Advances in Brief

Novel Magnetic Resonance Imaging Contrasts for Monitoring Response to Gene Therapy in Rat Glioma

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Abstract

Magnetic resonance imaging relaxation times, $T_{1p}$ and Carr-Purcell $T_2$ (CP-$T_2$), were measured in a glioma herpes simplex virus-thymidine kinase gene therapy model. In treated tumors with >50% cell death by histology, $T_{1p}$ and CP-$T_2$ measured with short spacing ($\tau_{CP}$) between centers of adiabatic refocusing pulses showed similar enhanced sensitivity to cytotoxic cell damage over CP-$T_2$ measured with long $\tau_{CP}$ (long-CP-$T_2$): 54.3 ± 0.7 and 55.4 ± 1.2 ms, $P = 0.30$; short-$\tau_{CP}$ $T_2$: 61.3 ± 1.0 and 64.2 ± 1.1 ms, $P < 0.05$ before and day 2 of treatment, respectively. Without treatment, long-CP-$T_2$ provided the most pronounced contrast between tumor and normal cerebral tissue. These data demonstrate that endogenous $T_2$ contrast can be modulated and extended in a manner likely to be clinically important.

Introduction

Recent studies indicate that gene therapy is a promising approach for treating malignant gliomas (1). The HSV-tk-ganciclovir approach has shown therapeutic potential both in experimental settings and in the clinical treatment of glioma patients (2, 3). Gene therapy protocols would undoubtedly benefit from the availability of a reliable noninvasive means of detecting favorable treatment response. MRI and magnetic resonance spectroscopy have proven to be valuable noninvasive tools for detecting treatment response. Together, they provide a multiparametric approach for assessing treatment response in a single study by yielding information about tumor size, perfusion, molecular tumbling, chemical exchange, diffusion, and metabolite levels (4). Exogenous MRI contrast agents such as Gd-DTPA are increasingly used to detect early changes in vascular properties (perfusion and permeability) that result from treatment. In addition, MRI methodologies that yield sensitivity to molecular diffusion processes have shown potential to detect early treatment response both in experimental tumor models (5, 6) and in patients with brain (7) and rectal (8) cancers. Studies have shown that the apparent water diffusion coefficient and the cellular density are inversely correlated (7, 9).

Detection of early cellular responses to therapies can also be accomplished with certain MRI methodologies that sense specific relaxation processes of the endogenous tissue water. Recently, MRI contrast based on longitudinal relaxation in the rotating frame ($T_{1p}$) has been shown to provide an early surrogate marker for treatment response in tumors receiving gene therapy (10) and chemotherapy (11). One study showed that $T_{1p}$ MRI can reveal favorable treatment response even before diffusion MRI (10). Unfortunately, clinical exploitation of $T_{1p}$ may be limited by the need to use levels of RF energy that in certain applications (e.g., when volume coverage of whole head is required) easily exceed current Food and Drug Administration guidelines for allowed tissue heating in human magnetic resonance scanning.

The process of transverse relaxation, as described by the time constant $T_2$, is a commonly exploited source of MRI contrast, yet current $T_2$ MRI methods generally reveal pathological processes only in advanced stages and thus offer only limited utility for monitoring disease evolution. A superposition of several physicochemical factors dictate the value of the measured $T_2$, including dipolar interactions, proton exchange, and diffusion or exchange of water molecules between different tissue compartments. From experimentation on in vitro and ex vivo preparations, it is known that the magnitude of these effects depends on the length of time between $\pi$ pulses when the measurement is performed with a CP spin-echo sequence (12, 13). Varying the $\pi$ pulse spacing in a CP sequence represents a novel way to modify MRI contrast in vivo, but in practice this approach has been limited by the accuracy of conventional $\pi$ pulses. Adiabatic pulses provide a robust way to produce an accurate $\pi$ rotation (14) and thus make the CP approach practical in vivo, even in human applications (15). By decreasing the time interval ($\tau_{CP}$) between centers of adiabatic $\pi$ pulses, the pulse sequence becomes more sensitive to dipolar relaxation processes.

Here it is shown that $T_2$ MRI using an adiabatic CP sequence with short-$\tau_{CP}$ reveals cytotoxic treatment response in a rat glioma as early as $T_{1p}$ MRI. The sequence parameter $\tau_{CP}$ adjusts the sensitivity of the method to different $T_2$ relaxation processes in the tissue and thus provides a novel way to tailor the MRI contrast to the relevant processes taking place in vivo.

Materials and Methods

BT4C gliomas transfected with HSV-tk were induced by implanting 10$^4$ HSV-tk+ cells to a depth of 2.5 mm into the corpus callosum of female BDIX rats ($n = 12$) as described previously (10, 16). Rats in the treatment group ($n = 10$) were injected with GCV (25 mg/kg, i.p., twice daily) for the duration of the study. Untreated tumor-bearing animals ($n = 2$) served as controls. After 8 days of treatment, rats were sacrificed for histology. All animal experiments were performed according to the guidelines approved by the ethical Committee of the National Laboratory Animal Center, Kuopio, Finland.

During MRI measurements, animals were anesthetized with 1–1.5% halothane in 7:3 N$_2$O:O$_2$. Core temperature was maintained close to physiological level (36.5–37.5°C) by circulating warm water through a water jacket beneath the rat inside the magnet bore. MRI was performed in a 4.7 T magnet interfaced to a Varian console. To match the MRI data with histology, rats were fixed into a custom-built head holder from the front teeth and using ear

Received 6/5/03; revised 9/23/03; accepted 9/25/03.

Grant support: Academy of Finland, Finnish Cancer Foundation, Research Foundation of Leiras, and NIH Grants RR08079 and CA92004.

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1 The abbreviations used are: HSV-tk, herpes simplex virus-thymidine kinase; MRI, magnetic resonance imaging; RF, radiofrequency; CP, Carr-Purcell; GCV, ganciclovir; TE, echo time; SAR, specific absorption rate.

10.1158/0008-5472.CAN-03-1214
bars and tilted so that the tilt angle in the axial images corresponded to that used in the stereotactic rat brain atlas. An axial 1-mm-thick imaging slice, with preselected reduced field of view of 15 × 25 mm covered with 32 × 128 points, was positioned in the middle of the tumor, and data for CP-T$_2$ maps were acquired using an adiabatic CP-type spin-echo sequence, described in detail previously [Ref. 15; four different TE values in the range 36.8–134.9 ms; repetition time (TR), 2 s; number of acquisitions (NEX), 2–4]. For CP-T$_2$ measurements, different TE values were obtained by two different approaches. The first approach used increased time between centers of π pulses (τ$_{πp}$, 6.1–22.5 ms), and the CP-T$_2$ measured in this manner is referred to as long-τ$_{πp}$ T$_2$. The second approach used an increasing number of π pulses from 6 to 22 ms, whereas τ$_{πp}$ remained constant at 6.1 ms, and the CP-T$_2$ measured in this manner is referred to as short-τ$_{πp}$ T$_2$. These two approaches were expected to yield different sensitivities to diffusion, exchange, and dipolar interactions, including cross-relaxation. The shapes of the RF amplitude and frequency modulations of the adiabatic π pulses were based on hyperbolic secant and hyperbolic tangent functions, respectively (14, 17), using frequency-sweep amplitude (A) of 35,000 rad/s, peak RF amplitude (B$_{1max}$) of 0.4 G, and pulse length (T$_p$) of 0.0025 s. To further reduce the likelihood of pulse imperfections, the initial phases of adiabatic π pulses were prescribed according to the MLEV scheme (18). T$_{1q}$ was measured using variable length adiabatic spin-lock pulses (SLT, 10–70 ms; B$_1$, 0.2 G and 0.8 G) in front of the same imaging sequence with prelocalization of the reduced field of view with six adiabatic π pulses (τ$_{πp}$, 6.6 ms). The on-resonance spin-lock was a single RF pulse consisting of three segments: a 4-ms-long adiabatic half-passage followed by the CW spin-lock period and a reverse adiabatic half-passage pulse, which returned magnetization back to the z-axis. Tumor volumes were quantified from standard T$_1$ weighted multislice spin-echo data sets covering the entire volume of tumor [TE, 60 ms; TR, 2 s; field of view, 35 × 35 mm$^2$; data matrix, 128 × 256; slice thickness of 1 mm without gap between slices].

SAR was computed with the conservative assumption that all RF energy was deposited into the 5 g of tissue. The inhomogeneity of the surface-coil complicates SAR estimations but does not influence comparisons of SAR values between CP-T$_2$ and T$_{1q}$ measurements.

Parametric maps for CP-T$_2$ and T$_{1q}$ were calculated, and regions of interest in tumor and contralateral normal tissue were analyzed, carefully avoiding liquid contribution from CSF or visible vacuoles inside the tumor. Maps representing τ$_{πp}$-dependent changes were calculated by subtracting the long-τ$_{πp}$ image from the short-τ$_{πp}$ image with the same TE (134.9 ms) and normalizing to the long-τ$_{πp}$ image. Statistically significant differences were estimated using Student’s t test. Results are indicated as mean ± SE.

Animals were sacrificed with CO$_2$ and transcardially perfused with PBS for 15 min (20 ml/min), followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 15 min (20 ml/min). The fixed brain was removed from the skull, rinsed in PBS, and embedded in OCT compound (Miles, Elkhart, IN) for cryosectioning. Histological sections were stained with the Nissl method, which stains the ribonucleic acids within the cell body, rough endoplasmic reticulum, and nucleus, allowing for assessment of cytoarchitecture and cell counts. Cell counting was performed using Stereo Investigator software in a NeuroLucida morphometry system (MicroBrightField, Colchester, VT). Only cells with intact, well-defined margins were included.

Results

Before treatment, tumors showed uniform cellularity in Nissl-stained histological sections (Fig. 1A), and the long-τ$_{πp}$ T$_2$ provided the most pronounced contrast between tumor and normal cerebral tissue (Fig. 2A). CP-T$_2$ values measured with long τ$_{πp}$ were 53.5 ± 0.7 and 58.6 ± 0.6 ms in tumor and normal parietal brain cortex, respectively. With short-τ$_{πp}$, corresponding CP-T$_2$ values were 60.6 ± 0.7 and 64.4 ± 0.8 ms. T$_{1q}$ contrast between untreated tumor and normal brain tissue was marginal, and tumors could be detected mainly because of dark-appearing borders (Fig. 2, C and D). Furthermore, T$_{1q}$ contrast between tumor and brain did not vary significantly with spin-lock field strength. For glioma and normal cortex, T$_{1q}$ values were 69.0 ± 0.7 ms and 69.9 ± 0.6 ms with B$_1$ of 0.2 G, and 86.0 ± 1.0 ms and 88.6 ± 0.7 ms with B$_1$ of 0.8 G, respectively.

In nontreated control animals, a significant increase in tumor volume was evident. Specifically, by day 8 the tumor volume was 210 ± 53% (P < 0.01) of value measured on day 0. No significant change in CP-T$_2$ and a trend toward a 2–3-ms decrease in T$_{1q}$ values were detected during the 8-day-long observation period. No significant cell loss was detected in histology. In the GCV-treated animals, histology revealed varying response, which also was reflected in the MRI tumor volumes. Four of the treated animals showed moderately increasing tumor volume (on day 8, 130 ± 20%; P < 0.05) and were characterized after the 8-day treatment period with cell loss by <20% as quantified from Nissl-stained slices (Fig. 1B). These animals were grouped together for subsequent analysis and called “non-responding animals”. The rest of the GCV-treated animals responded well (n = 6, “responding animals”), as indicated by decreased tumor volumes as compared with baseline (on day 8, 76 ± 16%; P < 0.01) and by histologically detected cell loss (>50%) by day 8 (Fig. 1C).

Examples of T$_{1q}$ and CP-T$_2$ maps from a responding animal are shown in Fig. 2. When comparing the images acquired before and on day 8 of treatment, it can be seen that the most obvious changes occurred in T$_{1q}$ and short-τ$_{πp}$ T$_2$ in the rim of the tumor.

![Fig. 1. Nissl-stained histological sections from nontreated tumor (control, A), nonresponding tumor (B), and responding tumor (C) at day 8 of GCV treatment. Bar, 100 μm.](cancerres.aacrjournals.org)
In nonresponding animals, only an increasing trend was detected in all relaxation times studied (Fig. 3A). By contrast, in responding animals, short-\(\tau_{\text{CP}}\) \(T_2\) was significantly increased by treatment day 2, from a pretreatment value of 61.3 ± 1.0 to 64.2 ± 1.1 ms (\(P < 0.05\)), and increased further to 76.7 ± 3.6 ms (\(P < 0.01\)) by the end of treatment (Fig. 3B). Conversely, from long-\(\tau_{\text{CP}}\) \(T_2\) treatment response was not apparent until day 6, when decreased tumor volume was evident and cell loss had been shown to take place (10) in this glioma model. Sensitivity of \(T_{1p}\) MRI to detect GCV treatment-induced cell death was comparable to that of short-\(\tau_{\text{CP}}\) \(T_2\) (Fig. 2, F–H, and Fig. 4B), being significant as early as day 2 of treatment. Interestingly, the sensitivity of \(T_{1p}\) contrast to treatment-induced cell death was independent of \(B_1\) amplitude, and similar contrast was obtained with \(B_1\) of 0.2 and 0.8 G (Fig. 2, G and H, and Fig. 3B).

To visualize \(\tau_{\text{CP}}\)-dependent signal changes, relative difference images were calculated by subtracting the long-\(\tau_{\text{CP}}\) image from the short-\(\tau_{\text{CP}}\) image and then normalizing to the long-\(\tau_{\text{CP}}\) image. Both the long-\(\tau_{\text{CP}}\) and short-\(\tau_{\text{CP}}\) images were acquired with the same TE (134.9 ms). Only weak contrast between glioma and normal brain tissue was visible before treatment, but the contrast intensified greatly from day 4 onward in animals that responded to treatment (Fig. 4).

**Discussion**

Noninvasive methods to obtain surrogate markers of neoplastic cell death are important in drug development research and to evaluate treatment responses in patients. The current study described a novel MRI method that allowed early detection of microstructural changes associated with gene therapy-induced programmed cell death (10, 16). Specifically, in responding tumors a significant change in the CP-T2 measured with short-\(\tau_{\text{CP}}\) having occurred by day 2 of treatment. Although the sensitivity of the short-\(\tau_{\text{CP}}\) \(T_2\) was found to be roughly equivalent to that of \(T_{1p}\), the CP-T2 method had the significant advantage of depositing less RF energy than the \(T_{1p}\) method. High RF energy deposition has been a limiting factor in the clinical exploitation of the spin-lock (\(T_{1p}\)) methods. Although a study showed the feasibility of \(T_{1p}\) imaging of human head at 4 T within Food and Drug Administration guidelines for RF power deposition, this approach required long repetition times and RF transmission via a nonstandard, half-head coil (19). Although a significant amount of RF power is also delivered with the CP-T2 method, the average RF power deposited was 48% of that required by the \(T_{1p}\) experiment using the lower \(B_1\) (0.2 G). In principle, the RF power required with the CP-T2 approach can be reduced further by using more efficient adiabatic \(\pi\) pulses or by lengthening the pulses while reducing \(B_{1\text{max}}\) (14). Adiabatic CP-T2 measurements have been performed previously in human head at field strengths up to 7 T (15) and therefore should be feasible in clinical settings.

Before treatment, contrast between glioma and normal cerebral cortex was weakest with \(T_{1p}\) and highest with long-\(\tau_{\text{CP}}\) \(T_2\). This finding contradicts an earlier report showing similar contrast in \(T_{1p}\) and \(T_2\)-weighted images between implanted murine brain tumor and surrounding tissue (20). However, the disagreement might be explained by a difference in the nature of these two tumor models. The mechanisms responsible for the high sensitivity of \(T_{1p}\) and short-\(\tau_{\text{CP}}\) \(T_2\) to detect treatment response are different from those responsible for the high contrast between tumor and brain of long-\(\tau_{\text{CP}}\) \(T_2\), and they may be dependent on the tumor model used. In tissue, a substantial contribution to \(T_2\) signal decay arises from the loss of phase coherence that can occur as water protons exchange or diffuse between sites with locally different magnetic fields, a phenomenon referred to as dynamic averaging. In tumor tissue, measurements with a spin-lock pulse and a short \(\tau_{\text{CP}}\) are expected to minimize the effects of dynamic averaging on the observed \(T_2\). Consequently, the detected relaxation processes are expected to be dominated more by dipolar interactions, including possible cross-relaxations, which are sensitive to changes in molecular tumbling rates and distances between interacting molecules. Cytoarchitecture, cellularity, and pH are just some of the differences between brain and tumor that possibly influence the relative dynamic averaging and dipolar interactions reflected in the different relaxation time constants measured in this study.

A finding deserving further discussion is the \(B_1\) dependency of \(T_{1p}\) contrast. In an experimental stroke study, enhancement of contrast was reported with increasing \(B_1\) in the range of 0.16–1.6 G, and dispersion of \(T_{1p}\) as a function of \(B_1\) was found to be the most sensitive indicator of acute postischemic alterations (21). However, in the present study the contrast was found to be independent of the \(B_1\) used. Clearly, water proton dynamics in the glioma and stroke models are different. BT4C glioma treated with ganciclovir-HSV-\(\alpha\)-k gene therapy shows several features of apoptosis, including substantial terminal deoxynucleotidyl transferase-mediated nick end labeling positivity preceding cell death (10, 16), lack of either microglial activation or extensive invasion of monocyte-macrophages (22), and

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Fig. 3. Relative changes in MRI relaxation times of nonresponding (A) and responding (B) tumors. Statistically significant difference from day 0 was evaluated using paired Student’s \(t\) test. *, \(P < 0.05\).

Fig. 4. Parametric maps before (A) and after (B) 8 days of successful GCV treatment calculated by subtracting the long-\(\tau_{\text{CP}}\) image from the short-\(\tau_{\text{CP}}\) image with the same TE and normalizing to the long-\(\tau_{\text{CP}}\) image. For more anatomical details, compare this figure with Fig. 2. Maps represent \(\tau_{\text{CP}}\)-dependent changes. C. Relative changes in subtraction images compared to day 0 in nontreated, nonresponding (cell death, <20%) and responding animals (cell death, >50%). **, \(P < 0.01\).
accumulation of polyunsaturated lipids (23). Whether the differing MRI observations in the glioma and acute stroke models are related to different cell death processes remains to be studied.

Importantly, the present data demonstrate that $T_2$ contrast can be modified to detect unique cellular/tissue alterations, revealing early treatment response to GCV gene therapy before actual cell loss or tumor volume changes. The long-$\tau_{CP}$ $T_2$ contrast obtained with six $\pi$ pulses and incremented $\tau_{CP}$ (6–22 ms) closely resembled the time course and sensitivity reported previously for Hahn $T_2$ contrast with echo spacing in the range of 20–110 ms (10, 16). The time course of CP-$T_2$ contrast obtained with constant, short-$\tau_{CP}$ was comparable to $T_{1p}$ MRI, which is known to precede diffusion MRI changes (10) and is thus one of the most sensitive contrasts to detect cytotoxicity-induced cell death in experimental tumor models.

To summarize, we have shown that CP-$T_2$ MRI exploiting an adiabatic CP sequence can reveal cytotoxic treatment response in a rat glioma much earlier than reported for conventional T2 MRI (10, 16), and is therefore one of the most sensitive contrasts to detect cytotoxicity-induced cell death in experimental tumor models.

Acknowledgments

Professor Asla Pitkänen’s expertise in histology is highly appreciated. We thank Dr. Shalom Michaeli for help in pulse-sequence implementation and for fruitful discussions concerning adiabatic CP $T_2$ contrast.

References


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