Evidence of Reperfusion Injury, Exacerbated by Thrombolytic Therapy, in Human Focal Brain Ischemia Using a Novel Imaging Marker of Early Blood–Brain Barrier Disruption

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Abstract—Loss of integrity of the blood–brain barrier (BBB) resulting from ischemia and reperfusion is a hypothesized precursor to hemorrhagic transformation (HT) and worse clinical outcome than would be expected from the beneficial effects of reperfusion. We used a novel magnetic resonance imaging marker to characterize early BBB disruption in acute focal brain ischemia and tested associations with reperfusion, HT, and poor outcome (modified Rankin score $>$2).

The BBB disruption was evident as delayed gadolinium enhancement of cerebrospinal fluid space on fluid-attenuated inversion recovery (FLAIR) images and, for convenience, has been termed hyperintense acute reperfusion marker (HARM). HARM was found in 47 of 144 (33%) ischemic stroke patients. Reperfusion was found to be the strongest independent predictor of early BBB disruption ($P=0.018$) in multivariate analysis. HARM was associated with HT and worse clinical outcome (after adjustment for initial severity). It was also associated with more severe strokes at onset and greater age. Because the timing of the disruption was early enough (median estimate 3.8 hours from onset) to make it relevant to acute thrombolytic therapy, early BBB disruption as defined by HARM may be a promising target for adjunctive therapy to reduce the complications associated with thrombolytic therapy, broaden the therapeutic window, and improve clinical outcome. (Stroke. 2004;35:000-000.)

Key Words: acute care ■ blood–brain barrier ■ stroke, hemorrhagic

Reperfusion of ischemic brain by the recanalization of occluded arteries is the most effective therapy for acute stroke in humans.$^{1,2}$ However, reperfusion can also contribute to brain damage through the mechanisms of reperfusion injury,$^{3}$ the occurrence of which has not been demonstrated in human brain, and through hemorrhagic transformation (HT) of ischemic parenchyma. Reperfusion injury and HT share the common substrate of an abnormally permeable capillary bed resulting from a disruption of the blood–brain barrier (BBB).$^{4}$ Injury resulting from reperfusion and damage to the microvasculature is a focus of investigation in stroke models; however, the relevance of such studies to the pathology and treatment of acute stroke in humans has yet to be established. We summarize our results from recent studies that demonstrate early BBB opening is related to reperfusion, risk of HT, worse clinical outcome, and treatment with thrombolytic drugs.$^{5-7}$

During the course of an observational study of magnetic resonance imaging (MRI) features of evolving stroke, we unexpectedly observed postgadolinium enhancement of the cerebrospinal fluid (CSF) space on fluid-attenuated inversion recovery (FLAIR) images in 2 patients undergoing intra-arterial recombinant tissue plasminogen activator (rtPA) therapy. These patients showed delayed and slow clinical recovery, despite complete recanalization and reperfusion at the time of the procedure. This observation led us to perform an analysis of a general sample of stroke patients: 213 ischemic stroke patients at our center who had been studied with MRI over a 22-month period. We aimed to describe the unique features of this enhancement and hypothesized that it was related to: (1) greater clinical severity at onset; (2) reperfusion; (3) risk of hemorrhagic transformation; and (4) poor clinical outcome. For convenience of reference, we have termed this observation hyperintense acute reperfusion marker (HARM). We subsequently confirmed aspects of these observations in an independent sample undergoing endovascular acute stroke therapy.

Patients were first imaged using MRI within 24 hours of onset (last known to be free of symptoms) and before any treatment. Patient evaluations and management were standardized. Thrombolytic therapy with rtPA was given to 38 patients; 3 of the patients were treated intra-arterially. Baseline clinical severity was defined as mild if the National Institutes of Health Stroke Scale score was $\leq 6$. Clinical outcome was defined as poor if the modified Rankin score obtained at 30 or 90 days after onset was $>2$. Follow-up

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Imaging was scheduled at 5 days and 90 days for all patients and at 3 hours, 24 hours, and 30 days for patients who were treated with rtPA or who had a focal perfusion defect on the initial scan. Because of clinical care requirements, patient death, or patient requests, follow-up research scans were sometimes performed outside of the target range of times or not at all. MRI was performed using a 1.5-Tesla clinical MR system. The scanning protocol was standardized for sequence parameters and order of acquisition to include: diffusion-weighted imaging, $T_1^*$-weighted gradient recalled echo (GRE), FLAIR, and perfusion-weighted imaging. Perfusion-weighted imaging was obtained using the bolus passage of contrast method by injecting Gd-DTPA at 0.1 mmol/kg dose via power injector. Image analyses of FLAIR (for HARM), of perfusion-weighted imaging (for ischemia–reperfusion), and of GRE (for hemorrhage) were performed independently and blind to clinical information. A reduction in the volume of the perfusion deficit by $>\approx50\%$ by qualitative judgment blinded to clinical and other imaging data was evidence of reperfusion.

**General Observations**

HARM is delayed gadolinium enhancement of CSF space. A total of 213 patients were imaged on presentation with MRI; 5 did not have FLAIR imaging on the first examination. Enhancement of the CSF space on FLAIR imaging was never observed before administration of Gd-DTPA ($n=208$). Follow-up FLAIR images after Gd-DTPA were available in 144 patients. In this subset of patients, the median time from symptom onset to first imaging was 2.9 hours; 98 were first scanned within 6 hours. Evidence of BBB disruption was observed in 47 of 144 (33%) patients. Only on follow-up examination after contrast administration on the previous scan session and only within the CSF space was the presence of Gd-DTPA evident. HARM was not evident within 10 minutes after contrast injection. The mean time from onset of ischemia to the observation of BBB disruption was 12.9 hours (median $=10.1$, SD $=10.3$). CSF enhancement was observed focally in the sulcal space in the vascular territory of the acute stroke in 21 patients (Figure 1), both focally and diffusely within the ventricles in 20 patients, and only diffusely in the ventricles in 6 patients. Sulcal enhancement never corresponded to either hypointensity on GRE or hyperattenuation on computed tomography (CT), which would have suggested the presence of blood as the cause of the increased signal. CSF enhancement was observed as late as 5 days after the previous Gd-DTPA administration.

HARM is associated with reperfusion. Of all stroke patients who underwent perfusion MRI ($n=178$), most (85%) had focal ischemia on their initial examination, and of those with focal ischemia who underwent follow-up perfusion imaging ($n=105$), nearly two thirds (63%) subsequently had evidence of reperfusion within 1 week. BBB disruption was more common in patients with reperfusion (45%) than in patients without reperfusion (18%) ($P=0.006$). In multiple logistic regression, among clinical, demographic, and imaging variables, reperfusion was the strongest independent predictor of early BBB disruption (OR, 4.09; 95% CI, 1.28 to 13.1; $P=0.018$).

HARM is associated with subsequent HT. Acute and follow-up GRE imaging was performed on 121 of the 144 patients with multiple FLAIR scans. The GRE scans were evaluated for any evidence of HT. Two patients had HT on their acute examination and 22 (18%) showed evidence of HT on follow-up, with a mean time from stroke onset to observation of HT of 31.3 hours (median $=18.9$, SD $=27.7$), which is significantly longer than time to observed BBB disruption. Early BBB disruption was more common in patients with HT (73%) than in patients without HT (25%) ($P<0.001$).

In this study population, 36 (25%) patients were treated with rtPA as part of standard acute care. Both HT and early BBB disruption were more common in patients treated with rtPA (31% and 55%) than those not treated (14% and 25%) ($P=0.057$ and $P=0.001$, respectively). In the subgroup of rtPA–treated patients, HT (both symptomatic and nonsymptomatic) was associated with BBB disruption ($P=0.01$); 8 of the 9 patients who bled after rtPA therapy also had early BBB disruption.

HARM is associated with poor clinical outcome. Modified Rankin scores obtained at 30 or 90 days were available in 110 of 144 patients. In univariate analysis, BBB disruption ($P=0.001$), but not reperfusion, was significantly associated with poor outcome as indicated by Rankin $>2$. Patients with HARM were more likely to have poor outcome in a multivariate logistic regression analysis that adjusted for baseline National Institutes of Health Stroke Scale score and HT. For the subgroup of patients with reperfusion, poor outcome was observed more frequently in patients with early BBB disruption (63%) as compared with those without (25%) ($P=0.003$).

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**Figure 1.** A typical case of HARM. The initial MRI obtained 3 hours after onset of symptoms shows an early evolving lesion adjacent to the posterior horn of the right lateral ventricle on diffusion-weighted imaging. FLAIR at 3 hours shows negligible CSF signal (precontrast). Arrows point to comparable sulci in all images. On the scan at 7 hours after onset (4 hours after gadolinium injection), enhancement is observed in the CSF spaces throughout the right middle cerebral arterial territory, notably in the sulci and on cortical surface.
1. Focal ischemia \(\rightarrow\) reperfusion \(\rightarrow\) tissue salvage \(\rightarrow\) better outcome

2. Focal ischemia \(\rightarrow\) reperfusion \(\rightarrow\) BBB opening \(\rightarrow\) HT \(\rightarrow\) worse outcome

\(\ast\) effect may be amplified by thrombolytic drug

Figure 2. HARM hypothesis. Reperfusion has a net positive effect on clinical outcome, but adverse effects of reperfusion mitigate that benefit and independently increase the risk of worse outcome relative to patients without evidence of BBB opening.

Discussion

We observed an association of reperfusion and early BBB disruption. Early BBB disruption was associated with HT and with poor clinical outcome, but reperfusion was not independently associated with these adverse outcomes. These data are evidence of adverse effects of early BBB disruption in focal cerebral ischemia. Furthermore, treatment with thrombolytic drugs was associated with a greater prevalence of early BBB disruption (Figure 2). The hypothesis of causality may be tested by prospectively defined serial measurements or in experimental clinical trials to pharmacologically block BBB disruption in patients undergoing reperfusion therapy of stroke.

The BBB opening indicated by CSF enhancement is distinct from enhancement of the leptomeninges and from the parenchyma enhancement that is observed during the later stage (days to weeks) of cerebral ischemia. Because of the short plasma distribution and elimination half-life of Gd-DTPA, 0.2±0.13 hours and 1.6±0.13 hours, respectively, it is likely that the opening in the BBB actually occurred before or soon after administration of the contrast agent. Assuming the Gd-DTPA crossed the BBB within this first 1.6 hours after administration, then the estimate of time from onset to evidence of BBB opening is a median of 3.8 hours. This places the timing of the BBB opening close to the treatment time window of acute thrombolytic therapy and makes this event relevant to the development of therapies to prevent HT and improve outcome after thrombolysis or other reperfusion therapy.

Reperfusion injury has been defined in numerous ways, including activation of the endothelium, excess production of oxygen free radicals, inflammatory responses, and leukocyte recruitment, increases in cytokine production, and edema formation. Common to all these mechanisms is concomitant changes in the microvascular structure. By identifying individuals who have sustained injury to the microvasculature, independent of symptomatic HT, early Gd-DTPA extravasation may serve as a marker for injury mediated by reperfusion.

The comparison of mechanical embolectomy with intra-arterial thrombolysis indicates that BBB disruption with reperfusion may be exacerbated in the presence of thrombolytic drugs. Exogenous plasminogen activators administered for clot lysis exacerbate injury to the microvasculature through activation of the proteolytic cascade and pathways that contribute to the dissolution of the basal lamina.\(^8\)

The results of these retrospective studies require confirmation in a prospective study. Nonetheless, evidence of early BBB disruption was common in these samples and associated with reperfusion, hemorrhagic transformation, and poor clinical outcome. The timing of the disruption is early enough to make it relevant to acute thrombolytic therapy, and the higher proportion of patients exposed to thrombolytic drugs having HARM suggests that early BBB disruption in humans may identify an important target population for adjunctive therapy to reduce the complications associated with acute thrombolysis, broaden the therapeutic window, and improve clinical outcome.

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This work reviews data originally presented in other publications and at other scientific conferences.

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