Serial Proton MR Spectroscopy of Contrast-enhancing Multiple Sclerosis Plaques: Absolute Metabolic Values over 2 Years during a Clinical Pharmacological Study

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BACKGROUND AND PURPOSE: **The time courses of total creatine (Cr), *N*-acetylaspartate (NAA), choline (Cho), and *myo*-inositol have not previously been investigated in the follow-up of contrast-enhancing multiple sclerosis (MS) plaques. Therefore, over a period of 2 years, we compared the absolute concentrations of these metabolites between patients treated with a placebo or 15 ± deoxyspergualin (DSG) and between clinical groups with relapsing-remitting or secondary-progressive MS.**

METHODS: **Sixteen patients, recruited from a pharmacological study of DSG, and 11 healthy control subjects were investigated by a stimulated-echo acquisition mode sequence (TR/TE = 3000/20). The selected volume initially contained a contrast-enhancing plaque, which was followed up for a period of 2 years.**

RESULTS: **In contrast-enhancing plaques, Cho was significantly elevated and showed a significant reduction after both 3 and 12 months. The initially normal Cr significantly increased between 3 and 12 months, and was negatively correlated with plaque volume on T1-weighted MR images. NAA initially showed normal values, a significant decrease at 1 month, and a slow recovery over 2 years. *Myo*-inositol did not show a clear tendency. The placebo group did not differ from the treated group, nor did the relapsing-remitting group differ from the secondary-progressive group.**

CONCLUSION: **The contradictory time courses of Cr and NAA show that an absolute quantification in proton MR spectroscopy in MS is necessary to avoid a false interpretation of reduced NAA/Cr ratios. The increase in Cr is probably due to remyelination. The initial dip and later recovery of NAA seem to be related to diminishing edema and remyelination.**

Proton MR spectroscopy has previously been used to examine multiple sclerosis (MS) plaques at different stages of development (1–17). Serial studies have also been performed to investigate the time course of metabolism (18–32). In most serial studies, the results were expressed as metabolic ratios relative to creatine (Cr) (19–23, 26–28, 30, 31), to the sum of the metabolites (18), to a contralateral voxel (24), or to tissue water (29). A reduction in the *N*-acetylaspartate (NAA)/Cr ratio was reported by the majority of these authors and interpreted as neuronal or axonal loss (19–24, 26–31). Two authors found a subsequent recovery of NAA/Cr over time, leading to the suggestion that axonal loss is not the only mechanism of reduction in the NAA/Cr ratio (23, 24). An increase in the choline (Cho)/Cr ratio (19–21, 23–25, 29) and its subsequent normalization (23, 29) were described and interpreted as a temporarily increased membrane turnover in the demyelination process.

The use of metabolic ratios has the disadvantage of always being reliant on two variables of unknown concentrations. The concentration of Cr is not definitely proved to be stable with time: in a serial MS study, De Stefano et al (24) found a reduction of Cr relative to a contralateral voxel in acute lesions and a normalization in the follow-up

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period. Thus, an absolute quantification is essential for an adequate description of metabolic changes in serial proton MR spectroscopy of MS. Davie et al (25) and Sarchielli et al (32) performed absolute quantification of the metabolites in serial studies and found unchanged concentrations of Cr and Cho and reduced NAA over time; however, they did not investigate contrast-enhancing plaques.

Because changes in metabolic concentrations are thought to be related to the different stages of plaque development, the purpose of our study was to investigate and follow up the spectral changes of acute contrast-enhancing plaques with quantitative proton MR spectroscopy. Our aim was to ascertain the time course of the changes in Cr, NAA, Cho, and *myo*-inositol during the acute and chronic phases of plaque development to discover whether any changes in metabolic concentrations are related to the acute contrast-enhancing stage or to later stages.

We took advantage of the opportunity to join a clinical pharmacological study on the effect of the immunomodulating drug 15 ± deoxyspergualin (DSG) to investigate and to follow up a carefully selected cohort of patients with either relapsing-remitting or secondary-progressive MS (33, 34) over a period of 2 years. Our aim was to use proton MR spectroscopy to determine whether modifications in cerebral metabolic concentrations differed between the placebo group and the treated group and between the two clinical groups (relapsing-remitting or secondary-progressive MS) in the series.

Methods

Patients and Control Subjects

Sixteen patients (mean age, 38 ± 9 years) with clinically definite MS were investigated during a European multicenter double-blind trial on the effect of DSG (33) over 2 years. Eleven healthy volunteers (mean age, 30 ± 9 years) served as control subjects. All patients included in this study fulfilled the following inclusion criteria: clinically definite MS (35); relapsing-remitting or secondary-progressive course (34); clinically active disease (ie, at least two relapses with residual deficits in the last 2 years or a deterioration in the Kurtzke Expanded Disability Status Scale score of at least one grade within the last year) (36); no clinical relapse for at least 30 days before entry into the study; no steroid use or other immunosuppressive treatment on entry into the study; at least one contrast-enhancing lesion on cranial MR images on entry (month 0); and age between 18 and 50 years.

Ten patients had the relapsing-remitting form of the disease; six had the secondary-progressive form. Six patients received a placebo, six received 2 mg/kg body weight of DSG, and four received 6 mg/kg body weight of DSG.

MR Imaging

All measurements (MR imaging and MR spectroscopy) were performed at 2.0 T on a whole-body system using a circularly polarized head coil. In every session, a complete image set was obtained, consisting of axial contiguous T2- and T1-weighted spin-echo images, the latter without and with 0.1 mM/(kg body weight) Dotarem (Laboratoire Guerbet, Aulnay-sous-Bois, France). The slice thickness was 6 mm, the matrix was 256 × 256, and the field of view was 230 mm. Slices were oriented in the AC-PC line (the line between the anterior and posterior commissure), and repositioning was carefully performed by orienting the images with respect to anatomic landmarks, such as the AC-PC-line, on a sagittal plane to always produce nearly identical slices.

MR Spectroscopy

Single-voxel localized proton MR spectroscopy was performed using a stimulated-echo acquisition mode (STEAM) sequence (37–39) with parameters of 3000/20/256 (TR/TE/excitations) and a mixing time of 30 milliseconds. Three chemical-shift selective pulses with a bandwidth of 45 Hz were applied for water suppression (40). Field homogeneity was optimized by global and local shimming. The full width at half-maximum intensity was 10 Hz after global shimming and 6 Hz after local shimming, which is equivalent to a spectral resolution of less than 0.1 ppm at 2.0 T.

An 8-cm^3 voxel was centered on the MS lesion of interest, which had shown contrast enhancement on the initial MR images. The first proton MR spectroscopic examination was performed 24 to 48 hours after intravenous contrast administration to prevent possible influences of gadolinium-containing compounds on the spectra. Follow-up proton MR spectroscopy was always performed before intravenous contrast administration. For repositioning of the voxel, a plain radiograph indicating the exact position of the volume of interest (VOI) on at least three slices was obtained in all patients and used as a reference for each follow-up session. Repositioning of the VOI was performed with reference to adjacent anatomic structures (eg, the foramen of Monroe, sulci, and gyri) and to the lesion itself.

Among the 16 patients, the acute plaque was located in the frontal lobe in five patients, in the parietal deep white matter in seven, in the occipital lobe in two, and in the temporal periventricular white matter in two. Figure 1A shows a typical contrast-enhancing plaque, and Figure 1B shows a representative position of a VOI. In the 11 control subjects, the VOIs were located in the frontal lobe in four individuals, in the parietal deep white matter in three, and in the occipital periventricular white matter in four. All investigators were blinded to the type of disease and the mode of treatment throughout the study.

Time Course of the Investigations

After initial MR imaging and MR spectroscopy (month 0), the follow-up sessions for serial proton MR spectroscopy were performed in months 1, 2, 3, 4, 5, 6, 12, and 24 in eight of the 16 patients and in months 1, 3, 6, 12, and 20 in the remaining eight patients. Medical treatment was started after the first spectroscopy session and continued until the fifth month.

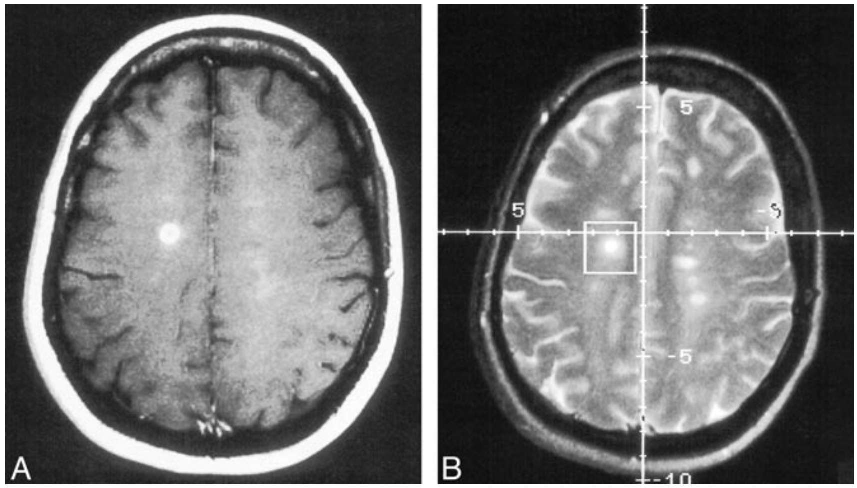
Postprocessing

Spectra were analyzed using the LC model, including corrections for residual eddy currents and coil load effects (41–43). A further correction was performed to compensate for transmitter instabilities (44). All statistical calculations were performed using the data determined by the LC model. In short TE spectra, the region of 0.9 to 1.3 ppm is dominated by a broad resonance caused by lipids, macromolecules, and lactate. Lactate is a well-investigated metabolite in proton MR spectroscopy, and we expected no new information about it. Because the spectral region of 0.9 to 1.3 ppm can be affected by lipid contamination (45, 46), we did not analyze this spectral region. The plaque volume was estimated on contrast-enhanced T1-weighted images and on T2-weighted images after superimposing the VOI, using a manual segmentation procedure.

Statistics

For the statistical description of the data, an analysis of variance (ANOVA) model was applied. The investigations of

FIG 1. *A* and *B*, Two representative sections, one showing an acute MS plaque on a contrast-enhanced T1-weighted image (*A*) and another indicating the position of the VOI superimposed on a T2-weighted image (*B*).



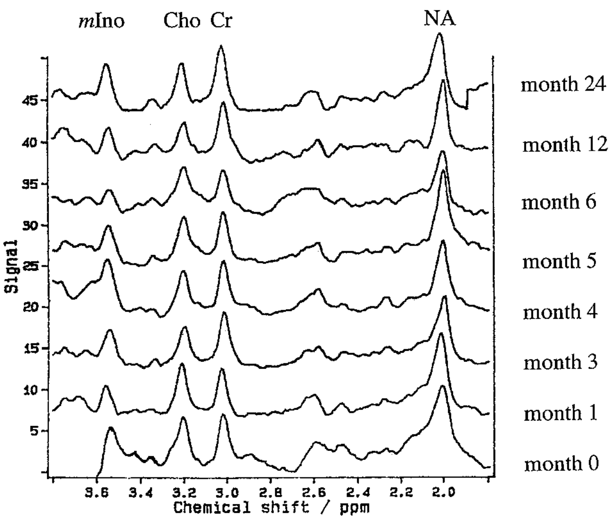


FIG 2. Series of STEAM spectra (TE = 20) of an initially contrast-enhancing plaque over a period of 2 years (TR = 3000, mixing time = 30 milliseconds). No baseline correction has been applied. The indicated resonances are NAA, Cr, Cho, and *myo*-inositol (*m*-Ino). Cho shows a clear reduction at month 3; Cr shows an elevation in month 12.

Absolute concentrations of metabolites and metabolic ratios in patients and control subjects

	Absolute Concentrations, in mM/L (SEM)			
	myo-Inositol/Cr	Cho	Cr	NAA
Patients at month 0	9.63 (2.15)	2.62 (0.13)	8.28 (0.64)	10.46 (0.59)
Healthy control subjects	7.58 (0.69)	1.90 (0.13)	7.07 (0.54)	11.86 (0.43)
Significance level	NS	$P < .001$	NS	NS
	myo-Inositol/Cr	Cho/Cr	NAA/Cr	NAA/Cho
Patients at month 0	0.75 (0.14)	0.99 (0.05)	1.32 (0.08)	1.36 (0.09)
Healthy control subjects	0.86 (0.10)	0.84 (0.06)	1.77 (0.13)	2.18 (0.17)
Significance level	NS	NS	$P < .01$	$P < .001$

Note.—NS indicates not significant.

months 20 and 24 were pooled together as a "month 22" investigation, as we found no substantial changes in the spectra between months 20 and 24. To compare the data by months, a paired Student's *t*-test was used. All data are given in mean ± SEM. The study protocol was approved by the Ethics Committee of the University of Basel. All subjects were informed of the purpose of the study and gave their written consent.

Results

Serial spectra (STEAM, TE = 20) from a representative patient are displayed in Figure 2. These spectra demonstrate the good spectral resolution of about 0.1 ppm at a field strength of 2.0 T. Some occasional baseline disturbances may have arisen from insufficient water suppression, but the fitting algorithm of the LC model was always able to create a reasonable baseline. The relative plaque size within the voxel estimated on T2-weighted images ranged from 6% to 80% (mean, 26%), which corresponded to a mean plaque size of 2.1 cm^3.

Initial Metabolic Concentrations and Metabolic Ratios

As shown in the Table, at the beginning of the study (in month 0), the absolute concentration of metabolites and metabolic ratios differed between healthy volunteers and MS patients. During the early acute stage of a newly formed plaque, the absolute concentration of Cho was significantly increased relative to that in the volunteers. The metabolic ratios of NAA/Cr and NAA/Cho were significantly decreased, although neither the absolute value of NAA nor that of Cr were significantly different.

Changes in Metabolic Concentrations and Metabolic Ratios over the Investigated Time Period

Using ANOVA for statistical analysis and taking all patients into consideration, the absolute concen-

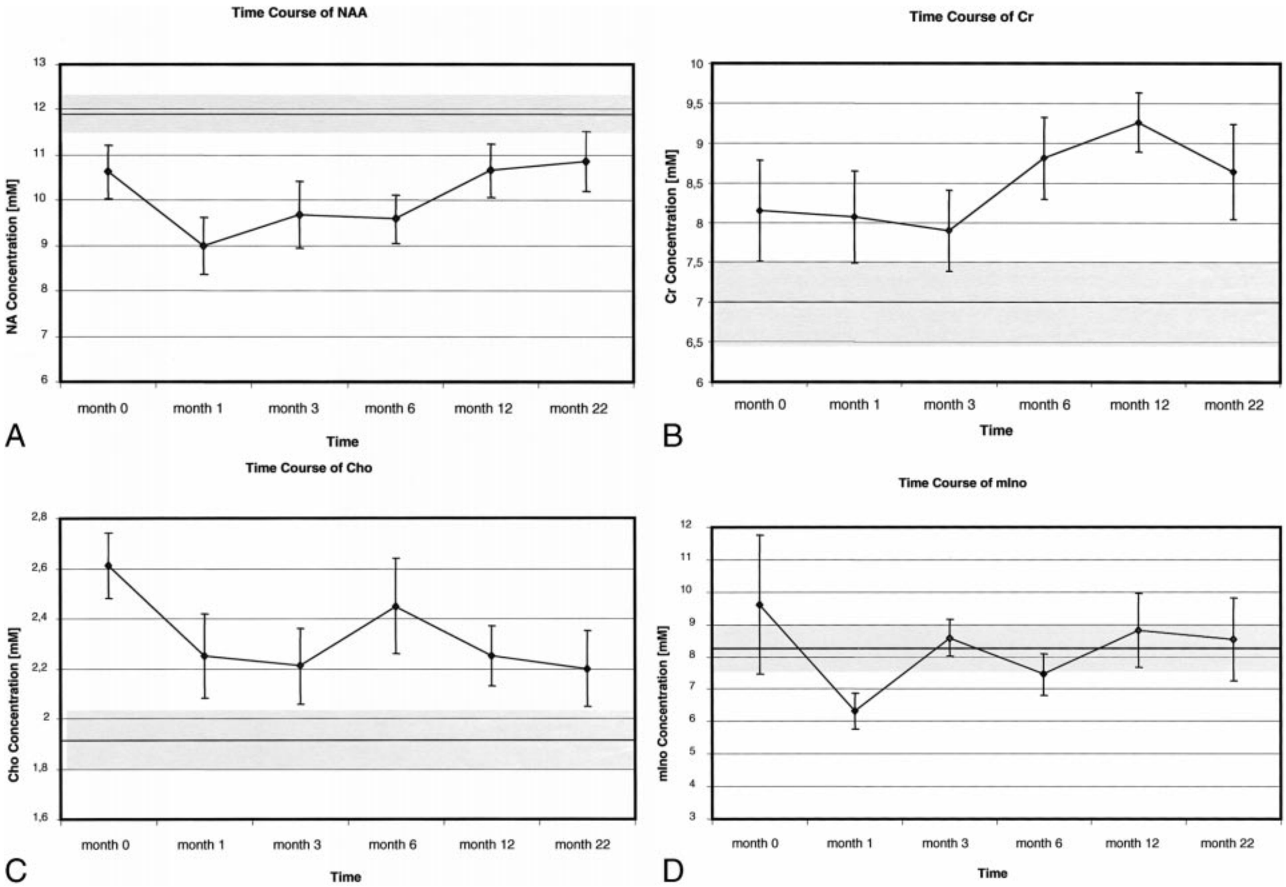


FIG 3. The absolute concentrations of the metabolites as a function of time. The mean of the healthy control subjects ± 1 SEM is indicated by the *horizontal bars.*

A, Time course of NAA. After a reduction in month 1, a slow recovery is visible until month 22.

B, Time course of Cr. There is a significant elevation from month 3 to months 6 and 12.

C, Time course of Cho. Note the significant decrease from month 0 to month 3.

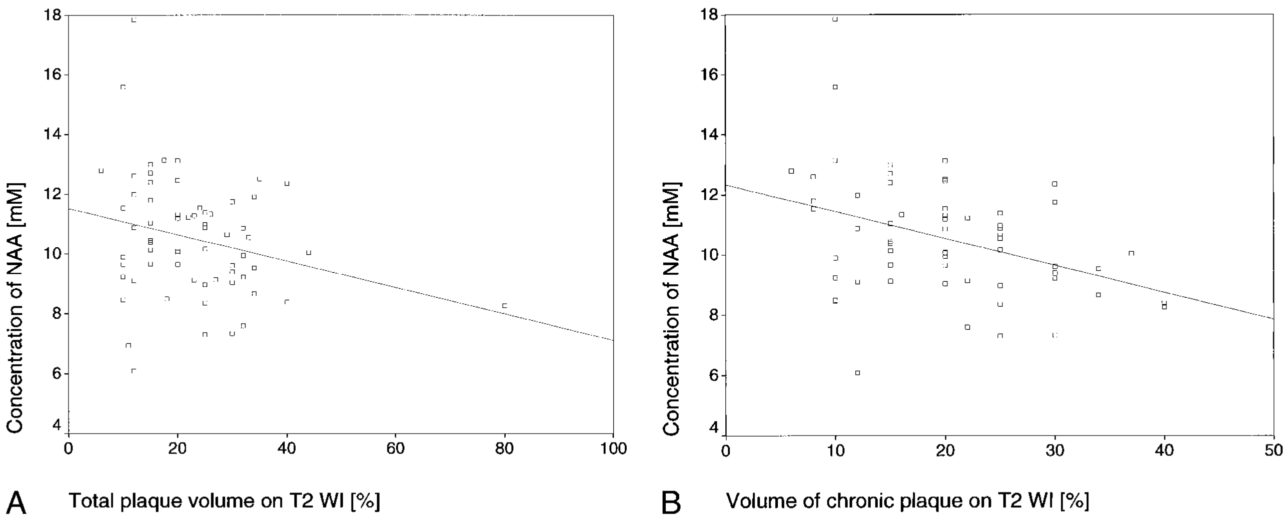
D, Time course of *myo*-inositol. No systematic changes are recognizable.

tration of NAA showed a significant variation in its time course ($P < .04$), with a final increase over 2 years (see Fig 3A). A significant reduction ($P < .003$) of NAA in MS patients (8.96 ± 0.63 mM/L) relative to that in healthy control subjects (11.86 ± 0.43 mM/L) was observed in month 1. The concentration of NAA correlated with the total relative plaque volume within the VOI on T2-weighted images ($r = -0.26$, $P < .05$) (see Fig 4A). However, when only chronic plaques were considered (not contrast-enhancing plaques within the VOI), there was a stronger negative correlation in the concentration of NAA relative to (chronic) plaque volume ($r = -0.38$, $P < .01$) (see Fig 4B).

The time courses of the absolute concentrations of Cr, Cho, and *myo*-inositol are displayed in Figure 3B–D. According to ANOVA calculations, changes in these concentrations during the specified time courses were not significant. To investigate the changes in metabolism during the early periods of plaque development, we compared these results month by month, using a paired *t*-test. The Cr concentration in month 12 (9.04 ± 0.37 mM/L) was significantly elevated relative to that in control subjects (7.07 ± 0.54 mM/L, $P < .001$) and decreased significantly ($P < .02$) (but to still-elevated values) after 2 years (8.65 ± 0.59 mM/L). The initially increased Cho level (2.62 ± 0.13 mM/L) was significantly decreased after 3 months (2.21 ± 0.15 mM/L, $P < .04$) and after 1 year (2.25 ± 0.12 mM/L, $P < .04$), approaching normal levels after 2 years (2.20 ± 0.15 mM/L).

Spectral Changes during the Stage of Contrast-enhancement

Owing to the course of the disease, some of the investigated VOIs contained new contrast-enhancing plaques at more than one investigation. In 16 patients, a total of 35 voxels containing a contrast-enhancing plaque were found. To reiterate, the initial proton MR spectroscopy was performed 1 or 2 days after diagnostic imaging with gadolinium-containing compounds. The follow-up investigations were done before contrast administration. There was a significant negative correlation ($r = -0.9$, $P < .03$) between the size of the contrast-enhancing plaque on T1-weighted images and the absolute concentration of Cr (see Fig 4C). The concentration of Cho and *myo*-inositol were not correlated with



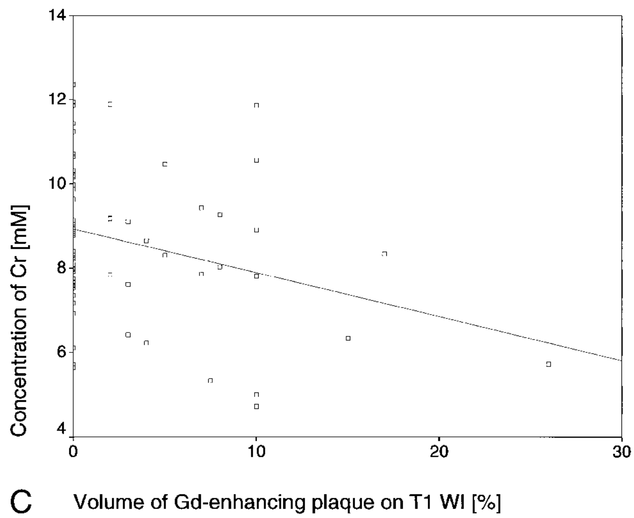


FIG 4. Absolute concentration of metabolites as a function of plaque size.

A, The concentration of NAA (in mM/L) correlated with total plaque volume on T2-weighted images, expressed as a percentage of the VOI (in %). There is a negative correlation between NAA and total plaque volume on T2-weighted images ($r = -0.26$, $P < .05$).

B, The concentration of NAA (in mM/L) correlated with chronic plaque volume on T2-weighted images, expressed as a percentage of the VOI (in %). There is a stronger negative correlation between NAA and chronic plaque volume on T2-weighted images than in *A* ($r = -0.38$, $P < .01$).

C, The concentration of Cr (in mM/L) correlated with the volume of acute contrast-enhancing plaques on T1-weighted images, expressed as a percentage of the VOI (in %). Note the negative correlation between Cr and acute contrast-enhancing plaque volume on T1-weighted images ($r = -0.9$, $P < .03$).

plaque size, as assessed on contrast-enhanced T1-weighted images and on noncontrast T2-weighted images. Elevated marker peaks in the region of 2.1 to 2.6 ppm (2, 4, 12) were not found.

Differences among the Treated Groups

Neither at month 0 nor during follow-up were significant differences found in the metabolic concentrations among the groups treated with placebo, 2 mg DSG, or 6 mg DSG. For this reason, all three groups were pooled together for statistical analysis.

Differences between the Clinical Groups

There was no difference between the relapsing-remitting and secondary-progressive MS groups. The time courses of the groups were not significantly different over the period of investigation.

Discussion

Quantitative proton MR spectroscopy is essential for the unambiguous determination of the metabolites considered here. Serial studies of MS are important for estimating the role of metabolites during the different stages of plaque development. Several investigators have undertaken serial studies to ascertain an absolute quantification of metabolites (although they did not investigate contrast-enhancing plaques), and found unchanged concentrations of Cr and Cho and reduced levels of NAA over time (25, 32).

In our study, the time courses of the metabolites Cr, NAA, Cho, and *myo*-inositol in acute contrast-enhancing plaques were determined with quantitative proton MR spectroscopy to investigate whether any changes in the metabolic concentrations were related to the acute contrast-enhancing stage or to later stages of plaque development. Fortunately, we were able to join a clinical pharmacological study investigating the effects of the immunomodulating drug DSG, which enabled us to study differences in cerebral metabolic concentrations over time between the placebo group and the treated group and between the relapsing-remitting and secondary-progressive MS groups.

The reproducibility of metabolic signals in proton MR spectroscopy has been a matter of controversy in the literature (47–49). We performed a fully automated postprocessing procedure using the LC model, as recommended by Simmons et al (49), who reported a reliable long-term in vivo precision. Reproducibility of the position of the VOI is also a critical issue, and in this study, the VOI was carefully repositioned with regard to a plain radiograph, obtained in each patient, indicating the exact position of the VOI in relation to adjacent anatomic structures and to the lesion itself.

We found a mean plaque volume of 2.1 cm^3, a value comparable to that reported in another serial clinical study (32). Nevertheless, we still have to assume that a variable amount of normal-appearing white matter (NAWM) contributes to the spectra. The metabolic concentrations of healthy control subjects, as measured in our study, are in agreement with previous studies (45, 49–54), suggesting that the spatial registration and quantification of the spectra are reliable.

Time Course of Cr

The initially normal absolute concentration of Cr is in agreement with previous studies (25, 32) and is supported by a proton MR spectroscopy study showing a normal phosphocreatine/(total phosphorus) ratio in MS plaques (5). In one study using MR spectroscopy techniques comparable to ours (14), the Cr concentration was elevated in two acute MS plaques, one showing contrast enhancement. The reasons for this discrepancy are not clear.

The main finding in our study was the significant increase in Cr from month 3 to month 12, during the subacute and chronic course of plaque development (see Fig 3B). A time course of the progression of demyelinating MS plaques has been described by Prineas et al (55), in which remyelination of acute plaques started at 4 weeks and achieved significant proportions after 10 weeks. This was preceded by a repopulation of the plaques with oligodendrocytes. Thus, the increase of Cr might be related to the presence of oligodendrocytes. Our observations could be explained by the following theory: Cr was reduced in acute plaques; owing to the contribution of NAWM to our spectra, the concentration of Cr in months 0 through 2 was normal; and at month 3, when repopulation of the plaques with oligodendrocytes began, a ''synergistic effect'' of remyelination of the plaque and microgliosis of NAWM led to increased Cr in the spectra.

Time Course of NAA

NAA has been investigated extensively, and a reduction in absolute NAA concentration has been seen in the majority of these studies (2, 6, 14, 25, 29, 32). Our results at month 1, showing a significant reduction of NAA relative to that in healthy control subjects, are in agreement with these earlier findings. At month 0, however, we did not see any significant changes in NAA concentration. Thus, we conclude that at month 0, we observed hyperacute MS plaques, in which no changes in NAA have yet occurred.

We found a significant variation in the time course of NAA concentration over 2 years, with a decrease until month 1 and an increase up to the initial values. Simple edema or an anatomic loss of axons or neurons alone cannot account for these reversible changes. Several factors have to be considered. First, edema may lead to a decrease in the relative number of axons in and around the lesion. A recent study showed a reduced axonal density in three histologically examined demyelinating lesions; and even in the late remyelinating lesions, some edema was described (56). Our results are in agreement with these findings, as the concentration of NAA was negatively correlated with plaque size on T2-weighted images ($P < .05$). However, the fact that this phenomenon showed a stronger significance when applied only to the chronic (not contrast-enhancing) parts of plaque volume on T2-weighted images ($P < .01$) suggests that the negative correlation between NAA concentration and plaque volume is also an issue of the chronic stage of disease and not of edema alone. Second, reversibly altered relaxation times of NAA could account for the observed changes, especially if long TEs and/or short TRs are used. Animal studies, however, using the model of acute experimental allergic encephalomyelitis, did not reveal any changes in the T1 or T2 relaxation times of NAA (57). Third, the role of NAA is still not completely understood. Possible biochemical functions of NAA include involvement in lipid and/or protein synthesis, in the storage form of aspartate, or in the breakdown product of NAA glutamate (58). Because NAA and the state of myelination during normal brain maturation are closely related (59), one has to take into consideration that NAA and remyelination may also be related. According to Prineas et al (54), the remyelination of acute plaques starts at 4 weeks and achieves significant proportions after 10 weeks. This is about the time that the concentration of NAA started to increase again in our study (see Fig 3A), and would thus support this argument.

Time Course of Cho

The significantly elevated Cho concentration at the initial stage of our study ($P < .001$) is consistent with findings in the literature (29), and is thought to be due to an increase in the steady-state concentrations of phosphorylcholine and glycerophosphorylcholine during active myelin breakdown (18, 23, 29). During the time course of our investigation, Cho significantly decreased after 3 and 12 months and normalized after 2 years, indicating that Cho is related to the acute contrast-enhancing stage of plaque development. Its concentration, however, is not related to plaque size. Thus, Cho might be related to changes within the plaque and the surrounding NAWM during the first months.

Time Course of myo-*Inositol*

An increase in *myo*-inositol in MS plaques relative to that in healthy volunteers and/or NAWM has previously been described (14, 32). In our study, the time course of *myo*-inositol did not reveal a clear tendency. As there was no significant correlation between plaque size (as estimated on T2-weighted or contrast-enhanced T1-weighted images) and the concentration of *myo*-inositol, the amount of NAWM within the VOI seems not to play an important role. Because *myo*-inositol is the metabolite most sensitive to side effects of the water resonance, its evaluation and interpretation are more difficult than for the other metabolites, and final conclusions about its time course and its relevance are not possible at present.

Effects of Treatment with DSG

There were no effects of DSG on metabolic concentrations or on their time courses. This is in agreement with clinical observations, which also revealed no statistically significant effects (33).

Differences between Clinical Groups (Relapsing-remitting and Secondary-progressive MS)

No differences were found between the relapsing-remitting and secondary-progressive MS groups. In the literature, differences between these groups are expressed as ratios, and the alterations in single metabolites (eg, reduction of NAA or increase of Cr) are not significant, although they may reach a significant level in combination (8, 9, 16, 19, 26, 30, 31). Using absolute concentrations, Davie et al (15) found reduced NAA concentrations in both groups, which supports our results.

Conclusions

This is the first serial quantitative proton MR spectroscopy study of acute contrast-enhancing plaques. One prominent finding of our study is that Cr is not stable over time. The initially unchanged values of Cr increased from month 3 through month 12 and remained elevated after 2 years. This observation has several implications: that the creation of metabolic ratios relative to Cr is influenced by the altered concentration of Cr and should be avoided; that the importance of an absolute quantification has to be emphasized; and that Cr itself may be related to a repopulation of plaques with oligodendrocytes and to remyelination. A decrease in NAA was found in month 1, with recovery starting at 3 months and proceeding until the end of the second year. Again, the necessity of absolute quantification has to be stressed, because metabolic ratios can be misleading, as none of the metabolites remained stable over time. The reasons for the recovery of NAA remain unclear, as does its biochemical role. Diminishing edema over time may play a role, as the concentration of NAA was weakly correlated with plaque size on T2-weighted images. However, the strong correlation ($P < .01$) of NAA with the amount of chronic plaques within the VOI on T2-weighted images shows that the recovery of NAA concentration is not due to edema alone: a relationship with remyelination must also be considered.

The time course of the initially elevated Cho showed a reduction after 3 and 12 months and normalized after 2 years. Cho is obviously related to the acute stage of plaque development and not to plaque size, indicating that the surrounding NAWM may also be involved during acute inflammation. No effect of DSG on the spectra was found and there were no spectral differences between the clinical (relapsing-remitting and secondary-progressive MS) groups.

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