Iron Oxide Particle-Enhanced MRI Suggests Variability of Brain Inflammation at Early Stages After Ischemic Stroke

Andreas Saleh, MD; Michael Schroeter, MD; Adrian Ringelstein, MD; Hans-Peter Hartung, MD; Mario Siebler, MD; Ulrich Mödder, MD; Sebastian Jander, MD

Background and Purpose—Inflammation contributes to brain damage caused by ischemic stroke. Ultrasmall superparamagnetic iron oxide (USPIO)-enhanced MRI allows noninvasive monitoring of macrophage recruitment into ischemic brain lesions. In this study, we determined the extent of USPIO enhancement during early stages of ischemic stroke.

Methods—Twelve consecutive patients with typical clinical signs of stroke underwent multimodal stroke imaging at 1.5-T within 24 hours of symptom onset. They received intravenous USPIO (ferumoxtran) infusion at 26 to 96 hours (mean, 44 hours) after stroke. A total of four follow-up MRI scans were performed 24 to 36 hours, 48 to 72 hours, 7 to 8 days, and 10 to 11 days after USPIO infusion.

Results—Nine patients were included in the final analysis. Parenchymal USPIO enhancement occurred in 3 of 9 analyzed patients and was mainly evident on T1-weighted spin-echo images. USPIO-dependent signal changes were spatially heterogeneous, reflecting the distinct patterns of hematogenous macrophage infiltration in different lesion types.

Conclusions—Our findings suggest a variable extent and distribution of macrophage infiltration into early ischemic stroke lesions. USPIO-enhanced MRI may help to more specifically target antiinflammatory therapy in patients with stroke.

(Key Words: inflammation • macrophages • magnetic resonance imaging • stroke • USPIO)

Brain inflammation is a potentially important pathomechanism of ischemic brain injury in a subacute time window after stroke onset. Postischemic inflammation is dominated by cells of the mononuclear phagocyte system comprising resident microglia/brain macrophages as well as hematogenous macrophages. Microglia activation occurs rapidly within minutes after the insult, whereas hematogenous macrophage infiltration is delayed. Precise time window varies between different studies and models. Findings in a rat model of permanent microvascular stroke indicated a delay of at least 3 days, which was confirmed in transient middle cerebral artery (MCA) occlusion in mice.

Previous approaches to antiinflammatory therapy of ischemic stroke in the clinical setting have failed. Possible reasons are the heterogeneity of underlying pathomechanisms and the uncertain time window when inflammation could be targeted in the human disease situation. Therefore, techniques for the noninvasive detection of inflammation hold considerable promise for the development of new therapies. Iron oxide nanoparticles such as ultrasmall superparamagnetic iron oxide (USPIO) are macrophage-specific contrast agents that can be injected intravenously and induce typical signal changes in MRI. USPIO-enhanced MRI allowed noninvasive monitoring of macrophage infiltration in animal models of ischemic stroke and autoimmune demyelinating disease. Furthermore, in a previous pilot study confirmed by others, we provided proof of principle that macrophage imaging by means of USPIO-enhanced MRI is also possible in human ischemic stroke. In that previous study, we injected USPIO contrast medium at a subacute stage of 5 to 7 days after symptom onset and observed enhancement in all patients studied. In our present study, we extended these investigations to an earlier stage of 2 to 3 days after stroke. We show that USPIO enhancement after early injection is more variable suggesting interindividual variability of postischemic brain inflammation with potential therapeutic implications.

Methods

Subjects
A total of 12 consecutive adult neurological inpatients (7 male, 5 female; mean age, 64 years; age range, 34 to 79 years) presenting...
with typical clinical signs of stroke were enrolled in this study. Oral and written informed consent was obtained from all patients before inclusion. Major exclusion criteria were cerebral hemorrhage evident on initial MRI (see subsequently), ambiguous time of symptom onset, lesion size below 1 cm$^3$ at diffusion-weighted imaging on initial stroke imaging, enrollment into other clinical studies, having received gadolinium complexes within 24 hours or iron particles within 6 months before, known allergy to dextran or drugs containing iron salts, and contraindications to MRI. The study was approved by the ethics committee of the medical faculty of the Heinrich-Heine-University, Duesseldorf.

**MRI Protocol**

All MRI examinations were performed on a 1.5-T whole-body MR system with a conventional gradient system (Magnetom Vision; Siemens Medical Solutions, Erlangen, Germany) using a standard quadrature head coil operating in the receive mode. The trial schedule is displayed in Figure 1. A first multimodal MR examination was undertaken within 24 hours of symptom onset with the aim to (1) delineate the extent and location of ischemic brain damage through diffusion-weighted imaging (DWI), perfusion-weighted imaging (PWI), and T2-weighted imaging (turbo spin-echo gradient-echo sequence and fluid-attenuated inversion recovery sequence); (2) assess the integrity of the blood–brain barrier through diffusion-weighted imaging (DWI), perfusion-weighted imaging (PWI), and T2-weighted imaging (turbo spin-echo gradient-echo sequence and fluid-attenuated inversion recovery sequence); (3) exclude endogenous iron deposition in the infarct area through T2*-weighted gradient-echo images (fast low-angle shot). If considered necessary, additional CT scanning was performed to exclude hemorrhage. The initial MRI was followed by a single USPIO infusion 24 to 36 hours later. The USPIO contrast agent (ferumoxtran, AMI-227, Sinerem) was provided by Guerbet (Roissy, France). It was administered intravenously in a single dose (2.6 mg iron/kg body weight) by drip infusion as described elsewhere. A total of 4 follow-up MRI scans were performed 24 to 36 hours, 48 to 72 hours, 7 to 8 days, and 10 to 11 days after USPIO infusion. These scans included T1-weighted spin-echo and T2*-weighted gradient-echo images (Figure 1). The sequence parameters are described elsewhere.

**Image Analysis**

All examinations were read independently by 2 of the investigators (A.S., M. Schroeter) with identical results. Any signal elevation on USPIO-enhanced T1-weighted images compared with nonenhanced T1-weighted images resulting in a signal intensity (SI) exceeding the SI of normal white matter was rated as USPIO-induced signal increase. Any signal loss on USPIO-enhanced T2*-weighted images compared with nonenhanced T2*-weighted images resulting in a SI drop below the SI of normal white matter was rated as USPIO-induced signal loss. Patients exhibiting signal alterations suggesting hemorrhage at any time during the study were excluded from final analysis.

**Results**

USPIO contrast agent was infused at 26 to 96 hours (mean, 44 hours) after stroke and was well tolerated by all patients. For at least 48 hours after the infusion, patients stayed hospitalized under neurological observation. No unexpected deterioration of their clinical status was observed. The baseline characteristics of the study population are given in the Table. None of the patients had clinical evidence of inflammatory or infectious disease during the observation period. Three patients had to be excluded from analysis attributable to...
withdrawal (case 1), evidence of hemorrhagic transformation (case 5), or protocol violation (case 11).

Similar to our previous study, we observed distinct patterns of USPIO-induced signal changes on T1- and T2*-weighted images. On T1-weighted images, a gradual increase of parenchymal hyperintensity was observed from the first to the second USPIO-enhanced scan (Figure 2E–F). On the third and fourth T1 scans obtained at 7 to 11 days after infusion, USPIO enhancement decreased. On T2*-weighted images, we observed mainly vessel-associated hypointense signal alterations that were most pronounced on the first scan after USPIO infusion and decreased already on the second scan.

From the 9 patients included in the final analysis, only 3 showed consistent signal changes on the post-USPIO MRI scans. None of the patients showed gadolinium enhancement. Interindividual heterogeneity of USPIO enhancement was particularly evident from the comparison of 2 cases with cortical MCA infarction in the posterior parietal cortex (Figure 2). Despite the otherwise similar appearance of the

### Table. Clinical Characteristics of the Patients

<table>
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<tr>
<th>No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Medication*</th>
<th>Territory</th>
<th>Infarct Type</th>
<th>Etiology</th>
<th>Therapy</th>
<th>Delay, h†</th>
<th>Gadolinium Enhancement</th>
<th>USPIO Enhancement</th>
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<tr>
<td>1</td>
<td>56</td>
<td>Male</td>
<td>ASA, statin</td>
<td>MCA</td>
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<td>Heparin</td>
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<td>MCA</td>
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<td>Arterioarterial embolism</td>
<td>Alteplase</td>
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<td>None</td>
<td>Deep gray matter</td>
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</table>

* Prior intake of potentially antiinflammatory drugs.
† Delay of administration of USPIO contrast agent relative to stroke onset. Stroke etiology was defined according to Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria.25

ASA indicates aspirin; BA, basilar artery; ACA, anterior cerebral artery.
lesions, significant USPIO-related T1 enhancement was seen in case 6 (Figure 2D–F, compare table) but not in case 7 (Figure 2A–C).

Apart from interindividual differences, our findings also indicated regional heterogeneity of USPIO enhancement within a given infarction. Figure 3 shows images from a large MCA infarction caused by proximal MCA occlusion (case 4). In this patient, significant T1 enhancement was only present in the subcortical core of the infarction (Figure 3C, E). By contrast, the cortical part of the infarct did not enhance.

Discussion

Previous studies suggested that the extent of signal alterations in iron oxide particle-enhanced MRI is critically dependent on the timing of contrast agent injection relative to both stroke onset and the delay of the subsequent MRI scans. In the rat model of cortical photothermalbosis, the presumed migration of iron oxide particle-laden macrophages into the lesions occurred mainly between days 5 and 6 after ischemia but not at earlier time points.16 This corresponds to the peak of hematogenous macrophage influx into the infarctions.6,8 Accordingly, in our previous human pilot study using USPIO injection between days 5 and 7 after stroke,21 USPIO enhancement was found in all 10 patients studied.

In contrast to this uniform response at the late stage of our present investigation of USPIO, enhancement at earlier stages of lesion development revealed a far more heterogeneous pattern. First, USPIO enhancement was present in only 3 of 9 patients included in the final analysis, which is overall consistent with the experimental findings of a more delayed time course of hematogenous macrophage recruitment into ischemic brain lesions. However, compared with the experimental situation, greater variability may be present in patients explaining the early enhancement in 3 patients in our study. We speculate that the extent and kinetics of USPIO enhancement depends on the individual predisposition to mount an inflammatory reaction to brain ischemia.22 Second, we also observed spatial heterogeneity of USPIO enhancement. In a case of proximal MCA occlusion, consistent enhancement was present in subcortical but not cortical parts of the infarction. This is in line with experimental findings in transient MCA occlusion. In this model, both ischemic tissue damage and macrophage infiltration progress more rapidly in the subcortical core of the infarction, whereas cortical areas undergo delayed and incomplete damage with predominant activation of resident microglia.9,24 Our data suggest that USPIO-enhanced MRI may have the potential to reflect these distinct cellular responses by means of a noninvasive imaging procedure.

Limitations of our study arise from the relatively small sample size. Furthermore, attributable to the selective uptake of the USPIO agent by cells of the mononuclear phagocyte system, the contribution of other cell types such as polymorphonuclear granulocytes cannot be assessed by USPIO-enhanced MRI. As an additional note of caution, the assumption that circulating phagocytes are the principal cell type responsible for iron particle uptake after intravenous injection is so far only based on indirect evidence. Therefore, we cannot exclude that USPIO enhancement at the early stages reflects microglia activation rather than or in addition to hematogenous macrophage recruitment. However, this would require prior passage of free USPIO through the blood–brain barrier, which appeared to be intact in all patients in this study, at least based on the observation of the absence of gadolinium enhancement.

Taken together, our present data are in line with experimental observations that macrophage responses to brain ischemia...
ischemia are variable between different lesion types and perhaps also depending on the genetic predisposition of the afflicted individual. USPIO-enhanced MRI has the potential to detect these distinct cellular responses and may thereby help to more specifically target antiinflammatory therapy.

Acknowledgment

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Disclosures

None.

References


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