Identification of Reversible Disruption of the Human Blood–Brain Barrier Following Acute Ischemia

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Background and Purpose—Animal models of acute cerebral ischemia have demonstrated that diffuse blood–brain barrier (BBB) disruption can be reversible after early reperfusion. However, irreversible, focal BBB disruption in humans is associated with hemorrhagic transformation in patients receiving intravenous thrombolytic therapy. The goal of this study was to use a magnetic resonance imaging biomarker of BBB permeability to differentiate these 2 forms of BBB disruption.

Methods—Acute stroke patients imaged with magnetic resonance imaging before, 2 hours after, and 24 hours after treatment with intravenous tissue-type plasminogen activator were included. The average BBB permeability of the acute ischemic region before and 2 hours after treatment was calculated using a T2* perfusion-weighted source images. Change in average permeability was compared with percent reperfusion using linear regression. Focal regions of maximal BBB permeability from the pretreatment magnetic resonance imaging were compared with the occurrence of parenchymal hematoma (PH) formation on the 24-hour magnetic resonance imaging scan using logistic regression.

Results—Signals indicating reversible BBB permeability were detected in 18/36 patients. Change in average BBB permeability correlated inversely with percent reperfusion (P=0.006), indicating that early reperfusion is associated with decreased BBB permeability, whereas sustained ischemia is associated with increased BBB disruption. Focal regions of maximal BBB permeability were significantly associated with subsequent formation of PH (P=0.013).

Conclusions—This study demonstrates that diffuse, mild BBB disruption in the acutely ischemic human brain is reversible with reperfusion. This study also confirms prior findings that focal severe BBB disruption confers an increased risk of hemorrhagic transformation in patients treated with intravenous tissue-type plasminogen activator. (Stroke. 2016;47:2405-2408. DOI: 10.1161/STROKEAHA.116.013805.)

Key Words: acute stroke ▪ blood–brain barrier ▪ hemorrhagic transformation ▪ magnetic resonance imaging ▪ permeability imaging

Animal models of stroke have established a timeline for disruption of the blood–brain barrier (BBB) in acute cerebral ischemia and subsequent reperfusion. BBB disruption begins at the onset of ischemia and increases with sustained hypoperfusion.1 BBB integrity is thought to recover after reperfusion. Subsequently, a biphasic pattern of BBB disruption has been described comprising an early reversible phase and a late irreversible phase.2

Prior studies of BBB disruption in human acute stroke have found that severe BBB disruption is associated with intracranial hemorrhage (ICH), whereas mild BBB disruption is not.3 We hypothesized that mild diffuse BBB disruption measured in stroke patients is consistent with reversible BBB dysfunction in the setting of cerebral reperfusion, whereas severe focal BBB disruption is indicative of BBB rupture and an increased risk of ICH.

Methods

Patients enrolled in the institutional review board–approved National Institutes of Health Natural History of Stroke Study during 2013 and 2014 were considered for inclusion. Inclusion criteria were magnetic resonance imaging (MRI) with perfusion-weighted imaging (PWI), demonstrating a perfusion deficit before treatment with intravenous tissue-type plasminogen activator, MRI with PWI performed >2 hours after treatment, and an MRI with gradient echo imaging at 24 hours. An ischemic region of interest (ROI) was defined on the pretreatment MRI based on a time-to-peak threshold of 4 seconds beyond normal. All images were coregistered to generate transformation matrices that allowed ROIs to be moved between time points. Percent reperfusion was defined as the fraction of the acute ischemic ROI that no longer had time-to-peak >4 seconds at the 2-hour time point.

Blood–brain permeability images (BBPIs) were generated from the T2*-weighted dynamic susceptibility contrast sequence used for PWI.1 BBPIs provide a voxel-by-voxel relative measure of BBB permeability expressed as a percent leakage of gadolinium on the PWI.
source images. An arrival time correction was performed to adjust for regional differences in perfusion. An arrival time correction uses a curve fit between normal tissue and tissue with possible BBB disruption. Patients whose PWI at either time point was too noisy to perform an accurate curve fit, defined by an average $r^2<0.85$, were excluded.

The average permeability was calculated within the ROI on the pretreatment and 2 hour time points by averaging the BBPI voxels above a noise threshold of 1%. Change in average BBB permeability was defined by subtracting the pretreatment value from the 2-hour value. Focal maximal BBB permeability was defined as the mean of the 10 highest BBPI voxel values within the ROI on the pretreatment scan.

Permeability analysis was performed by one author (R. Leigh) who was blinded to the 24-hour gradient echo scan. Identification of parenchymal hematomata (PH) on the 24-hour gradient echo was based on ECASS (European Cooperative Acute Stroke Study) criteria by one author (A.N. Simpkins) who was blinded to the permeability results. Change in average permeability was compared with percent reperfusion using linear regression. Focal maximal BBB permeability from the pretreatment MRI was compared with the presence/absence of PH on the 24-hour gradient echo using logistic regression.

### Results

Of the 131 patients who had an MRI followed by intravenous tissue-type plasminogen activator and were enrolled during

Table. The Clinical and Demographic Data Are Shown for the Whole Population as Well as the Subsets of Patients With and Without Reversal of BBB Disruption

<table>
<thead>
<tr>
<th></th>
<th>All Patients (n=43)</th>
<th>Patients With Reversal of BBB Permeability (n=18)</th>
<th>Patients Without Reversal of BBB Permeability (n=18)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>70</td>
<td>67</td>
<td>69</td>
<td>0.68</td>
</tr>
<tr>
<td>Percent female</td>
<td>37%</td>
<td>39%</td>
<td>39%</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension</td>
<td>77%</td>
<td>83%</td>
<td>78%</td>
<td>0.67</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>23%</td>
<td>33%</td>
<td>17%</td>
<td>0.25</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>35%</td>
<td>44%</td>
<td>33%</td>
<td>0.49</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>35%</td>
<td>22%</td>
<td>50%</td>
<td>0.08</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>16%</td>
<td>22%</td>
<td>11%</td>
<td>0.37</td>
</tr>
<tr>
<td>Median pretreatment NIHSS</td>
<td>8</td>
<td>8.5</td>
<td>7.5</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean pretreatment PWI volume</td>
<td>64.4 mL</td>
<td>60.6 mL</td>
<td>78.4 mL</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean pretreatment DWI volume (ADC&lt;600)</td>
<td>14.1 mL</td>
<td>15.8 mL</td>
<td>15.6 mL</td>
<td>0.98</td>
</tr>
<tr>
<td>Large vessel occlusion</td>
<td>28%</td>
<td>22%</td>
<td>39%</td>
<td>0.28</td>
</tr>
<tr>
<td>Mean time to MRI</td>
<td>104 min</td>
<td>111 min</td>
<td>97 min</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean time to treatment</td>
<td>131 min</td>
<td>137 min</td>
<td>125 min</td>
<td>0.54</td>
</tr>
<tr>
<td>Percent reperfusion</td>
<td>66%</td>
<td>79%</td>
<td>53%</td>
<td>0.008</td>
</tr>
</tbody>
</table>

$P$ values reflect the comparison of patients with and without BBB reversal using $t$ tests for continuous data and chi-squared test for categorical variables. ADC indicates apparent diffusion coefficient; BBB, blood–brain barrier; DWI, diffusion-weighted image; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; and PWI, perfusion-weighted imaging.

Figure 1. A, A scatter plot of the average permeability before treatment on the $x$ axis and the average permeability 2 hours after treatment on the $y$ axis. Dots falling on the blue line showed no change, whereas those below the line showed reversal of BBB permeability and those above the blue line show an increase in blood–brain barrier (BBB) permeability. B, A scatter plot of the change in average BBB permeability vs the percent reperfusion. The red curve fit line demonstrates that when there was a high percent of reperfusion, there was a decrease in BBB permeability, whereas in the absence of reperfusion, BBB permeability was more likely to increase. The $P$ value reflects the significance of this association from their linear regression.
the study period, 48 patients had PWI before, and \( \approx 2 \) hours after, treatment. Of these, 36 patients had adequate PWI source images to perform the change in BBB disruption analysis, and 42 patients had adequate imaging to perform the PH risk analysis based on successful curve fitting in the BBB analysis. Eighteen of 36 patients showed decreased permeability at 2 hours. The table shows the clinical and demographic information for all patients as well as the subsets of patients with and without reversal of BBB disruption. The only significant difference between the groups was the percent reperfusion. Figure 1A shows a scatter plot comparing the average BBB permeability acutely versus 2 hours later. Patients with data points that are below the blue line showed reversal of BBB permeability, whereas those above the line showed increased permeability at 2 hours. Figure 1B shows a scatter plot of the change in average permeability versus percent reperfusion. Early reperfusion was associated with reversal of BBB disruption (negative values), whereas sustained ischemia was associated with increasing BBB permeability \( (P=0.006) \).

Higher focal maximal BBB permeability on the pretreatment scan was associated with an increased risk of PH formation 24 hours after treatment with tissue-type plasminogen activator \( (P=0.013) \), with an odds ratio of 1.63 for every 10% increase in the focal maximal BBB permeability. Elevated average permeability on the pretreatment MRI did not confer such a risk \( (P=0.871) \). Figure 2 shows an example of a patient with severe focal BBB disruption on the pretreatment scan who subsequently suffered a PH after treatment. Although only one of the 4 patients who suffered a PH was noted to have a clinical deterioration at the time of the hemorrhage, 3 of the 4 were deceased 90 days after the stroke.

**Discussion**

This is the first study to demonstrate diffuse, reversible BBB disruption in human ischemic stroke. Such reversible BBB disruption correlated with increased reperfusion, implying an association with a shorter period of cerebral ischemia. Our results help explain prior studies that reported conflicting findings about the relationship between BBB disruption and hemorrhagic transformation. Specifically, our findings support the hypothesis that diffuse mild BBB disruption is potentially reversible, whereas focal severe BBB dysfunction signals the risk of BBB rupture.

**Figure 2.** An example of a patient with severe focal blood–brain barrier (BBB) disruption who went on to suffer a parenchymal hematoma is shown. **A–C.** The pretreatment scan. **D.** The 24-hour scan. **A.** The diffusion-weighted image with early ischemic changes. **B.** The ischemic lesion on the time-to-peak map of the perfusion-weighted imaging (PWI). **C.** The blood–brain permeability map overlain on the source image from the PWI acquisition; the color code represents increasing BBB permeability going from green, to yellow, to orange, to red (greatest). **D.** The gradient echo images 24 hours later with a large parenchymal hematoma centered on the area of focal BBB disruption.
The method used in this study—dynamic susceptibility contrast (T2*) imaging—allowed us to measure BBB permeability on a continuous scale. This is in contrast with prior studies, such as those using fluid attenuated inversion recovery (FLAIR) hyperintense reperfusion marker, that have reported BBB disruption as a binary measure, an approach that may fail to differentiate diffuse mild BBB disruption from focal severe BBB disruption. Unlike dynamic contrast-enhanced permeability imaging, which uses T1-weighted imaging to detect changes in the T1 signal and may be confounded by low signal-to-noise ratio, dynamic susceptibility contrast (T2*) imaging only detects T1 signal when gadolinium has leaked through the BBB. Furthermore, dynamic contrast-enhanced imaging requires a lengthy collection of serial images (≈15 minutes) that is unrealistic in the acute stroke setting, whereas dynamic susceptibility contrast typically takes only 60 to 80 seconds.

Our findings are in accord with experimental studies of BBB disruption occurring with acute cerebral ischemia and reperfusion: such studies emphasize the multiphasic nature of the process. After increased BBB permeability at the time of resumption of cerebral perfusion, a biphasic permeability pattern is classically observed, each component being attributed to the role of specific metalloproteinases. Our current findings of reversible BBB permeability do not allow us to identify a temporal homolog for humans—serial scans would be required to establish such a time line. However, our evidence does indicate that reversible BBB permeability occurs in humans having stroke who regain cerebral perfusion. Furthermore, in those patients with focal, severe BBB disruption, PH was more likely to occur.

Examination of BBB disruption in the management of acute stroke is an emerging field. Our results suggest that distinguishing between BBB dysfunction and BBB rupture will be important if BBB permeability is to be used to guide clinical care. Although MRIs are not typically acquired in the evaluation of acute stroke patients because of time constraints, rapid evaluation with MRI is possible at specialized centers. The methods used in this study can be applied to typical MRI scans acquired as part of routine clinical care. However, if BBPI is to become a clinical tool, MRI scanner manufacturers will need to adopt BBB permeability analysis into their existing PWI workflow.

Appendix

This research was possible because of contributions from the National Institutes of Health Natural History of Stroke Investigators: Richard T. Benson, Amie W. Hsia, Lawrence L. Latour, Richard Leigh, Marie Luby, John K. Lynch, Jose G. Merino, Zurab Nadareishvili, and Steven J. Warach (see also online-only Data Supplement).

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Disclosures

None.

References

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