INTRODUCTION

Deficits or abnormalities in cerebral metabolism and perfusion have been linked to many brain disorders and diseases. Direct assessment of the cerebral metabolic rate of oxygen (CMRO₂) and perfusion (CBF) can provide invaluable information and biomarkers for diagnosis and treatment of the diseases. An in vivo ¹⁷O MRS imaging (MRSI) approach at high/ultrahigh fields has been developed recently for non-invasive mapping of CMRO₂ and CBF in animals such as rats and/or cats [1-4]. Mouse models of the brain diseases have played a crucial role in preclinical research. Imaging CMRO₂ in the small mouse brain is challenging for the ¹⁷O-MR based CMRO₂ imaging technique. In this study, we explored the feasibility of mapping brain CMRO₂ of mice undergoing a middle cerebral artery (MCA) occlusion using the ¹⁷O-MRS imaging approach at 11.7T. Our results suggest that imaging the rates of the oxygen metabolism and the blood flow in the tiny mouse brain is feasible; offering a new utility using the high field ¹⁷O-MRS imaging technique to study the oxygen metabolism in various brain diseases in a non-invasive way.

METHOD

Four male C57BL/6 mice (18-30g) with 60-min right middle cerebral artery (MCA) occlusions prepared 5-7 days prior to MR scanning were used in this study. Mice were anesthetized with ketamine (87 mg/kg) / xylazine (13 mg/kg) cocktail solution via intraperitoneal injection followed by constant subcutaneous infusion at 2.6 mL/kg/hr of the same cocktail during the MR scanning. The heads of the mice were immobilized with tooth and ear bars. Spontaneous respiration and body temperature of the animal were monitored throughout the MR experiment; warm water and hot air were used to maintain the body temperatures of the mice at ~37°C. ¹⁷O₂ (60% ¹⁷O enrichment) gas was supplied 2-3 minutes to each mouse for imaging both CMRO₂ and blood perfusion. All experimental procedures and protocol were conducted under the guidelines of the National Institutes of Health and the Institutional Animal Care and Use Committee of the Washington University.

The ¹⁷O MRS/MRSI data and ¹⁷H anatomic brain images were acquired on 11.7T/26-cm clear bore horizontal animal magnet (Magnex Scientific, UK) interfaced with Varian INOVA console (Varian Inc., Palo Alto, CA). An RF coil probe consisting of a single loop ¹⁷O surface coil (diameter ~12 mm) and a larger quadrature ¹⁷H coil was built for the ¹⁷O-MRS and ¹⁷H MR imaging of the mouse brain. T₂-weighted fast spin echo and/or proton density gradient echo sequences were used for acquiring the ¹⁷H MR images. Three-D ¹⁷O-MRS imaging data were acquired using the Fourier Series Window (FSW) technique [5] with the following acquisition parameters: 10 ms TR, 50-μs hard pulse for a nominal 90° excitation, spectral width=30 KHz; 2×2×2 cm³FOV; 9×9×5 phase encodes; 0.04ml (or 15μl nominal) voxel size; and ~11-s temporal resolution (for one 3D MRSI dataset). A 17x17x9 matrix of FIDs was generated from the original 9x9x5 phase encode data for each 3D ¹⁷O image. The linear fitting model, which has been established in the rat study [6], was applied to quantify the absolute CMRO₂ values in these mice. The measured metabolic H₂¹⁷O washout time course after the cessation of ¹⁷O₂ inhalation was regressed to an exponential decay function. The fitted decay rate was defined as constant k, proven to be proportional to the CBF value [4].

RESULTS and CONCLUSION

Figure 1 displays multi-slice T₂-weighted ¹⁷H MRI and the corresponding 3D-CSI images of the natural abundance H₂¹⁷O in a representative stroke mouse brain. The acquisition time was 8 min and 11 s for ¹⁷H anatomical and 3D ¹⁷O images respectively. The anatomical brain structure and the lesion area due to the MCA occlusion at the right side of the brain are evident from the ¹⁷H images. The spatial distribution of the ¹⁷O-MR signals of the natural abundance H₂¹⁷O water in the mouse brain is clearly shown from the 3D ¹⁷O chemical shift images. The improved ¹⁷O-MR sensitivity at 11.7T readily transformed into the excellent spatial and temporal resolution of the 3D CMRO₂ and/or CBF imaging in this study.

Figure 2 illustrates an expanded region of interest in a representative mouse brain with a strong B₂ field of the ¹⁷O coil covering both ischemic and normal sides of the brain. Three voxels located in the ischemic (Voxel 1), middle (Voxel 2) and control (Voxel 3; contralateral homologous region) regions of the brain are identified with red, black and blue colored circles in the T₂-weighted ¹⁷H image (Fig 2A, the corresponding CMRO₂ (Fig. 2B), and the washout decay rate constant (the putative perfusion measure; Fig. 2C) images. The time course of the H₂¹⁷O water signal intensity in these three voxels before, during (shaded region), and after a 2.5 min inhalation of ¹⁷O₂ gas are displayed in Fig. 2D. Close examination of the time courses revealed that (1) the rate of metabolically generated H₂¹⁷O water contents during the ¹⁷O₂ gas inhalation was much slower in Voxel 1, and (2) the exponential decay rate of the ¹⁷O-labeled metabolic water washout after cessation of the ¹⁷O₂ inhalation in Voxel 1 was also much slower than that of the contralateral control Voxel 3. The results suggest a clear deficiency in oxygen metabolism and impaired tissue perfusion in the ischemic infarction. The quantitative analysis suggested an approximately 25% decrease in CMRO₂ in the lesion voxel compared to the control (Fig 2B) and approximately a 60% decrease in tissue perfusion (Fig. 2C). Voxel 2 located between Voxels 1 and 3 in the brain region without obvious ischemic lesion judged by the ¹⁷H image. Interestingly, a minimally impaired CMRO₂ with a more significant perfusion deficit was observed (Fig. 2B and C). In other mouse brains with more severe ischemia, we observed not only the alteration in the tissue oxygen metabolism and perfusion, but also the onset time of the metabolic H₂¹⁷O water production and/or washout processes lagged significantly due to the severe shortage of the blood supply.

In conclusion, this preliminary ¹⁷O-MRS imaging study of stroke mice demonstrated the feasibility of 3D CMRO₂ and perfusion mapping in the mouse brain. It presents an exciting opportunity for assessing physiological measures of oxygen metabolism and their alterations when diseases are induced through the use of mouse models. In addition, the rapid and noninvasive nature of the 3D ¹⁷O-MR based CMRO₂ imaging approach at high fields will allow longitudinal study following the progression of the disease and recovery after treatment. Therefore, the new utility of the ¹⁷O-MR technique will provide a great tool for understanding the mechanisms of brain diseases, and for advancing the biomedical research and application.

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REFERENCES