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Proton MR Spectroscopic Measurement of Neurometabolites in Hepatic Encephalopathy during Oral Lactulose Therapy

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BACKGROUND AND PURPOSE: **MR imaging and MR spectroscopy are increasingly being used to determine response to pharmacologic therapy. Hepatic encephalopathy (HE) is characterized by abnormal cerebral metabolites, yet the response to lactulose and other anti-HE measures is still primarily determined by using arbitrary categorical clinical rating scales, rather than MR spectroscopy. The purpose of this study was to determine whether MR spectroscopy could demonstrate relevant neurometabolic changes associated with lactulose therapy and thereby provide further support for the use of MR spectroscopy in clinical trials.**

METHODS: **Ten control subjects and 23 patients with grades I to III HE were studied by proton MR spectroscopy with imaging parameters of 2000/26 (TR/TE). Metabolic ratios were calculated for *myo*-inositol (mI)/creatine (Cre), choline (Cho)/Cre, (glutamine + glutamate) (Glx)/Cre, *N*-acetylaspartate (NAA)/Cre, and (Cho + mI)/Glx. A time series design trial was used in which eight patients with HE were compared before and after lactulose therapy (60 mL by mouth three times per day).**

RESULTS: **Relative to control subjects, HE was characterized by 43%, 64%, and 5% reductions, respectively, in mI/Cre, (Cho + mI)/Glx, and Cho/Cre. In comparison, Glx/Cre was increased by 75% and NAA/Cre was not changed. Therapy with lactulose was associated with increases of 29%, 37%, and 7%, respectively, in mI/Cre, (Cho + mI)/Glx, and Cho/Cre, as well as respective decreases of 15% and 42%, respectively, in Glx/Cre and HE grade. NAA/Cre did not change with lactulose therapy.**

CONCLUSION: **MR spectroscopy detects neurometabolic changes associated with pharmacologic therapy for HE. The metabolic ratios mI/Cre and (Cho + mI)/Glx are the most sensitive measures of lactulose effect. These data support the expanded use of MR spectroscopy as an adjunctive technique in pharmaceutical development and clinical trials for HE.**

Hepatic encephalopathy (HE) is a reversible metabolic delirium of major clinical importance that complicates end-stage liver disease (1, 2). Because of compromised liver function, the brain is exposed to a variety of potential neurotoxins, including increased levels of ammonium ion, glutamine, mercaptan, octopamine, phenolic compounds, and γ-aminobutyric acid (3). Disturbances in neurotransmitter balance, cerebral metabolism, blood-brain barrier integrity, and sodium-potassium–adenosine triphosphatase activity have been implicated (4–7). Findings of proton MR spectroscopy have established marked metabolic abnormalities in the brain, including reduced choline (Cho) and *myo*-inositol (mI), and increased glutamine (7–10). In humans, both HE and brain metabolic abnormalities can be reversed with hepatic transplantation (11, 12). Oral lactulose, which has a number of beneficial effects on nitrogen balance and excretion in HE, is usually the initial medical therapy for HE, but the precise effects of lactulose on brain neurometabolites have yet to be determined (13–16).

MR imaging and MR spectroscopy are increasingly being used to determine response to pharmacologic therapy in a number of disease states. Ross and colleagues have unequivocally demonstrated that MR spectroscopy is a powerful technique in the assess-

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ment of HE (8–12). However, MR spectroscopy is not widely used to assess HE, rather HE is still accessed by using clinical categorical scales, which are arbitrary, nonparametric measures for a disease process that is certainly not categorical but rather continuous in nature (17). Theoretically, parametric variables should more accurately represent a noncategorical process, and there has been considerable speculation that measurement of neurometabolites might provide parametric data that more accurately reflect a disease process that is a continuum like HE (8–12, 17). The purpose of this study was to determine whether MR spectroscopy could demonstrate relevant neurometabolic changes associated with lactulose therapy and thereby provide further support for the use of MR spectroscopy in clinical trials. We hypothesized that lactulose therapy would be associated with improvement of brain neurometabolic abnormalities as measured by MR spectroscopy. In the present study, we measured the brain metabolic ratios in healthy subjects and patients with HE, and then determined the response of brain metabolic ratios to oral lactulose therapy in a subset of this group.

Methods

Subjects

Twenty-three patients (29 to 56 years old) with chronic end-stage liver disease of various causes (eight with alcoholic cirrhosis, five with chronic obstructive jaundice, three with hepatitis B chronic active hepatitis with cirrhosis, four with hepatitis C chronic active hepatitis, and three with cryptogenic cirrhosis) were studied with MR spectroscopy and compared with 10 healthy control subjects (23 to 45 years old). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional human research review committee. Informed consent was obtained in writing from all subjects or their legal guardian before they participated in the study.

In a subset of patients, a time series design was used in which individual patients served as their own control subjects before and after therapy. A time series design controls specifically for innate variables, including age, sex, chronic disease, cultural differences, genetic factors, and individual habits, including diet, in a situation in which the outcome variable responds rapidly to intervention (18). To assure that spontaneous changes unrelated to therapy were minimized, the patients remained on a stable protein-restricted diet before and during the study, and only patients with a demonstrated stable HE grade were entered into the protocol. A randomized blinded placebo-controlled trial with and without lactulose was considered unethical in these ill patients, since lactulose has already been shown to be effective in HE and these patients required therapy. Moreover, the present trial was designed to identify neurometabolic changes associated with lactulose therapy rather than the clinical efficacy of lactulose therapy that has already been established (13–16).

HE was diagnosed using the criteria of Parsons-Smith et al (17) as follows: grade 0, no detectable abnormality; grade I, patient is unable to perform serial 7s and is unable to copy intersecting pentagon; grade II, above abnormalities and asterixis; grade III, above abnormalities and disorientation (advanced confusion); and grade IV, patient is stuporous but responsive. After a period of 1 week to determine stability of the HE grade, patients with stable HE were entered into the study. Eight patients with grades II to III HE fit these requirements and were selected for the treatment protocol. These patients and their families were instructed not to make any changes in diet (all were already on protein-restricted diets) before and during lactulose therapy. On day 1, the patients were studied with MR spectroscopy, placed on lactulose therapy (60 mL by mouth three times per day), and then restudied on day 8. Since this was a time series design in which individual patients served as their own control subjects before and after therapy, and a period of 7 days had been chosen to establish stability of HE grade, a period of 7 days (day 1 to day 8) was subsequently appropriate to study the response of HE grade and neurometabolites to lactulose therapy. This symmetry in time design minimized confounding time-dependent factors associated with nontherapy-related changes in outcome that might occur with shorter or longer periods of observation.

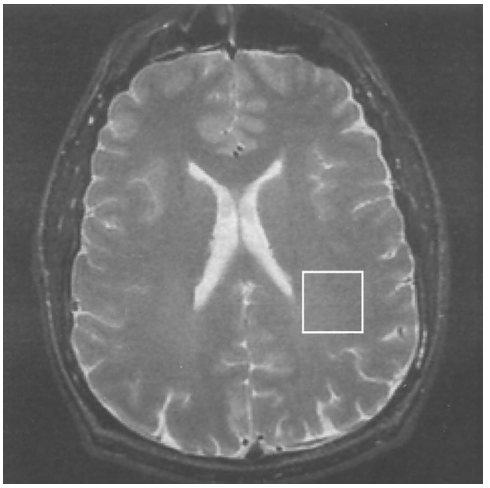

FIG 1. MR localizing axial T2-weighted image (2800/80/1) shows $2 \times 2 \times 2$-cm^3 voxel (volume of interest) in deep normal-appearing occipitoparietal white matter.

Proton MR Spectroscopy

Localized proton MR spectroscopy was performed on a 1.5-T system. A $2 \times 2 \times 2$-cm^3 voxel was selected in deep white matter of the parietal lobe from preliminary axial images (Fig 1). Localization was achieved using point-resolved spectroscopy (PRESS) (19), with imaging parameters of 2000/26/128 (TR/TE/excitations) and a spectral width of 1 kHz, to define the volume of interest. To facilitate highly accurate relocalization on repeat studies, local anatomic landmarks were identified; specifically, the gray-white interface and sulcal patterns adjacent to the spectroscopic voxel. This required an initial T1-weighted sagittal series (800/20/2), careful selection of surface sulcal patterns and internal landmarks on this series to reproducibly prescribe a standard axial proton density– and/or T2-weighted series (2800/80/20/1), and then careful section selection based on local sulcal patterns to reproducibly prescribe the location of the spectroscopic voxel in parietal white matter. Because subtle differences in the scanning angle or positioning of the section based on internal structures may result in major differences in section position of the peripheral portions of the brain, use of local adjacent sulcal patterns to relocate the spectroscopic voxel was important and far more reliable than the use of internal gross brain features (ie, ventricles) for relocalization. This compulsive technique minimized variation induced by errors in section angle, section selection, and repositioning of the voxel in local tissues, resulting in maximum reproducibility of spectroscopic measurements.

A total of 128 averages were summed, zero-filled, and treated with an exponential filter corresponding to 1 Hz of line-broadening before Fourier transformation. A baseline sim-

ulation method, similar to the spline-fitting baseline correction (20), was used to remove underlying broad resonances based on points defined by the bases of the Cho, creatine (Cre), *N*-acetylaspartate (NAA), and the absolute baseline point at 0 ppm, as previously described (21, 22). Metabolic resonance peaks were integrated and metabolic ratios were calculated for mI/Cre, Cho/Cre, (glutamate + glutamine) (Glx)/Cre, and NAA/Cre. Identical objective criteria were used for analyzing every spectrum, ensuring that the same assumptions regarding inclusion or exclusion of ambiguous line shapes were used across the whole data set. Although curve-fitting of the resonance peaks could have been used, the integrative method has excellent reproducibility (22, 23), and simultaneous analysis of peaks using the integrative and curve-fitting methods has previously established no significant statistical difference (24). The resonances of Glx were overlapping and not independently resolvable and were thus integrated for a composite Glx/Cre ratio. It has been found that Glx/Cre corresponds well to alterations in the true glutamine concentration (8, 25, 26). At the time this study was performed, absolute metabolic concentration protocols had not been instituted; thus, the data are reported as ratios.

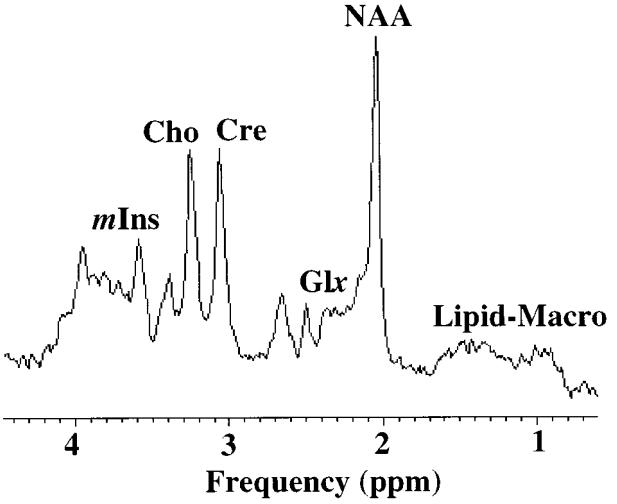

FIG 2. Normal proton MR spectrum (PRESS [2000/26/128]) shows the characteristic resonances of mI (*m*Ins), Cho, Cre, NAA, Glx, and lipid macromolecules (*Lipid-Macro*).

Statistical Analysis

Statistical analyses were conducted with SPSS 6.1.1 for Macintosh (SPSS Inc, Chicago, IL). Means of groups were compared with the two-tailed *t*-test. Paired data obtained before and after lactulose therapy were compared with the paired difference *t*-test. Abnormal neurometabolic values were defined as those exceeding the mean metabolic ratio of the control group ±2 SD. Categorical data were analyzed using nonparametric methods.

Results

The proton spectrum in Figure 2 is normal. Resonances from NAA, Glx, Cho, Cre, and mI are observed. The spectrum in Figure 3A represents a patient with HE before lactulose therapy and shows a massive increase in Glx and decreases in Cho and mI. The collated data from control subjects and the HE cohort are presented in Table 1. The number of patients falling outside the normal range (±2 SD beyond mean control values) is indicated. The most common abnormalities were increases in Glx/Cre (96% of patients), decreases in mI/Cre (83% of patients), and increases in (Cho + mI)/Glx (96% of patients). Depletion of Cho/Cre and NAA was not as common (22% and 17%, respectively). Figure 3B shows the patient's brain spectrum in HE after 1 week of lactulose therapy. Table 2 displays the HE grade and metabolic ratios before and after therapy. Therapy with lactulose was associated with significant increases in mI/Cre (prelactulose mI/Cre: 0.45 ± 0.16; postlactulose mI/Cre: 0.58 ± 0.18; n = 8; $P = .03$) and (Cho + mI)/Glx (prelactulose [Cho + mI]/Glx: 0.27 ± 0.06; postlactulose [Cho + mI]/Glx: 0.37 ± 0.09; n = 8; $P = .03$). HE grade, Cho/Cre, and Glx/Cre improved with lactulose therapy, but at lesser significances ($P = .14$, .31, and .14, respectively).

Discussion

Our results confirm the previously reported metabolic abnormalities associated with HE (8–12). We

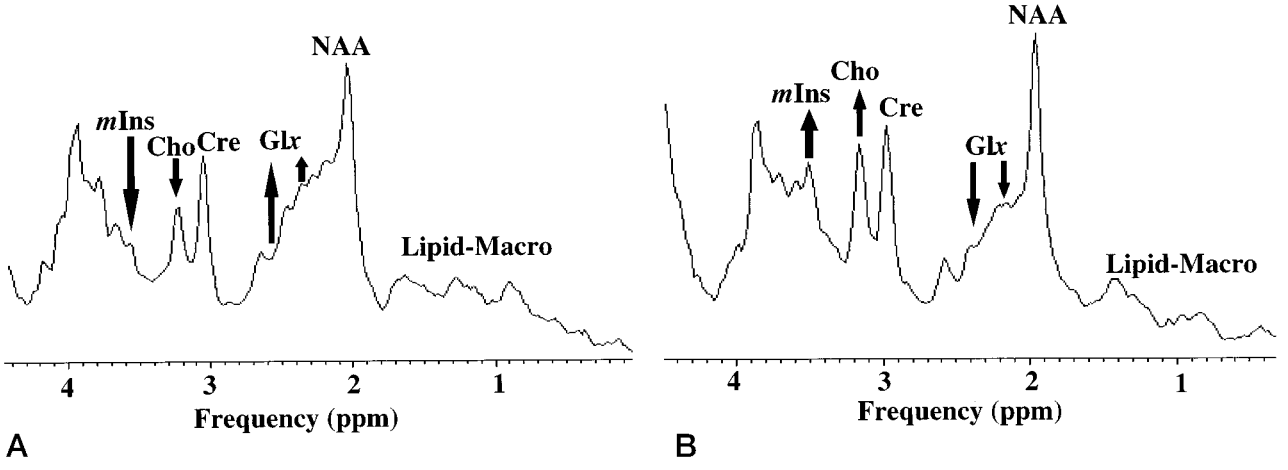

FIG 3. *A*, Proton MR spectrum in patient with HE (PRESS [2000/26/128]) shows a significant increase in Glx (*upward pointing arrows*), and marked decreases in Cho and mI (*m*Ins) (*downward pointing arrows*).

B, Proton MR spectrum after lactulose therapy (PRESS [2000/26/128]) shows a definite increase in mI (*m*Ins) after therapy, as well as an increase in Cho (*upward pointing arrows*) and a reduction in Glx (*downward pointing arrows*).

TABLE 1: MR spectroscopic metabolite ratios in 23 patients with hepatic encephalopathy and 10 control subjects

Metabolite	Control Subjects (n = 10)	Hepatic Encephalopathy		No. (%) Outside ±2 SD
		(n = 23)	*P* Value	
mI/Cre	1.10 ± 0.16	0.62 ± 0.28	.00002	19/23 (83)
Cho/Cre	0.80 ± 0.05	0.76 ± 0.16	.45	5/23 (22)
Glx/Cre	2.26 ± 0.14	4.00 ± 0.96	.00003	22/23 (96)
NAA/Cre	2.29 ± 0.09	2.47 ± 0.57	.33	4/23 (17)
(Cho + mI)/Glx	0.84 ± 0.21	0.37 ± 0.16	.00003	22/23 (96)

Note.—mI indicates *myo*-inositol; Cre, creatin; Cho, choline; Glx, (glutamine + glutamate); NAA, *N*-acetylaspartic acid.

TABLE 2: MR spectroscopic metabolite ratios in eight patients with hepatic encephalopathy before and after lactulose therapy

Metabolite	Prelactulose Therapy (n = 8)	Postlactulose Therapy		No. (%) of Patients Responding to Therapy
		(n = 8)	*P* Value	
HE grade	0-grade 0 0-grade I 5-grade II 3-grade III	2-grade 0 2-grade I 3-grade II 1-grade III	.14	4/8 (50)
mI/Cre	0.45 ± 0.16	0.58 ± 0.18	.03	7/8 (88)
Cho/Cre	0.67 ± 0.14	0.72 ± 0.16	.31	4/8 (50)
Glx/Cre	4.30 ± 0.48	3.63 ± 0.90	.14	6/8 (75)
NAA/Cre	2.49 ± 0.32	2.48 ± 0.46	.93	0/8 (0)
(Cho + mI)/Glx	0.27 ± 0.06	0.37 ± 0.09	.03	6/8 (75)

Note.—HE indicates hepatic encephalopathy; mI, *myo*-inositol; Cre, creatine; Cho, choline; Glx, (glutamine + glutamate); NAA, *N*-acetylaspartic acid.

found a mean 43% decrease in mI/Cre, a 5% decrease in Cho/Cre, and a 75% increase in Glx/Cre, which compare favorably with the respective values of 45%, 14%, and +50% reported in the literature for patients with similar stages of HE (8–10). These results confirm the previous findings, originating primarily from Ross and colleagues, that gross cerebral metabolic abnormalities are present in HE (8–10, 12, 26). The findings of the present study also establish that MR spectroscopy can detect the neurometabolic changes associated with pharmacologic therapy of HE.

The pathophysiology of HE has been attributed to a variety of causes, including the effects of systemic ammonia toxicity; however, considerable controversy remains (1–3, 14, 27–33). Clearly, neurometabolic disturbances (including increased Glx/Cre, decreased Cho/Cre, and decreased mI/Cre) are prevalent with HE, but it is uncertain how these metabolic disturbances are related to the observed neurologic dysfunction. Levels of mI and Cho in HE may be reduced due to osmotic mechanisms associated with glutamine accumulation, decreased hepatic synthesis, decreased dietary intake, or increased consumption of metabolites in an attempt to compensate for hepatic failure (1, 31–33). The results of the present study confirm the central findings of increased Glx/Cre and depressed mI/Cre in HE and further suggest that the neurometabolic ratios mI/Cre and (Cho + mI)/Glx may be more sensitive measures of lactulose effect than are Glx/Cre, Cho/Cre, or HE grade.

Conventional medical therapy for HE includes removal of factors that are toxic to the liver, correction of concomitant medical conditions that might be precipitating or exacerbating HE (infection, dehydration, gastrointestinal hemorrhage), and lowering nitrogen-containing proteins and other products from the gut, as reflected in changes in blood ammonia (14). This is accomplished with protein restriction, judicious use of poorly absorbable antibiotics, and administration of lactulose. The beneficial effects of lactulose on HE have been postulated to be attributable to a decrease in gut transit time, a decrease in ammonia absorption by conversion of ammonia to the poorly absorbable ammonium ion, a decrease in ammonia production in situ by bacteria, and a decrease in the production and absorption of neurotoxic 3–6-carbon fatty acids (1, 13–16). The beneficial effect of lactulose on cerebral metabolic ratios could be related to reduced absorption of neurotoxins at the level of the gut, reduced serum ammonia levels, reduced concentrations of glutamine in astrocytes, improved osmotic balance, or restoration of the biosynthesis and transport of mI (32–42). Alternatively, lactulose may change the bacterial milieu of the gut, diminishing production of various toxins and permitting greater absorption of dietary mI and other necessary nutrients (15).

Hepatic transplantation results in complete resolution to normal glutamine and mI levels (11, 12), but restoration of normal Cho levels is somewhat delayed (43, 44). This indicates that changes in glutamine and mI are closely linked, whereas the changes in Cho may involve a structural, inducible, or deficiency state that requires time to fully equilibrate in response to restoration of normal hepatic function. These findings of previous reports are consistent with those of the current study, which did not establish a significant

increase in Cho/Cre despite a significant increase in mI/Cre and (Cho + mI)/Glx with lactulose therapy. The findings of the present study suggest that changes in brain metabolic ratios, especially mI/Cre, are directly susceptible to manipulations of the gut, emphasizing the important role of diet, enteric organisms, and nitrogen-containing compounds in the induction of HE.

Conclusion

Lactulose therapy of HE is associated with improved levels of brain neurometabolites as measured by MR spectroscopy. The metabolic ratios mI/Cre and (Cho + mI)/Glx appear to be the most sensitive measures of lactulose effect, although HE grade, Glx/Cre, and Cho/Cre may also be useful with larger numbers of patients. These results suggest the possibility that lactulose may improve HE by modulating levels of neurometabolites. These data also establish that MR spectroscopy detects neurometabolic changes associated with pharmacologic therapy for HE and support the expanded use of MR spectroscopy as an adjunctive technique in clinical trials. MR spectroscopy may be particularly useful for providing noncategorical data specific to the brain to support the more conventional categorical techniques used customarily to develop new pharmaceutical agents and to establish drug efficacy in clinical trials of HE.

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