Quantitative cerebral and retinal blood flow using continuous arterial spin labeling MRI and fluorescent microsphere

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INTRODUCTION Arterial spin labeling (ASL) MRI is widely used to measure blood flow (BF) because it is non-invasive, provides value in classical unit of milliliter per gram tissue per minutes, and makes repeated measurements every few seconds. High-resolution ASL MRI has been extended to image BF of the rat brain and retina [1,2]. High resolution BF MRI could provide invaluable information for preclinical and clinical studies of brain and eye diseases. However, BF MRI quantitation remains to be validated. The goal of this study was to use an established microsphere technique to cross-validate ASL MRI BF measurement in the rat brain and retina. By using a mixture of the two different sized fluorescent microspheres with two different colors, the cerebral, retinal and choroidal BF can be measured simultaneously in the same subject.

METHODS Adult male Sprague Dawley rats (n = 41, 250–300 g) were anesthetized with 1-1.2% isoflurane, ventilated, and paralyzed. The respiratory rate was set between 57-60 stroke/min. Body temperature, end-tidal CO_2 and arterial pCO_2 were maintained within normal physiological ranges. MRI cerebral BF measurement (n=14) was performed on a 7T magnet and ocular BF measurement (n=6) was performed on an 11.7T magnet (Bruker, Billerica, MA). Quantitative BF MRI was acquired using continuous ASL technique in the brain and retina as described elsewhere [2,3].

Microsphere BF measurement employed a mixture of 2.5 million 8 μm green and 0.5 million 10 μm blue or red fluorescent microspheres. The microsphere technique of the eye was similar to that described previously [3,4]. Microsphere cerebral BF was measured in a subgroup of rats (n=6). The brains were freshly removed at the end of the microsphere experiments and preserved in 5% formalin. To minimize ice crystals from forming during rapid freezing process, brain tissue was transferred to 20% sucrose in phosphate buffered saline (PBS), and then transferred to 30% sucrose in PBS. Brain tissue was allowed to be fully impregnated with sucrose in each step before rapid freezing. Brains were then frozen in nitrogen cooled isopentane and sliced at 20μ with cryostat. Two adjacent 20 μm slices were collected for 3 slice locations corresponding to MRI data. Locations of slices were selected using rat brain atlas as a reference. Brain slices were sealed with fluorescence mounting medium and cover slipped. Microspheres were counted on fluorescent pictures using imageJ software. BF was converted to ml/g/min using a brain wet weight of 1.993±0.11 g and brain volume of 1.849±0.06 ml measured from three animals with matched age and body weight. The volume of each brain regions was defined by the area of ROIs on the atlas in a unit of mm^2 multiplying the slice thickness (0.04 mm). Statistical analysis used paired t-tests or ANOVA with Bonferroni posthoc test. A probability value was set at $p<0.05$. All data in text are expressed as mean ± SD.

RESULT & DISCUSSION In 18 rats, invasive arterial pCO2, pH, and MABP were measured and these parameters were 38.5±3.5 mmHg, 7.46±0.04, and 101±12 mmHg, respectively. **Figure 1a** shows basal ocular BF map at 44x44 μm in plane resolution by MRI. The averaged BF values in the retina and the choroid were 1.18±0.57 and 8.14±1.8 ml/g/min, respectively (n=6). The retina and the choroid BF values were significantly different from each other (p=0.00008). The ratio of MRI retinal to choroidal BF (μl/g/min) was 1:6.9. **Figure 1b** shows representative fluorescent images of the retina and choroid. Green (8 μm) microspheres were counted in the retina and blue (10 μm) microspheres were counted in the choroid. The retinal and choroidal BF values were 9.12±2.8 and 73.38±45.2 μl/min per tissue, respectively (n=18). The retina and the choroid BF values were significantly different from each other (p=0.0008). The ratio of microsphere retinal to choroidal BF (μl/min) was 1:10.8. Using the average wet weight of the rat retina reported previously (7.5 mg) [5-7], the retinal BF by microspheres was 1.22±0.36 ml/g/min (n=18), which is not significantly different from retinal BF measured by MRI (p=0.85). The choroidal BF cannot be converted into ml/g/min unit because the wet weight of the choroid is unknown. Cerebral BF maps at 200x200 μm in plane resolution by MRI are shown in **Figure 2a**. Total microsphere distributions (n=6) in the brain are shown in **Figure 2b**. The ROIs used for BF analysis are shown in **Figure 2c**. Group-averaged BF values by MRI and microsphere are compared in **Figure 2d**. BF in the cerebral cortex was 0.91±0.29 by MRI and was not significantly different from that of the 10 μm (1.09±0.37, p=0.99) and the 8 μm (1.38±0.44, p=0.09) microsphere data. BF in the striatum by MRI was not significantly different from that of the 10 μm microsphere data (p=0.55), but significantly lower than 8 μm microsphere data (p=0.001). This may be due to different vessel size between cortical and subcortical structures and the microsphere size was not optimized. The present study demonstrated that basal BF values measured by MRI are in good accordance with the microsphere technique in the rat retina and cerebral cortex.

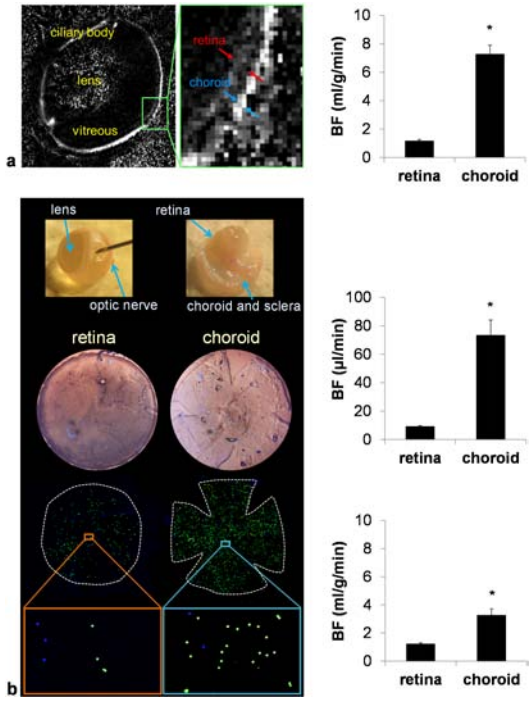

Figure 1. Quantitative basal retinal and choroidal blood flow by high-resolution MRI and fluorescent microspheres. (**a**) A typical ocular blood flow map and group-averaged blood flow values (n=6). Images were acquired at 44x44 μm and scaled from 0–7 ml/g/min. (**b**) A representative fluorescent microsphere images of the retina and choroid and group-averaged blood flow values by microsphere in two different units (n=18). *p<0.05. Error bars are S.E.M.

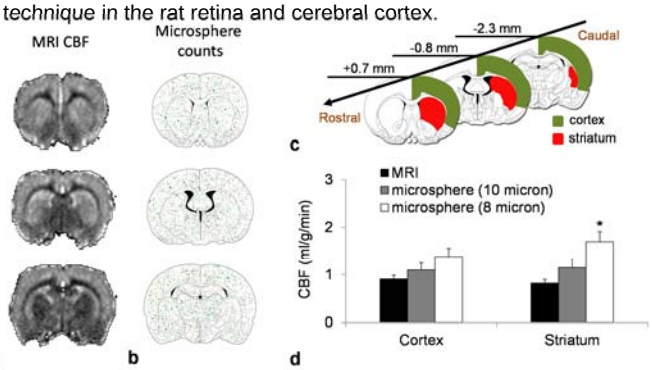

Figure 2. Quantitative basal cerebral blood flow measurement by high-resolution MRI and fluorescent microspheres. (**a**) A representative cerebral blood flow map acquired at 200x200 μm resolution. Images were scaled from 0–2 ml/g/min. (**b**) Distribution of total fluorescent microspheres (n=6). Green dots represent 8 μm and red dots represent 10 μm microspheres. (**c**) Region of interest on the brain atlas. (**d**) Quantitative cerebral blood flow measured by MRI (n=14) and microsphere (n=6). *p<0.05, significantly different from MRI data. Error bars are S.E.M.

REFERENCE *[1] Zhang et al., MRM, 1995, 33:370. [2] Li et al., Exp Eye Res, 2012, 101:90. [3] Shih et al., JCBFM, 2011, 31:832. [4] Wang et al., Exp Eye Res 2007, 84:108. [5] Tilton et al., IOVS, 1988, 26:861. [6] Tilton et al., IOVS, 1999, 40:689. [7] Winkler et al., IOVS, 1997, 38:62.*