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Early Experience of translating pH-weighted MRI to image human subjects at 3 Tesla

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Abstract

Background and Purpose—In acute stroke, mismatch between lesions seen on diffusion-(DWI) and perfusion-weighted (PWI) MRI has been used to identify ischemic tissue prior to irreversible damage. Nevertheless, the concept of PWI/DWI mismatch is oversimplified and the ischemic tissue metabolic status and outcome are often heterogeneous. Tissue pH, a well-regulated physiological index that alters upon disrupted tissue metabolism, may provide a surrogate metabolic imaging marker that augments the DWI and PWI for penumbra imaging.

Methods—pH-weighted MRI was obtained by probing the pH-dependent amide proton transfer between endogenous mobile proteins/peptides and tissue water. The technique was validated using animal stroke models, optimized for human use, and preliminarily tested for imaging healthy volunteers.

Results—pH-weighted MRI is sensitive and specific to ischemic tissue acidosis. pH MRI can be optimized for clinical use, and a pilot human study showed it is feasible at a standard 3 Tesla MRI scanner.

Conclusions—Ischemic acidosis can be imaged via an endogenous pH-weighted MRI technique, which complements conventional PWI and DWI for penumbra imaging. pH-weighted MRI has been optimized and appears feasible and practical in imaging human subjects. Additional study is necessary to elucidate the diagnostic use of pH MRI in stroke patients.

Keywords

Acidosis; Acute Stroke; DWI; PWI; pH

Introduction

The development of diffusion and perfusion MRI has improved our understanding of acute ischemic tissue damage, and is increasingly utilized to help guide stroke treatment [1–3]. Specifically, while thrombolytic therapy can restore blood flow and improve patient outcome, the clinic usage of tissue plasminogen activator (tPA) is still limited due to its narrow three-

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Conflicts of Interest/Disclosures

The authors have no conflict of interest.

hour (or, in some locations, 4.5 hour) treatment window [4–8]. One approach to overcoming this problem is to develop non-invasive penumbra imaging that might identify patients who may potentially benefit from late tPA treatment with minimal adverse effects [9–14]. Because ischemic tissue damage is heterogeneous, salvageable ischemic penumbral tissue may be present well beyond the conventional tPA treatment window [15]. Perfusion-weighted (PWI) and diffusion-weighted (DWI) MRI are the most well-established imaging techniques being used to detect regions of reduced blood flow and cytotoxic edema, respectively [16, 17]. As such, the PWI/DWI mismatch is postulated to represent ischemic tissue that has not yet undergone severe tissue damage, and sometimes used operationally to define the ischemic penumbra [1, 18, 19].

Whereas the PWI/DWI mismatch provides important pathophysiological insight and can be readily identified, ischemic tissue damage is complex and multifactorial, and the PWI/DWI mismatch provides only an approximation of the penumbra [20, 21]. It has been observed that the final infarct volume is generally smaller than the initial PWI lesion, yet larger than the acute DWI lesion. In addition, the DWI abnormality is energetically heterogeneous and portions of some DWI lesions may reverse if treated promptly [22–24]. Hence, despite being the most practical method at the present, the concept of PWI/DWI mismatch may be somewhat oversimplified. New imaging markers could augment existing penumbral imaging and provide greater insight into disease pathophysiology and perhaps, if validated, serve to help guide treatment decisions such as late thrombolysis.

As energy production is vital for cell viability, monitoring tissue metabolism may offer additional insights about ischemic tissue damage and outcome [10]. It has been shown that cerebral oxygen and glucose metabolism are disrupted at blood flow levels higher than those that cause infarction, and therefore, measurement of oxygen and/or glucose metabolism may provide a sensitive index of early ischemia prior to irreversible damage. Specifically, in ischemic tissue lactic acid is produced due to anaerobic glycolysis, causing tissue acidosis (decreased pH) [25]. The cellular energy imbalance is exacerbated due to the reduced buffering capacity of bicarbonate at acidic pH, hypoperfusion, and disrupted oxygen and glucose metabolism, hence, tissue pH may fall even further. As a result, essential ATP-dependent functions such as the critical enzyme Na/K-ATPase are compromised, and without prompt treatment, ischemia will eventually lead to cell death and irreversible tissue damage. Therefore, tissue pH imaging may serve as an important physiological biomarker for tissue viability and dysfunction, complementing the conventional hemodynamic and structurally-based MRI. However, historically, non-invasive *in vivo* pH imaging has been quite challenging. While ^{31}P and lactate magnetic resonance spectroscopy (MRS) may reflect tissue metabolic state and therefore have been actively investigated, their sensitivity and spatiotemporal resolution are not yet adequate for imaging acute stroke [26].

To address this unmet biomedical need, chemical exchange saturation transfer (CEST)-based pH MRI has been recently developed [27, 28]. As CEST MRI probes pH via the abundant tissue water signal, its pH sensitivity is significantly higher than that of the conventional MRS-based methods, and remains promising for *in vivo* use. Amide proton transfer imaging, a specific form of CEST MRI that utilizes the composite amide protons from endogenous mobile proteins and peptides, is particular suitable for *in vivo* pH imaging [29, 30]. Specifically, endogenous amide proton exchange is dominantly base-catalyzed, and its exchange rate and hence, pH MRI signal, decreases at the acidic pH present during ischemia. Our preclinical animal stroke studies have demonstrated that pH MRI deficit detects not only the same tissue as evident on DWI, but also additional hypoperfused tissue with altered oxygen metabolism, strongly suggesting that it may serve as a novel metabolic imaging marker for the ischemic penumbra that does not require injection of an exogenous contrast agent. [31]. These observations highlight the potential value of performing pH imaging in acute stroke patients. Toward this goal, we have optimized an *in vivo* pH-weighted MRI protocol and developed necessary image processing tools, and preliminarily tested the pH-weighted MRI at 3 Tesla [32]. Here, we describe this methodology and present pilot human pH imaging.

Materials and Methods

Animal Model

Animal studies were conducted following an institutionally approved protocol, Partners. Adult male Wister rats were anesthetized using the isoflurance regimen (1–1.5% during study, 70% N_2O/30% O_2), and had standard physiological monitoring throughout the study. Global ischemia was induced in three animals via KCl injection through the femoral artery, and additional four animals were subjected to filament middle cerebral artery occlusion (MCAO).

Healthy Volunteer

All studies were approved by institutional review board, Partners (IRB). A healthy male volunteer 34 year old was scanned at 3 Tesla, and consent forms were obtained prior to study.

MRI

Animals were imaged at Bruker 4.7T. The pH-weighted MRI includes continuous wave (CW) saturation prior to single slice spin echo echo planner imaging (EPI) readout (slice thickness of 2.5 mm). The image matrix was 64 by 64, with an isotropic in-plane resolution of 0.5 mm. The repetition time and echo time were TR/TE=6500ms/32 ms, and 16 signal averages (NA) were obtained. The irradiation RF amplitude was 0.75 μT (~30 Hz) with offset varied serially from −6 to 6 ppm per 0.5 ppm. In addition, standard T_1, T_2 and diffusion images were acquired. For the global ischemia model, MRI was acquired both before and immediately after cardiac arrest. For the focal ischemia model, point resolved solvent suppressed spectroscopy (PRESS) magnetic resonance spectroscopy (MRS) was also obtained, with two regions of interest (ROIs) of 4 mm^3 each positioned in the ipsilateral ischemic lesion and contralateral normal areas (TR/TE=1000ms/144 ms, and NA=1024).

Clinical implementation was done on a 3T Siemens Tim Trio scanner (Siemens, Erlangen Germany), using a 32-channel RF receiver head coil. RF irradiation was composed of a train of π pulses, each with a duration of 20 ms with an interval of 20 ms delay (50% duty cycle), interleaved between single shot EPI readout. We used TR/TE=5000ms/12 ms, NA=4 and the total scan time was 2.5 min. The RF offsets were −3.5, 2, 2.75, 3.5, 4.25 and 5 ppm. In addition, standard field mapping was acquired (TR=100ms, ΔTE=2.46 ms), with a scan time of 12 s. Eight slices were acquired and each slice thickness was 6 mm, distance factor of 25%. The FOV was 192 × 192 mm with image matrix being 64 by 64, for both pH MRI and field map to facilitate co-registration, and zero-filled to 128 by 128. Motion artifact was corrected using the standard motion correction FMRIB's linear image registration tool (MCFLIRT) [33, 34].

Results

Fig. 1 shows that the pH-weighted MRI measurements changed noticeably upon global ischemia. Specifically, Z-spectrum was obtained by monitoring tissue water signal while the RF irradiation was swept around water resonance. While this technique is similar to magnetization transfer (MT) MRI, it is important to note that its RF irradiation has been optimized for sensitizing proton exchange of endogenous proteins/peptides [30]. Notably, the magnetization transfer ratio (MTR) was 68.6 ± 1.1% under normal condition, and increased to 72.4 ± 0.4% postmortem, consistent with the notion that endogenous amide proton exchange is dominantly base-catalyzed around physiological pH (Fig. 1a). The maximal change in Z-spectral intensity was 3.8% peaked at 3.5 ppm, representing the composite amide proton chemical shift (Fig. 1b). This suggested that tissue pH can be assessed via the pH-weighted CEST/APT MRI. In addition, the apparent diffusion coefficient (ADC) decreased from 0.84 ±0.01 to 0.61±0.02 $\mu m^2/ms$. In addition, T_1 and T_2 changed from 1.41±0.03 s and 58.8±0.7 ms to 1.36±0.13 s and 56.3 ± 0.9 ms, respectively, likely attributable to the slightly decreased body temperature and edema during global ischemia.

We also preliminarily compared pH-weighted MRI with lactate MRS using the focal ischemia animal model. The pH-weighted MRI was calculated as MTR asymmetry, (i.e., $I_{ref} - I_{label}/I_0$), where I_{ref} and T_{label} are reference and label with RF applied at −3.5 and 3.5 ppm, respectively, and I_0 is the scan without RF irradiation. The pH MRI showed a large pH deficit across the hypoperfused right MCA territory (Fig. 2a). In addition, the MRS showed that the lactate level in the ipsilateral stroke lesion was greatly elevated, but lactate was nearly absent in the contralateral normal region, similar as the findings of Jokivarsi et al. [35] (Fig. 2b). Moreover, a subtle NAA decrease was observed, consistent with early neuronal damage during acute ischemia.

Fig. 3 shows a pilot pH-weighted MRI of a normal volunteer. It is important to point out that the field inhomogeneity was compensated based on the co-registered field map. In addition, pH-weighted image was calculated by taking the difference between the label scan (3.5 ppm) and mean of two reference scans (2 and 5 ppm) instead of the commonly used MTR asymmetry analysis. This alternative method was significantly less susceptible to the intrinsic MTR asymmetry shift. The pH-weighted MRI appeared reasonably homogeneous within the brain, which should facilitate detection of subtle pH lesion in acute stroke patients.

Discussion

Our work confirms reports of pre-clinical pH-weighted MRI [31]. We extend earlier work by translating this method to the clinical setting, and obtained promising pilot data from a healthy volunteer. Given that pH is well regulated under normal physiological conditions, it may serve as a specific surrogate biomarker for altered tissue metabolism, particularly useful for characterizing heterogeneous ischemic tissue damage. Our preclinical study showed that abnormalities detected on pH-MRI correlate with much of the PWI/DWI mismatch [31]. While these pre-clinical studies have been crucial to verify the methodology, considerable effort has been required for clinical translation. Specifically, we have optimized the image acquisition and processing, including optimizing the pulsed-RF irradiation, correcting motion and field inhomogeneity artifacts, and compensating the concomitant intrinsic MTR asymmetry shift [36]. The scan time has been significantly reduced so that patients may be imaged with only minimal or no interference with their clinical care. It is important to point out that additional fast multi-slice pH MRI pulse sequence and image processing algorithms are currently being evaluated, which may further facilitate routine clinic use of pH MRI. For instance, tissue pH MRI may serve as an imaging metabolic biomarker for guiding late thrombolytic treatment and evaluating novel therapeutics that aims to improve tissue metabolism [37].

Our study utilized the endogenous amide proton signal and provided only pH-weighted information. However, pH-weighted MRI contrast could change slightly in the presence of changes in relaxation times, such as in the setting of vasogenic edema. While such issues are presumably not a major problem for hyperacute stroke patients, these confounds might be present in the setting of subacute or chronic ischemia. In addition, our current study utilized reference images around the composite amide proton offset instead of the conventional reference scan at −3.5 ppm to minimize concomitant MT asymmetry effect. In summary, our goal is to fully develop pH MRI and evaluate its diagnostic utility in clinic, and ultimately to augment our diagnostic capability of stroke and other debilitating diseases.

Summary

Our study demonstrated a non-invasive endogenous pH-weighted MRI technique in an animal model of cerebral ischemia, and translated it to initial human use. This work confirms that pH-weighted MRI is feasible in the clinic, which may ultimately provide complementary information to the routine PWI and DWI scans. Additional study is needed to test whether pH MRI may augment routine clinical MRI and ultimately help guide stroke treatment.

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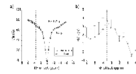

Fig. 1.

Evaluation of *in vivo* pH-weighted MRI intensity. Z-spectra were acquired shortly before and immediately after postmortem, which showed signal increase around 3.5 ppm, the composite amide proton chemical shift (Fig. 1a). The difference of Z-spectra clearly showed pH-induced decrease of exchange rate, consistent with the fact the amide proton exchange is dominantly based catalyzed.

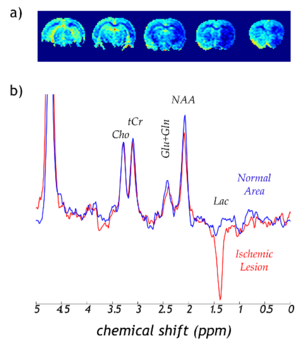

Fig. 2.

MRI and MRS characterization of acute focal ischemia. a) pH-weighted MRI showed acute pH deficit in the hypoperfused brain region. b) MRS detected significantly elevated lactate, suggesting ischemic acidosis. In addition, subtle decrease of NAA was observed, an early maker of neuronal damage.

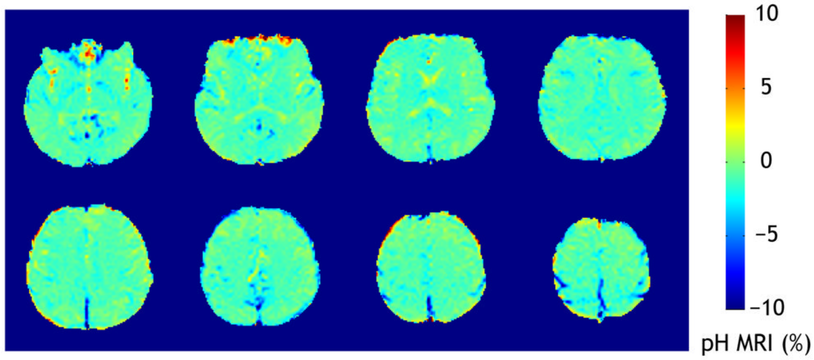

Fig. 3.
pH-weighted MRI of a normal subject at 3 Tesla. The B0-inhomogeneity compensated pH-weighted MRI appeared reasonably homogeneous within the brain, which should facilitate detection of pH lesion in stroke patients.