Comparison of Cerebral Blood Flow as Measured by Arterial Spin Labeling and Cerebral Blood Volume as Measured by Micro-CT Imaging over Regions of the Mouse Brain

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

[1]Toronto Centre for Phenogenomics, Mouse Imaging Centre, The Hospital for Sick Children, Toronto, Ontario, Canada

Introduction: Resting state measurements of microvessel density have been found to correlate with cerebral blood flow (CBF) over regions of the rodent brain [1]. This relationship can be studied more extensively in mouse models of neurovascular disease by comparing images of cerebral blood volume (CBV) with images of CBF. We were interested in studying this relationship between CBV and CBF over multiple regions of the entire mouse brain using an efficient imaging protocol that, in future, can be applied to phenotype models of neurovascular disease. As outlined below, we performed a study that compared normative values of CBV and CBF over 13 mouse brain regions and over the cerebral cortex. The CBF was imaged using 3D arterial spin labeling (ASL) and these images were co-registered to CBV images determined by a micro-CT protocol.

Materials and Methods: All animal procedures described below were approved by the Hospital for Sick Children Animal Care Committee.

Determination of Cerebral Blood Flow: Eight female C57Bl/6 mice, anesthetized with 1.4% isoflurane, were scanned at 7.0-T using continuous arterial spin labeling. Flow-induced adiabatic inversion was applied using a 3.125 s long Hanning-weighted pulse of amplitude 90 mG and a 1.3 G/cm gradient positioned perpendicular to the common carotid arteries using vascular landmarks to guide alignment [3], which was previously found to invert 80% of the blood [4]. A post-label delay of 300ms was used to reduce transit time dispersion artifacts and to clear the intravascular signal [5]. The whole brain was imaged using a 3D fast spin echo pulse sequence (echo train length = 30) with 250 micron isotropic resolution followed by a 3.0s relaxation delay. To correct for magnetization transfer, interleaved amplitude modulated control images [6] were acquired with a B1of 127mG and a modulation frequency of 400Hz, leading to a net spin labeling efficiency of 0.50. The total ASL scan time was 50 minutes. CBF was quantified using a two compartment model [5] assuming a T1=1.6 s (grey matter) or T1=1.5 s (white matter) [7], T1=3.2 s (blood) and a partition coefficient of 0.9. Each 3D-CBF image was registered to an MRI anatomical brain atlas[8], based on an average of 40 C57BL/6 images of 32-micron isotropic resolution, using a rigid body registration based on four anatomical landmarks identified in the control scans and in the atlas.

Determination of Cerebral Blood Volume: Using a previously described procedure [9], nine 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 heparinized-PBS with inflow through the left ventricle and outflow through a slit in the right atrium. Next, the vasculature was perfused with a radio-opaque silicone rubber (Microfil®, Flow-Tech Inc.) at 150mmHg. With the pump off, the right atrium was sealed with superglue. With the pump set to 30mmHg, which is approximately the mean capillary pressure, the Microfil was left to polymerize over the next 90 minutes. The dissected skulls were then fixed in 10% formalin, decalcified with 5% formic acid and mounted in 1% agar. Each vascular image volume was acquired using 720 views through 360° rotation on a GE eXplore Locus SP Specimen Scanner at 20 µm isotropic resolution over 2 hours. Each 3D image was registered to the MRI anatomical brain atlas using a rigid body registration based on four vascular bifurcations defined on both the micro-CT images and the atlas. Voxels were scaled into CBV units by treating them as a mixture of Microfil and water density equivalent tissue.

Correlation Analysis: The average values of CBV and CBF were determined for the thirteen largest brain regions delineated by the MRI anatomical brain atlas. Specifically, the regions studied included the amygdala (A), the arbor vitae (AV), the corpus callosum (CC), the cerebellar cortex (CE), the cerebral cortex (C), the hippocampus (HI), the hypothalamus (HY), the midbrain (MI), the olfactory bulb (O), the pons (P), the medulla oblongata (ME), the striatum (S) and the thalamus (T). The mean ± SEM of CBV and CBF for the respective groups of mice were compared as shown in Fig. 1. To determine maps of CBV and CBF over the cerebral cortex, we solved Laplace's equation [10,11] 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; both the CBV and the CBF values were averaged over these field lines for the entire cerebral cortex to generate maps, the group average of which is shown in Fig.2.

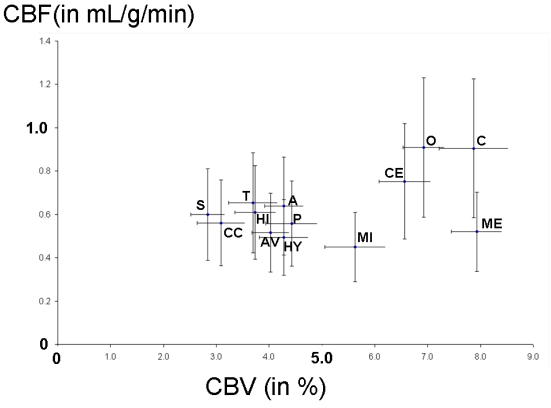

Fig.1: Regional analysis indicated that CBV and CBF were significantly correlated (r=0.5, p~0.05) for 13 brain regions

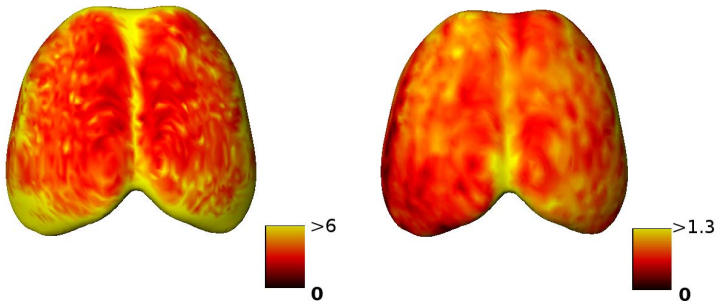

Fig.2: Group average cortical maps of CBV (left) in percentage units and CBF (right) in units of mL/g/min.

Results and Discussion: The CBV and CBF were found to be significantly correlated (r=0.5, p~0.05) over the 13 brain regions examined. Similar analysis over the group average cortical maps yielded no significant correlation. The network of major vessels running along the surface of the cortex, which are reflected more in the CBV maps than in the CBF maps, may be in part responsible for the lack of correlation. This method to map out both CBF and CBV could be useful for characterizing regional differences in vascularity in mouse models of cerebrovascular disease.

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