^{1}H-MRS in patients with multiple sclerosis undergoing treatment with interferon β-1a: results of a preliminary study

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Abstract

***Background*—In vivo magnetic resonance spectroscopy (MRS) has been widely used to assess biochemical changes which occur in demyelinating lesions in white matter of patients with multiple sclerosis. It has been suggested that metabolic variations evidenced by MRS are sensitive indicators of the effects of immunomodulatory treatments in this disease.**

Given the recent finding of an increase in the disease activity in patients with multiple sclerosis treated with interferon (IFN) β-1a in the first period of treatment, ^{1}H MRS was used to investigate further the modification in brain metabolic indices, particularly in the first phase of IFN β treatment.

***Methods*—A ^{1}H MRS study was performed on five patients with relapsing-remitting multiple sclerosis who were being treated with intramuscular IFN β-1a (6 million units/week) for six months and on five untreated patients. The mean age, duration of the disease, and expanded disability status scores (EDSS) of the two groups were similar. Patients were evaluated at the beginning of the study and in the first, third, and sixth months of treatment.**

***Results*—In the multiple sclerosis white matter lesions, N-acetylaspartate (NAA), choline (Cho), inositol (Ins), and creatine (Cr) peaks did not vary significantly over the entire period of the study in the untreated group.**

In the treated group there was a significant increase in the Cho peak area at the first month compared with the pretreatment period, and this increase continued in the third and sixth months ($p<0.001$). A slight but not significant rise in the Cho peak was also found in normal appearing white matter in the patient group undergoing treatment with IFN β-1a. The increase in Cho and the lack of significant changes in Cr and NAA peaks induced a significant rise in Cho/Cr and Cho/NAA ratios over the entire period of treatment compared with those at the beginning of the study ($p<0.02$ and $p<0.005$ respectively).

In the treated group there was a slight but significant increase in the Ins peak in the first month ($p<0.05$) but in the third and sixth months of treatment the Ins values returned to the pretreatment range.

***Conclusions*—IFN β-1a has an impact on metabolite concentrations in multiple sclerosis lesions measured by proton MRS. The increase in Cho, Cho/NAA, and Cho/Cr ratios in multiple sclerosis lesions reinforces the view that they are an index of active or recent demyelination and could support the clinical, neuroradiological and immunological evidence showing an increase in disease activity during the first period of treatment with IFN β-1a. On the other hand, the increase in the Cho peak could be indicative of a rise in membrane turnover in multiple sclerosis lesions or a remodelling of plaques which is not necessarily due to a de novo immune mediated demyelination.**

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In the past few years, the development of localised proton magnetic resonance spectroscopy (^{1}H MRS) has allowed the in vivo study of some cerebral metabolites in various diseases of the CNS, including multiple sclerosis.[1–6] The recent progress in MRS study, with localisation of small volumes of interest, permits a more accurate assessment of changes in cerebral metabolites in lesional areas, reducing, at least in part, the contamination of spectroscopic results by white and grey matter surrounding the demyelinating lesions.

The principal finding with ^{1}H MRS in patients with multiple sclerosis was a decrease in the N-acetyl aspartate (NAA) peak area, whereas contrasting results were obtained for other metabolites.[7–13]

Although the results of some proton MRS studies on patients with multiple sclerosis were contrasting, a common issue was a decrease in axonal density in lesional areas, particularly in chronic lesions, due to the combination of axonal loss and gliosis associated with demyelinating lesions, as well as the presence of biochemical abnormalities in the normal appearing white matter of patients with multiple sclerosis, not detectable with standard spin echo MRI.

The discrepancy among the results of ^{1}H MRS studies in multiple sclerosis could be due to the different patient selection and inclusion criteria, the different techniques used, the different areas selected (active or chronic

lesions), and the difference in the size of selected volumes of interest which do not completely exclude surrounding normal white and grey matter. Despite the difficulties in interpreting data obtained in vivo, MRS provides direct information concerning metabolic variations and damage to or integrity of myelin and axons, which cannot be evidenced with traditional MRI, and it has been suggested that it may play an important part in studies on the natural history of the disease and the assessment of immunosuppressive or immunomodulatory treatment in clinical trials.[11 14 15]

In particular, metabolic variations evidenced by MRS have been proposed as sensitive indicators of the effects of immunomodulatory treatment in multiple sclerosis.

Until now, few data have been available regarding the cerebral metabolic changes evidenced by ^{1}H MRS due to immunosuppressive or immunomodulatory treatments, including IFN β, in patients with multiple sclerosis.[16]

The purpose of the present research was to use ^{1}H MRS to assess the modifications in the cerebral metabolite concentration in lesional areas and in the normal appearing white matter of patients with multiple sclerosis treated with IFN β-1a in the first six months of treatment, comparing them with the same indices obtained in untreated patients with multiple sclerosis matched for age and disability.

Patients and methods

PATIENTS

The ^{1}H MRS analysis was performed on 10 patients with relapsing-remitting multiple sclerosis. Five of them (four women and one man) had undergone IFN β-1a treatment, and five (three women and two men) were untreated. For both treated and untreated patients the inclusion criteria was definite multiple sclerosis[17] for at least two years, a baseline expanded disability status score (EDSS)[18] of 1.0 to 3.5 inclusive, at least two documented exacerbations of the disease in the two years before the study, but no exacerbation in the past three months. None of the patients had undergone immunosuppressant therapy within the six months before the study, or adrenocorticotrophic hormone or corticosteroid treatment within two months before the beginning of the study. Other exclusion criteria were pregnancy or nursing, an unwillingness to practice contraception, or any other disease other than multiple sclerosis.

Interferon β-1a (Serobif, Ares Serono; a glycosylated recombinant IFN β analogous to the natural sequence) was given intramuscularly at a dosage of 6.0 million units (30 μg) weekly, using the study design of Jacobs *et al*,[19] for up to 24 weeks.

At the beginning of the study, the untreated patients with multiple sclerosis were matched to the treated group for mean age, duration of the disease, and EDSS.

The mean age of the treated patients was 34.5 (SD 3.6) years, the disease duration two to seven (mean: 3.2 (SD 1.9) years, and the EDSS ranged from 1 to 3.5 (mean: 2.5 (SD) 1). The mean age of untreated patients was 37.6 (SD 4.7) years; the disease duration was three to eight (mean: 3.9 (SD 1.8)) years; and the EDSS ranged from 1.5 to 3 (2.0 (SD 1.0)). The mean relapse rate was 0.8/year and 0.9/year in treated and untreated multiple sclerosis groups respectively.

Patients were evaluated at the beginning of the study and in the first, third, and sixth months of treatment. All patients signed informed consent forms according to the declaration of Helsinki.[20]

At the beginning of the study MRI and ^{1}H MRS were also performed on six healthy, age matched control subjects (mean age 33.5 (SD 3.7)) years with no systemic or neurological diseases.

MRI

Evaluations with MRI and ^{1}H MRS were performed at the above mentioned times in a single session with a clinical, 1.5T, whole body MR system (Signa Advantage, GE Medical Systems) with a standard head coil.

To quantify the lesional load and number of active lesions, MRI examination was performed in a separate session preceding ^{1}H MRS. T1 weighting (650/15 TR ms/TE ms), proton density (2000/15 TR ms/TE ms), and T2 weighting (200/70 TR ms/TE ms) images were obtained in the axial plane. Gd-DTPA was given intravenously in a dose of 0.2 ml/kg body weight (0.1 mmol/kg) followed by a postinjection flush with 10 ml saline. T1 weighted sequences were obtained starting five to 10 minutes after Gd-DTPA injection, with an in plane resolution of 1.0×1.3 mm^2 and a slice thickness of 5 mm (gap 1.25 mm).

The quantification of MRI abnormalities was performed as previously described.[21] The assessment of lesions was performed on 15 anatomically defined brain sites (seven periventricular and separated from the ventricles). An arbitrary scoring system weighted for lesion size was used to estimate the regional lesional load. One point was given for each lesion with a diameter≤5 mm; two points for 6 –10 mm lesions, and three points for lesions >10 mm. Confluent lesions were scored one extra point. Scores of the 15 sites were then added up to determine the cumulative lesional load score. Areas of greatly increased signal intensity not related to a physiologically enhancing structure and consisting of at least three pixels, were considered Gd enhancing lesions. The number, not the size, of Gd-DTPA enhancing lesions was used as the study index.

^{1}H-MRS DATA ACQUISITION AND PROCESSING

In each patient spectra acquisition was performed in two areas with demyelinating lesions (white matter lesions) and in one area of normal appearing white matter. In a further imaging session, sagittal and axial views were taken to ensure the correct volume of interest position and accurate repositioning in the subsequent follow up period. The imaging was performed by fast spin echo (FSE) sequences with echo train=8, repetition time (TR)=4000 ms, echo time (TE)=18 ms and 100 ms, field of view (FOV)=24×24 cm^2,

Table 1 Metabolite values (relative to the unsuppressed water peak) of control subjects and patients with multiple sclerosis at the beginning of the study

	Ins (Mean (SEM))	*Cho (Mean (SEM))*	*Cr (Mean (SEM))*	*NAA (Mean (SEM))*
Treated patients	4.73 (0.28)*	5.25 (0.22)	5.69 (0.33)	7.49 (0.31)**
WM lesions	4.80 (0.19)*	5.41 (0.17)	5.55 (0.20)	7.15 (0.19)**
Untreated patients	4.88 (0.28)*	5.56 (0.28)	5.41 (0.26)	6.82 (0.18)**
Treated patients	4.19 (0.35)	5.43 (0.23)	5.60 (0.15)	8.48 (0.10)
NAWM	4.15 (0.21)	5.33 (0.23)	5.95 (0.19)	8.63 (0.14)
Untreated patients	4.12 (0.31)	5.23 (0.43)	6.29 (0.27)	8.77 (0.27)
Control subjects	3.97 (0.19)	5.01 (0.24)	5.77 (0.26)	8.99 (0.40)

*p < 0.05; ** p < 0.0001 *v* control subjects.
WM = white matter; NAWM = normal appearing white matter.

acquisition matrix=256×256, slice thickness=3 mm, interslice distance=0.5 mm.

Depending on the location of white matter lesions in each treated and untreated patient, normal appearing white matter volumes of interest were identified in frontal white matter in five patients, in the parietotemporal white matter in three, and in the periventricular white matter in two of them. In the control subjects the same areas identified in patients were assessed for the white matter measurement of metabolite concentration, and the values were averaged and compared with the mean metabolite values in the normal appearing white matter of treated and untreated patients with multiple sclerosis.

The typical voxel size ranged from 2 to 4 ml. Only volumes of interest wherein either normal appearing white matter entirely occupied the voxels or multiple sclerosis lesions predominately occupied the voxels (at least 90%) were selected. The mean size of lesions in the volumes of interest was 2.7 mm^3 in untreated patients with multiple sclerosis and 2.5 mm^3 in treated patients with multiple sclerosis. The size of each individual lesion chosen did not vary over a six month time period of the study in both groups. Moreover, all selected white matter lesions were not enhancing at the beginning of the study.

The homogeneity of the magnetic field over the volume of interest was optimised by observing the ^{1}H MRS signal of tissue water, measured with the spatially selective STEAM (stimulated echo acquisition mode) sequence. Typical line widths (full width at half maximum) of 4–5 Hz were achieved in this way for the unsuppressed water peak.

The same sequence (STEAM) was used to acquire spectra, after water peak suppression (CHESS sequence). Acquisition indices were: TR=2600 ms, TE=35 ms, TM=13.7 ms; band width=2500 Hz, number of points=4096; phase-cycle=8, number of averages=256.

Spectra were elaborated by eddy current correction[22]; Lorentzian-Gaussian apodisation; zero filling to 8192 points; fast Fourier transform (FFT); and Gaussian fit (Levenberg-Marquardt method).[23]

The signal intensities of NAA, choline (Cho), inositol (Ins), and creatine (Cr) were quantified by normalisation to the unsuppressed water peak acquired in the same conditions. Whereas most MRS studies reported ^{1}H MRS results as ratios between the cerebral metabolites, we expressed our results both as values relative to water and as ratios.

STATISTICS

Data were expressed as mean (SEM). Analysis of variance (ANOVA) was used to compare the values of the cerebral metabolites of the control group with those of both patient groups at the beginning of the study. The same test for repeated measures and Fisher's least significant difference (LSD) were also used to compare the values of all the metabolite peaks examined at each stage of the study for both treated and untreated groups.

Five per cent for two tailed tests was chosen as the level of significance.

Results

INITIAL STAGE

Both treated and untreated patients with multiple sclerosis examined at the start of the study showed significantly lower values of NAA in the white matter lesions compared with the controls (treated multiple sclerosis *v* controls p<0.0001, untreated multiple sclerosis *v* controls p<0.0001). No significant differences were found between the Cr and Cho peak signal intensities of patient groups and control subjects (table 1).

The reduction of NAA peaks in patients with multiple sclerosis without significant differences in Cr and Cho compared with the healthy subjects induced significantly lower NAA/Cr and higher Cho/NAA ratios in patients than in controls (NAA/Cr: treated multiple sclerosis *v* controls p<0.01, untreated multiple sclerosis *v* controls p<0.01; Cho/NAA: treated multiple sclerosis *v* controls p<0.05, untreated multiple sclerosis *v* controls p<0.01) (table 2).

Moreover the values of Ins peaks in white matter lesions were significantly higher in both patient groups than in the control subjects (treated multiple sclerosis *v* controls p<0.05, untreated multiple sclerosis *v* controls p<0.05) conditioning higher values of Ins/Cr ratio

Table 2 Metabolite ratios of control subjects and patients with multiple sclerosis at basal time

	Ins/Cr (Mean (SEM))	*Ins/NAA (Mean (SEM))*	*Cho/Cr (Mean (SEM))*	*Cho/NAA (Mean (SEM))*	*NAA/Cr (Mean (SEM))*
Treated patients	83.67 (3.65)*	63.15 (2.87)**	92.05 (4.46)	68.96 (2.39)*	133.39 (4.42)**
WM Lesions	86.98 (2.50)**	67.51 (2.84)**	97.79 (3.35)	75.39 (2.78)**	130.76 (3.53)**
Untreated patients	90.28 (3.28)**	71.87 (4.64)**	103.54 (4.48)	81.81 (4.22)**	128.13 (5.63)**
Treated patients	75.24 (7.35)	49.42 (4.16)	97.32 (6.17)	64.11 (3.38)	151.62 (3.42)
NAWM	69.29 (4.81)	48.32 (2.49)	86.89 (5.52)	62.75 (2.81)	142.72 (4.38)
Untreated patients	63.35 (5.53)	47.21 (3.29)	82.52 (8.23)	61.39 (4.91)	133.82 (4.99)*
Control subjects	68.89 (1.92)	44.24 (1.64)	86.89 (1.02)	55.83 (1.40)	156.07 (3.19)

*p < 0.05; ** p < 0.01 *v* control subjects. WM = white matter; NAWM = normal appearing white matter.

Table 3 Untreated and treated patients with multiple sclerosis

	Untreated patients				*Treated patients*			
	Lesional load		*No of active lesions*		*Lesional lood*		*No of active lesions*	
Patients	*Basal time*	*6 th month*	*Basal time*	*6th month*	*Basal time*	*6th month*	*Basal time*	*6th month*
1	31.0	30.0	3.0	2.0	26.0	29.0	3.0	4.0
2	34.0	36.0	4.0	5.0	28.0	29.0	3.0	5.0
3	26.0	27.0	2.0	2.0	34.0	36.0	2.0	3.0
4	28.0	30.0	4.0	2.0	28.0	29.0	3.0	2.0
5	24.0	27.0	1.0	2.0	29.0	30.0	3.0	1.0
Mean (SEM)	28.8 (1.77)	30.0 (1.64)	2.8 (0.58)	2.6 (0.60)	29.0 (1.34)	30.0 (1.49)	2.8 (0.60)	3.0 (0.70)

compared with the controls (Ins/Cr: treated multiple sclerosis *v* controls $p<0.05$, untreated multiple sclerosis *v* controls $p<0.01$) (tables 1 and 2).

There were no significant differences between the metabolite peaks of the treated and untreated patient groups at the initial [1]H MRS examination.

It should be noted that the concentration of Cho was slightly lower in treated patients with multiple sclerosis at the beginning of the study than in the untreated group, but the difference was not significant. This finding should not be interpreted as an error of randomisation, but may rather be attributed to the slightly smaller size of three out of 10 lesions examined in the treated group before treatment, which in any case occupied at least 90% of the volume of interest.

In the normal appearing white matter no significant differences emerged between patient groups and the control subjects.

FOLLOW UP

No significant clinical deterioration was found and no relapses occurred in either treated or untreated patients during the follow up period. In addition, no significant changes were found in all multiple sclerosis lesion sizes during T2 weighted scans preceding [1]H MRS examination, performed in the first, third, and sixth months in both treated and untreated patients. Table 3 shows the individual values of lesional scores and the number of Gd-DTPA enhancing lesions of the untreated and treated patients at the beginning of the study and in the sixth month of examination are shown. None of these active lesions had been included in the volumes of interest chosen for the MRS examination. All untreated and treated patients had enhancing lesions at the sixth month. No significant difference were found in lesional loads at each time of observation between the two groups of patients.

Figures 1 and 2 display the values of metabolite peaks in white matter and normal appearing white matter of both untreated and treated patients with multiple sclerosis evaluated at each stage of the study.

In multiple sclerosis white matter lesions, NAA, Ins, Cho, and Cr peaks did not vary significantly over the entire period of the study in the untreated group. This was also true for the normal appearing white matter. In this group the maximum variation of metabolite peak values was always <5% in the white matter lesions and always <3% in normal appearing white

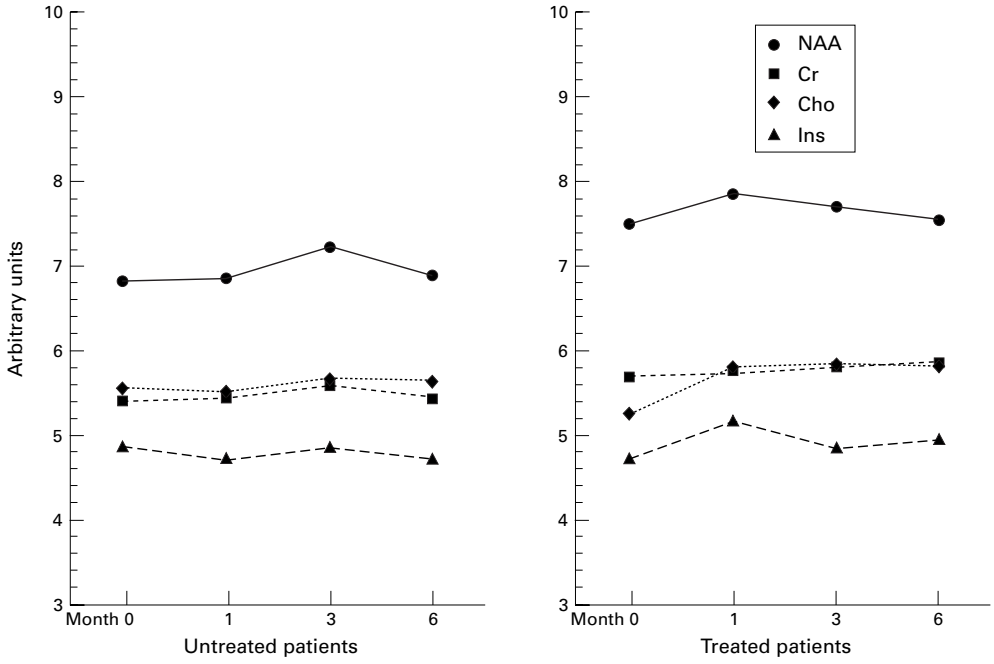

Figure 1 Mean values of NAA, Cho, Cr ,and Ins peaks in white matter lesions of patients with multiple sclerosis treated with IFN β-1a and in untreated patients with multiple sclerosis at the beginning of the study and in the first, third, and sixth months.

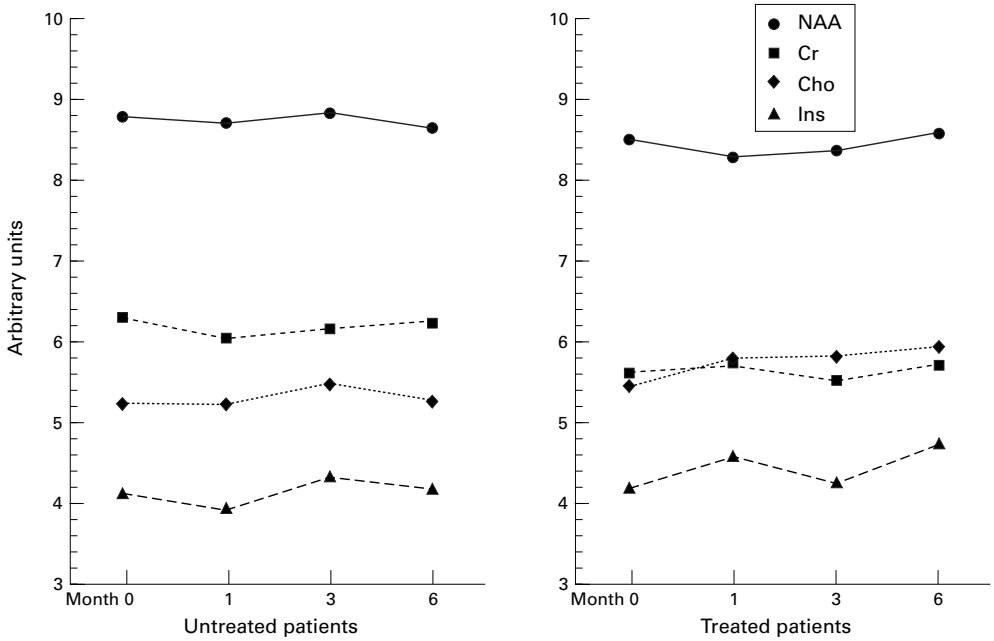

Figure 2 Mean values of NAA, Cho, Cr, and Ins peaks in normal appearing white matter of the patients with multiple sclerosis treated with IFN β-1a and in untreated patients with multiple sclerosis at the beginning of the study and in the first, third, and sixth months.

matter in each stage of the study compared with the initial values.

In the treated group, no significant variations were found in the values of the Cr and NAA peaks in both white matter lesions and normal appearing white matter during the six months of the treatment compared with the pretreatment period. On the other hand, in the first month there was a significant increase in the Cho peak in the white matter lesions in the treated group (5.79 (0.26)) compared with the pretreatment period (5.25 (0.22)), and this was maintained in the third (5.84 (0.27)) and sixth months (5.82 (0.14)) (ANOVA: $p<0.001$; LSD: initial stage *v* first month $p<0.007$, initial stage *v* third month $p<0.004$, initial stage *v* sixth month $p<0.005$). A slight but not significant rise in the Cho peak was also found in normal appearing white matter in the patient group undergoing treatment with IFN β-1a.

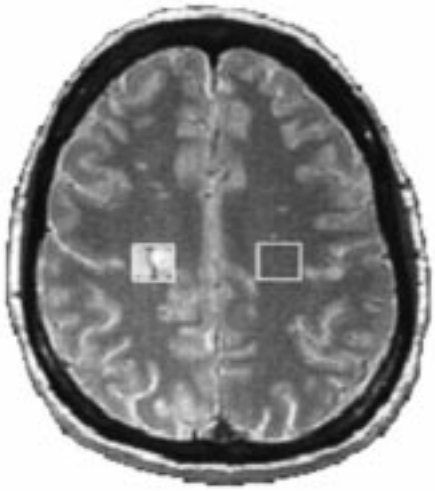

Figure 3 Volumes of interest chosen in a patient with multiple sclerosis treated with IFN β-1a relative to a white matter lesion and an area of normal appearing white matter which were re-evaluated at each stage of the study.

In the treated group, a slight but significant increase in the inositol peak in the white matter lesions was also found in the first month (start of study 4.73 (0.28), first month 5.18 (0.28), $p<0.05$). The Ins intensity signals at the third and sixth months returned to the range of initial values before treatment. No significant changes in Ins signals were obtained in normal appearing white matter in the treated group at any stage of the study.

Figure 3 shows the volumes of interest chosen for a treated patient corresponding to one lesion and normal appearing white matter area sampled at each time of the study.

Figure 4 displays the MRS spectra obtained from the same white matter volume of interest traversing the demyelinating lesion of the same patient examined at the beginning of the study and in the first month of treatment with IFN β-1a.

In the treated group the increase in Cho and the lack of significant changes in Cr and NAA peaks induced a significant rise in Cho/Cr and Cho/NAA ratios over the entire period of treatment (ANOVA $p<0.02$ and $p<0.005$ respectively; LSD Cho/Cr initial stage *v* first, third, and sixth months $p<0.04$; Cho/NAA initial stage *v* first month $p<0.08$, initial stage *v* third month $p<0.01$, initial stage *v* sixth month $p<0.002$) (fig 5).

A trend toward higher values of Cho/Cr and Cho/NAA was also found in the normal appearing white matter of the treated group, but the increase in these ratios did not reach the level of significance (fig 6).

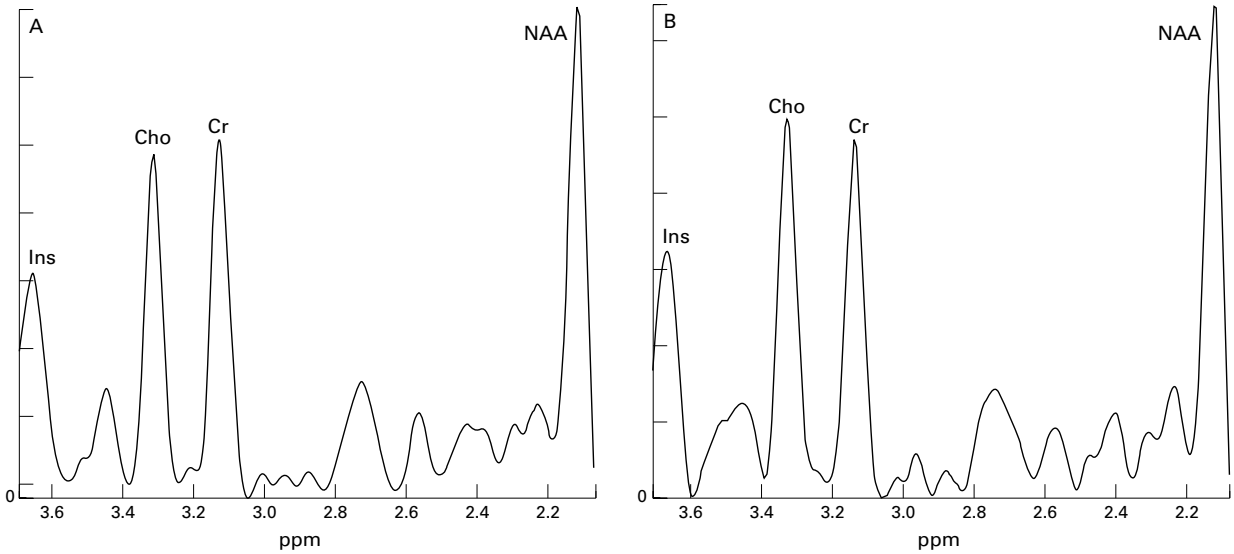

Figure 4 MRS spectra of the same white matter lesion shown in figure 3 in the same patient assessed (A) at the beginning of the study and (B) in the first month of the treatment.

Discussion

In this preliminary study, variations in cerebral metabolites were assessed during treatment with IFN β-1a in a small cohort of patients with relapsing-remitting multiple sclerosis and were compared with those of untreated patients with multiple sclerosis matched for age and disability. Metabolites were quantified in relation to the unsuppressed water peak, and ratios among them were also calculated. The absolute quantification, which needs T2 measurements of metabolites and water and allows data to be expressed as mmol, was not possible due to the lengthy duration of examination, which consisted of three spectra acquisitions (two lesions and one normal appearing white matter). We were more interested in investigating metabolite changes in the same volumes of interest over the test time period rather than absolute concentrations. For the above reason, the T2 variations which may occur between different volumes of interest should not have significantly influenced our results.

Our research confirms previous [1]H MRS studies showing changes in metabolite signal intensities in white matter lesions of patients with multiple sclerosis compared with controls and points out the impact of IFN β-1a on the levels of some of these metabolites in multiple sclerosis lesions, which is evident in the first six months of the treatment.

At the beginning of the study all patients, from both the treated and untreated groups, showed a decrease in NAA peak areas in white matter lesions compared with the control subjects. These results concur with those of previous studies, and can be indicative of an axonal injury or loss within white matter. NAA is con-

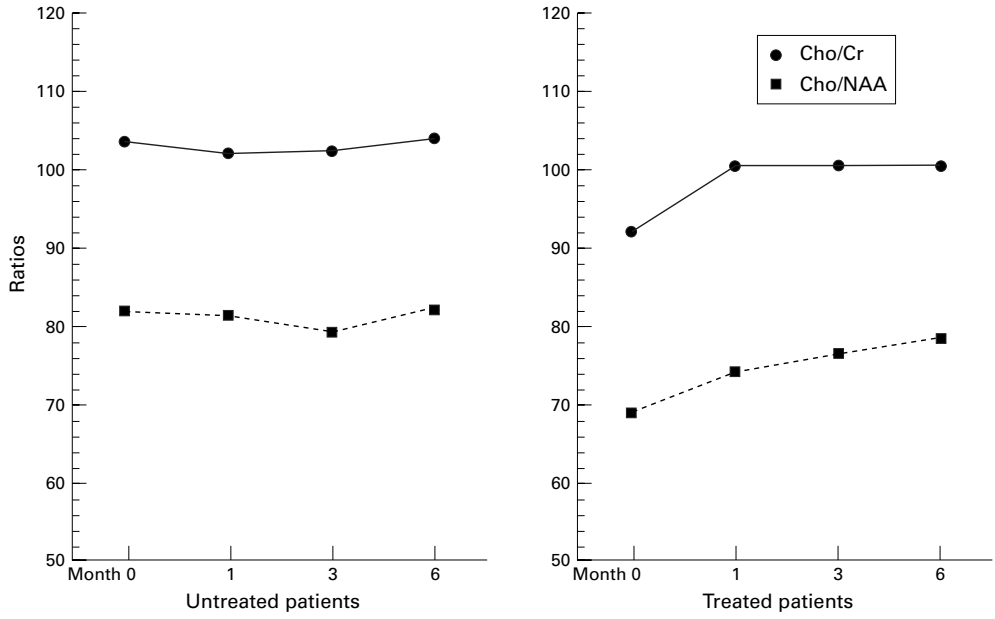

Figure 5 Mean values of Cho/NAA and Cho/Cr ratios in white matter lesions of the patients with multiple sclerosis treated with IFN β-1a and in untreated patients with multiple sclerosis at the beginning of the study and in the first, third, and sixth months.

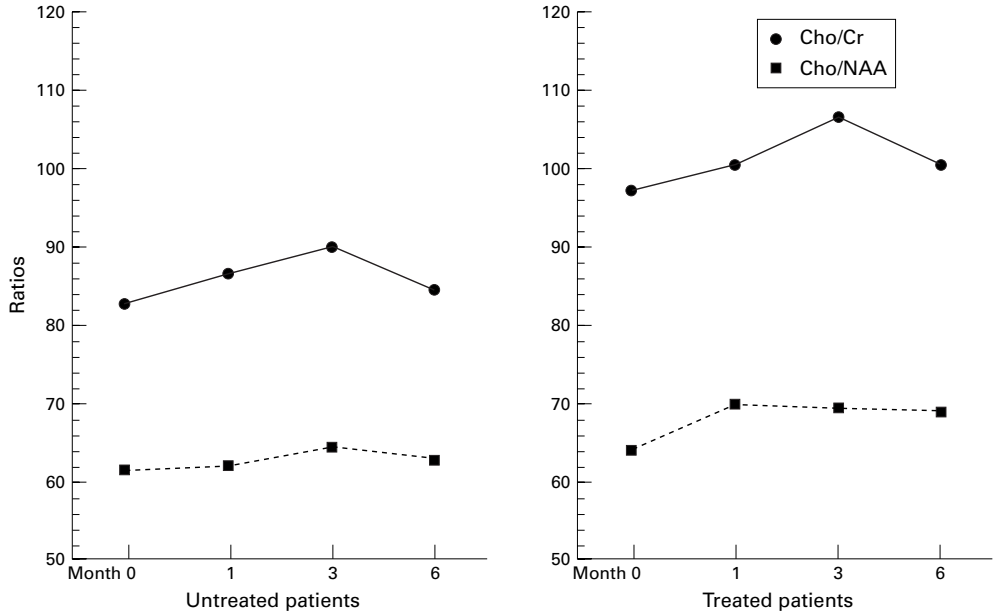

Figure 6 Mean values of Cho/ NAA, and Cho/Cr ratios in normal appearing white matter of the patients with multiple sclerosis treated with IFN β-1a and in untreated patients with multiple sclerosis at the beginning of the study and in the first, third, and sixth months.

sidered a neuron specific molecule because it is largely present within neurons, being absent in both mature glial cultures and tumours of glial origin.[24–27]

According to Matthews *et al*[15] MRS studies indicated that axons as well as myelin are damaged in multiple sclerosis lesions, further evidence of the heterogeneity of the pathological lesions in multiple sclerosis suggested by McDonald *et al.*[28] On the other hand, as the axonal component does not constitute the greatest portion of the white matter, the NAA signal variations in white matter may also be due to changes in the levels of other N-acetyl moieties which could be influenced by the demyelinating process.[29]

Our study also shows an increase in the Ins peak area in the multiple sclerosis lesions of both treated and untreated patients with multiple sclerosis at the start of the study, confirming the finding of previous studies showing an increase in the Ins concentrations in multiple sclerosis plaques.[30]

The principal effect in this study which seems to be due to IFN β-1a is an increase in the total Cho peak, which is evident in the first month of treatment and persists in the third and sixth months. The significant increase in the Cho/Cr and Cho/NAA ratios seems to be mainly due to the rise in Cho because the mean values of the Cr and NAA peaks did not vary significantly over the entire study period.

The change in Cho and in Cho/Cr and Cho/NAA ratios seems be attributed to an IFN effect rather than to patient intervariability or poor spectroscopic measure reproducibility. As mentioned before, in white matter lesions the maximum variation in the metabolite values in the untreated patients during the study time always seems to be less than 5%, whereas the variation in mean Cho peak values between pretreatment and the first months in the treated group was more than 10%.

The increase in Cho, Cho/NAA, and Cho/Cr ratios in multiple sclerosis lesions can be interpreted as an index of active or recent demyelination.[7 8 11] This was suggested by the finding of an abundance of Cho containing compounds in myelin and in all cell membranes, including those of inflammatory cells.[31] This increase in Cho may occur without significant clinical deterioration or disease activity, as it also emerged in our study that no significant modifications in disability or relapses and lesional load were observed in treated patients over the follow up period.

However, if the increase in Cho observed in our study is interpreted as an index of active demyelination, it might have been expected to see the presence of lipid peaks in multiple sclerosis lesions. However we did not find these peaks in any lesion VOI of treated patients during the entire period of the study.

Because the relation between Cho variations and modifications in underlying lesions is not known at the moment, it cannot be excluded that the rise in this metabolite could express an increased turnover of myelin in the plaques examined, which may not necessarily be related to a new demyelination but rather to a remodelling of plaques and even to a remyelination process.

A conclusive clarification of the significance of changes in Cho could be obtained by the contemporary evaluation of the gadolinium enhancement and variation in metabolite peaks in the white matter lesion volumes of interest in a greater number of patients and in longer term follow up studies.

Although the function of myoinositol is uncertain, the transient but significant increase in the Ins peak evidenced in patients with multiple sclerosis treated with IFN β-1a in the first month of treatment should also be noted: it could be attributed to changes in metabolic pathways involved in the polyphosphoinositol

second messenger cascade or transient local changes in osmolysis or reactive gliosis.[32 33]

Given the recent clinical and MRI finding of an increase in the disease activity in patients with multiple sclerosis treated with IFN β-1a[34] and some immunological results indicating an increased activity in the first phase of IFN β treatment,[35 36] it could be hypothesised that the immunological alterations found in the peripheral blood could reflect themselves in the CNS and induce changes in the metabolic pattern of white matter lesions and normal appearing white matter, even if a central effect of IFN β is considered improbable in the absence of a breakdown of the blood-brain barrier.

Many studies performed in vitro and in vivo suggest an immunomodulatory effect of IFN β (particularly 1b) on cytokine secretion, adhesion molecule and MHC expression, and suppressor function.[37–46]

Perhaps long term treatment with IFN β (both 1a and 1b) may induce a down regulation of the early immunological activation which could be reflected in variations of brain metabolites, indicating a reduction of the pathological components of the demyelinating process as recently suggested by Pan *et al*[16] who found a decrease in cho peak after one year of treatment with IFN-β. This reduction may accompany the well known effects on the lesional load or the number and volume of active lesions and the clinical course of the disease.[19 47–49] On the other hand, the direct or indirect action of the drug on the remyelinating process has yet to be shown, but for the time being it cannot be discounted.

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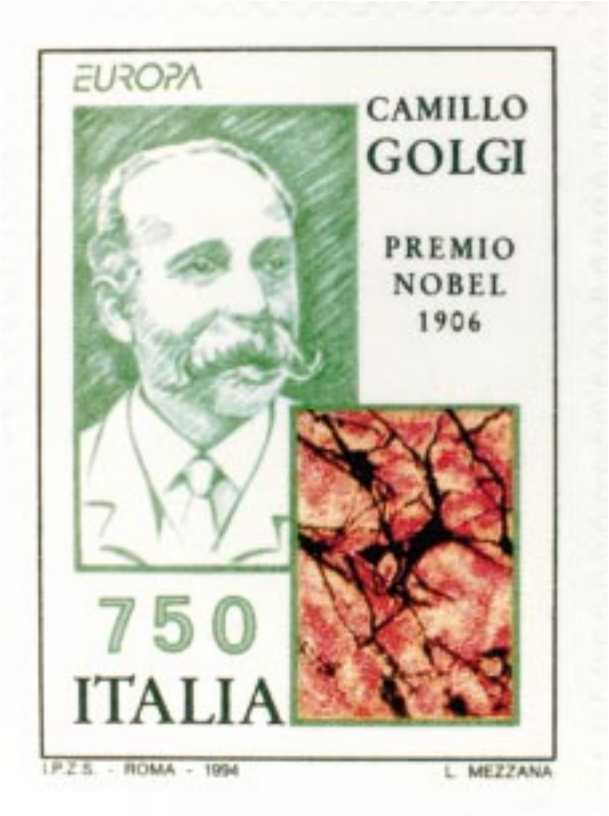
NEUROLOGICAL STAMP

Camillo Golgi (1843–1926)

The Italian physician and biologist Camillo Golgi was born in Corteno (now Corteno Golgi) a tiny village in northern Lumbardy. He graduated in Medicine at the University of Pavia in 1865 with a thesis on somatic and hereditary factors in mental illness. Thereafter he became assistant at the Hospital of San Matteo where he worked at the psychiatric clinic headed by Cesare Lombroso. Meanwhile Golgi began to learn histological techniques under the direction of Giulio Bizzozero at the Institute of General Pathology. In 1872 he moved to Abbiategrasso as chief of the "Pio Luogo degli Incurabili" (a hospital for chronic diseases) where, probably at the beginning of the 1873, he obtained the "black reaction" that was a breakthrough for brain structure research. While in Abbiategrasso Golgi discovered, with this technique, the branching of the axon and the fact that dendrites are not fused in a reticular network; furthermore he performed studies on the structure of the cerebellum and olfactory lobe and noted striatal and cortical lesions in a case of chorea. He returned to Pavia as professor of histology and general pathology and chief of a medical ward at the San Matteo Hospital and made a series of important discoveries that still bear his name: the Golgi tendon organ, the Golgi Mazzoni corpuscles, the Golgi method with potassium dichromate and mercuric chloride, the Golgi-Muller tubules of the peptic glands, the Golgi-Rezzonico myelin's annular apparatus, the cycle of malarian parasites in human blood (Golgi cycle), and finally, the most important, the cytoplasmic Golgi apparatus (or Golgi complex). It is not generally known that Golgi was also a skilled physician who always refused private activity and published important papers on peritoneal blood transfusions, on intestinal worm infection, and on pathological changes of kidney.

Golgi was elected Dean of the medical faculty and Rector of the University of Pavia, Senator of the Italian Reign, honorary doctor of the Universities of Cambridge, Geneva, Kristiania (Oslo), Athens, and Paris (Sorbonne). In 1906 he was awarded the Nobel prize together with the Spanish scientist Santiago Ramon y Cajal. During the first world war Golgi directed a military hospital in Pavia. After the war he continued to teach histology, to perform morphological research, and to publish papers until 1923. Camillo Golgi died in Pavia on 21 January 1926. The European Community commemorated him in 1994 with a stamp.



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