The Effects of Ultrasonically Induced Hyperthermia on Experimental Tumors in the Rabbit Eye

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Greene's melanomas, implanted in the anterior chamber of 31 eyes of 19 Dutch rabbits, were treated with hyperthermia induced by high intensity focused ultrasound (HIFU). The percentage necrosis 24 hr after treatment (N) as a function of steady-state intratumor temperature (T) could be described with N(T) = -181.5 + 4.77T (P < 0.05). The most frequent side effect was a local hemorrhagic keratitis.

The acoustic absorption coefficient and thermal conductivity of the tumor tissue were 0.08 cm⁻¹ X MHz⁻¹ at 36°C and 0.011 W, cm⁻¹ X °C⁻¹, respectively. With these parameters a simple thermal model was established that enabled us to predict the ultrasonic intensity needed to reach a desired intratumor temperature. Invest Ophthalmol Vis Sci 30:835-844, 1989

In ophthalmology, interest in the use of hyperthermia to treat cancer has recently been aroused, since hyperthermia potentiates the therapeutic effect of ionizing radiation and can nowadays be applied locally. Beneficial effects of hyperthermia in intraocular tumors have been reported, alone or in combination with irradiation, in both experimental animals and humans. To achieve a synergetic tumoricidal action, ionizing radiation is usually applied directly after a hyperthermic treatment, that is, in the phase of acute hyperthermic damage.

For systematic (pre)trement of intraocular malignancies, the acute effects of hyperthermia should be quantitatively predictable within certain limits. Quantitative information on the acute effects of hyperthermia on intraocular malignancies is lacking. Only qualitative information on the long-term effects of hyperthermia on intraocular tumors is available.

The purpose of this study is therefore to quantify the amount of acute damage to tumor tissue as a function of temperature and to quantify the amount of heat generated in the tumor tissue as a function of energy input.

To this end we treated Greene's amelanotic hamster melanoma, implanted in the anterior chamber of the rabbit eye, with hyperthermia by means of high intensity focused ultrasound (HIFU).

Materials and Methods

Animal Model/Drugs

Experiments were performed on 31 eyes of 19 Dutch rabbits of either sex, mean weight 1750 ± 250 (SD) grams. About 9 days prior to the experiment a ±1 mm³ specimen of the Greene's amelanotic hamster melanoma was implanted transcorneally onto the iris of both eyes at the 12 o'clock position. The experiments were carried out when the tumor had reached a diameter of about 7 mm in order to preclude spontaneous necrosis. The latter is due to the tumor's high growth rate and occurs when diameters exceed about 11 mm.

During both implantation of the tumor and the experiment the animals were anaesthetized with a 1:1 mixture (1.5 ml/hr i.m.) of ketamine 10% (Aescoket®, Aesculaap, Boxte, Holland) and 2-(2.6 xylyidine)-5.6-dihydro-4H-1.4-thiazine 2% (Rompun®, Bayer, Barcelona, Spain).

The animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Experimental Set-up/Recordings

The therapeutic ultrasound beam requires a fluid coupling bath between the transducer (Sonocare CST-100/4.6 MHz, Ridgewood, NJ) and the eye of the rabbit. To achieve this, the coupling cone of the transducer was filled with degassed, distilled water and closed with a latex membrane, while around the proposed eye of the rabbit a fluid bath was constructed that was filled with phosphate-buffered saline. The saline was circulated through a heat exchanger by a pump. The eye bath temperature was

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Fig. 1. Experimental set-up. 1: pump, 2: heat exchanger, 3: inlet tube, 4: metal ring holding fluid bath, 5: thermistors (one for measuring temperature of the tumor, the other for measuring temperature of the fluid bath), 6: fluid bath constructed from Steridrape around the proposed eye of the rabbit, 7: outlet tube, 8: electronic thermometer, 9: ultrasound transducer.

Thus kept at about 36°C, monitored by a thermocouple probe (Ellab, A-K19, Rødvre, Denmark) in the eye bath (Fig. 1).

Before filling the eye bath, the cornea was perforated near the limbus, parallel to the iris plane, by a 30.5-gauge needle. Through this perforation the tip (diameter 0.2 mm) of a thermocouple probe (Ellab, A-ISC; diameter 0.4 mm) was inserted into the center of the tumor.

After filling the eye bath, the therapeutic transducer was aimed perpendicularly at the tumor by the coaxial diagnostic A-mode transducer and by the central fiber optic channel in alignment with the central axis of the treatment beam. The appropriate distance (S) from focus to tumor was determined by:

$$S = 0.5d/\tan(0.5\beta)$$

where $\beta$ is the beam angle and $d$ is the largest tumor diameter on the frontal aspect of the tumor (broadbeam insonification) (Fig. 2).

During the experiment both intratumor and eye bath temperature were monitored with an electronic thermometer (Ellab, CTD 85) and recorded on a chart recorder for later analysis.

Protocol

After the radiation force was calibrated against a mass balance (Mettler, PE 60, Greifensee, Switzerland), the power setting was determined at which the desired intratumor temperature, ranging from 43 to 49°C, was reached. When this power setting was established the tumor was treated for 30 min. Deviations from the desired intratumor temperature level during treatment were offset by manual control of the power level (vernier dial).

Twenty-four hours after treatment the rabbits were sacrificed by an i.v. injection of 5 ml sodium pentobarbital (60 mg/l), and the eyes were enucleated and fixated in buffered formaldehyde 4.0%.

One tumor-containing rabbit eye was not treated by HIFU-induced hyperthermia and served as a control.

Histological Evaluation

The eyes were dehydrated and embedded in celloidin. Serial sections of 15 μm (Jung Microtome, Heidelberg, W. Germany) were cut in a plane parallel to the main axis of the ultrasound bundle. About 25 sections per eye were examined after hematoxylin/eosin staining. Damage to nontumorous tissue was evaluated qualitatively. Tumor-containing sections were also examined planimetrically by a grid (10 X 10 mm) inserted in one of the ocular tubes of the microscope (Carl Zeiss, Leipzig, East Germany magnification X20). The number of grid crossings covering the total tumor tissue and those covering the necrotic parts of the tumor were counted in five to nine sections evenly spread over the tumor width. The mean quotient of the counts in the necrotic parts and the...
total counts in the tumor was used as a quantitative measure of the tumor-destructive effect of HIFU. The counting was performed independently by two observers masked to temperature. All calculations were performed by a desk-top computer (Olivetti PC M24, Ivrea, Italy). For statistical operations a probability level of \( P < 0.05 \) was adopted.

**Results**

In the control experiment, without hyperthermia, necrosis was absent (Fig. 3A). Areas with necrosis and hemorrhage were observed in the tumor after treatment by hyperthermia (Fig. 3B). At high magnification the necrotic tumor cells exhibited pyknotic or disintegrated nuclei and hypochromatic cytoplasm.

**Effect of Hyperthermia as a Function of Temperature**

The mean percentage necrosis as a function of steady-state intratumor temperature is shown in Figure 4. The amount of necrosis increases with temperature. The relation between the mean percentage necrosis (±SEM) (N) and intratumor temperature (\( T \)) can be described by: \( N(T) = -181.5 + 4.7T \) \((P < 0.05)\). At 45 and 46°C the SEM is larger than the SEM at the other temperatures.

The side-effects of HIFU in the nontumorous tissues are summarized per temperature in Table 1. Damage to the nontumorous ocular tissues always had a local character. Hemorrhagic keratitis was a constant finding after HIFU-induced hyperthermia.

**Prediction of the Ultrasonic Intensity Needed to Reach a Desired Intratumor Temperature**

The intratumor temperature depends on the amount of incident power that is absorbed by the tumor. The effect of stepwise increases of incident ultrasonic power on the intratumor temperature is shown in Figure 5A. Upon each power step the temperature exhibits a transient and reaches a steady state in about 15 sec. From temperature transients the necrotic tumor cells exhibited pyknotic or disintegrated nuclei and hypochromatic cytoplasm.

With knowledge of the absorption coefficient and the dimensions of the tumor, the absorbed power density in the tumor (corrected for the cornea) could be calculated (see Appendix). The relation between the absorbed power density (Q) and the resulting steady-state intratumor temperature (T), derived from the experiment shown in Figure 5A, is linear (Fig. 5B): \( T(Q) = 35.3 + 9.7Q \) \((P < 0.05)\).

The temperature axis intercept of the T–Q relation (\( T_0 = 35.3°C \)) indicates the estimated value of the intratumor temperature at zero power and corresponds well with the measured value of the intratumor temperature at zero power (35.4°C; Fig. 5A) and with the eye bath temperature (35.5°C). The slope of the T–Q relation \( \Delta T/\Delta Q = 9.7°C \times W^{-1} \times cm^{-1} \) indicates the steady-state temperature rise with respect to \( T_0 \) per unit absorbed power density.

In all experiments the T–Q relations were significantly linear (mean corr. coeff. 0.995 ± 0.007 (SD) \( n = 28 \)). From the slope \( \Delta T/\Delta Q \) of these relations and the radius of the tumors the thermal conductivity (\( \lambda \)) of tumor tissue could be estimated (see Appendix). Furthermore, there was a significant correlation (corr. coeff. 0.881 \( n = 28 \)) between \( T_0 \) (mean: 36.0°C ± 1.6 (SD)) and the eye bath temperature (mean: 35.9°C ± 1.6 (SD)).

Eventually, with the following relation (Appendix eq. (8)) the (spatial average) ultrasonic intensity (\( I_o \)) can be predicted that is needed to reach a desired intratumor temperature: \( I_o = x_i \times (T - T_0) \times 2 \times \lambda \times R^{-2} \times (exp(-\alpha_c \times x_i) \times (1 - exp(-\alpha_t \times x_i))^{-1} \) where \( x_i \) is the tumor thickness (0.11–0.28 cm; mean 0.17 cm ± 0.03 (SD)), \( T \) is the desired intratumor temperature (eg, based on Fig. 4), \( T_0 \) is the eye bath temperature (eg, chosen to be 36°C), \( \lambda \) is the thermal conductivity (0.011 W × cm⁻¹ × °C⁻¹), R is the tumor radius (0.25–0.49 cm; mean 0.37 cm ± 0.17 (SD)), \( \alpha_c \) is the absorption coefficient of the tumor tissue (0.37 cm⁻¹ at 4.6 MHz), \( \alpha_i \) is the corneal absorption coefficient (1.06 cm⁻¹ at 4.6 MHz), and \( x_c \) is the corneal thickness (0.034 cm).

As a check on the predictive value of the above relation the predicted (spatial average) intensity (\( I_o \)) was plotted against the measured (spatial average) intensity (\( I_m \)) (Fig. 6). The regression line drawn through the data points was forced through the origin. The relation between predicted (Appendix eq. (8)) and measured (Appendix eq. (3)) intensity could be described as \( I_o \propto I_m (P < 0.05) \) (corr. coeff. 0.962). The hypothesis that the slope (1.05) is equal to 1 (line of identity) could not be rejected \( (P < 0.05) \).

**Discussion**

**Critique of Methods**

The animal model: Although the Greene's amelanotic hamster melanoma in the anterior chamber of the rabbit eye differs in many respects from the melanoma in the human eye, it is suitable for hyperthermia experiments. The control tumor in this study showed no evidence of spontaneous necrosis 9 days after implantation, at the moment the other tumors were treated by HIFU. This confirms earlier observations by our laboratory in a control group of Greene's melanomas implanted in the anterior...
chamber of the rabbit eye.9 Tumor necrosis, therefore, must have been due to HIFU treatment.

Thermometry: Both invasive and noninvasive thermometry are used in measuring intratumor temperature. In noninvasive techniques, especially suitable for superficial tumors, temperature is measured indirectly, for example, by microwave radiometry, X-ray tomography or NMR.11 Another good alternative might be fluorescence techniques.12 In invasive techniques, also suitable for deep tumors, temperature is measured directly by thermistors or thermocouples (cf thermal mapping).13 Intratumor temperature may also be predicted from a thermal model (analytically or numerically) if the model parameters are known. For validation of such a model (non)invasive thermometry is necessary. We used thermal modeling to acquire knowledge of the parameters that govern the relation between incident ultrasonic energy and the resulting heat in intraocular tumor tissue.

The use of thermocouples therefore seemed a logical choice because it is direct, simple, reliable and inexpensive. A disadvantage is that it produces some tissue damage, albeit little. For this reason we used only one thermocouple probe per tumor, since tumor volume was small (mean 0.08 cm³ ± 0.03 (SD)). We used an electronic thermometry system with constantan thermocouple probes having an accuracy of 0.1 °C and a temporal resolution in the order of 1 sec. These features meet the general requirements for a thermometer for hyperthermia studies.11 In the model calculations we used the central tumor temperature since the tip of the thermocouple probe was placed in the center of the tumor.

Effect of HIFU-Induced Hyperthermia

Among the direct effects of hyperthermia are damage to the cell membrane and nuclear structures, and changes in the tumor blood flow.15 Indirect effects are leakage of ions to the extracellular space, decreased repair of DNA strands14 and decrease in tissue pH and tissue oxygenation.15 All these effects occur within 24 hr and lead to cell necrosis. In this acute phase, a good impression of the amount of cell death could be expected at all examined temperatures.16 We chose to determine the degree of necrosis as a function of temperature 24 hr after insonification. For intraocular malignancies, such a functional relationship has not been described before. Parameters often used in oncological studies, such as tumor growth delay or cessation could not be referred to, since they pertain to long-term therapy effectiveness.

When tissue is treated with HIFU the ultrasound energy is converted into heat.17-20 Tumor cells are heat-sensitive above 42°C.21 This sensitivity also depends on the exposure time.15 We chose a temperature range of 43-49°C with a fixed exposure time of 30 min since this period would be clinically applicable.

The results of this study demonstrate a significant linear dose–response correlation between temperature and percentage necrosis, within the temperature range studied. The parameters estimated by the linear regression analysis (N (T) = −181.5 + 4.7T, see Results) would predict 0% necrosis at about 38°C and 100% necrosis at about 60°C. Although this is not an unrealistic prediction, it does not prove that the temperature–necrosis relation is also linear outside the measured temperature range. Higher order regression analysis or nonlinear fitting of the data points of the temperature–necrosis relation gave statistically nonsignificant results. In feline brain tissue Lyons et al
Table 1. Histological evaluation of effects of HIFU-induced hyperthermia on nontumorous ocular structures

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>Cornea</th>
<th>Iris</th>
<th>Lens</th>
<th>Retina and choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>43 (3)</td>
<td>Local hemorrhagic keratitis (2)</td>
<td></td>
<td>Local cataract (1)</td>
<td>Local edema (2)</td>
</tr>
<tr>
<td>44 (4)</td>
<td>Local hemorrhagic keratitis (3)</td>
<td>Hemorrhage (1)</td>
<td>Local cataract (1) with damaged lens epithelium (1)</td>
<td>Local edema and choroidal hyperemia (1)</td>
</tr>
<tr>
<td>45 (5)</td>
<td>Local hemorrhagic keratitis (5)</td>
<td>Iris (3), edema (1)</td>
<td>Local cataract (3) with damaged lens epithelium (1)</td>
<td>Local edema and hemorrhage (3)</td>
</tr>
<tr>
<td>46 (4)</td>
<td>Local hemorrhagic keratitis (3)</td>
<td>Iris (2), hyperemia (1)</td>
<td>Local cataract (2) with damaged lens epithelium (1)</td>
<td>Local necrosis (1), hemorrhage (3), local edema (2), local exsudative retinal detachment (1)</td>
</tr>
<tr>
<td>47 (4)</td>
<td>Local hemorrhagic keratitis (3)</td>
<td>Iris (1)</td>
<td>Local cataract with loss of lens epithelium (2)</td>
<td>Local edema (2)</td>
</tr>
<tr>
<td>48 (4)</td>
<td>Local hemorrhagic keratitis (3)</td>
<td>Iris (3)</td>
<td>Local cataract (3) with damaged lens epithelium (2)</td>
<td>Local necrosis and choroidal hyperemia (2), local edema (3), scleral perforation (1)</td>
</tr>
<tr>
<td>49 (3)</td>
<td>Local hemorrhagic keratitis (2)</td>
<td>Iris (2), edema (1)</td>
<td>Local cataract (3) with damaged lens epithelium (3)</td>
<td>Local edema (3), local necrosis and choroidal hyperemia (3)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of rabbit eyes.

also found a linear dose–response relationship.16 In analyzing the tissue damage produced by heat, however, they used the histologic specimens that contained the maximum lesion size, whereas we calculated an average necrosis from sections over the whole tumor.

Although we observed 100% necrosis in individual tumor sections, the necrosis per tumor was always subtotal; the non-necrotic tumor tissue was usually located at the tumor’s edge, in a plane perpendicular to the ultrasound beam. An explanation for the subtotal necrosis may therefore be the fact that the beam width was chosen equal to the largest tumor width (see Materials and Methods), resulting in an insufficiently steep temperature gradient towards the tumor’s periphery.

The variance in the percentage necrosis at 45 and 46°C is large compared to the variance at the other temperatures. This discrepancy might be due to the variable time-dependent effect of different degrees of hyperthermia on the tumor blood flow.15 During the 30 min treatment time we made the overall observation that for 43 and 44°C the power needed almost no correction, that for 45 and 46°C correction was necessary by either lowering or raising power and that for 47, 48 and 49°C the power usually had to be lowered after about 12 to 18 min after the start of the treatment. This observation seems to indicate that at temperatures of 45 and 46°C blood flow may sometimes have decreased or stopped, and sometimes not.

To further evaluate this observation fluorescence angiograms were made in five rabbits before and after treatment with HIFU at 43, 44, 45, 46 and 48°C. All five tumors showed a diffuse fluorescence before treatment. After treatment at 43 and 44°C tumor fluorescence was unchanged, but fluorescence in the tumor treated at 46°C was strongly diminished, while in those treated at 45°C and 48°C no fluorescence could be demonstrated. Our observations confirm those of Dudar and Jain, that for 30 min treatment time the temperature at which tumor blood flow stops varies between 45 and 46°C.22 Although this unpredictable behavior of the blood flow at 45 and 46°C may explain the large variance in the percentage necrosis it still leaves unexplained why the maximum percentage necrosis at 46°C (79.6%) exceeds the maximum percentage necrosis at 49°C (62.3%).

The histopathological findings in the tumors 24 hr after hyperthermic treatment agree well with what Lyons et al found: pyknosis of the cell nuclei, loss of cytoplasmic integrity and minimal migration of in-
flamatory cells. A constant finding was hemorrhage, which is a pathologic feature common to most tissues after hyperthermia.

Hemorrhagic keratitis was a constant side-effect. In the control eye, however, keratitis was also present, albeit less severe than in the treated eyes, and without hemorrhage. The keratitis in the control eye was predominantly located at the site of tumor implantation (cf Franken et al). Cataract formation due to HIFU was sometimes seen and has also been described by Lizzi et al as an effect of hyperthermia induced by HIFU. Damage to the retina was usually confined to edema. Scleral perforation, observed once, may partially have been due to intensity amplification caused by reflection of the ultrasound on the orbit.

In almost all treated tumors and in the control tumor chronic inflammatory infiltration was found in the iris, which might be due to an immune response. All side-effects had a local character, and may be regarded as reversible, except for perforation, necrosis and cataract. From the analysis of the data of Table 1 it may therefore be inferred that great care should be taken in aiming the ultrasound beam to avoid cataract formation. Necrosis and perforation of sound tissue will not occur at temperatures below 45°C.

Prediction of Ultrasonic Intensity

We predicted the ultrasonic intensity from the desired treatment temperature and tumor dimensions by means of a thermal model. As a first-order approximation we used an analytical solution of a reduced form of the bioheat transfer equation. We assumed a spherical geometry of the tumor, modified according to Lagendijk for calculating (and measuring) central tumor temperature (Appendix eq. (7)). This implies that the axial thermal deposition was averaged over the tumor depth. This is not an unreasonable assumption since tumor thickness was low. The surrounding of the tumor could be regarded as an infinitely large conductor of constant temperature. Heat generation due to metabolic activity was assumed negligible compared to the HIFU-induced heat generation.

Since the equation proposed by Swindell neglects heat flow due to convection, the estimation of the thermal conductivity resulted in an effective value (λ = 0.011 W × cm⁻¹ × °C⁻¹) higher than reported for water (λ = 0.006 W × cm⁻¹ × °C⁻¹), or rabbit lens (λ = 0.004 W × cm⁻¹ × °C⁻¹). The estimated value of the absorption coefficient (α = 0.08 cm⁻¹ × MHz⁻¹) of the tumor tissue shows good agreement with the value of Goss et al, who found 0.09 cm⁻¹ × MHz⁻¹ (mean value measured in six different tissues by means of the thermoelectric method). Although we used a simple thermal model the measured and predicted intensity show a significant correlation. A simple model has the advantage of being easily comprehensible. We are, however, aware of the fact that an intraocular tumor will usually not assume an ideal spherical geometry and that central point temperature will not ideally represent all thermal events in the tumor. On the other hand, small Greene's amelanotic hamster melanomas contain

![Fig. 5. (A) Intratumor temperature resulting from stepwise increases of ultrasonic power as a function of time (Exp. 26). The baseline temperature (at zero power) is 35.4°C. The numbered arrows indicate power increments: (1) 0.47 W, (2) 0.84 W, (3) 1.25 W and (4) 1.73 W. After each power increment the temperature reaches a steady state value within 10–20 sec. (B) Relation between absorbed power density (Q) and steady-state intratumor temperature. The correlation coefficient of the linear regression line drawn through the data points amounted to 0.998. R = tumor radius.](image)
quite homogenous tissue and are evenly vascularized (no large heat sinks) so that inhomogenous thermal deposition is improbable. Furthermore, the use of small tumor volume and broad-beam insonification provides a relatively uniform thermal deposition over the tumor width. Beam width, however, should probably exceed tumor width (see above).

We conclude that the amount of HIFU-induced necrosis increases linearly with temperature in the range 43-49°C, and that a safe treatment temperature should not exceed 45°C. By a simple thermal model, the ultrasonic intensity needed for a desired treatment temperature can be predicted. The thermal conductivity and the absorption coefficient of the specific tumor tissue are thereby important parameters. Although this is only a preliminary study, we feel that HIFU-induced hyperthermia could be a valuable (additive) means in the treatment of intraocular malignancies.

Key words: hyperthermia, focused ultrasound, Greene’s amelanotic hamster melanoma, rabbit, absorption coefficient, thermal conductivity, intraocular tumor

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References


Appendix

Estimation of the Absorption Coefficient of the Tumor Tissue

Inherent to the experimental setup, the so-called transient thermoelectric technique could be applied to determine the absorption coefficient of tumor plus cornea. It can be shown for tissue in which no heat is lost by thermal conduction or convection that the absorption coefficient $\alpha$ (in cm$^{-1}$) is given by:

$$\alpha = \rho \times C \times \frac{dT}{dt} \times \frac{\Delta T}{\Delta t}^{-1} \quad (1)$$

where $\rho$ is the tissue density (taken to be 1.05 g X cm$^{-3}$), C is the tissue heat capacity (taken to be 4.18J X g$^{-1}$ X °C$^{-1}$) and $\frac{dT}{dt}$ is the linear temperature rise in the first second after insonation (Appendix Fig. 1).

In seven experiments the absorption coefficient was determined directly from the chart recording, yielding a mean value of $1.43 \pm 0.9$ cm$^{-1}$ at 4.6 MHz and 36°C. If for the cornea the same absorption coefficient is taken as for the sclera, namely $\alpha_c = 0.23$ cm$^{-1}$ X MHz$^{-1}$, which may be linearly extrapolated to 1.06 cm$^{-1}$ at 4.6 MHz according to Goss et al., then the absorption coefficient for the tumor ($\alpha_t$) is 0.37 cm$^{-1}$ at 4.6 MHz and 36°C (0.08 cm$^{-1}$ X MHz$^{-1}$).

Calculation of the Absorbed Power Density

The incident ultrasonic power ($P_0$) is calculated as:

$$P_0 = RF \times c \times 0.01 \quad (2)$$

where RF is the measured radiation force (in grams), c is the ultrasound velocity in water (taken as 1500 m X s$^{-1}$) and 0.01 is a proportionality constant to express $P_0$ in watts. The incident (spatial average) intensity ($I_0$) of the ultrasonic beam is calculated as:

$$I_0 = P_0 \times \pi \times R^{-2}, \quad (3)$$

where $R$ is the measured radius of the tumor (in cm). $I_0$ is expressed in W X cm$^{-2}$.

The ultrasonic intensity after passing the cornea ($I_c$) is calculated as:

$$I_c = I_0 \times \exp(-\alpha_c \times X_c), \quad (4)$$

where $\alpha_c$ is the absorption coefficient of the cornea and $X_c$ is the measured thickness of the rabbit cornea (0.034 cm). Since tissue thickness was small effects of focusing were discarded.
Appendix Fig. 2. Relation between the tumor radius (R) and the slope of the T-Q relation (ΔT/ΔQ). ΔT is the steady state temperature increase above baseline temperature. The line drawn through the data points (n = 28), that each represent an experiment, is the best least squares fit. From this fit procedure the parameter λ (thermal conductivity) was estimated.

The ultrasonic intensity in the tumor \( I_r \) is calculated as:

\[
I_r = I_c \times \exp(-\alpha_c \times x_c),
\]

where \( \alpha_c \) is the absorption coefficient of the tumor tissue (in \( \text{cm}^{-1} \)) and \( x_c \) is the thickness of the tumor tissue (in cm).

The absorbed power density \( Q \) was calculated as:

\[
Q = (I_c - I_r) \times x_c^{-1} = I_0 \times \exp(-\alpha_c \times x_c) \\
\times (1 - \exp(-\alpha_c \times x_c)) \times x_c^{-1}
\]

\( Q \) is expressed in \( \text{W cm}^{-3} \).

Absorbed power density \( Q \), (central tumor) temperature \( T \) and tumor radius \( R \) are related as:

\[
\frac{\Delta T}{\Delta Q} = R^2 \times (2\lambda)^{-1}
\]

where \( \Delta T \) (in °C) is the steady-state temperature rise above baseline \( (T_0) \) and \( \lambda \) (in \( \text{W cm}^{-1} \times \text{°C}^{-1} \)) is the thermal conductivity. By combining eqs. (4) through (7), the spatial average intensity \( I_0 \) can be predicted from:

\[
I_0 = x_c \times (T - T_0) \times 2 \times \lambda \times R^{-2} \times (\exp(-\alpha_c \times x_c) \\
\times (1 - \exp(-\alpha_c \times x_c)))^{-1}
\]

Estimation of the Thermal Conductivity Coefficient of the Tumor Tissue

Since the slopes of the T-Q relations (ΔT/ΔQ) and the radii \( R \) are known for all experiments, \( \lambda \) can be estimated by means of a least squares parameter estimation procedure\(^{29} \) according to eq. (7). Appendix Figure 2 shows the slopes plotted as a function of radius for all experiments. The drawn line gives the best fit and \( \lambda \) is estimated to be 0.011 \( \text{W cm}^{-1} \times \text{°C}^{-1} \) ± 0.001 (SD) (\( P < 0.05 \)).