The Existence and Evolution of Diffusion–Perfusion Mismatched Tissue in White and Gray Matter After Acute Stroke

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Background and Purpose—Although white matter is a potential target of acute stroke therapy, there is uncertainty about its relative resistance to ischemia and whether it is capable of mounting a penumbral response. To explore these issues further, we examined the differential effects of ischemia on gray and white matter using magnetic resonance (MR) perfusion–diffusion mismatch after acute stroke.

Methods—MR imaging studies were performed within 12 hours in patients with initial hemispheric ischemic stroke. “At-risk” tissue was defined as tissue with abnormal diffusion-weighted imaging/perfusion-weighted imaging or infarction on follow-up image. Tissue was segmented using a probabilistic atlas generated from age-matched controls. The proportions of “at-risk” tissue, which was penumbral at the time of imaging, were compared between gray and white matter.

Results—Thirty-two patients had diffusion–perfusion mismatched penumbral tissue present in both gray and white matter compartments. Although the absolute mismatch volumes were greater in gray (median 42 cm³, interquartile range 18 to 70 cm³) than in white matter (39 cm³, 17 to 49 cm³; P<0.001), the proportion of “at-risk” tissue, which was penumbral at the time of imaging (median 3.7 hours, range 1.5 to 9.9 hours) was greater in white (69%, 49% to 86%) than gray matter (62%, 52% to 75%; P=0.026). However, the proportions spontaneously salvaged by 3 months were similar in both compartments.

Conclusions—These findings are consistent with white matter being able to mount an ischemic penumbral response in humans and being more resistant to cerebral ischemia than gray matter. They also raise the possibility that the therapeutic window is longer for white matter and may require alternative therapeutic strategies. (Stroke. 2005;36:2132-2137.)

Key Words: cerebral ischemia ■ diffusion-weighted imaging ■ perfusion-weighted imaging ■ white matter

Over the last decade, neuroprotectants have been shown to be effective in animal models of acute ischemic stroke but have failed when tested in humans.1 Although the reasons for this failure are likely to be multifactorial,1 one issue of importance is the gray matter mechanism of action of these compounds and their failure to ameliorate ischemic damage to white matter.

The target of any neuroprotective therapy is the ischemic penumbra, brain tissue that is functionally impaired but structurally intact and which has the potential to be salvaged therapeutically.2 The importance of ischemic injury to cerebral white matter is that this may be a major contributor to the functional disability experienced after stroke.3 Hence, the failure of some neuroprotection trials may be explained by ineffectiveness of the neuroprotective agents in protecting against white matter axonal damage. This may, in part, be the result of differing cellular constituents and ischemic neurochemical cascades in gray and white matter compartments which, in turn, may confer a differential vulnerability to ischemia. However, the relative vulnerability of white matter compared with gray remains a controversial issue with conflicting evidence from different animal models.4,5

Although it has been possible to image the ischemic penumbra using a number of different techniques, there has been uncertainty about the presence of a penumbral response in white matter. However, recent advances in imaging techniques such as the use of the mismatch between perfusion-weighted images (PWI) and diffusion-weighted images (DWI) using magnetic resonance imaging (MRI) as a penumbral marker has allowed investigators to obtain more

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precise images in patients with recent ischemic stroke. By using this approach, we aimed to establish if the ischemic penumbra exists in white matter in patients within 12 hours of ischemic stroke and, by determining its dynamic outcome, examine the differential effects of ischemia on gray and white matter compartments. We recently used the hypoxic penumbral marker 18 F-F misonidazole (FMISO) with positron emission tomography (PET) imaging to test the same hypotheses, but wanted to confirm our findings using a different imaging modality and shorter time windows from stroke onset.6

**Methods**

**Subjects**
The inclusion criteria were age 18 years or greater and symptoms of first-ever acute hemispheric ischemic stroke. Only patients with over 5 cm$^3$ of diffusion-perfusion mismatch were enrolled to minimize artifact and resolution problems. Patients were excluded for the following reasons: those who had received thrombolytic therapy, women who were pregnant or lactating, medically unstable patients, and patients with a contraindication to MRI scanning. The National Institute of Health Stroke Scale (NIHSS) and affected vascular territories (anterior cerebral artery, middle cerebral artery, and/or posterior cerebral artery) were recorded on admission. The study was approved by the Austin Health Human Research Ethics Committee, and informed consent was obtained from all patients and volunteer subjects or their next of kin.

**Imaging Protocol**
After an initial computed tomography (CT) scan, MRI studies comprising T1-weighted sagittal localizer, conventional T2-weighted axial fast spin-echo sequence, diffusion-weighted single-shot echo planar, and perfusion-weighted sequence were performed within 12 hours of onset on a 1.5-Tesla (T) whole-body scanner (Signa Horizon Echospeed; General Electric). The total imaging time was approximately 25 minutes. The T2 sequence contained 19 slices (thickness 5 mm) with an interslice gap of 1.7 mm, TR/TE/TE 3500/10/60 ms, a field of view (FOV) of 24×24 cm, and a matrix of 256×256 pixels. Each of axial slices obtained with spin-echo DWI (slice thickness 5 mm, interslice gap 1.7 mm, TR/TE 10000/100 ms, FOV 40×20 cm, and matrix 256×128) was acquired with b values of 0 and 1000 s/mm$^2$; the high b value measurements were performed with diffusion gradients in the 3 orthogonal (x, y, z) directions in space. Apparent diffusion coefficient (ADC) maps were calculated using standard methods. The perfusion study (gradient echo, 12 slices, slice thickness 6 mm, interslice gap 1.0 mm, TE 60 ms, FOV 40×20 mm, and matrix 256×128) consisted of 40 T2*-weighted measurements obtained at intervals of 2 seconds. The contrast agent (Gd-DTPA 0.2 mmol/kg) was injected with a power injector followed by 15 mL saline. Patients had follow-up T2-weighted MRI (approximately 3 months after onset) or CT scan (1 week of onset). Abnormal voxels were identified using purpose-written MATLAB 6.0 software.

**Perfusion-Weighted Image Indices**
Gd-DTPA time concentration curve was calculated from the signal time curve at each voxel using standard formulae. Transit time (MTT) was estimated from the ratio of the first moment of the Gd-DTPA concentration curve to its zero moment. MTT thresholds were calculated using contralateral mirror voxels and a >4-second difference to define abnormal perfusion.7

**Diffusion-Weighted Image Indices**
Abnormal DWI was identified using a semiautomated algorithm. First, a large region of interest was drawn to include all voxels with an abnormal appearance on the b1000 image, which was then transposed onto the ADC image created from b0 and b1000 images in the same coordinate space. Voxels within the region of interest were included within the final volume of abnormal DWI based on high signal intensity on the b1000 image and then further thresholded for ADC values of less than 800 mm$^2$/sec. The latter was derived from the mean of normal brain from volunteer subjects.

**T2 and Computed Tomography Indices**
The final infarct was manually segmented on the late T2 image or follow-up CT image after coregistration. To assist in manual segmentation, reference was made to a subtraction image identifying differences between normalized early and late T2 scans.

**Probabilistic Maps and Image Analysis**
To allow segmentation of ischemic tissue into gray (including subcortical gray) and white matter compartments, a probabilistic atlas of both compartments was created using high-resolution T1-weighted images from 37 approximately age-matched (mean±standard deviation [SD] age 73.5±8.4 years), neurologically normal subjects. MR images from volunteer subjects were registered into a standardized stereotaxic coordinate space using a template image created by the Montreal Neurological Institute (Figure 1A).8,9 Image registration was performed using the Automated Image Registration software package. Segmentation into tissue type was performed using SPm-99 (Wellcome) software (Figure 1B, 1C). All MRI images from patients were transformed into same standardized stereotaxic coordinate space to allow voxel-based determination of tissue fate based on baseline and final images (Figure 1D to 1G). Membership of a voxel in each tissue class was assessed using a probabilistic threshold (r) with this value set at 0.50 because this gives the best compartmental discrimination.10 Unclassified voxels were not analyzed further.

**Definitions**
Time of stroke onset was determined from the patient or witness or, if the patient awoke, the midpoint between the time deficit observed and the last time patient known to normal. Stroke risk factors were defined based on known history. New-onset atrial fibrillation was documented on electrocardiography.

“At-risk” tissue was the total tissue “at risk” of infarction at onset of the ischemic process (Figure 1, ART). It was defined as tissue with abnormal DWI (Figure 1D), abnormal MTT (Figure 1P), or final infarct (Figure 1I). Penumbral tissue was defined as diffusion-perfusion mismatch in voxels with abnormal MTT and normal DWI on the first MRI scan (Figure 1M). Infarcted mismatch was defined by abnormal MTT and normal DWI on the initial scan and infarct on the late 482 T-weighted image or CT scan after coregistration (Figures 1 and 2).

**Statistical Analysis**
To examine the differential effects of hyperacute ischemia on gray and white matter, the proportion of “at-risk” tissue with abnormal DWI and which was penumbral at the time of first imaging was compared between gray and white matter using the nonparametric Wilcoxon signed-rank test because the data were both paired and not normally distributed. For the same reasons, the same test was used to compare proportions of “at-risk” tissue and penumbral tissue that subsequently infarcted in each compartment. Statistical significance was set at P<0.05.

**Results**
Thirty-two patients (mean±SD age 74.0±12.1 years) fulfilled the entry criteria between January 2000 and March 2003 and had diffusion-perfusion mismatch with scans performed at a median interval 3.7 hours (range 1.5 to 9.9 hours) after onset. Associated risk factors were hypertension 13 patients (41%), diabetes mellitus 6 patients (19%), hypercholesterolemia 9 (28%), smoking 8 (25%), and atrial fibrillation 8 (25%). The median NIHSS score on admission was 12 (range 2 to 27). All patients had
Figure 1. (A) Schemata of standardized stereotaxic coordinate space and probabilistic maps of (B) gray matter and (C) white matter with probability threshold (r) set at 0.50. (D) DWI. (E) PWI. (D) An example of abnormal DWI lesion on acute DWI, (E) abnormal mean transit time (MTT) lesion on acute PWI (MTT delayed over 4 seconds compared with contralateral side), (F) diffusion-perfusion mismatch lesion, (G) final infarct on follow-up scan, and (H) and coregistered datasets on each compartment.
nonlacunar hemispheric stroke; 29 (91%) had middle cerebral artery, and 3 (9%) had posterior cerebral artery territory infarcts. Twenty-seven patients had follow-up T2-weighted MR scans and 5 patients had CT scans.

**Absolute Tissue Volumes**
Acute DWI revealed at least one lesion in all patients; the median volume of “at-risk” tissue as defined was 159 cm$^3$ (interquartile range 63 to 222 cm$^3$). The median abnormal DWI lesion volume was 20 cm$^3$ (7 to 40 cm$^3$). The median volume of diffusion–perfusion mismatch was 114 cm$^3$ (43 to 142 cm$^3$). The median final infarct volume on follow-up scan was 79 cm$^3$ (22 to 123 cm$^3$). The median volume of salvaged mismatch was 61 cm$^3$ (37 to 100 cm$^3$).

Diffusion–perfusion mismatch was able to be identified in both gray and white compartments of brain. The summary data for absolute volumes of “at-risk” tissue, abnormal DWI, diffusion–perfusion mismatch, final infarct (infarcted “at-risk” tissue) and salvaged mismatch in gray and white matter are shown in Table 1. Median 26 cm$^3$ (17%) of “at-risk” tissue was unable to be classified. An example of segmented abnormal DWI, abnormal MTT, diffusion–perfusion mismatch, final infarct, and salvaged mismatch tissue superimposed on the probabilistic map is shown in Figure 1D to 1H.

**Fate of Tissue in Each Compartment**
Although the absolute diffusion–perfusion mismatch volume was greater in gray than white matter ($P<0.001$; Table 1), the proportion of “at-risk” tissue with abnormal DWI at the time of first imaging was greater in gray matter (23%, 10% to 30%) than white matter (16%, 7% to 29%; $P=0.029$), and the proportion of “at-risk” tissue that was penumbral on acute MRI was greater in white matter (69%, 49% to 86%) than in gray matter (62%, 52% to 75%; $P=0.026$; Table 2). However, the proportion of penumbral tissue that was subsequently spontaneously salvaged was similar in both gray matter (70%, 56% to 84%) and white matter (67%, 57% to 79%; $P=0.822$; Table 2). To determine as to whether the proportion of tissue unclassified influenced the result, a sensitivity analysis was undertaken whereby 100% of uncertain tissue was sequentially attributed to either white or gray matter compartments. On reanalysis, the evolution and fate of tissue in each compartment remained statistically unchanged.

**Discussion**
These results confirm our recent finding using FMISO PET that potentially salvageable tissue exists in human white matter and that the dynamics of the penumbral evolution to infarction or salvage would be consistent with a greater resistance to ischemia of the white matter compartment compared with gray.$^6$ However, in our PET study, the median time to imaging was 16.5 hours and the rate of spontaneous penumbral salvage approximately 50%, unlike the current study in which the time to imaging was only 3.7 hours and the rate of spontaneous penumbral salvage 70%. The demonstration that penumbra was present and had characteristics consistent with a greater resistance to ischemia for white matter than gray using PET and MR, and during different time periods of penumbral evolution, suggests that the findings are robust. Interestingly, resistance to cerebral ischemia

![Figure 2. Example of early mismatch of DWI/PWI in the left subcortex extending out to the left parietal region. There was some expansion of initial DWI to final T2 at 3 months, but a significant proportion was salvaged in gray and white matter compartments.](image)

**Table 1. At-Risk Tissue, Abnormal DWI, Diffusion–Perfusion Mismatch, Infarct and Infarcted Mismatch Volumes in Gray and White Matter (n=32)**

<table>
<thead>
<tr>
<th></th>
<th>Gray Matter</th>
<th>White Matter</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART volume, cm$^3$</td>
<td>73.5 (27.9–113.7)</td>
<td>53.2 (19.0–81.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abnormal DWI volume, cm$^3$</td>
<td>8.6 (4.1–28.4)</td>
<td>5.7 (1.9–15.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diffusion–perfusion mismatch volume, cm$^3$</td>
<td>41.7 (18.1–69.7)</td>
<td>38.9 (16.5–49.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infarct volume, cm$^3$</td>
<td>38.4 (11.9–60.0)</td>
<td>22.7 (7.0–38.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salvaged mismatch volume, cm$^3$</td>
<td>24.8 (11.3–38.5)</td>
<td>22.5 (8.4–34.7)</td>
<td>0.092</td>
</tr>
</tbody>
</table>

Volumes are presented as median (interquartile range).

*Wilcoxon signed-rank test was used for comparisons.
ART indicates at-risk tissue.
is tissue-dependent in animal models. Furthermore, the fact that similar proportions of diffusion-perfusion mismatch, a penumbral marker, and “at-risk” tissue in both studies ultimately infarcted or were salvaged would also suggest that the resistance to ischemia is likely to be quite dynamic, with the differential in vulnerability extending for at least 12 hours or so, but for an uncertain period thereafter. Hence, a different time window for therapy may exist for gray and white matter, although their outer limits are uncertain. Interestingly, the 3.7-hour median time to imaging in the current study was near the 3-hour limit of the most effective form of therapy, tissue plasminogen activator (tPA), although up to 6 hours with prourokinase. Whether differing tissue compartment vulnerabilities to ischemia within this therapeutic time window translates to differing responses to therapy is uncertain and warrants further study.

There are some limitations to our study that deserve comment. There have been difficulties in establishing upper and lower thresholds to identify penumbral tissue using MR and an absolute gold standard does not exist. However, DWI/PWI mismatch provides a practical means to identify the ischemic penumbra in the acute stroke setting despite the fact that DWI volumes may decrease with early recanalization. Events before and after the time of imaging may introduce apparent errors of tissue coregistration, which cannot be controlled. Although ADC and perfusion thresholds have been identified that differentiate between penumbra and other tissue with modest degrees of accuracy, there is no real difference in ADC thresholds of gray and white matter. Despite lower CBF in white compared with gray matter based on PET studies, there is no evidence to suggest an MTT difference, and it seems reasonable to use an MTT delay of 4 seconds for both compartments. Furthermore, because we used relative MTT values compared with each mirror contralateral voxel rather than absolute values, threshold differences between compartments are eliminated. Similarly, any inaccuracies associated with the 15% of cases in which CT was registered to MR would be statistically accommodated by the intrasubject comparisons of tissue compartments. Other issues relate to the technique of tissue segmentation. Because of the need to set the probability threshold for the probabilistic map at 0.50, some tissue remains unclassified and cannot be included in the analysis. This could be minimized by individual patient segmentation based on high-resolution MR T1, but this is difficult to obtain in the acute phase. Finally, small diffusion-perfusion mismatch volumes of 5 cm³ were excluded to minimize artifact and resolution problems. Hence, we have no information about lacunar infarcts despite their importance in white matter ischemia.

The issue of a differential vulnerability to ischemia of white compared with gray matter has been controversial. The traditional view was based on work done in baboons in which white matter was found to be more resistant, perhaps because gray matter requires greater metabolic activity to maintain structural integrity. Counter to this is the more recent work of Pantoni et al who showed that, in a rat model of focal cerebral ischemia, ischemic changes occurred in white matter several hours before those in gray matter. Interestingly, there has been no contribution to this conundrum from the clinical literature until now, and our data supports the findings in baboons. This is also true for work performed in cats in which Graf et al found that functional impairment caused by transient white matter ischemia was more readily reversed than that caused by transient cortical ischemia. After the onset of ischemia in this model, extracellular Ca²⁺ concentrations were maintained longer and adenosine was involved in an autoprotective process in white matter, both findings consistent with increased resistance. Other known differences in the neurochemical response to ischemia may help explain resistance imbalances: gray matter ischemia involves the neurotoxic influences of glutamate culminating in Ca²⁺ influx across membranes through excitotoxin-gated channels. However, in white matter, damaging Ca²⁺ entry occurs through reversal of the Na⁺–Ca²⁺ exchanger and axonal depolarization. Oligodendrocytes express non-N-methyl-D-aspartate type (AMPA) receptors and their blockade might protect axons from hypoxic injury.

When investigating the effectiveness of neuroprotective drugs in animal stroke models, the ratio of gray to white matter may also be an important issue. The most commonly used animal model is the rat of which only approximately 14% of the brain is white matter. The neuroprotective effects of most compounds in the literature are not specifically tested for white matter because the predominantly gray matter rat model is in almost universal use. Given that the volume of white matter in humans is approximately 50% of total brain volume, this could be an important oversight.

Although we should interpret our findings cautiously because of possible confounding by other factors such as collateral circulation and ischemic cascade events, the clinical implications are important. First, by demonstrating that salvageable penumbral tissue exists in white matter, reperfusion and neuroprotective strategies can be developed. Second, the finding that white matter is more resistant to ischemia than gray matter means that more specific neuroprotective strate-
gies for each compartment are needed. Third, given that the differing dynamic evolution of the penumbra in each compartment, we may be able to set different therapeutic time windows for each.

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