The case of the missing glutamine

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Introduction

Proton magnetic resonance spectroscopy (^{1}H MRS) is uniquely positioned to investigate non-invasively the biochemical state of tissue. In particular, there is increasing interest in glutamatergic dysregulation in mood disorders, with changes in glutamine (Gln) due to anti-glutamatergic treatment in bipolar disorder being reported [1]. Unfortunately, *in vivo* Gln concentration measurements through ^{1}H MRS at clinical field strengths (≤4T) have proven unreliable and yielded contradictory results. Not only that repeatability results in such studies are little encouraging (ranging from 20-40% [2]), but absolute Gln concentration values measured *in vivo* tend to belong to one of two distinct categories. Some ^{1}H MRS studies [3] report Gln concentration values in the 4 mM range, correlating relatively well with *ex vivo* studies [4]. Yet other groups report Gln concentrations in the 2 mM range [5]. As measurement accuracy is impossible to determine *in vivo*, and as *ex vivo* studies are difficult to perform, it is only a theoretical study that can explain the experimental results. It is the goal of this study to clarify through simulations the performance of a number of pulse sequences in quantifying Gln concentration. Three of the simulated sequences are also used for *in vivo* validation of results. Consistent, but lower than expected Gln concentrations, are obtained with all 3 sequences. An explanation for the mismatch between the *in vivo* ^{1}H MRS and *ex vivo* results is attempted.

Methods

The methodology used in the current study is similar to the one recently documented [6]. Briefly, the GAMMA set of libraries [7] was used to compute the response of 14 individual brain metabolites to a number of pulse sequences proposed in the past for Gln detection. PRESS, STEAM, and Carr-Purcell PRESS with 2 and 4 extra refocusing pulses (CPRESS2 and CPRESS4) were included in our comparative study. The resulting spectra, weighted according to their concentration reported *in vivo*, were added together to simulate a human brain. Gln concentrations of 2mM and 4.5mM were considered. A residual water signal and a number of broad Gaussian peaks, simulating macromolecule (MM) signals, were also added to complete an ideal brain spectrum. A penalty factor of exp(-TE/T_2), with T_2(MM)=35ms, and T_2(metabolite) =250ms, multiplied all resulting spectra. Noise was added to the idealized brain signal; the resulting data set was fit using LCModel. The noise level and the acquisition bandwidth were kept constant for all pulse sequences. The SNR of the spectra was equivalent to the one of a spectrum acquired from a 16 cm^3 voxel in 5 minutes. The process of adding noise/ fitting the signal was repeated 1000 times for each pulse sequence/ acquisition parameters, while using different noise seeds. The metabolite concentrations were saved for each run. Three of the simulated pulse sequences were implemented in a clinical GE, 3T scanner, and 5 volunteers were scanned (using identical parameters as for the simulations) with each of these 3 sequences, three times in a row for each sequence.

Results and Discussions

Figure 1 depicts a typical *in vivo* voxel location, containing mostly gray matter. Table 1 presents the simulation results for the coefficient of variation (% CV) and absolute error for the Gln concentration for all acquisitions considered. Table 1 also contains the average intra-volunteer, intra-session % CV's from the *in vivo* acquisitions. The last column of the table (column 8) represents a division of the absolute concentration measured *in vivo* (column 7) by a correction factor accounting for the expected bias of each pulse sequence (column 5 of the table). Note a few very remarkable results from this table. First, there is a good correlation between the *in vivo* % CV's and the ones predicted by the simulations for the 2mM Gln case (which is closer to the Gln concentration measured by us in this study). Second, most of the pulse sequences tend to have a very large bias (absolute error) in measuring Gln concentrations (note column 5 of Table 1). This bias, which may depend on Gln concentration (as evidenced by the comparison of columns 3 and 5 of the table), can explain some of the higher Gln concentrations previously reported in vivo [2]. Third, repeatability measures for Gln concentration vary significantly across pulse sequences. From the sequences considered by us, a reasonable choice, providing good accuracy and repeatability, is a PRESS, TE=80ms sequence. Last, but not least, when accounting for the expected error associated with each pulse sequence, absolute Gln concentrations provided by the 3 sequences tested by us in vivo tend to be relatively close to each other. The average Gln concentration resulting from the 3 sequences is 1 mM, significantly below other published values (in particular considering the fact that the majority of the tissue in the voxel was gray matter, already expected to have higher Gln concentration than white matter [2]).

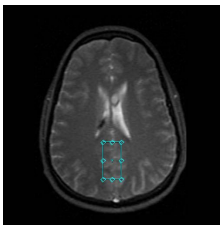

Figure 1: Typical voxel location.

	SIMULATIONS						
	c_Gln=4.5 mM		c_Gln=2 mM		In vivo data		
Pulse Sequence	%CV Gln	Abs error Gln [%]	%CV Gln	Abs error Gln [%]	%CV Gln	c_Gln [mM]	c_Gln / expected error [mM]
PRESS							
TE=35ms	14.7%	-7.7%	52.2%	-28.6%	122.0%	0.6mM	0.84 mM
TE=45ms	8.7%	17.9%	16.4%	41.2%			
TE=80ms	6.8%	-3.3%	14.8%	0.6%	12.7%	1.18Mm	1.17 Mm
STEAM							
TE/TM=5/5ms	7.1%	34.4%	12.4%	70.6%			
TE/TM=72/6ms	15.6%	21.4%	30.6%	43.1%			
Carr Purcell echo train							
CPRESS 2 (TE=45ms)	8.8%	19.7%	16.6%	35.3%	16.9%	1.4mM	1.03 mM
CPRESS 4 (TE=67ms)	14.1%	13.1%	30.0%	23.3%			

Table 1: Simulations and in vivo data

Conclusions

A simulation study is presented, investigating the bias and repeatability of multiple pulse sequence in measuring Gln concentration. Good agreement between simulations and *in vivo* data indicate that a TE=80ms PRESS sequence may provide a good choice for Gln measurements at 3T. A smaller Gln concentration than expected from other studies, in the 1mM range, emerges from this study. This concentration is very close to the ~0.5mM extra-cellular Gln concentration reported in the literature [8]. The rest of the brain Gln should be located in the mitochondria of the glial cells [8]. As high viscosity of (at lest certain fractions of) the mitochondria was previously reported, restricting free diffusion [9], it is possible that the intra-cellular Gln is characterized by low T_2, thus making it mostly invisible in the *in vivo* MRS exams.

References: **1.** Frye et al, Neuropsychopharmacology, 32:2490(2007); **2.** Kaiser et al, Neurobiol Aging, 26:665(2005); **3.** Pouwels et al, Magn Reson Med, 39:53(1998); **4.** Omori et al, Biol. Psychiatry, 42:359(1997); **5.** Schubert et al, NeuroImage, 21:1762(2004); **6.** Hancu, NMR Biomed, 22:426(2009); **7.** Smith et al, J Magn Reson, 106:75 (1994). **7.** Oja et al, 2007, Handbook of neurochemistry and Mol Neurobiology ; **8.** Lopez et al, 1996, J Biol Chem, 271: 10648-10653.

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