In vivo Hydrogen-1, Sodium-23, Phosphorus-31, and Potassium-39 Magnetic Resonance Imaging after Middle Cerebral Artery Occlusion

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INTRODUCTION: ^{23}Na-MRI has only recently been established for investigating stroke in pre-clinical stroke models [1-3] and stroke patients [4,5]. However, to fully understand the pathological transition of still-viable underperfused tissue to permanently-damaged tissue recording of additional parameters such as the changes of local Phosphorus-31 (^{31}P) and Potassium-39 (^{39}K) content may prove worthwhile since (i) ^{31}P relates directly to the cell's energy metabolism and (ii) the ^{39}K ion concentration is much higher in the intracellular space. Thus, both the ^{39}K and the ^{31}P signal could provide important information about the maintenance of transcellular ion gradients and Na/K ATPase activity. The aim of this project was to develop single-tuned X-nuclei resonators for ^{23}Na, ^{31}P, and ^{39}K MRI of the living rat brain in order to explore the metabolic and ion concentration changes within ischaemic stroke tissue following middle cerebral artery occlusion (MCAO).

METHODS: All experiments were carried out on a 9.4T MRI system (BioSpec, Bruker Biospin GmbH, Germany). For rat brain MRI a linear volume resonator was used in conjunction with either a commercial receive-only four-element ^{1}H array, or one of the home-built transceiver X-nuclei (^{23}Na, ^{31}P, or ^{39}K) surface resonators. The X-nuclei resonators were inductively-coupled two-winding surface coils (i.d.: 20 and 30 mm) with variable tuning facilities. The orthogonal arrangement of the surface and volume resonator's B_1-field (as depicted in Figure 1) allowed for interleaved ^{1}H ADC and X-nuclei measurements without the need to exchange coil systems during the experiment. All *in vivo* experiments were carried out under appropriate animal license according to the institutional guidelines and state laws. Focal ischaemia was induced by left MCAO in male Sprague Dawley rats (bodyweights ~300g, n = 3) using the intraluminal thread model. Two permanent and one transient MCAO experiments were performed, which are furthermore named as MCAO 1 to 3. MCAO 1 represents a transient occlusion experiment (120 min of MCAO followed by 22 h of reperfusion), while MCAO 2 and 3 represent permanent occlusion experiments with the MRI measurements performed during the early phase (appr. 4 h; MCAO2) or during the late phase (48 h after MCAO). Heart rate, body temperature, and respiration were monitored and maintained within normal limits. ^{23}Na images were acquired using a 3D Fast Low Angle Shot (FLASH) sequence, with: TR/TE=21/3.2ms, 10% partial echo acquisition, BW=4kHz, voxel resolution (after two-fold 3D zerofilling) = (0.5x0.5x2)mm^3, 5 minute acquisition time. ^{1}H DWI images were acquired using an EPI sequence with TR/TE=5000/20.2ms, 3 orthogonal directions; b-values=100 and 1000s/mm², (0.2x0.2)mm^2 in-plane resolution, 1mm slice thickness, 15 slices, and 4 segments. ^{1}H T_2-weighted images were recorded with Rapid Acquisition with Relaxation Enhancement (RARE) sequence: TR/TE=2500/30ms, RARE factor 4, BW=19kHz, TA=2min10s, 2 averages, FOV=(40x40)mm², in-plane resolution=(0.156x0.156)mm², 15slices, 2 mm inter-slice distance. A 2D-Chemical Shift Imaging (CSI) sequence was used for ^{31}P-MRI with TA=34min8s, TR=2000, 6 averages, 200 FID samples, in-plane resolution=(4x4)mm², 4 slices, and 4cm slice thickness. A 3D-CSI sequence was used for ^{39}K-MRI with a weighted k-space sampling scheme, TA=24min10s, TR=11ms, 3 averages, voxel resolution=(4x4x4)mm³, 45 FID samples, and 8 slices. The CSI data was reconstructed offline using a routine written in MATLAB. Regions of interest (ROI) were manually drawn in ischaemic and normal tissue in the ^{1}H, ^{23}Na,^{39}K, and ^{31}P images. The signal within the ischaemic tissue was subsequently computed as a percentage signal of contralateral tissue.

RESULTS: The ^{31}P NMR spectrum in normal and ischaemic tissue is presented in Figure 2 together with a ^{31}P image corresponding to the phosphor-di-ester (PDE) peak at -5ppm relative to the phosphocreatinine (PCr). Furthermore, the PDE image was superimposed with a ^{1}H edge image. An Apparent Diffusion Coefficient (ADC) map is shown right beside the PDE image indicating the extent of ischaemic hemispheric damage. The ATP and PCr peaks were clearly reduced in stroke tissue while the integral of the PDE peak was slightly higher than in normal tissue. During the acute stroke phase (~4hrs after MCAO) the ADC was decreased from an early time point in subcortical tissue, while ^{1}H T_2 appeard normal. The ^{23}Na signal was slightly increased (to 156%) and the ^{39}K was slightly decreased (to 81%) relative to the non-ischaemic hemisphere. During the chronic phase (MCAO 3) the T_2 was high (at 180%) as measured by ^{1}H MRI, while the ^{23}Na was high (at 250%) and the ^{39}K was low (at 49%). Furthermore, the area of significantly-increased ^{23}Na signal (i.e. compared to contralateral) closely matched the area of infarcted tissue in each rat.

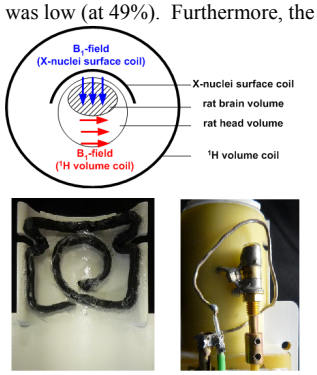

Figure 1: arrangement of X-nuclei surface resonators with ^{1}H volume resonator and below two photographs of the surface resonator (left) and the variable inductive coupling system with variable tuning trimmer (right.

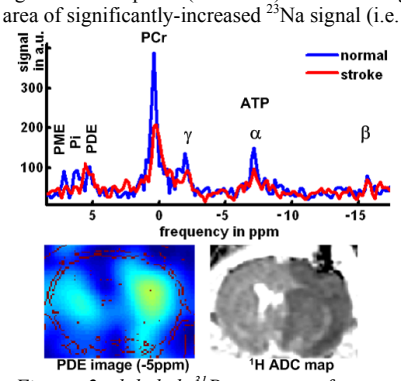

Figure 2: labeled ^{31}P spectrum for one representative rat at ~24hrs after MCAO and below the phosphor-di-ester (PDE) image (left) and the corresponding ADC map (right) of the same rat. Note the decrease in ATP, PCr, inorganic phosphate (Pi), and phspho-mono-ester (PME) peaks, while the integrated PDE peak gains higher signal in ischaemic tissue.

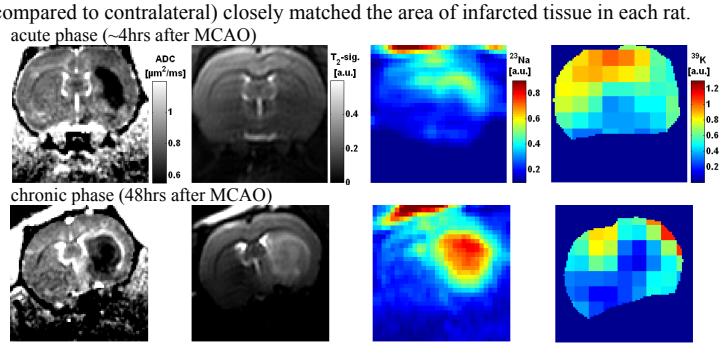

Figure 3: ADC maps, T_2-weighted ^{1}H-images as well as ^{23}Na- and 39K-images for two different rats which were scanned during the acute and chronic phases. Note the slight increase in ^{23}Na and slight decrease in 39K signal within the area of restricted ADC (below 0.55µm²/ms), while the T_2-weighted signal appears normal during the acute phase. High T_2-weighted signal as well as much higher ^{23}Na and much lower 39K signal was measured in the region of low ADC during the chronic phase.

DISCUSSION: A decrease of the energy rich phosphorus compounds, i.e. PCr and ATP as well as an increase of the PDE compounds have been reported previously [6]. These changes probably reflect on the decreased energy metabolism and the accompanying cell membrane decomposition. During the acute phase ^{23}Na was slightly increased, and ^{39}K was slightly decreased in tissue with low ADC, while the T_2 was normal during this phase. During the chronic phase the T_2 was high, and as expected a strong increase in ^{23}Na and strong decrease in ^{39}K was successfully measured non-invasively. These alterations are in good agreement with results obtained from invasive measurement methods [7]. Our results suggest that the multi-parametric recording of ^{23}Na, ^{39}K, and ^{31}P changes using MRI methodology may be a worthwhile approach for studying the time course of ischaemic cell death non-invasively in individual animals. These results will help to better understand the pathophysiology of ischaemic brain damage and allow for the development of new therapeutic concepts.

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