Modest MRI Signal Intensity Changes Precede Delayed Cortical Necrosis After Transient Focal Ischemia in the Rat

Santiago Rojas, MSc; Abraham Martín, MSc; Carles Justicia, PhD; Carles Falcón, PhD; Núria Bargallo, MD, PhD; Ángel Chamorro, MD, PhD; Anna M. Planas, PhD

**Background and Purpose**—Diffusion-weighted imaging (DWI) hyperintensities and apparent diffusion coefficient (ADC) hypointensities are MRI features of acute stroke. DWI alterations during ischemia recover with early reperfusion, but they can reappear later. Pronounced signal abnormalities early after stroke are associated with infarction, but the significance of subtle changes is unclear. Here we evaluated the degree and time course of regional signal intensity changes during the first 24 hours of reperfusion after transient ischemia, and we related them to the progression of the histopathological damage.

**Methods**—Rats (n=54) were subjected to 1-hour intraluminal middle cerebral artery occlusion to assess the dynamics of MRI signal intensity changes during the initial 24 hours and their correspondent histopathological features: 2,3,5-triphenyltetrazolium chloride (TTC) staining, and hematoxylin and eosin, and immunoreactivity to 70-kDa heat shock protein and to astroglial and microglial markers.

**Results**—This model of ischemia caused early striatal infarction but delayed necrosis in the cortex. The striatum showed marked MRI changes from 4 hours of reperfusion. By 12 hours, the striatal ADC signal intensity ratio to the homologous contralateral region was 30% reduced, and the TTC staining evidenced infarction. Contrarily, the cortical ADC ratio was only 15% reduced, and TTC staining was normal at 12 hours. After this time, the cortex showed sudden and pronounced (>30%) ADC signal intensity changes coincidentally with the manifestation of infarction, accompanied with severe vacuolation and unambiguous signs of neuronal and astroglial death.

**Conclusions**—These findings suggest that minor changes in ADC signal intensity early after ischemia should not be underestimated because they may be harbingers of delayed infarction. *(Stroke. 2006;37:1525-1532.)*

**Key Words:** magnetic resonance imaging, diffusion-weighted imaging, middle cerebral artery occlusion, neuronal death
[GE]) and provided with a QDWRIST coil (quadrature coil to wrist explorations; GE). Acquisition parameters were: TE=102 ms, TR=4200 ms, field of view (FOV)=8×8 cm, matrix=224×160, number of excitations (NEX)=6, slice thickness=2 mm for spin-echo T2-weighted (T2w) images; and TE=91 ms, TR=10 000 ms, FOV=8×8 cm, matrix=128×128, NEX=4, slice thickness=2 mm, and spacing=0.5 mm for DWI images. The b-values were 0 and 1000 s/mm². ADC maps were produced with Functool2 software (GE). Perfusion studies were performed by injecting a contrast agent (gadodiamide; Omniscan; Amersham Health) as an intravenous bolus (0.3 mmol/kg of body weight). Acquisition parameters were: TR=80 ms, TR=1200 ms, FOV=8×8 cm, matrix=128×128, NEX=1, and slice thickness=2 mm.

Magnetic Resonance Imaging

Two regions of interest (ROIs) of 15 mm² each were defined in the ipsilateral cortex and striatum in a coronal section at the level of Bregma. ROIs were placed in dorsal striatum and in parietal cortex within zones showing the highest incidence of infarction according to previous findings. ADC signal intensity was calculated for each time point was the mean (n=10). SD of the percent value of the ROIs (MRIcro software) and expressed as the ratio to homologous contralateral region. ADC signal intensity ratio is the mean of several explorations; GE). Acquisition parameters were: TE=8 cm, matrix=4200 ms, field of view (FOV)=224×160, NEX=4, slice thickness=2 mm, and spacing=0.5 mm for DWI images. The b-values were 0 and 1000 s/mm². ADC maps were produced with Functool2 software (GE). Perfusion measures were performed in similar ROIs (7 mm²) using Functool2 software (GE). The perfusion value for each time point was the mean (n=4)±SD of the percent value of the ipsilateral versus the corresponding contralateral ROL, which was taken as 100%.

Measure of Infarct Volume and Assessment of Histological Damage

The brain was cut in 2-mm-thick sections that were stained with 2,3,5-triphenyltetrazolium chloride (TTC), and infarct volume was measured. Two sections were embedded in paraffin and stained with hematoxylin and eosin (H&E). Another section was cut in a vibratome for immunohistochemistry. The antibodies used were: 72-kDa heat shock protein (Hsp72) (#Ab-1; Oncogene) diluted 1:1000; glial fibrillary acidic protein (GFAP; #8140369; Boehringer Mannheim) diluted 1:500 for astroglia; and anti-CD11b (clone 1:1000; glial fibrillary acidic protein (GFAP; #8140369; Boehringer Mannheim) diluted 1:500 for astroglia; and anti-CD11b (clone MRC-OX42; #MCA275G; Serotec) diluted 1:500 for microglia. Tissue vacuolation was assessed by measuring the percent area of unstained tissue in H&E sections (×200 magnification) using AnalySIS software (Soft Imaging System). The percent area of unstained tissue was automatically determined by setting an intensity gray-value range: right threshold =255 gray value; and left threshold varied from 180 to 200 gray value, depending on each staining batch. The left threshold was set in the contralateral cortex and was applied to all measures in the same section. For each rat, values were the mean of 3 different measures within the ROI used for imaging studies. The percent area of unstained tissue in contralateral cortex was taken as background because it was caused by spaces surrounding vessels that were normally seen in paraffin-embedded tissue. Background was subtracted from ipsilateral hemisphere value to calculate the percent area of unstained tissue attributable to pathological vacuolation.

Statistics

Results were compared with 1-way ANOVA followed by post hoc analysis (Bonferroni test). Two-way ANOVA was used to examine the effect of regions and time or presence of infarction.

Results

Physiological Parameters

Body weight (mean±SD) was 309±26 g. Physiological parameters (mean±SD) during MCAO were: mean arterial blood pressure 102.2±9.5 mm Hg; rectal temperature 36.3±0.5°C; blood PO2 121.0±37.8 mm Hg; PCO2 46.3±18.6 mm Hg; and pH 7.42±0.09.

Time Course of Tissue Infarction

TTC staining showed no signs of infarction in cortex (Figure 1A) up to 12 hours of reperfusion despite that a large cortical infarction was manifested at 24 hours (Figure 1A). In contrast, striatal lesion was already apparent at 12 hours (Figure 1A). A time course study (Figure 1B) illustrated the delayed progression of cortical in relation to striatal lesion. Microscopic examination showed signs of cortical injury at 12 hours evidenced by tissue vacuolation, which strongly increased after 12 hours (Figure 1C).

Figure 1. Progression of the cortical and striatal infarcts. A, TTC-stained coronal sections at 12 and 24 hours after 1-hour MCAO. Signs of infarct (pale zones) are detected in striatum (str) at 12 hours and 24 hours and in cortex (ctx) at 24 hours, whereas no signs of infarct are apparent in cortex at 12 hours. B, The time course of cortical and striatal infarct volumes shows that manifestation of cortical infarction is delayed compared with striatal infarction. Points are mean±SEM of n rats per time (n=2 at 8 hours; n=5 at 12 hours; n=1 at 15 hours; n=3 at 18 hours; and n=5 at 24 hours). Curves were fit to a Bolzman sigmoidal equation with GraphPad Prism software. r² illustrates the goodness of the fit. C, Quantification by image analysis of the % area of vacuolation in cortex shows a small increase at 8 to 12 hours and a further increase later. & and # indicate P<0.05.
Histopathological Features: Delayed Cortical Necrosis

The striatum showed necrosis at 12 hours (Figure 2B and 2C). Comparatively, the histological lesion was less severe in cortex because neurons with normal morphology were accompanied in their immediate vicinity by shrunken neurons surrounded by swelling astrocytic processes (Figure 2G through 2I). Tissue vacuolation increased \( (P<0.001) \) in relation to control tissue (Figure 2O). Cortical alterations became more severe after 12 hours, showing signs of cell death and cytotoxic edema (Figure 2K through 2N). These features correspond to delayed cortical necrosis.

Neurons showing Hsp72 expression, which is a sign of neuronal viability,\textsuperscript{15–17} were rare in striatum and were restricted to the margins of infarction. However, strongly Hsp72-stained healthy-looking neurons were seen within the
cortex at 12 hours (Figure 3A through 3C). By 24 hours, the core of the cortical infarct did not show neuronal Hsp72 (Figure 3D and 3F), which, in contrast, was intensely expressed in the periphery (Figure 3D and 3E).

Astroglial damage was not observed in cortex up to 12 hours (Figure 3G and 3H). However, vacuolation and disintegration of astrocytic processes were detected later (Figure 3I and 3K) versus controls (Figure 3J). By 24 hours, GFAP staining was not seen in the core (Figure 3L), whereas strongly reactive astrocytes are seen at the periphery. M through O, OX-42 staining of microglia. Progressive stages of increased microglia reactivity is observed from control (M) to 12 hours (N) and 18 hours (O). Bar=2.5 mm in A and D; bar=100 μm in B; bar=50 μm in C; bar=30 μm in E through I, and M through O; bar=15 μm in J and K; bar=120 μm in L.

MRI Alterations During Ischemia Normalized at Reperfusion, but They Reappeared Later

During MCAO, we observed DWI hyperintensity and ADC hypointensity but no alteration in T₂w images (Figure 4A). T₂w hyperintensities appeared in the same animals at 24 hours and concomitant infarction (Figure 4A). However, the altered MRI signals during MCAO normalized after 30 minutes of reperfusion (Figure 4B). Then MRI alterations progressively reappeared first in striatum and later in cortex (Figure 4C). Progression of the cortical ADC signal in the same animals (n=4) evidenced the shift from modest to high signal intensity alteration from 12 to 18 hours (Figure 4D).

Time Course of the ADC Signal Intensity Changes at Reperfusion

At 1 hour of reperfusion, the mean±SD signal intensity ratio to the contralateral region was unaltered in striatal (1.013±0.021; n=4) and cortical (0.994±0.053; n=4) ROIs (Figure 5A). At 4 hours, striatal ADC ratio was 20% reduced (P<0.001) and further decreased to 38% at 24 hours (Figure...
In contrast, significant reductions in ADC ratio were not detected in cortex until 8 hours (Figure 5C). At 12 hours, cortical ADC ratio was modestly reduced (15%; \( P < 0.001 \)), but it dropped to lower values at 15 hours (31%; \( P < 0.001 \)), 18 hours (36%; \( P < 0.001 \)), and 24 hours (39%; \( P < 0.001 \)). A significant (\( P < 0.05 \)) difference between the 2 regions was found at 12 hours (Figure 5D). Raw ADC data for the different time points are shown in the Table. The ADC shift after 12 hours was not attributable to secondary perfusion alteration, as assessed with perfusion studies showing similar results of semiquantification of residual flow in ipsilateral and contralateral hemispheres in cortex (Figure 5E) and striatum (Figure 5F). The percentage of perfusion in the ipsilateral versus the contralateral ROI was (mean±SD): 54.7±20.5 in cortex and 37.5±16.5 in striatum during ischemia; 95.4±10.4 in cortex and 98.2±12.4 in striatum at 12 hours; and 94.4±5.1 in cortex and 97.4±10.5 in striatum at 18 hours.

Modest Reduction in Cortical ADC Signal Intensity Preceded the Manifestation of Cortical Infarct

We then studied a group of rats (\( n = 16 \)) by MRI at 12 hours and killed the animals at 24 hours to examine the histopathological outcome of early MRI signal intensity changes. Striatal infarction was found in 13 rats; 11 of these also developed cortical infarction. Rats with striatal infarction showed conspicuous striatal DWI hyperintensity and ADC hypointensity. However, only subtle cortical DWI and ADC alterations were seen in rats with cortical infarct (Figure 6A). The mean striatal ADC ratio was 30% lower (\( P < 0.001 \)) in rats with striatal infarct than in rats without, whereas the cortical ADC ratio was only 15% lower in rats with cortical infarct (\( P < 0.01 \)). Rats that did not develop lesion at 24 hours showed no alteration of ADC signal intensity ratio (mean±SD ADC ratio 0.999±0.01 [\( n = 3 \)] in striatum and 0.994±0.02 [\( n = 5 \)] in cortex).
Figure 5. Time course of changes in ADC signal intensity after ischemia. A. ROIs are defined in cortex (ROI-ctx) and striatum (ROI-str).
B. In striatum, ADC ratio in relation to the homologous contralateral region was unmodified at 1 hour of reperfusion, but it was progressively reduced from 4 hours onward. C. In cortex, a significant ADC reduction was not found until 8 hours. ADC values at 12 hours are significantly higher than at 15, 18, and 24 hours. One-way ANOVA by time, followed by post hoc Bonferroni test, was performed in B and C. D. Comparison of cortex and striatum showed that at 12 hours, cortical ADC value was significantly higher than corresponding striatal value, as evaluated with 2-way ANOVA by time and region. One symbol indicates $P < 0.05$; 2 symbols, $P < 0.01$; and 3 symbols, $P < 0.001$. E and F. Representative contrast-based perfusion measurements at 12 and 18 hours show the effect of bolus passage on the MRI signal and illustrate that the ADC drop after 12 hours is not associated to secondary perfusion alterations in cortex (E) and striatum (F).
Discussion

MRI alterations during MCAO were followed by normalization of signals at early reperfusion to reappear at follow-up imaging. This is in agreement with previous reports showing that reversal at early reperfusion of DWI abnormalities during MCAO did not always reflect cell viability.\(^9\)–\(^10\) DWI hyperintensity during ischemia may be attributed to tissue metabolism disturbances,\(^2\),\(^18\) ATP loss, and increased extracellular potassium.\(^19\) Accumulation of diffusion-restricted water in the intracellular space (cytotoxic edema) may cause reappearance of hyperintense DWI signals at reperfusion.\(^18\) DWI changes at reperfusion were delayed in cortex compared with striatum. It is feasible that better collateral circulation and milder hypoperfusion in the former underlies this effect.\(^20\)

Delayed maturation of the cortical lesion is likely an intrinsic tissular effect, but we cannot exclude a contribution of the previous striatal lesion to triggering manifestation of the cortical lesion.

Here we identified subtle changes in DWI and ADC signal intensities in the cortex between 8 and 12 hours of reperfusion that mirrored minor cellular damage and vacuolation, without TTC alteration, which illustrates that TTC is not an adequate method to assessing damage during the early hours after MCAO. We found indications that during this period, cortical neurons are not dead (histological features and Hsp72 expression) but metabolically functional (because mitochondria can oxidize TTC). However, after 12 hours, the minor early MRI changes in cortex became severe and were accompanied by widespread signs of necrosis. Therefore, this quantitative shift in cortical DWI and ADC signals was correlated with substantial tissue damage, and the subtle DWI signaling alterations at reperfusion were harbingers of delayed cortical infarction.

In humans, transient neurological symptoms can be associated with infarction (TSI), as assessed with MRI.
condition appears to have unique features distinguishable from transient ischemic attack and ischemic stroke, including a higher risk of recurrence mainly in the first 48 hours.21 In TSI patients, the decrease in ADC signal intensity is not as pronounced as in stroke patients.22 Therefore, identification of the different intensity change might be of clinical predictor value. Based on our findings, we argue that modest MRI alterations in signal intensity evidence ongoing histopathological disturbances of evolving infarction. Long-term MRI follow-up studies of TSI patients would help to unravel whether higher risk of early recurrence translated delayed neuronal death.

We propose that the degree of changes in DWI and ADC signal intensity at reperfusion correlate with specific neuro-pathological features (ie, modest changes reflect signs of ongoing lesion, whereas severe drop of ADC corresponds to cell death). Further studies will establish whether very early therapeutic treatments may reverse these modest MRI changes.

Acknowledgments

This work was supported by grants from Comisión Interministerial de Ciencia y Tecnología (SAF2002-01963 and SAF2005-05793-CO2-01), Fondo de Investigaciones Sanitarias (FIS2004-1104-O), and by the EC-FP6-project DiMI (LSHB-CT-2005-512146). A.M. and S.R. have fellowships from FIS and MEC, respectively. We thank P. Palau and C. Garrido for MRI technical work and N. Montoya and E. Gómez for assistance with histological techniques.

References

Modest MRI Signal Intensity Changes Precede Delayed Cortical Necrosis After Transient Focal Ischemia in the Rat
Santiago Rojas, Abraham Martín, Carles Justicia, Carles Falcón, Núria Bargalló, Ángel Chamorro and Anna M. Planas

Stroke. 2006;37:1525-1532; originally published online May 4, 2006; doi: 10.1161/01.STR.0000221713.06148.16
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/37/6/1525

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/