Identification of 4-vessel occlusion in rat using MR angiography and ^{1}H MRS at 14.1T

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TARGET AUDIENCE: Whoever is interested in studying metabolic evolution in stroke models using non-invasive MR techniques

PURPOSE: Studying metabolic evolution of ischemic cortex remained challenging in filament induced focal middle cerebral artery occlusion due to incomplete vessel occlusion. It's been shown that a 4-vessel occlusion (both vertebral arteries and both common carotid arteries, 4VO) delivers bilateral hemispheric ischemia (1) only when excluding the potential incomplete occlusion of vertebral artery (VAs) using the laser Doppler. Alternatively, MR angiography (MRA) has been shown of capable of identifying ischemic attack (2,3). On top of that, we've shown that localized ^{1}H MRS can be applied on murine stroke model to deliver non-invasive diagnostic biomarkers for identifying ischemia subtypes, such as transient ischemic attack, minor stroke, moderate stroke and permanent ischemia (4). Thus, the aim of this study was to evaluate whether both MR angiography and localized ^{1}H MRS could be applied for diagnosis purpose on rat after global ischemia induced by 4VO at 14.1T.

METHODS All studies were approved by the local animal care and use committee. We applied one-stage anterior approach (1) to achieve 4VO in adult male Wistar rats (300-350g). In brief, two rats were anesthetized with 4% isoflurane mixed with air and oxygen (50:50) and were placed in the supine position. Surgical preparation was performed under 2.5% isoflurane anesthesia. A 2.5 cm midline skin incision was made on the neck with the aid of a pair of surgical magnifying glasses. Subcutaneous connective tissue and muscles were gently retracted to expose the cervical vertebral bodies. Both VAs were carefully occluded between cervical diapophysis C2 and C3 using a cauterizer. Common carotid arteries (CCAs) were isolated and encircled loosely with 3-0 silk surgical sutures. Once CCAs were ligated, 4VO can be achieved. Immediately, animal heads were carefully fixed using a bite piece and two ear bars to avoid motion, placed in prone position and transferred in a horizontal 14.1T magnet. Once the field inhomogeneity was adjusted, 3D gradient echo sagittal images (TE/TR=1.5/6ms, 45×35×35mm^3, 160×96×96, SW=100kHz, nt=8) were acquired to cover the entire head and some of the neck. Flip angles were set to 90° to enhance signal to noise ratio for MR angiography (maximum intensity projection (MIP), generated by vnmrj, Agilent Inc, USA) due to the use of a home-built quadrature surface coil (two 16-mm-inner-diameter geometry decoupled loops). The surface coil was placed on the top of cortex and used for localized ^{1}H MRS. Localized ^{1}H MR spectra (TE/TR=2.8/4000ms, nt=160) were acquired within 1hr after occlusion using SPECIAL, as previously applied on murine models (4). Bilateral cortical tissue (45-55µl) was defined based on anatomical images (FSE/GRE). ^{1}H MR spectra were quantified using LCMODEL referencing the endogenous water signal from the identical voxel (80% water contents in cortex). In order to illustrate reliability in identifying successful 4VO, three rats were operated as in the 4VO model, but only one VA was occluded (3VO).

RESULTS AND DISCUSSION

Using the surface coil, the 3D GRE images generated MIP images with an excellent quality (Figure 1). The MIP images illustrated the typical vascular structure of rat head, i.e. CCAs, VAs, BA and the circle of willies etc (Figure 1). After 4VO, the MIP images showed that nearly all signals originated from major vessels, including CCAs, VAs, and BA etc, were significantly remained. Alternatively, some signal intensities were remained in VAs, BA and towards the circle of willies after 3VO. Since the signal intensities of BA were remained visually observed after 3VO, CNR was evaluated between a reference tube containing 10mM $MnCl_2$ (SNR=556±17) and the BA. The preliminary CNR results were 277±21 in control, 432±8 in 3VO and 478±4 in 4VO. The acquired MR spectrum of cortex showed a strong increase in lactate (Lac) and γ-amino-butyrate (GABA) after 4VO (Figure 2C). Quantification of the ^{1}H MRS data revealed additional metabolic changes i.e. increase in glycine and creatine (Cr) and decrease in glucose (Glc) and phosphate creatine (PCr) etc (Figure 3). However, ^{1}H MR spectra remained mostly unaltered after 3VO (Figure 2D) when compared to control (Figure 2B & Figure 3). Indeed, MRA techniques illustrated that the vascular signal intensities globally decreased significantly during both 4VO and 3VO, but a complete reduction was reached in 4VO only. Thus, the incomplete occlusion, i.e. 3VO, would be sufficient in maintaining normal cortical metabolic profiles and possibly attenuate ischemic attack in cortex. ^{1}H MRS data suggested modification in cortical tissue occurs only in case of permanent global ischemia. Similar results were obtained previously in ipsilateral striatum (4). We also noticed that some metabolites changes were slightly different from murine studies (4), e.g. myo-inositol (Ins) and taurine (Tau), which might due to different brain regions and species, and remained to be studied. Nonetheless, the striking increase of Cr and GABA and the decrease of PCr were observed in rat cortex after 4VO.

Therefore, we conclude that a complete 4VO can be visualized by MRA and metabolic changes in cortex can be successfully assessed by ^{1}H MRS after brain stroke.

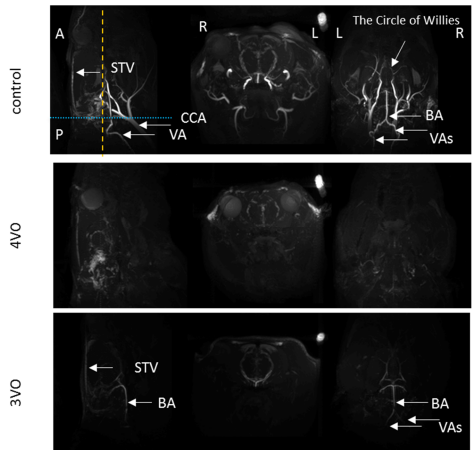

Figure 1 Representative MIPs in sagittal (at the midline of brain), coronal (the blue dotted line) and axial (the orange dashed line) planes of one control rat (top row), one after 4VO (2nd row) and one after 3VO (bottom row). BA, Basilar artery; VA, vertebral artery; CCA, common carotid artery; STV, superficial temporal vein. Orientation: A, anterior; P, posterior; R, animal right; L, animal left. The circle of willies (BA towards to the anterior of the head) can be visualized in both control and after 3VO. The bright spot at the animal left side was a reference tube containing 10mM $MnCl_2$.which was attached to the side of the surface coil and with a final position at the animal left side.

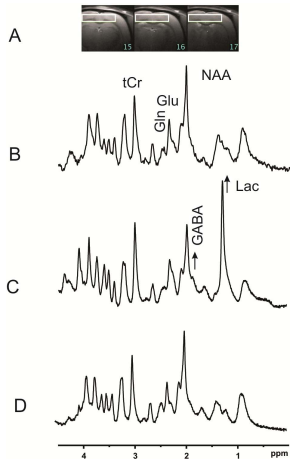

Figure 2 Typical localized ^{1}H MRS of rat cortex (white squares in FSE images, A) from one control (B), during 4VO (C) and during 3VO (D) at 14.1T. GABA, γ-amino butyrate; tCr, total creatine; Glu, glutamate; NAA, N-acetyl-aspartate; Lac, lactate. Arrows indicated visually observed spectrum changes when comparing to that was acquired before occlusion.

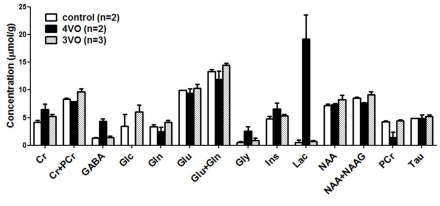

Figure 3 Summary of selected metabolites quantified from localized ^{1}H MRS of rat cortex from control (white), 4VO (black) and 3VO (gray). NAAG, N-acetyl-aspartate-glutamate; Gly, glycine.

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