Objective: To determine the highest safe treatment temperature, at 30- and 60-second exposure durations, for transscleral thermotherapy (TSTT) of choroidal melanoma.

Methods and Design: Transscleral conductive heating was performed in 15 rabbits at 50°C to 70°C for 30 or 60 seconds. The thermal lesions in the ocular fundus were monitored for 4 months with ophthalmoscopic, photographic, and fluorescein angiographic examination. Histologic examination included polarized light microscopy.

Results: The effect of TSTT was similar for both exposure durations. Vascular occlusion in the retina and choroid developed at temperatures of 55°C and higher. After heating at 60°C, scleral collagen fibers developed a minimal undulation; at 65°C, they became clearly undulated. The undulation resolved in the 3 to 4 months after heating. Heating at 70°C caused persistent severe damage to the sclera. Retinal tears developed after heating at 65°C and 70°C.

Conclusions: A temperature of 65°C was found to be the highest temperature that did not cause permanent damage to the sclera at both exposure durations. A temperature of 60°C may be the optimal temperature for TSTT of choroidal melanoma because retinal tears may develop at 65°C.

Clinical Relevance: In TSTT, the temperature levels reached are cytotoxic for choroidal melanoma as well as intrascleral tumor cells. Occlusion of choroidal vessels induced by TSTT may contribute to tumor necrosis because these vessels serve as feeder vessels for the tumor.

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Transscleral Thermotherapy

Short- and Long-term Effects of Transscleral Conductive Heating in Rabbit Eyes

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METHODS

Experiments were performed in both eyes of 15 pigmented rabbits: 8 to study the early effects and 7 to study the late effects of transscleral conductive heating. General anesthesia was induced with 0.75 mL of 10% ketamine hydrochloride (Eurovet, Bladel, the Netherlands) and 0.75 mL of 2% xylazine hydrochloride (Bayer, Leverkusen, Germany) injected intramuscularly; local anesthesia was induced with 0.5 mL of 2% prilocaine hydrochloride (Astra Pharmaceutica, Zoetermeer, the Netherlands) injected subconjunctivally. The head of the rabbit was placed in a fixation device. The eyelids were spread with a speculum, and the pupils were di-
lated with 1% atropine sulfate eye drops. A limbal suture was placed at the 12-o’clock position for downward rotation of the eye. The superior conjunctiva was incised, and the temporal and nasal superior surfaces of the sclera were exposed.

For transscleral conductive heating, a hollow metal disk attached to a handle was placed on the sclera (Figure 1). It was heated with a continuous flow of water through the disk. This allowed accurate maintenance of its temperature by ±0.25°C. The temperature of the closed system of circulating water was monitored using thermocouples in the inflow and outflow channels of the applicator; the difference in temperature between both channels did not exceed 0.5°C. The applicator (with a temperature of 30°C, 55°C, 60°C, 65°C, or 70°C) was placed on the sclera for 30 or 60 seconds. The temperature at the scleral surface, measured with a thermocouple, reached the treatment temperature within 5 seconds. On each eye, 2 heat applications were performed: 1 located nasally superior and 1 temporally superior, with the exception of temperatures of 65°C and 70°C. At these temperatures, heat was applied only at the temporally superior location. After the heat application, the surface of the sclera was inspected. Then the conjunctiva was closed because scleral exposure may induce scleral necrosis; tetracycline ointment was applied to the eye.

An ophthalmoscopic examination was performed in all rabbits before and directly after heat application and in 7 rabbits after 3 to 4 months. Photographs of the fundus were taken 3 and 30 minutes before and directly after heat application and in 7 rabbits after 3 to 4 months (late stage).

The animals were euthanized either 2 days or 3 to 4 months after the experiment. The eyes were enucleated, fixed in buffered 4% formalin, dehydrated, and embedded in paraffin. Sections were stained with hematoxylin-eosin for histologic examination. Polarized light microscopy was used because it enhances the visibility of scleral collagen fibers and shows a loss of birefringence when the collagen fibers are damaged.

For both early- and late-stage experiments, each temperature-time combination was performed in duplicate. Treatment of the animals was in full compliance with the guidelines of the National Academy of Sciences.

RESULTS

There were no signs of heat-induced lesions after transscleral conductive heating at 50°C. On ophthalmoscopic, fluorescein angiographic, or histologic examination, both in the early and late stages (Table). At higher temperatures, the effect of heat on the retina, choroid, and sclera was the same after 30 and 60 seconds of application time. Therefore, exposure time is mentioned only in specific cases. Damage to the ocular fundus was confined to the heated area.

OPHTHALMOSCOPIC EXAMINATION OF THE OCULAR FUNDS

The fundus lesions were light gray directly after transscleral heating at 35°C, gray with fine retinal folds after heating at 60°C (Figure 2A), gray-white with coarse retinal folds after heating at 65°C (Figure 2B), and white after heating at 70°C. The whiteness of the lesions increased during the first 2 days after heating. At 60°C and higher, the retinal arteries showed fragmentation of the dark-red hypoxic blood column, a sign of occlusion of the vessel (Figure 2A). Crescentic retinal tears developed in half of the experiments along the margin of the lesion within 2 days after heating at 65° and 70°C (Figure 2C). At these temperatures, preretinal exudates developed in the vitreous. At 70°C, the lesions were surrounded by a serious retinal detachment.

Scars formed within 2 to 3 weeks of heating. The atrophic, irregularly pigmented scar had a well-defined border (Figure 2D-F). After heating at 55°C, the structure of nerve fibers remained largely intact (Figure 2D). In the range of 60°C to 70°C, the retina in the lesion shrank to a small white mass (Figure 2E and F). In 1 lesion after heating at 70°C for 1 minute, the retinal pigment epithelium and choroid ruptured, exposing the bare sclera (Figure 2F).

FLUORESCEIN ANGIOGRAPHIC EXAMINATION OF THE RETINA

After heating at 50°C, the angiographic findings were normal. At 55°C, a few retinal vessels became occluded in the central part of the lesion (Figure 2G). At 60°C, the retinal vessels showed perfusion up to but not beyond the center of the lesion (Figure 2H). At 65°C and 70°C, all retinal vessels in the heated area were occluded (Figure 2I). Hemorrhages were not observed at any of the temperatures. The area of occlusion of retinal vessels 3 to 4 months after heating was similar to that at day 2.

HISTOPATHOLOGIC EXAMINATION OF THE RETINA

In the early stage, after heating at 55°C, the retina in the lesion was slightly swollen. Retinal cells are pyknotic, but the structure of the 2 nuclear layers could still be distinguished even after heating at 70°C. In the late stage, the retina was atrophic after heating at 55°C to 70°C (Figure 3A). After heating at 70°C, the vitreous contained turbid epiretinal veils. Retinal hemorrhages did not develop at any of the temperatures.

FLUORESCEIN ANGIOGRAPHIC EXAMINATION OF THE CHOROID

Angiographic examination of the choroid was not informative in the first 2 days after heating because the fluo-
resonance was reduced by the opacity of the whitened retina. In the late stage, after heating at 55°C, most of the choroidal vessels were occluded; a few large, irregularly dilated choroidal vessels remained perfused (Figure 2G), which is consistent with the histologic findings (Figure 3A). After heating at 60°C, perfusion was retained in certain very small choroidal vessels. In 1 experiment, a triangular patch of the choriocapillaris remained perfused. The latter was attributed to insufficient contact between the applicator and sclera (Figure 2H). Even at 65°C and 70°C, a few small choroidal vessels remained perfused (Figure 2I).

**HISTOPATHOLOGIC EXAMINATION OF THE CHOROID**

In the early stage, after heating at temperatures of 55°C and higher, choroidal vessels in the lesion were dilated and densely packed with erythrocytes; hemorrhages developed profusely throughout the choroid but not beyond its inner and outer borders or outside the treatment area (Figure 3B). In the late stage, the choroid was atrophic after heating at temperatures of 55°C and higher (Figure 3A), and it showed marked fibrosis after heating at 70°C. The hemorrhages were totally resorbed. At 65°C to 70°C, a few small choroidal blood vessels remained patent; these contained some erythrocytes. In the early stage, at 55°C and higher, the melanocytes lost their pigment, which was dispersed throughout the choroid. In the late stage, after heating at temperatures of 60°C and higher, the melanocytes formed pigmented clumps (Figure 3A and B).

**HISTOPATHOLOGIC EXAMINATION OF THE SCLERA**

In the early stage, after heating at 55°C, the structure of the scleral collagen was not affected by heat. However, after heating at higher temperatures, structural changes were seen. At 60°C, collagen fibers in the heated area showed a minimal undulation. At 65°C, the sclera was slightly thickened, and the undulation of the collagen fibers was more evident (Figure 3B). At 70°C, the sclera was slightly thickened; the collagen fibers showed tortuosity and fragmentation, and birefringence decreased.

The sclerae in both eyes of a young rabbit were perforated in the center of the lesion when the intraocular pressure increased during enucleation, 2 days after heating at 65°C for 1 minute and at 70°C for 30 seconds. Perforation did not occur when the experiment was repeated in an adult rabbit.

In the late stage, the scleral collagen structure was not affected after heating at temperatures up to 65°C (Figure 3A, C, and D). However, after heating at 70°C, the scleral thickness was reduced by two thirds, and the birefringence of collagen fibers decreased. A layer of new, fine collagen fibers was seen on the inner surface of the sclera. The outer surface of the sclera was covered with fibrotic tissue (Figure 3E).

Sclerocytes were slightly shrunken in the early stage after heating at 55°C to 60°C but were normal in the late stage. At 65°C to 70°C, they were pyknotic; both in the early and late stages, their number was reduced in the inner layers of the sclera (Figure 3B and C).

**COMMENT**

Diathermy was the first technique of transscleral heating for the treatment of choroidal melanoma, originally performed by Weve in 21 patients. Three eyes had to be enucleated, and there were no recurrences. Diathermy did not become generally accepted as a treatment for choroidal melanoma, presumably because of the often severe burning of the sclera. In addition, interest became focused on radiotherapy, mainly with iodine 125 and ruthenium 106 applicators, which are still widely used. The main complication of brachytherapy is radiation retinopathy; radiation outside the target area causes loss of visual acuity in about 50% of patients. In a study on thermoradiotherapy, the radiation dose could be reduced by half when radiotherapy was synergistically combined with hyperthermia at 47°C to 50°C for 45 minutes. A visual acuity of 20/200 in the treated eye was maintained in 69% of patients compared with 44% in studies using radiation alone.26

For the treatment of choroidal melanoma, TTT differs from hyperthermia by its shorter exposure time of 1 minute and a higher temperature, up to 65°C; this temperature-time combination exerts a direct necrotizing effect on tumor cells. When combined with TTT, TSTT may...
have the potential to replace brachytherapy in the treatment of choroidal melanoma. The aim of TSTT is to destroy tumor cells in the basal layers of the choroidal melanoma and in the overlying sclera, which contains tumor cells in 27% to 92% of patients. In a study on TSTT for melanoma in hamsters, the tumor could be destroyed up to a depth of 4.4 mm without damage to the sclera.

The effect of transscleral conductive heating in the eye was similar at exposure times of 30 and 60 seconds on ophthalmoscopic, angiographic, and histologic examinations. Transscleral conductive heating of the sclera at 50°C for 1 minute did not cause ophthalmoscopically visible fundus lesions. Directly after heating at 55°C, the fundus showed a light-gray lesion (Figure 2A and B), and after heating at 70°C, a white lesion developed. This corresponds with the finding in rabbit eyes in which whitening of the retina occurred at 51°C to 52°C when heated transsclerally with a microwave applicator for 10 seconds.
onds. The temperature threshold for producing lesions in monkey eyes was found to be 62°C after 10 seconds of exposure and 57°C for an extrapolated exposure time of 60 seconds. For clinical evaluation of the effect of TSTT, a white lesion in the fundus directly after heating is considered to be a sign of overtreatment and entails a risk of complications, such as the development of retinal tears or breakdown of the scleral collagen.

In the late stage, 3 to 4 months after heat application, the scar in the fundus was sharply defined and demarcated from the surrounding normal fundus on fluorescein angiographic and histologic examinations (Figure 2E and 2G-I). This corresponds with histologic findings of choroidal melanoma in humans after experimental TTT and in the rabbit fundus after microwave hyperthermia. The restriction of the lesion to the heated area is relevant with respect to sparing the foveal area and preventing loss of visual acuity when melanomas near the posterior pole are treated.

Heat-induced occlusion of the feeder vessels of the tumor may promote tumor necrosis, as observed after photocoagulation treatment of the fundus surrounding a melanoma and retinoblastoma. We found that most of the blood vessels in the heated area became occluded after transscleral heating starting at 55°C. This corresponds with the findings of the occlusion of vessels in the fundus after heating at 52°C for 10 seconds as well as the occlusion of tumor vessels after heating at 60°C for 1 minute with TTT in choroidal melanoma and TSTT in hamster melanoma. However, even after heating at 65°C and 70°C, tiny choroidal vessels remained perfused, as revealed with fluorescein angiography (Figure 2G-I). Their patency may be related to the strong cooling effect of the high blood flow in the choroidal circulation. Comparable dose-
related vascular occlusion in the ocular fundus was observed after ruthenium 106 episcleral radiation at a dose of 20000 to 80000 rad (200-800 Gy).20

The retinal and choroidal vasculature responded differently to heating. After heating at temperatures of 55°C and higher, the retinal blood vessels became occluded but did not bleed. The choroidal blood vessels became maximally dilated, were engorged with packed erythrocytes, and bled profusely. This difference in hemorrhagic susceptibility between the 2 vascular systems may be related to structural variations in the vessel wall, which is evident in their differences in permeability.30

The sclera appears to be resistant to heat at temperatures up to 65°C; after heating at 65°C, undulation of the collagen fibers, a sign of minimal scleral damage, was transient (Figure 3B-D). Our results concerning the heat tolerance of the rabbit sclera in vivo correspond with those of our in vitro study with samples of the human sclera, in which 62°C to 63°C was the threshold temperature for thermal damage.31 In another study, after transscleral heating in rabbits at 65°C for 10 seconds, there was a wavy appearance of the scleral collagen fibers,32 which corresponds with our finding of minimal undulation of the fibers at this temperature. At 70°C, the sclera was severely damaged; new collagen fibers were formed on the inner surface of the sclera in the course of several months after heating (Figure 3E). In 1 young rabbit, the sclera became perforated during enucleation 2 days after heating at 65°C and 70°C. This complication may be attributed to an increased vulnerability of the scleral collagen to heat at a young age, which has also been found in sclerae heated in vitro.33 The existence of a possible repair mechanism of the sclera is supported by the finding that after diathermy, the loss of scleral strength was transient.31 In the sclera32,33 and joint capsulae,34,35 the thermally damaged structure of collagen fibers showed signs of repair by invaded fibroblasts.

In this study, a temperature of 65°C for 1 minute was well tolerated by the sclera. This temperature is effective for inducing necrosis of tumor cells because the destruction of these cells already starts at 45°C when heated for 1 minute.17,36,37 For TSST of the area around the tumor, when tumor cells may have invaded the choroid, the scleral temperature should not exceed 60°C because retinal tears may develop at temperatures of 65°C and higher. These findings and those of our in vitro experiments8 suggest that the parameters derived from these studies may be applicable to the use of experimental TSST in patients. When TSST proves to be effective, it should be combined with TTT to necrotize the tumor both from the base and the top.

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