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Measurement of creatine kinase reaction rate in human brain using magnetization transfer image-selected *in vivo* spectroscopy (MT-ISIS) and a volume $^{31}\text{P}/^1\text{H}$ radiofrequency coil in a clinical 3-T MRI system

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Abstract

High-energy phosphate metabolism, which allows the synthesis and regeneration of adenosine triphosphate (ATP), is a vital process for neuronal survival and activity. In particular, creatine kinase (CK) serves as an energy reservoir for the rapid buffering of ATP levels. Altered CK enzyme activity, reflecting compromised high-energy phosphate metabolism or mitochondrial dysfunction in the brain, can be assessed using magnetization transfer (MT) MRS. MT ^{31}P MRS has been used to measure the forward CK reaction rate in animal and human brain, employing a surface radiofrequency coil. However, long acquisition times and excessive radiofrequency irradiation prevent these methods from being used routinely for clinical evaluations. In this article, a new MT ^{31}P MRS method is presented, which can be practically used to measure the CK forward reaction rate constant in a clinical MRI system employing a volume head ^{31}P coil for spatial localization, without contamination from the scalp muscle, and an acquisition time of 30 min. Other advantages associated with the method include radiofrequency homogeneity within the regions of interest of the brain using a volume coil with image-selected *in vivo* spectroscopy localization, and reduction of the specific absorption rate using nonadiabatic radiofrequency pulses for MT saturation. The mean value of k_f was measured as $0.320 \pm 0.075 \text{ s}^{-1}$ from 10 healthy volunteers with an age range of 18–40 years. These values are consistent with those obtained using earlier methods, and the technique may be used routinely to evaluate energetic processes in the brain on a clinical MRI system.

Keywords

ISIS; Creatine-Kinase; magnetization-transfer ^{31}P MRS; MT-ISIS; MT- ^{31}P -MRS

INTRODUCTION

Both the synthesis and utilization of adenosine triphosphate (ATP) are vital processes for neuronal survival and activity (1). However, the cellular pool of ATP is relatively small, even in cells with high and fluctuating energy requirements (2,3). Indeed, during the activation of excitable neuronal tissues, pre-existing ATP levels are able to fuel only a very brief period before complete ATP depletion. Fortunately, brain tissues have a temporal energy buffer with large intracellular phosphocreatine (PCr) stores and a high creatine kinase (CK) activity that replenishes ATP with the phosphorylation of adenosine diphosphate (ADP) at a sufficiently rapid rate to cope with the high energy demand (4,5). This 'PCr circuit' is crucial for appropriate neuronal activity and cell functioning. Tightly coupled to the metabolism of ATP, this circuit involves the reversible transfer of a high-energy phosphate group between PCr and ADP by the enzyme CK (6–8). This reaction can be described by the forward and reverse transitions between PCr and ATP, known as the 'CK reaction':

Although many energetic reactions are critical for optimal brain function, the flux of the CK reaction has been reported to be much faster, approximately five-fold, than that of the Pi–ATP reaction (9). It is possible to directly interrogate enzyme kinetics by studying the CK reaction, which has been correlated directly with brain function in rat brain (10). In addition, changes in CK gene expression indicate oxidative and bioenergetic stress in a cell (11). Furthermore, the CK forward reaction in the brain has been found to be decreased in several animal models of neurodegeneration and abnormal altered energy metabolism, such as Huntington's disease, dementia and diabetes mellitus (4). This important *in vivo* relationship between the CK reaction rate and brain activity can be measured using the magnetization transfer (MT) MRS technique. The selective saturation of the γ -ATP resonance has been generally used to measure the forward transition rate constant (k_f) of the CK reaction in brain (12,13), skeletal muscle (14) and heart (15,16) in humans, as well as in animal brain (10,17,18).

k_f is measured by acquiring a series of NMR signals with various recovery times whilst applying a train of MT saturation radiofrequency (RF) pulses (MT saturation) centered on the γ -ATP resonance frequency. Most previous studies of k_f have been performed using extended acquisition times, surface RF coil and free induction decay (FID)-type acquisitions, because of the relatively high signal-to-noise ratio (SNR) (15,19) afforded by this approach.

The assessment of FID is commonly used in *in vivo* ^{31}P MRS with a surface RF coil, the localization of which is accomplished using the B_1 profile of the coil. Although the SNR of the measurement using the surface coil is superior to that obtained using a volume RF coil, important disadvantages of a surface RF coil include the limitation in the spatial coverage only to tissues adjacent to the coil because of the extremely poor B_1 homogeneity. Thus, an adiabatic RF pulse is generally preferred for all RF pulses for excitation, refocusing, inversion and MT saturation. However, the large B_1 amplitude, which is an intrinsic requirement for the adiabatic RF pulse, can induce an excessive amount of specific absorption rate (SAR). The large number of RF pulses required for MT saturation can cause substantial local heating, which should not be ignored as a potential safety concern, particularly in human brain.

In this article, an MT-ISIS (image-selected *in vivo* spectroscopy) (20,21) sequence is presented, which can be used to measure k_f in the CK reaction in 30 min, employing a volume RF coil and ISIS. ^{31}P MRS using a volume coil with ISIS localization is less susceptible to contamination from the scalp muscle, bone and averaged lipid than measurements made using a surface RF coil. High phosphorus concentrations in scalp muscle and bone, combined with the high sensitivity near the surface coil, may easily contaminate brain ^{31}P spectra. An advantage of using a volume RF coil for the MT experiment is that a train of sinc RF pulses with constant amplitude can be employed instead of adiabatic RF pulses, thereby reducing RF deposition because of the low B_1 amplitude. This technique was applied to a limited number of healthy human volunteers to measure the CK reaction rate constant k_f , pH and free Mg^{2+} level (pMg) in the brain.

THEORY: STEADY-STATE MT EXPERIMENT

The separation in chemical shift between PCr and γ -ATP is only 2.5 ppm (125 Hz at 3 T). It is difficult to suppress the γ -ATP line only without affecting the PCr peak. Under the influence of the RF bleed-over, the longitudinal magnetization of PCr in the CK reaction process is described by the modified Bloch equation:

$$\frac{dM_z(t)}{dt} = \frac{M_0 - M_z(t)}{T_2} - k_f M_z(t) - \eta M_z(t) \quad [2]$$

where η is the rate of loss at the PCr peak by an imperfect RF saturation pulse. The solution of this modified Bloch equation becomes:

$$M_z(t) = M_0 \frac{R_2}{R_1'} \left[1 - \left(1 - \frac{M_z(0) R_1}{M_0 R_1'} \right) e^{-R_1' t} \right] \quad [3]$$

with the apparent relaxation rate $R_1' = R_1 + k_f + \eta$, where R_1 is the intrinsic relaxation rate $1/T_1$. In eqn [3], the steady-state longitudinal magnetization $M_z(\infty)$ with $t_{\text{sat}} \rightarrow \infty$ is reduced by R_1/R_1' from the equilibrium magnetization M_0 as:

$$M_z(\infty) = M_0 \frac{R_1}{R_1 + k_f + \eta} = M_0 \frac{R_1' - k_f'}{R_1'} \quad [4]$$

$$k_f' = R_1' \left(1 - \frac{M_z(\infty)}{M_0} \right) \quad [5]$$

and

$$R_1 = R_1' \frac{M_z(\infty)}{M_0} \quad [6]$$

To accurately measure k_f , the RF bleed-over effect η must be removed from the rate measurement. The loss factor η can be estimated using fully recovered equilibrium signals with the saturation irradiation centered at the opposite side of γ -ATP, i.e. +2.5 ppm. With the control irradiation, i.e. +2.5 ppm, the signal intensity for the magnetization under the RF

bleed-over is reduced to the true equilibrium signal intensity as: $M_0' = M_0 \frac{R_1}{R_1 + \eta}$. Therefore, the loss factor η by the RF imperfection to the measured k_f can be estimated using:

$$\eta = R_1 \left(\frac{M_o}{M_o'} - 1 \right) = R_1' \frac{M_z(\infty)}{M_o'} \left(\frac{M_o}{M_o'} - 1 \right) \quad [7]$$

The CK forward reaction rate k_f can be obtained by substituting eqn [7] into eqn [5]:

$$\begin{aligned} k_f &= k_f' - \eta = R_1' \left(1 - \frac{M_z(\infty)}{M_o'} \right) - R_1' \frac{M_z(\infty)}{M_o'} \left(\frac{M_o}{M_o'} - 1 \right) \\ &= R_1' \left(1 - \frac{M_z(\infty)}{M_o'} \right) \end{aligned} \quad [8]$$

Equation [7] indicates that k_f can be measured using the apparent relaxation rate R_1' , longitudinal magnetization $M_z(\infty)$ of PCr with steady-state MT saturation on γ -ATP (-2.5 ppm) and thermal equilibrium magnetization M_o' with control MT irradiation at $+2.5$ ppm.

MATERIALS AND METHODS

MRS experiments were performed on 12 healthy subjects using a 3-T clinical MRI system (Tim-Trio, Siemens Medical Solutions, Erlangen, Germany), with a custom-made $^{31}\text{P}/^1\text{H}$ double-tuned volume head coil (Clinical MR Solutions, LLC, Brookfield, WI, USA). The proton channel was used for localization, anatomic imaging and shimming. Signed informed consent, which was approved by the Institutional Review Board of the University of Utah, was collected from each subject prior to participation. A saturation recovery process was used to measure the apparent relaxation time T_1' . The initial magnetization before starting MT saturation was nulled by three pairs of 110° hard RF and spoiler gradient pulses, so that the measurement time was greatly reduced for the same number of data points compared with the use of a decay process with large initial magnetization.

MT-ISIS pulse sequence

Figure 1 shows the schematic timing diagram of the MT-ISIS pulse sequence, which was developed using the Siemens pulse sequence development tool (IDEA, Siemens Medical Solutions). The MT-ISIS sequence consists of M_z saturation, MT saturation and ISIS acquisition.

A hyperbolic secant RF pulse with a bandwidth of 4 kHz and duration of 5.12 ms was designed for three adiabatic inversion pulses for ISIS localization (20,22,23). A 90° hard RF pulse with a pulse width of 400 μs was used for excitation. Six spatial saturation (OVS) pulses and gradients precede the ISIS inversion pulses to prevent contamination from the ^{31}P signal residing at the outer volume.

Three pairs of 400- μs hard RF pulses with a nominal flip angle of 110° and a spoiling gradient were applied to saturate all longitudinal magnetizations (M_z saturation) at $t_{\text{sat}}=0$. One MT saturation RF pulse was applied just before M_z saturation to further suppress γ -ATP, so that the reverse transfer from γ -ATP to PCr was prevented at early t_{sat} . The delay was inserted between the end of data sampling and the next M_z saturation pulse to keep SAR within the Food and Drug Administration guideline.

A 66-ms-long sinc RF pulse with a bandwidth of 75 Hz was used for MT saturation by repetitive application during the entire MT saturation duration t_{sat} . Each saturation RF was followed by a spoiler gradient to disperse any coherence in the transverse magnetization. One additional MT saturation pulse was also applied between the OVS and ISIS inversion pulses to limit the regrowth of γ -ATP magnetization during the duration of OVS and ISIS inversion pulse application, which was about 50 ms. Variable amplitude BISTRO (B_1

insensitive train to oblique signal) saturation was also implemented in the same pulse sequence for SAR comparison (14,24).

³¹P NMR spectroscopy experiment

To test the performance of M_z saturation, ISIS with three pairs of hard RF and spoiling gradient pulses with zero t_{sat} was applied on a spherical phantom (diameter, 20 cm) (GE Braino, Milwaukee, WI, USA). The size and orientation of the volume of interest was the same as that for human brain application, i.e. $80 \times 110 \times 30 \text{ mm}^3$. The flip angle of the M_z saturation pulses for optimal saturation was empirically found to be 110° . The residual signal intensity was compared with that obtained without M_z saturation.

A three-dimensional inversion recovery magnetization-prepared rapid gradient echo (MP-RAGE) sequence was performed to obtain T_1 -weighted images with an inversion time of 1.1 s and an isotropic resolution of 1 mm^3 . The MP-RAGE images were used to localize the ISIS voxel and OVS bands. For each subject, three-dimensional global shimming was performed several times until the full width at half-maximum of the water resonance of the selected voxel was less than 30 Hz. The carrier frequency of ³¹P NMR was set to the PCr resonance frequency. The ³¹P MRS experiments in healthy volunteers were performed using MT-ISIS with a receiver bandwidth of 2.5 kHz and 1024 acquisition points. Each spatial saturation band for OVS was positioned parallel to each face of the rectangular voxel with a gap of 5 mm.

The MT experiment was performed using sinc RF saturation pulses on the γ -ATP line (-2.5 ppm). To measure the apparent relaxation rate R_1' and the steady-state magnetization $M_z'(\infty)$, seven spectra datasets were acquired using different saturation durations t_{sat} with different numbers of signal averages of the eight ISIS phase cycles, as eight 0.25 and 0.5 s, six 1.0 and 2.0 s, and four 4.0 and 9.0 s. To measure the signal intensity for the thermal equilibrium magnetization M_0' under RF bleed-over, MT-ISIS was applied with two average ISIS cycles at $+2.5 \text{ ppm}$ and $t_{\text{sat}} = 20 \text{ s}$, which is about three times the expected intrinsic T_1 of PCr (25). The MT-ISIS acquisition was repeated with the saturation RF at $+20 \text{ ppm}$ to measure the signal for M_0 without RF bleed-over effect. These two datasets were used to estimate the RF bleed-over effect on the rate measurement, as well as to measure pH and pMg (26). The total acquisition time for k_1 measurement was 32 min.

All FID data were processed using a public-domain MRS processing software jMRUI v4.0 (Magnetic Resonance User Interface, <http://www.mrui.uab.es/mrui/>) (27) that performs 10-Hz Gaussian broadening, time-domain baseline correction and fast Fourier transformation. The spectrum was fitted using AMARES (28) in the jMRUI package to a pre-existing database of peak location and width to obtain the area under each NMR line. To obtain the apparent recovery rate R_1' and the steady-state saturation magnetization $M_z'(\infty)$ under RF bleed-over, the PCr signals were fitted to an exponential recovery equation $A + Be^{-R_1' t_{\text{sat}}}$, where the coefficient A corresponds to the steady-state magnetization $M_z'(\infty)$ in eqn [7]. pH and pMg were obtained using the spectrum with $\text{TR} = 20 \text{ s}$ and 20 ppm MT saturation.

RESULTS

RF energies for V-shaped BISTRO with 32 adiabatic RF pulses and for 98 sinc RF pulses with a flip angle of 100° were estimated in the IDEA pulse sequence simulation tool using identical imaging parameters, i.e. a saturation bandwidth of 75 Hz, saturation duration of 6.6 s and $\text{TR} = 8 \text{ s}$. The B_1 amplitude of the largest adiabatic RF pulse in BISTRO was set to half of the 90° excitation hard RF pulse with a pulse duration of $400 \mu\text{s}$. The calculated RF

energy using V-shaped BISTRO saturation was about 100 times that obtained using sinc saturation.

The NMR spectrum in Fig. 2a is a typical ^{31}P spectrum obtained using ISIS with a repetition time of 20 s from a rectangular ISIS voxel with dimensions of $80 \times 110 \times 30 \text{ mm}^3$, which is overlaid on the T_1 -weighted three-dimensional MP-RAGE image in Fig. 2b.

Figure 3a shows a series of MT-ISIS spectra with different MT saturation durations t_{sat} , which indicate the apparent relaxation process of PCr. No residual γ -ATP signal is noticeable. The spectra in Fig. 3b show the thermal equilibrium NMR spectra with and without RF bleed-over, i.e. with MT saturation at +2.5 and +20 ppm. The decrease in the PCr amplitude with +2.5 ppm MT saturation is caused by the imperfect MT saturation. In this example, the fractional decrease caused by the RF bleed-over effect was less than 3%.

The signal intensities of the PCr line in Fig. 3a are plotted in Fig. 4 to extract the apparent relaxation rate R'_1 and the steady-state signal $M'_z(\infty)$. The plot indicates that the magnetization of PCr almost reaches steady-state saturation magnetization at $t_{\text{sat}} = 9 \text{ s}$. For this specific example, the apparent relaxation rate was measured as 0.410 s^{-1} . The plot indicates that the initial longitudinal magnetization is almost zero. The means and standard deviations of k_f , pH and pMg were measured as $0.320 \pm 0.075 \text{ s}^{-1}$, 7.037 ± 0.020 and 0.159 ± 0.015 , respectively, for 10 subjects with a mean age of 25.4 ± 0.51 years (five women and five men). The average SNR of all subjects was 20.94. If the measured SNR at the peak of PCr on the MT data with $t_{\text{sat}} = 9 \text{ s}$ was lower than 15, the data were excluded.

DISCUSSION

The BISTRO (14,24) technique, which applies a series of adiabatic RF pulses with varying amplitude to randomly disperse the magnetization vectors, has been proven to be effective for MT suppression using a surface RF coil. However, in our preliminary application of MT-ISIS using BISTRO saturation, the SAR monitoring algorithm suggested that TR should be increased to 30 s or longer even with the B_1 amplitude of the largest adiabatic RF pulse half that of the hard excitation RF pulse, which resulted in nonfeasible scan times for measurements in humans.

^{31}P MRS using a surface RF coil and B_1 -independent rotation RF pulses for excitation benefits in obtaining local high SNR (24,29). However, it may be difficult to completely remove the contamination from the scalp muscle or skull bone. Because the volume RF coil produces a relatively homogeneous RF field, a hard RF pulse can be used for small voxel excitation.

Although the volume RF coil provides superior RF homogeneity over that obtained using a surface coil, it still generates nonuniformity across the large spectral voxel. This incomplete M_z saturation may induce error in k_f measurement by allowing the reverse transfer from the residual γ -ATP magnetization to PCr magnetization during the early short duration of t_{sat} . Using three pairs of 110° hard RF and spoiler gradient pulses, the longitudinal magnetization was suppressed to 4%, which was at the noise level. The data with insufficient M_z saturation, i.e. the signal intensity for $t_{\text{sat}} = 0$ deviates substantially from zero, tended to give a decreased k_f value compared with the data with good M_z saturation. Therefore, the data with poor M_z saturation and low SNR (< 15) were excluded from the calculation of the average value of $k_f = 0.032 \text{ s}^{-1}$. These data were mostly the result of the poor shimming and coil performance, not the pulse sequence.

Spectral misregistration during OVS saturation and ISIS localization may introduce errors into the rate measurement. Fortunately, γ -ATP is located at 2.5 ppm (125 Hz at 3 T) from the PCr peak. Because the bandwidth of ISIS inversion, spatial saturation and excitation was 4 kHz, the fractional shift in the position is only 3.4%, which translates to 3.7 mm for the 110 mm in the anteroposterior direction of the ISIS voxel.

The mean k_f of 0.320 s^{-1} is comparable with the values of 0.30 s^{-1} and 0.16 s^{-1} for predominantly gray matter and white matter regions in the brain reported by Cadoux-Hudson (12) and of 0.29 s^{-1} in monkey brain reported by Mora *et al.* (17,18), but lower than the value of 0.42 s^{-1} for the entire human brain presented by Bottomley and Hardy (19). Because our ISIS voxel includes both gray and white matter, the measured rate constant reflects all tissue types enclosed within the voxel.

CONCLUSIONS

An MT-ISIS method is presented, which can be practically used to measure the CK forward reaction constant in a clinical MRI system employing a volume head ^{31}P coil. The acquisition time was 32 min and the resulting ^{31}P spectra were free of contamination from the scalp muscle and skull bone. The technique employs the advantages associated with a volume head ^{31}P coil, which include RF homogeneity and coverage, for ISIS localization and reduction of SAR by using nonadiabatic RF pulses for MT saturation. The mean value of k_f was measured as $0.320 \pm 0.075 \text{ s}^{-1}$ from 10 normal volunteers with an age range of 18–40 years (mean age, 25.1 ± 0.51 years). The technique may be used routinely to evaluate the high-energy phosphate process in the brain on a clinical MRI system.

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Abbreviations used

ADP	adenosine diphosphate
ATP	adenosine triphosphate
BISTRO	B1 insensitive train to oblique signal
CK	creatine kinase
FID	free induction decay
ISIS	image-selected in vivo spectroscopy
MP-RAGE	magnetization-prepared rapid gradient echo
MT	magnetization transfer
PCr	phosphocreatine
RF	radiofrequency
SAR	specific absorption rate
SNR	signal-to-noise ratio

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Figure 1.

Pulse diagram of magnetization transfer image-selected *in vivo* spectroscopy (MT-ISIS). ACQ indicates the data acquisition window. The sequence starts with an MT saturation radiofrequency (RF) pulse and three 110° hard RF pulses to saturate the entire longitudinal magnetization, followed by a small delay and a train of MT saturation pulses. The first MT RF pulse is to suppress γ -adenosine triphosphate (γ -ATP) and to prevent reverse transfer because of the incomplete M_z saturation at early times. An additional MT saturation RF pulse is applied between the OVS spatial saturation RF pulses and the ISIS inversion pulses to minimize the growth of γ -ATP. Hard RF pulses are used for excitation and M_z saturation. The transmit bandwidths of the hard RF, OVS and ISIS inversion pulses are 4 kHz. All gradient pulses are to spoil the transverse magnetization, except for three slice-selective gradients (labeled x, y, z) for ISIS localization.

Figure 2.

(a) Typical magnitude *in vivo* ^{31}P MR spectrum for a rectangular image-selected *in vivo* spectroscopy (ISIS) voxel with dimensions of $80 \times 110 \times 30 \text{ mm}^3$ (b) and outer volume suppression on a human subject. The spectrum was acquired with four averages of eight-phase-cycled ISIS with a recovery time of 20 s. Lorentzian apodization of 5 Hz was applied. Various metabolites were identified: ATP, adenosine triphosphate; PCr, phosphocreatine; Pi, inorganic phosphate.

Figure 3.

(a) *In vivo* brain ^{31}P MR spectra vs magnetization transfer (MT) saturation time t_{sat} , whilst saturating γ -adenosine triphosphate (γ -ATP), to measure the apparent relaxation rate R_1' . (b) Spectra for thermal equilibrium magnetization with and without radiofrequency (RF) bleed-over effect. γ -ATP is fully suppressed down to the noise level (a). The signal intensity of the phosphocreatine (PCr) line is plotted with respect to the saturation time to measure the apparent relaxation rate R_1' .

Figure 4.

Plot of the phosphocreatine (PCr) signal with respect to the saturation time t_{sat} . The signal intensities were fitted to a single-exponential recovery function to extract the apparent relaxation rate and steady-state magnetization under magnetization transfer (MT) saturation.