Magnetic Resonance Diffusion Imaging Detects Structural Damage in Biological Tissues upon Hyperthermia

Kwan Hon Cheng and Marissa Hernandez

Abstract

The use of quantitative nuclear magnetic resonance (MR) imaging to investigate the extent and mechanism of hyperthermic damage in biological tissues has been studied. By using the multiple delay–multiple echo and pulsed-gradient spin echo MR imaging sequences, multiple frame MR images of freshly harvested rabbit tissues (brain, kidney, and muscle) and intact duck embryos in shells were obtained before and after heat treatment (45°C for 30 min) using a clinical 1.5-Tesla whole-body superconducting MR scanner. Based on the relaxation and diffusion models, maps of the proton spin density, relaxation times, and various self-diffusion parameters of tissue water were generated from these multiple frame MR images. Our results indicated that the values of the diffusion barrier size and fractal parameter of the tissues and the self-diffusion coefficient of tissue water increased significantly, i.e., approached that of free water, after the heat treatment. In comparison, only slight changes in the spin density and relaxation times of the tissue water were found after the identical heat treatment. We concluded that the significant changes in the self-diffusive behavior of the tissue water are due to the denaturation of macromolecules (e.g., protein and fiber) within the tissues at elevated temperatures. We further suggested that MR diffusion imaging represents a powerful tool to investigate the extent and mechanism of heat damage of biological tissues in vivo and therefore bears important potential in the clinical assessment of the therapeutic efficacy of hyperthermia in cancer therapy.

Introduction

The therapeutic efficacy of hyperthermia by itself, or in combination with radiotherapy and/or chemotherapy, in the treatment of tumors has been investigated for many years. As compared with adjacent normal tissues, some tumors are more sensitive to heat damage in the range of 42–46°C, owing to their low pH, nutrition deprivation, low blood flow, and chronic hypoxia (1, 2). At present the major mechanism of heat damage in either normal tissues or tumor is still speculative. In addition, the extent of heat damage in biological tissues is quite difficult to assess in vivo.

Although MR3 imaging is becoming a standard modality in the clinical diagnosis of pathological disorders in human tissues (3, 4), the use of MR imaging as a quantitative tool to assess hyperthermic damage in either normal tissues or tumors has not been fully explored. Recent advances in MR imaging technology have allowed one to generate synthetic images or maps of important biophysical parameters of the imaging tissues based on different imaging sequences and mathematical models (3, 4). These physical parameter maps form the basis for the quantitative characterization of tissues at the molecular level. The major goal of this pilot study is to explore the potential of using these new quantitative MR techniques to assess the extent of biological tissue damage due to hyperthermia.

Denaturation of macromolecules (e.g., protein and fibers) within the tissues is believed to be the major factor contributing to the damage of tissues upon hyperthermia (5). Water in biological tissues is mostly bound to macromolecules, e.g., protein, fibers, and membranes, as well as to ions. As a result, the values of the relaxation times (T1 and T2) of the tissue water, which are related to the translational and rotational rates of water, are significantly reduced to the millisecond range as compared to that of free water (~2 s) (6). In addition, water undergoes restricted diffusion in tissues due to the highly hindered microenvironment of the tissues. Water molecules in the tissues travel an average distance of a few micrometers, the size range of a cell, during the millisecond MR imaging time scale, and the rate of self-diffusion of tissue water is about 2–3 times slower than that of free water. Therefore, the proton relaxation times (T1 and T2) and self-diffusion parameters of tissue water represent the intrinsic probes for investigating the structural changes in the tissues at elevated temperatures. Specifically, an understanding of the detailed changes in these physical parameters, in terms of T1, T2, and diffusion parameter maps, is relevant to quantifying the extent of tissue damage due to hyperthermia.

In this model study, we used freshly harvested rabbit tissues (kidney, muscle, and brain) and intact duck embryos in their shells. The values of the relaxation times and diffusion parameters of water in those tissues are very similar to those found in human tissues (7). Moreover, a clinical whole-body 1.5-Tesla MR scanner was used in this study. The imaging sequences used in this study are already available in several commercial MR scanners or can easily be installed on other scanners. Therefore this model study on animal tissues may also be extended to future investigations on live animals and human subjects.

Materials and Methods

Imaging Objects

Freshly harvested rabbit tissues (whole brain, kidney, and thigh muscle), intact duck embryos in shells, and control solutions (0.5 mm CuSO4-doped water, 30% glycerol, and 1% agarose doped with 1 mm CuSO4) were used as imaging objects. New Zealand white rabbits were sacrificed, and the tissues were harvested and immersed immediately in a storing buffer (50 mM Tris-HCl, 250 mm sucrose, 25 mm KCl, 5 mm MgSO4, pH 7.4) at 4°C. All rabbit tissues were used for imaging after 8 h after the rabbits were sacrificed. Duck embryos (2–3 weeks old) in their shells were obtained from a local farm and stored at 4°C before use.

Heat Treatment

The heat treatment was performed by heating the rabbit tissues in the beakers containing the storing buffer and the intact duck embryos in distilled water using a water bath with temperature regulated to within ±0.5°C. In some studies, a home-built air-circulating heating device, which was placed into the MR scanner, was used. Here, regulated house compressed air (10–15 psi) was heated in a remote heat exchanger and led into an insulated plexiglass chamber where the imaging objects were located. A small nonmagnetic heater was also placed near the air input.
of the chamber for fine temperature adjustment. The temperature fluctuation inside the control solution was around 0.5°C for temperatures lower than 45°C. The temperature of the samples was monitored by nonmagnetic probes in the storing buffer of the harvested tissues and inside the duck embryos (for several experiments). This in vivo heating device may also be used for small living animals.

MR Scanner

Proton MR imaging measurements were performed on a 1.5-Tesla (64 MHz) Magnetom (Siemens, NJ) superconducting whole-body MR scanner using a head coil. All objects were imaged in the coronal plane with a slice thickness of 8 mm. The field of view was 12.8 x 12.8 cm². A matrix size of 64 x 128 was used for storing all of the images. A VAX11/750 MR computer was used for performing the two-dimensional Fourier transform and generation of the raw MR images.

Image Acquisitions

Two different kinds of raw MR images were obtained. They are the MDME images used for the generation of N(H), T₁, and T₂ maps, and the PGSE images for the diffusion parameter maps.

For the MDME image acquisition, Carr-Purcell-Meiboom-Gill multiple spin echo sequences with five different Tₑₛ of 2000, 1000, 600, 400, and 300 ms were used (see Fig. 1A). For each Tₑₛ, 32 echoes were used. A total of 160 MDME raw images with different combinations of Tₑ and Tᵣ were therefore obtained. A detailed description of this MDME sequence can be found elsewhere (8).

For the PGSE image acquisition, pairs of balanced diffusion-sensitizing pulsed magnetic field gradients parallel to all three imaging directions were used (see Fig. 1B). These diffusion-sensitive gradients were sent from the existing whole-body gradient coils of the MR scanner. The pulse width (δ) or gradient duration was fixed at 30 ms, while the diffusion time (Δ) was varied from 40 to 150 ms in four steps. The strength of each gradient (G) was also varied from 0 to 10 mT/m in five steps. A total of 20 PGSE raw images, with different combinations of Δ and G, were therefore obtained. A detailed description of this PGSE sequence can be found elsewhere (4).

Mathematical Models for Image Analysis

The intensity of the spin-echo signal as a function of Tᵣ and Tₑ for the MDME pulse sequence is given by (8):

\[ I(T_r, T_e) = N(H) e^{-\frac{T_r}{T_1}} \left[ 1 - e^{-(T_r + 2M + \frac{2M^2}{T_1})} \right] \cos(r/T_1) - 1] \]

where M is the total number of echoes (= 32 in our case) and 2r is the time interval between two consecutive echoes (= 15 ms in our case) (see Fig. 1A). From the raw images of different Tₑ and Tᵣ, the N(H), T₁, and T₂ parameters were calculated simultaneously based on a nonlinear least-squares procedure.

The intensity attenuation (I/I₀) of the spin-echo signal for the PGSE sequence is given by the following diffusion models:

**Einstein Model**

\[ I/I_0 = \exp(-bD_ₑ) \]  

where

\[ b = \gamma^2 G^2 \left( \Delta - \frac{\delta}{3} \right) \]

Here I and I₀ are the signal intensity in the presence and absence of the pulsed field gradient, respectively, Dₑ is defined as the self-diffusion coefficient of the tissue water based on the Einstein model, and γ is the nuclear gyromagnetic ratio of proton. The b factor (Equation C) contains all the pulsed-gradient parameters used in the PGSE measurements. This Einstein model assumes that water undergoes self-diffusive motion in an unrestricted and isotropic medium, and its mean square displacement in time t is directly proportional to t (9).

**Non-Brownian Diffusion Model**

\[ I/I_0 = \exp \left[ -\gamma^2 G^2 \left( \Delta - \frac{\delta}{3} \right) \right] \]

This model (10, 11) suggests that the structures of the tissues exhibit fractal geometry on a microscopic scale and that the mean square displacement of tissue water in time t is proportional to t². In model studies, the value of b is less than 1 for diffusion in fractal systems such as porous media, gels, polymeric solutions, and biological tissues (10–12). Note that Equation D is equivalent to Equation B when b = 1. In this case, the water diffusion obeys the Einstein model.

**Barrier Model**

\[ I/I_0 = (1 - R) \exp(-bD_ₑ) + R \]
where $R$ is the residual attenuation and $D_B$ is defined as the water self-diffusion coefficient based on the barrier model. As seen in Equation D, $I/I_0$ approaches $R$ as the $b$ factor approaches infinity. Equation E is an asymptotic approximation of a more complicated equation which involves an infinite series (13). This barrier model assumes that the translational motion of water in the tissues is hindered or restricted. The water molecules encounter geometrical barriers (13) and compartments (14) within the tissues. In the case of an impermeable spherical cavity barrier, the value of $R$ can also be associated with the radius ($\rho$) of the cavity by the following expression (13):

$$R = \exp \left[ -\frac{1}{5} \rho^3 G_{12}^2 \right]$$

(F)

Note that Equation F is equivalent to Equation B when $R = 0$. In this case, the water undergoes unhindered diffusion.

Synthetic Image Calculations and Display

Synthetic images were generated from the raw MDME and PGSE MR images by performing pixel-by-pixel calculations on a VAX vector computer based on the mathematical models (Equations A–F). The calculated images in the form of real numbers were transformed into the 128 x 128 x 8 bit image formats and subsequently displayed on a Macintosh IIXI computer. All image analysis programs were developed by our research group, while the digital image display program (IMAGE v.1.43) was kindly provided by Dr. Wayne Rasband from the NIH.

RESULTS

Before performing the intensive pixel-by-pixel synthetic image calculations, the mean intensities of several ROI of the tissues as well as control solutions (CuSO$_4$-doped water, 30% glycerol, and 1% agarose doped with 1 mM CuSO$_4$) were determined from the raw MDME and PGSE MR images. The ROI of the tissues were the combined cerebrum and cerebellum of the rabbit brain, the cortex of the kidney, the central region of the thigh muscle, and the body region of the tail. The mean intensity values were then used to calculate the values of $N(H)$, $T_1$, and $T_2$ based on Equation A and various self-diffusion parameters of water based on Equations B–E.

Table 1 shows the calculated values of $N(H)$, $T_1$, $T_2$, and $D_E$ for the ROI of control solutions. The calculated $T_1$ and $T_2$ parameters for the CuSO$_4$-doped water and the agarose solution conform with the published values for a 60-MHz spectrometer (15, 16). Also, the calculated values of $D_E$ for the CuSO$_4$-doped water and 30% glycerol are close to the published values for a 2.35-T imaging/spectroscopy system (17) and a 2.3-T spectrometer (18), respectively.

Table 2 summarizes the heat responses of the values of $N(H)$, $T_1$, $T_2$, and $D_E$ for the ROIs of various tissues due to the heat treatment (45°C for 30 min). The values of $N(H)$ and $T_1$ for all tissues, except $N(H)$ of muscle, were found to remain essentially unchanged after the heat treatment. For the brain tissue, $T_2$ decreased by ~6% and $D_E$ increased by 120% after the heat treatment. For the kidney tissue, $T_2$ remained unchanged and $D_E$ increased by ~23% after the heat treatment. For the muscle tissue, $T_2$ increased by ~30% and $D_E$ increased by ~60% after the heat treatment. For the embryonic tissue, $T_2$ remained unchanged and $D_E$ increased by ~40% after the heat treatment. The above observations suggested that the values of the proton relaxation time parameters increase, decrease, or remain unchanged after the heat treatment, depending on the type of tissue. Yet a consistent and significant increase in the value of $D_E$ was found for all of the tissues after the heat treatment. The percentage increase of $D_E$ for the tissues can be arranged according to the following order: brain > muscle > embryo > kidney.

Fig. 2 shows the log of the signal attenuation ($I/I_0$) of the ROI for the control solutions and embryonic tissues as a function of the $b$ factor (see Equation C). Straight lines were used to fit the data using the Einstein model (Equation B). As seen in Fig. 2A, a linear relationship between the log of $I/I_0$ and $b$ was observed for the solutions; and the value of the slope for the 30% glycerol solution was found to be smaller than that for the water. As shown in Equation B, the slope is equal to the diffusion coefficient $D_E$ of water in the imaging object. Therefore, our result demonstrated that the measured value of $D_E$ decreases as the viscosity of the solution increases. On the other hand, a good linear behavior was not found for the data of the embryonic tissues either before or after the hyperthermic treatment as shown in Fig. 2B. The value of the slope for the tissues after the hyperthermia was found to be significantly higher than that before the hyperthermia. Therefore, the $D_E$ values of the tissues increased significantly as a result of the heat treatment. The data from all of the rabbit tissues we studied did not exhibit a linear behavior as well (results not shown). This observed

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Table 1 Calculated spin density, relaxation time, and diffusion parameters of water in control solutions

<table>
<thead>
<tr>
<th>Control solutions</th>
<th>Proton spin density $N(H)$</th>
<th>Longitudinal relaxation time $T_1$ [ms]</th>
<th>Transverse relaxation time $T_2$ [ms]</th>
<th>Diffusion coefficient $D_E$ $[10^{-5} \text{ cm}^2/\text{s}]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mm CuSO$_4$</td>
<td>2106 ± 17</td>
<td>1093 ± 23</td>
<td>1258 ± 4</td>
<td>1.99 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(1823 ± 18)</td>
<td>(1266 ± 58)</td>
<td>(763 ± 30)</td>
<td>(2.10)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2316 ± 40</td>
<td>1081 ± 47</td>
<td>735 ± 30</td>
<td>1.10 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>(1751 ± 34)</td>
<td>(664 ± 37)</td>
<td>(664 ± 34)</td>
<td>(1.22)</td>
</tr>
<tr>
<td>Agarose</td>
<td>2072 ± 50</td>
<td>725 ± 32</td>
<td>93.1 ± 0.7</td>
<td>0.63 ± 0.92</td>
</tr>
</tbody>
</table>

a Diffusion coefficient of water as determined from the Einstein diffusion model.

b Double-distilled water doped with 0.5 mm CuSO$_4$.

c 30% glycerol in water.

d 1% agarose, 1 mm CuSO$_4$ in water.

e 60-MHz spectrometer (15).

f 50-MHz spectrometer (16).

g 2.35-T imaging/spectroscopy system (17).

h 2.3-T spectrometer (18).
Fig. 3 shows the fitting of the PGSE data for the embryonic tissue based on the non-Brownian (Fig. 3A) and barrier (Fig. 3B) models. For the barrier fit, a single curve was enough to fit the data with different diffusion times ($\Delta$) and gradient strengths ($G$). For the non-Brownian fit, multiple straight lines were used to fit the data. Each straight line corresponded to a fixed value of $\Delta$ (see Equation D). Since four different values of $\Delta$ were used for the PGSE measurements, four straight lines were therefore plotted. A significant improvement in the value

nonlinear behavior suggested that the self-diffusive behavior of water in the tissues fails to obey the simple Einstein diffusion model as described by Equation B, and therefore other non-Einstein models are required to properly analyze the PGSE data.

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Fig. 2. Log of MR signal attenuation ($I/I_0$) versus pulsed gradient parameter $b$ factor for control solutions (A) and the body region of intact duck embryonic tissues (B) at 20°C. The PGSE measurements were performed with different diffusion times (C, 39 ms; D, 70 ms; O, 99 ms; □, 132 ms) and different strengths of pulsed gradients (4.47, 7.75, 8.94, and 10.00 mT/m). The solutions are 0.5 mM CuSO$_4$-doped water (G0) and 30% glycerol in water (G30). For the tissues, the data before and after the heat treatment (45°C for 30 min) are shown. For the case of solutions, no detectable changes were found after the same heat treatment. The straight lines were obtained by fitting the data using the Einstein model (Equation B). The $x^2$ values for the solutions G0 and G30 were 0.399 and 0.427, respectively. The $x^2$ values for the embryonic body tissues, before and after the heat treatment, were 0.901 and 0.567, respectively.

Fig. 3. Fitting of the log of MR signal attenuation ($I/I_0$) versus pulsed gradient parameter $b$ factor for the embryonic body tissues at 20°C based on the non-Brownian (Fig. 3A) and barrier (Fig. 3B) models. A single curve was obtained based on the barrier model (Equation E); however, multiple straight lines, each corresponding to a particular value of diffusion time, were formed based on the non-Brownian model (Equation D). The $x^2$ values of the non-Brownian and barrier model fitting were 0.407 and 0.243, respectively. The conditions of PGSE measurements have been described in the caption of Fig. 2.
of the imaging objects are shown in the footnotes. Numbers in parentheses correspond to the physical parameters of tissue water after a heat treatment of 45°C for 30 min. All measurements were performed at 20°C.

Non-Brownian coefficients DN and DR, which provide information pertaining over the Einstein fit (0.901) was clearly observed. Again, similar observations were made for all of the rabbit tissues (results not shown).

As for the structural parameters, β increases while R decreases significantly after the heat treatment. On the basis of the images of the objects due to the heat treatment. Here C and H are synthetic images of the objects before and after the heat treatment, respectively.

In the synthetic N(H), T1 and T2 maps of the duck embryo, the embryonic body tissues, fluid, and yolk were clearly resolved. The yolk was found to have the shortest relaxation times and the fluid the longest (results not shown). No significant changes were found in all regions of the embryo before and after the heat treatment (results not shown), in agreement with the ROI results (Table 2). In the DE map of the duck embryo, as shown in Fig. 4A, the values of DE were significantly lower in the embryonic tissues than in the surrounding embryonic fluid. The egg yolk has the smallest DE in the embryos. After heating, the values of DE in the embryonic tissues increased significantly, in agreement with the ROI calculation (see the percentage change map, i.e., Fig. 4B). In comparison, the values of DE for the yolk, embryonic fluid, and the control 0.5 mM CuSO4 solution remained unchanged after the heat treatment. Similar to the trend of DE, the diffusion coefficient DN calculated from the non-Brownian model increased after the heat treatment. However, the diffusion coefficient DB calculated from the barrier model exhibited no significant change due to the heat treatment. The above trends in DN and DB maps again agreed with the ROI results.

Using the barrier and non-Brownian models, the structural maps R and β were calculated and are shown in Fig. 4 (C and E, respectively). The high values of β and the small values of R in the embryonic fluid region and in the control solution suggested that no structural features exist in the embryonic fluid or isotropic solution. In comparison, the embryonic tissues exhibited significantly lower values of β and higher values of R, in agreement with the ROI calculations. In addition, the structural maps appeared to provide better contrasts than the dynamic maps to separate the body tissues from their surrounding isotropic fluid. After the heating (see the percentage change maps, i.e., Fig. 4D and F), β increased and R decreased significantly, indicating that the fractal nature and the structural hindrance imposed by the tissues on the water diffusion are significantly reduced due to heat damage in the tissues.

### DISCUSSION

Using the quantitative PGSE MR imaging technique, we have demonstrated for the first time that significant irrever-

<table>
<thead>
<tr>
<th>Biological tissues</th>
<th>Non-Brownian model</th>
<th>Barrier model</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Diffusion coefficient DN [10^-5 cm^2/s]</td>
<td>Fractal parameter β</td>
</tr>
<tr>
<td></td>
<td>R [10^-3 cm^2]</td>
<td></td>
</tr>
<tr>
<td>Brain a</td>
<td>0.14 ± 0.04</td>
<td>0.17 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(0.24 ± 0.04)</td>
<td>(0.67 ± 0.06)</td>
</tr>
<tr>
<td>Embryo c</td>
<td>0.42 ± 0.05</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>(0.76 ± 0.13)</td>
<td>(0.86 ± 0.07)</td>
</tr>
<tr>
<td></td>
<td>Diffusion coefficient DB [10^-3 cm^2]</td>
<td>Residual attenuation R</td>
</tr>
<tr>
<td></td>
<td>Barrier size β</td>
<td>Barrier size [µm]</td>
</tr>
<tr>
<td>Embryo</td>
<td>0.65 ± 0.17</td>
<td>0.51 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(0.72 ± 0.11)</td>
<td>(0.26 ± 0.02)</td>
</tr>
<tr>
<td></td>
<td>1.19 ± 0.01</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(1.24 ± 0.04)</td>
<td>(0.09 ± 0.02)</td>
</tr>
</tbody>
</table>

a Combined cerebrum and cerebellum of harvested whole rabbit brain.

b The difference is significant (P < 0.01).

c Body region of intact duck embryos.

![Table 3](attachment://table3.png)
Fig. 4. Synthetic images of $D_e$ (A), $R$ (C), and $\beta$ (E) for an intact duck embryo and one vial of CuSO$_4$-doped water at 20°C. On the intensity scale used, the maximum values for $D_e$, $R$, and $\beta$ are $2.0 \times 10^{-5}$ cm$^2$/s, 0.5 and 1.0, respectively, while the minimum values are $0.1 \times 10^{-5}$ cm$^2$/s, 0.0, and 0.2, respectively. The percentage change of synthetic images, $|H - C|/C \times 100\%$, of $D_e$ (B), $R$ (D), and $\beta$ (F) are also shown for the same objects at 20°C but after an in vivo heat treatment of 45°C for 30 min. Here $C$ and $H$ are the synthetic maps before and after the heat treatment, respectively. The conditions of PGSE measurements are given in Fig. 2.

Possible changes in the self-diffusion parameters of water in the rabbit and duck embryonic tissues occur after hyperthermia. In addition, these changes in the diffusion parameters are much greater than those observed in the conventional $T_1$ and $T_2$ parameters under an identical heat treatment. The use of percentage change maps also allows us to directly quantitate the extent of heat damage in different parts of the imaging objects in vivo.

Synthetic $T_1$ and $T_2$, as well as $T_1$- and $T_2$-weighted MR images, are commonly used to detect pathological disorders in tissues (3, 8). Now the question of whether the lack of significant changes in $T_1$ and $T_2$ after hyperthermia is due to the selection of the MR imaging sequence needs to be addressed. In this study, a MDME MR sequence was used for the $T_1$ and $T_2$ measurements. This is to be compared with the conventional spin echo MR sequence in which the $T_1$ map was generated by...
measuring two images with different $T_E$ but with the same $T_R$, and the $T_2$ map by measuring two images with different $T_E$ but with the same TR. The accuracy and sensitivity of these two-image spin echo measurements depend strongly on the selected values of $T_E$ and $T_R$ (3, 8). For the case of MDME, $T_1$ and $T_2$ are calculated simultaneously from the raw images measured with a wide range of $T_R$ and $T_E$ values that are sensitive to all possible values of $T_1$ (200–2000 ms) and $T_2$ (30–500 ms) found in the biological tissues. The MDME method is therefore more accurate than the conventional two-image spin echo method in measuring the relaxation times in biological tissues (8, 14).

In agreement with the previously reported in vitro PGSE measurements on various biological tissues (19, 20), our results indicated that the simple Einstein model for isotropic solutions obviously fails to provide a good fit to the raw PGSE images for the rabbit and embryonic tissues. In general, we observed that the diffusion coefficients, fractal parameter, and barrier size increase significantly after the heat treatment (see Tables 2 and 3). These observations led us to conclude that the water in the tissues moves faster, the fractal behavior of the tissues becomes less significant, and the structural hindrance imposed on the water motion diminishes as a result of tissue damage at elevated temperatures. In other words, the self-diffusive behavior of tissue water approaches that of free water after hyperthermia.

How do the diffusion parameters of tissue water provide insights into understanding the mechanisms of tissue damage due to heat? Denaturation and the irreversible rearrangement of macromolecules are thought to be the major factors contributing to the functional damage of tissues due to heat. The observed higher diffusion constants of the tissue water suggested a weaker interaction of water with the protein, fibers, and intracellular organelles in the tissues after the heat treatment. This may be due to a change in the hydration properties of the macromolecules as a result of the heat-induced irreversible conformational change (or denaturation) of the macromolecules. The structural parameters, particularly the barrier size, strongly suggested that the tissues provide a less structured and hindered microenvironment for water after the heat treatment. This can be explained by the rearrangement of the intracellular compartments within the tissues as a result of the denaturation of macromolecules. Here the possibility of separating the dynamic and structural features of the self-diffusive behavior of tissue water by using the non-Brownian and barrier models allows us to understand more detailed changes in the physiology of water within the tissues upon hyperthermia than by using the Einstein model alone.

The quantitative MR imaging techniques described in this model study may also be applied to future clinical investigations on the efficacy of hyperthermia in cancer therapy. In the diffusion imaging of human subjects, some technical problems still need to be overcome. The presence of coherent microcirculation, perfusion, and pulsatility of the tissues are the major factors that have to be considered (21–27). Also, our present conclusion that the N(H), $T_1$, and $T_2$ maps of the tissues exhibit less significant changes than do the diffusion maps after the heat treatment still awaits to be verified in future human or live animal studies (28, 29).

Finally, it is important to mention that Le Bihan and his coworkers (21, 22) have proposed that the diffusion coefficient image, i.e., the $D_B$ map in our case, can be used to map the temperature profile of the soft tissues during the hyperthermia treatment. Our results demonstrated that irreversible changes in the diffusion coefficients of the tissues occur due to the heat damage of tissues. However, we also observed that the values of $D_B$ calculated from the raw PGSE images obtained with a short diffusion time (<30 ms) are quite insensitive to heat treatment as compared with those with a long diffusion time (e.g., 150 ms). This is also reflected by the insensitivity of the value of $D_B$ after the heat treatment. As seen in Equation E, the value of $D_B$ is related to the rate of water diffusion at a very short diffusion time. This is probably because the water molecules measured with a short diffusion time cover shorter distances and therefore fail to probe the subtle change in the structural hindrance of the tissues as compared with those measured with a long diffusion time. This dependence of $D_B$ on diffusion time has also been shown by in vitro PGSE measurements in several animal tissues (19) and recently by PGSE imaging measurements in the human brain (23). Therefore, the diffusion coefficient map generated by fast MR imaging methods, e.g., echo-planar (24, 25) and stimulated echo (26, 27), should be a valuable means of generating a thermal map of the imaging object during hyperthermia therapy; and the diffusion maps based on a range of diffusion times, as suggested in this study, will be useful for assessing the irreversible structural damage of tissues after hyperthermia therapy.

ACKNOWLEDGMENTS

We thank Dr. Lloyd K. Mark at the TTUHSC for allowing us to use the clinical MR scanner and facilities in the magnetic resonance imaging center at TTUHSC. The enlightening discussions of Drs. Michal Neeman, Laurel O. Sillerud, and James P. Freyer at the Los Alamos National Laboratory; Richard D. Nathan at TTUHSC; Robert H. Posteraro at the University of Texas at San Antonio; and James R. Lepock at the University of Waterloo are kindly acknowledged. We are also grateful for the research coordination of Lisa C. Lemen at TTUHSC and the valuable technical support from Gary R. McNeal and Richard Vigil at Siemens Medical Systems, Inc.; from Rodney Trotter, Alma J. Wood, and Julie Crane at TTUHSC; and from David N. McGaughey and David F. Coons at the Texas Tech University Academic Computing Services.

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MR IMAGING IN HYPERTHERMIA
Magnetic Resonance Diffusion Imaging Detects Structural Damage in Biological Tissues upon Hyperthermia

Kwan Hon Cheng and Marissa Hernandez


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