in the inhibition of neovascularization [32,33]. The HSP hyperexpression observed in our study could be one of the first steps toward production of cytokines.

Since this study aimed to analyze the presence or absence of HSP hyperexpression after TTT, no quantification of the thermotolerance could be established from our data. Some authors have analyzed the intensity of thermotolerance after heat shock, showing that HSP70 hyperexpression follows a time–temperature history increase [34–36]. Since a certain amount of HSP above a given threshold may be necessary to have a clinical significance, further experimental studies must be performed to quantify HSP production for short exposures. From a more clinical point of view, following the results of the TTT4CNV study, if there is an identification of subgroups of patients where the technique is more efficient, then a clinical study could be performed with short laser exposure duration TTT.

CONCLUSION

Transpupillary laser irradiations lasting 15, 30, or 60 seconds induce an hyperexpression of HSP on choroidal layers. This could be a basis for the use of TTT with "short" laser exposures.

ACKNOWLEDGMENTS

This work has been presented at the annual meeting of the American Society of Lasers in Medicine and Surgery in April 2003. The authors are grateful to Quantel Medical® (France) for the loan of their equipment, technical assistance, and funding of this study. The authors also thank Professor Martin Mainster for his advices during the study. The technical assistance of Philippe Maury was particularly appreciated.

REFERENCES

1. Reichel E, Berrocal AM, Ip M, Kroll AJ, Desai V, Duker JS, Puliafito CA. Transpupillary thermotherapy of occult subfoveal choroidal neovascularization in patients with age-related macular degeneration. Ophthalmology 1999;106:1908–1914.
2. Miller-Rivero NE, Kaplan HJ. Transpupillary thermotherapy in the treatment of occult and classic choroidal neovascularization. Invest Ophthalmol Vis Sci ARVO Abstr 2000;41:S179.
3. Petrone S, Staurenghi G, Migliavacca L, Ottochian M, Orzalesi N. Transpupillary thermotherapy for subfoveal choroidal neovascularization in age-related macular degeneration. Invest Ophthalmol Vis Sci ARVO Abstr 2000; 41:S320.

4. Newsom RS, McAlister JC, Saeed M, McHugh JD. Transpupillary thermotherapy (TTT) for the treatment of choroidal neovascularisation. Br J Ophthalmol 2001;85:173–178.
5. Kim SH, Kim JE, Connor TB, Wirostko WJ, Han DP. Transpupillary thermotherapy for the treatment of occult subfoveal choroidal neovascularization in age related macular degeneration. Invest Ophthalmol Vis Sci ARVO Abstr 2002;43:4418.
6. White MF, Mason JO, Feist RM, McGwin G, Emond TL. Transpupillary thermotherapy of occult choroidal neovascularization: 18 month follow-up. Invest Ophthalmol Vis Sci ARVO Abstr 2002;43:4415.
7. Sanders JB, Hoskins JC, Funderburk RL, Googe JM, Miller JH, Stone JL, Gunn JM, McMillan TA, Cummings HL, Franklin AJ. The treatment of predominantly occult choroidal neovascularization secondary to age related macular degeneration using transpupillary thermotherapy. Invest Ophthalmol Vis Sci ARVO Abstr 2002;43:4406.
8. Salinas Alaman A, Garcia Layana A, Juberias Sanchez JR, Sanchez Tocino H, Sadaba Echarri LM, Moreno Montanes J. Transpupillary thermotherapy in occult subretinal neovascularization in age-related macular degeneration. Preliminary results. Arch Soc Esp Oftalmol 2002;77:617–622.
9. Robertson DM, Salomao DR. The effect of transpupillary thermotherapy on the human macula. Arch Ophthalmol 2002; 120:652–656.
10. Yamaji H, Shiraga F, Endo J, Kato M, Nomoto H, Ohtsuki H. Retinal changes after transpupillary thermotherapy for choroidal neovascularization. Invest Ophthalmol Vis Sci ARVO Abstr 2002;Perdrizet GA:4414.
11. Lanzetta P, Michieletto P, Pirracchio A, Bandello F. Early vascular changes induced by transpupillary thermotherapy of choroidal neovascularization. Ophthalmology 2002;109: 1098–10104.
12. Benner JD, Ahuja RM, Butler JW. Macular infarction after transpupillary thermotherapy for subfoveal choroidal neovascularization in age-related macular degeneration. Am J Ophthalmol 2002;134:765–768.
13. Welch AJ, Wissler EH, Pricbe LA. Significance of blood flow in calculations of temperature in laser irradiated tissue. IEEE Trans Biomed Eng 1980; BME 27:164–166.
14. Welch AJ, Polhamus GD. Measurement and prediction of thermal injury in the retina of the rhesus monkey. IEEE Trans Biomed Eng 1984;31:633–643.
15. Mainster MA, Reichel E. Transpupillary thermotherapy for age-related macular degeneration: Long-pulse photocoagulation, apoptosis, and heat shock proteins. Ophthalmic Surg Lasers 2000;31:359–373.
16. Desmettre T, Maurage CA, Mordon S. Heat shock protein hyperexpression on chorioretinal layers after transpupillary thermotherapy. Invest Ophthalmol Vis Sci 2001;42:2976–2980.
17. Thermotolerance. In: Perdrizet GA, editor. Heat shock response and organ preservation: Models of stress conditioning. Chapter 4. Austin, TX: Landes Bioscience; 1996. pp 71–97.
18. Rice LC, Urano M, Maher J. The kinetics of thermotolerance in the mouse foot. Radiat Res 1982;89:291–297.
19. Law MP. Induced thermal resistance in the mouse ear: The relationship between heating time and temperature. Int J Radiat Biol Relat Stud Phys Chem Med 1979;35:481–485.
20. Law MP. The induction of thermal resistance in the ear of the mouse by heating at temperatures ranging from 41.5 to 45.5 degrees C. Radiat Res 1981;85:126–134.
21. Venard V, Carret AS, Pascal N, Rihn B, Bordigoni P, Le Faou A. A convenient semi-quantitative method for the diagnosis of Epstein–Barr virus reactivation. Arch Virol 2000;145:2211–2216.
22. Holden AL, Hayes BP, Fitzke FW. Retinal magnification factor at the ora terminalis: A structural study of human and animal eyes. Vision Res 1987;27:1229–1235.
23. MA Pak. Ocular refraction and visual contrast sensitivity of the rabbit, determined by the VECP. Vision Res 1984;24: 341–345.
24. Ibarra MS, Madjarov B, Glazer-Hockstein C, Mainster MA, Maguire AM, Bennett J, Tolentino MJ. Determination of retinal thermal dosimetry from transpupillary thermotherapy. Invest Ophthalmol Vis Sci ARVO Abstr 2002;43:4412.
25. Capon A, Souil E, Gauthier B, Sumian C, Bachelet M, Buys B, Polla BS, Mordon S. Laser-assisted skin closure (LASC) using a 815 nm diode-laser system accelerates and improves wound healing. Lasers Surg Med 2001;28:168–175.
26. Souil E, Capon A, Mordon S, Dinh Xuan AT, Polla BS, Bachelet M. Treatment with an 815 nm diode-laser induces long-lasting expression of 72 kDa heat shock protein in normal rat skin. Br J Dermatol 2001;144:260–266.
27. Bernstein SL, Liu AM, Hansen BC, Somiari RI. Heat shock cognate-70 gene expression declines during normal aging of the primate retina. Invest Ophthalmol Vis Sci 2000;41:2857–2862.
28. Barbe MF, Tytell M, Gower DJ, Welch WJ. Hyperthermia protects against light damage in the rat retina. Science 1988; 241:1817–1820.
29. Gohdo T, Ueda H, Ohno S, Iijima H, Tsukahara S. Heat shock protein 70 expression increased in rabbit Muller cells in the ischemia-reperfusion model. Ophthalmic Res 2001;33:298–302.
30. Park KH, Cozier F, Ong OC, Caprioli J. Induction of heat shock protein 72 protects retinal ganglion cells in a rat glaucoma model. Invest Ophthalmol Vis Sci 2001;42:1522–1530.
31. Yu Q, Kent CR, Tytell M. Retinal uptake of intravitreally injected Hsc/Hsp70 and its effect on susceptibility to light damage. Mol Vis 2001;7:48–56.
32. Ogata N, Ando A, Uyama M, Matsumura M. Expression of cytokines and transcription factors in photocoagulated human retinal pigment epithelial cells. Graefes Arch Clin Exp Ophthalmol 2001;239:87–95.
33. Lutty G, Grunwald J, Majji AB, Uyama M, Yoneya S. Changes in choriocapillaris and retinal pigment epithelium in age-related macular degeneration. Mol Vis 1999;5:35.
34. Fujitomi Y, Kashima K, Ueda S, Yamada Y, Mori H, Uchida Y. Histopathological features of liver damage induced by laser ablation in rabbits. Lasers Surg Med 1999;24:14–23.
35. Blom DJ, De Waard-Siebinga I, Apte RS, Luyten GP, Niederkorn JY, Jager MJ. Effect of hyperthermia on expression of histocompatibility antigens and heat-shock protein molecules on three human ocular melanoma cell lines. Melanoma Res 1997;7:103–109.
36. Ritchie KP, Keller BM, Syed KM, Lepock JR. Hyperthermia (heat shock)-induced protein denaturation in liver, muscle, and lens tissue as determined by differential scanning calorimetry. Int J Hyperthermia 1994;10:605–618.