MRM of Perfused Alzheimer's Mice Hippocampal Slices

P. Tek[1], S. F. Othman[1], O. M. Abdullah[1], O. Lazarov[2], C. Fall[2], and R. L. Magin[1]

[1]Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, United States, [2]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL, United States

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by severe memory loss and cognitive decline. Care and clinical cost is estimated to be over $100 billion a year and it is predicted that by 2050, AD will affect more than 15 million Americans (1). The hallmarks of the disease are neurofibrillary tangles and β-amyloid deposits that accompany massive neuronal cell death in specific brain areas. Changes in the brain may start more than 20 years before the diagnosis of early AD stages. However, there are no diagnostic tools or biomedical markers available to detect such early changes in the brain. Early prediction of future neuropathology would be invaluable in terms of our ability to provide a strategic plan, treat or even prevent a devastating future outcome. The use of mice or rat brain slices has been exploited by neuroscientists for many reasons; which include the ability to manipulate the extracellular environment, the ability to focus on specific regions of the brain, the elimination of the blood-brain barrier, and the ability to retain the *in situ* functionality. With the introduction of a MR compatible perfusion chamber, Blackband and Shepard were able to report diffusion coefficients in *live* rat hippocampal brain slices (2). With the advent of genetic engineering, transgenesis has made it possible to create animal models for these diseases. For instance, a disease like Alzheimer's can be mimicked by creating mice that express specific proteins. The familial, early form of the disease (FAD) is caused by mutations in amyloid precursor protein (APP), presenilin-1 (PS1) and presenilin 2 (PS2). No previous investigation has been reported to compare MR parameters of live transgenic and nontransgenic brain slices placed in a perfusion chamber *in vitro*. In this study, we use transgenic animal model of AD to test the feasibility of examining the correlation between modulation of physical measures/tissue characteristics following expression of FAD-linked mutant proteins with MRI parameters such as spin-spin relaxation times (T_2) and apparent diffusion coefficient (ADC).

METHODS

All mice used in this study were maintained according to protocols approved by the University of Illinois at Chicago. Hippocampii were isolated from 4-5 month old FAD-linked APPswe/PS1ΔE91 transgenic and age matched nontransgenic male mice using standard procedures (5). MR experiments were conducted using a 56-mm vertical bore (Oxford Instruments, Oxford, UK) 11.74 T (500 MHz for proton) magnet equipped with Bruker DRX Avance spectrometer controlled by a Silicon Graphics SGI 02 workstation (Mountain View, CA, USA). The transgenic and nontransgenic brain slices were loaded into a perfusion chamber that holds the tissue and allows for perfusion with oxygenated ACSF. The chamber was placed into a test tube that fits in a 10-mm diameter RF saddle coil and was inserted into the Bruker Micro5 imaging probe equipped with a tri-axial gradient set with a maximum strength of 200 G/cm. The setup is shown in Fig. 1. For quantitative analysis, the spin–spin relaxation time (T_2) and the apparent diffusion coefficient (ADC) were measured for a specific region of interest localized in the hippocampal section of each sample. T_2 was measured by applying a spin echo imaging pulse sequence to acquire 32 echoes with a 7 ms echo spacing (TE) from the chosen axial slice (TR = 4 s, TE = 7 ms FOV = 1 cm, slice thickness = 300 µm, matrix = 128 × 128, in-plane resolution = 78 µm x 78 µm, and NEX = 1). The ADC was measured using a standard diffusion-weighted imaging (DWI) spin echo sequence. The diffusion gradient was applied along the read direction by linearly varying its strength in 16-steps giving a maximum b-value of 4854 s/mm^2 (TR = 1 s, TE = 30 ms, FOV = 1 cm, slice thickness = 300 µm, δ = 3 ms, Δ = 18 ms, matrix = 128 × 128, in-plane resolution = 78 µm x 78 µm, and NEX = 1).

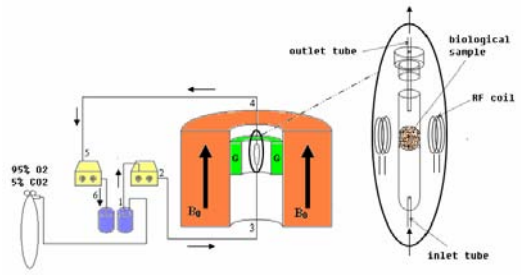

Fig. 1 The schematic drawing of the customized perfusion system. Starting at point 1, oxygenated culture medium is pumped into the peristaltic system through one pump. At point 2, the medium is being pumped out of the peristaltic system towards the magnet. Point 3 indicates the location where medium enters the magnet. After the medium is pumped into the test tube, it is pumped out of the magnet at point 4. At point 5, the medium is pumped into another peristaltic system. At point 6, the medium is pumped into a waste compartment.

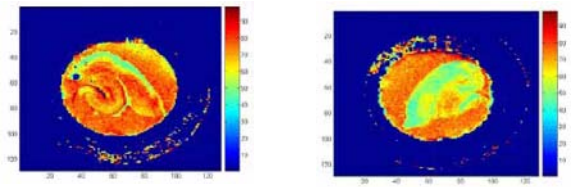

Fig. 2 T_2 maps (ms) of hippocampus excised from an age matched nontransgenic mouse (Left) and a FAD-linked APPswe/PS1ΔE91 transgenic mouse brain (Right).

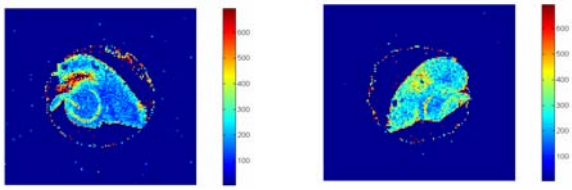

Fig. 3 ADC maps (µm^2/s) of hippocampus excised from an age matched nontransgenic mouse (Left) and. FAD-linked APPswe/PS1ΔE91 transgenic mouse brain (Right).

RESULTS

Fig.2 shows T_2 maps for both the transgenic mouse and the nontransgenic mouse. The T_2 value for 49 pixels ROI in the hippocampus is (56.8 ms) and (76.5 ms) for the transgenic and nontransgenic; respectively. Fig.3 shows ADC maps for the same ROI above, the average ADC values is (276 µm^2/s) and (168 µm^2/s) for the transgenic and nontransgenic mice, respectively. ADC was measured in the surrounding ACSF solution (prepared from deionized water) at 32±1 ºC to be (1900 µm^2/s).

DISCUSSION AND CONCLUSION

T_2 and ADC analysis was able to show differences in average values between nontransgenic and trangenic mice hippocampal slices. The T_2 values of the diseased tissue show an average decrease in value, which agrees with the decrease in average T_2 values for fixed transgenic and nontransgenic mice (3). This decrease in T_2 can be associated with the iron formation in the hippocampal region causing morphological changes. For future studies, it would be possible to observe changes in MR parameters for transgenic and nontransgenic living brain samples while manipulation the extracellular environment, such as neurostimulators [4], constrast agents, or other pharmacological molecules. In this study, the feasibility of observing differences in T_2 and ADC values in living transgenic and non-transgenic mice brain slices was illustrated

REFERENCES: [1] Brown. Environmental Health Perspective. 13(9):1250-1256 (2005). [2] Shepard T.M. Magnetic Resonance in Medicine 48:565–569 (2002). [3] Zhang. Magnetic Resonance in Medicine 51:452-457 (2004). [4] Bui .Neuroscience. 93(2):487-90 (1999)

ACKNOWLEDGEMENT: The authors would like to thank Mr. Michael Demars and Mr. Adam Beagley from the Department of Anatomy and Cell Biology at the University of Illinois at Chicago for the preparation of the samples.