Inflammatory Response After Ischemic Stroke
A USPIO-Enhanced MRI Study in Patients

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Background and Purpose—The intensity of the inflammatory response may be related to the volume of acute infarction. Ultra-small superparamagnetic particles of iron oxide (USPIO) may enable assessment of neuroinflammation. We aimed to assess whether the intensity of the inflammatory response might be related to the subacute ischemic lesion volume.

Methods—We enrolled patients who presented with acute anterior circulation stroke. MRI was performed at day 0, day 6, and day 9. The MRI protocol included T1-weighted imaging, gradient-echo T2*-weighted imaging, diffusion-weighted imaging, perfusion-weighted imaging and MR angiography. Blood-brain barrier disruption was defined as post-gadolinium enhancement on T1-weighted images. USPIO was administered after day 6 MRI. USPIO enhancement ratios were defined as the ratio between USPIO-related signal volume on day 9 T1-weighted imaging (respectively T2*-weighted imaging) and day 6 diffusion-weighted imaging infarct volume. The relationship between day 6 infarct volume and the enhancement ratio was assessed using Pearson and Spearman correlation tests.

Results—The protocol was completed in 10 patients. Signal alterations after USPIO injection was observed in 9/10 patients on day 9 T1-weighted imaging and in 5/10 patients on day 9 T2*-weighted imaging. USPIO-related MRI enhancement was heterogeneous. Lesion volume on day 6 diffusion-weighted imaging had no impact on USPIO enhancement at day 9 according to the Pearson correlation test ($P = 0.39$) or Spearman test ($P = 0.25$). There was no relationship between blood-brain barrier disruption and USPIO enhancement.

Conclusions—USPIO MRI enhancement is heterogeneous and not clearly related to subacute lesion volume. (Stroke. 2007;38:303-307.)

Key Words: acute stroke • brain infarction • inflammation • MRI

Experimental data have shown that focal cerebral ischemia induces a time-dependent activation of granulocytes, lymphocytes, and macrophages. Macrophage activity through the early activation of resident microglia and the infiltration of hematogenous monocytes starts after a delay of 1 to 5 days and persists for a longer time period.1 A relationship between the volume of brain tissue damage and the inflammatory process has been demonstrated in experimental models and clinical studies.2,3 In addition to positron emission tomography methods using the ligand [11C] (R)-PK11195,4 ultra-small superparamagnetic particles of iron oxide (USPIO) may also assess neuroinflammation. USPIO remain for several days in the lysosomes of macrophages, where they induce a signal increase in T1 and a signal decrease in T2*-weighted images.5–7 MRI data evaluating neuroinflammation in stroke patients are scarce.5 Our aim was to assess the relationship between the subacute ischemic lesion volume and the intensity of the inflammatory response.

Methods

Patients

We enrolled patients in this study who presented with documented acute anterior circulation stroke, with a National Institutes of Health Stroke Scale (NIHSS) score $\geq 8$, and who were not eligible for thrombolytic therapy. Oral and written informed consent was obtained before inclusion. Major exclusion criteria were: (1) brain hematoma on initial brain CT scan or MRI; (2) initial MRI performed $>24$ hours previously; (3) ambiguous time of symptom onset; (4) lesion size below 1 cm$^3$ on diffusion-weighted imaging on initial stroke imaging; (5) enrolment into other clinical studies; (6) past history of neoplasia or known active liver disease; (7) administration of gadolinium complexes within 24 hours or iron particles within 6 months before this study; (8) known allergy to dextran or drugs containing iron salts; and (9) current contraindications to MRI.

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The study design was approved by our ethical committee (CCPPRB Lyon B).

MRI Protocol
A sequential MRI approach was designed. MRI was performed at day 0, day 6, and day 9 on a 1.5-T clinical magnetic field (Philips Intera). The MRI protocol included gradient-echo T2*-weighted imaging (T2*WI), diffusion-weighted imaging (DWI), perfusion-weighted imaging, fluid-attenuated inversion recovery, T1-weighted imaging (T1WI), and 3-dimensional time-of-flight angiography, with the following parameters:

1. T2*WI sequence: repetition time (TR), 800 ms; echo time (TE) 28 ms; flip angle, 20°; field of view (FOV), 230 mm; matrix, 512; 22 slices; slice thickness, 5 mm.
2. DWI sequence: 3-directional, single-shot echoplanar sequence; TR, 3082 ms; TE, 74 ms; flip angle, 90°; 3 values of b (0, 500, and 1000 s/mm²); echo-planar imaging factor 69; FOV, 230 mm; matrix, 256; 22 slices; slice thickness, 5 mm.
3. Perfusion-weighted imaging: gradient-echo echoplanar-imaging was obtained over the whole brain, at day-6, with the bolus tracking method (0.1 mmol/kg dose of gadopentate dimeglumine injected with a 5 mL/s through a 0.9-mm access diameter into an antecubital vein); TR 17 s; TE 25 ms; echo-planar imaging factor 17, 22 slices; 5-mm slice thickness; FOV 220 mm; matrix 128, angle 7°, 40 dynamic scans, scan matrix 64. Perfusion maps were calculated from the signal-time curve. Time-to-peak refers to the time between the first T2*-weighted measurement and the bolus peak.
4. Fluid-attenuated inversion recovery imaging: fluid recovery-turbo-spin-echo sequence; TR 9000 ms; TE 105 ms; inversion time 2200 ms; 20 transverse sections parallel to the base of the skull; slice thickness, 5 mm; 1.5-mm intersection gap; 180° flip angle; FOV 230 mm; matrix 173×256; total imaging time 3 minutes 8 s.
5. T1WI spin-echo sequence: TR, 764 ms; TE, 15 ms; FOV, 230 mm; matrix, 512; 22 slices; slice thickness, 5 mm.
6. Time-of-flight angiography: TR 25 ms; TE 3.1 ms, slice thickness: 0.5 mm, 140 slices, FOV: 160 mm, scan matrix 360, reconstruction matrix 512.

USPIO (ferumoxtran, AMI-227, Sinerem) were kindly provided by Guerbet (Roissy CDG Cedex, France) and reconstituted according to the manufacturer’s instructions. It was administered in a single dose infusion (2.6 mg iron/kg body weight) through a 0.22-μm pore filter at a rate of 4 mL/min immediately after the day 6 MRI. A close monitoring of adverse events related to Sinerem infusion was performed.

Image Analysis
Image analysis was performed by consensus by 2 senior neuroradiologists (M.H., Y.B.). Infarct volume was assessed by manual contouring of signal abnormalities on day 6 DWI obtained at b=1000 s/mm². Lesion volume was determined by multiplying the area of diffusion hyperintensity by the sum of the slice thickness and the interslice gap. Volume of signal changes attributed to USPIO were assessed both on T1WI and T2*WI at day 9. Volumes of USPIO-related signals were delineated manually, by the consensus of 2 neuroradiologists, in cerebral parenchyma, excluding large vessels. Because the analysis of USPIO-related signal changes could be affected by hemorrhagic transformation of brain infarction, the volume of hemorrhagic transformation–related signal changes was assessed at day 6 T1WI or T2*WI, and was removed from the day 9 USPIO signal change volume. Blood-brain barrier (BBB) disruption was assessed at day 6 before USPIO administration on T1-weighted sequence. BBB disruption imaging was obtained using Gd-DTPA at 0.1 mmol/kg. BBB disruption was classified as absent, mild (<1/3 of the middle cerebral artery [MCA] territory), or severe (>1/3 of the MCA territory). USPIO enhancement ratios were defined as the ratio between USPIO-related signal volume on day 9 T1WI (respectively T2*WI) and day 6 DWI infarct volume.

Statistical Analysis
The descriptive statistics of the NIHSS score and MRI characteristics are given as mean values with standard deviation or median values and range. Two-tailed paired Student t test was used to compare DWI lesion volume at day 6 and day 9. Pearson and Spearman correlation tests were used to assess the relationship between the volume of the lesion at day 6 and USPIO enhancement ratio at day 9. Probability values of 0.05 or less were considered to indicate statistical significance. Statistical analysis was performed with SPSS 11 (SPSS Science) statistical software package for Windows.

Results
Eleven consecutive patients (3 women and 8 men; mean age, 64±11; range, 50 to 77 years) were recruited from May 2005 to February 2006. One patient was excluded because movement artifacts precluded MRI analysis. Clinical and imaging patient data are listed in the Table. Mean NIHSS score on admission was 13±3. Mean time delay between symptom onset and baseline MRI was 10±2 hour (range, 8 to 14 hour). Ischemic strokes were localized to the MCA territory (superficial MCA territory, n=6; the lenticulostriate territory, n=3; the entire MCA territory, n=1). An arterial occlusion was observed at baseline magnetic resonance angiography in 7 patients, involving the proximal MCA (n=3) and the MCA insular branches (n=4). A spontaneous recanalization was observed in 67 patients on day 6 magnetic resonance angiography. At day 6, we did not observe a significant perfusion defect between the 2 hemispheres with regard to time-to-peak maps. A mild BBB disruption was observed in 6 cases, and a marked BBB disruption in 1 case (Table). There was no relation between BBB disruption and USPIO-related signal changes.

Median lesion volume was 59.5 cm³ on day 6 DWI (range, 2 to 240 cm³) and 39.5 cm³ on day 9 DWI (range, 2 to 210 cm³). The difference in lesion volume between day 6 and day 9 was not significant (P=0.59). Median USPIO-enhanced volume on day 9 T1WI was 2.5 cm³ (range, 0 to 42 cm³). Median USPIO-signal loss volume on day 9 T2*WI was 0.5 cm³ (range, 0 to 9 cm³). USPIO signal alterations are illustrated in 3 cases in Figure 1. Enhancement after USPIO injection was observed in 9/10 patients on day 9 T1WI and in 5/10 patients on day 9 T2*WI. T1WI and T2*WI USPIO signal alterations were confined within the ischemic area assessed at day 6 DWI. We did not observe any USPIO enhancement remote from the day 6 DWI ischemic area. A heterogeneous distribution of ratios was found (Figure 2). Values for the T1 enhancement ratio (USPIO enhanced volume on day 9 T1WI/d 6 DWI lesion volume) ranged from 0 to 0.93 (median, 0.07). Values for the T2* ratio (USPIO-enhanced volume at day 9 T2*WI/d 6 DWI lesion volume) ranged from 0 to 0.20 (median, 0.02). The day 6 DWI lesion volume had no impact on USPIO enhancement at day 9: there was no statistically significant relationship, according to the Pearson correlation test (r=0.39) or Spearman test (r=0.25; Figure 2). No adverse effects were observed after USPIO infusion.
Clinical and Imaging Patient Data

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<th>Lesion Volume at Day 9 USPIO Enhanced T1WI (cm³)</th>
<th>Lesion Volume at Day 9 USPIO Enhanced T2*WI (cm³)</th>
<th>Ratio Between USPIO Enhanced Volume at Day 9 T1WI and Day 6 DWI Lesion Volume</th>
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Discussion

To date, many systemic biomarkers of inflammation involved in ischemic injury have been identified. The systemic inflammatory response after stroke likely results from a response to the necrotic tissue itself. Audebert et al have shown that an increase in inflammatory parameters correlates significantly with lesion volume and stroke severity. Necrotic tissue is eliminated by cellular, humoral, or metabolic mechanisms, which are all part of the inflammatory reaction. The inflammatory response after stroke is a heterogeneous process. Though further investigation involving larger groups of patients is warranted, USPIO-enhanced MRI appears as a promising technique to

USPIOs have recently been introduced as a cell-specific MRI contrast agent taken up by macrophages. The proof of concept that USPIO can visualize macrophage infiltration has been confirmed in animals and patients in several applications. The dynamics of polymorphonuclear leukocyte accumulation in acute cerebral infarction, and their correlation with the volume of brain tissue damage, have been demonstrated using technetium-99m hexamethylpropyleneamine oxime (99m Tc HMPAO)-labeled leukocyte brain single-photon emission computed tomography (SPECT) in a previous clinical study. Therefore, we speculated that postischemic neuroinflammation as demonstrated by USPIO enhancement may be correlated with subacute volume assessed with DWI. In our study, the presence or absence of USPIO enhancement could not be predicted by known clinical or MRI parameters. Indeed, USPIO enhancement was heterogeneous, and the overall volumes of USPIO signal alterations were smaller than the ischemic lesion volumes.

USPIOs have been recently introduced as a cell-specific MRI contrast agent taken up by macrophages. The proof of concept that USPIO can visualize macrophage infiltration has been confirmed in animals and patients in several applications. Carotid atherosclerotic lesions, stroke, brain tumors and multiple sclerosis. The USPIO signal alterations observed in ischemic areas of stroke patients is probably related to the visualization of inflammatory macrophage recruitment because animal experiments in such models showed close matching of USPIO-induced signal alterations with the distribution of iron-laden macrophages on histological brain sections.

We designed our protocol according to literature data. The time window used for acute and pre- and post-USPIOs imaging was derived from a study investigating the inflammatory response in stroke patients that had died 15 hours to 18 days postinjury, showing that macrophages accumulated in the infarcts at 5±9 days, and from the single available clinical data, in which the potential of USPIO-enhanced MRI was demonstrated in human stroke by infusing USPIO at day 6 postinjury and imaging patients twice between 24 and 72 hours post-USPIO administration. In this study, T1 effects were maximal on the second scan (48 to 72 hours). Therefore, in the present study patients were imaged at day 6 before USPIO administration and at day 9, ie, 72 hour postinjection. We found that parenchymal enhancement was more extended on T1WI compared with T2*WI, in agreement with the latter report. The main difficulty in validating the USPIO-enhanced MRI technique as a marker of neuroinflammation is to rule out the possibility of nonspecific USPIO brain uptake: for instance, passive leakage through a damaged BBB. However, USPIO-related signal alterations differed from gadolinium-enhanced regions. Moreover, 3 patients had USPIO enhancement on T1WI without BBB disruption, whereas the patient with severe BBB disruption did not show any USPIO-related enhancement. The signal alterations observed in the parenchyma must therefore be assigned to an active mechanism, most likely macrophage uptake.

There are several limitations in our study: (1) the small sample size; (2) USPIO are selectively captured by macrophagic cells; this contrast agent may thus underestimate the intensity of the inflammatory response, because the dynamic of the postischemic neuroinflammation process involves several cell types, including granulocytes and T lymphocytes; (3) differing degrees and timing of reperfusion may influence the results. However, magnetic resonance angiography showed a spontaneous recanalization before USPIO injection in 6 of 7 patients in whom an arterial occlusion was found, and we did not find any significant perfusion defect between both hemispheres.

In summary, this study confirms that brain inflammation after stroke is a heterogeneous process. Though further investigation involving larger groups of patients is warranted, USPIO-enhanced MRI appears as a promising technique to
Figure 1. A, A 52-year-old man (patient 10) developed an acute left hemiplegia related to a right proximal MCA occlusion. Top, from left to right: (1) Day 6 DWI; (2) T2*WI; and (3) T1WI without contrast showed a large right MCA infarct. Bottom, from left to right: (4) Day 9 DWI; (5) T2*WI; and (6) T1WI 72 hours after UPSIO injection: a mild USPIO enhancement was observed (arrow). B, A 55-year-old woman (patient 1), with a past history of transient confusional state 2 months before admission, which was likely related to a right posterior MCA stroke, was admitted for acute left MCA stroke with nonfluent aphasia and severe right hemiplegia. From left to right: (1) Day 6 DWI without contrast; (2) Day 9 T2*WI; and (3) Day 9 T1WI 72 hours after USPIO infusion. Right, old posterior superficial MCA ischemic infarction and recent left superficial MCA stroke with a large USPIO enhancement (arrow) compared with DWI lesion volume. C, A 77-year-old man (patient 9) developed right hemiplegia and aphasia. In the present case, BBB disruption was not associated with USPIO uptake. (1) Day 6 DWI: left superficial MCA territory stroke; (2) Cortical enhancement (arrow), consistent with BBB disruption, was observed on day 6 T1WI after intravenous gadolinium injection; (3,4) No signal change related to USPIO was present on day-9 T2*WI and on day-9 T1WI.
provide new in vivo information about neuroinflammation in human stroke.

Acknowledgments
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Disclosures
None.

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