A Novel Technique to Study Mouse Cerebrovasculature Using MR Guided Micro-CT

B. P. Chugh[1,2], L. X. Yu[1], R. M. Henkelman[1], J. G. Sled[1]

[1]Mouse Imaging Centre (MICe), Toronto, Ontario, Canada, [2]Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

Introduction

Micro-computed tomography (micro-CT) can be used to quantitatively study the 3D architecture of blood vessels in the mouse brain. An obstacle to obtaining neurologically useful information from micro-CT has been the inability to relate the vascular architecture to corresponding neurological regions. Although MR images can provide excellent anatomical landmarks for segmenting the brain, registration of MR and micro-CT is difficult due to the dissimilar contrast. To overcome this problem, we developed a novel landmark-based technique for registering micro-CT images to MR images of the same brain using an intravascular contrast agent that is visible in both modalities. Using this new method, we were able to compute a blood density difference between the cerebral cortex and the entire brain. This could lead to an effective way to quantitatively compare neurovascular phenotypes of the mouse brain.

Materials and Methods

Three male CBA mice, 24-*26* weeks old, were anesthetized with an i.p. injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). To purge the cardiovascular system, a 100 ml solution of heparinized-PBS (1 unit heparin/ml) at 37°C was injected using a 23-gauge butterfly needle though the left ventricle of the heart at a constant pressure of 160 mmHg. The circulation was then filled with a radiopaque silicone rubber (Microfil® MV-122, Flow-Tech Inc.) and left to polymerize for 90 minutes, as previously described for the kidney[1].

Next, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures were allowed to postfix in 4% paraformaldehyde at 4°C for 12 hours. Following a further incubation period of 5 days in phosphate buffered solution (PBS) and 0.01% sodium azide at 15°C, the skulls were transferred to a PBS and 2mM Prohance® (Bracco Diagnostics Inc., Princeton, NJ) solution for at least 7 days at 15°C. MR imaging occurred 12 to 21 days postmortem.

A four-channel 7.0-T MR scanner (Varian Inc, Palo Alto USA) with a 6-cm inner bore diameter gradient set was used to acquire anatomical images of brains within skulls. Prior to imaging, the skulls were removed from the contrast agent solution and placed into plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp., St. Paul, MN). Custom-built, 12-mm over-wound uniform solenoid coils were used to image three brains in parallel. The parameters used in the scans were optimized for grey/white matter contrast: T2-weighted, 3D fast spin-echo sequence, with TR/TE= 325/32 ms, four averages, field-of-view 12 x 12 x 25 mm and matrix size = 780 x 432 x 432 giving an image with 32 μm isotropic voxels. The total imaging time was 14 hours.

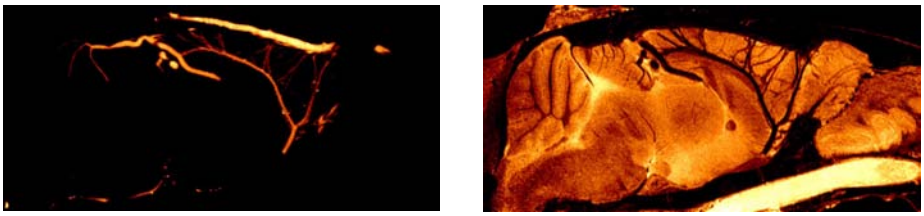

Figure 1: Mid-sagittal slices of a registered microCT image(left) and MR image (right).

In preparation for scanning with micro-CT, the brains were removed from the skulls and mounted in 1% agar. Each vascular image volume was acquired over 720 views through 360° rotation using a GE eXplore Locus SP Specimen Scanner at 20 μm isotropic resolution. Each brain scan took 2 hours.

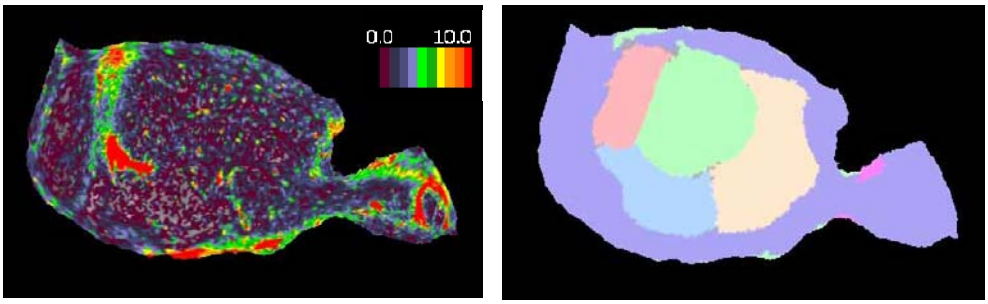

Figure 2: Comparison of the average percentage of blood over the right cortical surface of the mouse brain (right) with the segmentation of the same surface into cortical lobes, namely, frontal (yellow), parietal (green), occipital (pink), temporal (blue), limbic (purple) and olfactory (dark pink).

The ability to locate voxels in the MR images where several blood vessels intersected, permitted each MR image to be accurately registered to the corresponding micro-CT image (see Fig.1). For each brain, a 12 parameter affine transformation was performed between the micro-CT image and the corresponding MR image by selecting four points, one as anterior as possible and the other three as posterior and distal as possible. Each MR image was non-linearly registered by selecting 8-10 landmarks, followed by a thin-plate-spline transformation onto a C57Bl6 mouse brain atlas[2], whose cortical surface has been flattened and segmented into lobes[3]. Thus, each of the three micro-CT images could be described in the coordinate system of the cortical surface of the atlas. For each micro-CT image, the average intensity of all voxels in the 400μm cortical column perpendicular to the surface was projected to the surface using the software Caret[4]. This blood density map, showing the percentage of micro-CT vasculature / MR volume, was compared with the segmented cortical lobes (see Fig.2).

Results and Discussion

Aside from the blackening of blood vessels in the MR images caused by the presence of Microfil, no artifacts were observed when compared to MR images taken without the presence of Microfil. The blood density of the entire brain was measured to be 2.7%, whereas the blood density of the cerebral cortex was 1.4%. This was likely because the volume of the blood vessels projected to the cortical surface did not contain the major superficial vessels. By expanding this study to a larger population of mice, some of which have vascular defects, this technique could provide an effective method for quantifying neurovascular phenotypes.

References

1. Bentley MD, et al. The use of microcomputed tomography to study microvasculature in small rodents. Am J Physiol Regulatory Integrative Comp Physiol. 2002, 282: R1267-1279.
2. Van Essen DC. Surface-based atlases of cerebellar cortex in the human, macaque, and mouse. Ann NY Acad Sci. 2002, 978: 468-479.
3. http://brainmap.wustl.edu:8081/sums/directory.do?id=636032
4. Van Essen DC, et al. An integrated software system for surface-based analysis of cerebral cortex. J Am Med Infor Assoc. 2001, 41: 1359-1378.