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“Molecular” MR Imaging at High Fields

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Abstract

Magnetic resonance imaging (MRI) and spectroscopy (MRS) have contributed considerably to clinical radiology, and a variety of MR techniques have been developed to evaluate pathological processes as well as normal tissue biology at the cellular and molecular level. However, in comparison to nuclear imaging, MRI has relatively poor sensitivity for detecting true molecular changes or for detecting the presence of targeted contrast agents, though these remain under active development. In recent years very high field (7Tesla and above) MRI systems have been developed for human studies and these provide new opportunities and technical challenges for molecular imaging. We identify 5 types of intrinsic contrast mechanisms that do not require the use of exogenous agents but which can provide molecular and cellular information. We can derive information on tissue composition by (i) imaging different nuclei, especially sodium (ii) exploiting chemical shift differences as in MRS (iii) exploiting specific relaxation mechanisms (iv) exploiting tissue differences in the exchange rates of molecular species such as amides or hydroxyls and (v) differences in susceptibility. The increased signal strength at higher fields enables higher resolution images to be acquired, along with increased sensitivity to detecting subtle effects caused by molecular changes in tissues.

Keywords

molecular imaging; high field; endogenous contrast; CEST; magnetization transfer; chemical exchange

The formal definition of molecular imaging is “the visualization, characterization and measurement of biological processes at the molecular and cellular level in humans and other living systems.” (1) As such molecular imaging provides information that is beyond regional anatomy and physiology related to tissue composition and molecular changes within tissues. MR images have high signal to noise ratio and spatial resolution ($\approx$ mm) because water protons are abundant in roughly 80 – 90 Molar concentrations in tissues. Unfortunately, conventional MRI has poor molecular specificity or sensitivity compared, for example, to positron emission tomography (PET). This is because most MRI methods acquire signals from protons in water molecules and their interactions with the local environment are only indirect reporters of molecular events within tissues. Conversely, magnetic resonance

spectroscopy (MRS) has exquisite molecular specificity (indeed, it reports the presence of specific molecular structures more directly than other modalities) but suffers from relatively poor sensitivity, so that typically millimolar concentrations of metabolites must be present to be detected in reasonable times from relatively large ($\approx \text{cm}^3$) volumes of interest. Over several years there has been a great deal of effort to improve the specificity of MRI to molecular events via targeted or smart agents which affect MRI water signals and which might be used as tracers in similar manner to the use of radioactive ligands in nuclear imaging (for review see (2)). In reality these agents have limited potential in practical applications because relatively high concentrations of materials such as paramagnetic ions are required for detection (typically, concentrations of 10s or 100s micromolar metal must be achieved within a voxel (3)). Thus the question arises whether there are other ways to improve the molecular specificity of MRI, making use of endogenous contrast mechanisms, and in particular, whether there are new opportunities as we move to higher fields.

The advent of higher magnetic fields such as 7 Tesla has potential advantages for enhancing our ability to exploit endogenous contrast mechanisms. For example, the higher intrinsic signal-to-noise ratio obtainable at higher fields makes smaller changes in signal more detectable, so we can detect more subtle changes in composition as well as acquire images of other nuclei that are less abundant than protons, such as sodium. Using higher fields also provides greater dispersion of magnetic resonance spectra which improves spectral resolution and also increases the effects of chemical exchange processes on transverse magnetization. A third benefit is that contrast effects from variations in magnetic susceptibility increase, including signal variations related to local concentrations of deoxyhemoglobin as exploited, for example, in BOLD (blood oxygenation level dependent) imaging. In general, molecular information may arise from images when the contrast is based on either the nucleus imaged, the chemical shift or resonance frequency of the nucleus, the influence of specific relaxation processes, the exchange rate between molecular species, and the presence of susceptibility variations.

Multinuclear Imaging

Realistically, sodium is the only element other than hydrogen present in tissues in sufficient abundance to be able to be imaged in reasonable times at a reasonable spatial resolution. At 3T and beyond, sodium imaging is quite practical and there is considerable interest in measuring spatial maps of sodium concentrations to assess, for example, storage and metabolism of sodium in skin and muscle as well as in neural tissues of the brain. Figure 1 shows a comparison of proton and sodium images of a normal cross section of a lower leg in which the sodium image was acquired at 3T in about 15 minutes with spatial resolution of about 3 mm. More sophisticated acquisition sequences also have the potential to distinguish sodium contained in different tissue compartments (4).

Magnetic Resonance Spectroscopy

Molecular information is also contained in the chemical shift and spin-spin (J) couplings of protons. One of the simplest types of spectral imaging is the separation of the proton signal into fat and water components to create separate fat and water images. A common approach

is based on the method of Dixon (5) which allows the frequency difference between lipid and water resonances to evolve until there is a significant (e.g. 180 degree) phase difference in their separate components. Comparisons of images at different phase offsets allows separate imaging of fat and water. Images of lipids are of considerable interest for the quantification of the visceral fat deep in the body which is considered a risk factor for various metabolic disorders such as diabetes and which is a potential biomarker of the effects of various dietary and pharmacological interventions (6). Current more advanced approaches to fat imaging make use of the information contained in multiple gradient echoes and more sophisticated analyses of gradient echo trains to obtain greater specificity about the precise nature of hydrocarbons present in the lipid signals. For example it is now possible to separately image the saturated fat fraction, the mono-unsaturated fraction, and the poly-unsaturated fraction present in a composite signal, thereby giving us information on the different bond structures in a mixture of lipids (7).

Proton magnetic resonance spectroscopy and spectral imaging have been implemented for many years but the greater sensitivity and spectral resolution available at higher fields increases the quality of data available *in vivo*. For example, high resolution spectra can be acquired for every voxel within a two-dimensional slice with spatial resolution less than 0.5 cm³ in a few minutes at 7T. These spectra can then be used to produce maps of individual metabolites within planes. There is some evidence that the increased spectral resolution at 7T enables subtler spectral distinctions to be made, for example between N-acetylaspartate (NAA) and NAA-glutamate. Figure 2 shows an example of the type of data obtainable.

MR spectroscopy has also been previously used to detect and measure the neurotransmitters GABA and glutamate, which are the major inhibitory and excitatory neurotransmitters in the brain. Their levels can be measured using appropriate acquisition editing sequences, and they change in various disorders and in response to drugs (8). At 7T reasonably high quality GABA or glutamate spectra can be obtained from volumes of order 4 mL in under 10 minutes from anywhere in the brain. Figure 2 shows a typical result obtained at 7T.

High field MRI suffers from the effects of non-uniform RF fields as higher frequencies are attenuated and produce dielectric effects within tissues, which make proton MRI and MRS challenging. These restrictions are less important for other nuclei such as phosphorus-31 or carbon-13, so higher fields are especially advantageous for multi-nuclear acquisitions. At 7T the carbon-13 resonance frequency is about 75 MHz, not too dissimilar to protons at 1.5T (64 MHz). For example, measurements of glycogen at natural abundance can be obtained from muscle in a few minutes, while the metabolism of carbon-13 labeled substrates such as glucose can be followed with temporal resolution of a few minutes acquiring data from several milliliters.

Contrast Based on Magnetization Transfer

Proton relaxation processes in general are considered to be not very specific for obtaining molecular information as they represent the integrated effects of water interactions with multiple tissue components. There are, however, several mechanisms by which molecular

information can be extracted from appropriate measurements that exploit relaxation times and mechanisms that differ between tissues of different composition. Protons may relax through inter- and intramolecular dipolar interactions that are modulated by their motions, and by cross relaxation between mobile water and relatively immobilized protons within macromolecules. In addition, at higher fields, chemical exchange between sites of different resonant frequencies becomes more important and can reduce relaxation times. These processes may in turn depend on the physical exchange of water between different environments e.g. on and off a protein surface or between intra - and extra-cellular spaces. Thus although macromolecules themselves may remain invisible in MRI they may be sensed by different processes which collectively constitute magnetization transfer (MT).

We can distinguish three types of magnetization transfer. The first is the non-specific exchange of magnetic spin states between water and the so-called semi-solid or immobile protons present within, for example, large proteins. A second form of magnetization transfer is chemical exchange between water and specific labile groups such as amides, amines or hydroxyls which have different chemical shifts compared to water. A third type of magnetization transfer is the nuclear Overhauser enhancement (NOE) mediated saturation transfer which occurs between water and protons undergoing cross relaxation which themselves have specific chemical shifts. Each type of magnetization transfer conveys different information on the molecular content of the tissue.

Quantitative magnetization transfer (qMT) methods involve acquisitions of several images with different MT weightings that are combined to derive quantitative information about macromolecules in tissue. For example, the transient recovery of the magnetization following an inversion pulse that selectively affects only the mobile proton pool can be analyzed as the sum of two exponential recoveries, from which maps of the macromolecular-to-water pool size ratio (PSR) can be calculated (9). PSR represents the total macromolecular content interacting with water via MT and is sensitive to, for example, the total protein content of tumors, or amount of myelin in white matter. Figure 3 shows a calculated map of the PSR in a human brain. Usually PSR and the longitudinal relaxation rate R_1 are strongly correlated.

Chemical exchange processes between water and labile protons on smaller molecules have been exploited to reveal molecular information. For example, the imaging method CEST (chemical exchange saturation transfer) involves the application of frequency selective saturating RF pulses which reduce the longitudinal magnetization of specific labile protons such as amides (at around 3.5 ppm from water) and hydroxyls (at around 1 ppm). The saturated protons then exchange with water protons and this reduces the available water signal. Thus the signals from low concentrations of metabolites are magnified by their CEST effects on the much larger water resonance, allowing maps of each exchanging moiety. Figure 4 shows CEST z-spectra, which represent the water signal for different applied frequencies of the saturating RF in which negative peaks in the curve represent specific resonance absorptions. Figure 5 shows maps of amides and hydroxyls in a human breast derived from an appropriate analysis of specific dips in a z-spectrum. In tissues such as cartilage, hydroxyls are mainly associated with proteoglycans, whereas amides may be present largely in proteins and amino acids. A specific form of CEST, amide proton transfer

or APT, has been shown to reveal molecular changes within tumors, possibly as the abundance of mobile proteins and peptides increases (10).

NOE-mediated saturation transfer effects also may be identified in z-spectra but represent a transfer of energy via cross-relaxation when the saturating energy has the same frequency of a specific chemical group whose resonance may be too broad to be resolved by regular magnetic resonance spectroscopy. Figure 4 shows two small NOE dips in z-spectra from animal brain that occur on the opposite side of the water resonance to CEST effects. There is, for example, an NOE effect at -1.6 ppm that is potentially an indicator of choline phospholipids in brain (11). Recent reports have shown that these two NOE signals decrease in brain tumors (Figure 6) (12). NOE images complement the role of conventional MRS as illustrated in Figure 7. This shows the z-spectra and high resolution MR spectra of the brain metabolite NAA in a simple solution and when immobilized in a cross-linked protein (BSA, bovine serum albumin). The liquid solution shows narrow lines on a conventional spectrum and no discernible sign on the z-spectrum, whereas the immobile NAA gives very broad lines that are not resolvable on high resolution MRS but gives very clear and characteristic dips on a z-spectrum at the frequencies corresponding to the high resolution resonances. Clearly these effects are related and complementary but detectable by different types of MT. Moreover, the magnitude of the NOE effect will depend on different parameters such as molecular mobility and degree of immobilization.

Contrast Based on Exchange Rates

When protons exchange between different groups such as between water and amides, the modulation of the local resonance frequencies results in a contribution to the transverse relaxation rate, $1/T_2$. This exchange broadening of lines reduces T_2 to a degree that increases with magnetic field strength, as pointed out by Zhong et al. (13) who showed that the relaxivity (i.e. the relaxation rate contribution per concentration of solute) of diamagnetic proteins has a biphasic behavior with magnetic field. From 0 to ≈ 100 MHz the relaxivity decreases as expected for dipolar interactions, whereas from ≈ 100 MHz to higher fields the relaxivity increases remarkably, consistent with an expected quadratic dependence on chemical exchange processes. An important consequence is that at lower fields e.g. 1.5T, the contrast between tissues may represent differences in the number and sizes of macromolecules causing water relaxation, whereas at higher fields (3T, 7T) the contrast depends on the number and exchange rates of labile protons, a very different physico-chemical feature of tissue composition.

One approach to exploiting exchange processes makes use of the ability to refocus some of the transverse magnetization lost by exchange by the application of a locking pulse. Spin-lock relaxation (or spin-lattice relaxation in the rotating frame) may be quantified by the rate of signal decay during application of a transverse locking pulse of a specific amplitude on resonance after a 90-degree excitation (14). The rate of measured signal decay is $R_{1\rho} = 1/T_{1\rho}$ where $T_{1\rho}$ has a value between T_1 and T_2 . When the locking field is very weak the decay rate is higher and $\approx 1/T_2$, but it decreases with increasing locking field strength, thereby giving rise to a dispersion of values as a function of locking field. From this dispersion, the exchange rate between protons can be explicitly calculated, as shown for

hydroxyls exchanging within cartilage by Wang et al. (15). Moreover, by judicious selection of the locking fields used in combinations of images, parametric images that emphasize contents that show specific ranges of exchange rates (rather than frequency offsets as in CEST) can be constructed (16).

Contrast Based on Susceptibility Variations

MRI signals decay in the presence of magnetic field inhomogeneities so that T_2 and T_2^* are reduced. Magnetic field inhomogeneities may arise from the presence of both macroscopic and microscopic variations in tissue susceptibility such as occur in bone and air, which distort the applied field and induce gradients that in turn cause signal dephasing. Of major interest is the difference in susceptibility between oxygenated and deoxygenated blood such that tissues with higher levels of venous blood have faster transverse signal decay rates. These susceptibility contrast effects are increased at higher fields and form the basis, for example, of blood oxygenation level dependent (BOLD) signal changes that are exploited in functional MRI of the brain (17). Intrinsic susceptibility effects are also seen in tissues rich in iron such as the basal ganglia or the liver in various disorders, so that measures of T_2^* form an indirect metric of tissue molecular composition.

Summary

High field systems are proliferating - at least in a research setting - and their wider use is limited mainly by cost. Experiences with high field MRI scanners to date have been encouraging and images of higher SNR, spectra of better quality from smaller voxels, and larger BOLD contrast effects have all been realized. Several weak effects become more detectable for use in practical imaging e.g. NOE effects. Some effects increase dramatically - e.g. BOLD and CEST - providing better opportunities for more specific molecular imaging. Thus although MRI does not enjoy the same molecular sensitivity as other modalities such as PET, it is possible to derive some knowledge of molecular changes within intact biological tissues at relatively high spatial resolution using only endogenous contrast mechanisms.

Acknowledgments

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Figure 1.

Proton and sodium images of the lower leg of a 25y old male subject. Four calibration phantoms (NaCl aqueous solutions) were placed under the leg to serve as reference standards and were scanned together with sections through the calf muscles. Sodium concentrations in the solutions (from left to right) were 40mM, 30mM, 20mM, and 10mM, respectively. (A) Proton image acquired by the scanner body coil of a Philips 3T Achieva system (Philips Healthcare, Best, the Netherlands) with a Turbo Spin Echo (TSE) sequence, parameters: FOV=192×192mm², thickness=5mm, pixel size=1×1mm², TR/TE=4000ms/30ms, NEX=2, scan time=2min 28sec. (B) Sodium image acquired with a 3T ²³Na quadrature knee coil (Rapid Biomedical GmbH, Rimpf, Germany) using a 3D gradient echo (GRE) sequence, parameters: FOV=192×192×210mm³, voxel=3×3×30mm³, 7 slices, TR/TE/FA=50ms/0.99ms/87°, BW=434Hz/pixel, NEX=64, scan time=15min 3sec. The proton and sodium images were aligned in the middle of their imaging slabs.

Fig 2a

Fig 2b

Fig 2c

Figure 2.

(a) Chemical shift imaging of brain obtained using a Philips Achieva 7 Tesla scanner (Philips Healthcare, Best, Netherlands) in 6 minutes with resolution $9 \times 9 \times 9$ mm, superimposed on reduced scale conventional proton image. (b) A typical ^1H spectrum from within one voxel. Maps of individual peaks may also be produced. (c) ^1H spectra from 6 mL volume in anterior cingulate obtained in 8min. Top spectrum shows glutamate from a PRESS sequence, lower spectrum shows GABA from a J-difference acquisition. Glx is the resonance from the glutamate/glutamine complex.

Figure 3.

Parametric images of two sections through brain obtained by analysis of qMT data at 7T showing (left to right) (i) conventional T_1 -weighted image, (ii) map of pool size ratio (PSR), (iii) map of derived $R_1 (= 1/T_1)$ for free water pool, and (iv) map of k_{mf} the exchange rate of magnetization between macromolecular and free water pools. Black arrows show location of a multiple sclerosis lesion.

Figure 4.

(a) CEST z-spectra obtained from tumor and contralateral normal tissue in a rat brain bearing 9L tumor. (b) CEST z-spectra from white matter (WM) and gray matter (GM) in a healthy rat brain. Experiments were performed with a continuous wave CEST sequence with irradiation power of 1 μ T at 9.4 T. Note that four main CEST dips (APT, Amine, NOE at -1.6 ppm, and NOE at -3.5 ppm) can be identified.

Figure 5. CEST imaging of amides (an APT map) and hydroxyls (mainly a GAG or glycosaminoglycan map) in a human breast at 7T.

Figure 6. CEST images derived from the NOE peak at -1.6 ppm (a) and NOE at -3.5 ppm (b) at 9.4 T in a rat brain bearing a 9L tumor. Both NOEs are reduced in the tumor, with greater loss at -1.6 ppm.

Figure 7.

(a) CEST z-spectra and (b) conventional high resolution ^1H NMR spectra of immobile and mobile NAA. Experiments were performed with a continuous wave CEST sequence with irradiation power of $0.25\ \mu\text{T}$ at 9.4 T. The ^1H NMR spectra were obtained using PRESS on the same voxel. The CEST NOE negative peaks appear only in the immobilized sample, whereas the converse is true for conventional NMR spectra.