Background and Purpose—It is hypothesized that tissue plasminogen activator rescues brain tissue by improving perfusion. In this study, we aimed to examine acute regional perfusion changes and how they influence infarction and clinical outcome.

Methods—Three sequential MR scans were performed in 15 tissue plasminogen activator-treated patients within 3.5 (tp1), at 6 hours (tp2), and at 1 month (tp3) after stroke onset. “Hypoperfusion” was defined if mean transit time prolongation was more than a threshold (4 thresholds: 3, 4, 5, and 6 seconds). Four regions of interest were classified: (1) “reperfusion”—hypoperfused at tp1, normal at tp2; (2) “nonreperfusion”—hypoperfused at tp1 and tp2; (3) “normal perfusion”—normal at tp1 and tp2; and (4) “new hypoperfusion”—normal at tp1 and hypoperfused at tp2. Risk of infarction was calculated within each region of interest. Associations between tissue perfusion changes and clinical variables were evaluated using stepwise multiple linear regressions. Moreover, the association between National Institutes of Health Stroke Scale changes and perfusion alterations was assessed using linear mixed effect models.

Results—Regardless of the mean transit time threshold chosen, the risk of infarction in nonreperfused regions (40% to 68%, thresholds 3 to 6 seconds) was higher than reperfused regions (9% to 30%, \( P < 0.05 \)), and it was higher in new hypoperfusion regions (9% to 33%) than normal perfusion regions (3% to 4%, \( P < 0.05 \)). Volume of new hypoperfusion was significantly associated with onset-to-treatment time and initial hypoperfused volume. Overall relative reperfusion was significantly associated with National Institutes of Health Stroke Scale improvement.

Conclusion—Early tissue perfusion changes influenced final tissue fate. The development of new hypoperfusion may result from delay in tissue plasminogen activator and a large initial lesion. (Stroke. 2011;42:65-72.)

Key Words: cerebral perfusion • hypoperfusion • ischemic stroke • reperfusion • tPA

Restoration of blood flow to ischemic regions in a timely manner enhances clinical improvement.1,2 Moreover, tissue reperfusion is thought to be the leading mechanism underlying the efficacy of intravenous recombinant tissue plasminogen activator (tPA).3–9 If reperfusion occurs early, it may promote survival in vulnerable tissue that would otherwise progress to infarction without reperfusion.9,10 Despite the importance of time-to-reperfusion on clinical outcome after stroke, few studies have critically examined tissue perfusion changes within the first several hours after stroke onset.

Although recanalization or reperfusion in acute stroke may occur either by medical intervention or spontaneously up to several days after stroke onset, clinical benefit from reperfusion-promoting therapies has only been found if it is given within 6 hours from symptom onset.2,9,11 Thus, to understand clinically relevant reperfusion, studies should be conducted within this 6-hour window. The Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET)9 and Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE)10 studies examined diffusion–perfusion mismatch at 2 time points during acute ischemic stroke. The 2 studies differed with respect to the time for assessing reperfusion with a median of 9 hours for DEFUSE and a median of 3 days for EPITHET. Given the delayed assessment of reperfusion beyond the clinically relevant reperfusion time window, these studies may have underestimated the impact of early reperfusion. In this study, we hypothesize that there are substantial temporal and spatial heterogeneities in tissue reperfusion within 6 hours from
stroke onset and the extent to which tissue reperfusion may depend on baseline clinical variables and initial lesion volumes. Voxel-based analysis, using a 6-hour assessment for reperfusion in a cohort of hyperacute tPA-treated patients with ischemic stroke, we measured the effects of perfusion alterations on risk of infarction at 1 month. Moreover, associations between perfusion alterations and clinical variables such as onset-to-treatment time (OTT) and change in the National Institutes of Health Stroke Scale (NIHSS) were evaluated.

Methods

Patients and Inclusion Criteria
All protocols were approved by the Institutional Review Board. Consecutive patients within 3 hours of stroke onset were considered for enrollment based on the following prespecified inclusion criteria: clinically suspected cortical acute ischemic stroke; age ≥18 years; NIHSS ≥5; could be imaged immediately after tPA bolus (within approximately 3.5 hours of stroke onset); and patient or patient’s next of kin capable of informed consent. Exclusion criteria included bilateral strokes or any acute endovascular or surgical intervention. In this study, only tPA-treated patients were included for subsequent data analysis. Patients were given intravenous tPA according to the National Institutes of Neurological Disorders and Stroke tPA trial protocol. The study imposed no delay in time-to-tPA treatment and no deviation from standard monitoring practices. The NIHSS was obtained on admission and at all imaging time points.

MRI Protocol
Patients were scanned with MRI at 3 time points: within 3.5 hours (tp1), at 6 hours (tp2), and at 1 month (tp3) after stroke onset. The tp1 scan was performed as soon as possible, usually at the time tPA was infusing. MR images were acquired on a 3-T Siemens whole-body Trio system (Siemens Medical Systems, Erlangen, Germany) with a circular polarized head coil. The imaging protocol included diffusion-weighted images, T2-weighted fluid-attenuated inversion recovery images (TR/TE=10000/115 ms; inversion time=2500 ms; matrix=512×416; 20 slices, slice thickness=5 mm), and dynamic susceptibility contrast perfusion imaging (a T2*-weighted gradient echoplanar imaging sequence; TR/TE=1500/43 ms; 14 slices, slice thickness=5 mm, zero interslice gap; matrix=128×128). The dynamic susceptibility contrast sequence was repeated 50 times with contrast injection on the fifth measure using 0.1 mmol/kg gadolinium diethylenetriamine penta-acetic acid at a rate of 5 mL/s with a power injector followed by a 15-mL bolus of saline. MR angiography was not performed.

Data Analysis
For dynamic susceptibility contrast perfusion imaging, we followed established procedures for the estimation of mean transit time (MTT) by using the ratio between cerebral blood volume and cerebral blood flow as MTT=cerebral blood volume/cerebral blood flow. Voxels within the middle cerebral artery of the contralateral hemisphere were manually chosen to obtain an arterial input function.
Six parameter rigid image registration was performed to align dynamic susceptibility contrast and fluid-attenuated inversion recovery images across all scans for each patient using a well-established registration package, FSL 3.2 (FMRIB, Oxford, UK). Accuracy of image registration was evaluated manually by checking multiple structural landmarks such as the ventricle and brain boundaries in all registered images and their corresponding template image for each patient.

MTT was chosen to define perfusion status because MTT is cerebral blood volume/cerebral blood flow. For each voxel, MTT was defined as “hypoperfused” if MTT prolongation was greater than or equal to a predefined threshold or normal otherwise. Because the abnormal lesion volume varies depending on the MTT threshold chosen, 4–11 MTT prolongation thresholds (3, 4, 5, and 6 seconds) were tested to determine if results were consistent across a range of putative thresholds.

Table 1. Definitions of Altered Perfusion*

<table>
<thead>
<tr>
<th>Definition</th>
<th>tp1</th>
<th>tp2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reperfusion</td>
<td>Hypoperfused</td>
<td>Normal</td>
</tr>
<tr>
<td>Nonreperfusion</td>
<td>Hypoperfused</td>
<td>Hypoperfused</td>
</tr>
<tr>
<td>Normal perfusion</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>New hypoperfusion</td>
<td>Normal</td>
<td>Hypoperfused</td>
</tr>
</tbody>
</table>

*Normal: MTT prolongation=chosen MTT threshold; hypoperfused: MTT prolongation>a given MTT threshold.

Nonparametric repeated-measures analysis of variance (Friedman test) was used. Moreover, to assess whether MTT values differed between reperfused and nonreperfused regions at tp1 and between new hyperperfused regions and normal perfused regions at tp1, nonparametric unpaired comparison (Mann-Whitney test) was performed. The risk of infarction within the regions of reperfusion and nonreperfusion were compared as well as between normal perfusion and new hypoperfusion using nonparametric unpaired comparisons (Mann-Whitney test).
Table 2. Patient Information

<table>
<thead>
<tr>
<th>Patients</th>
<th>n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean±SD)</td>
<td>61±15</td>
</tr>
<tr>
<td>Women, no. (%)</td>
<td>5 (33%)</td>
</tr>
<tr>
<td>Stroke syndrome</td>
<td>Right MCA: 8, left MCA: 7</td>
</tr>
<tr>
<td>NIHSS Findings on admission</td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>14±6</td>
</tr>
<tr>
<td>tp1</td>
<td>14±6</td>
</tr>
<tr>
<td>tp2</td>
<td>12±7</td>
</tr>
<tr>
<td>tp3</td>
<td>7±7</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>164±24</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82±12</td>
</tr>
<tr>
<td>Blood glucose level, mg/dL</td>
<td>143±56</td>
</tr>
<tr>
<td>Time from symptom onset, hours</td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>0.9±0.6</td>
</tr>
<tr>
<td>Treatment (OTT)</td>
<td>1.8±0.5</td>
</tr>
<tr>
<td>tp1</td>
<td>2.7±0.7</td>
</tr>
<tr>
<td>tp2</td>
<td>6.4±0.5</td>
</tr>
</tbody>
</table>

MCA indicates middle cerebral artery.

Association Between Change of Tissue Perfusion and Baseline Clinical Variables

Baseline clinical variables, including OTT, age, systolic and diastolic blood pressure, and glucose level, were obtained from all patients. To examine the association between the clinical variables and the absolute or relative volumes of reperfusion, new hypoperfusion, and net perfusion change, a stepwise multiple linear regression analysis was performed (SAS 9.2; SAS Institute Inc, Cary, NC). Stepwise linear regression was used to select the independent variables that might yield a potential association (F statistic, P<0.15). In the final linear regression model, a probability value <0.05 was considered significant.

Association Between Clinical Outcome and Change of Tissue Perfusion

Four linear mixed effect models (SAS 9.2) were used to examine the association between temporal NIHSS change and Vreperf, Vnetchange, %Vreperf, and %Vnetchange, respectively. Overall change in NIHSS (from scores obtained at admission, tp1, tp2, and tp3, constructed as a vector) was modeled as the dependent variable, whereas baseline clinical variables and each of the Vreperf, Vnetchange, %Vreperf, and %Vnetchange were considered as fixed effects in each mixed effect model. Within-subject correlations of repeated NIHSS measurements at various times were accounted for.

Results

Fifteen patients with ischemic stroke treated with intravenous tPA (1.8±0.5 hours from symptom onset) were imaged with MRI at 2.7±0.7 hours (tp1) and 6.4±0.5 hours (tp2) after onset. Thirty patients had a 1-month follow-up scan (tp3) to determine the final infarct. The tp1 scan was performed at the time tPA was still infusing in 12 patients and begun shortly after the completion of tPA in the remaining 3 patients. The time interval between the end of tPA infusion and the tp2 scan was 3.6±0.7 hours. Patient demographics including baseline clinical information are shown in Table 2. All patients had middle cerebral artery strokes with mean NIHSS equal to 14.

Background susceptibility effects manifested as signal void and geometric distortion were occasionally observed in the anterior portion of brain in the dynamic susceptibility contrast images but did not localize within the ischemic lesion. Moreover, unlike the nonlinear transformation, the rigid image registration was insensitive to these local signal changes. The coregistered images were well-aligned with the template images yielding inaccuracies <3 voxels in any direction.

Perfusion Alterations Between tp1 and tp2

Changes in perfusion status between tp1 and tp2 were studied by examining regions of reperfusion, nonreperfusion, normal perfusion, and new hypoperfusion (Table 1) using 4 MTT thresholds (3, 4, 5, and 6 seconds). Several MTT thresholds were used to evaluate whether the major findings depend on a specific threshold. As anticipated, the initial ischemic volume decreased significantly with each increase in MTT threshold (P<0.001). Across different MTT thresholds, several findings were consistently detected (Table 3): (1) concurrent existence of reperfused and new hypoperfused regions were observed; (2) absolute volumes of new hypoperfusion and net change in perfusion did not differ across thresholds (P=0.18 and P=0.28, Friedman tests); (3) tp1 MTT values in nonreperfused regions were higher than tp1 MTT values in reperfused regions (P<0.001), suggesting that regions with severe ischemia were less likely to reperfuse after tPA; and (4) tp1 MTT values in the new hypoperfused regions were higher than tp1 MTT values in normal perfused regions (P<0.05). The constancy of these points regardless of MTT threshold is illustrated in the scatterplots (Figure 1), which show the absolute and relative volumes of reperfusion, new hypoperfusion, and net change obtained using the 4 MTT thresholds.

MTT maps from 2 representative patients are shown in Figure 2. For Patient 1 (upper panel), the majority of the initially hypoperfused brain region (Figure 2A) reperfused at tp2 (Figure 2C–D, green), whereas a small region had no reperfusion (Figure 2B, yellow). A small region of new hypoperfusion (or delayed hypoperfusion) was observed at tp2 (Figure 2C–D, red). In contrast, Patient 2 (lower panel) showed only a small region of reperfusion at tp2 (Figure 2G–H, green) but had a large new hypoperfused region at tp2 (Figure 2G–H, red). For all patients, the areas of new hypoperfusion were found within the affected middle cerebral artery ischemic territory and were not seen within other vascular distributions such as anterior or posterior cerebral artery territories.

Risk of Infarction

The interoperator similarity index for manually defined final lesion areas was 0.87±0.1, suggesting good agreement between the operators. One set of regions of interest (A.F.) was used for final infarction analysis. On tp2 or tp3 imaging, there were no new areas of restricted diffusion or infarction outside of the initially affected territory. For brain tissue that was hypoperfused at tp1, the risk of infarction in nonreperfused regions was higher than the reperfused regions (P<0.05, Mann-Whitney test). For tissue that was normally perfused at
Association Between Tissue Perfusion Changes and OTT and Initial Hypoperfusion

Although no association was found between the absolute volume of reperfusion ($V_{\text{reperf}}$) and other baseline variables, including OTT or $V_{\text{hypo,tp1}}$ (Figure 3A), a negative association was found between the relative volume of reperfusion ($\%V_{\text{reperf}}$) and the volume of hypoperfusion at tp1 ($V_{\text{hypo,tp1}}$; $r = -0.67$, $P < 0.01$, Figure 3B). The volume of new hypoperfusion ($V_{\text{newhypo}}$) correlated positively with the tp1 volume of hypoperfusion ($V_{\text{hypo,tp1}}$) and OTT ($r = 0.76$, $P < 0.05$, Figure 3C). Taken together, these findings suggest that large strokes are less likely to reperfuse and more likely to develop new regions of hypoperfusion. Moreover, a shorter OTT may minimize the development of new hypoperfusion. In agreement with these results, the relative volume of new hypoperfusion ($\%V_{\text{newhypo}}$) correlated positively ($r = 0.57$, $P < 0.05$, Figure 3D) and volume of net change in perfusion ($V_{\text{netchange}}$) correlated negatively with OTT ($r = -0.59$, $P < 0.01$, Figure 3E). Additionally, both volume of hypoperfusion at tp1 ($V_{\text{hypo,tp1}}$) and OTT correlated negatively with relative volume of net change ($\%V_{\text{netchange}}$; $r = -0.82$, $P < 0.05$, Figure 3F), suggesting that a large region of early ischemia and a prolonged OTT may result in less overall improvement in perfusion status.

Association Between Tissue Perfusion Changes and Clinical Outcomes

Across the study cohort, NIHSS decreased from admission to tp3 ($P < 0.0001$). When comparing the 4 different MTT thresholds, an interesting pattern emerged. MTT prolongation thresholds of 3 and 4 seconds showed an association between temporal NIHSS change and $\%V_{\text{reperf}}$ ($P < 0.05$, threshold $= 3$ seconds, $P = 0.066$ threshold $= 4$ seconds). In addition, a similar association was found between temporal NIHSS and $\%V_{\text{netchange}}$ ($P < 0.05$ for both thresholds of 3 and 4 seconds). No significant association was detected when using thresholds of 5 and 6 seconds. These findings may suggest that MTT thresholds $< 4$ seconds delineate both reversibly and irreversibly injured tissue, whereas MTT thresholds $> 5$ seconds delineates only irreversibly injured tissue. Thus, applying the more strict thresholds (MTT $> 5$ seconds), reperfusion does not correlate well with clinical improvement because regions with moderate perfusion deficit were excluded.

Discussion

The current voxel-by-voxel analysis of serial images focuses on tissue perfusion alterations after tPA treatment within 6 hours after stroke onset. We have observed substantial spatial and temporal heterogeneity of tissue perfusion in 15 tPA-treated patients. We demonstrated that tissue outcome depends on the evolution of tissue perfusion in the hyperacute phase of ischemic stroke with nonreperfused regions showing the highest infarction rate. Moreover, we have found that...
many patients with stroke develop regions of new hypoperfusion, or “delayed hypoperfusion”; these regions increase infarct probability compared with normally perfused tissue. Finally, NIHSS improvement was significantly associated with overall change in perfusion status ($\%V_{\text{net change}}$).

New Hypoperfusion
Regardless of the MTT threshold chosen, we observed the phenomenon of delayed or new hypoperfusion: normally perfused brain tissue at tp1, which becomes hypoperfused at tp2 (Table 1). All regions of new hypoperfusion fell within or were contiguous with the same arterial territory that was initially affected. No distinct regions to suggest emboli or recurrent strokes as the cause of new hypoperfusion were identified. Potential mechanisms that may result in new hypoperfusion include disorders of the macrocirculation such as failure of collaterals,$^{22}$ clot progression,$^{23}$ and recanalization followed by reocclusion.$^{24}$ In addition, disorders of the microvasculature may also be implicated. For example, after severe ischemia, injured edematous capillary endothelial cells may lead to microvascular occlusion accompanied by fibrin, platelet, and leukocyte plugging. Thus, even with recanalization, distal vessels may not reperfuse, termed “no reflow.”$^{25–28}$ This procoagulant and inflammatory process may also lead to new areas of hypoperfusion. In addition, microemboli of atherosclerotic debris may be released into the microcirculation causing further distal embolization after thrombolysis.$^{29}$ Finally, peri-infarct depolarizations may also contribute to new hypoperfusion. Peri-infarct depolarizations are waves of spreading depression adjacent to ischemic

Figure 1. Scatterplots of absolute (mL) and relative volumes (%) of reperfusion, new hypoperfusion, and net change in perfusion from all patients using MTT thresholds of 3 seconds (A–B), 4 seconds (C–D), 5 seconds (E–F), and 6 seconds (G–H).
tissue, which result in progressive decreases in blood flow and infarct growth. This novel phenomenon has been studied in animal models\textsuperscript{30,31} and has been reported in human subjects during early stroke.\textsuperscript{32}

Association Between Perfusion Alterations and Baseline Clinical Variables and Outcomes

We found that delay to tPA treatment was independently associated with new hypoperfusion, suggesting that earlier...
tPA treatment may limit its development. This finding along with the results that new hypoperfusion increases the risk of tissue infarction having clinical relevance because it has been demonstrated that late tPA treatment leads to a lower chance of favorable outcome after stroke.\textsuperscript{4,5} We found that the initial hypoperfused volume correlated positively with the volume of new hypoperfusion and negatively with relative volume of reperfusion. Furthermore, we found that the nonreperfused regions have significantly higher tp1 MTT values than the reperfused regions. These findings may suggest that larger regions and more severe early ischemia are less likely to reperfuse, resulting in a lower probability of tissue salvage. This is consistent with previous clinical studies that have demonstrated that baseline clinical stroke severity, baseline hypoperfusion severity, and large vessel occlusion are the strongest predictors of stroke evolution and early neurological deterioration.\textsuperscript{13,34}

For MTT thresholds $\leq 4$ seconds, NIHSS reduction as a function of time was associated with relative volume of improved perfusion but not with absolute volume. In this study, the clinical improvement over time is a relative measure for a specific patient and it correlated with how much of the initial ischemic volume improved proportionally (relative improvement). We also expect that the location of the initial lesion may play an important role in affecting the NIHSS change. A larger patient population may allow such investigation in the future. Furthermore, we did not find an association between NIHSS change and reperfusion for MTT thresholds of $\geq 5$ seconds. These data may corroborate prior studies, which have found similar perfusion thresholds for distinguishing between functionally impaired, reversibly injured tissue and structurally impaired irreversibly injured tissue.\textsuperscript{35,36}

Our study has several limitations. Due to the small sample size in our study, the multiple associations between perfusion alterations and baseline clinical variables tested (Figure 3) may not have optimal statistical power. Given the limited number of patients in this study, 3 associations (Figure 3C, D, F) with a $P<0.05$ should be considered as marginally significant, whereas the other 2 associations (Figure 3B, E) with a $P<0.01$ showed stronger correlations. Future study with a larger sample size is needed for further investigation. In addition, our study population did not include untreated patients (only patients treated with intravenous tPA). Therefore, we cannot be certain of how tPA may affect the development of delayed hypoperfusion given the lack of a control group. Moreover, we were unable to assess how tissue perfusion alterations affect clinical outcomes in our tPA-treated group relative to patients who do not receive tPA. Our study included moderate to large strokes with mean NIHSS of 14 and thus our results may not apply to patients with smaller strokes. Limited tissue reperfusion may be attributed to the short time interval between tPA administration and tp2. However, the majority of reperfusion after tPA is thought to occur within 2 hours in some studies.\textsuperscript{24,37,38} Moreover, given the known narrow therapeutic time window, this analysis of early reperfusion is likely to be more clinically meaningful. Finally, image coregistration was performed to align images across all time points and imperfect alignment could inaccurately result in the appearance of new hypoperfusion. To prevent this, we manually checked multiple structural landmarks for each sequence within every time point for every patient and found misalignment $<3$ voxels in any direction. In addition, isolated regions $<1$ mL were removed from the analysis to prevent inclusion of regions of misregistration or noise-induced variations.

Conclusions

We observed spatial heterogeneity of tissue perfusion in 15 tPA-treated patients with dynamic perfusion between tp1 and tp2. Reperfusion, nonreperfusion, normal perfusion, and new hypoperfusion were concurrently observed. Both nonreperfused and new hypoperfused tissue adversely influenced tissue infarction risk. Our results suggest that delay to tPA treatment and a large initial perfusion lesion may increase the risk of developing new hypoperfusion, leading to a worsened overall perfusion status after tPA treatment.

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Disclosures

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References


Early Changes of Tissue Perfusion After Tissue Plasminogen Activator in Hyperacute Ischemic Stroke
Hongyu An, Andria L. Ford, Katie Vo, Cihat Eldeniz, Rosana Ponisio, Hongtu Zhu, Yimei Li, Yasheng Chen, William J. Powers, Jin-Moo Lee and Weili Lin

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