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*Stroke.* 2001;32:2797-2802
doi: 10.1161/hs1201.099414

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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Spectroscopic Assessment of Alterations in Macromolecule and Small-Molecule Metabolites in Human Brain After Stroke

Glenn D. Graham, MD, PhD; Jong-Hee Hwang, PhD; Douglas L. Rothman, PhD; James W. Prichard, MD

Background and Purpose—We sought to measure the temporal evolution and spatial distribution of lesion macromolecules and small molecules (lactate, N-acetyl compounds, creatine, and choline) in stroke patients by using short echo time in vivo proton MR spectroscopy.

Methods—Single-voxel spectra with TE=22 ms were obtained with and without inversion recovery suppression of small-molecule resonances from 30 examinations of 24 patients 3 to 214 days after stroke. Subtraction of the suppressed from the unsuppressed spectra yielded metabolite spectra without overlap from macromolecules. Two-dimensional spectroscopic images were acquired with macromolecule and small-molecule suppression from 5 additional patients.

Results—Macromolecule signals were elevated in lesions relative to normal brain and tended to increase in the subacute period, even as lactate peaks declined. Regions of increased lactate, increased macromolecule signal at 1.3 ppm, and decreased N-acetyl compounds were closely correlated in the 2D spectroscopic images.

Conclusions—Short echo time spectra can be acquired in vivo in a manner that improves signal-to-noise ratio over long echo experiments and resolves overlapping macromolecule and small-molecule signals. The prominent macromolecule signals seen in the subacute period in association with persistently elevated lactate may represent mobile lipids in macrophages or other cells. (Stroke. 2001;32:2797-2802.)

Key Words: nuclear magnetic resonance ■ lipids ■ lactic acid ■ cerebral infarction ■ cerebrovascular accident

In vivo proton magnetic resonance spectroscopy (MRS) has consistently detected elevated brain lactate after human stroke that may persist for many months.1-3 The origin and significance of this lactate may vary at different times during the evolution of the infarct. While lactate is produced acutely by ischemia, lactate seen beyond the first 72 hours may reflect the presence of macrophages and other leukocytes.6,7 Previous studies2,8 used long echo times (TEs) of 270 ms to optimize lactate and eliminate coresonant lipid signals. However, the use of long TEs has several drawbacks. Signal magnitudes for all peaks are decreased due to T2 relaxation during the long echo interval, and signals from rapidly relaxing compounds, such as lipids and proteins within injured tissue, may disappear entirely.

Recently introduced short TE methods can measure both small-molecule peaks from lactate, N-acetyl compounds (NA), creatine, and choline, and macromolecule signals in vivo.9 We have applied these methods to record changes in the small-molecule and macromolecule spectrum after stroke using single volume techniques. We have also used a 2-dimensional spectroscopic imaging (2D-SI) implementation of these methods to assess the spatial extent and coincidence of the biochemical changes.

Subjects and Methods

Patients
This study was reviewed and approved by the Human Investigation Committee of the Yale University School of Medicine. Informed consent was obtained from all subjects before MR examination. A total of 33 single-voxel studies were performed on patients with cortical or deep white matter ischemic strokes identified on a scout image, from which 30 usable spectra were obtained. Twenty-one subjects (11 men and 10 women; average age 64 years, range 38 to 87 years) underwent MRS within 10 days of presentation, and 6 follow-up examinations on days 11, 19, 32, 34, 67, and 214 after infarction were carried out on 5 subjects. Three other stroke patients were examined once on postinfarct days 13, 23, and 125 (2 men and 1 woman, aged 56, 66, and 67 years, respectively). In 13 of the subjects, a spectrum from a homologous region of the contralateral cerebral hemisphere was also acquired.

Five additional patients (4 men and 1 woman; average age 52 years, range 36 to 65 years) underwent 2D-SI an average of 5.4 days after symptom onset (range 2 to 9 days), and follow-up studies were

Received February 15, 2001; final revision received June 11, 2001; accepted July 10, 2001.
From the Departments of Neurology, Radiology, and Neuroscience, University of New Mexico School of Medicine and Albuquerque VA Hospital (G.D.G.), Albuquerque, NM; Department of Medicine, Albert Einstein College of Medicine (J.-H.H.), New York, NY; and Departments of Diagnostic Radiology (D.L.R.) and Neurology (J.W.P.), Yale University School of Medicine, New Haven, Conn.
Correspondence to Glenn D. Graham, MD, PhD, Neurology/127, VA Medical Center, 1501 San Pedro Drive SE, Albuquerque, NM 87108. E-mail graham@unm.edu
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completed in 2. Four had cortical and 1 had a large subcortical infarct.

**Spectroscopy**

The studies were performed on a 2.1-T magnet (Oxford Magnet Research) using a birdcage head resonator. An 8-slice axial T2-weighted MRI (TR/TE=2050/95 ms) was used to identify the location of the stroke in all studies. In the single-voxel spectroscopy experiments, an 8 cm³ cube within the infarct and a second volume in a homologous region of normal brain, or in 2 cases two 2×1.6×2 cm³ regions from a 1D spectroscopic image, were selected for study. Localized shimming over the selected regions of interest was performed before MRS in all cases, and outer volume suppression pulses were positioned in the anterior-posterior and left-right dimensions to eliminate lipid signals arising from outside of the brain. A total of 256 iterations of the stimulated echo pulse sequence were then acquired (TR/TE=3700/22 ms) from the voxel containing the stroke by using a narrow, frequency-selective hyperbolic secant (sech) inversion recovery (IR) pulse as well as sinc pulses and crusher gradients applied during the mixing time (TM) to achieve water suppression. A second spectrum was acquired under identical conditions but used a wide-band hyperbolic secant IR pulse to produce suppression of the long T1 small-molecule metabolites as well as water, based on differences in relaxation with the macromolecule resonances, which have short T1 times. Where patient compliance permitted, a single unsuppressed spectrum was also acquired from the preselected contralateral brain region.

The raw spectra were zero-filled, exponentially line-broadened by 3 Hz, and Fourier transformed. Spectra were phased and baseline corrected, if necessary, to compensate for residual water. Because line widths were similar in all spectra, resonances were measured as peak heights to eliminate the effect of overlapping signals. Curve fitting is another method of resolving overlapping MR signals, but it assumes that the identities and line shapes of the component signals are known. Because a major goal of this research was to study macromolecule signals not yet fully characterized, we felt that such assumptions were not justified. To compare data across studies, the peak heights were scaled by the radiofrequency voltage required to generate a 90° pulse.

In the 2D-SI experiments, 4 outer volume sinc pulses were positioned in the anterior-posterior and left-right dimensions to suppress contaminating signals from lipids arising outside the selected region. The power to generate a 90° pulse was determined, localized first-order shims were adjusted, and the water inversion pulse power was set for each experiment. On the basis of the scout image, an 8×8×1 cm³ (for metabolites) or 8×8×1.5 cm³ (for macromolecules) axial slice was selected for 2D-SI. Phase encoding along the anterior-posterior and left-right axes divided the volume into voxels with a nominal in-plane size of 1×1 cm². Localized short TE spectra (TE/TM=24/28 ms, 256 iterations) were then obtained by using the stimulated echo (STEAM) pulse sequence, with each requiring about 13 minutes. To separate macromolecule from small-molecule resonances, 2D-SI data sets were acquired in 2 ways: with narrow band sech pulse IR water suppression and broadband suppression of short T1 signals from macromolecules (TR/TIR=2940/179 ms) to produce the small-molecule spectra, and with broadband IR suppression of water and long T1 small molecules (TR/TIR=3045/1060 ms) to yield the macromolecule spectra. If patient tolerance permitted, 2 sets of 2D-SI data were acquired with metabolite and with macromolecule suppression, for a total experiment time of 80 minutes. The 2D-SI data were apodized, and the metabolite (but not macromolecule) data were zero-filled in the spectral dimension from 1024 to 2048 points. All spectra were then Fourier transformed and modulus corrected. The area under each peak was determined by numerical integration and used to generate maps of lactate, NA, and the macromolecule signal at 1.3 ppm. Full details of the 2D-SI acquisition and processing methods used are given elsewhere.9

**Results**

Figure 1 shows the lesion and selected region of interest for a 67-year-old woman studied 23 days after subcortical stroke.

The single-voxel spectra acquired from this region are depicted in Figure 2. Subtraction of the metabolite-suppressed from the corresponding narrow sech pulse “raw” spectrum yields a difference spectrum containing only small-molecule peaks uncontaminated by lipid or protein resonances. The macromolecule signals (peaks a through c in Figure 2B) were measured directly from the metabolite-suppressed spectrum.
Figure 2C shows lactate, NA, creatine, and choline on a flat baseline after removal of the broader signals originating from macromolecules. Spectra from within an infarcted region of a 72-year-old man’s brain examined at 3 different times after stroke are shown in Figure 3A, scaled to compensate for signal-to-noise improvements produced by an instrumental upgrade performed during the follow-up period. Lactate was acutely increased and declined over time, although a lactate doublet was clearly present even 7 months after stroke. The macromolecule signal at 1.3 ppm was greatest at 11 days, and then declined by the final study. Neither lactate nor macromolecules appear in the 2 spectra acquired from contralateral, noninfarcted brain (Figure 3B).

Combining all single-voxel MRS data acquired at all of the time points, the average macromolecule signals occurring at 1.3 and 2.1 ppm from all 30 spectra were increased in the infarct by 86% ($P=0.063$, $t$ test) and 296% ($P<0.0002$, $t$ test) compared with control normal brain regions (averaged from 13 spectra); in the latter case the increase was statistically significant. Comparing only the paired data available from 13 experiments on 11 different subjects, in which spectra were recorded from both the infarct and the contralateral hemisphere on the same day, macromolecule signals at 0.9, 1.3, and 2.1 ppm were increased in the lesion by 28% ($P=0.081$, paired $t$ test), 157% ($P=0.024$), and 358% ($P<0.0001$), respectively. Figure 4 shows the average lesion lactate and 0.9 ppm and 1.3 ppm macromolecule signals for all of the spectra acquired, grouped by poststroke day. While lactate was greatest in the earliest studies ($P<0.0001$, 1-way ANOVA), the macromolecule peaks at 1.3 ppm were greatest in the subacute period ($P<0.037$), whereas changes in the 0.9-ppm signal were not statistically significant ($P=0.119$). On average, the macromolecule signal at 1.3 ppm increased over the first several weeks after stroke before declining in the latest studies.

Again combining all the single-voxel MRS data, lesion lactate was elevated within the stroke in all cases, and declined with time in those patients studied more than once. NA, creatinine, and choline were significantly decreased by 56%, 46%, and 34%, respectively ($P<0.002$, $t$ test), within the lesion (average from 30 spectra) compared with studies within the uninfarcted hemisphere (average from 13 spectra). Unresolved amino acid resonances at about 2.3 and 2.7 ppm were also routinely measured within the stroke and were decreased by 47% and 48% ($P<0.0001$) from levels in normal brain.

We examined the possible effect of patient age on the single-voxel MRS results. While subjects ranged from 38 to...
87 years of age, nearly two thirds of the subjects were between the ages of 60 and 80 years. Also, because MRS from voxels contralateral to the stroke were used as controls, we have compensated (at least in part) for any changes in normal brain metabolite levels occurring with aging. Our primary classification was by poststroke day, and our sample size is too small to also stratify by patient age. However, a comparison of the early MRS studies (days 3 to 5) performed on patients aged 65 years or less (6 patients, average age 52) with those performed on patients over age 65 (8 patients, average age 75) revealed no statistically significant differences in mean lesion metabolite or macromolecule signals between the groups (individual probability value 0.15 to 0.85).

In the 2D-SI experiments, all subjects had elevated lactate and decreased NA signals in the region of the stroke, corresponding to the location of increased signal on the T2-weighted scout image. The peak at 1.3 ppm in the macromolecule 2D-SI was also markedly elevated in the region of the infarct in these studies, which were performed in the subacute period between 2 and 9 days after stroke. In all the cases studied, the zones of increased lactate and macromolecule and decreased NA signal were closely correlated in the spectroscopic images (see Figure 5). Correlations between elevated lactate, elevated macromolecule signal at 1.3 ppm, and reduced NA were examined by tabulating concordance or discordance of these abnormalities on a voxel by voxel basis in the initial study of 4 patients. The fifth subject moved between the small-molecule and macromolecule SI acquisitions and had to be excluded from this analysis. All of the correlations were found to be highly statistically significant (P<0.0001) by Cohen’s kappa test (κ=0.60 to 0.77). In the patients undergoing repeat studies, lactate declined but the macromolecule signal at 1.3 ppm further increased within the lesion over time as measured by the follow-up spectra.

**Discussion**

The ability to separate overlapping lactate and macromolecule signals in short TE MRS represents a significant improvement in the spectroscopic evaluation of stroke patients. The degree of elevation of macromolecules in acute stroke, and their variability between individual patients, underscores the importance of characterizing the macromolecule baseline independently for each subject if short echo MRS is to be used to quantify poststroke metabolites. While the origin of these rapidly relaxing signals remains to be determined definitively, several lines of evidence suggest that they may arise from mobile lipids within the infarct. The macromolecule peaks match lipid resonances at 0.9 and 1.3 ppm recorded from activated white cells and other cell lines containing cytoplasmic lipid droplets in vitro. Presence of elevated mobile lipid has been confirmed by MRS in a brain slice model after trauma in juvenile rats, and in preliminary experiments in an adult rat after temporary middle cerebral artery occlusion. In these studies, 2D correlation (COSY) spectroscopy was used to confirm that the elevated signals arise from fatty acyl groups in lipid and not from protein.

This study cannot directly ascertain the biochemical identity or origin of the cellular localization of the lipid resonances measured. Pathology from a patient who died shortly after MRS examination as well as neuropathology studies of the natural evolution of a cerebral infarct suggest that, at least in the subacute period, the lipids may reside within macrophages. Leukocytes are known to produce lactate even under aerobic conditions and contain prominent lipid peaks when activated. If borne out by further studies, the combined assessment of both lactate and lipid within infarcted brain may provide a noninvasive way to monitor the inflammatory response after stroke. Because modulation of inflammation during stroke recovery represents a potential target for therapeutic intervention, the ability to track leukocyte presence and activity by MRS may have important clinical implications.

We have also shown that abnormalities in metabolites and macromolecules occurring after stroke can be successfully detected and monitored by 2D-SI in human subjects, and their spatial distribution can be mapped and correlated. In the subacute period, spatial concordance of the lactate and macromolecule resonance at 1.3 ppm supports the hypothesis that these peaks may originate from the same histological source. Use of 2D-SI in the acute postinfarct period before...
macrophage infiltration might detect lactate not associated with lipid, which should reflect production at the time of the infarction or ongoing ischemia. Other signals detected by 2D-SI or the use of 13C-labeled glucose to probe the metabolic activity of early lactate may be able to distinguish these lactate pools.

Saunders et al used similar metabolite suppression single-voxel MRS methods to measure lipid, macromolecule, and metabolite signal changes in 6 stroke patients followed serially. They detected elevations in lipid resonances at 0.9 and 1.3 ppm that followed a different time course from changes in lactate or other metabolites, in good agreement with our findings. They reported the largest changes in the 1.3-ppm peak, which increased in magnitude in several of their patients during the subacute period. Their observation that lactate declines over the first few weeks while the lipid signals generally increase during the same time interval caused them to conclude that the elevated lipid signals could not arise from macrophages. Numerous studies have demonstrated that lactate declines in the weeks following infarction but remains detectable above normal values for many months. It is probable that most, if not all, of the early lactate is produced by direct tissue ischemia. As ischemic regions either progress to completed infarction or are reperfused, ongoing lactate production ceases, and lactate diffuses out of the lesion. This likely explains the declines in lactate seen over the first days after infarction. Lactate from macrophages is likely to be of a lesser magnitude and appear later than that produced by brain infarction. This may explain the different time courses observed for lactate and the macromolecule signal at 1.3 ppm, even though both are correlated spatially. While lipids may arise from postischemic changes within the brain parenchyma, such as membrane breakdown, results to date cannot exclude a macrophage source. The measurement of lipid resonances performed within the first few days after stroke may help to estimate the concentration of nonmacrophage lipid, since leukocyte infiltration is minimal before 48 hours after infarction.

In this study, only a limited number of patients underwent serial scans. No subject was examined before the third poststroke day, and only a few experiments were performed on patients in the chronic recovery period. Multiple studies of the same cohort of patients, while logistically challenging, may reduce the inherent intersubject variability caused by differences in infarct size and location, as well as idiosyncratic differences between individuals. Serial spectra beginning in the first 48 hours after stroke, before the onset of significant lesion infiltration by leukocytes, and continuing through the chronic recovery period will be required to define further the clinical significance of the macromolecule resonances we have observed. Although we found no obvious, large differences in MRS results with patient age, our ability to detect such a difference was limited by the sample size. Possible variations in subacute macromolecule and metabolite levels with age would be worth examining in a systematic way in a separate study. While 2D-SI provides data on the spatial distribution of metabolite and macromolecule signals in an examination time comparable to that of the 1D experiments, the signal-to-noise resolution of the individual spectra is reduced by the smaller voxels employed, limiting quantitative accuracy. The qualitative correlation of changes in MRS peaks, however, is important to a more complete understanding of the clinical and pathophysiological significance of these resonances.

Many of the recent advances in the MR evaluation of stroke have focused on diffusion and perfusion imaging. MRS measurements of lactate and NA within the first days after stroke have been shown to have prognostic value for clinical outcome measures, as has the size of the acute lesion on the initial diffusion-weighted image (DWI). In a recent study, the addition of data from long echo spectroscopic measurements within the lesion, as defined by diffusion-weighted MRI, has been shown to improve the prediction of outcome beyond the DWI lesion volume alone. The advent of diffusion imaging should spur a reassessment of the clinical role of MRS in acute stroke, because the region of interest can now be acutely visualized. There is reason to hope that the additional information provided by the measurement of macromolecule signals in short echo spectroscopy will further enhance the power of MRS to assess the severity of acute injury as well as monitor the evolution of stroke-related pathological changes.

Acknowledgments
This study was supported by a grant from the National Institutes of Health. The authors thank Charles Gasparovic, PhD, and Clifford Qualls, PhD, for helpful discussions.

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