Imaging Inflammation in Acute Brain Ischemia
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Abstract—Brain inflammation holds promise as a therapeutic target in subacute stages of ischemic stroke. At the cellular level, posts ischemic inflammation is dominated by cells of the innate immune system with resident microglia/brain macrophages and blood-derived monocytes/macrophages being the most important cell types involved. Iron oxide nanoparticles such as ultrasmall superparamagnetic iron oxide (USPIO) are novel cell-specific contrast agents for MRI. 

After intravenous injection USPIO is taken up by circulating phagocytic cells. USPIO-laden macrophages cause typical signal changes in MRI of infarcted brain parenchyma, which has been demonstrated in studies of both experimental ischemia and human stroke. USPIO-enhanced MRI may therefore represent an important tool to address the role of macrophages for hemorrhagic lesion development both in basic science and clinical studies. (Stroke. 2007;38[part 2]:642-645.)

Key Words: acute stroke ■ animal models ■ basic science ■ inflammation ■ magnetic resonance

Ischemic stroke is the most frequent cause of persistent neurologic disability in modern Western societies. Therapeutic intervention through systemic or local thrombolysis is currently only possible in a narrow time window of 3 to 6 hours after stroke.1-3 However, a large body of evidence suggests that stroke-induced brain damage progresses during subacute stages up to several days after the insult,4 causing delayed expansion of the infarctions4,5 along with clinical worsening or impaired recovery. In addition, a considerable proportion of stroke patients reaches hospital treatment well beyond the early time window for thrombolysis therapy. Thus, there is an urgent need for novel therapeutic strategies that could be applied in later stages of ischemic lesion development.

Local brain inflammation is a pathologic hallmark of ischemic stroke lesions6-7 and is spatiotemporally related to the occurrence of delayed apoptotic cell death.8 At the cellular level, posts ischemic inflammation is dominated by cells of the innate, nonspecific immune system with resident microglia/brain macrophages and blood-derived monocytes/macrophages being the most important cell types involved (Figure 1). Microglia is activated within minutes of ischemia onset and produces a plethora of inflammatory mediators, which exacerbate tissue damage,9-11 but may also protect the brain against ischemic and excitotoxic injury.12-14 In contrast to the rapid microglia response, blood-derived macrophages are recruited with a delay of at least 24 to 48 hours.15-17 At these later stages, the synthesis of proinflammatory cytokines is already downregulated,18 whereas various antiinflammatory and protective factors are progressively expressed.19,20 Thus, inflammatory responses to brain ischemia are heterogeneous with respect to temporal pattern, the cell types involved, and the pathophysiological implications.

Several approaches for antiinflammatory or immunomodulatory treatment have proven effective in animal stroke models.21-23 However, attempts to translate this into successful clinical application have failed so far.24 Possible reasons are the heterogeneity of underlying pathomechanisms and the uncertain time window at which inflammation could be targeted in the human disease situation. In other inflammatory central nervous system diseases such as multiple sclerosis, monitoring of inflammatory lesion activity through gadolinium-enhanced MRI has greatly contributed to the development of immunomodulatory therapy.25 However, work in experimental stroke models showed that multimodal MRI comprising diffusion- and perfusion-weighted as well as gadolinium-enhanced imaging is not able to discriminate inflamed from noninflamed infarct subareas.26 Thus, there is considerable interest in the development of novel techniques for the noninvasive detection of brain inflammation in stroke and other central nervous system pathologies.

Iron Oxide Nanoparticle-Enhanced MRI: 
Experimental Findings

Already several years ago, superparamagnetic iron oxide nanoparticles have been introduced as cell-specific contrast agents that can be injected intravenously and are taken up by cells of the mononuclear phagocyte system.27 Based on particle size, one can distinguish superparamagnetic iron oxide (SPIO; ~60 to 150 nm diameter) from ultrasmall superparamagnetic iron oxide particles (USPIO; ~20 to 50 nm). SPIO particles are rapidly phagocytosed by cells of the reticuloendothelial system in liver and spleen leading to rapid clearance from the blood pool after intravenous injection. In contrast, circulation times of USPIO are considerably longer which, at least in theory, favors the interac-
Brain ischemia, central nervous system autoimmune disease, and traumatic nerve lesions. Additional applications include the tracking of in vitro prelabeled stem cells on transplantation into the lesioned central nervous system.

In experimental brain ischemia, Rausch and coworkers were the first to use USPIO for macrophage imaging in a model of permanent middle cerebral artery occlusion (pMCAO). In this study, ferumoxtran-10 was injected intravenously into rats at 5 hours after pMCAO followed by repeated MRI on days 1, 2, 4, and 7. On T2-weighted images, patchy areas of signal loss in the infarctions were found until day 4 and decreased thereafter. Essentially similar findings were obtained in a transient ischemia model. Photochemically induced ischemia is another model of ischemic stroke characterized by the permanent occlusion of cortical microvessels, which has the advantage of circumscribed, highly reproducible lesions. Using this model, several studies showed accumulation of iron oxide particles in the infarct border zone during subacute stages of lesion development.

Importantly, iron-related signal changes on MRI were paralleled by macrophage-associated iron deposition detected histochemically on postmortem brain sections. In the study by Kleinschnitz et al., SPIO injection between days 5 and 6 after ischemia, but not at earlier time points, caused typical signal loss on T2*-weighted images obtained 24 hours after injection. Thus, based on the assumption that SPIO was primarily taken up by circulating phagocytes, this study suggests that infiltration of SPIO-laden macrophages occurred to a significant extent only at the end of the first week after ischemia. This is in line with earlier results suggesting that the recruitment of hematogenous macrophages likewise occurs in a narrow time interval between days 3 and 6 after ischemia. Collectively, these findings demonstrate that appropriate timing of contrast agent injection and subsequent MRI is of critical importance for iron oxide particle-based macrophage imaging in brain ischemia.

Microglia and macrophage responses are segregated not only with respect to time, but to some extent also spatially. After focal cortical ischemia, there is secondary involvement of the ipsilateral thalamus attributable to retrograde degeneration of thalamocortical projection fibers. In these areas of delayed degeneration, strong and longlasting microglia activation develops, whereas hematogenous macrophages are largely excluded. In the studies reported so far, iron oxide particle-related signal changes were restricted to the primary lesion site and not observed in the thalamus. These observations further support the concept that particle uptake occurs peripherally with subsequent infiltration of iron-laden cells into the lesioned central nervous system parenchyma. Thereby, iron oxide particle-enhanced MRI potentially differs from positron-emission tomography with [11C]PK11195 as a radioactive ligand to peripheral-type benzodiazepine-binding sites on mononuclear phagocytes. Previous studies showed increased binding of [11C]PK11195 in the thalamus ipsilateral to a cortical MCA infarction indicating that [11C]PK11195–positron-emission tomography detects microglia responses in the degenerating areas. USPIO uptake and [11C]PK11195 binding may therefore reflect distinct aspects of the cellular inflammatory response to brain ischemia.

Clinical Trial of Ultrasmall Superparamagnetic Iron Oxide-Enhanced MRI in Human Stroke

Based on the experimental findings, we conducted an open-label clinical phase II pilot trial of USPIO-enhanced MRI in 10 consecutive patients with early ischemic stroke. In our study, USPIO contrast agent was infused 5 to 6 days after stroke onset, which corresponds to the presumed period of hematogenous macrophage recruitment and infant expansion shown in quantitative MRI analysis. In line with preclinical safety studies, USPIO infusions were well tolerated by all patients. To delineate lesion extension, all patients underwent initial stroke MRI, including diffusion- and perfusion-weighted imaging, within 24 hours after stroke. To define the pattern of gadolinium-based contrast medium enhancement, a first follow-up gadolinium-enhanced MRI was performed 4 to 5 days after symptom onset (ie, 24 hours before USPIO infusion). Two additional scans were obtained at 24 and 48 hours after USPIO infusion. As the main finding, we observed consistent USPIO-related signal changes in all 10 patients, whereas gadolinium...
enhancement only occurred in three patients. Thus, USPIO enhancement was not a mere epiphenomenon of blood–brain barrier breakdown. Interestingly, our study revealed two distinct components of USPIO-related signal changes, one associated with blood vessels and one representing parenchymal enhancement (Figure 2). Vessel-associated changes appeared as signal loss on T2/T2*-weighted images and decreased from the first to second scan after USPIO infusion, most likely reflecting a transient blood pool effect of the contrast agent. Conversely, parenchymal enhancement was mainly evident on T1-weighted images, increased over time, and matched with the expected distribution of macrophages. We therefore suggest that the increasing USPIO enhancement on T1-weighted images indicates brain infiltration by USPIO-laden macrophages. Similar to our findings, Dousset and colleagues recently reported prominent signal changes on T1- rather than on T2*-weighted images in patients with multiple sclerosis. The reasons for the distinct USPIO-related signal patterns on T1- versus T2*-weighted images are currently unknown.

Open Questions and Perspectives
The studies summarized here raise the perspective that USPIO-enhanced MRI may provide a novel in vivo surrogate marker of cellular inflammation in stroke and other central nervous system pathologies. There are a number of open questions that require further study. First of all, the assumption that circulating monocyte-derived phagocytes act as the principal cell type responsible for iron particle uptake after IV injection is so far only based on indirect evidence. Furthermore, even circulating mononuclear phagocytes can be subdivided into subpopulations with distinct phenotypic and functional profiles. Thus, USPIO enhancement may reflect the infiltration of a specific macrophage subpopulation, which remains to be identified. Finally, inflammation may not only have deleterious consequences for ischemic lesion progression, but may also mediate beneficial effects such as lesion demarcation, wound healing, and tissue regeneration. So far, the specific functional contribution of macrophages for the development of brain infarction is essentially unknown. USPIO-enhanced MRI may represent an important tool to address this issue both in basic science and clinical studies.

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Disclosures
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