Differences and similarities in brain metabolites observed by high resolution magic angle spinning 1H NMR of intact tissue and solution 1H NMR of tissue extracts from a primate model of neuroAIDS

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Introduction:

Proton NMR spectroscopy has become a powerful technique for the study of biological systems both *in vivo* and *ex vivo*. Quantification of cellular metabolites and their changes during the progression of a disease provide biochemical markers of changes in metabolism, as well as pathological conditions. In the past, the primary focus of *ex vivo* 1H NMR studies was on the analysis of chemical extracts of tissue. However, one drawback to the preparation of tissue extracts for analysis by high resolution NMR by chemical extraction is that histopathological structures are not preserved prohibiting pathological analysis of the same sample. Another disadvantage is that important molecular components may be lost by the extraction procedure. With the development of high resolution magic angle spinning (HRMAS) NMR, intact tissue can now be measured directly with spectral resolution comparable to that observed with extract solutions. A better understanding of the relative merits of the two methods is needed. The goal of this study was to directly compare the two approaches. *Ex vivo* HRMAS NMR spectra obtained from intact brain tissue were compared to those collected by from 1H NMR of brain extracts from the same sample.

Methods:

Tissue samples: Brain tissue samples were obtained from both the frontal cortex and putamen from SIV-infected rhesus macaques that were necropsied at 11 days post inoculation and two months post inoculation with SIVmac251. The samples were snap frozen at necropsy, and stored at –70 ºC until NMR analysis. From each tissue sample, two to three pieces (23 to 40 mg) were dissected to yield a total of 16 tissue samples.

HRMAS 1H NMR: HRMAS measurements were performed at 4 ºC on an MSL400 NMR spectrometer (1H frequency 400.15 MHz) equipped with a BD-MAS probe (Bruker Instruments), using a 7 mm zirconium oxide MAS rotor and a sample spinning rate of 2.5 kHz. Data was acquired using a rotor synchronized, T2 filtered CPMG pulse sequence with a short T2 of 20 ms and water pre-saturation.

Extraction procedure: Extraction of brain tissue was performed using a methanol/chloroform procedure as previously described modified for small samples [1]. A FastPrep FP 120 cell disrupter (Thermo-Savant) was used to pulverize the sample tissue.

Solution 1H NMR: High resolution proton nuclear magnetic resonance studies were performed at room temperature on a Bruker Avance 600 NMR spectrometer (Bruker Instruments) (1H frequency 600.45 MHz) equipped with a 5 mm probe. A one-pulse experiment was used with a recycle delay of 20 s.

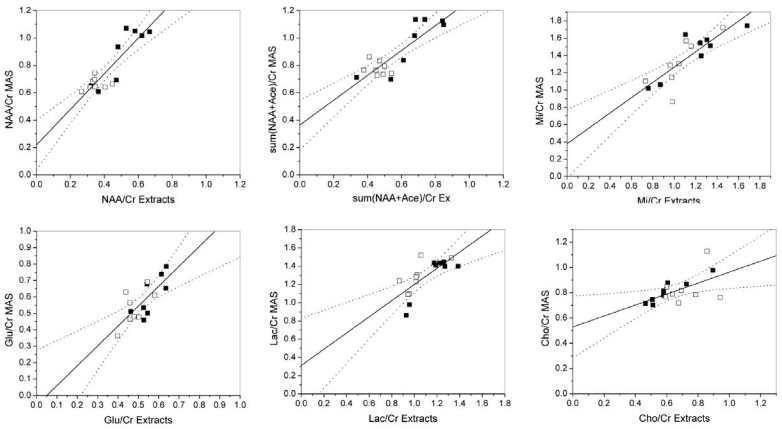
NMR data analysis: All spectra were processed off-line using the commercially available programs Peak Research NMR software (PERCH solutions) and NMR Utility Transform Software (Acorn, NMR Inc.) to determine the quantities of N-acetylaspartate (NAA), acetate (Ace), myo-inositol (MI), lactate (Lac), glutamate (Glu), choline-containing compounds (Cho) and creatine and phosphocreatine (Cr). The ratios of individual metabolites were calculated with respect to Cr. Statistical analysis was performed by linear regression between measurements of metabolite ratios in intact tissues and tissue extracts.

Results:

Table summarizes the results of the linear regression analysis shown in Figure 1.

	R^2	P	Slope	Intercept
NAA	**0.77**	**< 0.0001**	1.30 ± 0.19	0.21 ± 0.08
NAA in GM	**0.76**	**0.0051**	1.43 ± 0.33	**0.15 ± 0.17**
NAA in Put		N.S.		
NAA+Ace	**0.73**	**< 0.0001**	**0.91 ± 0.15**	0.36 ± 0.08
NAA+Ace in GM	**0.55**	**0.049**	0.69 ± 0.27	0.554 ± 0.18
NAA+Ace in Put		N.S.		
MI	**0.75**	**< 0.0001**	**0.87 ± 0.14**	**0.37 ± 0.17**
MI in GM	**0.86**	**0.0008**	**0.91 ± 0.14**	**0.27 ± 0.19**
MI in Put	**0.67**	**0.0125**	**0.82 ± 0.23**	**0.30 ± 0.29**
Cho	0.32	**0.02**	0.43 ± 0.16	0.52 ± 0.11
Cho in GM	**0.87**	**0.0007**	0.62 ± 0.10	0.43 ± 0.61
Cho in Put		N.S		
Lac	**0.54**	**0.0011**	**0.89 ± 0.21**	**0.31 ± 0.24**
Lac in GM:	**0.79**	**0.0031**	**1.33 ± 0.28**	**-0.27 ± 0.33**
Lac in Put:	**0.50**	**0.049**	**0.83 ± 0.34**	**0.43 ± 0.34**
Glu	**0.54**	**0.0012**	**1.21 ± 0.29**	**-0.06 ± 0.15**
Glu in GM	**0.64**	**0.0121**	**1.54 ± 0.49**	**-0.26 ± 0.28**
Glu in Put	0.38	(0.09)		

Bold-faced data indicate significant correlation $p<0.05$, $R^2 > 0.5$, a slope of 1.0 and an intercept of 0 considering the standard deviation.



Linear regression analysis for individual metabolite ratios: Solid squares represent gray matter samples; putamen is represented by open squares. The dotted line reveals the 95% confidence band.

Conclusion:

Using all 16 brain specimens we found overall significant correlation between the two methods. The results could be segregated into three patterns.

A) The correlation between NAA/Cr and the sum NAA+ Ace/Cr in intact versus extracted tissue is excellent, but the intercept is not at the origin. The slope for NAA is slightly greater than one, but when including its degradation product Ace the 95% confidence intervals would include a slope of unity. Linear regression analysis of MI/Cr ratios obtained from intact tissue and tissue extracts exhibit a strong correlation. The slope is slightly below unity and the intercept is approximately 0.4. The pattern we observed for NAA/Cr (Naa+Ace)/Cr and MI/Cr may be best explained by macromolecular contributions to the NAA and MI resonances in the brain that do not vary with time post infection with SIV. **B)** Glu/Cr and Lac/Cr show a fairly good correlation between HRMAS data of intact tissue and NMR data of extracts. The slope was near unity and the intercept was near the origin. This pattern suggests that both NMR methods are measuring the same molecular contribution to the resonance. That is, there is no significant macromolecular contribution to increase the HRMAS ratios compared to the extract solution ratios. This is supported by a lack of macromolecular resonances observed in these spectral regions as we previously reported [2]. **C)** The pattern in the relationship between tissue HRMAS and extract solution NMR for Cho/Cr was distinct from the other metabolites. We found a weak correlation between Cho/Cr obtained by MAS NMR of intact tissue versus NMR of tissue extracts. Furthermore, the slope was much less than unity and the intercept was well above the origin even when considering the 95% confidence limits. One possible explanation for the observations is that the extraction procedure partially removes choline-containing compounds from the tissue. Another possibility is that there is a major contribution from macromolecules that are also removed by the extraction procedure. A slope that is substantially different from unity could be explained by biological factors coupled with the chemical and physical factors. A detailed view will be discussed in the presentation. Interestingly, metabolites from frontal cortex samples demonstrate significantly better correlations than putamen samples, particularly for NAA/Cr and Cho/Cr. There may be a biological explanation for these observations. Putamen has a significant white matter contribution with myelinated axons traversing this structure, while frontal cortex has none.

References:

[1] Tracey et al. *J Acquir Immune Defic Syndr Hum Retrovirol*, 1997. **15**(1): p. 21-7.

[2] Cheng et al. *Cancer Res*, 1998. **58**(9): p. 1825-32.