Localized Proton NMR Spectroscopy in the Striatum of Patients with Idiopathic Parkinson's Disease: A Multicenter Pilot Study

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Single voxel proton MRS was used to study brain metabolism in the striatum of patients diagnosed with idiopathic Parkinson's disease (PD). Peak metabolite ratios in long echo time spectra were evaluated in 151 patient spectra and 97 age-matched control spectra collected at four participating institutions using identical hardware and clinical protocols. Combining data from all ages (27–83 years old) showed no significant difference between patient and control ratios. However, in an elderly subset of patients (51–70 years old), a significant decrease in striatal *N*-acetylaspartate (NAA)/choline (Cho) was observed. Also, a significant decrease in the mean NAA/Cho ratio was observed in patients versus controls for patients not being treated with Sinemet (Du Pont Pharm, Wilmington, DE) (hereafter referred to as levodopa/carbidopa). This result is consistent with the hypothesis that NAA may provide a reversible spectroscopic marker for neuronal dysfunction, although a prospective follow-up study will be needed to confirm this. Quantitation of MRS would be useful to exclude the possibility that a change in Cho levels affected the NAA/Cho ratios.

Key words: MRS; Parkinson's disease; basal ganglia; levodopa

INTRODUCTION

Idiopathic Parkinson's disease (PD) is a progressive, degenerative disease of the extrapyramidal system leading to specific motor symptoms. Clinically, PD is characterized by akinesia, rigidity, and tremor. The major pathological process involves the degeneration of heavily pig-

mented dopaminergic neurons of the substantia nigra (SN) and adjacent midbrain. The neuronal loss underlies the catastrophic decrease in the concentration of striatal dopamine where the axons of the involved neurons terminate. Depletion of the neurotransmitter dopamine seems central in accounting for the attending motor symptoms. Neuroimaging studies using positron emission tomography have facilitated visualization of the severe loss of dopamine in the caudate nucleus and especially in the putamen of PD patients by quantitating the spatiotemporal fate of injected ^{18}F -dopa, a precursor of dopamine. Positron emission tomography studies also have shown an increase in regional cerebral blood flow and hypermetabolism in the basal ganglia of PD patients (1, 2).

The technique of localized *in vivo* proton MRS (^1H -MRS) has recently become practical within the clinical environment through the judicious use of commercial high-field (1.5T) MR systems in conjunction with software for MRS. This new application opens up the possibility of observing major brain metabolites that contribute to the three major resonances seen in proton MR spectra of the brain: *N*-acetyl-L-aspartate (NAA), creatine/phosphocreatine (Cr), and choline-containing compounds (Cho). Information may be obtained about local cellular metabolism by determining peak metabolite ratios of the neurochemicals detected in the spectra. These data may have important implications regarding the neurophysiological status of patients with PD and patients suffering from other degenerative neurological disorders. Such data must be obtained relative to an appropriate control population.

The aim of this study was to use localized ^1H MRS for the characterization of metabolism in the striatum of patients diagnosed with idiopathic versus age-matched control subjects. Our present study used a large data base of human proton spectra provided from a multi-institutional cooperative trial. These data were suitable for extensive statistical analysis. Particular attention was given to the use of detailed quality control and quality assurance procedures to enable the determination of baseline levels of metabolites.

METHODS

The MRS facilities at four institutions participated in this study (Loma Linda University Medical Center, Universität Münster, University Hospital of Nijmegen, and Turku University Central Hospital), and all applied iden-

MRM 33:589–594 (1995)

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Received September 12, 1994; revised January 3, 1995; accepted January 4, 1995.

This work was supported, in part, by grants from the National Parkinson Foundation (JMT) and the National Science Foundation (BNS-9016469) (JMT), support from The Department of Veterans Affairs and the Loma Linda Veterans Association for Research and Education and by a grant from the JANIVO foundation, Breda, the Netherlands.

0740-3194/95 \$3.00

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tical hardware, clinical protocols, and spectral postprocessing. MR imagers (Siemens 1.5T MAGNETOM 63 SP and NUMARIS version A2.3) with a standard circularly polarized head coil and spectroscopy package were used for all MRI and MRS acquisitions. Transverse spin echo, proton density, and T_2 -weighted images ($TR/TE/TE2 = 2700/22/90$ ms, 5 mm thickness, 192×256 matrix size, 1 acquisition, 230 mm field of view) and additional gradient echo coronal and sagittal images were acquired for volume localization.

After shimming, a double spin echo sequence with water suppression ($TR/TE = 1600/135$ ms, 256 acquisitions, 1024 data points) was used to acquire two spectra of cubic 8 ml volumes, each centered on the right and left globus pallidus. Spectra without water suppression but the same TR , TE , and data points with eight acquisitions and four prescans served as a reference for a standardized eddy current compensation, supplied with the spectroscopy package (3). Approximately 10–20% of the voxel volume was estimated to be outside the striatum. The SN, of obvious importance to this study, could not be examined, because its small volume (1–2 ml) and geometry would produce a large partial volume effect with the single voxel technique used.

Data were postprocessed using zerofilling to 2K, Gaussian filtering (approximately 0.8 Hz line broadening), Fourier transformation, and phase correction. Each spectrum was visually inspected by members of all participating institutions (quality control criteria: water signal line width below 10 Hz, signal-to-noise ratio of NAA, Cr, and Cho better than 3, total lipid intensity below that of the NAA signal, absence of remaining eddy current artifacts). Spectra were selected for the final analysis only after acceptance by all sites. A standardized method of calculating peak areas was supplied with spectroscopy software and was performed manually.

After giving informed consent, all subjects passed standard eligibility screening criteria for these institutional review board-approved MRI studies. Each patient was diagnosed by a neurologist as having PD using criteria of the United Kingdom Parkinson's Disease Society Brain Bank (4) and completed a questionnaire detailing patient history, symptoms, and medication. Most examinations were performed on patients being treated with levodopa/carbidopa. Controls were screened in order to rule out neurological and psychiatric illness.

RESULTS

Although MRS examinations of patients with movement disorders potentially might be influenced by motion artifacts, proton spectra of PD patients suggested little evidence that this was a problem. Spectra were routinely acquired uneventfully, taking about 1 h/patient. Eighty-one percent of all patient and control spectra were determined to be of acceptable quality and were included in the data for subsequent analysis.

Within 6 months of initiating the present study, a total of 151 spectra from 90 PD patients (53 males, 37 females) and 97 spectra from 61 age-matched controls (26 males, 35 females) were collected at the four participating sites. Ages ranged from 37–83 years (mean 63 ± 10 years) for

the patients and from 27–80 years (mean 59 ± 12 years) for the controls. Metabolite ratios and standard deviations from the control group were all within the experimental errors established in a larger baseline study in which 38 institutes participated and a total number of 258 volunteer examinations were performed using standardized protocols (5). Examples of typical spectra from PD patients and controls recorded at different sites for this study are presented in Figs. 1a and 1b.

Table 1 shows the combined results of mean metabolite ratios for patients compared with controls. The patient data includes spectra from patients treated with levodopa/carbidopa and those with no treatment. No statistical corrections were applied to these data for differences in geographical sites, in subject's age, or in any other variables. Although the mean ratios seem indicative of lower NAA/Cho and NAA/Cr for PD patients

FIG. 1. (a) Typical proton spectra (Double Spin Echo; $TR/TE = 1600/135$) from the striatum of age-matched controls from each institution. (b) Proton spectra from the striatum of Parkinson's patients. m, male; f, female; gp, Globus pallidus; ppm, parts per million.

Table 1
Mean Metabolite Ratios for Combined Data from All Sites
Comparing PD Patients with Age-matched Controls

	Patient spectra (<i>n</i> = 151)	Control spectra (<i>n</i> = 97)	<i>P</i>
NAA/Cho	1.70 ± 0.46	1.83 ± 0.62	.076
NAA/Cr	1.62 ± 0.44	1.70 ± 0.56	.204
Cho/Cr	0.98 ± 0.22	0.98 ± 0.26	.920

when compared with age-matched controls and thus seem indicative of a tendency of NAA reduction in the striatum of PD patients, these differences were not statistically significant when analyzed by *t* tests.

More sophisticated statistical data may be obtained with a meta-analysis using the Winer combined test (6), which takes into account the data obtained at the different sites participating in the present study. The meta-analysis again showed a tendency toward NAA reduction in patients, but the results were not statistically significant (*P* = 0.06 for NAA/Cho and *P* = 0.3 for NAA/Cr).

No clear lactate signals could be detected in patients or controls. An estimation of the lower detection limit of observing lactate under the experimental conditions of this study is 2 mM.

Analysis of data from the patient questionnaires yielded additional results of potential interest. The patients (including untreated patients) and age-matched controls were separated into age ranges yielding numbers of cases large enough to analyze statistically as determined by power analysis (0.85). Using a *t* test, the data in Table 2 showed that no significant differences between PD patients and age-matched controls were seen in the younger age range (27–50 years; mean age = 44 ± 5 years) in which the average duration of the disease was 3 years. The NAA/Cho ratio tended toward significance (*P* = 0.079), with patients showing a much lower mean value. However, at the older age range, (51–70 years; mean age = 62 ± 5 years), a significant decrease was observed in the NAA/Cho ratio (*P* = 0.030) between PD patients and age-matched controls. The average duration of disease was 6 years in the 51–70-year age group. Data from patients above the age of 70 were not analyzed in this way because of a lack of sufficient control data to maintain age-matching. Control subjects at the upper ages were more difficult to recruit.

Another interesting and significant result was found when treatment with levodopa/carbidopa was analyzed as a function of metabolite ratios. A subgroup of patients (*n* = 27; mean age = 64 ± 9 years) either had never used levodopa/carbidopa because they were newly diagnosed or had stopped using this medication very early in treat-

ment because of untoward side effects. Using *t*-tests, a significant decrease in the NAA/Cho ratio (*P* = 0.012) was seen in patients not using levodopa/carbidopa when compared with age-matched controls (*n* = 96; mean age = 59 ± 12 years). The impact of levodopa/carbidopa treatment on the NAA/Cho ratio is shown in Table 3. The average duration of disease (based on time of diagnosis) in the subgroup of patients not using levodopa/carbidopa was 6 months. No significant differences in metabolite ratios were found comparing the patients being treated with levodopa/carbidopa (*n* = 91; mean age = 64 ± 10 years) with ratios observed in age-matched controls (*n* = 89; mean age = 62 ± 9 years). The average duration of disease for patients taking levodopa was 7 years. The number of controls varied to maintain age-matching. Also, for comparison, the ages of patients being treated with levodopa/carbidopa were matched with the older subset (51–70 years) of PD patients (including untreated). Using a standard Student's *t*-test, no significant difference was found in the mean ages of these two groups (*P* > 0.9). No other significant results were obtained when (a) effects of other medications, (b) sex, or (c) data derived from right versus left striatum were analyzed.

DISCUSSION

Defects in oxidative phosphorylation have been reported from investigations in different tissues of PD patients including platelets (7), skeletal muscle (8, 9), and brain (10, 11). In most cases, deficiencies of the first enzyme-protein complex (Complex 1) of the mitochondrial respiratory chain were noted, specifically those involving a reduced activity of the enzyme reduced nicotinamide-adenine dinucleotide (NADH) coenzyme Q₁ (CoQ₁) reductase (11). However, the relationship to the pathogenesis and progression of PD is still unknown. An inhibition of electron transport and a concomitant increase in free radical generation due to primary defects in oxidative phosphorylation could account for the observed dopaminergic cell death observed in PD (9, 11). Alternatively, reduced enzyme activity might be secondary to mitochondrial damage from free radicals produced by redox cycling of some environmental or endogenous neurotoxic process in the SN related to dopamine and/or neuromelanine synthesis (10, 11).

A common consequence of oxidative phosphorylation abnormalities appreciated through recent *in vivo* ¹H-MRS data obtained from children with inherited disorders of mitochondrial function is an increase in cerebral lactate (12, 13). Recently, there was a report of increased cerebral lactate derived from occipital lobe ¹H-MRS in 12 PD patients (14). This result is not consistent with our

Table 2
Mean Metabolite Ratios for Combined Data as a Function of Age Comparing Patients with Age-matched Controls

	Age (27–50 years)			Age (51–70 years)		
	Patient spectra (<i>n</i> = 15)	Control spectra (<i>n</i> = 14)	<i>P</i>	Patient spectra (<i>n</i> = 96)	Control spectra (<i>n</i> = 73)	<i>P</i>
NAA/Cho	1.51 ± 0.36	1.82 ± 0.57	0.079	1.70 ± 0.46	1.89 ± 0.63	0.030
NAA/Cr	1.58 ± 0.32	1.82 ± 0.52	0.150	1.61 ± 0.47	1.71 ± 0.54	0.233
Cho/Cr	1.09 ± 0.21	1.02 ± 0.22	0.494	0.97 ± 0.22	0.94 ± 0.24	0.427

Table 3
Mean Metabolite Ratios for Combined Data as a Function of Patient Treatment

	No treatment			Treatment with levodopa/carbidopa		
	Patient spectra (n = 27)	Control spectra (n = 96)	P	Patient spectra (n = 91)	Control spectra (n = 89)	P
NAA/Cho	1.60 ± 0.33	1.83 ± 0.62	0.012	1.80 ± 0.48	1.81 ± 0.62	0.901
NAA/Cr	1.59 ± 0.40	1.69 ± 0.53	0.360	1.67 ± 0.46	1.67 ± 0.52	0.924
Cho/Cr	1.01 ± 0.19	0.98 ± 0.26	0.535	0.95 ± 0.18	0.97 ± 0.26	0.556

findings in the striatum, in which no lactate was observed above the estimated detection limit of 2 mM in a large number of patients relative to control subjects. The basal ganglia are the most affected brain areas in PD, whereas there is no degeneration in the occipital lobe. No evidence for the presence of increased lactate levels was also obtained in a recent MRS study on PD patients and patients for whom vascular Parkinsonism was suspected, in which the striatum and deep white matter were investigated (15). Additionally, in a subgroup of 15 patients investigated at one site (Universitat Münster) for this study, occipital lactate levels were below detectability (unpublished data). The localized double spin echo sequence applied in our study is especially sensitive for the lactate resonance at 1.33 ppm, which easily can be identified as a doublet, 180° out of phase compared with noncoupled singlets. Furthermore, our data agree well with analysis of mitochondrial enzyme function in different brain areas of PD patients, indicating a marked reduction of only SN NADH CoQ₁ reductase activity (11). Therefore, the hypothesis that a uniform somatic impairment of energy metabolism is involved in the biochemical etiology of PD is difficult to reconcile with the anatomical specificity of dopaminergic cell loss.

Considerable effort has been invested in attempting to elucidate the role of NAA in cerebral metabolism (16, 17, 18). Although the exact function is still unknown, there is strong evidence, especially from investigations of human brain tumors (19–22), tumor extracts (23), and studies of extracts from cultured cells (24), that suggest this compound is limited solely to neurons. Furthermore, it has been shown that lesions produced by specific neurotoxic agents lead to reductions in NAA (25). Therefore, NAA has been used as a marker of neuronal integrity, and reductions in NAA seen in proton MRS have been suggested to be secondary to neuronal loss in cerebral pathology as exemplified by stroke (26), acquired immunodeficiency syndrome (AIDS) (27), multiple sclerosis (28, 29, 30), demyelinating diseases (27), and diabetes mellitus (31). Reductions in NAA attributed to degeneration of neurons has been further verified by a large data base using primary (32) and metastatic brain tumors (33), temporal lobe epilepsy (34), AIDS (35), and Alzheimer's disease (36), also investigated in another facet of our multi-institutional clinical trial.

Our analysis of the combined data from all patients and age-matched controls from each facility (Table 1) shows a trend toward slightly decreased NAA/Cho and NAA/Cr ratios in the striatum of PD patients, whereas Cho/Cr is within the normal range. These findings may indicate slightly decreased NAA and unaffected Cho and Cr levels, or alternatively, they may indicate increased

Cho and Cr levels to give decreased NAA/Cho and NAA/Cr ratios with a normal Cho/Cr ratio. Absolute quantitation of the metabolites would resolve this question. For the present, however, we cannot, exclude that there might be sufficient neuronal degradation in the striatum of PD patients to produce significant decreases in NAA upon MRS examination.

An additional analysis of our data presented in Table 2 shows that the difference in NAA/Cho between PD patients and controls reaches significance if the data are restricted to those obtained from older subjects (51–70 years). Because these patients had a markedly longer average duration of disease, we suggest that advanced cases of PD might be associated with more pronounced neuronal damage in the striatum, as detected using *in vivo* proton MRS. However, we must be careful when interpreting these results. The mean NAA/Cho ratio in the younger age group patients (27–50 years) was noticeably lower than the older patient age group mean, although the results were not significant using *t* test. This might be due to statistical error associated with the smaller number of patients available for calculating the younger age group mean.

MRS alterations have also been observed in patients with Huntington's disease, for whom the initial damage accompanied with neuronal loss is known to occur in the striatum. Recently, *in vitro* (37) and *in vivo* (38) MRS data demonstrated a decrease of NAA in the striatum of Huntington's disease patients. To the extent that reduction in NAA reflects neuronal cell loss, this reduction might be irreversible, inasmuch as current paradigms hold that central nervous system neurons do not readily regenerate. However, significant differences in NAA/Cho ratios of PD patients were apparently affected by their treatment with levodopa/carbidopa, (Table 3), indicative of the possibility that NAA may provide a reversible marker for neuronal dysfunction. Lowest NAA/Cho ratios were observed in patients who did not use levodopa/carbidopa. The Cho/Cr ratio was also slightly increased (not significantly) in this group of patients, so that the possibility that increased Cho may have had an affect on the NAA/Cho ratio cannot be excluded. This significance cannot be attributed to age, inasmuch as the mean age of the untreated patients was the same as that of the treated patients. Evidence for NAA recovery indicative of reversible neuronal damage has recently been observed in neurochemical studies of patients after occipital cortical infarction (39), relapsing remitting multiple sclerosis (40), and in induced diabetic ketoacidosis in a rat model (41). In a recent study, Kamada *et al.* concluded that the correction of NAA/Cr ratios by their corresponding *T*₂ values negates the significance of the decline in NAA/Cr in

their own data of areas of the brain studied in which edema is present (42). Although no large areas of edema were present in our own patients, in the future, T_2 relaxation time corrections may need to be incorporated when calculating metabolite ratios and/or quantifying metabolites. A prospective, follow-up study will be important to test the hypotheses that NAA recovery in PD patients receiving levodopa/carbidopa therapy is, in fact, due to their treatment.

CONCLUSIONS

The present investigation provides evidence that single voxel proton MRS is a robust technique capable of providing reliable data on cerebral metabolism and appropriate to the noninvasive investigation of neurochemical variables in patients suffering from PD. There was no evidence that defects in oxidative phosphorylation leads to an elevation of striatal lactate, which should have been detectable by ^1H -MRS were it present in high enough concentrations. A trend in NAA/Cho reduction, being statistically significant in a subgroup of older patients, might be indicative of neuronal cell death in the striatum in the progression of PD. Significant differences in NAA/Cho ratios depending on levodopa/carbidopa may have clinical application if NAA recovery can be used as a marker of neuronal function for monitoring the pharmacotherapy of PD. Because quantitation of metabolites was not done, we cannot exclude the possibility that changes in Cho affected the NAA/Cho ratio.

ACKNOWLEDGMENTS

The authors thank all who have contributed to this study from the following centers: Loma Linda University Medical Center, Loma Linda, California; Jerry L. Pettis, Veterans Affairs Medical Center, Loma Linda, California; Universität Münster, Münster, Germany; University Hospital of Nijmegen, Nijmegen, the Netherlands; Turku University Central Hospital, Turku, Finland; and Siemens AG, Medical Engineering Group, Erlangen, Germany.

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