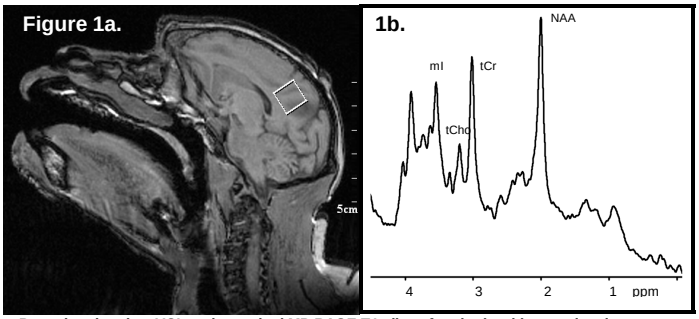
In vivo proton MRS changes in geriatric Rhesus monkey brain: Similarities to Human Alzheimer's Disease

R. BLENMAN-ABANGE[1], R. BAUMGARTNER[2], M. HOLAHAN[1], J. COOK[1], R. HARGREAVES[1], AND D. WILLIAMS[1]
[1]MERCK & CO INC., WEST POINT, PA, UNITED STATES, [2]MERCK & CO INC., RAHWAY, NJ, UNITED STATES

INTRODUCTION

Geriatric non-human primates develop behavioral and brain anomalies similar to those that occur in persons with Alzheimer's disease (1-2). Alzheimer's disease is characterized by deposits of neurofibrillary tangles and amyloid plaques, and loss of neuronal connectivity with reduced synaptic density in the mesiotemporal structures, parietal and frontal association cortex. All geriatric (>24 years of age) monkeys exhibit diffuse amyloid deposits, congophilic angiopathy, and neuronal degeneration (3-6). Unpublished histological data confirm that all monkeys >24 years of age from the in-house colony have amyloid deposits. These animals provide the unique opportunity to examine biochemical changes that occur with the deposition of amyloid and subsequent neuronal death. Since in vivo MRS has consistently revealed metabolic changes in AD brain, i.e. ↓ NAA (N-acetylaspartate) and ↑ mI (Myoinositol) (7-8), in vivo proton MRS was proposed in a study to [i] examine the feasibility of localized in vivo proton MRS in the brain of the Rhesus monkeys, and [ii] measure any differences in metabolites (implicated in AD) in young adult versus geriatric Rhesus monkeys.



Posterior cingulate VOI on the sagittal MP-RAGE T1 slice of geriatric with associated spectrum

Figure 2.

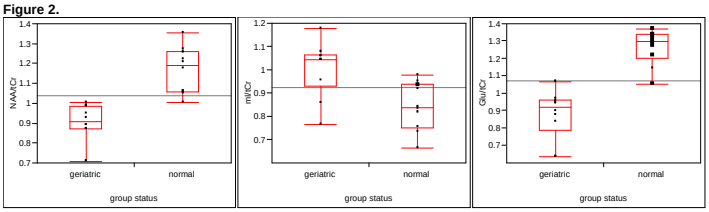


Table 1: Correlations between/among Rhesus monkey brain ratios and age

	NAA/tCr		mI/tCr		tCho/tCr		Glu/tCr	
	Correlation	(p-value)	Correlation	(p-value)	Correlation	(p-value)	Correlation	(p-value)
mI/tCr	-0.4969	(0.03)						
tCho/tCr	0.5682	(0.009)*	-0.2129	(0.3675)				
Glu/tCr	0.7883	(<0.0001)*	-0.4441	(0.05)	0.6271	(0.003)*		
Age	-0.8501	(<0.0001)*	0.5415	(0.01)*	-0.5607	(<0.01)*	-0.8518	(<0.0001)*

Table 2: Mean accuracy of the two classifiers used

Biomarkers (features)	LDA Mean (std)	SVM Mean (std)
NAA, mI, tCho	0.92 (0.04)	0.90 (0.06)
NAA, mI, tCho, Glu	0.94 (0.05)	**0.99 (0.02)**
NAA, mI, Glu	0.94 (0.03)	0.94 (0.02)
NAA	0.90 (0.04)	0.82 (0.09)
tCho	0.78 (0.03)	0.72 (0.08)
mI	0.75 (0.04)	0.72 (0.05)
Glu	0.80 (0.02)	0.94 (0.02)

MATERIALS & METHODS

All experiments were conducted on a Siemens Trio 3T scanner using a vendor-supplied ^{1}H extremity coil. To evaluate within-subject variability, three adult Rhesus monkeys were scanned repeatedly. The measured variability in the NAA/mI ratio (≈8%) was used to determine the sample size (n=7) required to reliably detect a 15% change in brain metabolites with 90% statistical power. In vivo water-suppressed MR spectra were then acquired for 10 geriatric (≥ 24 yrs) and 10 young adult (≤ 15 yrs) Rhesus monkeys per IACUC guidelines. The animals were anesthetized with 1.0-1.5% isoflurane and physiologically monitored for temperature, respiration and blood O_2 levels. After fast ^{1}H MR 3-plane localizers were obtained for ROI placement and shimming, all first- and second- order shim currents were adjusted on tissue water with an automated shim protocol. Linewidths of the H_2O resonance observed from the 1 cm^3 voxel were approximately 20Hz. Spectra were acquired from water suppressed PRESS voxels consistently placed posterior and superior to the splenium of the corpus callosum in the posterior cingulated (Figure1a). Pulse sequence parameters were as follows: TR=5s, TE=30ms, NEX=128 scans, N=2048 and SW = 1200Hz. The data (no line broadening applied) were fitted with LCModel (9) and metabolite quantification was obtained by using tCr (total creatine) as an internal standard. Statistical analysis was performed using JMP 6.0 (SAS Institute, Cary, NC). The ratios of NAA, tCho (total choline), Glu (glutamate) and mI to tCr, and NAA to mI were compared between young adults and geriatrics with two-tailed two-sample t-tests (α = 0.05). One-tailed tests were also adopted for NAA/tCr, mI/tCr, Glu/tCr and NAA/mI on the basis of prior MRS findings in human AD. Associations between age and metabolite ratios (as well as interrelationships among ratios) were measured with Pearson correlation. Results of two linear classifiers - the Linear Discriminant Analysis (LDA) and Support Vector Machines (SVM) classifiers – used to classify the subjects into two classes (young and geriatric) were compared.

RESULTS & DISCUSSION

The spectrum in Figure 1b is representative of the quality consistently achieved in this study. Rhesus geriatrics have significantly decreased NAA/tCr (0.91±0.0.03 vs. 1.17±0.03, p<0.001)*, increased mI/tCr (1.01±0.04 vs. 0.84±0.04, p=0.004)*, decreased tCho (0.11±0.05 vs. 0.13±0.05, p=0.009)* and decreased Glu/tCr (0.88±0.04 vs. 1.27±0.04, p<0.0001)*. Group data are shown in Figure 2. Pearson correlations in Table 1 show that NAA/tCr, tCho/tCr and NAA/mI correlated negatively with age, mI/tCr correlated positively with age, and tCho/tCr correlated positively with NAA/tCr and NAA/mI. Classification results are depicted in Table 2 for the two types of linear classifiers used. The classifiers were evaluated by ten runs of 5 fold cross-validation. Overall, high accuracy is obtained for both LDA and SVM. Notably, 99% accuracy is achieved for the panel of 4 biomarkers with the SVM classifier. This study not only shows the feasibility of localized in vivo quantitative proton MRS in Rhesus monkeys, but uses human AD biomarkers to distinguish between groups of young and geriatric monkeys. As with humans, these primates have been consistently shown to develop amyloid plaques as a normal part of the aging process. Since global brain atrophy associated with normal aging is not expected to alter metabolite ratios, their altered biochemical profiles suggest neuronal loss (NAA, ≈20% ↓, p<0.001), gliosis (mI, ≈20% ↑, p=0.004), and decreased excitatory function (Glu, ≈30% ↓, p<0.0001) in the posterior cingulate. These changes in Rhesus brain correspond with changes (both in direction and magnitude), observed in MRS biomarkers of human AD (10-12). In vivo proton MRS in Rhesus monkey brains offers a potential tool to elucidate AD mechanisms and assess potential therapeutic strategies.

REFERENCES

1. Bachevalier et al. *Neurobiol. Aging* 12:99-111 (1991) **2.** Walker et al. *Neurobiol. Aging* 9:657-666 (1988) **3.** Martin et al. *Proc Natl Acad Sci USA.* 88(4):1461-1465 (1991) **4.** Cork et al. *Am J Pathol.* 137(6):1383-92 (1990) **5.** Walker et al. *Brain Res Brain Res Rev.* 25(1):70-84 (1997) **6.** Sani et al. *Acta Neuropathol* 105:145-156 (2003) **7.** Shonk et al. *Radiol.* 195:65-72 (1995) **8.** Soher et al. *Neuroimg Clin N Am.* 15:847-852 (2005) **9.** Provencher SW. *Magn Reson Med.* 30(6):672-679 (1993) **10.** Shonk et al. *Radiology* 195:65-72 (1995) **11.** Soher et al. *Neuroimag Clin N Am* 15:847-852 (2005) **12.** Blenman et al. *Procs. ISMRM 2008*