Early Time Course of N-Acetylaspartate, Creatine and Phosphocreatine, and Compounds Containing Choline in the Brain After Acute Stroke
A Proton Magnetic Resonance Spectroscopy Study

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Background and Purpose: The early time course after acute stroke of cerebral N-acetylaspartate, creatine and phosphocreatine, and compounds containing choline was studied in vivo by means of localized water-suppressed proton magnetic resonance spectroscopy.

Methods: Eight patients with acute stroke were studied serially in the acute phase, 1 week after, and 2-4 weeks after the onset of clinical symptoms. Ten healthy volunteers served as controls. A stimulated echo (STEAM) sequence was used for measurement of the brain metabolites in a volume of interest located within the infarcted area as visualized by magnetic resonance imaging. For quantification, the unsaturated water signal was used as the internal standard. Regional cerebral blood flow in the infarcted area was measured relative to a symmetrically located unaffected area by means of single-photon emission computed tomographic scanning, using $^{99m}$Tc-labeled d,l-hexamethylenepropyleneamine oxime as the flow tracer.

Results: Relative regional cerebral blood flow was considerably reduced in the infarcted area in the acute phase. After 1 week, hyperemia was seen in all but one patient. The N-acetylaspartate content was significantly reduced, with the loss appearing to occur between 6 and 24 hours after the stroke incident. The reduction in N-acetylaspartate content was greater in the central part than in the peripheral part of the infarcted area. Creatine and phosphocreatine were also reduced in the infarcted area, whereas no significant change was seen in the choline content.

Conclusions: Assuming that N-acetylaspartate content reflects neuronal survival or loss, our results may suggest that treatment procedures with restoration of blood flow to severely ischemic areas should be initiated within the first 6 hours after stroke onset. (Stroke 1992;23:1566-1572)

KEY WORDS • cerebral blood flow • cerebral ischemia • metabolism • nuclear magnetic resonance

By means of proton magnetic resonance spectroscopy (1H-MRS), it has become possible to detect a number of metabolites in the brain, including N-acetylaspartate (NAA), creatine and phosphocreatine (Cr+Pcr), and compounds containing choline (Cho). Using the unsaturated water signal as the internal standard, average concentrations of these metabolites in a volume of interest (VOI) can be approximated by assuming an average fractional water concentration in brain tissue. These studies suggest concentrations in normal brain tissue of 10-12 mM for NAA, 7-10 mM for Cr+Pcr, and 1-2 mM for Cho.

The role of NAA in the brain is largely unknown. Koller et al showed that NAA existed in higher concentrations in gray matter than in white matter and disappeared after injection of the neurotoxin kainic acid into the neurons. A number of studies indicate that NAA is largely present within neurons. Thus, NAA may be used as a neuronal marker to study the number of viable neurons in the brain tissue.

Phosphocreatine serves as a reserve for high-energy phosphates most prominently in neurons and muscle cells and buffers cellular ATP reservoirs. The proton resonance peak in vivo represents the composite signal of Cr+Pcr. The Cho peak observed probably represents the major water-soluble Cho-containing compounds in the brain, including choline, glycerophosphocholine, and phosphocholine.

Animal studies of ischemic brain using phosphorous magnetic resonance spectroscopy ($^{31}$P-MRS) have shown a rapid reduction in the PCr/inorganic phosphate ratio.
(Pi) ratio and acidic pH after the onset of ischemia, after which 31P-MRS spectra quickly returned to normal. Proton spectroscopy has demonstrated rapid formation of lactate during ischemia after clipping of the carotid artery, and a gradual reduction of lactate content to normal values after restoration of blood supply. Studies of rat brain indicate that NAA, Cr+PCr, and Cho levels are markedly decreased after the induction of ischemia. It should be emphasized, however, that such animal models may not be directly comparable to human pathology; differences in collateral perfusion may constitute a major problem.

Studies of brain infarction in humans using 31P-MRS have shown reduction in the PCR/Pi ratio only within the first week and acidic pH only within the first 32 hours after stroke, after which pH became alkalotic in the subacute phase and subsequently returned to normal. This is in agreement with earlier positron emission tomographic studies. No changes in metabolic ratios or pH were found in human chronic infarcted tissue; however, a decrease of up to 40% of the total phosphorous signal of infarcted tissue was observed.

Preliminary studies using 1H-MRS in humans indicate that acute stroke is followed by loss of NAA in the infarcted brain tissue. Recent studies indicate a decrease of NAA, Cr+PCr, and Cho after acute stroke. These studies, however, do not permit quantification because the T1 and T2 relaxation rates of the metabolites in question were not taken into account.

Therapeutic approaches aimed at reducing the neurological deficits after acute stroke depend on a better understanding of the pathophysiological mechanisms operating during severe cerebral ischemia. Thus, estimation of the early time course of neuronal death or survival after acute stroke in humans, as suggested by changes in the NAA content, may be of particular clinical importance.

To our knowledge, no studies have been reported describing serial measurements of early changes of NAA, Cr+PCr, and Cho in infarcted brain tissue, including measurement of regional cerebral blood flow (rCBF) after acute stroke in humans. This study therefore focuses on the following issues: early changes in NAA, Cr+PCr, and Cho contents after acute stroke; the regional variation of metabolite contents within the infarcted area; and the time course of relative rCBF in the infarcted area.

**Subjects and Methods**

Our study comprises eight patients with acute stroke (age range, 27–80 [mean±SD, 58.4±19.7] years). 1H-MRS and rCBF measurements were carried out between 6 and 52 hours after the onset of symptoms, with the time between the 1H-MRS measurement and the rCBF measurement ranging between 6 and 24 hours. The measurements were repeated 1 week and 2–4 weeks after the stroke incident. For comparison, 10 age-matched (50.1±22.9 [range, 23–80] years) healthy volunteers were examined with 1H-MRS. The study was approved by the local ethics committee, and informed consent was obtained in all cases.

**rCBF Measurements**

The rCBF was measured in eight slices using a brain-dedicated single-photon emission computed to-
FIGURE 1. T2-weighted magnetic resonance image (top panel) and perfusion (single-photon emission computed tomography [SPECT]) image (bottom panel) obtained in acute phase. Selected volumes of interest are indicated in top panel. Perfusion image shows large perfusion defect on left side corresponding to occlusion of left middle cerebral artery.

FIGURE 2. T2-weighted magnetic resonance image (top panel) and perfusion (SPECT) image (bottom panel) obtained 1 week after stroke in same patient as in Figure 1. Perfusion image now shows a partial reperfusion, with areas of hyperemia.
The Friedmann two-way analysis of variance by ranks was used for test of significance of the relative metabolite signals with time within the patient group. The Mann-Whitney rank sum test for unpaired samples was used for test of significance between the metabolite signals from the patient group in the acute phase and the control group. The level of significance was set at p<0.05.

**Results**

Examples of T2-weighted MRI and perfusion (SPECT) images in the same patient obtained 1 day and 1 week after clinical symptoms are shown in Figures 1 and 2.

**rCBF**

The mean relative rCBF was reduced to about 51% (range, 28–73%) at the center of the infarcted areas at the time of the first examination. All but one patient developed reperfusion hyperemia during the following 2-4 weeks. The average values are summarized in Table 1.

**1H-MRS**

Examples of 1H-MRS spectra obtained at day 1 and after 1 week are shown in Figure 3. Resonances were assigned, according to Michaelis et al,7 as follows: 1) Cr+PCr-methylene singlet at 3.94 ppm; 2) inositols at 3.56 ppm; 3) Cho at 3.22 ppm; 4) Cr+PCr-methyl singlet at 3.03 ppm; 5) NAA-aspartyl group at 2.48, 2.60, and 2.66 ppm; 6) NAA-methyl singlet at 2.01 ppm; and 7) lactate at 1.33 ppm. Peak 7 is known to be lactate; spectra were also obtained at TE=135 msec and 270 msec, and the peak exhibited the phase changes characteristic for lactate.

**Acute Phase**

In the acute phase, the NAA signal relative to water of 1.8×10^-4 (range, 0.0–4.1) was lower than that of 4.0×10^-4 (range, 3.1–4.9) observed in the control group (p<0.01; Figure 4). The measured NAA content versus time in the acute phase is shown in Figure 5. It is seen that the NAA content decreased from the normal range to very low levels between 6 and 52 hours after the onset of symptoms. In Figure 5, the estimated NAA concentrations are shown, assuming a fractional water content of 0.75.24 Using this assumption, the mean NAA concentration was 11.1 (range, 8.6–13.6) mM in the control group and 5.0 (range, 0.0–11.4) mM in the patient group.

The Cr+PCr concentrations in the patient group in the acute phase were significantly lower than those in the control group (p<0.02), whereas no significant difference in Cho concentrations was seen between the two groups (p>0.1; Figure 4). Assuming a fractional water content of 0.75, the mean Cr+PCr concentration was 7.2 (range, 5.8–9.5) mM in the control group and 5.6 (range, 2.8–7.8) mM in the patient group in the acute phase. For Cho, the mean concentration was 1.8 (range, 1.1–2.4) mM in the control group and 1.3 (range, 0.7–1.9) mM in the patient group.

Lactate content was found in all patients in the acute phase.

**Time Course**

No significant change in the NAA content was observed 1 week or 2-4 weeks after acute stroke, and no significant changes in relative signals with time were seen for Cho or Cr+PCr. The lactate content decreased to undetectable levels 2-4 weeks after stroke in all patients.

**Figure 3.** Example of proton spectrum obtained during acute phase (left panel) and 1 week after stroke (right panel) from same patient. Repetition time, 1.5 sec; echo time, 46 msec; and TM, 30 msec. Resonance peaks are as follows: 1, creatine and phosphocreatine (Cr+PCr); 2, inositols; 3, choline; 4, Cr+PCr; 5, N-acetylaspartate (NAA); 6, NAA; and 7, lactate. Vertical scales are identical. There is a marked decrease of NAA, Cr+PCr, and lactate signals and a smaller decrease in the choline signal.
FIGURE 4. Metabolite signals relative to unsaturated water signal in patients (P) obtained at examination in acute phase compared with control subjects (C). Horizontal bars represent mean values. N-Acetylaspartate (NAA) content is considerably reduced within the infarcted area. Cr + PCr, creatine and phosphocreatine; CHO, choline.

Regional Variation

In six patients, regional variation of the NAA content was estimated during the acute phase and 2–4 weeks after. As shown in Figure 6, the NAA signal was lower in the central part than in the outer part of the affected area during the acute phase in five of the six patients studied (p < 0.05). In the sixth patient, no NAA signal was seen in the total volume measured. The same trend was seen at 2–4 weeks after the clinical incident.

Discussion

The main results of the present study are that the NAA content in infarcted brain tissue is considerably reduced compared with normal brain tissue; this is in agreement with earlier reports.15–19 The decrease appears to occur mainly between 6 (in one patient) and 24 hours after the clinical incident. The loss of NAA seems to be greater in the central than in the outer part of the VOI, suggesting a heterogeneous distribution of NAA within the infarcted area as visualized by MRI. The Cr + PCr content also appears to be reduced in the infarcted area, whereas no significant changes occurred in the Cho content.

Methodological Considerations

Quantification of the metabolite concentrations in the brain using the unsaturated water signal as the internal standard depends on conditions related to experimental as well as biological factors.2 The experimental factors include signal linearity, with the amount of metabolites and good volume selection avoiding signals from the brain tissue outside the VOI. Moreover, the spectral resolution must be sufficient to avoid overlapping of peaks in the spectrum.

The biological factors include the water concentration remaining constant during the actual changes in pathophysiological conditions and known for absolute quantification. The metabolites studied should be fully MRS visible, and the T1 and T2 relaxation behavior must be known.

Even with all these conditions fulfilled, it is possible to calculate only the average concentration of the metabolites in the selected VOI.

The instrumental factors have been tested in a series of phantom studies using water solutions of NAA at different concentrations within the biological range.

The results show an excellent linearity of the NAA signal with selected volume size as well as with NAA concentration. The absolute error in calculation of NAA concentration was approximately 1 mM. In vitro studies on a two-compartment phantom showed less than 1% contamination by signals from outside the selected volume.

The same linearity of metabolite signals versus volume size (8 ml, 15.6 ml, and 27 ml) was found in the occipital lobe in five healthy volunteers.2 This finding indicates that the fractional water content of brain tissue does not vary significantly under the present measurement conditions.

When using an external standard,1 variations in signal amplitudes due to B1 field inhomogeneity and static field inhomogeneity may occur. Furthermore, the use of an external standard is dependent on instrumental settings, including receiver gain, coil sensitivity, and radio-frequency inhomogeneity.12

Concerning the biological factors, the T1 and T2 relaxation behavior was estimated at each investigation. The T1 and T2 relaxation rates of the three metabolites did not differ significantly from those measured in the control group; furthermore, no significant changes were seen during the time course of infarction.25 The T2 relaxation rate of water in the acute phase was not significantly longer in the patient group compared with the control group; however, at the later examinations the calculated T2 relaxation rates of water were significantly longer in the patient group, indicating edema.25

FIGURE 5. N-Acetylaspartate content (Estimated NAA conc mM) measured in acute phase at first examination of each patient plotted against time after onset of clinical symptoms. Note that value obtained after approximately 6 hours is within normal range.
The changes in the T2 relaxation rates of water with time in the patient group were not significant. We used the metabolite ratios in the acute phase, in which the T2 of water was not significantly longer, in the comparison with the control group, as the same assumed fractional water content could reasonably be used for the calculation.

It is not known at present whether the metabolites measured in this study are all fully MRS visible. However, the measured NAA concentrations tend to be higher than those measured by direct chemical analysis, which suggests that NAA at least may be fully MRS visible.

With regard to the fractional water concentration, we used a value of 75% (representing a mean value of white matter [71%] and gray matter [81%]) in the calculation of the absolute concentrations. The water content may increase due to formation of edema during the hyperemic period in the first week after stroke. The maximal error is 33%, if the water concentration is 100%, assuming that all water is MRS visible. The error is probably less than 10% underestimation of the concentrations, as we did not observe any significant change in the T2 relaxation behavior of the water signal with time.

Regarding spectral resolution, we cannot exclude that other N-acetyl moieties may contribute to the resonance peak observed at 2 ppm. Frahm et al have found a resonance peak at 2.045 ppm in a human brain region consisting mainly of white matter and suggest that this accounts for up to 20% of the peak area assigned to NAA. They tentatively assigned this peak to N-acetylneuraminic acid. Thus, the concentration of NAA may be overestimated by 20% in normal white matter corresponding to about 2 mM.

Based on these considerations, we suggest that even though the absolute concentration values can be regarded only as first-order approximations with an uncertainty of approximately 20%, methodological errors do not hamper the main conclusions indicated above.

The calculated metabolite concentrations in the age-matched control group in this study are in agreement with the values reported earlier.

With regard to the rCBF measurements, we calculated the relative perfusion as the ratio of the count rate in the infarcted and symmetrically located nonaffected area, corrected for early washout of the tracer according to Lassen et al. However, our results may be influenced by scatter of radiation, yielding an overestimation of the amount of tracer in the infarcted area in the acute hypoperfusion phase. On the other hand, in the later phase the hyperemic perfusion may be underestimated due to initial washout of the tracer. Thus, the calculated relative rCBF in the acute phase reflects maximum values, and those obtained in the hyperemic phase represent minimum values. All in all, this indicates that the observed increase in relative perfusion with time represents a minimum value.

Pathophysiological Implications

Heterogeneous distribution of NAA within the infarcted area. Our results indicate a heterogeneous distribution of NAA within the affected volume shown by MRI. Partial volume effects are probably of minor importance because we found a clearly reduced NAA content in the outer volume as well as in the inner volume. The tendency toward a greater loss of NAA in the central part of the lesion is compatible with the finding that the reduction in blood flow is greater in the central part as well (Table 1). Thus, these findings support the concept of a "penumbra zone" with viable neurons in the ischemic periphery of the lesion.

Time course of NAA content. Our results may suggest that the loss of NAA occurs between 6 and 24 hours after the onset of clinical symptoms. Ideally, repetitive studies on the same patients during the acute phase within 24 hours should be carried out. This, however, is almost impossible to accomplish in practice. Because there is evidence suggesting that NAA for all purposes is located exclusively within neurons, the observed decrease in NAA concentration indicates neuronal loss. Assuming that the NAA concentration correlates with the number of surviving neurons, our results suggest that neuronal damage or loss occurs after 6 hours of severe ischemia. This is in agreement with experimental findings in animals. The question now is whether the neuronal damage is reversible when the blood flow is reestablished during the reperfusion hyperemia. It must be emphasized that we did not observe any increase of the NAA content during hyperemia in the patients who showed no NAA content during the ischemic phase. We therefore believe that the loss of NAA is mostly irreversible.

Creatine + phosphocreatine and choline. We observed a significant decrease in the Cr+PCr content at the first examination. One important methodological implication is that this peak does not remain stable and is therefore unsuitable as an internal standard for in vivo quantification of metabolite concentrations in the brain by MRS.

In the acute phase, the decrease in Cr+PCr may reflect changes in energy metabolism with reduced uptake or increased loss of Cr. However, in the later phase, during hyperemia the persistent reduction in the Cr+PCr content supports earlier findings that gliotic tissue contains less Cr+PCr than does neuronal tissue. The Cho content was within the normal range, and we did not observe significant changes with time. Duijn et al in a recent study, found that Cho was reduced approximately 54% compared to the contralateral hemisphere.
Clinical Implications

Assuming that NAA concentration in fact reflects the number of intact neurons, ¹H-MRS may become an important clinical tool for assessment of the indication for treatment and follow-up studies of the effect. Our results support earlier findings that treatment procedures, in order to prevent neuronal damage after severe ischemia by restoration of blood flow, should be initiated within 6 hours after stroke. It should be emphasized, however, that the prognostic information of the recorded NAA loss in terms of clinical outcome remains to be clarified in controlled clinical studies.

References

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