Metabolic Counterpart of Decreased Apparent Diffusion Coefficient During Hyperacute Ischemic Stroke
A Brain Proton Magnetic Resonance Spectroscopic Imaging Study

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Background and Purpose—Recent studies have shown that the brain ischemic area defined by the map of decreased apparent diffusion coefficient (ADC) obtained by diffusion-weighted imaging (DWI) during the first hours of ischemic stroke includes a significant part of ischemic penumbra. We hypothesize that the misjudgment of the final infarct size by ADC mapping may be related to a restricted ability of DWI to capture variations in the intensity of cellular suffering. In an attempt to characterize metabolically the hypoperfused brain parenchyma, we studied the relationship between ADC values and brain metabolic parameters measured by proton MR spectroscopic imaging (SI).

Methods—Six patients with hyperacute ischemic stroke were explored within the first 7 hours after onset with the use of a MR protocol including T2*-weighted MRI, DWI, SI, perfusion-weighted imaging, and MR angiography.

Results—This study demonstrates, for the first time, a wide gradient of ischemia-related metabolic anomalies within the abnormal area delineated by DWI during hyperacute ischemic stroke. In the narrow range of decreased mean ADC values (0.60 to 0.40 × 10⁻⁹ m² · s⁻¹), a 33% decrease in mean ADC is associated with a 122% increase in lactate/N-acetyl aspartate ratio. Mean ADC values never fall below 0.40 × 10⁻⁹ m² · s⁻¹ within the severely affected ischemic tissue, while SI still detects a large metabolic heterogeneity inside areas showing similar decreased mean ADC values close to this threshold.

Conclusions—Our results indicate that the region of very low mean ADC values observed during hyperacute ischemic stroke contains areas of various tissue damage intensity characterized by SI in relation to different stages of cellular metabolic injury. This observation may explain why ADC mapping does not reliably predict final infarct size. (Stroke. 2003;34:e82-e88.)

Key Words: magnetic resonance imaging, diffusion-weighted spectroscopy, nuclear magnetic resonance stroke, acute
MTT maps were calculated as described by Smith et al. Three formed at a rate of 1 acquisition per second (echo time 60 ms; flip angle/110°). Isotropic ADC maps were reconstructed with the use of one third of the MCA territory. No brain hemorrhage was detected for intracranial arteries and 17 seconds for cervical arteries). The duration of the conventional MRI protocol was 10 minutes (T2*: 135 ms/1600 ms; slice thickness = 15 mm, resulting in a voxel volume of 5.7 mL, as explained in the report of Galanad et al.9 This spatial resolution is close to that usually obtained by the single-voxel spectroscopy technique at 1.5 T: 8 mL (20×20×20 mm3) or 3.37 mL (15×15×15 mm3). None of the patients had lesions <5.7 mL in volume or 22 mm in dimension within a single 5-mm-thick ADC slice. The strict immobility of the patient’s head during SI acquisition was helped by the use of pads and pillows and was continuously monitored by a video system. Under these conditions, we did not observe any head movements in the cohort of 6 patients. The duration of the conventional MRTI protocol was <10 minutes (T2*: 128 seconds; DWI: 48 seconds; PWI: 65 seconds; MRA: 40 seconds for intracranial arteries and 17 seconds for cervical arteries). The delay between the end of the DWI and the end of the SI was approximately 15 minutes. Images from DWI and PWI parameters and metabolic images from SI were transformed in 256×256 matrix images with the use of a Fourier interpolation. This interpolation optimizes the informational content of spectroscopic images by increasing their apparent resolution via a 64% increase in measuring accuracy of the intensity and localization of MR signals registered during the SI acquisition. This Fourier interpolation allows a quantitative analysis of individual regions of interest (ROIs) centered on each pixel of the 256×256 matrix spectroscopic image, but the diameter of these ROIs cannot be <22 mm.9

The apparent distortion of metabolic map contours was related to the use of a mask corresponding to the skull signal and to outer volume saturation slices applied to eliminate the lipid signals from the scalp. Moreover, peripheral artifacts on metabolic maps caused by residual lipid contamination or magnetic field heterogeneity were also removed with the use of AMARES-MRUI FORTRAN code.12 After spatial realignment of images obtained by the various modalities, correlations between mean ADC values and metabolic ratios were measured in cylindric ROIs of 22 mm in diameter and 15 mm in thickness corresponding to the SI resolution before Fourier interpolation. These ROIs were defined inside the ipsilateral hyperperfused area delineated by MTT values equal or superior to the contralateral average MTT values +3 SDs and thus including the abnormal area delineated by the ADC map. The use of this rigorous statistical approach was possible owing to the low SD among the quasi-uniform MTT values measured in the parenchyma of the unaffected contralateral hemisphere. The coordinates of each ROI selected within the hyperperfused area were used to position the corresponding ROI of the averaged ADC map and of the spectroscopic image to perform appropriate correlations. The relationship between ADC values and metabolic parameters was not analyzed in anterior frontal regions owing to the presence of magnetic field inhomogeneities in these regions, which cause distortion artifacts in the echo-planar images and degrade the quality of spectroscopic images. Finally, 73 ROIs were defined in brain of patients with MCA occlusion (18, 17, 21, and 17 ROIs, respectively) (Figure 1) while, in relation to a smaller lesion, only 9 ROIs (1 and 8 ROIs, respectively) were defined in brain of the 2 patients without MCA occlusion. Data from SI (Figure 2) were expressed as the ratio of the resonance area of each detected metabolite over the sum of metabolites (S): N-acetyl aspartate (NAA), choline-containing compounds (Cho), and creatine-phosphocreatine (Cr). The use of metabolic ratios eliminates the bias related to a dilution effect caused by edema or cerebrospinal fluid contamination. Regression analysis between mean ADC values and metabolite ratios was performed with a linear or nonlinear regression model (Statview 5.0 software, Abacus Inc). The choice of the mathematical function performing the best-fit regression was based on the highest corresponding r value with a probability value <0.05. The use of this parametric statistical analysis was justified by the gaussian distribution of mean ADC values and metabolite ratios.
as demonstrated by a Kolmogorov-Smirnov test (probability values >0.1 indicated a gaussian distribution) (GraphPad InStat 3.0 software, GraphPad Software).

Results

The decrease in ADC is not correlated with Cho/S ratio or Cr/S ratio whether or not MCA is occluded (data not shown). In patients with MCA occlusion, there is a significant linear relationship between mean ADC values and the increase in lactate (Lac)/S ratio ($r^2=0.47; P<0.0001$; data not shown), as already described in experimental studies.13 Correlation is significant but more complex with the decrease in NAA/S ratio (exponential regression; $r^2=0.48; P<0.0001$) (Figure 1). The Lac/NAA ratio, expressing a combined index of the intensity of anaerobic glycolysis and neuronal suffering, is also correlated with mean ADC values (Figure 1). This correlation is polynomial (second-order polynomial regression; $r^2=0.60; P<0.0001$) in relation to a wider dispersion of values of Lac/NAA ratios in the narrow range of severely decreased mean ADC values ($0.60$ to $0.40\times10^{-9}$ m$^2$·s$^{-1}$) compared with the hypoperfused area with normal mean ADC values (Figure 1, right part of curve). Indeed, in this narrow range of very low mean ADC values, a $33\%$ decrease in mean ADC values is associated with a $122\%$ increase in Lac/NAA ratio. The metabolic heterogeneity inside the area of severely decreased mean ADC is clearly illustrated with the use of SI, whether or not a diffusion-perfusion mismatch is present (Figures 3 to 5). In the 2 patients without MCA

Figure 2. MR spectra recorded during SI acquisition presented in Figure 3. An exponential filtering, a zero-filling (512 zero points filled to 1024), and a zero-order phase correction were applied. Spectra are displayed at the same scale without baseline correction or postacquisition water suppression. a, Spectrum recorded from ROI located inside the infarct core. b, Spectrum recorded from the contralateral ROI. On 135-ms echo time MR spectra, the lactate signal appears as a negative doublet, which facilitates its differentiation from the positive signal of lipids resonating in the same frequency range.

Figure 3. Multimodal MR maps of a patient brain (57-year-old man; NIHSS score, 19) with an acute proximal MCA occlusion explored within 5 hours of onset without a diffusion-perfusion mismatch. ADC units are expressed in $10^{-9}$ m$^2$·s$^{-1}$. TTP and MTT units are expressed in seconds. The area delineated with a dotted line was manually defined from the corresponding averaged ADC map and represents the area of decreased ADC values. The heterogeneity of ADC values visible inside that area clearly did not match well the metabolic heterogeneity demonstrated by SI.
occlusion but explored in the same time window as patients with MCA occlusion, the relationship between mean ADC values and NAA/S ($r^2=0.57; P=0.02$) or Lac/NAA ($r^2=0.63; P=0.01$) ratios is linear in relation to a slighter decrease in mean ADC values that does not reach the minimal threshold observed in the group of patients with MCA occlusion (data not shown).

### Discussion

The wide dispersion of values of Lac/NAA ratios in the narrow range of severely decreased mean ADC values may reflect the large heterogeneity in tissue damage inside the ischemic area that cannot be evaluated with DWI alone. Indeed, mean ADC values do not decrease below a threshold of approximately $0.40 \times 10^{-9}$ m$^2$ · s$^{-1}$, representing for water molecules the maximal possible restricted state of the extracellular space relative to the acute ischemic cytotoxic edema.\(^\text{14}\) In fact, this lowest value of mean ADC during severe ischemia was also observed in experimental studies in which a similar ROI analysis was performed.\(^\text{14,15}\) A limitation of our study is that analysis of correlations between ADC values and brain metabolic parameters needs to merge the ADC pixel values into relatively large ROIs, thereby blurring potentially important ADC heterogeneities at the pixel level. This heterogeneity of ADC values in the narrow range of low ADC values characterizing the ischemic area is demonstrated on 3 individual ADC slices with a pixel-based analysis and on the corresponding averaged ADC map measured on a patient with MCA occlusion explored within 6 hours of onset (Figure 5). This observation is in excellent agreement with the mean ADC value of $0.40 \times 10^{-9}$ m$^2$ · s$^{-1}$ measured in the severely affected ischemic tissue. Nevertheless, despite their higher spatial resolution, these nonaveraged ADC images failed to detect the gradient of the metabolic consequences of ischemia on brain parenchyma demonstrated by SI (Figure 5). In addition, moderate ischemic injury defined by low Lac/NAA ratios was observed in areas characterized by very low values of mean ADC (Figure 1), demonstrating the limitations of DWI to evaluate the severity of cellular suffering. Recent data confirm that these lowest ADC values observed during the hyperacute phase do not correspond to maximal tissue injury.\(^\text{16}\) Indeed, the early decrease in ADC is almost complete as early as 1.5 hours after the onset of ischemia. This time is shorter than the accepted threshold of irreversible tissue injury (3 to 6 hours), whereas the brain parenchymal density evaluated by CT shows a continuous linear decrease long after this period.\(^\text{16}\)

Whether or not the short 15-minute delay between the end of DWI acquisition and the end of SI acquisition in patients with MCA occlusion may be a confounder in our results is difficult to appreciate. Indeed, the duration of ischemia is one of the most important factors to consider when the reversibil-
ity of tissue injury is predicted. One can assume that a 15-minute delay is probably sufficient to observe significant changes in ADC values when DWI is performed during the first 2 hours of ischemia. However, this assumption probably does not hold beyond the initial 2-hour period because the decrease in ADC is then almost complete. This is the case in our patients who were explored between 3.5 and 7 hours after the onset of ischemia, when mean ADC had probably already reached its asymptotic lower values. Moreover, after the first 3 hours of ischemia, the short 15-minute delay is probably not sufficient to observe significant measurable changes in lactate and NAA signals owing to their respective kinetics of evolution, as analyzed in an experimental model of focal ischemia. Under these conditions, the delay between DWI and SI acquisitions is unlikely to be a significant confounder in our results.

The metabolic heterogeneity that we found in the ischemic area is probably related to different stages of tissue injury and metabolic dysfunction, ranging from an anaerobic glycolysis without detectable neuronal injury (isolated increase in lactate level) to a more severe metabolic degradation defined by an anaerobic glycolysis associated with a moderate or severe neuronal injury (combination of different levels of increase in lactate and decrease in NAA). The metabolic heterogeneity within the ischemic area is a well-known feature that has been related to the severity and the duration of hypoperfusion in human and animal studies. The slighter decrease in mean ADC value observed in the 2 patients without MCA occlusion but explored in the same time window as that for patients with MCA occlusion is in accordance with experimental studies that demonstrate moderately low ADC values inside the ischemic area after early reperfusion.

The mismatch between diffusion and metabolic parameters brings novel insight to the understanding of the pathophysiology of ADC variations during hyperacute ischemic stroke, highlighting the added value of SI over DWI (Figures 3 to 5). As illustrated in Figure 1 (right part of curves), SI also discriminates between 2 metabolically distinct hypoperfused areas with subnormal to normal mean ADC and normal NAA/S level, modulated by the

Figure 5. Multimodal MR maps of a patient brain (50-year-old man; NIHSS score, 24) with a right acute proximal MCA occlusion explored within 6 hours of onset. Note the brain atrophy and the presence of an old left occipital infarct. ADC units are expressed in $10^{-9} \text{m}^2 \cdot \text{s}^{-1}$. MTT units are expressed in seconds. The top row of images corresponds to the 3 consecutive diffusion MRI slices averaged to obtain the ADC map matching the spectroscopic image, presented on the left and on the right of the corresponding MTT map, respectively. A cerebrospinal fluid mask was applied to the averaged ADC map. However, as for Figures 3 and 4, we chose a high upper ADC threshold value for the display of the averaged ADC map to preserve as much as possible the definition of image contours. The visualization of ADC heterogeneity inside the ischemic area is then optimized, and the visual comparison with other imaging modalities is facilitated. To define precisely the area of abnormal ADC decrease inside the affected hemisphere on the 3 consecutive diffusion MRI slices, a histogram comparison of ADC ranges from the affected and unaffected hemispheres was performed, as described in Reference 2. Then a similar semiautomatic segmentation technique was applied with a higher resolution (narrower range) to detect the 4 principal levels of ADC variations within this area and to tag the corresponding pixels on each diffusion MRI slices with the color code described in Reference 2, in which the warmer the color, the lower are the ADC values in the corresponding area.
presence or the absence of lactate. The first hypoperfused area characterized by a subnormal to normal mean ADC value, a normal NAA/S level, and the presence of lactate matches well the diffusion-perfusion mismatch area (Figure 4), while the other hypoperfused area characterized by a subnormal to normal ADC mean value, a normal NAA/S level, and the absence of lactate (Lac/NAA = 0) might possibly correspond to an oligemic zone. However, further studies with combined DWI and SI performed before and after early reperfusion using thrombolysis are needed to confirm this hypothesis. In addition, modern advances in SI techniques using an echo-planar sequence should soon provide fast 3-dimensional SI covering the entire brain in the same acquisition time as 2-dimensional SI.17 This new sequence might be of interest in the clinical assessment of acute ischemic stroke.

The present study demonstrates the potential of SI to evaluate early consequences of acute ischemia on brain cells and the existence of a gradient of cellular metabolic injury within the area of decreased mean ADC values. Interestingly, this metabolic gradient supports the idea that heterogeneity of ADC within infarcts5 is not only related to a difference in ADC reduction between gray and white matter18,19 but is also associated with a heterogeneity of the tissue metabolic injury, which in turn may explain why ADC is not a reliable predictor of final infarct size.

Although further studies of hyperacute ischemic stroke performed before and after thrombolysis are needed to definitely assess the prognostic value of SI, it is clear that brain evaluation by SI in ischemic patients provides a new metabolic dimension that is already presenting valuable information on the heterogeneity and gravity of lesions identified by DWI.

References

Editorial Comment

ADC and Metabolites in Stroke: Even More Confusion About Diffusion?

Recently, permanent and transient early ADC renormalization has been reported in stroke patients. Several other articles with different focus report on patients with substantial decrease of lesion volume from initial DWI to outcome T2-weighted imaging. Differences in the reported rates of patients showing ADC normalization may be due to varieties in imaging time points and application of thrombolytic therapy. These reports resulted in some confusion among stroke physicians about the clinical value of the ADC.

The accompanying article by Nicoli et al provides novel insights into the pathophysiological basis of DWI in patients with acute ischemic stroke. Using spectroscopic imaging, the authors describe a heterogeneous cellular metabolic injury within the region of decreased ADC values. This finding may
help us to understand why severe ADC decreases do not necessarily predict irreversible tissue damage in stroke patients.\textsuperscript{1,2}

What do we know about the ADC? Diffusion in tissues is referred to as random walk of water molecules restricted by fibers, membranes, and macromolecules. Despite some lack of understanding about the biophysical background of ADC decrease, we have learned much about its pathophysiological correlates from stroke models.\textsuperscript{3} It has been shown by different experimental modalities that cerebral ischemia results in failure of ion pumps and anoxic cell membrane depolarization. Water shifts from the extracellular to the intracellular space with consecutive cell swelling. The increase of the intracellular osmolarity as a result of lactate accumulation is considered the reason for cell swelling beyond depolarization. The time course of these changes corresponds to the evolution of ADC decreases,\textsuperscript{4} even when the precise biophysical link to ADC is not exactly known. The fundamental association between ion homeostasis and ADC has been shown by blocking the Na\textsuperscript{+} channels, which results in a delay of the rapid ADC decrease.\textsuperscript{5} The association between the degree of the perfusion deficit and the severity of ADC decrease has been confirmed in humans\textsuperscript{6} and recently has been shown to be time dependent.\textsuperscript{7}

Unfortunately, the relationship between ADC and prediction of final tissue injury is less explicit. The degree of ADC decrease has been related to the location and extent of neuronal injury.\textsuperscript{8} On the other hand, similar ADC values may reflect various histological states of ischemic tissue changes.\textsuperscript{9,10} However, recent animal studies show that ADC mapping performed before thrombolysis provides reliable risk assessment of brain injury but, as a result of uncertainties of postischemic reperfusion, does not allow precise outcome prediction.\textsuperscript{11} The link between severe absolute ADC decreases and progress to infarction is also missed in individual stroke patients.\textsuperscript{2} The observations of Nicoli et al suggest that the use of ADC thresholds as parameter of tissue viability should be discussed critically at least; regions with comparable ADC values may be associated with heterogeneous metabolic injury and, possibly, heterogeneous fate.

How can we exploit ADC values for stroke imaging? The degree of ADC decreases during stroke evolution tells us much about pathological tissue properties related to age, degree, and duration of the perfusion deficit, modulated by the local susceptibility to ischemia. These changes are associated with the local tissue impairment but not necessarily with tissue outcome. One should keep in mind that hyperacute lesions in ADC maps may reverse transiently or permanently, especially after thrombolytic treatment. However, most acute stroke patients will show the time course of further ADC decrease if reperfusion is not achieved in a timely manner.

ADC is just one side of the coin and should be interpreted in context with time after stroke onset, perfusion- and T2-weighted imaging, localization, occlusion type, and clinical data. Multiparametric prediction maps may facilitate the review of this complex information in the future.

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