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Time course of NAA T2 and ADC_w in ischaemic stroke patients: ^1H MRS imaging and diffusion-weighted MRI

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Abstract

Background and purpose: Proton spectroscopy and quantitative diffusion-weighted imaging (DWI) were used to investigate the pertinence of *N*-acetyl aspartate (NAA) as a reliable marker of neuronal density in human stroke. *Methods:* The time courses of tissue water apparent diffusion coefficient (ADC_w) and metabolite T2 were investigated on a plane corresponding to the largest area of cerebral infarction, within and outside the site of infarction in 71 patients with a large cortical middle cerebral artery territory infarction. *Results:* Significant reductions are seen in NAA T2 deep within the infarction during the period comprised between 5 and 20 days postinfarction; the relaxation times appearing to normalise several months after stroke. After an acute reduction in ADC_w, the pseudonormalisation of ADC_w occurs at 8–12 days after the ischaemic insult. The minimum in *N*-acetyl aspartate T2 relaxation times and the pseudonormalisation of ADC_w appear to coincide. *Conclusions:* The data suggest that modifications in the behaviour of the observed proton metabolites occur during the period when the vasogenic oedema is formed and cell membrane integrity is lost. For this reason, NAA may not be a reliable marker of neuronal density during this period.

Keywords: Cerebrovascular stroke; Magnetic resonance spectroscopy; Diffusion-weighted magnetic resonance imaging; Apparent diffusion coefficient; *N*-acetyl aspartate; Metabolite T2

1. Introduction

Magnetic resonance spectroscopy (MRS) and MRI are potent tools to investigate the anatomical and metabolic alterations induced by ischaemic stroke. Diffusion-weighted imaging (DWI) has become an important MRI method for the early diagnosis and characterisation of ischaemic stroke [1–3]. Early reports showed that acute infarcts appear hyperintense on DWI because of a reduction in the apparent diffusion coefficient (ADC_w) of water within minutes after the onset of ischaemia, reflecting early disruption of energy metabolism and cytotoxic oedema.

Over the past decade, magnetic resonance spectroscopy has made important contributions towards an understanding of the physiological manifestations of ischaemic brain injury leading to stroke. In particular, proton (^1H) MRS allows an appreciation of the changes occurring during the different phases of ischaemic brain injury, including the relative depletion of neurochemicals and an accumulation of lactate [4–11]. In the ischaemic core, MRS spectra typically show a reduction in the *N*-acetyl aspartate (NAA), creatine–phosphocreatine (Cr–PCr) and choline signals, albeit to a lesser extent, and the presence of lactate. As NAA, in the mature brain, is considered to be confined to neurons [12–14], a reduced NAA level in brain should reflect decreased neuronal density—thereby suggesting that NAA could be used as a putative neuronal marker. Indeed, in normal healthy tissue, the extracellular NAA content is considered to be insignificant [12,14,15].

However, animal studies on the relationship between NAA decline and neuronal survival in focal brain ischaemia would seem to be in contradiction with this latter statement.

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Studies on the early time course of NAA in the brain during focal ischaemia [13] have shown that within the infarct, [NAA] is only reduced by 20–30% after several hours of ischaemia. It would therefore appear that [NAA] yields an underestimation of the extent of neuronal damage. Sager et al. [13] demonstrated in a mouse model that a significant proportion of NAA is trapped in cell debris and that its use as a marker of neuronal density was thus restricted.

Many animal studies have concentrated on the early effects of focal ischaemia on the NMR-derived brain metabolite concentrations. In humans, both short-term and long-term studies have principally evaluated the variations in metabolite ratios (NAA/Cho etc). Unfortunately, these data do not give a clear picture as to the behaviour of the metabolites during the different phases following the ischaemic insult. In the event that the biochemical environment of the metabolites were to be modified, i.e., interaction with large proteins or macromolecules, their NMR behaviour may be modified, and these modifications could be quantified via the motion-sensitive transverse relaxation time, T2. A few studies, using single voxel techniques, have investigated the variations of proton metabolite relaxation times, essentially T2, in ischaemic tissues [10,16–20]. However, the results have been somewhat heterogeneous. Thus, the aim of this study was to investigate the time course of ^{1}H brain metabolite T2 relaxation times in human ischaemic stroke. The ADC_w was also acquired via DWI in order to simultaneously follow tissue water modifications commonly associated with the ischaemic lesion.

2. Materials and methods

2.1. Study population

Seventy-seven patients (42 M, 35 F: 61.2 ± 16.1 years) with a large cortical middle cerebral artery (MCA) territory infarction were included in this study. The diagnosis was established according to clinical and radiological criteria. The clinical criteria were those outlined by the National Institute of Neurological stroke and disease [21]: acute onset of cortical or subcortical functions in the MCA territory, with hemiplegia mainly affecting the brachiofacial territory and sensory deficit, hemianopia and aphasia being more or less associated. In all cases, the neurological disorders lasted over 24 h. In all studied patients, CT scan had been performed (Elscint 2400) to identify cerebral infarction. A second CT scan was performed 24 h later and showed a hypodense image in all cases, corresponding to the MCA territory. Patients with obvious haemorrhage or tumour seen on CT scan or MRI were excluded from the study. At the time of the MRS examination, the infarction age varied between 2 days and 12 months. The study had been approved by the local ethics committee.

2.2. Diffusion-weighted magnetic resonance imaging (DW-MRI)

A 1.5 T Magnetom Vision (Siemens, Germany) whole body scanner was used. diffusion-weighted magnetic resonance imaging (DW-MRI) was performed using a multislice echo planar sequence with the Stejskal–Tanner diffusion-encoding method [22]. The imaging parameters were TR 4000 ms, TE 100 ms, matrice 96×128, FOV 256 mm. Twenty 5-mm slices were acquired in 56 s. The trace DW sequence used three diffusion b values (0–1000 s/mm^2) in each of the three orthogonal directions. An apparent diffusion coefficient (ADC_w) map was generated from these data. The apparent diffusion coefficient was measured directly on the ADC_w maps generated through the manufacturer's software and expressed in the units of mm^2/s.

2.3. Magnetic resonance spectroscopic (MRS) imaging

Images acquired in the three orthogonal planes using a double echo T2-weighted turbo spin echo (TSE) sequence (TE 14/85, TR 3500) for diagnostic purposes were used for spectroscopy localisation. Spectra were acquired using a chemical shift imaging (CSI) sequence based on the point-resolved spectroscopy (PRESS) technique. Spectroscopy was performed in the transversal plane and with a slice thickness of 15 mm. Sixteen by 16 partitions were acquired over a field of view of 240 mm, giving voxel dimensions of $15 \times 15 \times 15$ mm (~ 3.4 ml). Water suppression was achieved by applying chemical shift-selective (CHESS) saturation pulses. Shimming was performed automatically used the manufacturer's programme 3DSHIM.

For the purpose of metabolite T2 measurements, the patients were studied at TEs of 80, 135 and 270 ms (TR 1500 ms) with water suppression. One average was performed in each case leading to a total acquisition time of approximately 20 min for the three CSI sequences.

The CSI data were processed using the spectroscopy analysis package MRUI 99.1b (Magnetic Resonance User Interface: http://www.mrui.uab.es/mrui). The residual water resonance was removed using the HSVD filter routine [23]. Peak detection and quantitation were performed in the time domain using a VARPRO-like algorithm called AMARES [24], which allows an inclusion of a large amount of prior knowledge. The resonances quantified in each metabolite spectrum were the NAA peak at 2.02 ppm, the Cr–PCr peak at 3.02 ppm, the choline peak, at 3.20 ppm and the lactate doublet at 1.33 ppm. Peak integrals were quantified by fitting to a gaussian line shape.

2.4. T2 estimation from MRS data

The spin–spin relaxation times of the proton metabolites were estimated from the peak integrals corresponding to each echo time and a single exponential curve was used to fit the data. For the analysis of patient data, the voxels were

classed according to a simple visual classification scheme based on the T2-weighted TSE images. Central voxels were defined as those voxels within the centre of the lesion and entirely surrounded by abnormal peripheral voxels. Peripheral voxels were defined as those voxels within the lesion and situated on its periphery. Apparently healthy tissue voxels were defined as those voxels directly adjacent, but outside the lesion. These voxels have no or very little abnormal signal on T2-weighted images. Normal voxels were defined as those voxels situated either within the contralateral hemisphere or at least distant from the lesion, and in tissue presenting no abnormal signal on T2-weighted images and being free of excessive CSF contamination.

2.5. Statistical analysis

The statistical tests were performed using the statistical package SYSTAT 7.0 for Windows (SPSS, Evanston, IL, USA). An analysis of variance (ANOVA) with a Tukey ad hoc test was used to compare the data in the different groups. Statistical significance was accepted for $p < 0.05$.

3. Results

3.1. Patient data

Of the 77 patients included in this study, the data sets from six patients could not be used (three subjects were too agitated, three data sets were of too poor quality). The results presented below concern the remaining 71 patients.

3.2. ADC_W measurements

The time course of the ADC_w (Fig. 1) is typical of that already illustrated in the literature; an initial drop in the ADC_w value followed by a pseudonormalisation at 8–12 days postinfarction. The ADC_w continues to rise and, at the chronic phase, reaches values four times those encountered in normal tissue. Quantitatively, similar values were obtained for both the central portion of the lesion and on the periphery.

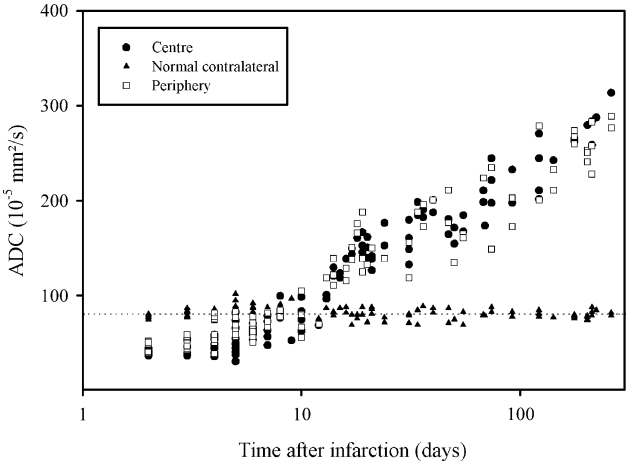

Fig. 1. The time course of the ADC_w deep within the lesion (centre), on its periphery and in normal contralateral tissue.

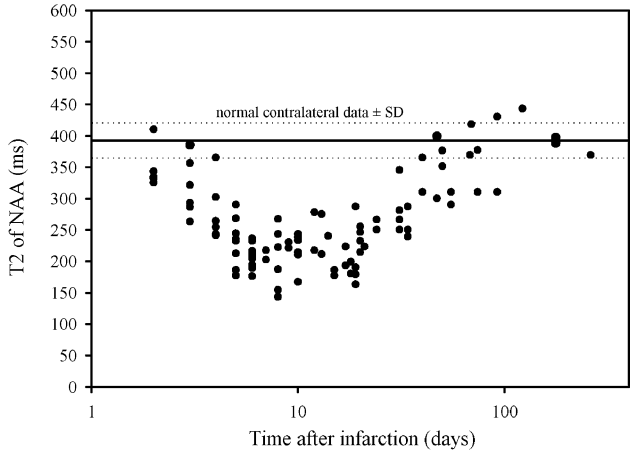

Fig. 2. The time course of NAA transverse relaxation times within the centre of the lesion.

3.3. In vivo metabolite T2 measurements

Fig. 2 illustrates the time course of NAA transverse relaxation times within the central voxels of the infarction. NAA T2 exhibits a significant reduction during a temporal window starting from approximately the fifth day after stroke up to 20 days after the insult—mean NAA T2 during this period: 226 ± 48 vs. 393 ± 27 ms in normal tissue ($p < 0.05$). NAA T2 is also significantly depressed (317 ± 28 ms) in peripheral voxels (although to a lesser extent) during the same period ($p < 0.05$). In both cases, the NAA T2 appear to normalise in chronic lesions (>3 months after stroke): 396 ± 41 ms (centre) and 381 ± 37 ms (periphery), respectively. Choline shows a similar tendency towards lower T2, albeit much less pronounced than for NAA, and this only within the centre of the lesion (T2: 235 ± 53 vs. 291 ± 34 ms in normal tissue; $p < 0.05$). Creatine does not show any significant variation even within the central voxels during this same period (T2: 187 ± 47 vs. 197 ± 23 ms in normal tissue; NS).

4. Discussion

To our knowledge, this is the first paper comparing, simultaneously, the time courses of ADC_w and ^1H metabolite transverse relaxation times; both parameters being sensitive indicators of molecular motion within the cellular environment.

Our in vivo study has clearly identified modified spin–spin relaxation times of cerebral ^1H metabolites in cerebral infarction. The data would also suggest a transient nature in

their variation, the T2 values shortening substantially at the subacute phase (5–20 days postinfarction) and then appearing to normalise at the chronic infarction stage. The in vivo NAA resonance appears to be the most affected at the centre of the lesion. The voxels situated at the periphery of the lesion follow a similar tendency, but to a lesser extent. Choline also observes a similar, but less pronounced behaviour in the centre of the lesion. It is worth noting that our normative T2 data are close to those cited in previous methodological studies [25–27].

The chronology of the ADC_w values observed in this study is similar to that described in the literature, whether it be in absolute terms or relative to contralateral data [2,28,29]. After an acute reduction in ADC_w due to a shift of water from the extracellular space into cells, it reaches a minimum within less than 24 h [30]. The subsequent gradual increase of ADC_w to pseudonormal and then elevated values is thought to reflect a sequence of histopathological changes beginning with vasogenic oedema and progressing through loss of cell membrane integrity and gliosis [30].

In our study, the pseudonormalisation of ADC_w occurs at roughly 8–12 days after the ischaemic insult in both the central and peripheral voxels within the lesion. It is worth noting that the pseudonormalisation occurs at the same time as a minimum in the NAA T2 time course curve. This suggests that modifications in the behaviour of the observed proton metabolites occur during the period when the vasogenic oedema is formed and cell membrane integrity is lost.

The chronology of cerebral stroke has been described by many different biochemical, histological and imaging modalities. Few studies have, however, addressed the serial variations of metabolite NMR relaxation times during acute, subacute and then chronic stroke. Using a single voxel STEAM approach (27 ml), Gideon et al. [10] studied six patients with ischaemic stroke during the acute and chronic phases. They concluded that metabolite relaxation times remained within the ''normal range'', whereas tissue water T2 increased significantly from 111 ms in the acute stage to 264 ms in the chronic stage. In the acute phase of focal ischaemia in rat brain, Dreher et al. [31] observed a tendency towards a higher Cr–PCr spin–spin relaxation time. In a transient global ischaemia model in rat brain, Fujimori et al. [17] observed no significant early changes in metabolite T2, whereas water ADC_w values were significantly depressed, 2 h postreperfusion, indicating cytotoxic oedema. In a paper from van der Toorn et al. [18], concerning cerebral ischaemia in the rat, the authors demonstrated a significant trend towards a falling NAA transverse relaxation time at least 24 h postischaemia. Sappey-Marinier et al. [19] observed an increased T2 of the creatine resonance in chronic infarction, although their metabolite T2 normal data were generally much lower than those encountered in the literature. The most striking example of metabolite T2 variations has been given by Kamada et al. [20]. Their single voxel study showed dramatically reduced metabolite T2 values in both acute stroke and peritumour oedema. In their study, NAA T2 was seen to fall from c 350 to c 160 ms, while Cho and Cr followed a similar pattern (Cho: c 320 to c 200 ms; Cr: c 230 to c 150 ms). In the present study, our observations appear to corroborate those of Kamada et al., although we have observed the most significant reduction in NAA during the subacute period (393 ± 27 to 226 ± 48 ms at 5–20 days postinfarction). However, very little change was noted in the Cre resonance. Interestingly, they observed a normalisation of T2 times in peritumour oedema corresponding to an improvement of the oedema. Two nonstroke articles have also quoted reduced NAA T2 in pathology. In a single voxel study, Hanstock et al. [32] observed lower NAA and Cre T2 times in the brain stem of patients suffering from amyotrophic lateral sclerosis compared with control subjects. Similarly, Isobe et al. [33] found lower T2 times for NAA and Cre in low- and high-grade glioma.

Several authors have attempted to explain the variations in the metabolite spin–spin relaxation behaviour. It had been suggested that cell swelling (cytotoxic oedema) at the acute phase of ischaemia might provoke a shortening of metabolite T2. Several studies have investigated the behaviour of metabolite ADC during acute focal ischaemia [34–36]. van der Toorn et al. [34], working on a rat brain model, observed reduced ADC values for both NAA and Cr-PCr in the acute phase of ischaemia. Abe et al. [36] found all the three principal metabolite ADC values reduced within a few hours of ischaemia, although ADC(Cho) to a lesser extent. However, as mentioned above, Fujimori et al. [17] have shown that during the acute phase of ischaemia, the reduction in ADC_w, observed 2 h postischaemia, is not accompanied by a significant drop in metabolite T2. Our own data corroborate the absence of a significant drop in NAA T2 in the very acute phase of ischaemia.

During cell swelling, it has been demonstrated via in vitro experiments that intracellular NAA is extruded from the cell into the extracellular space. It has been suggested that the redistribution of intracellular and extracellular NAA could affect the NMR visibility of this amino acid [12,37]. However, despite a significant extrusion of NAA out of the cell during this phase, the extracellular population still remains very small [38].

Some authors have suggested that the tumbling time of the amino acid could in some way be altered, thereby affecting the spin–spin relaxation time [34]. Little is known about the nonspecific binding of NAA to large macromolecules or proteins. The binding of lactate and alanine to BSA have been studied by Chatham and Forder [39]. The authors concluded that while the alanine resonance was unaffected, '... decreased NMR visibility of lactate in proteinaceous solutions is due to nonspecific binding ...'. Moreover, a methodological study from Murphy et al. [40] demonstrated a significant reduction in NAA T2 in aqueous solutions of NAA with bovine serum albumin (BSA). Our own phantom experiments (unpublished data) using BSA

revealed a very important reduction in NAA T2 from 751 ± 11 ms in absence of BSA to 267 ± 44 ms (BSA 0.44 mmol l^{-1}). It is worth noting that NAA T1 was not significantly modified. In addition, in the Kamada et al. study, the presence of a vasogenic oedema appeared to be the common factor relating falling T2 times in peritumour oedema and stroke lesions. The presence of large proteins or macromolecules within the oedema somehow entering in contact with the proton metabolites may provoke severe reductions in T2. The pathogenesis of cerebral vasogenic oedema is also believed to share a common pathway with that of gliomatous tumour cysts, in that they are the result of a breakdown of the blood-brain barrier [41]. The major findings of studies on tumour cysts have been a very high serum protein content. In a study of 39 tumour cysts, Lohle et al. [41] found an average plasma protein concentration of c 35 g/l.

A few recent articles have put into question the authenticity of NAA as a reliable marker of neuron viability. A non-NMR study on global ischaemia in gerbils by Nakano et al. [42] has shown that for a twofold decrease in the absolute concentration of NAA, the corresponding fall in neuronal density (cells/mm), after 1 week, was in fact 10-fold. The surprisingly high NAA content with respect to a 10-fold drop in neuronal density could be interpreted through the hypothesis proposed by Sager et al. [13] in their recent article that a significant proportion of NAA is trapped in cell debris (i.e., eosinophilic neurons) or located extracellularly.

In conclusion, our study of the metabolite T2 variations in stroke patients confirms other works depicting variations in spin–spin relaxation times. These variations are most marked towards the centre of the lesion, becoming less important towards the periphery. The variations would also appear to be transitory with a reestablishment of normal metabolite T2 values in chronic lesions, although the transient nature still needs to be confirmed by serial measurements on the same patient.

Irrespective of the phenomenon provoking reduced NAA T2, one practical implication of these data is that when using long TE spectroscopic imaging and reporting either simple NAA ratios or absolute concentrations of NAA from within stroke lesions, care must be taken when interpreting data from ischaemic lesions of different ages. Ideally, a T2 correction should be carried out. Neglecting such a correction may lead to gross underestimations of [NAA], and therefore, of potentially viable neurons. In the event that no T2 correction were to be applied, then the only practical solution would be to acquire data at the shortest possible echo time, thereby alleviating the need for metabolite T2 correction. To this end, recent technical improvements in spectroscopic imaging [43] have allowed shorter and shorter TE to be implemented. However, when data acquisition is performed using a short TE, the baseline of spectra is often distorted by eddy current effects and lipid/macromolecule signals, thus making peak analysis difficult.

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