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Spectroscopic data following stroke reveal tissue abnormality beyond the region of T2-weighted hyperintensity

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Abstract

Cerebral tissue with T2 magnetic resonance imaging (MRI) abnormalities following stroke is generally considered infarcted, while surrounding regions with normal MRI appearance are believed to be healthy. To assess whether these surrounding regions consist of normal tissue, we explored the distribution of *N*-acetylaspartate (NAA) and lactate within and around the hyperintense area on T2-weighted MRI using proton MR spectroscopy. The study was carried out in 25 patients with middle cerebral artery occlusion imaged between 1 and 42 days after stroke onset. NAA/choline (Cho) ratios were significantly reduced in both areas of T2 hyperintensity and in surrounding tissue. The reduction was greater in the region of T2 hyperintensity than in the surrounding region (−50% vs. −28%, respectively) and was unrelated to the delay after the ictus. Lactate/Cho ratios increased massively within the abnormal T2 area, but did not differ from control values beyond the margin of hyperintensity. Overall data indicate that T2 visible lesions on MRI do not infer the entire injured tissue.

Keywords: *N*-Acetylaspartate; Magnetic resonance imaging; Spectroscopy; Stroke

1. Introduction

Magnetic resonance imaging (MRI) enables the visualization of early changes in total water content and distribution in brain tissue and is increasingly used for assessing the extent and location of cerebral damage following brain infarction [1]. From a practical point of view, T2-weighted MRI hypersignal is usually considered to represent the infarcted area, so that the term of infarcted size is often indiscriminately used to characterize the size of the area of hyperdensity. Proton magnetic resonance spectroscopy (^{1}H MRS) is another powerful MR technique that can be used to non-invasively evaluate brain metabolism in a wide variety of diffuse and focal cerebral diseases [2]. Combined in the same session, these two complementary techniques have been used in recent years to localize brain infarction and predict the clinical outcome in stroke patients.

Typical conventional ^{1}H MRS spectra in areas of T2 hyperdensity following stroke reveal elevated lactate signal and decreased level of *N*-acetylaspartate (NAA) [3]. The rise of lactate indicates inadequate oxidative metabolism occurring in all surviving cells. Therefore, NAA is considered as a better indicator of neuronal damage and prognosis than lactate [4,5] since it is almost exclusively located in neurons [6,7]. Many previous studies combining MRI and ^{1}H MRS techniques in stroke management have been widely performed in the region of T2 hyperdensity. However, in recent years, the ^{1}H MRS technique has evolved from a single-voxel to a multi-voxel technique, thus permitting the simultaneous acquisition of metabolite signal intensities in successive volume elements. Interestingly, data showing that the reduction in NAA signal is greater in the core than in the peripheral area of the infarcted region [8,9] suggests that NAA measurements may be helpful for visualizing early neuronal dysfunction in the penumbra.

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However, the tissue-surrounding areas of T2 abnormality have not retained much attention, as they are usually considered as normal. In the present work, we focused particular attention on this border region. Our purpose was twofold: (i) to evaluate whether it can be considered as healthy, (ii) to investigate whether MRI and ^{1}H MRS give consistent information on the extent of the cerebral lesion. Indeed, if the T2 visible lesion covers the entire tissue damage, there should be no reduction in NAA outside of the region of T2 abnormality.

2. Materials and methods

This work was performed in our research program on cerebral ischemia in humans from the Dijon Stroke Registry [10], using ^{1}H MRS in middle cerebral artery infarction [11]. The protocol of the study was approved by the Medical Ethics Committee of the Dijon University Hospital, and each patient gave an informed consent to participate in the study.

2.1. Clinical and radiological evaluation of the middle cerebral artery infarction

Twenty five consecutive patients were included according to the following criteria: cerebral infarction diagnosed on clinical criteria (acute focal cerebral deficit) and on imaging hypodensity on the vascular territory of the middle cerebral artery on CT scan or T1-weighted hyposignal and T2-weighted hypersignal on MRI according to the Damasio's template mapping [12]. The mechanism of cerebral infarction was defined as being either due to cardioembolic origin, atheroma of large vessels or carotid artery dissection. The neurological score of Orgogozo [13], dedicated to the middle cerebral artery territory infarction was used in all cases. A normal score is 100 and a severe deficit is below 50.

2.2. Magnetic resonance spectroscopy

MRI was performed once, prior to the ^{1}H MRS examination. ^{1}H MRS was performed using a 1.5 T Magnetom Vision (Siemens, Germany) whole body scanner between day 1 and day 42 after the onset of the stroke, without prior knowledge of the Orgogozo values (PW, AL, FB). Scout images were acquired in the three orthogonal planes using a double echo T2-weighted turbo spin echo (TSE) sequence (TE 14/85, TR 3500). Using the commercial quadrature head coil, spectra were acquired using a chemical shift imaging (CSI) sequence based on the point resolved spectroscopy (PRESS) technique. Spectroscopic imaging (TE=270 ms/TR=1.5 s) was performed in the transversal plane and with a slice thickness of 15 mm.

Table 1
Individual data from spectroscopy

Patient	Time since stroke (day)	NAA/Cho			Lactate/Cho		
		A	B	C	A	B	C
1	1	1.6	0.7	0.9	0.1	1.0	0.4
2	2	1.0	0.0	1.1	0.0	2.5	0.6
3	2	1.9	1.2	1.6	0.0	0.2	0.0
4	2	1.4	1.4	1.6	0.0	1.8	0.2
5	3	2.3	0.7	1.1	0.0	1.6	0.4
6	3	1.7	1.1	1.8	0.0	1.3	0.7
7	4	1.7	1.2	1.4	0.0	0.7	0.1
8	4	2.0	1.3	1.6	0.0	0.4	0.3
9	4	1.8	1.4	1.9	0.2	0.6	0.0
10	4	1.6	1.1	1.4	0.0	0.8	0.2
11	5	1.6	0.9	1.0	0.0	1.1	0.5
12	5	1.5	1.0	1.5	0.0	1.0	0.2
13	5	1.9	0.9	1.3	0.1	2.7	0.8
14	6	1.9	1.0	0.8	0.0	0.3	0.0
15	7	2.2	0.8	1.3	0.0	6.0	0.7
16	7	1.5	1.2	1.0	0.0	5.0	2.9
17	7	1.6	0.8	1.2	0.0	1.1	0.0
18	7	2.4	0.9	1.1	0.5	0.9	1.4
19	7	1.6	1.3	1.7	0.2	1.5	0.2
20	8	1.8	0.8	1.1	0.1	0.3	0.6
21	8	2.0	0.9	1.5	0.0	4.8	0.0
22	10	1.9	0.7	1.6	0.0	4.5	1.2
23	14	2.3	1.4	1.4	0.3	0.7	0.2
24	30	2.5	0.8	1.3	0.3	0.8	0.5
25	42	2.0	0.5	0.8	0.2	1.6	0.2
Means±S.D.	7.9±9.1	1.8±0.3	1.0±0.3	1.3±0.3	0.1±0.1	1.7±1.6	0.5±0.6

(A) The spectroscopy voxel is placed in normal tissue in the contralateral hemisphere, symmetrically to the region of interest. (B) The voxel closest to the center of the lesion. (C) A voxel as close as possible to the afore-mentioned voxel, but within apparently normal tissue and at the periphery of the lesion.

Partitions (16×16) were acquired over a field of view of 24 cm, giving voxel dimensions of 15×15×15 mm (≈3.4 ml). Water suppression was achieved by applying chemical-shift-selective (CHESS) saturation pulses. Shimming was performed automatically using the manufacturer's program MAPSHIM. Multi-voxel spectroscopic imaging was primordial with regard to the purpose of our study. A long-echo time was chosen rather than a short-echo time although it decreased the signal-to-noise ratio (S/N) of the measured resonances. Indeed, short time echo multi-voxel spectroscopic imaging is not easily applicable and the results are often disappointing due to poor water suppression and lipid contamination.

In order to quantify changes in metabolites levels, we have chosen to use the choline peak as an internal reference. It has been shown [14] that the choline peak does not vary significantly during the acute and subacute phases of stroke. Moreover, no significant drop was observed up to 70 days post-accident. This makes the choline moiety an ideal reference for comparisons of NAA variation during the early stages of stroke.

After fast Fourier transformation into the frequency domain, the spectra were manually phase corrected. The baseline was corrected using a polynomial spline function on those parts of the spectrum known to be relatively free of significant resonances. Peak integrals were then qualified by fitting the major peaks to a Gaussian lineshape [15].

2.3. Voxel selection

In order to compare the multi-voxel data we chose three voxel locations according to the following criteria: (i) the voxel closest to the center of the lesion, (ii) a voxel as close as possible to the aforementioned voxel, but within apparently normal tissue and at the periphery of the lesion, (iii) a third voxel located in the contralateral hemisphere, symmetrically to region of interest. T2-weighted images were used to differentiate apparently normal tissue and the lesion.

2.4. Statistical analysis

Statistical differences between the multi-voxel data were determined by one-way analysis of variance (ANOVA) followed by a Student Newman–Keul's test. A value of $p \leq 0.05$ was considered significant.

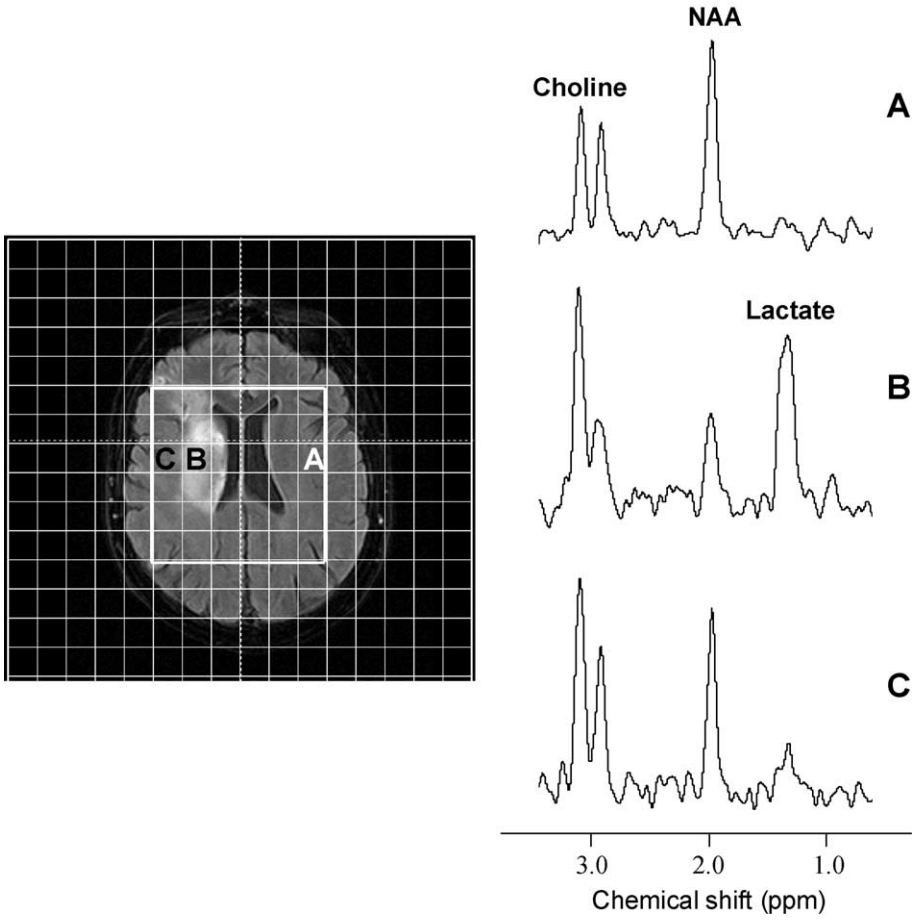

Fig. 1. Representative T2-weighted image showing the location of the voxels used for spectroscopic analysis and the corresponding spectra in contralateral tissue (A), within (B) and outside of the area of T2 abnormality (C).

3. Results

Table 1 reports the individual data from spectroscopy in the 25 patients included in the study (15 men: mean age 57.0±20.3 years and 10 women: mean age 53.4±20.5 years, mean±S.D.). The time range post-ictus was 7.9±9.1 days (mean±S.D.). This relatively late time value was mainly due to the last four entries (10–42 days) and the mean time for the bulk of the data (21 of 25 entries) was about 5 days (4.8±2.2, mean±S.D.). Evidence of infarction of the middle cerebral artery territory was demonstrated in all cases. Cardioembolic infarct was diagnosed in six cases, large vessel atheroma infarct in seven cases and carotid artery dissection in seven cases. All patients had motor deficit. Aphasia was present in eight cases and Orgogozo score at day 1 after stroke onset was 53.8±24.7 (mean± S.D., range 20–90).

Fig. 1 shows a typical T2-weighted image and multivoxel spectra in contralateral tissue (A), within (B) and outside (C) the abnormal area on T2-weighted MRI. Table 1 presents the individual NAA/Cho and lactate/Cho ratios and indicates no dependency of these ratio on time after stroke.

In the contralateral hemisphere, three prominent resonances were consistently detected: Choline (Cho), NAA and total creatine (phosphocreatine + creatine), while the lactate resonance was insignificant. A small signal was observed in some patients' contralateral hemisphere at or around the lactate resonance. We tentatively attributed this signal to lactate but we could not explicitly rule out the presence of other lipid resonances given the low S/N. As shown in Fig. 2, NAA/Cho ratios were significantly reduced in areas of T2 hyperintensity (−50%) and a massive increase in lactate/Cho ratios was observed (~35-fold). NAA/Cho ratios outside the abnormal MRI areas were still reduced as compared with contralateral tissue (−28%, $p<0.05$). Nevertheless, the values were significantly higher (+42%) than those observed in areas of T2 abnormality. Lactate/Cho ratios peripheral to the lesion area were not significantly elevated as compared with contralateral ratios ($p=0.48$), although some lactate was detected in most cases. However, only three patients exhibited marked elevated lactate/Cho ratios over 1.0 in that area (entries 16, 18 and 22, see Table 1).

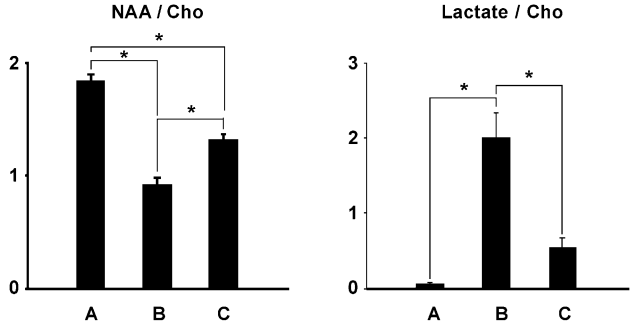

Fig. 2. Spectroscopic data (means±S.E.M.) in contralateral tissue (A), within (B) and outside (C) the area of T2 abnormality. *($p<0.05$).

4. Discussion

NAA/Cho ratios in areas of T2 hyperintensities were markedly reduced, while lactate/Cho ratios were increased, and this is in agreement with many earlier reports (see Introduction). However, the main result of the present study is that the NAA/Cho ratios in areas adjacent to those of T2 abnormalities were significantly reduced as compared with contralateral tissue. The reductions were unrelated to the delay after the onset of the ictus and were indiscriminately observed in patients with acute, subacute and late infarction. Conversely, lactate/Cho ratios in areas adjacent to those of T2 abnormalities did not significantly differ from those observed in the contralateral hemisphere. Now, the question arises about the significance of the observed changes in both areas of T2 abnormality and in surrounding areas.

T2-weighted MRI measurements in early stroke reflect a local increase in brain water content and visualize the extent of brain edema. Clearly, early rises of lactate in regions of T2 hyperintensity indicate glycolytic disturbances while early decreases in NAA likely reflect the presence of both nonfunctional and dysfunctional neurons. Nonfunctional neurons are expected to contain no NAA, presumably because they are badly injured (even dead) and incapable of synthesizing and storing NAA. Dysfunctional neurons are damaged and contain less than normal NAA due to some combination of less synthesis [16] or greater breakdown. These neurons may only be reversibly injured at this time. Accordingly, we have recently demonstrated that experimental mitochondrial dysfunction in vivo is associated with a marked decrease in NAA even in the absence of neuronal death [17] and there are an increasing number of animal and clinical studies indicating that reversible decreases in NAA can occur in situations associated with neurological recovery [17–24]. In addition, it has been recently reported that the absence of NAA in brain tissue was not associated with extensive loss of viable neurons in a child with a rare enzymatic abnormality of NAA metabolism and neurodevelopmental retardation [25]. Thus, in our study, areas B and C probably contain both kinds of damaged neurons. It is possible, however, that only dysfunctional neurons are initially present in areas B and C.

Obviously, the situation is different after several days post-ictus. Then, T2-weighted MRI measurements initially relate to both edema and tissue necrosis and only reflect necrotic tissue after several weeks. This is another circumstance in which lactate level may be elevated. Indeed, previous ^{1}H MRS studies of infarcts showed elevated lactate signals after several weeks [26] to several months after stroke [27,28]. In focal ischemia, the pattern of ischemic

damage is characterized by successive stages of glial cell reactions [29]. The initial phase is characterized by the presence of activated resident microglia. Later, microglia become further activated while activated astroglia become visible, mainly in the border of the lesion. An exogenous inflammatory response takes place. Infiltration and accumulation of blood-derived macrophages are important and macrophages are thought to be responsible for the lactate accumulation [30]. On the other hand, delayed NAA depletion in the region of T2 abnormality reflect neuronal destruction. This is corroborated by data obtained in longitudinal studies showing that the reduction in NAA in this region was irreversible [27,31]. However, as evidenced by present and by other previous clinical [9,11,30] and experimental reports [17,32], the reduction of NAA was modest even in situations in which it is believed that most neurons were destroyed. This means that neuronal loss was largely underestimated. One explanation might be the inability of the lesioned tissue to eliminate NAA from the interstitial fluid. Other possible reasons might be the trapping of NAA in cell debris [33], the redistribution of NAA in the glial cells [34,35] or NAA synthesis by activated astrocytes [33].

Present data indicate that lactate/Cho ratios outside of the region of T2 abnormality were almost normal values in most patients, indicating no apparent glycolytic disturbance. Only three patients exhibited elevated lactate signals over 1.0 in that region. In these patients, the rise of lactate was unrelated to the delay after stroke onset and possibly originated from intervoxel signal contamination [36] even though impaired oxidative metabolism cannot be excluded. Present data also indicate that the NAA/Cho ratios were decreased outside of the region of T2 abnormality. This is in disagreement with the observations by Gillard et al. [36] who reported that NAA was not significantly different from normal values in the regions adjacent to those of T2 abnormality within 24 h of stroke onset. Importantly, in that study, ^{1}H MRS measurements in these regions were performed in only six patients, which may weaken the conclusion that NAA was not altered. Our results are also in disagreement with those by Wild et al. [9] who recently reported no reduction in NAA beyond the margin of T2 hyperintensity in 11 patients imaged 24–72 h after stroke onset. However, it can be pointed out that, in this latter study, the differences between the outer areas and normal tissue were near to statistical significance ($p=0.06$). Therefore, it possible to speculate that a significant reduction might be observed in a larger group of patients.

As illustrated by histological studies, the evolution of ischemic damage differs among the anatomical sites of the lesion, showing two distinct patterns of damage [37]: (i) complete infarction consisting in necrosis of all tissue structures (neuronal and glial cells, nerve fibers and blood vessels), and (ii) selective loss of neurons in the periphery of the infarct with preservation of the tissue structure and morphological integrity of the surrounding cells. Areas exhibiting only mild ischemic damage at a distance from the occluded vessel may be therefore invisible to MRI. In addition, although necrotic cell death has been traditionally related to focal ischemia and referred to brain infarction, two different forms of cell death can be distinguished morphologically: necrotic and apoptotic death [38]. Apoptosis is an energy-requiring cell death process and is therefore believed to be less decisive than necrosis in focal ischemia. However, it may be speculated that it becomes of greater importance far from the core of the lesion. Therefore, both scattered necrotic and/or apoptotic neurons can, at least partly, account for a decrease NAA signal in distant areas.

In summary, the results of this study do not support the notion that the extent of the cerebral lesion is directly reflected in the appearance of the brain on T2 MRI at that particular moment. Present data clearly show that MRI underestimates the volume of abnormal tissue as compared with spectroscopy. This appeared as a constant feature and was indiscriminately observed whatever the delay before spectroscopy. It should be pointed out that longitudinal studies would surely provide more reliable information on the evolution of ischemic damage as assessed by MRI and ^{1}H MRS. However, our study was performed in patients with a large panel of delays after the onset of the ictus, so that it can be hypothesized that it gives a global picture of the different stages after infarction. Within recent years, the use of diffusion-weighted imaging associated with contrast media-based perfusion imaging has provided more powerful tool for the precocious study of the stroke lesion and the surrounding (potentially jeopardized) tissue. Clinical studies, carried out within hours of stroke insult and based on the "mismatch" between areas of perfusion defects and anomalous diffusion, have allowed a differentiation between infarct and salvageable peripheral tissue (often denoted as the penumbra) [39,40]. In our study, the average time post-ictus was approximately 5 days for the bulk of the data. At this time, the lesion depicted by diffusion-weighted images roughly matched that defined by the conventional T2-weighted images. Considering that NAA is a marker of brain dysfunction, we postulate that early multi-voxel ^{1}H MRS measurements could also provide valuable information on the ischemic penumbra, an area difficult to demonstrate. This will be of particular importance for testing pharmacological treatments aiming at limiting neuronal injury in stroke. Clearly this needs to be confirmed and requires further investigations.

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