Heat Shock Protein Hyperexpression on Chorioretinal Layers after Transpupillary Thermotherapy

Thomas Desmettre,[1,2,3] *Claude-Alain Maurage,*[4] *and Serge Mordon*[1]

Purpose. To assess a biological effect induced by temperature elevation during transpupillary thermotherapy (TTT).

Methods. Six pigmented rabbits were anesthetized, and TTT was performed on the right eye using an 810-nm diode laser installed on a slit lamp (spot size, 1.3 mm; duration, 60 seconds; power, 92–150 mW). A series of laser pulses were aimed at the posterior pole of the retina. The left eyes were used as the control. Twenty-four hours after laser irradiation, a histologic study was performed on the chorioretinal layers. Tissue samples were fixed in formalin and embedded in paraffin. A monoclonal antibody was used to detect heat shock protein (Hsp)70 immunoreactivity, followed by a biotinylated goat anti-mouse antibody, revealed by the avidin-biotin complex and the 3-amino-9-ethyl-carbazole (AEC) chromogen. Retinal structures were further identified by hematoxylin erythrosin saffron (HES) coloration.

Results. The photocoagulation threshold was found to be at the 150-mW laser power. Under this threshold, Hsp70 immunostaining was the strongest at the 127-mW power, with staining of some choroidal cells, including capillary endothelial cells. No Hsp70 immunoreactivity was observed on the retina. At the 107-mW power, Hsp70 reactivity was observed only in occasional choroidal cells. At the 98-mW power, only mild, diffuse Hsp70 immunoreactivity was observed in the choroid. At the 92-mW power, as in nonirradiated eyes, no Hsp70 immunoreactivity was detected.

Conclusions. Subthreshold transpupillary 810-nm laser irradiation induces choroidal Hsp hyperexpression. This confirms that choroidal Hsp hyperexpression can be induced during TTT, as has been recently hypothesized by several investigators. (*Invest Ophthalmol Vis Sci.* 2001;42:2976–2980)

Thermotherapy is the induction of a modest thermal elevation, usually between 4°C and 10°C above basal temperature.[1] For several years, it has been used in ophthalmology as transpupillary thermotherapy (TTT) for the treatment of small selected choroidal melanomas, alone or in conjunction with radiotherapy.[2,3] Recently, this therapeutic procedure has been evaluated for the treatment of subfoveal occult choroidal neovessels of age-related macular degeneration (AMD) in pilot studies.[4–6]

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 (1 minute).[5] The advantage of infrared light is its deeper chorioretinal penetration compared with visible light.[7] In contrast with photocoagulation, one of the features of TTT is that it does not change the tissue architecture—that is, there is no effect or faint whitening of the area of laser irradiation in response to the laser pulse.[5,7] TTT is therefore a subthreshold procedure.[7,8]

For this reason, devising a precise protocol remains difficult. The power and duration of the laser pulse cannot be modulated during the treatment, as it can in photocoagulation. Some overdoses have been reported, leading to a decrease in visual acuity related to a central scotoma.[9] This lack of visual control becomes a drawback for using TTT to safely treat subfoveal neovessels or for calculating optimal laser doses that could differ according to the type of choroidal neovessel. Also, interindividual variations in fundus pigmentation and media transparency make it difficult to reconcile the use of laser parameters that would be identical for all patients and the concept of a modest temperature increase just below the photocoagulation threshold. Finally, the rationale of TTT has not yet been clearly established, mainly because this technique is new but also because of the difficulty in obtaining reproducible effects.

Some investigators have suggested that apoptosis and heat shock proteins (Hsps) may play a role in the decreased exudation from the choroidal neovessels of AMD.[7] Hsps are ubiquitous proteins whose role is to maintain basic cellular functions both under basal conditions and after various cellular stresses.[10,11] These Hsps are preferentially expressed in response to a variety of insults, including hyperthermia, free oxygen radicals, inflammation, and infection.[12] Stress-related functions of Hsps include the refolding, translocation, and degradation of proteins, thus acting as chaperones to maintain cytoskeletal integrity and metabolic homeostasis of cells.[13] Hsp accumulation has long been used as a marker of cell and tissue damage, especially for mild hyperthermia. For this reason, this study was conducted to determine in an animal model of TTT whether Hsp hyperexpression in chorioretinal layers would reveal the biological effect of mild thermal elevation. If so, such a hyperexpression could help us to better understand the mechanisms of the therapeutic effect of TTT.

Materials and Methods

Animals

Animal procedures were performed 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. Laser pulses were performed on the posterior pole to limit focusing errors and near the optic disc so that they could be retrieved during the histologic study.

From [1]Unité Propre de Recherche de l'Enseignement Superieur (UPRES) EA2689, Institut National de la Santé et de la Recherche Médicale (INSERM), Institute Fédératif de Recherche (IFR) 22, Centre Hospitalier Universitaire (CHU), Lille, France; the [2]Imaging, Laser and Low Vision Rehabilitation Center, Lambersart, France; the [3]Department of Ophthalmology, Lariboisière University Hospital, Paris, France; and the [4]Department of Pathology, Centre Hospitalier Universitaire, Lille, France.

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, May 2001.

Supported by funding from Quantel Medical, Clermont-Ferrand, France.

Submitted for publication February 20, 2001; revised June 13, 2001; accepted July 26, 2001.

Commercial relationships policy: F

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Thomas Desmettre, UPRES-EA2689, INSERM IFR 22, University Hospital, Pavillon Vancostenobel, 59037 Lille Cedex, France. desmettre@lille.inserm.fr

Investigative Ophthalmology & Visual Science, November 2001, Vol. 42, No. 12

The homogeneous pigmentation of the fundus reduced the variation of laser light absorption. Six male pigmented rabbits weighing between 3.0 and 3.5 kg were lightly anesthetized with an intramuscular 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 throughout the procedure. Pupil dilation was achieved with tropicamide 0.5% and phenylephrine (10%) eye drops. After completing the experiments, the animals were killed with an overdose of ketamine and chlorpromazine.

Laser

An 810-nm diode laser (Iridis; Quantel Medical, Clermont-Ferrand, France) with a specific TTT adaptor was installed on a slit lamp (Takagi, Nagano, Japan). This diode laser system was used in continuous mode. A contact lens (SuperQuad; Volk Optical, Mentor, OH) was used to magnify the laser spot, counterbalance the minifying effect of the rabbit's eye, and obtain a large spot size comparable to the spot used for TTT in the clinical studies.[14,15]

To increase the reproducibility of the laser irradiation, the spot diameter and the pulse duration were kept constant (diameter, 1.3 mm, actual size on the retina; pulse duration, 60 seconds). The laser power ranged from 92 to 150 mW, and the fluence ranged from 386 to 629 J/cm^2. These parameters were determined during a preliminary study (70 mW, no effect; 150 mW, photocoagulation threshold). The power was checked with a meter (Laserstar; Ophir Optronics, Jerusalem, Israel) before each series of laser spots was applied.

Methods

Six male pigmented rabbits were anesthetized, and TTT was performed on their right eyes. A series of 9 to 12 adjacent laser pulses was delivered to the posterior pole region of the retina, inferior to the myelinated fiber layer. Five laser power settings were used: 150 mW (fluence, 629 J/cm^2; irradiance, 10.5 W/cm^2); 127 mW (fluence, 532 J/cm^2; irradiance, 8.9 W/cm^2); 107 mW (fluence, 450 J/cm^2; irradiance, 7.5 W/cm^2); 98 mW (fluence, 411 J/cm^2; irradiance, 6.8 W/cm^2); and 92 mW (fluence, 386 J/cm^2; irradiance, 6.4 W/cm^2). The duration of the laser pulse (60 seconds) and the size of the laser spot (1.3 mm) were kept constant during all the experiments. Left eyes were used as a control. Twenty-four hours after laser irradiation, the animals were killed, and a histologic study was performed on the retina.

Histologic Study

Routine Preparations. The eyes were immediately immersed after choroidal incision in 10% formalin for 48 hours and were then totally paraffin embedded. Sections corresponding to the area of laser irradiation were stained with hematoxylin erythrosin saffron (HES) to identify retinal structures. Further 5-μm-thick sections adjacent to selected areas were mounted on silane-prepared microscope slides.

Immunodetection of Hsp70 Reactivity. Slides were deparaffinized in xylene, followed by absolute ethanol, 95% ethanol, and distilled water. Before immunostaining procedures, sections were incubated for 5 minutes in citrate buffer (pH 6.0) in a pressure cooker at 112°C to enhance the immunoreactivity of samples. A monoclonal antibody was used to detect Hsp70 immunoreactivity (mouse IgG1; SPA-810; StressGen, Victoria, British Columbia, Canada), followed by a biotinylated goat anti-mouse antibody (Dako, Glostrup, Denmark), revealed by the avidin-biotin complex (Vectastain kit; Vector, Burlingame, CA) and the 3-amino-9-ethyl-carbazole (AEC) chromogen.

Control Samples

Human lymphocytes were used for immunocytochemistry. Lymphocytes were separated by a single-density gradient method (Ficoll; Pharmacia Upjohn, Uppsala, Sweden),[16] and kept in RPMI medium. Half was heated for 30 minutes at 40°C. Cytospins of heated and nonheated 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 nonheated lymphocytes (Fig. 1).

To control antibody specificity, the primary anti Hsp70 antibody was replaced by nonimmune horse serum applied on paraffin sections or cytospins. These slides did not display any immunoreactivity (data not shown).

Results

The photocoagulation threshold was obtained at the 150-mW laser power. With this power, faint whitening was observed within the laser spot after the 60-second pulse (data not shown). A 1.3-mm spot size was measured after enucleation.

At 127 mW the amount of laser energy was generally not sufficient to generate any ophthalmoscopically visible lesion, except in three of nine laser pulses. In these three spots, a slight whitening was observable a few minutes after the laser irradiation was stopped. In the remaining six spots, no modification of the irradiated areas was detected under biomicroscopy or on gross examination.

Under low-magnification light microscopy, only mild congestion was observed on the choroid. The capillary lumen was dilated by erythrocytes, and there was no blood extravasation or thrombosis. No hemorrhage, necrosis, atrophy, or detachment was observed after HES staining, and no tissue architecture modification was observed. Endothelial cells and vascular smooth muscle cells were not vacuolized. The nuclei appeared similar to the control cells. Chromatin was not abnormally clumped, and there were no pyknotic nuclei. Also, the neuroretina appeared remarkably undamaged, and no morphologic alteration of photoreceptor was observed.

We therefore assumed that this 127-mW power level corresponded to the highest subthreshold power that could be used during our experiments. In these six laser spots, the Hsp70 immunoreactivity was considered to be strong. This immunoreactivity was detected in choroidal nonpigmented cells but also in some choroidal capillary endothelial cells. No Hsp70 immunoreactivity was observed in the retina (Fig. 2A).

Three decreasing laser powers were then studied: 107, 98, and 92 mW. On biomicroscopy and gross examination, no modification of the irradiated areas was detected. Under low-magnification light microscopy, as was true at 127 mW, only mild congestion was observed, with a dilation of the capillary lumen by erythrocytes without blood extravasation or thrombosis. No hemorrhage, necrosis, atrophy, or detachment was observed after HES staining. The intensity and location of the hyperexpression of Hsp70 on the choroid were different at each laser dose. No Hsp70 immunoreactivity was observed on the retina.

At 107-mW laser power (n = 11), Hsp70 reactivity was observed in some choroidal cells. The staining was sometimes stronger on some choroidal, pigmented, or nonpigmented nonvascular cells. No staining was observed on the choroidal vessel walls (Fig. 2B). At 98-mW laser power (n = 9), only mild and diffuse Hsp70 immunoreactivity was observed in the choroid without immunoreactive spots (Fig. 2C). At 92-mW laser power (n = 12), as for the sections from nonirradiated eyes (n = 2), no Hsp70 immunoreactivity was detected on either the choroid or the retina (Fig. 2D). We therefore assumed that this 92-mW power level corresponds to a laser dose that is not powerful enough to induce thermal stress detectable by microscopy or Hsp70 hyperexpression.

Discussion

TTT uses the noninvasive potential of the infrared laser light to cause a limited increase in temperature that may achieve some

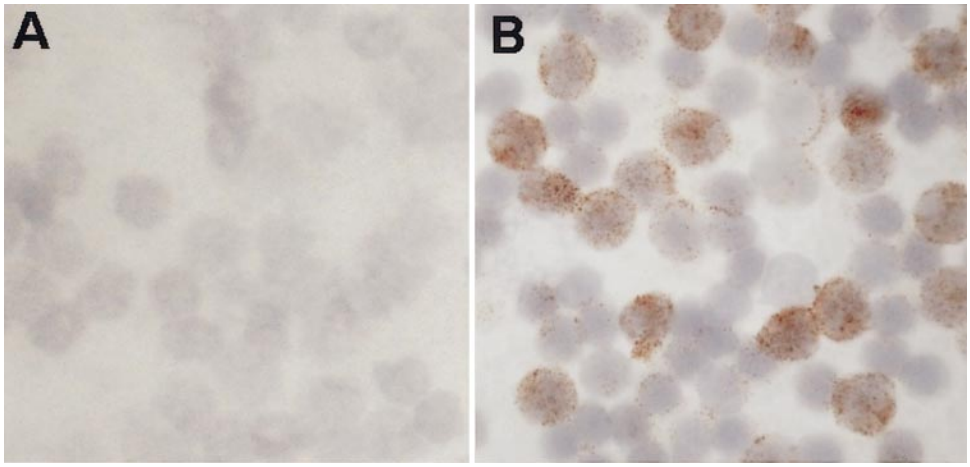

FIGURE 1. High magnification of nonheated lymphocytes (**A**) and heated lymphocytes (**B**). Hsp immunoreactivity was revealed by *red* staining that was present only in heated lymphocytes. AEC substrate, Mayer's hematoxylin; original magnification, ×900.

selective occlusion of choroidal neovascularization.[5,7] It has recently been hypothesized that TTT could modify the inflammatory response and play a major role in apoptosis and/or Hsp expression.[7]

During photocoagulation, the change in optical properties of the tissue (i.e., whitening) corresponds to a denaturation of tissue proteins, usually thought to occur at approximately 55°C, 18°C above basal temperature.[17] However, protein denaturation begins under the photocoagulation threshold with a change in the folding of proteins starting at 46°C to either 43°C or 49°C.[18–21] For this reason we started our study with a laser power that induced a faint whitening on the retina after a few minutes (150 mW). Because there is a latency of tissue denaturation after the laser is stopped, we assumed that this laser power of 150 mW corresponded to the photocoagulation threshold. During the experiments, a range of power from 92 to 127 mW was used for TTT (with fluences ranging from 400 to 550 mJ/cm^2). With these parameters, whitening was not observed, except for some pulses made with the laser set at 127 mW. This is consistent with TTT as proposed by Reichel et al.,[5] who used a variable spot size of 1.2, 2.0, or 3.0 mm and a duration of 60 seconds (maximum fluence, 680 mJ/cm^2). The

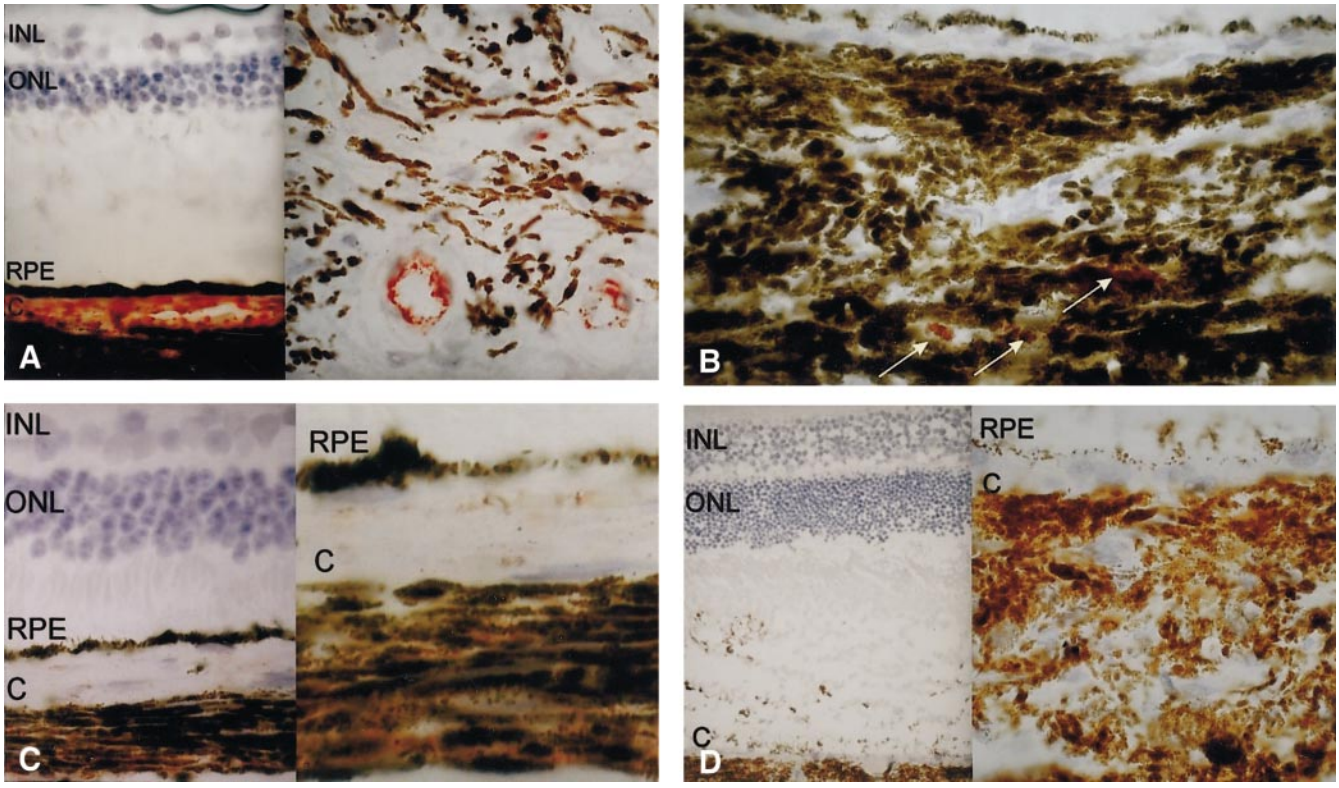

FIGURE 2. (**A**) Chorioretinal layers with a 127-mW laser pulse. Hsp70 immunoreactivity was detected in capillary endothelial cells. The *red* signal of the immunostaining was clearly different from the *brown* color of melanin, allowing better identification of the Hsp 70 hyperexpression zones. (**B**) Choroid irradiated at 107 mW with immunostaining of Hsp70. *Arrows*: a few choroidal pigmented or nonpigmented nonvascular cells, showing focal immunostaining, stronger than at lower laser power. (**C**) Chorioretinal layers irradiated at 98 mW showed mild diffuse Hsp70 immunostaining within the choroid without any reactivity in the neural retina. (**D**) Nonirradiated chorioretinal layers immunostained for Hsp70 were devoid of any signal. INL, inner nuclear layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium; C, choroid. Original magnification, (**A**, *left*; **B**; **C**, *left*) ×400; (**A**, *right*; **C**; **D**, *right*) ×1000; (**D**, *left*) ×200.

temperature increase corresponding to TTT has been recently calculated by Mainster and Reichel[7] to be approximately 10°C.[7] The basal body temperature of rabbit is 39.4°C, and some correlation between the onset temperature for protein denaturation and body basal temperature has been reported.[1] In our experiments, after this correlation, the maximal temperature reached after 60 seconds' irradiation can be estimated at approximately 50°C. However, for very long exposures, such as 60 seconds, because of choroidal blood flow, heat convection moderates the temperature increase and impedes a reliable modeling of the temperature increase throughout the duration of the laser pulse.[22]

Our study clearly demonstrates Hsp70 hyperexpression after a transpupillary thermal elevation induced by an infrared laser using 98 to 127 mW. Hsp70 immunostaining was less diffuse, stronger, and more often located on the vessel wall with increasing laser power. The mild and diffuse staining observed at 98 mW was restricted to the cytoplasm of both pigmented and nonpigmented choroidal cells. For higher powers, the endothelial cells of some capillaries were stained. Conversely, no Hsp immunostaining was observed at the power of 92 mW. This confirmed that a window of thermal stress termed hyperthermia can occur under the photocoagulation threshold.

The discrepancy of immunostaining between choroid and retina in our study could be due to a thermotolerance difference with relatively thermolabile choroid blood vessels and more thermotolerant neurosensory retina, in that tissue-specific variations in Hsp70 expression have already been observed.[23]

After hyperthermia, the presence of thermotolerant cells is partly due to inhibition or modulation of apoptosis.[24,25] In neural cells, it has been suggested that the induction of Hsp70 may be responsible for the protective effect of heat shock on apoptosis.[26] On fibroblasts, some investigators have reported that Hsp70 hyperexpression after heating may play a direct role in repairing heat-damaged cells.[27–29] Moreover, according to Polla et al.[11], Hsp70 is necessary to make cells evolve toward apoptosis instead of necrosis or toward survival instead of apoptosis.[12,30] Hsp chaperone functions are adenosine triphosphate (ATP) dependent. Necrosis, apoptosis, and cell survival respectively require increasing levels of ATP. Under particular stress conditions, ATP levels decrease, threatening cell homeostasis and integrity. Hsp70 synthesis and overexpression are among the mechanisms developed to protect cells. Furthermore, inflammation and neovascularization are amplified by cell necrosis, whereas apoptosis can limit inflammation and the subsequent release of cytokines.[11,12,30]

A series of tissue injuries such as hypoxia or inflammation can result in vessel destabilization, leading from vasculogenesis and angiogenesis toward neovascularization by the release of a series of cytokines, mainly the VEGF family, bFGF, and TGFβ.[31,32] An excess in necrosis and apoptosis compared with cell survival in AMD is generally admitted.[33,34] With this perspective, an interesting hypothesis would be that the increase of Hsp70 provoked by TTT would decrease the amount of necrosis and inflammation within the choroidal tissue, leading to a decrease of neovascularization and an associated increase in vascular permeability (Fig. 3). The continuous improvement in exudation that is observed for months after a TTT procedure in some patients would be consistent with the hypothesis that TTT may interrupt a vicious cycle.

The intensity of thermotolerance after heat shock has been analyzed by some investigators, showing that Hsp70 hyperexpression is related to time and temperature increase.[1,29,35]

Analysis of survival of mammalian cells after heating, by construction of Arrhenius plots has revealed an inflection point near 43°C with an increased proportion of cells that would be killed by heating to more than 43°C.[36] Thus, for temperatures between 37°C and 43°C, Hsp70 hyperexpression induces thermotolerance. This thermotolerance leads some proportion of cells toward survival or toward apoptosis instead of necrosis. Conversely, at temperatures higher than 43°C and depending on the duration of the laser pulse, Hsp70 hyperexpression is induced but the duration and temperature increases are too strong to induce thermotolerance.[36] Under extreme conditions, the stress level eliminates the capacity for regulated activation of apoptotic cascade, and the cells undergo necrosis.[12] It is then expected that the appropriate laser dosage leading to TTT (i.e., low thermal stress modulating apoptosis in a significant proportion of cells) is close to the laser dosage that induces predominant necrosis. Again, the heat convection related to choroidal blood flow and its possible variations throughout the long-duration spot can be a limit for reproducible laser dosages.

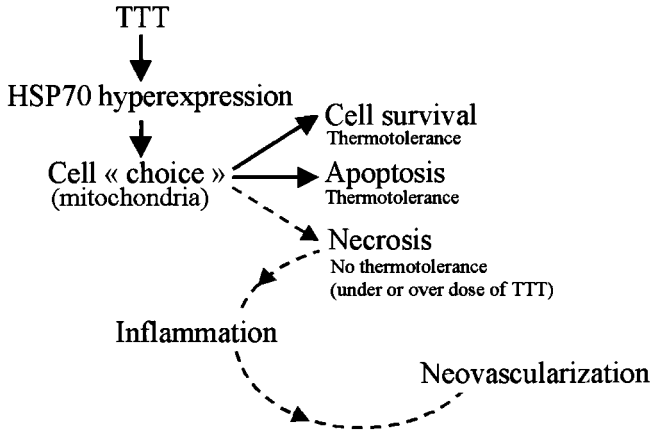

FIGURE 3. Schematic representation of the possible role of Hsp70 during TTT. Hsp70 could participate in the control of a "choice" of cell death: necrosis, apoptosis, or survival. Hsp70 is required to make cells evolve toward apoptosis instead of necrosis or toward survival instead of apoptosis. The increase of Hsp70 induced by TTT would then diminish the amount of necrosis and inflammation in the choroidal tissue, leading to a diminution of neovascularization and the associated vascular permeability.

A laser technique used in dermatology, laser-assisted skin closure, uses a similar diode laser (810 nm) with a low dose, below the photocoagulation threshold. This procedure does not induce visible modification of the targeted tissue during the laser irradiation.[37,38] In this study Hsp hyperexpression was demonstrated at short laser pulses (3 seconds). This observation could be considered an interesting perspective for reducing the heat convection effect related to the long duration of the TTT laser pulse.

Acknowledgments

The authors thank Quantel Medical, Clermont-Ferrand, France, for the loan of their equipment and Christine Warren for help with the English.

References

1. 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.
2. Overgaard J, Overgaard M. Hyperthermia as an adjuvant to radiotherapy in the treatment of malignant melanoma. *Int J Hyperthermia.* 1987;3:483–501.

3. Shields CL, Shields JA. Transpupillary thermotherapy for choroidal melanoma. *Curr Opin Ophthalmol.* 1999;10:197–203.
4. Petrone S, Staurenghi G, Migliavacca L, Ottochian M, Orzalesi N. Transpupillary thermotherapy for subfoveal choroidal neovascularization in age-related macular degeneration [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 2000;41(4):**S**320. Abstract nr 1689.
5. Reichel E, Berrocal AM, Ip M, et al. Transpupillary thermotherapy of occult subfoveal choroidal neovascularization in patients with age-related macular degeneration. *Ophthalmology.* 1999;106: 1908–1914.
6. Ip M, Kroll A, Reichel E. Transpupillary thermotherapy. *Semin Ophthalmol.* 1999;14:11–18.
7. 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.
8. Mainster MA, White TJ, Tips JH, Wilson PW. Retinal-temperature increases produced by intense light sources. *J Opt Soc Am.* 1970; 60:264–270.
9. Benner JD, Ahuja RM, Schwartz JC, Butler JW, Steidl SM. Macular infarction after transpupillary thermotherapy in the treatment of occult subfoveal choroidal neovascular membranes [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 2001;42(4):**S**444. Abstract nr 2397.
10. Tissieres A, Mitchell HK, Tracy UM. Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J Mol Biol.* 1974;84:389–398.
11. Polla BS, Stubbe H, Kantengwa S, Maridonneau-Parini I, Jacquier-Sarlin MR. Differential induction of stress proteins and functional effects of heat shock in human phagocytes. *Inflammation.* 1995; 19:363–378.
12. Samali A, Orrhenius S. Heat shock proteins: regulators of stress response and apoptosis. *Cell Stress Chaperones.* 1998;3:228–236.
13. Beckmann RP, Mizzen LE, Welch WJ. Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. *Science.* 1990;248:850–854.
14. 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.
15. Pak MA. Ocular refraction and visual contrast sensitivity of the rabbit, determined by the VECP. *Vision Res.* 1984;24:341–345.
16. 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.
17. Welch AJ. Laser irradiation of tissue. In: Shitzer A, Eberhart C, eds. *Heat Transfer in Medicine and Biology.* New York: Plenum Press; 1985:135–179.
18. Cain CP, Welch AJ. Measured and predicted laser-induced temperature rises in the rabbit fundus. *Invest Ophthalmol.* 1974;13:60–70.
19. Dewey WC. Arrhenius relationships from the molecule and cell to the clinic. *Int J Hyperthermia.* 1994;10:457–483.
20. Gabel VP, Birngruber R, Weinberg W, McCord R, Hillenkamp F. Comparison of temperature measurements and fundus reflectometry in laser coagulation. *Mod Probl Ophthalmol.* 1979;20:169–173.
21. Welch AJ, Pearce JA, Diller KR, Yoon G, Cheong WF. Heat generation in laser irradiated tissue. *J Biomech Eng.* 1989;111:62–68.
22. 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.
23. Krebs RA, Feder ME. Tissue-specific variation in Hsp70 expression and thermal damage in *Drosophila melanogaster* larvae. *J Exp Biol.* 1997;200:2007–2015.
24. Chen YC, Lin-Shiau SY, Lin JK. Involvement of heat-shock protein 70 and P53 proteins in attenuation of UVC-induced apoptosis by thermal stress in hepatocellular carcinoma cells. *Photochem Photobiol.* 1999;70:78–86.
25. Katschinski DM, Boos K, Schindler SG, Fandrey J. Pivotal role of reactive oxygen species as intracellular mediators of hyperthermia induced apoptosis. *J Biol Chem.* 2000;275:21094–21098.
26. Mailhos C, Howard MK, Latchman DS. Heat shock protects neuronal cells from programmed cell death by apoptosis. *Neuroscience.* 1993;55:621–627.
27. Ohtsuka K, Laszlo A. The relationship between Hsp70 localization and heat resistance. *Exp Cell Res.* 1992;202:507–518.
28. Laszlo A, Venetianer A. Heat resistance in mammalian cells: lessons and challenges. *Ann NY Acad Sci.* 1998;851:169–178.
29. 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.
30. Polla BS, Kantengwa S, Francois D, et al. Mitochondria are selective targets for the protective effects of heat shock against oxidative injury. *Proc Natl Acad Sci USA.* 1996;93:6458–6463.
31. Aiello LP. Vascular endothelial growth factor and the eye: biochemical mechanisms of action and implications for novel therapies. *Ophthalmic Res.* 1997;29:354–362.
32. D'Amore PA. Growth factors and cell-cell interactions in vessels growth and development. In: D'Amore PA, Adamis AP, Chang-Ling T, et al., eds. *ARVO Saturday Education Course: Angiogenesis in the Eye.* Bethesda, MD: FASEB; 2000:127–130.
33. Young RW. Pathophysiology of age-related macular degeneration. *Surv Ophthalmol.* 1987;31:291–306.
34. Green WR, McDonnell PJ, Yeo JH. Pathologic features of senile macular degeneration. *Ophthalmology.* 1985;92:615–627.
35. Blom DJ, De Waard-Siebinga I, Apte RS, Luyten GP, Niederkorn JY, Jagger 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. Bauer KD, Henle KJ. Arrhenius analysis of heat survival curves from normal and thermotolerant CHO cells. *Radiat Res.* 1979;78: 251–263.
37. 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 kD heat shock protein in normal rat skin. *Br J Dermatol.* 2001;144:260–266.
38. Capon A, Souil E, Gauthier B, et al. Laser-assisted skin closure (LASC) using a 815 nm diode-laser system accelerates and improves wound healing. *Lasers Surg Med.* 2001;28:168–75.