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Hyperpolarized ^{129}Xe spectra from C6 glioma cells implanted in rat brains

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***Abstract*— Tumor cell density is dramatically different from normal tissue. Since the chemical shift of hyperpolarized ^{129}Xe reflects local cell structure, we hypothesized that the presence of tumor cells could potentially be determined from ^{129}Xe spectra. Spectra and washout decay rate from three rats implanted with C6 glioma cells were compared with eight control rats. No significant differences between normal and tumor spectra were observed. The decay time of the C6 rats (mean 13.5 ± 1.9 s) was not significantly different from normal rats (mean 11.7 ± 1.8 s). These results suggest that hyperpolarized Xe may not be a superior tracer for detection of tumor cells in the intact brain**

I. Introduction

Hyperpolarized gas has been polarized so that it carries a magnetization that can be observed with NMR techniques [1,2]. The NMR signal is proportional to the magnitude of nuclear polarization and the density of the polarized nuclei. With proton NMR, the nuclear polarization is actually quite small, but a good signal can be observed because the number of tissue protons is very large. In the case of a tracer study, the number of tracer nuclei in the tissue is small. Hence, with normal polarization levels, the signal-to-noise is also very small. However, if the polarization of the nucleus is increased by several orders of magnitude (hyperpolarized), it becomes possible to detect a signal from a small number of tracer nuclei. The hyperpolarization process allows Xe to be used as a tracer for brain studies.

Research with hyperpolarized gases has been conducted over the past 60 years. Kastler first proposed the concept of alkali metal optical pumping in 1950 [2], and the first biological application of hyperpolarized gas was reported in rat lungs by Albert et al. in 1994 [3]. Over the past 20 years, techniques have improved to the point where it is possible to obtain excellent MR images of human lungs and other gas spaces using hyperpolarized He and Xe [4,5].

A chemical shift of hyperpolarized ^{129}Xe is thought to reflect local structure [6,7]. In porous silica-based materials, the correlation between Xe chemical shifts and pore size has been demonstrated [6]. In the rat brain *in vivo*, we have confirmed the presence of a Xe chemical shift in MR spectra [8,9]. Peak A (appearing at around 195 ppm in the rat head) was attributed to Xe dissolved in brain tissue, whereas peak B (around 189 ppm) was due to Xe in non-brain tissue [8,9]. Since the cellular density of tumors is dramatically different from normal tissue, we postulated that there would be a change in the dissolved peak of Xe in brain tissue that included tumor cells. Thus, the present pilot study was performed to evaluate differences in ^{129}Xe spectra between normal rat brains and rat brains implanted with C6 glioma cells. We also investigated the washout decay of Xe from both normal rats and tumor-implanted rats.

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II. Methods

A. Animal Preparation

All experimental procedures were performed in strict accordance with the guidelines of the Physiological Society of Japan and were approved by the Animal Care and Use Committee of the Akita Research Institute for Brain and Blood Vessels. Around 50,000 C6 glioma cells were injected into the caudate nucleus of male Wistar rats (n=3) under pentobarbital anesthesia. The glioma cells reached about 20% of the entire brain in two weeks. Eight male rats were used as controls. Before a Xe inhalation study was performed, an endotracheal tube was inserted into the trachea followed by a thinner tube, which was used to deliver hyperpolarized ^{129}Xe to the lungs of the animal from outside of the magnetic coil [10].

B. Measurement Procedure

The animals were placed in the bore of a 4.7 tesla Varian Inova MR spectrometer with a 3 cm ^{1}H and ^{129}Xe dual-tuned surface coil placed over the head. Then, T_1- and T_2-weighted ^{1}H images were acquired by spin-echo sequence for identification of C6 glioma cells in the brain. MRI images were obtained with a repetition time (TR) of 1.5 seconds and echo times (TE) of 18 ms for T_1-weighted images and 80 ms for T_2-weighted images. T_2-calculated images were obtained on a pixel-by-pixel basis with the use of linear least-squares regression from the two different TE images. The field of view and matrix size were 2.5×2.5 cm^2, and 128×128, respectively. Hyperpolarized, enriched ^{129}Xe gas (^{129}Xe 80% + N_2 20%) was produced in a commercially available polarizer (Toyoko-Kagaku Co. Ltd., Japan, Fig. 1). Spectra were acquired using a single hard-pulse sequence with a pulse width of 24 μsec and a bandwidth of 30 kHz. The RF pulse

(A) (B)

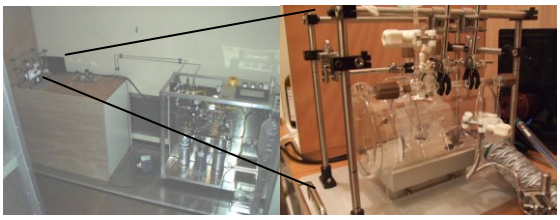


Figure 1. Commercially available polarizer (HPXE2104H: Toyoko-Kagaku Co. Ltd.)

was centered at approximately 150 ppm from the gas peak. For estimating the decay time, a strict two-pulse measurement protocol was applied [10]. A single spectrum acquired 4 s after the cessation of Xe delivery was followed by additional acquisitions at 8, 12 and 16 s. After the experiments were completed, the animals were euthanized and their brains were fixed by transcardial perfusion with paraformaldehyde for conventional histological study. Hematoxylin and eosin (H&E) staining was applied to cross sections of the entire brain for identification of tumor cells.

C. Analytical Procedure for decay estimation

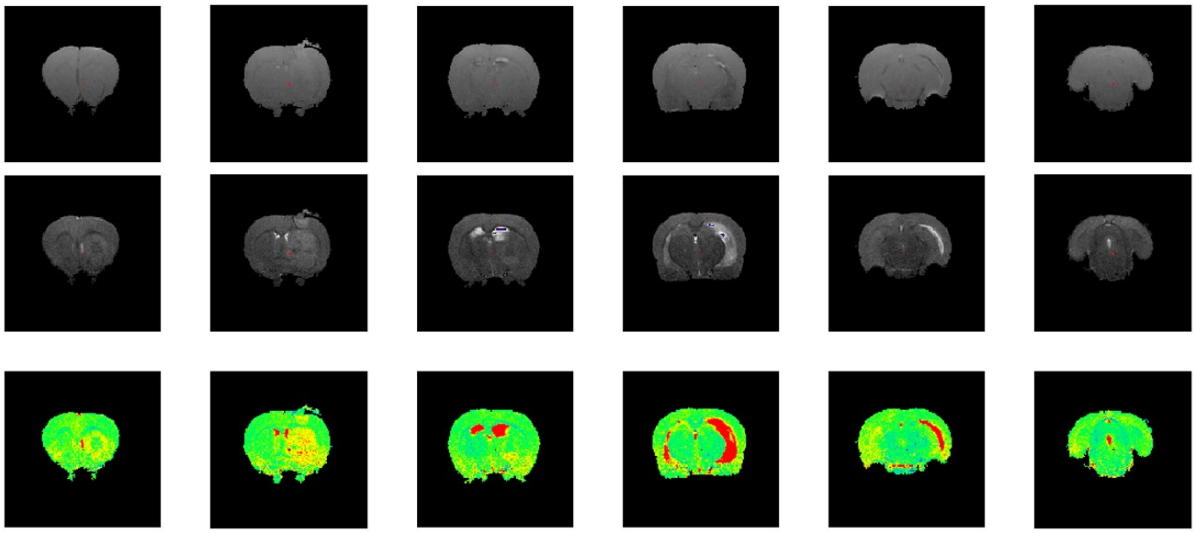
A surface coil was positioned over the rat head to maximize the sensitivity of the ^{129}Xe MR signals from the rat brain. Since this coil was also used as the pulse transmitter, it is possible that a systematic error might be introduced because of the inhomogeneous RF power distribution of the coil. The usual signal equation can be modified to avoid this problem; however, the modification means that the tissue relaxation time cannot be measured in the traditional way by taking repeated measurements as the signal relaxes. Alternatively, a two-pulse MR acquisition protocol was employed to measure signal decay during the Xe washout phase.

A detailed description of the two-pulse protocol has been described previously [10,11]. Briefly, it is assumed that the dominant peak in ^{129}Xe spectra arises from xenon atoms in a single tissue compartment that has a unique T_1, T_2 and resonance frequency. As noted above, a compartment model of Xe behavior *in vivo* predicts that during the washout phase the longitudinal magnetization decays exponentially: $M(t) = M(0)\exp(-t/\tau)$. If two spectra separated by a time (ΔTR) are acquired during the washout phase, then it can be shown that

$$\ln\left(\frac{S_2}{S_1}\right) = K - \frac{\Delta TR}{\tau} = K - \left(\frac{1}{T_1} + \frac{F}{\lambda}\right)\Delta TR$$

assuming that Xe atoms do not move a significant distance in the tissue compared with the scale over which the homogeneity of the RF field varies. In the above equation, S_1 and S_2 denote the amplitude of the dominant peak in the first and second spectra, respectively. In the case of a spatially uniform RF pulse, K would be equal to $\ln\cos\theta$, where θ is the flip angle; however, the non-uniform RF pulse of a surface coil means that the flip angle is not a well-defined quantity. Nevertheless, K may be regarded as a constant for the two-pulse protocol as long as the characteristics of the RF pulse do not vary across repeated experiments. The two-pulse protocol estimates a decay time of τ with changes in both T_1 and cerebral blood flow (CBF).

(A)



(B)

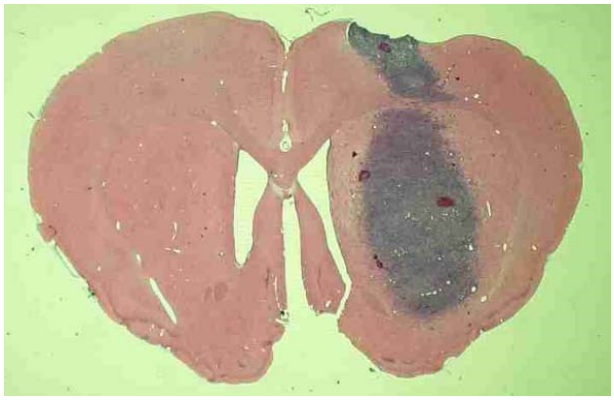


Figure 2. Typical magnetic resonance images from a C6 glioma rat brain (Rat02). (A) T_1-weighted (top line), T_2-weighted (middle line)and T_2-calculated (bottom line) images from six different slices are shown. (B) H&E staining of the 2nd slice from the MR images shown above.

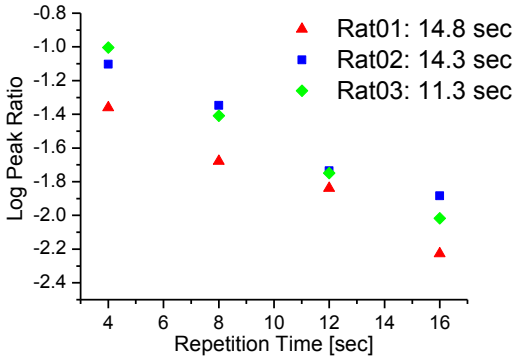


Figure 3. The relationship between the log of the peak signal ratio and the interpulse delay (ΔTR) was used to estimate the washout times of ^{129}Xe in the three C6 glioma rats. The washout times were calculated from the slope of each dataset and are shown in the legend.

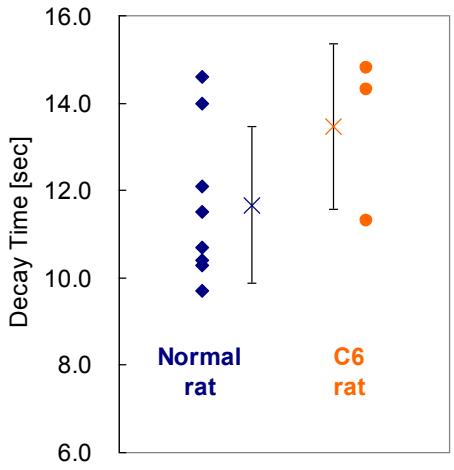


Figure 4. The decay time from both groups. The decay time in the C6 glioma rats (solid blue diamonds, mean 13.5 ± 1.9 s) was not significantly different from the normal rats (solid orange diamonds, mean 11.7 ± 1.8 s) .

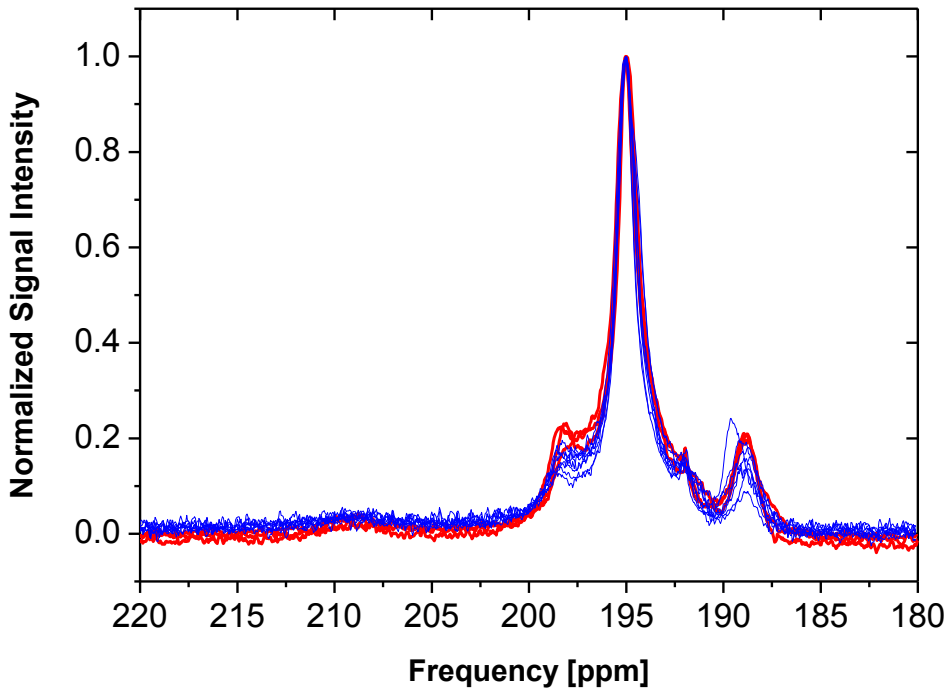


Figure 5. Spectra from three C6 glioma rats (red lines) and eight normal rats (blue lines). Each spectrum was normalized by the height of the largest peak.

III. Results

Fig. 2 shows an anatomical view of a C6 glioma-implanted rat. T_1- and T_2-weighted images and T_2-calculated images are shown. H&E staining from a C6 glioma-implanted rat are shown in Fig. 2(B). A large region of tumor cells was found in the right hemisphere. The size of the region was about 20% of the whole brain. Fig. 3 shows the relationship between the log of the peak signal ratio and interpulse delay that was used to calculate the washout decay of Xe in the three C6 glioma rats. Fig. 4 shows the decay times from both groups. The decay time of the C6 glioma rats (mean 13.5 ± 1.9 s) was not significantly different from that of the normal rats (mean 11.7 ± 1.8 s). Fig. 5 presents the spectra from the three tumor-implanted rats (red lines) and eight normal rats (blue lines). Each spectrum was normalized by the height of peak A. Five dissolved-phase peaks were observed in all spectra, as reported previously. No significant differences between the two groups of rats were observed.

IV. Discussion

The chemical shift of a Xe molecule is sensitive to the environment. The major contribution to this shift originates from van der Waals interactions between the solvent and Xe molecules [6,12]. Since tumor cell density is dramatically different from normal brain tissue, we expected that van der Waals interactions should have been different between normal tissue and tumor cells. Five dissolved-phase peaks were observed in all spectra, as shown in Fig. 5. We had shown previously that Peak A is due to Xe dissolved in brain tissue and peak B is due to Xe in non-brain tissue [8,9]. Therefore, we postulated thought that the presence of tumor cells would result in either an additional peak in the spectra or a frequency shift of peak A; however, neither of these changes were observed. The results indicate the van der Waals interactions for Xe molecules are similar in tumor and normal cells. Xe is an inert gas and therefore the van der Waals interactions were mainly affected by the molecular weight of the biological component of the cells. The weight of Xe molecules should be similar between tumor and normal cells. Another potential mechanism for a chemical shift is the size of the tumor cells. It may be that the tumor size was not large enough when spectra were acquired from the entire brain. Spectra obtained from a much smaller region using the chemical-shift imaging technique may be necessary in order to detect the presence of tumor cells using hyperpolarized ^{129}Xe spectra. Additional studies are needed to evaluate a possible image-based chemical shift focusing on tumor cells alone.

The washout decay times of hyperpolarized Xe are perturbed by both T_1 and CBF. A lower tissue concentration of oxygen results in a longer Xe T_1. In the C6 glioma cells, CBF and oxygen concentration were reduced. Therefore, the Xe washout decay should be prolonged. Although the decay time of the C6 glioma rats was slightly longer than the normal rats, a significant difference was not observed, as shown in Fig. 4.

In conclusion, these results suggest that hyperpolarized Xe may not be a superior tracer for detection of tumor cells in the intact brain.

Acknowledgment

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