Exploring the New Utility of the ^{17}O-MRS Imaging Technique for Studying $CMRO_2$ and Perfusion in Stroke Mice

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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 ($CMRO_2$) and perfusion (CBF) can provide invaluable information and biomarkers for diagnosis and treatment of the diseases. An *in vivo* ^{17}O MRS imaging (MRSI) approach at high/ultrahigh fields has been developed recently for non-invasive mapping of $CMRO_2$ 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 $CMRO_2$ in the small mouse brain is challenging for the ^{17}O-MR based $CMRO_2$ imaging technique. In this study, we explored the feasibility of mapping brain $CMRO_2$ of mice undergoing a middle cerebral artery (MCA) occlusion using the ^{17}O-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 ^{17}O-MRS imaging technique to study the oxygen metabolism in various brain diseases in a non- invasive way.

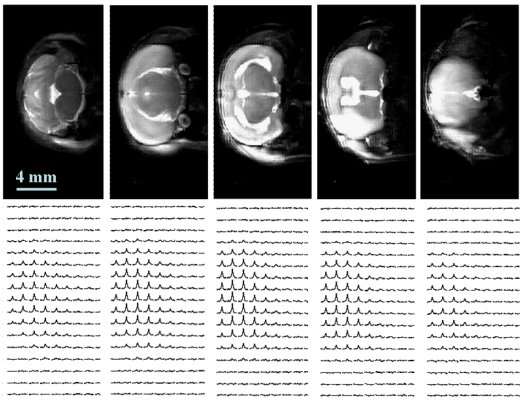

Fig. 1 The multi-slice T_2-weighted ^{1}H MRI obtained from a representative stroke mouse brain (top); and the corresponding natural abundance $H_2{}^{17}O$ images with 3D-CSI acquisition (bottom). The acquisition time for the ^{1}H anatomic images and the 3D ^{17}O chemical shift images are 8 minutes and 11 seconds, respectively.

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 itroperitoneal 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. $^{17}O_2$ (60% ^{17}O enrichment) gas was supplied 2-3 minutes to each mouse for imaging both $CMRO_2$ 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 ^{17}O MRS/MRSI data and ^{1}H 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 ^{17}O surface coil (diameter ~12 mm) and a larger quadrature ^{1}H coil was built for the ^{17}O-MRS and ^{1}H MR imaging of the mouse brain. T_2-weighted fast spin echo and/or proton density gradient echo sequences were used for acquiring the ^{1}H MR images. Three-D ^{17}O-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 cm^3 FOV; 9×9×5 phase encodes; 0.04ml (or 15μl nominal) voxel size; and ~11-s temporal resolution (for one 3D MRSI dataset). A 17×17×9 matrix of FIDs was generated from the original 9×9×5 phase encode data for each 3D ^{17}O image. The linear fitting model, which has been established in the rat study [6], was applied to quantify the absolute $CMRO_2$ values in these mice. The measured metabolic $H_2{}^{17}O$ washout time course after the cessation of $^{17}O_2$ 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 T_2-weighted ^{1}H MRI and the corresponding 3D-CSI images of the natural abundance $H_2{}^{17}O$ in a representative stroke mouse brain. The acquisition time was 8 min and 11 s for ^{1}H anatomical and 3D ^{17}O images respectively. The anatomic brain structure and the lesion area due to the MCA occlusion at the right side of the brain are evident from the ^{1}H images. The spatial distribution of the ^{17}O-MR signals of the natural abundance $H_2{}^{17}O$ water in the mouse brain is clearly shown from the 3D ^{17}O chemical shift images. The improved ^{17}O-MR sensitivity at 11.7T readily transformed into the excellent spatial and temporal resolution of the 3D $CMRO_2$ and/or CBF imaging in this study.

Figure 2 illustrates an expanded region of interest in a representative mouse brain with a strong B_1 field of the ^{17}O 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 T_2-weighted ^{1}H (Fig. 2A), the corresponding $CMRO_2$ (Fig. 2B), and the washout decay rate constant (the putative perfusion measure; Fig. 2C) images. The time course of the $H_2{}^{17}O$ water signal intensity in these three voxels before, during (shaded region), and after a 2.5-min inhalation of $^{17}O_2$ gas are displayed in Fig. 2D. Close examination of the time courses revealed that (1) the rate of metabolically generated $H_2{}^{17}O$ water contents during the $^{17}O_2$ gas inhalation was much slower in Voxel 1, and (2) the exponential decay rate of the ^{17}O-labled metabolic water washout after cessation of the $^{17}O_2$ 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 $CMRO_2$ 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 ^{1}H image. Interestingly, a minimally impaired $CMRO_2$ 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 times of the metabolic $H_2{}^{17}O$ water production and/or washout processes lagged significantly due to the severe shortage of the blood supply.

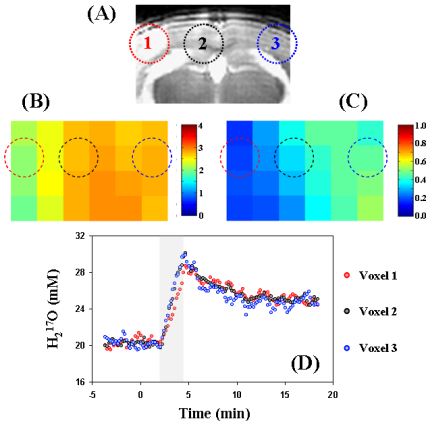

Fig. 2 (A) Anatomic brain Image of a stroke mouse showing three circular voxels located in the ischemic (voxel 1), middle (voxel 2) and control (voxel 3) areas; (B) $CMRO_2$ map and (C) perfusion map of the same brain region; (D) Time courses of the voxels 1-3 showing the dynamic change of the $H_2{}^{17}O$ water concentrations before, during and after 2.5 min $^{17}O_2$ inhalation (gray bar). The color bars in (B-C) display the scale of the $CMRO_2$ (μmol/g/min) and the $H_2{}^{17}O$ decay rate k (min^{-1}) maps, respectively.

In conclusion, this preliminary ^{17}O-MRS imaging study of stroke mice demonstrated the feasibility of 3D $CMRO_2$ and perfusion mapping in the mouse brain. It presents an exciting opportunity for assessing physiological measures of oxygen metabolism and perfusion and their alterations when affected by diseases through the use of mouse models. In addition, the rapid and noninvasive nature of the 3D ^{17}O-MR based $CMRO_2$ 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 ^{17}O-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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