TECHNICAL NOTE

¹³C MR Imaging of Methionine-rich Gliomas at 4.7T: A Pilot Study

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We explored the feasibility of using carbon-13 (¹³C) magnetic resonance imaging (¹³C-MRI) to depict ¹³C-labeled methionine-enriched gliomas at 4.7 tesla. We transplanted 2 types of glioma cells separately to 2 subcutaneous tissue sites on the backs of mice weighing 15 to 20 g. After confirming tumor growth, we used ¹³C-MRI and ¹H-MRI to scan 4 mice that had been administered ¹³C-labeled methionine and 2 control mice. ¹³C-MRI of all 4 transplanted mice administered with ¹³C-labeled methionine revealed 2 areas of hyperintensity that corresponded to the tumor sites on ¹H-MR images, but no such areas were visualized in transplanted controls. Our data suggest that ¹³C-MRI can show the accumulation of ¹³C-labeled tracer by gliomas.

Keywords: *¹³C-MRI, glioma, high-field MRI*

Introduction

Carbon-13 (¹³C) magnetic resonance imaging (¹³C-MRI) is expected to facilitate molecular imaging study of biologically important materials. However, the clinical use of ¹³C-MRI has been limited by the low natural abundance of ¹³C (1.1%) and its low magnetogyric ratio (the γ of ¹³C is 25% that of protons [¹H]).

Approaches to improve the sensitivity of ¹³C-MRI have involved proton decoupling and the use of ¹³C-labeled metabolic substrates.¹ The major safety limitation in human ¹H decoupled ¹³C-MRI is the specific absorption rate (SAR) of tissue exposed to ¹H frequency irradiation.² Replacing the ¹²C (98.9% natural abundance) with the ¹³C isotope at a specific carbon in a metabolic substrate does not affect its biochemistry. In cells, animals, and humans, the uptake of ¹³C-labeled substrate and the incorporation of ¹³C into metabolites can be monitored without interference from background signals.

Methionine positron emission tomography (Met-PET) has gained wide acceptance for its high sensitivity in evaluating malignancy grade, tumor ex-

tent, tumor progression, and differential diagnosis of gliomas.^{3–6} After delivery of ¹³C-labeled methionine to living organisms harboring tumors, ¹³C-MRI may depict uptake of methionine by tumor metabolites without interference from background signals, but such depiction remains to be investigated. We explored the feasibility of using ¹³C-MRI to depict gliomas enriched with ¹³C-labeled methionine at 4.7 tesla.

Material and Methods

MR unit

All experiments were performed using a 4.7T UNITY INOVA MR spectrometer (Varian Associates Inc., Palo Alto, CA, USA). The magnet system was a JASTEC Horizontal NMR magnet (Japan Superconductor Technology Inc., Japan) with a 33-cm horizontal bore. The unit's ¹³C frequency was 50.4 MHz, and ¹H frequency, 200.6 MHz. The probe was a 3-turn circular surface coil of one-inch diameter that could be tuned to either ¹³C or ¹H frequency. The coil system was mounted on a plastic cradle that contained a mouse placed in the lateral decubitus position (Fig. 1).

Animal preparation

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Development approved the animal study protocol. We raised 6 male mice with severe combined immune deficiency (SCID) to 6 weeks of age (weight 15 to 20 g) and transplanted each mouse with 2 types of glioma cells, U373 and U87MG, at 2 different subcutaneous tissue sites on the back (Fig. 2). In *in vitro* culture, U87MG cells divided faster than U373 cells. After we confirmed growth of both subcutaneous tumors to greater than 5 mm in maximum diameter by palpation and inspection, we divided the mice into 3 groups of two each. We in-

Fig. 1. Magnetic resonance coil and animal placement. A coil system (arrow) is mounted on a plastic cradle in which a mouse has been placed in lateral decubitus position.

jected the first (control) group intraperitoneally (i.p.) with 1 mL of saline and the second group with 10 mg of methyl- ^{13}C labeled methionine (Cambridge Isotope Laboratories Inc., USA) dissolved in 1 mL saline; the third group received the same dose of ^{13}C -labeled methionine i.p. once a day for 6 consecutive days. All mice were sacrificed by cervical dislocation one hr after the last injection and immediately cryopreserved at -80°C until MR imaging study.

^{13}C -MRI acquisition

We defrosted the mice to room temperature. We used a spin echo sequence with $300\ \mu\text{s}$ Gaussian pulses, diagrammed in Fig. 3; slice selection gradient magnetic field, 0.072 Gauss/cm for 90° pulse and 0.036 Gauss/cm for 180° pulse; field of view (FOV), 10×10 cm; 64 phase-encoding steps (0.078 Gauss/cm each step); 256 read-out gradient magnetic field (4.089 Gauss/cm, 3 ms); echo time (TE),

Fig. 3. Diagram of the spin echo sequence

Fig. 2. The third group of mice, treated 6 times with ^{13}C -labeled methionine. (a) Two types of glioma cells, U87MG (tumor 1) and U373 (tumor 2), were separately transplanted to 2 subcutaneous tissue sites (circles) on the back of each mouse. (b) ^1H -MR image shows 2 areas of marked hyperintensity (arrows) in the area of tumor transplantation. (c) ^{13}C -MR image reveals 2 hyperintense areas (arrows) that appear to coincide with the tumor sites. The more cranial (U373) of the 2 implants showed lower signal, possibly as a result of cell activity of the tumors.

7.8 ms; repetition time (TR), 200 ms; and 128 acquisitions. Acquisition time was approximately 27 min. We did not use a proton decoupling pulse in this sequence.

¹H-MRI acquisition

Without moving the mice, we scanned them in a plastic cradle using a spin-echo sequence with 1600 μ s Gaussian pulses and the same pulse sequence as for ¹³C-MRI. Parameters were: slice selection gradient magnetic field, 0.906 Gauss/cm with 90° pulse and 0.453 Gauss/cm with 180° pulse; FOV, 10 × 10 cm; 64 phase-encoding steps (0.019 Gauss/cm each step); 256 read-out gradient magnetic field (0.807 Gauss/cm, 4 ms); TE, 11 ms; TR, 1000 ms; and one acquisition. Acquisition time was approximately one minute.

Evaluations

By consensus, one radiologist and one toxicologist evaluated the safety of the administered ¹³C-labeled material based on the behavior and lifespan or death of the injected mice.

By consensus, 2 radiologists evaluated the location of the 2 transplanted tumors on ¹H-MR images and the presence and number of hyperintense areas in the region of the transplanted tumors on ¹³C-MR images.

Results

None of the 4 tumor-bearing mice with i.p. infusion of ¹³C-labeled methionine manifested acute intoxication or died.

¹H-MR images showed 2 markedly hyperintense areas in the region of tumor transplantation in all 6 mice (Fig. 2). ¹³C-MR images of the 2 control mice demonstrated a weak signal presumed to correspond to subcutaneous fat seen on ¹H-MRI. ¹³C-MRI images of each of the 4 mice in the second and third groups revealed 2 hyperintense areas that corresponded to the tumors on ¹H-MRI and much lower signal for the more cranial of the 2 implants (Fig. 2). Visual assessment indicated slightly higher signal intensity for the third group than the second.

Discussion

Although subcutaneous fat was depicted as a weak signal on ¹³C-MRI in control mice, we could identify 2 definite areas of hyperintensity in the regions of glioma cell transplantation in all mice injected with ¹³C-labeled methionine. Thus, methyl-¹³C labeled methionine accumulated in the glioma cells was visualized on ¹³C-MRI. We attribute the

good visualization to stronger signal from methyl-¹³C-labeled methionine accumulated in glioma cells than from subcutaneous fat; probable absence of chemical shift of ¹³C in methyl-¹³C-labeled methionine because the methyl base including ¹³C does not take part in the peptide bond in the protein synthesis after uptake into tumor cells; and high signal-to-noise ratio of ¹³C-labeled methionine that facilitated signal detection on the 4.7T scanner.

The more cranial of the 2 implants consistently showed much lower signal. In *in vitro* culture, cell division of the more cranial tumor (U373) was slower than that of the more caudal tumor (U87MG). Cell activity of the tumors may have affected tracer uptake in our study.

The appropriate dose of ¹³C-labeled compounds for visualizing their metabolism on ¹³C-MRI remains to be determined. We expected more intense signals in our group receiving 6 i.p. injections of ¹³C-labeled methionine than the group with single injection (10 mg), and results were near the expectation, with a slight signal difference between the 2 groups. There may have been an accumulation effect of methionine on the gliomas, with administration route, i.e. intraperitoneal, intravenous, or peroral delivery affecting the dose of ¹³C-labeled compounds accumulating in tumors. Studies that deliver ¹³C-labeled compounds via different routes are necessary to address this issue. Use of a higher magnetic field, such as 7T, would facilitate signal detection and might reduce the administrative dose of ¹³C-labeled compounds.

Unlike positron emission tomography (PET), ¹³C-MRI does not raise concerns with respect to ionizing radiation, radioactive isotopes, or short tracer half-life. Manufacture of a sophisticated head coil for human ¹³C-MRI⁷ lends promise for diagnostic use of ¹³C MRI using ¹³C-labeled substrates in some clinical settings, such as for glioma grading, diagnosis of malignant progression in glioma, and differential diagnosis of brain tumors. Further studies are required to clarify the clinical usefulness of this technique. In our study, we experienced no instance of acute intoxication or death after ¹³C-labeled methionine administration, and various ¹³C-labeled substrates have been applied to humans with no side effects reported.⁸⁻¹⁰ Though initial costs of synthesizing ¹³C-labeled metabolic substrates are high, their increased production will lower their cost, as was the case for D-glucose-¹³C₆.

Our study has some limitations. First, the animals were imaged *ex vivo*. We sacrificed, cryopreserved, and defrosted all mice before MR imaging study, a process that may affect biological condition but that preserves ¹³C-labeled methionine ac-

cumulated in the glioma cells. Therefore, we presumed that increased signal in the mice reflected accumulation of the ^{13}C -labeled substance in the glioma cells. Although questions remain about visualization *in vivo*, this pilot feasibility study proved that ^{13}C -MR imaging can depict ^{13}C -labeled methionine-enriched gliomas at 4.7T. Further ^{13}C -MR imaging studies are needed to clarify visualization *in vivo*. Second, we performed only qualitative image analysis, and a quantitative approach that would measure cellular density or other biological parameters would be more useful. Third, we directly compared the images on ^{13}C -MRI and ^1H -MRI, and more accurate integration of functional and anatomic information might be expected from image fusion of ^{13}C - and proton signals.

Conclusion

Our pilot study suggests that ^{13}C -MRI using an MR unit with high magnetic field can demonstrate the accumulation of ^{13}C -labeled substance by gliomas.

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