Correlation between High Field MR Images and Histopathological Findings of Rat Transplanted Cancer Immediately after Partial Microwave Coagulation

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Purpose: To investigate the immediate effects of microwave coagulation on rat tumors in various magnetic resonance (MR) images at high magnetic field strength using histopathological examinations as reference.

Materials and Methods: Tumors implanted in rat femurs were partially thermocoagulated by microwave. Immediately after, T1- and T2-weighted images, diffusion-weighted images (DWIs), and contrast-enhanced T1-weighted images (CE-T1WIs) were acquired with a 7-tesla MR scanner. After measurements, tumors were examined histopathologically with hematoxylin-eosin (HE) staining and histochemically for acid phosphatase activity.

Results: Without contrast, boundaries of coagulated areas were unclear on MR images, including apparent diffusion coefficient (ADC) maps. CE-T1WIs clearly showed immediate contrast enhancement of untreated areas of tumor, and the area of enhancement gradually enlarged in 5 min. Quantitative analyses were conducted by classifying tumor areas by contrast enhancement results. Signal intensities of the areas in the MR images showed no significant differences, but at the periphery, ADC values were significantly higher in areas with delayed enhancement than those with immediate enhancement. Compared with histopathological findings, with microwave thermocoagulation, increased ADC value seemed to derive from collection of extracellular fluid in the outer zone, where acid phosphatase activity was attenuated.

Conclusion: ADC values in the areas with delayed enhancement of CE-T1WIs were higher than those in non-affected areas, but MR images could not show areas of coagulation within tumors. Clear detection of the boundaries of coagulated areas required contrast enhancement, even at magnetic field strength of 7T.

Keywords: cancer, coagulation therapy, diffusion-weighted imaging, magnetic resonance imaging, microwave

Introduction

New imaging techniques and instruments have enabled minimally invasive therapies of malignancies under image guidance. For liver tumors, various thermoablation therapies using laser,1 radio frequency,2 microwave,3 and focused ultrasound4 have had promising results, comparable to those of surgical resection, especially in small tumors. Ultrasonography has been most commonly and conveniently used for image guidance because of its simplicity, portability, and safety, and microbubble-based contrast agents have been used effectively for diagnosis and ablation therapies.5,6 However, these agents cannot be applied with bone and air spaces, and it is difficult to evaluate their immediate therapeutic effects. Magnetic resonance (MR) imaging has many advantages for image guidance in surgery because it provides good soft tissue contrast, requires no ionizing irradiation, and permits flexible control of image plane, although access to patients and MR-compatible surgical instruments are limited.

In Japan, a microwave coagulator operating at 2.45 GHz has been used for hemostasis and tissue
coagulation in liver surgery for more than a quarter of a century. It has been utilized as well in image-guided minimally invasive therapy of liver tumors. The 2.45-GHz frequency causes little noise in MR images, even during microwave irradiation. Use of the proton resonance frequency method allows temperature monitoring. The combined use of microwave ablation and MR image guidance is quite feasible.

Therefore, we have combined microwave ablation and MR image guidance using a 0.5-tesla, vertically oriented, open-configuration MR scanning system and already performed MR-guided microwave coagulation therapy in over 200 patients with primary or metastatic liver tumors. MR temperature monitoring is useful in assessing therapeutic effects, but it is time consuming and difficult to apply to all sessions of repeated punctures and ablations during one procedure. In addition, ordinary T1-weighted images (T1WIs) and T2-weighted images (T2WIs) are not sufficiently sensitive to differentiate coagulated from untreated areas within tumor during the procedure with this system at 0.5T. For final treatment evaluation, contrast-enhanced T1-weighted images (CE-T1WIs) have been acquired at the end of the procedure in most cases. With CE-T1WIs, the region coagulated by microwave ablation and untreated residual tumor tissue can be precisely distinguished by their regional blood perfusion. However, the use of contrast media, such as gadolinium compounds, is problematic. CE-T1WI cannot be performed repeatedly while the contrast medium remains in the patient, dosage is limited, and adverse effects, such as nephrogenic systemic fibrosis, have been reported with gadolinium-based contrast agents. Thus, a substitute diagnostic method is desirable.

Recently, diffusion-weighted MR imaging (DWI) has been applied for detecting malignancies throughout the body. DWI detects the microdiffusion of water in intra- and extracellular environments. Signal intensities in DWI are relatively high in acute ischemic regions with cytotoxic edema or malignant tumors with high cellularity, where diffusion of water molecules decreases. DWIs may differentiate the coagulated and untreated regions, even immediately after treatment. Otherwise, ordinary T1WIs or T2WIs at high magnetic fields may be sensitive enough to differentiate them. In this study, we investigated the immediate effects of microwave coagulation on rat tumors with various MR images at 7T by comparison with histopathological examination.

Materials and Methods

Animal preparation

The Laboratory Animal Care and Use Committee at Shiga University of Medical Science reviewed and approved all animal examinations. We used 15 male nude rats (Fisher 344/N, rnu/rnu, CLEA Japan Inc., Tokyo, Japan), each weighing approximately 400 g. We performed all experimental procedures of tumor transplantation, microwave coagulation, and MR image acquisitions with animals under general anesthesia with 2% isoflurane carried by 50% O2 through a face mask. We injected cultured cancer cells (1 × 10⁶) of HCT-116 (American Type Culture Collection, Manassas, VA, USA), a cell line of human colon cancer, into the subcutaneous tissue on the femurs of the rats. Tumors grew to approximately 10 mm in diameter during 14 days. Using a Microtaze™ microwave tissue coagulator (Alfresa, Osaka, Japan) operated at a frequency of 2.45 GHz as a heating device, we inserted a needle-type electrode (0.3-mm diameter, 2.5-mm length) prepared specifically for tumors of this size into the tumors and performed microwave coagulation at 25 W output for 30 s.

MR imaging

Using a 7.0T UNITY INOVA™ System and quadrature volume coil 6 cm in diameter (Varian Inc., Palo Alto, CA, USA), we acquired MR images of the transplanted tumors immediately after microwave coagulation. Rats were fixed in a supine position on an acrylic cradle, and a water sample was placed on the abdomen to standardize the signal intensities. The tail vein was cannulated with a plastic needle. First, multi-slice T2WIs and DWIs were collected with a spin echo image sequence (2000/40; repetition time [TR]/echo time [TE] in ms) in the axial planes with 1-mm slice thickness, 0-mm gap, 60 × 60 mm² field of view (FOV), 256 × 128 matrices, and 4 acquisitions. Motion-probing gradients of 0, 500, and 1000 s/mm² b values (strength 0.0, 51.4, and 72.7 mT/m, duration 13 ms, interval 20 ms) were sequentially applied along the slice direction. Apparent diffusion coefficient (ADC) values were calculated by linear regression analysis of the natural log of the signal intensities versus the b values from T2WI (b = 0) and DWIs (b = 500, 1000) in the corresponding image planes. T2WIs were then acquired with spin echo sequence (500/15 TR/TE) in the same axial planes as with T2WIs and DWIs with one acquisition. In addition, without moving the animal, contrast enhancement was performed by injecting a bolus of 0.2 mmol/kg body weight gadodiamide hydrate (Omniscan,
Daiichi-Sankyo Inc. Tokyo, Japan) through the tail vein. Acquisition time for one T1WI dataset was 64 s. In preliminary experiments with continuous acquisition of T1WIs, the enhanced area gradually increased until 5 min after the injection, but scarcely increased thereafter up to 20 min. Therefore, we acquired early-phase CE-T1WIs at one minute after injection and late-phase CE-T1WIs at 5 min post injection, using the same acquisition parameters as for T1WIs. All MR image acquisitions, including animal preparation, were completed within 2 hours after microwave coagulation. The enhanced areas in the 2 phases were traced with reference to a histogram of signal intensities in the tumor area. We classified 3 areas, A, B, and C, according to traces obtained from early- and late-phase CE-T1WIs. Area A was enhanced in neither phase; Area B was enhanced in late but not early phase; and Area C was enhanced in both phases. We quantitatively assessed signal changes in the 3 areas of the tumor on the T1WIs, T2WIs, and DWIs. We conducted statistical analyses of signal intensities and ADC values in the 3 areas using one-way analysis of variance (ANOVA). When we detected significant difference, we performed multiple comparisons among the individual areas with Tukey test. \( P < 0.05 \) was considered significant.

**Histopathological examinations**

After CE-T1WI acquisition, we euthanized the rats with intravenously injected overdose of sodium pentobarbital, excised treated tumor, and used a cryostat (Reichert-Jung Cryocut 1800, Heidelberg, Germany) to prepare serial 3-μm frozen sections corresponding to the MR image slices. We stained one section with hematoxylin and eosin (HE) and another for enzyme histochemistry of acid phosphatase (AcP) activities. The enzyme histochemistry was performed by slightly modifying a previously reported method.\(^{19,20}\) In brief, we used a diazonium salt coupling method with a naphthol AS (3-hydroxy-2-naphthanilide) series of phosphate esters. We incubated cryostat sections in a solution of 200 mL of 0.2 M acetate buffer (pH = 5.2) containing 48 mg of naphthol AS-ABI phosphate (7-bromo-3-hydroxy-2-naphthol-0-anisidine), 1000μL of N,N-dimethyl-formamide, 120 mg of Fast Red TR salt (Sigma-Aldrich, St. Louis, MO, USA), and 16 mg of MgCl₂ for 30 min at room temperature. We counterstained the sections with hematoxylin and mounted with Aquatex (Merck KGaA, Darmstadt, Germany) mounting agent.

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**Fig. 1.** Various magnetic resonance (MR) images of a rat-transplanted tumor immediately after partial microwave coagulation. (a) T₂-weighted image; diffusion-weighted images with (b) 500- and (c) 1000-s/mm² b values; (d) apparent diffusion coefficient map; and T₁-weighted images (e) before and (f) 1 and (g) 5 min after contrast enhancement. Areas A (non-enhancement), B (delayed enhancement), and C (immediate enhancement) are indicated in (f) and (g).
Results

MR image assessments

After microwave coagulation, we observed no apparent difference in signal intensity within the tumor in T₁WI, DWIs, ADC map, or T₂WI (Fig. 1a–e). On the other hand, CE-T₁WIs permitted clear differentiation between contrasted and non-contrast areas. The enhanced area was clearly larger in late- than early-phase images (Fig. 1f,g). Figure 2 shows standardized signal intensities of tumor in the 3 areas before and at 1 and 5 min after contrast enhancement. As a matter of course, the signal intensities at 1 min were highest in Area C and at 5 min were lowest in Area A. No significant differences in signal intensity were observed among the 3 areas in T₁WIs before contrast enhancement or in T₂WIs, or DWIs (Fig. 3). ADC values were significantly higher in Area B (range, 0.341–1.883; standard deviation [SD], 0.341 × 10⁻³ mm²/s) than in Area C (range, 0.281–0.887; SD, 0.149 × 10⁻³ mm²/s) (Table). There was no significant difference between Area A (range, 0.480–0.134; SD, 0.256 × 10⁻³ mm²/s) and either B or C.

Histopathological findings

In pre-fixed specimens, the coagulated region appeared white (Fig. 4a, arrow), but the boundary between the treated and untreated regions was not clear. Neither was the boundary clear in HE-stained sections by macroscopic observation (Fig. 4b). A coarse region (arrows in Fig. 4b) surrounded the heat center. Microscopy showed the coagulated area divided into 2 types of tissues (Fig. 5a). One zone near the electrode (I: inner zone) showed thermo-fixation and disrupted erythrocytes. Tumor cell structures were generally preserved, and nuclei were well stained with HE, but in 5 cases, tumor cell structures were destroyed. The adjacent zone (O: outer zone) showed extracellular edema, disrupted erythrocytes in blood vessels, and extravasation. The residual unaffected tumor tissue (N: non-irradiated zone) contained uninjured tumor cells.

Table. Apparent diffusion coefficient (ADC) values in 3 areas of partially treated tumors

<table>
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<tr>
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<th>Area A</th>
<th>Area B</th>
<th>Area C</th>
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<tr>
<td>ADC value</td>
<td>0.774 ± 0.066</td>
<td>0.849 ± 0.088</td>
<td>0.604 ± 0.038</td>
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<tr>
<td>(× 10⁻³ mm²/s)</td>
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The ADC values are the mean ± standard error of the mean (SEM) for 15 animals. Classification of the areas is described in Materials and Methods.

* Significant difference between Areas B and C.
Fig. 4. Macroscopic findings of excised tumors after microwave coagulation. (a) A cross section before formalin fixation. The coagulated region is shown as a white area (arrow). (b) A cross section of the same tumor stained with hematoxylin-eosin (HE). In this specimen, the boundary between the treated and untreated regions is unclear. There is a surrounding coarse region (arrows) around the heat center.

Fig. 5. Hematoxylin-eosin (HE)-stained specimens (×100) of the central region of coagulation (a) and of the periphery of the tumor (b). Thermo-fixation in the inner zone (I) and extracellular edema in the outer zone (O) are shown in a No thermal injury is shown in the non-irradiated Zone (N) in b. Erythrocytes are disrupted in a (arrow) but preserved in b (arrow).

Fig. 6. Histochemical examination with acid phosphatase (AcP) activity of the same cross section of the tumor. The inner zone (I) showed complete loss of AcP activity; the outer zone (O) showed attenuated AcP activity; and the non-irradiated zone (N) showed normal AcP activity. and uninjured erythrocytes in blood vessels (Fig. 5b).

With AcP staining (Fig. 6), the inner, outer, and non-irradiated zones were classified more clearly than with HE staining. AcP activity was absent in I, attenuated in O, and normal in N. In 7 cases, however, preparation of frozen sections was difficult and observation of AcP activity was not successful.

Discussion

We expected that we could use MR images at 7T
to differentiate coagulated and non-coagulated regions within a tumor, but boundaries between the 2 areas were not clear on T1WI, T2WI, or DWI. Only in CE-T1WIs could the coagulated region be clearly distinguished based on tissue perfusion. Therefore, it seems reasonable at 0.5T to evaluate the therapeutic effect of microwave coagulation with CE-T1WIs at the end of the procedure. In many studies for evaluating thermal ablation at the acute phase, MR images are acquired several days after treatment. In our case, however, MR images were acquired within 2 hours after treatment, so tissue responses to ablation may be slight and MR findings may differ from those in other studies. The groups of Lee and Onishi also reported MR images of the liver in animal models from 2 hours after radiofrequency (RF) ablation. They classified 3 zones in coagulated liver tissue: Zone 1, tissue loss (needle tract); Zone 2, swollen hepatocytes; and Zone 3, sinusoidal congestion. Their Zone 3, in which slight contrast enhancement is observed and NADH activity is partially positive, seems to correspond to our Area B (outer zone). They report strong signal intensity in Zone 2 with T1WIs, and we observed similarly intense signals in coagulated normal liver tissue along the puncture route during the procedure with the 0.5T system. Such findings are not clear in clinical cases with liver cirrhosis or in transplanted tumors, as in this study. Changes in signal intensity immediately after coagulation seem to depend on tissue type.

We used CE-T1WI findings as the gold standard for effect of microwave coagulation. The extension of the enhanced area from the early to the late phase seemed to be caused not by perfusion, but by diffusion of contrast media to the areas with impaired blood supply. Such “delayed enhancement” originates from the diffusion of contrast media through the extracellular space. With the quantitative assessment of signal intensities in MR images, T1WIs and T2WIs could not be used to identify the coagulated region, even with a 7T MR scanner. Therefore, microwave coagulation is not as effective on relaxation times of T1 and T2 in tissue water. A significant difference among the 3 areas was found only with ADC values between Areas B and C. Water diffusion might be affected by injury of the cell membrane caused by microwave coagulation. In this study, ADC values were obtained from DWIs with 500- and 1000-s/mm² b values. Some studies of white matter of the brain have applied b values greater than 2000, but high b values in body diffusion for malignancies usually range from 800 to 1000. Actually, we tried b values higher than 1000 in the preliminary experiments but did not obtain sufficient signal-to-noise ratio for analysis. Therefore, we used these experimental conditions.

In previous reports of histopathological changes in liver tissues after microwave coagulation therapy, the region of fixation was classified as the inner zone, the region of extracellular edema as the outer zone, and the non-irradiated region as the non-irradiated zone. In this study, 5 cases with HE stain showed no fixation, but destruction of tumor cells. We excluded tumors with apparent central necrosis from our study. In some cases, however, the central regions of tumors may be partially damaged before treatment by tumor implantation in subcutaneous tissue, which may also account for difficulties in preparation of frozen sections for histochemistry. Although the approximate sizes of the 3 areas determined with MR images and histopathological findings are compatible, the precise identification of the areas, classified with 2 independent methods, is difficult. Among them, we are interested in the histopathological findings of the outer zone, where the disruption of erythrocytes in and out of blood vessels, which impairs blood supply, is observed. Vessel occlusion is a determinant of tumor cell destruction. The extracellular edema that occurs as an inflammatory reaction to thermal injury is thought to increase ADC value and contribute to the secondary distribution of contrast media with diffusion into the non-perfused tissue. Area B determined with MR images could correspond with the outer zone determined by histopathology. Extracellular edema will also increase T2 value. The signal intensities of T1WIs were slightly higher in Area B than C, but not significantly different. Increased ADC value in this area would decrease signal intensity in DWIs. As a result, a slight difference seems to be detected only with ADC values, not with the signal intensity of DWIs. In the inner zone, the apparent cell structure is maintained similarly to “fixation.” The ADC values in Area A seem consistent with MR images, being lower than those in Area B. The poor enhancement in CE-T1WIs at all phases might result from “fixation.” Finally, Area C, which showed immediate contrast enhancement, is considered the non-irradiated zone in histopathology. Thus, Areas A, B, and C determined with MR images appear identical to the inner, outer, and non-irradiated zones determined by histopathology.

Mukaisho and colleagues have reported that regions with decreased AcP activity will cause coagulation necrosis and be totally replaced by fibrous scarring within 3 months. A significant difference in ADC values was detected between Area B...
and C, and the detection of the boundary between these two is important for evaluating the treated area. Unfortunately, the boundary was not clearly visualized in ADC maps, because the variance of ADC values was too big. The calculation of ADC values, however, might enable differentiation of the effects on the tumor tissue immediately after microwave coagulation therapy. In this study, all MR observations were completed within 2 hours to evaluate the immediate effects of microwave ablation. Edematous changes in Area B might progress afterwards, and the difference in ADC values and other findings of MR images might be more clearly shown in the subacute phase.

Conclusion

At the periphery of tumors, the significantly higher ADC values in areas with delayed rather than immediate contrast enhancement were attributable to extracellular edema from thermoablation. However, immediately after microwave coagulation, the boundaries of these areas were not clear in various MR images other than CE-T1WIs, even with a 7T MR scanner.

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