Molecular Imaging and Stroke

Michael E. Moseley, PhD

Molecular imaging (MI) is a rapidly developing field that encompasses many new (and old) imaging modalities that seeks to present patient-specific and disease-specific molecular and genetic information in conventional 2-dimensional and 3-dimensional anatomic imaging readouts. The foundations of MI are based on the fusion of a “promoter” agent that will be altered in a particular environment or disease state with an observable “reporter” agent that would register any change in the signal or contrast from the promoter. Much of MI is being done in experimental models of cancer, but only some of the developments can be scaled up to a clinical reality for eventual diagnostic and prognostic usefulness in stroke. The most commonly used modalities in MI research have been optical-based near-infrared or visible light sensors, bioluminescence,1,2 cameras sensitive to the firefly luciferase–luciferin generation of green light, nuclear medicine (NM)-based single-photon and positron tomography, and now, most recently, magnetic resonance (MR).

Optical imaging techniques developed early for molecular and cellular biology using a wide variety of wavelengths. The noninvasive imaging in vivo with light photons has largely come from the advances in targeted bioluminescence probes, near-infrared fluorochromes, activated near-infrared fluorescence agents, and primarily from light emitted from the luciferase entity (reporter) in the presence of a substrate (luciferin).1,2 Optical techniques using multiple wavelength probes such as quantum dots holds the potential for multichannel imaging. However, most fundamental to the widespread use of in vivo optical imaging of stroke or ischemia in living subjects is the difficulty of detecting light from the brain, primarily because of the presence of the skull. Because of this, MI using optical techniques has been largely limited to nonneuro studies of cancer in rodent models. Nonetheless, optical imaging has a bright future in neuroimaging research. Advances in the use of near-infrared for diagnostic,3-5 prognostic,6,7 and, more recently, in potential stroke therapy applications8-10 have been exciting. Look for near-infrared to open research areas in mapping of edema, oxygenation dynamics, blood flow (with injected contrast agents), and NAD/NADH turnover in stroke applications.

Nuclear medicine molecular imaging methods such as single photon emission CT and PET are much more relevant and indeed have been used for decades in stroke and neuro applications such as brain mapping, flow mapping, metabolism, and so on. The advent of small single photon emission CT (micro single photon emission CT), PET (microPET), and CT scanners has made the NM methods the main focus of stroke research. Here, NM relies solely on the design and use of injected MI probes to provide the imaging signal contrast. The term “probe” is commonly used to refer to the tracer, beacon, smart probe, reporter agent, contrast agent, and/or nanoparticle used. The biggest advantages of NM PET aside from the near-picomolar probe sensitivity is that existing probes can be modified with a radiolabel while minimally perturbing the parent molecule, again indirectly related to the exquisite sensitivity of NM to the radiolabel.11 This has not been widely possible for optical or MR, because the need for the contrast agent has involved bulky, less sensitive probes such as gadolinium in MR. Conversely, because NM relies on injected tracers, soft tissue contrast and resolution was poor. With the advent of PET/CT hybrids, however, 2-dimensional and 3-dimensional fusions provide both agent sensitivity and tissue contrast. The PET/CT hybrid is a potent methodology in stroke. Aside from the excellent usefulness of CT in stroke with hemorrhage detection, CT angiography, CT perfusion as well as xenon-enhanced CT, the PET/CT hybrid will allow for the addition of PET detection of receptor ligands, cerebral metabolism and blood flow, neuronal integrity as well as hypoxia and apoptosis markers.12-14

MR, with superb specificity to the water hydrogen proton, lacks a relatively low sensitivity. MR using other nuclei such as carbon-13 or fluorine-19 is much less efficient. This occurs because of the Boltzmann Distribution; that is, that at 1.5 Tesla, only approximately 5 to 10 protons per million ever contribute to the MR signal. As we will see, new methods of dynamically increasing this inherently low signal magnetization by as much as 5 orders of magnitude are now available, termed “hyperpolarization.”15-17 Using metabolically active carbon-13 labeled sugars, hyperpolarized MR will one day be routinely used in patients with stroke for mapping dynamic glucose usefulness, pH, oxygen extraction, and flow all in one examination.

MR contrast for molecular imaging in stroke research is derived from 4 major sources: endogenous contrast from the water proton dynamics in the microenvironment, endogenous chemical shifts of proton-bearing metabolites such as lactate,
injection of exogenous contrast agents that report water proton changes, and finally from detection of nonproton nuclei such as hyperpolarized carbon-13-labeled agents such as sugars.

Mapping water proton dynamics in clinical stroke is the backbone of diffusion-weighted and perfusion-weighted imaging. Adding to this, arterial spin-labeling of vascular flow protons has become a clinical use in numerous neuro MR protocols needing noninvasive and quantitative blood flow maps.\(^{18,19}\) More recently, several novel MR measures of brain water have found potential in stroke imaging; VASO\(^{20,21}\) maps the vascular space occupancy in brain and can do so in 3-dimension and in near real-time. This may prove ideal for mapping intracranial pressures, sickle cell dynamics, vasospasm effects, and cerebral blood volume changes in neurovascular diseases. Another noninvasive MRI method maps amide–water proton exchange interactions and, considering that each nucleic acid contains amide protons, has led to a novel approach to pH imaging. The method, CEST or chemical shift saturation transfer, also called amide–proton transfer,\(^{22,23}\) takes advantage of the sensitivity of the transfer to the local pH. In particular, the chemical exchange between the bulk water protons and the amide protons from endogenous proteins and peptides has been shown sensitive to ischemic tissue acidosis and may serve as a new surrogate metabolic imaging marker for stroke. Studies suggest that ischemic tissue with amide–proton transfer deficit correlates with the final lesion measured on follow-up T2. The perfusion lesion tends to overestimate stroke infarction, whereas the diffusion lesion underestimates it.\(^{24}\) Neuroimaging has been the biggest T1-shortening effect (largest T1 relativity, r\(_1\)) of those tested. The gadolinium chelates can be designed to bind to various in vivo compounds (such as blood pool albumin or clot-rich fibrin), receptors, antibodies, and so on. Other designs provide specifically active gadolinium chelates that alter tertiary structure (and thus their proton relativity) in the presence of lactate, sugars, differing pH, calcium, and a host of other interesting agents.\(^{25}\) The idea of functionally active agents is a good one; however, the lowest concentrations of the gadolinium chelates needed is still in the micromolar range. Other T1-shortening agents noted are the manganese ions, which are small enough to enter the neuronal white matter tracts through the eye (for example) and provide a trace of not only white matter integrity and structure, but also differences in tract activations.\(^{26}\)

The use of T2*-shortening agents containing superparamagnetic iron particles can provide an order of magnitude larger contrast effect than corresponding concentrations of the T1-shortening gadoliniums. This has created a whole body of literature around the labeling of stem or progenitor cells with iron nanoparticles to visualize the time course of cell trafficking, for example.\(^{27–29}\) Although this approach has been popular, it is difficult to imagine the clinical usefulness of iron-laden cells, especially in the setting of stroke. Nonetheless, from the observation that iron nanoparticles can bind specifically over time (in lymph nodes for example), it is conceivable that receptor binding or iron collection in cells or clots can be guided for clinical applications.

Because of the ultrasmall particle size, iron can be conveniently labeled to various in vivo receptors, agents, micelles, liposomes, nanotubes, and so on. In addition, iron nanoparticles are easily dual- or triple-labeled for eventual PET/MR or optical/MR hybrid imaging. The major consideration of iron (and T2*-shortening agents in general) is the need for good T2* sensitivity in the MR sequence, which requires heavy T2* weighting with often inferior signal and numerous artifacts. This occurs largely because of the course structure of the iron particles. It recently became apparent that the microstructure of the nanoparticle could be engineered with nanotechnology to be able to alter the local magnetic field T2* effect across the particle.\(^{30}\) This would have the effect of providing a multispectral effect of the particle; in other words, the chemical shift, T1, and T2* effects of each particle may differ depending on the microenvironment. This would provide a novel multichannel approach to pH imaging, for example.

The observation that the structure of the chelate around a gadolinium nucleus can have a profound effect on the local proton T1-shortening effect has created the design of functionally specific (“sensing”) contrast agents that are sensitive to lactate, for example.\(^{31,32}\) The presence of the lactate alters the tertiary structure of the chelate, which in turn affects the ability of the water proton to approach the gadolinium and experience the T1 shortening. The result is an image enhancement related to the presence and concentration of the molecular being “sensed.” However, the issue again is that most T1-shortening agents operate down only to the micromolar range because of the small changes in T1 relativity of these agents. Nonetheless, the idea that the proton microenvironment can be altered by functionally specific molecules or receptors has led to a true new frontier in MR for MI. This involves the broad understanding that some lanthanide nuclei such as gadolinium are superior T1-shortening agents, whereas dysprosium is a superior T2-shortening agent and that thallium or europium are outstanding chemical shift agents. The reason for the excitement in MR today is the observation and the realization that shift agents such as europium have extremely large effects on the CEST transfer between amides and protons and the “sensing” effects can be extended to the nanomolar range for the “paraCEST”
agents. This has been coupled with several new “amplification” strategies for the development of both gadolinium-based T1-shortening agents as well as for paraCEST “sensing” contrast agents designed specifically to detect even smaller concentrations of molecules or enzymatic activity in living systems. The need for these sensing MR contrast agents is justified by efforts to visualize fine structures in living tissue and to increase the molecular specificity and contrast agent sensitivity of MRI.

Given the excitement over the paraCEST approach to MI, one newly emerging MR methodology that may soon rival PET for MI is that of MR “hyperpolarization” and the astonishing rapid development and broad potential of this idea. Hyperpolarization is accomplished by pumping microwave energy into supercooled carbon-13 (for example) nuclei sitting in a strong magnetic field. Why carbon-13? The hyperpolarization decays with a T1 half-life; C13 T1s can be several seconds long. In fact, most nuclei can be hyperpolarized, including water hydrogen protons. The energy pumping increases the carbon-13 magnetization approximately 100 000-fold and provides a strong C13 MR signal with even a single pulse. MR images of carbon can be achieved in a single shot with signals rivaling those of protons. These procedures were first applied to noble gases such as helium-3 and xenon-129 (prompting many to pursue xenon-enhanced MRI for cerebral blood flow similar to Xe-CT) and then to small carbon-13 labeled containing metabolically active molecules such as pyruvate and bicarbonate. The only considerations to the enormous potential of hyperpolarized MR are toxicity issues and the T1 half-life of the polarized compound.

Hyperpolarized pyruvate has been the most researched molecule because it is involved in the energy production of most tissues and is metabolized into lactate, alanine, and CO2 in equilibrium with bicarbonate. Initial studies have looked at monitoring pyruvate metabolism in organs (heart, kidney) during ischemic episodes, although brain ischemia would also be possible. Typically, after 15 minutes of occlusion in the pig heart, the bicarbonate signal level in the affected area is reduced by almost half. These pig studies demonstrate that metabolic imaging with hyperpolarized pyruvate is feasible such that the changes in concentrations of the metabolites within a minute after injection can be detected and metabolic maps constructed.

More importantly perhaps, because alterations in tissue pH occur in most pathological processes, the capability to clinically image tissue pH could offer new ways of detecting disease and response to treatment. In a recent study, tissue pH was imaged in vivo from the ratio of the signal intensities of hyperpolarized bicarbonate (polarized at 16%) and CO2 in equilibrium with bicarbonate. None.

References


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