In Vivo X-Ray Angiography in the Mouse Brain Using Synchrotron Radiation

Keiji Kidoguchi, MD; Masahiro Tamaki, MD; Takashi Mizobe, MD; Junji Koyama, MD; Takeshi Kondoh, MD, PhD; Eiji Kohmura, MD, PhD; Takashi Sakurai, MD, PhD; Koichi Yokono, MD, PhD; Keiji Umetani, MD

**Background and Purpose**—We, for the first time, performed in vivo x-ray angiography in the mouse brain using SPring-8, a third-generation synchrotron radiation facility.

**Methods**—A thin PE-50 tube was placed in the unilateral external carotid artery in adult male C57BL/6J mice. While maintaining the blood flow in the internal carotid artery, 33 µL of contrast agent was injected and then selective angiography of the hemisphere was performed.

**Results**—The average diameters of cerebral artery were as follows: 142.5±7.90 µm in middle cerebral artery, 138.3±9.35 µm in anterior cerebral artery, 120.5±5.53 µm in posterior cerebral artery, and 162.6±10.87 µm in internal carotid artery (n=5). To demonstrate the changes in diameter, we induced hypercapnia and detected the dilatation of the vessels between 121% and 124% of the original diameters (n=5). We also repeated angiography in the mice before and after intracarotid injection of vasodilatation drugs papaverine hydrochloride, ATP disodium, and fasudil hydrochloride hydrate and demonstrated the chronological changes in the diameters in each artery at 1, 5, 15, and 30 minutes after injection (n=1 for each drug).

**Conclusions**—Using only a minimum volume of the contrast agent, synchrotron radiation enables us to study x-ray angiography in the mouse brain. The morphology of the vessels can be clearly observed under physiological conditions. The diameters and their changes can also be successfully studied in vivo. *(Stroke. 2006;37:1856-1861.)*

**Key Words:** angiography ■ animal models ■ synchrotron

Transgenic mice have been used for experiments dealing with cerebrovascular disease. Such studies have tended mostly to focus on the expression of proteins, signal transduction, or changes in the ischemic area, whereas the morphology of cerebrovascular vessels has so far only rarely been studied. In rats, a different histology regarding the cerebral arteries has been observed between normotensive rats and spontaneously hypertensive rats. In mice, histology regarding the cerebral arteries has been observed between vessels has so far only rarely been studied. In rats, a different in the ischemic area, whereas the morphology of cerebrovascular

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**Furthermore, because of the relatively large volume of contrast agent (300 to 500 µL) required for such studies, imaging studies could not be repeated in each rat.**

**We recently developed an angiography technique using SPring-8, a third-generation synchrotron radiation facility. With highly monochromatic synchrotron radiation as an x-ray source and a newly developed x-ray direct-conversion type VIDICON camera, our previous phantom study showed that the limiting spatial resolution was ≈6 µm using a gold bar chart.**

**With this technique, angiography has demonstrated the severity of the arteriosclerosis lesions in the brachial artery in apolipoprotein E–knockout mice.**

**Reduced NO-mediated relaxation in the femoral artery of endothelial NO synthase–overexpressing transgenic mice was demonstrated.**

**Microangiography of the liver in mice has also been reported.**

**However, the x-ray generator system has limitations when providing images of small blood vessels of <50 µm because images cannot be magnified without focal spot blurring. Conventional video camera is not intended for detecting small vessels of ≈200 µm because it is designed for large-field digital angiographic imaging with a 1024×1204 pixel format.**

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Transgenic mice have been used for experiments dealing with cerebrovascular disease. Such studies have tended mostly to focus on the expression of proteins, signal transduction, or changes in the ischemic area, whereas the morphology of cerebrovascular vessels has so far only rarely been studied. In rats, a different histology regarding the cerebral arteries has been observed between normotensive rats and spontaneously hypertensive rats. In mice, the cerebrovascular anatomy is different between C57BL/6J and SV129. SV129 mice have markedly less severe histological damage than C57BL/6J mice with the same ischemic manipulation. Such vulnerability has been largely attributed to differences in the vascular anatomy. Using transgenic mice and laser Doppler flowmetry, an impaired autoregulation and decreased distension in the cerebral vessels have been suggested.

Compared with the laser Doppler technique, x-ray angiography with a contrast agent has the main advantage of being able to demonstrate the anatomy of the vessels and changes in the morphology simultaneously. In rats, morphological changes and different vasoreactivity have been reported previously using angiography; however, the spatial resolution was not previously sufficient to detect detailed changes in the vessel diameters. Furthermore, because of the relatively large volume of contrast agent (300 to 500 µL) required for such studies, imaging studies could not be repeated in each rat. We recently developed an angiography technique using SPring-8, a third-generation synchrotron radiation facility. With highly monochromatic synchrotron radiation as an x-ray source and a newly developed x-ray direct-conversion type VIDICON camera, our previous phantom study showed that the limiting spatial resolution was ≈6 µm using a gold bar chart. With this technique, angiography has demonstrated the severity of the arteriosclerosis lesions in the brachial artery in apolipoprotein E–knockout mice. Reduced NO-mediated relaxation in the femoral artery of endothelial NO synthase–overexpressing transgenic mice was demonstrated. Microangiography of the liver in mice has also been reported. However, the x-ray generator system has limitations when providing images of small blood vessels of <50 µm because images cannot be magnified without focal spot blurring. Conventional video camera is not intended for detecting small vessels of ≈200 µm because it is designed for large-field digital angiographic imaging with a 1024×1204 pixel format.
The animals were anesthetized with pentobarbital sodium intraperitoneally, and the rectal temperature was maintained at 36 ± 1.0°C throughout the study by means of feedback-regulated heating pad. Using an operating microscope, a midline incision on the neck was made. The right common carotid artery (CCA), the external carotid artery (ECA), and the right internal carotid artery (ICA) were isolated while carefully separating them from the adjacent vagus nerve. A 6-0 silk suture was loosely tied at the origin of the ECA, and the distal end of the ECA was coagulated with a bipolar coagulator. The CCA and the ICA were temporally clamped by microvascular clips. A thin PE-50 tube with outer diameter measuring ~0.4 mm (Figure 1A) was cannulated into the ECA lumen through a small puncture in the retrograde direction. The top of the tube was placed close to the CCA so that all the injected contrast media went into the CCA while the systemic arterial blood flow from the CCA to the ICA was maintained. The suture around the origin of the ECA was tightened around the tube to prevent any bleeding, and then the microvascular clips were removed (Figure 1B). In our pilot study, a PE-10 tube was used for the injection of contrast agent, but we failed to inject the agent because of a high back pressure. By means of firing and stretching the tube, we were thus able to modify the PE-50 tube and obtained a very thin inner lumen segment in a lot less time than using all PE-10 tubing. A tracheotomy was then performed with a 20-G indwelling needle placed for mechanical ventilation. Finally, the wound was roughly sewed back while tubes were kept in the same position. The mice were rigidly held on a frame with a special-shaped mouse head holder and ear bars. The frame was placed vertically in the beam line so that the mouse body was kept in the lateral position. The body axis and beam line crossed each other at right angles, and axial views of the cerebral arteries were obtained.

Materials and Methods

Animals

Thirteen male 10- to 12-week-old C57BL/6J mice (CREA Japan, Inc.; Tokyo, Japan) weighing 26 to 29 g were used in the following experiments. C57BL/6J mice were chosen because this strain is commonly used to produce genetically altered mice. All animals were kept in a temperature-controlled room with a 12-hour light/dark cycle and had free access to water and a standard laboratory diet (CB-2; CREA Japan, Inc.). All animal experiments were conducted according to the guidelines for animal experiments at Kobe University Graduate School of Medicine.

Surgical Procedures

The animals were anesthetized with pentobarbital sodium intraperitoneally, and the rectal temperature was maintained at 36 ± 1.0°C throughout the study by means of feedback-regulated heating pad.
were performed before the injection and at 1, 5, 15, and 30 minutes after the injection.

**Synchrotron Radiation System**
This study was performed at the second optical hatch of BL28B2 beam line at SPring-8 (Japan Synchrotron Radiation Research Institute) in Hyogo, Japan. Synchrotron radiation was derived from a storage ring of electrons with an accelerated energy of 8 GeV and an average beam current of 99 mA. This synchrotron radiation was monochromatized by a silicon crystal that was placed in front of the mice. The monochromatized x-ray film at an energy of 33.2 keV just above the iodine K-edge energy was used to produce the highest contrast image of iodine contrast medium. To detect high-resolution real-time imaging (9.5×7 mm field of view; 30 frames/s), an x-ray SATICON camera (Hitachi Denshi Techno-System and Hamamatsu Photonics) was used. The direct-sensing detector consisted of an x-ray direct-sensing pickup tube with a beryllium faceplate for x-ray incidence to the photoconductive layer. The absorbed x-ray films in the photoconductive layer were directly converted to photoelectrons, and then the signal charges were read out by electron beam scanning. The image signals from the camera were converted into a digital format and then were stored in a frame memory with an image format of 1024×1024 pixels and 10 bits per pixel. For 1 image, 10 frames were integrated to obtain a higher contrast and lower noise image, and these images were obtained every 0.33 s.

**Image Analysis**
The images were stored digitally. A temporal subtraction operation was performed for flat-field correction; the summation image taken before injection was subtracted from raw images taken after injection to eliminate the superimposed background structure. Then the diameter of the vessels were measured semiautomatically on the digital image with Image Pro Plus Ver. 5.0 (Media Cybernetics, Inc) combined with a program developed for this study. Differences between normocapnia group and hypercapnia group were evaluated with Student t test. To obtain a general view of the hemisphere and neck, several images were assembled using computer program Adobe Photoshop version 7.0 (Adobe Systems Inc). To evaluate the distensibility of the intracranial vessels under drug administration, a frame centering on the ICA bifurcation was chosen. This framing covers the anterior cerebral artery (ACA), middle cerebral artery (MCA), posterior cerebral artery (PCA), and the intra-portion and extra-portion of the ICA. The pterygopalatine artery (PPA), which originates from the ICA and runs extracranially, can also be observed.

**Results**
A general view of the cerebral vessels in the right hemisphere was created as shown in Figure 2 by means of tilting the frame for several images and assembling them. To demonstrate the proximal part of ICA, we injected 100 μL of contrast medium, and as a result, the aorta was filled. Otherwise, we used 33 μL of contrast medium, and the posterior fossa was not filled, indicating that angiography was performed selectively in the ICA territory. The ophthalmalic artery originated from the distal part of the PPA and the anastomosis of the ACA to nasal vessels via the ethmoidal artery were observed. The smallest detectable vessels were branches of the MCA that had a diameter of

![Figure 3](image-url)

**Figure 3.** The serial images from the arterial phase to the venous phase at 1.0, 1.66, 3.0, 5.0, and 8.0 s after the starting of contrast agent injection.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Mouse</th>
<th>Mouse</th>
<th>Mouse</th>
<th>Mouse</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA</td>
<td>155.6</td>
<td>142.1</td>
<td>134.5</td>
<td>138.9</td>
<td>141.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>142.5±7.90</td>
</tr>
<tr>
<td>ACA</td>
<td>152.3</td>
<td>134.4</td>
<td>130.0</td>
<td>131.5</td>
<td>143.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>138.3±9.35</td>
</tr>
<tr>
<td>PCA</td>
<td>120.0</td>
<td>120.4</td>
<td>111.8</td>
<td>123.7</td>
<td>126.5</td>
</tr>
<tr>
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<td>120.5±5.53</td>
</tr>
<tr>
<td>ICA</td>
<td>180.3</td>
<td>159.8</td>
<td>153.0</td>
<td>164.9</td>
<td>155.2</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>162.6±10.87</td>
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</tbody>
</table>

**TABLE 2. Diameters of Cerebral Vessels of Mice Under Hypercapnia (μm)**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Mouse</th>
<th>Mouse</th>
<th>Mouse</th>
<th>Mouse</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA</td>
<td>194.2</td>
<td>163.8</td>
<td>161.6</td>
<td>162.8</td>
<td>186.0</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>173.7±15.29</td>
</tr>
<tr>
<td>ACA</td>
<td>187.9</td>
<td>174.9</td>
<td>165.5</td>
<td>139.3</td>
<td>170.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>167.5±17.86</td>
</tr>
<tr>
<td>PCA</td>
<td>140.1</td>
<td>161.6</td>
<td>145.6</td>
<td>145.6</td>
<td>141.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>146.9±8.60</td>
</tr>
<tr>
<td>ICA</td>
<td>220.2</td>
<td>213.8</td>
<td>190.0</td>
<td>182.5</td>
<td>200.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>201.3±15.78</td>
</tr>
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</table>
blood gas levels were as follows (mean between 121% and 124% (Figure 4A through 4C). The arterial arteries under hypercapnia was observed in all at a range of under normocapnia were as follows (mean

and Hypercapnia (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Normocapnia Group</th>
<th>Hypercapnia Group</th>
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<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>pH</td>
<td>7.36±0.07</td>
<td>7.09±0.10</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>28.5±3.05</td>
<td>62.1±6.29</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>102.8±10.1</td>
<td>54.0±3.67</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>97.0±2.35</td>
<td>75.8±5.07</td>
</tr>
</tbody>
</table>

The diameters of each artery in the C57BL/6J mice (n=5) under normocapnia were as follows (mean±SD): 142.5±7.90 μm for the MCA, 138.3±9.35 μm for the ACA, 120.5±5.53 μm for the PCA, and 162.6±10.87 μm for the ICA (Table 1). The diameters under hypercapnia (n=5) were as follows (mean±SD): 173.7±15.29 μm for the MCA, 167.5±17.86 μm for the ACA, 146.9±8.60 μm for the ACA, and 201.3±15.78 μm for the ICA (Table 2). To make reproducibility clear, we repeated imaging up to 5 times under normocapnia and compared the diameter of ICA. The diameter at the first imaging was 162±10.87 μm at the first imaging and 159.4±8.76 μm at the fifth imaging, and there were no significant differences (P<0.05; Table 3). The distention of the arteries under hypercapnia was observed in all in a range of between 121% and 124% (Figure 4A through 4C). The arterial blood gases were as levels as follows (mean±SD): pH 7.36±0.07, Paco2 28.5±3.05 mm Hg, PaO2 102.8±10.1 mm Hg, SaO2, 97.0±2.35% under normocapnia, and pH 7.09±0.10, Paco2 62.1±6.29 mm Hg, PaO2 54.0±3.67 mm Hg, SaO2 75.8±5.07% under hypercapnia (Table 4). The arterial blood pressure and pulse rate are shown in Table 5.

The intercarotid injection of the drugs induced dilatation in the vessels. Papaverine hydrochloride induced a rapid vasodilatation of the arteries (Figure 5A), starting after 1 minute and peaking at 5 minutes after the injection (Figure 5B). The dilatation ratio at 5 minutes after injection was 136.7% in MCA, 149.4% in ACA, 130.2% in PCA, 136.5% in ICA, and 125.9% in PPA. At 30 minutes after the injection, all arteries returned to the preinjection diameters. ATP disodium showed similar changes with papaverine hydrochloride in cranial vessels but not in PPA that showed poor distention (Figure 5C). Fasudil hydrochloride hydrate showed vasodilatation only in the PPA but not in the intracranial arteries, and the effect continued for 30 minutes (Figure 5D).

Discussion

For the first time, mouse cerebral angiography was performed in this study using synchrotron radiation and a contrast agent. The anatomy of the vessels as well as the average diameters of each vessel were successfully measured in the territory of the ICA. It was also interesting to note that the volume of injected agent was as small as 33 μL, thus allowing us to perform repeated imaging in each mouse. Placing the catheter selectively in the ECA by microsurgery was also an important factor in the success of this study because this maintained the ICA blood flow from the CCA to the brain during the entire experimental time period. In literatures of rat cerebral angiography, the values of vessel diameter have been surprisingly varied (eg, basilar artery between 224 and 351 μm and MCA between 305 and 351 μm). Some of those studies used retrograde injection of large volume of the contrast medium, resulting in contrast media filling in both carotid and vertebral arteries. This could increase the pressure of the lumen rapidly in nonphysiological manner. Other studies used anterograde injection while occluding the blood flow of the vessel, which decreases the vessel tone abnormally during the
experiment. We consider that our method of selective injection of the contrast media into ICA while maintaining the ICA blood flow is the most physiological method.

The diameters of the cerebral arteries have been reported in rats, as measured by conventional histology or by the endocasting method using scanning electron microscopy.\(^3\),\(^1\)\(^6\) Demonstration of cerebral vessels by endocasting method in mice has never been performed. Comparing between angiography and corrosion casting (or conventional histological study), we consider that the casting method contains many artifacts (eg, lack of pulsatile flow that is related to physiological shear stress on the endothelial cells, and tissue shrinkage and smooth muscle contraction attributable to fixation procedure). Although semiquantification may be possible by corrosion casting, detecting rapid physiological change of the vessel diameter can be performed accurately only by angiography. We can show more physiologically accurate values than those obtained from histological measurement.

We chose C57BL/6J in this study because this strain is most commonly used for genetically engineered mice. Differences in the morphology among various transgenic mice can thus be studied using our technique. Recently, Beckmann et al reported a study series using high-resolution magnetic resonance angiography operating at 4.7 T in the mouse brain. They demonstrated strain differences in the vascular anatomy between C57BL/6J and SV129 mice.\(^1\)\(^7\) They also studied

<table>
<thead>
<tr>
<th></th>
<th>Normocapnia Group</th>
<th>Hypercapnia Group</th>
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<tbody>
<tr>
<td>BP (Sys, mm Hg)</td>
<td>90.4±7.37</td>
<td>90.8±7.53</td>
</tr>
<tr>
<td>BP (Dia, mm Hg)</td>
<td>71.8±7.26</td>
<td>72.4±6.50</td>
</tr>
<tr>
<td>BP (Mean, mm Hg)</td>
<td>81.8±6.26</td>
<td>81.6±6.39</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>547.6±19.6</td>
<td>528.2±18.1</td>
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</table>

**Figure 5.** A, Chronological angiography of the mouse brain before and after the intracarotid injection of papaverine hydrochloride. The changes of the diameter were measured and are summarized in B. Similar changes were observed by ATP disodium shown in C and by fasudil hydrochloride hydrate shown in D.
the imaging of permanent and transient brain ischemia in C57BL/6J mice and age-dependent abnormalities in APP23 transgenic mice. More recently, an abnormal vasculature of brain tumors has been reported in genetically engineered mice: TgT121 and p53\(^{+/-}\). Although magnetic resonance angiography is less invasive than in vivo x-ray angiography, the spatial resolution is not sufficient to detect physiological responses in the cerebral arteries. The time resolution is also not sufficient in magnetic resonance angiography; a few minutes are necessary to produce a single image, and thus different time phases that show the direction of the blood flow are impossible to obtain.

We also herein demonstrated vasodilation and its time course by drug manipulation in mice. The drugs used in this study are known to induce vasodilation. Distensibility can also be studied with cerebral arterioles by cannulation to isolated vessels in vitro. This in vitro technique can help us to avoid any confounding influences of the surrounding brain elements, whereas the physiological circumstances, such as neurogenic control and mechanical pulsatile flow stress on the arteries, can be completely neglected. Synchrotron radiation has the possibility of creating the biggest change in medical x-ray imaging. Without using any contrast agent, phase-contrast/edge-enhancement imaging with synchrotron radiation allows us to show the microscopic details of moving blood vessels in live animals. The present study clearly demonstrated that synchrotron radiation is a novel diagnostic modality for angiography of the mouse brain.

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References

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