Quantifying Cerebral Blood Volume Over the Mouse Cerebral Cortex Using Micro-CT Co-registered to an MRI Anatomical Atlas

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Introduction: Measurement of the cerebral blood volume (CBV) in local regions of the mouse brain will be useful to describe the phenotypes of models of neurodegenerative diseases that alter microvasculature including Alzheimer's disease. Furthermore, the CBV associated with the microvessels may be an indicator of changes in the chronic metabolic state of brain regions. A limitation of current methods to determine CBV in mice, including MRI methods sensitive to magnetic susceptibility changes, is that they do not provide absolute quantification of CBV over the entire mouse cerebral cortex. To overcome this difficulty, we developed a method to determine absolute CBV over the mouse cortex using micro-computed tomography (micro-CT) co-registered to an MRI anatomical brain atlas. This method measures CBV by assuming that the percentage of radio-opaque contrast agent in each voxel is a measure of the CBV. We found that whole brain CBV was in reasonable agreement with previously published radioisotope dilution measurements.

Materials and Methods:

Preparation of Micro-CT Images: Five female C57BL/6 mice were anesthetized i.p. with ketamine (100 mg/kg), xylazine (20 mg/kg) and acepromazine (3 mg/Kg). Using a pressure-controlled pump, the upper body vasculature was rinsed with 10 mL of heparinized-PBS with inflow through the left ventricle and outflow through a cut in the right atrium. This was followed by perfusion of 2.5mL of a radio-opaque silicone rubber (Microfil® MV-122, Flow-Tech Inc.) at 150mmHg for 10 minutes. With the pump off, the right atrium was then sealed with superglue. With the pump set to 30mmHg, which is approximately the mean capillary pressure, the Microfil polymerized over the next 90 minutes. The skulls were then dissected, fixed for 12 hours in 10% formalin and decalcified with 5% formic acid at 50°C for 24 hours. These decalcified skulls were mounted in 1% agar and 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 over 2 hours.

Registration of Micro-CT images to an MRI Anatomical Brain Atlas: The vessel trajectories and diameters of each micro-CT image were determined by applying software that automatically calculates the vessel centerlines and estimates the boundary of each vessel based on the intensity distribution of the micro-CT images[1]. From these centerline images, the coordinates of four branch points were determined, namely, the branch of the vertebral arteries and basilar artery, the branch of the medial orbitofrontal artery from the azygos of the anterior cerebral artery, the branch point of rostral rhinal veins from superior sagittal sinus and the branch point of the transverse sinuses from the superior sagittal sinus (see Fig 1a). The same vascular landmarks were also identified on an MRI anatomical brain atlas (Fig 1b), which has been segmented into 62 three-dimensional regions based on an average of 20 female and 20 male C57BL/6 images of 32-micron isotropic resolution[2]. These images were acquired using a 7.0T MRI scanner (Varian Inc., Palo Alto, CA) with a 6-cm inner bore diameter insert gradient using a T2-weighted 3D fast spin-echo sequence with TR/TE=325/32ms. We then applied a six parameter least square rigid body registration of each of the micro-CT data sets to the atlas (see Fig.1c).

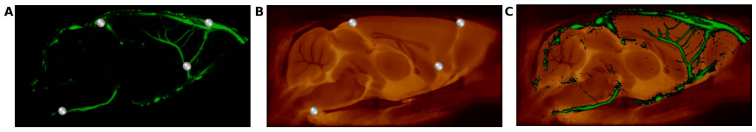

Figure 1: A slice from a micro-CT image (A) and MRI anatomical brain atlas (B) with suitably chosen landmarks (white markers) leads to accurate registration (C).

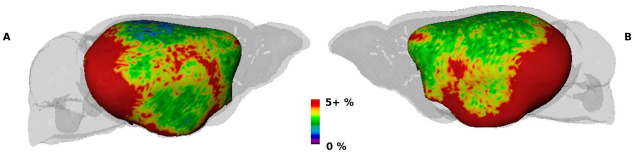

Figure 3: Mean CBV maps of right cortex (A) and left cortex (B)

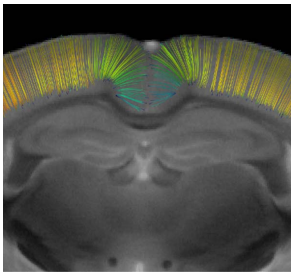

Figure 2: Coronal slice with field lines from inner to outer cortex.

Masking of the Major Blood Vessels: To reduce the bias of major blood vessels that do not contribute to local metabolism on regional value of CBV, we sorted the tracked model of the vessel segments and excluded those that were larger than 50 microns in diameter. The diameter of each vessel segment was dilated by 50% to account for any minor inaccuracies in the modeling. The voxels associated with these vessel segments were excluded from any subsequent CBV averaging.

Determination of the CBV: We assume that every voxel in each micro-CT dataset consists of a mixture of Microfil and water equivalent tissue. The percentage of Microfil present in each voxel was taken as a measure of the CBV. We have previously established that unperfused brain tissue has a radio-opacity to 80 kVp x-rays that is uniform and equivalent to 1% agar. As a calibration for CBV measurement, voxels located at the automatically tracked centerline positions with diameter between 0.1 and 0.2 mm were taken to be completely filled with Microfil whereas voxels in the surrounding 1% agar were taken to be completely unfilled. With these assumptions, all intensity values in the image were scaled into CBV units. The average CBV was measured over the entire brain and over the cerebral cortex. To determine maps of CBV over the cerebral cortex, we solved Laplace's equation to generate an image of field-lines from the inner surface of the cortex to the outer surface as defined on the MRI anatomical brain atlas (Fig.2). This method has previously been applied to map human cortical thickness[3] and has recently been adapted to determine cortical thickness in the mouse brain[4]. The CBV values were averaged over these field lines for right and left cortex (Fig.3).

Results and Discussion: The mean CBV (n=5) of the total brain was found to be 4.4±0.3% which is in reasonable agreement with previously made radioisotope dilution measurements that correspond to 3.5% to 4%[5]. The total cerebral cortex had a similar CBV to the whole brain, namely, 4.2±0.2%. These values may be different from CBV measurements made using MRI methodologies because the relationship between magnetic susceptibility changes and the size of vessels is complex. Using CBV maps based on micro-CT, a much higher degree of control can be gained over which vessels contribute to the measurement. As can be seen from the cortical maps in Fig.3, there was a high degree of symmetry in these maps. In spite of the effort to perform major vessel masking, there appeared to be a strip of high CBV around the region of the middle cerebral artery. The occipital regions were particularly high in CBV, which may be due to a specialization of visual function in the mouse. The proposed method to map CBV could be used to characterize regional differences of vascularity in mouse models of cerebrovascular disease.

References

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