Molecular MRI of Cerebral Venous Sinus Thrombosis Using a New Fibrin-Specific MR Contrast Agent

Christian P. Stracke, MD; Markus Katoh, MD; Andrea J. Wiethoff, PhD; Edward C. Parsons, PhD; Peter Spangenberg; Elmar Spüntrup, MD

Background and Purpose—Imaging of cerebral vein thrombosis is still challenging. Currently, diagnosis is based on CT venography and MRI including MRA and conventional digital subtraction angiography. However, especially in chronic cases, each method has shown its limitations. Newer strategies for MRI are found on molecular imaging using targeted contrast agents. The aim of this study was to prove the feasibility of a novel fibrin-targeted MR contrast agent (EP-2104R; EPIX Pharmaceuticals) for selective imaging of sinus venous thrombosis in an animal model.

Methods—Thrombosis of the superior sagittal sinus with human blood was induced in 6 pigs using a combined microsurgical and interventional approach. MRI was then performed before and up to 120 minutes after injection of 4 µmol/kg body weight EP-2104R. Molecular imaging was performed with a 3-dimensional high-resolution T1-weighted gradient echo sequence. Time courses of signal-to-noise ratio and contrast-to-noise ratio were analyzed. Thrombi were then surgically removed and the Gadolinium concentration was assessed.

Results—In all cases the thrombosis could be successfully induced; the complete MR protocol could be performed in 5 animals. In these cases the thrombi showed selective enhancement after injection of the molecular contrast agent. However, a continuous contrast-to-noise ratio increase was seen up to 120 minutes after contrast administration, achieving a contrast-to-noise ratio of 14.2±0.7 between clot and the blood pool.

Conclusion—The novel fibrin-targeted molecular MR contrast EP-2104R allows selective and high-contrast imaging of cerebral sinus vein thrombosis in an animal model. (Stroke. 2007;38:1476-1481.)

Key Words: cerebral venous thrombosis • molecular imaging • MR angiography • neuroradiology • venous thrombosis

Cerebral sinus vein thrombosis is a common disease with different clinical presentations.1 The clinical presentation varies with acute, subacute, or chronic symptoms. Symptoms vary between mild headache to severe intracranial bleeding and ischemic infarction.2,3 The mortality ranges between 5% and 15%.4

Since diagnostic procedures have improved, an increased incidence of sinus vein thrombosis has been noted. Imaging diagnosis relies on different modalities, such as CT, MRI, and x-ray digital subtraction angiography. In clinical routine, the first imaging modality used is usually cranial CT with and without intravenous contrast agent. In plain scans, thrombosed sinus and veins can appear hyperdense to the surrounding brain tissue, especially in acute thrombosis. After contrast injection, filling defects in the sinus can be observed like the classic empty triangle sign.5 With the increased availability of multi-row spiral CT scanners, CT venography has become a powerful diagnostic tool for the assessment of sinus thrombosis. Complete occlusion or extensive thrombosis is usually visible on CT venography. In patients with special anatomical features or suspicion of chronic cerebral venous thrombosis, the interpretation can be difficult.

MRI including MR venography are other broadly used methods to assess the cerebral venous system.4,6–8 Besides imaging with conventional T2- and T1-weighted sequences, MRA techniques play a major role. Time of flight angiograms can easily be obtained without contrast medium, but can lead to false results caused by signal changes arising from methemoglobin, which can mimic flow in the vessel.9 Phase-contrast MRA is another technique that can be applied with and without contrast medium. This technique is highly sensitive for slow blood flow and so excellently suitable for imaging of venous vessels. However, limitations include vessels with very slow flow or turbulent flow patterns.

Contrast enhanced MR venography offers the possibility for direct thrombus visualization as a filling defect in the contrast filled sinus or vein,10–13 but MRA techniques have the same problems in differentiating thrombosis from hyp-
oplasia or aplasia of a sinus. This problem can even occur in digital subtraction angiography, but x-ray angiograms offer additional indirect information, eg, the pathologic flow pattern in case of venous congestion. With the available techniques of CT venography and MRI and MR venography, cerebral venous thrombosis should be correctly diagnosed in the majority of cases. However, in a small number of patients the correct diagnosis remains difficult.

Consequently, it has been proposed that the incidence of sinus thrombosis is still underestimated.\textsuperscript{14}

A general solution to these limitations of all the diagnostic modalities may be the direct and selective high-signal visualization of the thrombus itself while the surrounding blood pool and soft tissue is signal-suppressed. Several recent developments in MR contrast media belong to the category of molecular imaging. These combine signal-generating gadolinium or iron oxide with tissue-specific chemical groups, which selectively bind to different molecular targets.

One new development in this field is EP-2104R, a fibrin-targeted gadolinium-based contrast agent (EPIX Pharmaceuticals). It exhibits highly specific binding to human fibrin. In various in vivo studies, it has been used to image human thrombi in the cardiac atrium, coronary or pulmonary vessels, or the carotid arteries.\textsuperscript{15–20} It has the further advantage of enabling imaging of acute, subacute, and chronic thrombi.\textsuperscript{15,19,20}

The aim of this study was to investigate the potential of EP-2104R for molecular imaging of sinus thrombosis using a recently developed minimal invasive animal model,\textsuperscript{21} with special consideration of the particular anatomical features of cerebral venous and surrounding anatomy.

### Methods

#### Sinus Thrombosis Animal Model

Sinus thrombosis was induced in a pig model with a combined surgical and interventional procedure as previously described.\textsuperscript{21} The experiments were approved by the government committee on animal affairs. The animals were obtained from the Institute of Animal Research, Aachen University, Germany.

After a cutaneous incision, a microsurgical opening of the skull bone was performed. After transdural puncture of the sinus a 2-French 4-mm balloon catheter (Syntel; Applied Medical). Simultaneous injection of human blood and thromboplastin (Dade Behring Inc; Newark, Del) induced thrombosis. The balloon was deflated and removed after 35 minutes. To prove thrombus formation, digital subtraction venograms were achieved repeatedly throughout the procedure by contrast injection through the 4-French sheath.

With only the sheath remaining, animals were transferred to the MR scanner. After finishing the MR measurements, direct x-ray venography was repeated to prove constancy of the clot. Subsequently, the animals were euthanized. The skull was opened and the clots were removed and analyzed. The clots were immediately weighed, and gadolinium content was later measured by inductively coupled plasma mass spectroscopy.\textsuperscript{15}

### MR Measurements

All measurements were performed on a 1.5-T scanner (Intera; Philips).

Animals were placed in a prone position and fixed to the MR table unit using tapes and pillows to minimize any potential movement artifacts. Scanning was performed using a small circular surface coil (CI; Philips). After a short gradient echo survey scan, initial MR examination was performed with standard structural and angiography sequences as used for imaging of the cerebral sinus in humans with parameters shown in Table 1.

For molecular imaging, a strongly T1-weighted, fat-suppressed, 3-dimensional gradient echo sequence was used. Repetition time was 14 ms, with an echo time of 5.6 ms, and flip angle of 40°. With a 512 matrix, a field of view of 360 mm was scanned with 150 contiguous slices of 0.5-mm thickness, resulting in a measured voxel size of 0.7×0.79×1 mm and reconstructed voxel size of 0.7×0.7×0.5 mm. The total measurement time was 5:26 minutes. After local shimming, a water-selective excitation pulse was applied to suppress signal from fat.

The entire MR protocol (phase-contrast MRA, structural scans, and the molecular imaging sequence) was performed before thrombus induction, and twice after thrombus induction, both before and after contrast media administration. The sequence for molecular imaging was repeated 5, 30, 60, and 90 minutes after contrast injection for all animals, and also after 120 minutes in 4 animals.

#### Contrast Agent

EP-2104R is a novel fibrin-targeted contrast agent based on gadolinium. It is composed as a small peptide with 4 gadolinium chelate moieties. It binds to fibrin, but not to circulating fibrinogen. A dose of 4 μmol/kg body weight was administered with slow infusion over 3 minutes.

### Data Analysis

Qualitative correlation of the clot extent in x-ray venography with that of the MR images was performed by 2 readers. Each reader assessed the extent of clot in the anterior, middle and posterior part of superior sagittal sinus first for x-ray venography images, second for the structural and angiographic MR images, and third for the molecular imaging MRI data sets.

Quantitative analysis of molecular imaging was performed with region-of-interest measurements. Signal time curves were measured in the anterior superior sagittal sinus, posterior superior sagittal sinus, an inner brain vein, the external carotid artery, the gray matter adjacent to the superior sagittal sinus, the white matter, and the muscle. Noise was quantified as the standard deviation of the signal measured in air. Signal-to-noise ratios for thrombus, nonthrombosed sinus, inner brain veins, skull bone, and gray and white brain tissue were calculated. Contrast-to-noise ratios were calculated over time for the difference between thrombus and nonthrombosed veins (blood pool) and between thrombi and the gray matter. Difference of signal-to-noise ratios for thrombus and nonthrombosed veins were statistically analyzed using a nonparametric Wilcoxon test (JMP software; JMP).

### Table 1. MR Protocols for Structural Imaging: T2 TSE and MRA

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Orientation</th>
<th>Field of View</th>
<th>Matrix</th>
<th>Slice Thickness</th>
<th>No. of Slices</th>
<th>TR</th>
<th>TE</th>
<th>Flip</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 TSE</td>
<td>cor</td>
<td>230</td>
<td>512×512</td>
<td>4</td>
<td>25</td>
<td>4522</td>
<td>100</td>
<td>90</td>
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<tr>
<td>Time of Flight MRA</td>
<td>cor</td>
<td>200</td>
<td>256×256</td>
<td>5</td>
<td>25</td>
<td>23</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>3-dimensional PCA</td>
<td>sag</td>
<td>230</td>
<td>256×256</td>
<td>0.8</td>
<td>220</td>
<td>16</td>
<td>6.5</td>
<td>10</td>
</tr>
</tbody>
</table>

TSE indicates turbo-spin-echo; TR, repetition time; TE, time to echo; PCA, phase contrast angiography.
Results

In all 6 animals the thrombosis could be successfully induced and verified by conventional x-ray venography (Figure 1). One animal died immediately after thrombus induction, presumably because of subdural hemorrhage. In all the remaining 5 animals, thrombus formations in the superior sagittal sinus were proven. In 2 animals additional thrombus in one of the transverse sinuses was visible (Table 2, Figure 1).

Thrombi could be explanted in these 5 animals from the superior sagittal sinus. The clot weights ranged between 57 and 560 mg (Table 2), with an average of 288 mg and SD of 186 mg. Gadolinium measurements were available only in 3 clots because of damage of 2 clots during transportation overseas to the institution where the gadolinium measurements were performed. Gadolinium concentration ranged between 0.3 and 0.65 mmol/L. Table 2 shows the thrombus weights and locations in the different animals.

Structural sequences and the angiographic sequences could be performed without any severe artifacts caused by the model used (Figure 2). The clot extent in direct x-ray venography correlated well to the signal changes in the MRA protocols (Table 2). In 3 cases, thrombus formations in the anterior superior sagittal sinus were suspected from MRI and time-of-flight and phase-contrast MRA, where it was not present in venography. This could be because of the fact that very slow flow can lead to pathologic signal behavior. In the inflow-sensitive gradient echo sequence (time-of-flight), thrombus formations showed intermediate signal intensity, comparable to brain tissue.

After contrast injection, signal elevation was apparent in the external carotid artery and the internal brain veins, similar to that seen with standard extracellular contrast agents. Within 60 minutes, the signal in the blood pool decreased over time. In all 5 animals, the thrombosed sinus showed significant signal increase, which was strongest after an average of 104 minutes (±23 minutes). Extension of the contrast enhancement in the thrombi in molecular MRI correlated strongly to the x-ray venography findings (Figure 1).

Differences in signal-to-noise ratios of the thrombosed sinus and nonthrombosed veins reached statistical signifi-

| TABLE 2. Clot Weights and Extent in Venography, Structural MRI, and MRA, and Enhancement in Molecular Imaging Sequence |
|---|---|---|---|---|---|
| Animal | Clot Weight, mg | Gadolinium Concentration, mmol/L | Thrombus Extent in Venography | Thrombus Extent in Structural MR and MRA | Thrombus Extent in Molecular MRI |
| 1 | 267 | … | mss, pss, rt | ass, mss, pss | mss, pss, rt |
| 2 | 57 | … | mss, pss, lt | ass, mss, pss | mss, pss, lt |
| 3 | 348 | 0.31 | mss | ass, mss | mss |
| 4 | 200 | 0.3 | ass, mss | ass, mss | ass, mss |
| 5 | … | … | … | … | … |
| 6 | 560 | 0.65 | mss, pss | ass, mss, pss | mss, pss |

ass indicates anterior superior sagittal sinus; lt, left transverse sinus; mss, middle third of superior sagittal sinus; pss, posterior part of the superior sagittal sinus; rt, right transverse sinus.

In structural MR and MR venography (PCA and TOF), a thrombosis is suspected in the anterior sagittal sinus in 3 cases, whereas it could not be proven in digital subtraction venography.
cance after 30 minutes (Figure 3). Contrast-to-noise between thrombus and nontrombosed vessels (ie, the blood pool) increased after contrast administration to an average of 14.21 (±0.76) after 120 minutes (Figure 4). Contrast-to-noise ratio between thrombus and the adjacent gray brain tissue also increased monotonically to 9.9 (SD ±3.14).

Figure 2. Fat-suppressed 3-dimensional gradient echo sequence. Left upper, Plain scan before surgery and interventional thrombus induction. Right upper, After thrombus induction there is a substance defect visible in the area of the microsurgical access to the sinus (double arrows). Signal in the adjacent superior sagittal sinus is slightly hyperintense (arrowhead). Lower row, Enhancement (arrows) in the thrombosed superior sagittal sinus 30 minutes after contrast (left) and 90 minutes after contrast administration (right). On both postcontrast images, the sinus thrombus can be seen as focal signal enhancement. However, contrast is superior after 90 minutes compared with 30 minutes after injection of the molecular agent.

Figure 3. Signal-to-noise ratios (n=5) for thrombus in the sinus (black squares) and inner brain vein (blood pool) after contrast administration; 5 minutes after contrast administration, there is no difference between the signal-to-noise ratio of thrombosed sinus and an inner brain vein. After 30 minutes the difference became statistically significant (*). SDs (n=4) are indicated with the error bars.

Figure 4. Contrast-to-noise ratios between thrombus and blood pool (black squares) and thrombus and gray brain tissue (open gray squares) up to 120 minutes after contrast administration.

Discussion
Cerebral venous thrombosis remains a diagnostic challenge. Combined imaging with CT, CTA, MRI, and MRA is often required. CT with CT venography and MRI with MR venography seem to have similar sensitivity for cerebral venous thrombosis. However, some authors consider MRI with MR venography as the imaging method of choice because of its superior information concerning parenchymal changes and the age of the thrombi. However, in a small group of patients the diagnosis itself remains uncertain and, often, digital
subtraction angiography is added to the diagnostic protocol. Molecular MRI allows for selective imaging of dedicated targets while the surrounding tissues show no significant enhancement. Hence, molecular imaging using a fibrin-specific contrast agent may be a new potential tool for specific diagnosis of thrombosis and clot imaging.

The novel contrast agent EP-2104R has already shown its potential to specifically bind to fibrin. Experimental studies from coronary, atrial, and pulmonary clot have shown the high-contrast achievable with this contrast agent. One study showed superiority of EP-2104R compared with a standard Gd-DTPA contrast agent for selective visualization of clots in the carotid arteries and proved the enhancement of the contrast agent in chronic clots up to 8 weeks. The aim of this study was to investigate the function of the contrast agent in the cerebral venous system in a sinus thrombosis model. In our animal model, cerebral venous thrombosis is induced in the superior sagittal sinus, which is significantly smaller than in humans (average maximum vessel diameter in our study: 2.6 mm [±0.42 mm]). Nevertheless, contrast enhancement with EP-2104R allowed discrimination between thrombus, blood pool, and brain tissue after 30 minutes. There was complete qualitative correlation of the clot extent in the superior sagittal sinus in the molecular imaging sequence as compared with the venographic gold standard. In comparison to structural MRI and MRA, molecular imaging additionally showed thrombi in the transverse sinus in 2 cases and did not bring any false-positive results, as structural and angiographic MR showed in 3 cases (Table 2). However, this difference can with regard to our small study not be considered as significant.

Signal kinetics of thrombus in the sinus showed nonambiguous enhancement, according to the behavior of contrast uptake in pulmonary and cardiac studies. Gadolinium content was measured in 3 thrombi. Concentrations were high and in the same range seen in previous studies. Contrasts between thrombi and surrounding tissues were high enough to distinguish between thrombosis and both brain and the blood pool between 1 and 2 hours after contrast administration. However, we have only investigated acute thrombi and no chronic thrombi, because our animal model seems not to be suitable for a chronic evaluation of cerebral venous thrombosis because it is rather invasive.

The molecular imaging sequence that was used is a simple and robust T1-weighted 3-dimensional gradient echo protocol available on routine clinical scanners from various vendors. Because of the sagittal slice orientation and the large field of view/thick 3-dimensional imaging slab covering the entire neurocranium, it shows no time-of-flight effects, so that flow phenomena cannot mimic specific contrast enhancement.

A potential complication with this sequence may be met-hemoglobin in subacute thrombi. This hemoglobin breakdown product occurs after a few days in clots and has short T1 relaxation times. Hence, it could therefore mimic contrast enhancement with signal elevation in our molecular imaging sequence. This pitfall should be overcome by measuring the sequence before and after contrast injection.

The molecular imaging protocol is simple, but clinical application may be further facilitated by other characteristics of the EP-2104R. It has 4 gadolinium moieties per molecule, and greater relativity when bound to fibrin. Thus, it is effective for thrombus visualization at a dose of 4 μmol/kg compared with usual Gd-DTPA in a dose of 0.1 mmol/kg. Moreover, its relativity in the blood pool, even at a low dose, may be sufficient to be used similarly to a standard extracellular contrast agent in a normal clinical MR examination; 60 minutes after injection, and after the wash-out, the specific thrombus enhancement could be imaged in a brief second examination that only includes the 3-dimensional gradient echo sequence. In clinical routine, this second examination could be performed in a few minutes.

Cortical vein thrombosis was not selectively induced in our study, so no statement about contrast behavior in the smaller cortical veins, which can be expected to be of submillimeter size can be made. However, the high contrast between thrombi and the blood pool may, in humans, allow us to distinguish smaller thrombi inside the sinus from arachnoid granulations or intrasinusoidal brain herniations, which are a frequent source of errors.

Our study shows the applicability of EP-2104R for selective thrombus imaging in the intracranial circulation. It would be valuable to study certain other applications for this contrast agent in similar anatomy. In stroke patients it may allow differential diagnosis of emboli or acute appositional thrombi in high-grade intracranial atherosclerotic stenoses or chronic vessel occlusions, providing valuable information with regard to thrombolytic therapy. In patients with intracranial or extracranial artery stenosis, EP-2104R could provide information concerning appositional clot before interventional therapy.

Conclusion
Molecular imaging with fibrin-specific EP 2104R of experimentally induced sinus thrombosis allows for high-contrast visualization of clots in small intracranial sinus.

Sources of Funding
This study was supported in part by the German Research Council (SP 634/2-1). The study was also funded in part by EPIX Pharmaceuticals.

Disclosures
None.

References


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Stroke. 2007;38:1476-1481; originally published online March 22, 2007; doi: 10.1161/STROKEAHA.106.479998

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/38/5/1476

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