Ultrafast High-Resolution In Vivo Volume-CTA of Mice Cerebral Vessels

Sebastian J. Schambach, MD; Simona Bag, MD; Volker Steil, MSc; Cristina Isaza; Lothar Schilling, MD, PhD; Christoph Groden, MD; Marc A. Brockmann, MD, MSc

Background and Purpose—Animal models developed in rats and mice have become indispensable in preclinical cerebrovascular research. Points of interest include the investigation of the vascular bed and the morphology and function of the arterial, capillary, and venous vessels. Because of their extremely small caliber, in vivo examination of these vessels is extremely difficult. In the present study we have developed a method to provide fast 3D in vivo analysis of cerebral murine vessels using volume computed tomography-angiography (vCTA).

Methods—Using an industrial X-ray inspection system equipped with a multifocus cone beam X-ray source and a 12-bit direct digital flatbed detector, high-speed vCTA (180° rotation in 40 s, at 30 fps) was performed in anesthetized mice. During the scan an iodinated contrast agent was infused via a tail vein. Images were reconstructed using a filtered backprojection algorithm. Image analysis was performed by maximum intensity projection (MIP) and 3D volume reconstruction.

Results—All mice tolerated i.v. injection of the iodinated contrast agent well. Smallest achievable voxel size of raw data while scanning the whole neurocranium was 16 μm. Anatomy of cerebral vessels was assessable in all animals, and anatomic differences between mouse strains could easily be detected. Mean vessel diameter was measured in C57BL/6 and BALBc mice. Changes of vessel caliber were assessable by repeated vCTA.

Conclusions—Ultrafast in vivo vCTA of murine cerebral vasculature is feasible at resolutions down to 16 μm. The technique allows the assessment of vessel caliber changes in living mice, thus providing an interesting tool to monitor different features such as vasospasm or vessel patency. (Stroke. 2009;40:1444-1450.)

Key Words: imaging • CT • animal experiments

Small rodents such as mice or rats are frequently used in preclinical cerebrovascular research.1–3 Investigations of the structure of the cerebrovascular network or the morphology and function of cerebral arterial, capillary, and venous vessels are of great interest and could be helpful in models of brain ischemia, tumor, or vasospasm.4–6 In the past, animals had often to be killed in order to obtain the pertinent information on the status of the vascular system.7–9 This method, however, does not allow repeated imaging of the same animal.

With the diameter of murine intracerebral arteries on the skull base ranging between 100 and 250 μm, a high resolution is required to allow valid analysis of these vessels. So far, different groups have demonstrated the feasibility of high-resolution μCT methodology for imaging cerebral arteries ex vivo.10,11 To our knowledge there are no publications demonstrating feasibility of CTA to study murine cerebral vessels in vivo.

The goal of the present study was to use high-resolution ultrafast volume-CT–based angiography (vCTA) to image murine extra- and intracerebral arterial and venous vessels in vivo. Using a micro-CT (μCT) system designed for industrial purposes we have developed a new scanning protocol taking less than 1 minute for data acquisition without the need for invasive intraarterial catheterization.

Materials and Methods

Volume-CT Hardware

An industrial X-ray inspection system in which the object is rotated was used (Yxlon Y. Fox; Yxlon International GmbH). The scanner theoretically provides X–Y feature recognition down to 500 nm and a geometric magnification level of up to 2720×. It features a 4-axis precision dimension manipulator with high-speed sample positioning, a rotation axis to hold the object, and a 6th axis for detector movement (ie, height adjustability).

The system uses a cone beam X-ray tube equipped with a multifocus X-ray tube allowing operation in a microfocus or a nanofocus mode. A hairpin-filament with a diameter of 200 μm is used to produce a voltage dependent 10 to 100 μm wide electron beam, which is focused onto a tungsten/beryllium transmission target resulting in a spot size of less than 1 μm. The opening angle of the beam is 10 degrees. The system is equipped with a 12-bit direct reconstruction.

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From the Departments of Neuroradiology (S.S., S.B., C.I., C.G., M.A.B.), Radiation Oncology (V.S.), and Neurosurgical Research (L.S.), University Hospital Mannheim, Germany.

Correspondence to PD Dr. med. Marc A. Brockmann, MSc, University of Heidelberg, Medical Faculty Mannheim, Department of Neuroradiology, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany. E-mail brockmann@gmx.de

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digital flatbed detector (PaxScan 2520 Amorphous Silicon Digital X-ray Detector; Varian Medical Systems) with a maximum of 30 frames per second (fps) in 2×2 binning mode (pixel matrix of 944×704) and a pixel pitch of 127 μm² (254 μm² binned; 1.97 Lp/mm at 30 fps).

Measurement of the Surface Radiation Dose

For the measurement of surface dose rates we used a PTW universal dosimeter (Unidos, PTW) with the ionization chamber type 23342 and an encapsulated 90Sr source T48010 for calibration. The readout of the dosimeter is provided in mGy/s. The ionization chamber was placed in a special adapter plate of the PTW slab phantom made of acrylic glass (Perspex; slab thickness, 2 cm). To determine the entry dose the dosimeter was placed under the X-ray at the same distance as the mice were positioned (3.5 cm). For determining the exit dose, the ionization chamber was placed straight underneath the slab phantom. The source-object-distance, tube voltage, and current were set to values typically used for vCT angiography in mice (see below). Because the animal was rotated by 180° during the 40 seconds scanning procedure the total surface dose (surface dose_{tot}) was estimated by the equation

\[ \text{surface dose}_{tot} = (20 \text{ seconds} \times \text{entry dose}) + (20 \text{ seconds} \times \text{exit dose}) \]

with the entry and exit dose given in mGy/s.

Animal Preparation

Institutional guidelines for animal welfare and experimental conduct were followed. Mice were anesthetized by intraperitoneal administration of a mixture of ketamine (100 mg/kg body weight) and xylazin (5 mg/kg body weight). A mouse-tourniquet as described previously by Hoff et al was placed over the tail root and tightened. A 23 Gauge venous catheter preflushed with a heparin-solution was connected to a syringe (1 mL) via a silicone tubing (1.5 m). Both syringe and tubing were filled with prewarmed contrast agent (Iomeron 300, Bracco Altana). Great care was taken to expel any air bubbles from the connecting sites to prevent air embolism. The syringe was inserted into a lateral tail vein 2 cm distal to the tourniquet and fixed with adhesive tape.

The mouse was transferred to a custom made acrylic fixation device (Figure 1) where it was placed in a supine position with the head resting on a rubber foam pillow. Two ear-bars made of radiolucent plastic and a sling over the incisors were used to achieve a 3-point fixation of the head. The lower parts of the animal’s body were held in the cradle by adhesive tape circled around the hips and the torso.

Acquisition Parameters and Scanning Protocol

The acrylic cradle with the mouse attached was mounted onto the three jaw drill chuck of the rotational axis of the CT device (Figure 1) and the animal’s neurocranium appropriately positioned within the X-ray beam. The source-object and object-detector distances were adjusted so that the skull of the mouse filled the field of view (FoV). The catheter in the tail vein was connected to a syringe (1 mL) via a silicone tubing (1.5 m). Both syringe and tubing were filled with prewarmed contrast agent (Iomeron 300, Bracco Altana). Great care was taken to expel any air bubbles from the connecting sites to prevent air embolism. The syringe was put on an infusion pump (PHD 2000; Harvard Apparatus) for administration of the contrast media at a constant speed.

For each scan a total of 350 frames per second (fps) in 2×2 binning mode (pixel matrix of 512×512 voxels). Vessel diameter of reconstructed images was measured using I-view software v. 1.0.0 (TeraRecon).

For 2D analyses and measurement of vessel diameter the reconstructed datasets were visualized using maximum intensity projection (MIP; Figure 2). The diameter of the vertebral artery (VA), superior cerebellar artery (SCA), basilar artery (BA), posterior (PCA), middle (MCA), and anterior cerebral artery (ACA) were measured on each side. We furthermore assessed the diameter of the intracranial part of the internal carotid artery proximal to the origin of the PCA (prox. ICA), and between the origin of the PCA and the MCA (dist. ICA). The vessel diameter was measured in 8 C57BL/6 mice. We additionally scanned and measured vessel diameter of 2 BALBc mice to demonstrate differences in the pattern of vascularization between different mouse strains.

To provide proof-of-principle that changes in vascular diameter of mice can be detected by repeated vCTA, we used a gas mixture containing 5% CO₂ and 12% O₂ (balance, N₂) to induce a state of hypoxic hypercapnia in a mouse. After 15 minutes breathing the gas mixture a CT scan was performed. Ventilation was then changed to room air and 20 minutes later a second scan was performed. Vessel diameters were measured offline as described above.
Results

Physiological Compatibility and Dosimetric Analyses
All animals tolerated the injection of 350 µL of iodinated contrast agent within the 40 seconds scanning period well. Even a repeated injection of 350 µL 20 minutes after the first one was tolerated. After the vCTA study the animals were returned to their home cages and kept for 3 weeks. During this time there were no clinical signs of renal failure. However, we did not perform measurements of blood or urine samples.

At the end of the 3-week observation period the animals displayed loss of hair in the neck and upper chest at the ventral side. Because these are the regions which were exposed nearest to the X-ray source during the vCTA study the hair loss was considered a long-term radiation effect. Therefore, dosimetric measurements were performed at different distances to the target of the X-ray tube yielding intensity values of 270 mGy/s and 2 mGy/s at the levels equivalent to the X-ray entry and exit sites in our experiments. From these values we estimated a total surface dose of 5.5 Gy for a 180° scan (40 seconds) using the equation given in the Materials and Methods section.

Image Quality and Visibility of Anatomic Structures
Anatomy of the extra- and intracerebral compartments of the vascular system could be assessed in all mice. Examples of 3D reconstructed pictures are shown in Figures 3, 4, and 5. In one animal (BALBc) the image was slightly blurred by artifacts because of minimal movement of the animal during the 180° rotation resulting from insufficient fixation. Differences in cerebroarterial anatomy between both strains studied, C57BL/6 and BALBc, could clearly be detected. Most notably, the posterior communicating artery connecting the PCA and the SCA was consistently observed on both sides in BALBc mice whereas it was not detectable in most of the C57BL/6 mice studied. These different expression patterns which are in accord with previous reports in the literature are exemplified in Figure 5.

Measurement of Vessel Diameters and Diameter Changes
Vessel diameters were measured in different intracerebral arteries using MIP reconstructed images. The results obtained
for each individual animal are provided in the Table. Mean vessel diameter (averaged for left and right side whenever appropriate) was for the BA 221 ± 27 μm in C57BL/6 versus 154 ± 4 μm in BALBc (P < 0.05), for the VA 191 ± 30 μm in C57BL/6 versus 118 ± 8 μm in BALBc (P < 0.05), for the SCA 164 ± 18 μm in C57BL/6 versus 109 ± 11 μm in BALBc (P < 0.05), for the proximal ICA (measured proximal to the origin of the PCA): 197 ± 27 μm in C57BL/6 versus 196 ± 32 μm in BALBc (P > 0.05), for the distal MCA (between the origin of PCA and MCA): 177 ± 24 μm in C57BL/6 versus 169 ± 22 μm in BALBc (P > 0.05), for the MCA 157 ± 25 μm in C57BL/6 versus 141 ± 10 μm in BALBc (P > 0.05), for the ACA 144 ± 27 μm in C57BL/6 versus 131 ± 27 μm (P > 0.05), and for the PCA 149 ± 17 μm in C57BL/6 versus 175 ± 16 μm in BALBc (P < 0.05). Thus, the arteries from the anterior part of the circulation did not differ in diameter between both strains, whereas the arteries belonging to the posterior part of the cerebral circulation were significantly smaller in BALBc than in C57BL/6. This finding is most probably attributable to the fact that in BALBc mice the SCA is supplied by the anterior cerebral circulation via the posterior communicating artery, thus reducing the territory supplied by the BA.

In an additional experiment in a C57BL/6 mouse, measurements of vessel diameter were performed under control conditions while breathing air and while challenged by breathing a gas mixture to induce hypoxic hypercapnia. Dilatation of vessels observed under these conditions was 37% in the VA, 57% in the BA, 16% in the SCA, 24% in the proximal ICA, 21% in the distal ICA, 9.2% in the MCA, 3.5% in the ACA, and 11% in the PCA. These results show the suitability of the vCTA for vasomotor studies, and they also suggest a marked regional heterogeneity in the vasomotor response of arteries in the anterior and posterior cerebral circulation. The changes of vessel diameters in the posterior circulation are illustrated in figure 6 showing a MIP projection imaging the VA, BA, and SCA.

**Discussion**

Recently, imaging modalities which are minimally or noninvasive such as MRI have become available and applied for in
vivo studies on the status of the cerebrovascular system in small experimental animals such as rats and mice.13–16 Unfortunately, dedicated small animal MRI scanners are very expensive, measurements are time consuming, and the acquired images still do not reach the level of resolution as provided by X-ray dependent techniques such as digital subtraction angiography (DSA) or computed tomography angiography (CTA). In fact, synchrotron radiation DSA has been reported to provide high-resolution images of the cerebral vasculature, even in animals as small as mice.17,18 However, it is invasive in that it requires insertion of a catheter and injection of contrast media into the arterial system to achieve sufficient contrast of the blood vessels. In addition, the method does not easily allow generation of 3 dimensional (3D) datasets. Therefore, in vivo CT-angiography appears to be the method of choice to obtain high-resolution 3D-datasets.

Micro-CT (μCT) devices are well suited for 3D imaging of small objects. In accord, this method has become a widely used technique in preclinical and experimental research. Initially developed for nonhuman studies, μCT combines the beneficial effect of 3D imaging and high spatial resolution, thus providing important new insights in different fields of biomedical research including bone structure and mineralisation, anatomy, oncology, and cardiovascular research.19–25 The resolution that can be achieved mainly depends on the pixel-resolution of the detector and the magnification level (ie, the distance of the object to the X-ray source on the one hand and the detector on the other).26 Thus, the highest resolution is achieved when the object is located as close as possible to the X-ray source and as far away as possible from the detector. In most CT devices used for small animal imaging the animal is kept in a fixed position while the X-ray source and the detector (the so-called gantry) rotate around the object during the scanning procedure. Although this helps to avoid soft tissue distortions, to minimize movement artifacts, and to facilitate connection to supplying and recording devices it results in a fixed and relatively low magnification level thus limiting the minimal voxel size. These systems currently do not provide resolutions sufficient for analysis of the murine cerebrovasculature.

Table. Mean Diameter of the Intracerebral Arteries in C57BL/6 (n=8) and BALBc Mice (n=2) as Measured by νCTA

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prox. indicates proximal; dist., distal; VA, vertebral artery; BA, basilar artery; SCA, superior cerebellar artery; ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior cerebral artery.

*p*Assessment of vascular diameter in one BALBc mouse was limited by artifacts.

Figure 6. Repeated νCTA illustrates diameter changes of cerebral arteries in a C57BL/6 mouse induced by a hypoxic hypercapnic challenge. In this figure the vessels of the posterior circulation are shown (labeling omitted for clarity) A, The arteries of the mouse are dilated by a hypoxic hypercapnic challenge. B, After breathing normal room air for 20 minutes the diameter of the arteries of the posterior circulation normalizes.
In the present study we used a system that allows the animal to be positioned at virtually any position between the X-ray tube and the detector. This is possible because the animal is rotated while the gantry is fixed. Thus, by positioning the animal’s head close to the X-ray tube while maintaining a large distance to the detector we achieved a high level of magnification resulting in a voxel size of 16 μm in our raw data. The fact that the voxel size of the reconstructed data are somewhat less (22 or 30 μm) is attributable to hardware restrictions. Currently, reconstruction of raw data for volume rendering relies on a 512×512 matrix. However, we are now modifying our system to allow reconstruction using a 1024×1024 matrix and generation of considerably smaller voxels in future studies. Nevertheless, even the 30-μm voxel size is much smaller than those achieved in many studies available in the literature. Thus, Beckmann et al using a small animal 4.7 T MR scanner for high-resolution studies on the mouse cerebrovasculature reported the pixel size of the raw data to be 150×100×100 μm. Using the method described in our article it is not possible to separate intracerebral arteries from veins. This, however, can be achieved by anatomic localization only, because the 40-second scanning time is much longer than the cerebral transition time in mice. Therefore it is currently not possible to perform cerebral in vivo CTA in mice in a purely arterial or venous phase.

In the past, high-resolution μCT studies required very long scanning periods of many minutes up to hours. However, such protocols are not suitable for in vivo studies because of restrictions resulting from the need to keep the animals anesthetized, to minimize movement artifacts, and to limit the radiation dose. We have, therefore, developed a completely new scanning protocol the hallmarks being (1) intravenous instead of intraarterial administration of the contrast medium, (2) rotation by 180° only, and (3) a high frame rate providing a sufficient number of images within a 40 seconds scanning time. Postprocessing using 3D reconstruction techniques resulted in high quality images that allow easy assessment of the anatomy of the murine cerebral circulation with the measurement of vessel diameter performed in images obtained from 2D analyses, ie, MIP pictures. Thus, we were able to consistently detect blood vessels in the diameter range of approximately 50 to 60 μm, eg, the small branches arising from the azygos ACA. In addition, we could quantify diameter changes induced by a hypoxic hypercapnic challenge of the major arteries on the base of the brain such as the ICA, the ACA, and the BA showing resting diameters in the range of 140 to 220 μm in individual animals. For the measurement of vasomotor responses the animals received two injections of contrast media with a volume of 350 μL each. The experimental protocol was designed to measure the baseline diameter, ie, with the animal breathing room air, after the challenge to account for possible volume effects by the repeated infusions. However, there was no apparent difference to the group of animals in which only one measurement was performed.

Therefore, one of our initial concerns—whether the animals would tolerate the injection of relatively large volume of a contrast agent—turned out to be not a major problem of the protocol. The large volume of 350 μL was required to achieve constant and sufficient contrast for vCTA. We did not observe any signs of obvious renal failure in the mice during a 3-week postinjection period, but we did not take blood samples for in-depth analysis of parameters reflecting the renal function.

In a preliminary study we injected a blood pool contrast agent (results not shown). However, in these experiments we could not obtain a sufficiently high contrast level within the vessels indicating that the concentration of 50 mg/mL iodine was not high enough. Improving the SNR by increasing the number of projections for reconstruction would help to increase contrast, but would also result in a higher radiation dose. Therefore, we did not pursue this approach.

In our setup the animals need to be placed in close proximity to the X-ray tube to provide the necessary resolution. Thus, relatively high radiation doses are applied. The radiation dose issue is clearly of relevance for all kinds of μCT studies, and accordingly has been intensively discussed in previous publications. Our observation of hair loss in animals 3 weeks after the examination is in accordance with the literature, where cumulative entrance skin doses of 3 Gy or higher are described to result in epilation. However with the increased resolution and sensitivity of future detectors, radiation doses might be reduced. First novel low-dose μCT scanners are currently being tested.

The number of 1200 projections per scan was not only given by the radiation dose but also because the software of our μCT was run on a Microsoft Windows XP 32-bit operating system, which is only capable of managing 2 gigabyte of random access memory per process. We are currently setting up studies to circumvent this limitation by performing repeated CT scans with subsequent overlay of the data of the single scans in order to increase SNR, although this is accompanied by an increase of radiation dose. Other possibilities would be to either modify the user interface so that it can run on a 64-bit operating system which does not suffer from the limitations of the windows-based systems or to program a “buffer-script” that allows transfer of data from the random access memory directly to the hard disk during image acquisition. We are currently engaged in comparing these 2 approaches for further studies.

Conclusion

In the present study we demonstrate that in vivo CT angiography is feasible in animals as small as mice using intravenous injection of a contrast agent. The smallest diameter of vessels that reliably can be imaged is in the range of 50 μm. Repeated measurements are possible and changes in vessel diameter can be monitored, making this technique an interesting tool for investigations of diameter or patency of cerebral arterial or venous vessels in vivo models of vasospasm or thrombolysis. The technique furthermore might be helpful to analyze the vascularization pattern before stroke experiments. The major limitation of the technique is the relatively high radiation dose applied, which currently limits observation periods to 2 to 3 weeks. Within this time range, however, the long-term effects of radiation do not appear to be a major obstacle.
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Disclosures
None.

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