Simultaneous Measurement of Glutathione and Other Metabolites in Stroke Patients by J-Difference Spectroscopy

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Introduction: As a free radical scavenger and non-enzymatic anti-oxidant, reduced glutathione (GSH) plays a major role in the defense against reactive oxygen species in the human brain. Acute ischemic stroke is associated with significant oxidative stress and subsequent precipitates changes in the glutathione system. Clinical studies have shown that subjects with risk factors for stroke have relatively low levels of GSH, and patients with acute ischemic stroke develop elevated blood GSH levels during the first hours to days post ictus [1]. However methodological issues mean that most previous studies of the GSH system in stroke have focused on changes in the blood or urine. In vivo measurement of GSH in stroke patients by magnetic resonance spectroscopy (MRS) can provide spatially localized GSH levels in lesion and normal brain tissue. We have demonstrated the feasibility of measuring GSH in stroke patients by using a J-difference spectroscopy technique [2] with an improved data post-processing algorithm [3]. In this study we used this technique to investigate the changes in brain GSH occurring after stroke, with respect to changes in other commonly measured metabolites: choline (Cho), creatine/phosphocreatine (Cr), N-acetyl-aspartate (NAA), and lactate (Lac).

Method: A J-difference spectroscopy pulse sequence [2,3] was implemented on a 3 T Philips Achieva scanner with a standard 30 cm diameter T/R volume head coil. The pulse sequence had a TR of 2 s, a TE of 131 ms, and 256 data acquisitions for each measurement. The total scan time for each measurement was 8.5 min. Among the 256 acquisitions, half were "on" acquisitions and the other half were "off" acquisitions. In an "on" acquisition, the editing pulses were tuned at the resonance frequency of cysteinyl α proton at 4.56 ppm to partially refocus the J-evolution of the GSH resonances of interest at 2.95 ppm (cysteinyl β protons). In an "off" acquisition, the frequency of the editing pulses was shifted to a higher value (+200 Hz) to allow the J-evolution of the GSH resonances at 2.95 ppm to fully develop. Subtraction between an "on" spectrum and an "off" spectrum cancelled the unedited NAA, Cr, and Cho singlet peaks and retained the edited GSH at 2.95 ppm and co-edited aspartate moiety of NAA signals. In order to minimize subtraction errors due to potential patient motion and system instabilities, a spectral alignment method [3] was developed to perform frequency, phase, and linear baseline corrections on the data. After this spectral alignment procedure, difference spectrum was computed as the difference between the averaged "on" spectrum and the averaged "off" spectrum (see Fig. 1). Meanwhile, the average of the averaged "on" and "off" spectra was taken as the averaged spectrum. For quantification purposes, basis spectra of Cho, Cr, NAA, Lac, and GSH were generated using GAMMA density operator simulations. The amplitude of the GSH basis spectrum was calibrated by two in vitro spectra obtained by scanning 20 mM GSH and Cr phantoms, respectively. Concentration levels of Cho, Cr, and NAA were obtained by fitting the Cho, Cr, and NAA basis spectra to the averaged spectrum. GSH concentration was obtained by fitting the GSH and NAA basis difference spectra to the difference spectrum. Lac concentration was obtained by fitting the Lac basis spectrum to the averaged "off" spectrum. Cr concentration in the contralateral normal tissue (8.0 mM) was used as an internal reference. Twelve stroke patients (five male, seven female, average age = 64) were studied, all of whom gave informed consent in accordance with procedures approved by the institutional review board. All twelve patients had large lesions (> 5cm in plane extent) within the middle cerebral artery (MCA) territory. Each patient received two GSH scans with one scan localized in the lesion and the other on the contralateral side as control. The same 5 x 3 x 3 cm^3 volume of interest (VOI) was used for all experiments.

Results and Discussion: Metabolite concentrations are given in table 1. The ratio between the GSH levels in lesion and control regions was computed and represented by rGSH. A correlation between rGSH and Lac level in the lesion was found to be significant at 0.05 level with Pearson's correlation coefficient r = 0.59 and p = 0.042 (two-tailed). In Fig. 2, the data point indicated by the arrow corresponds to patient # 6 in table 1, which was the only case in this sample that had extensive hemorrhagic transformation within the lesion and appears to be an outlier. Exclusion of hemorrhagic transformation leads to a stronger correlation between rGSH and Lac levels (r = 0.72, p = 0.013).

These findings not only highlight the important interplay between ischemia and oxidative stress but suggest a potential role for MR in assessing the anti-oxidant status of acute stroke lesions. High lactate levels, which can be associated with anaerobic glycolysis due to acute ischemia, tended to be associated with normal or increased levels of glutathione. This is consistent with findings from clinical studies where blood GSH levels were found to be increased in the first 48 hours post ictus. These preliminary data suggest the need for further study in a larger number of subjects where reperfusion status is known. Nonetheless they raise the possibility that the assessment of GSH in the brain of acute stroke patients may be useful in evaluating the effects of reperfusion or other acute stroke therapies.

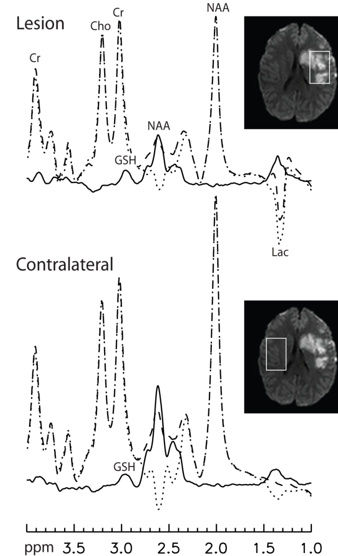

Fig. 1. *Averaged "On" spectra (dashed lines), averaged "off" spectra (dotted lines), and difference spectra (solid lines) in the lesion and contralateral normal tissue for a stroke patient. The VOI for each measurement is depicted on the corresponding diffusion weighted image (b = 1000 s/mm²).*

Table 1: Metabolite levels in lesion and contralateral normal tissue

Patient	Hours after onset	Cho (mM)		Cr (mM)		NAA (mM)		Lac (mM)		GSH (mM)		rGSH
		Lesion	Control	Lesion	Control	Lesion	Control	Lesion	Control	Lesion	Control	
1	30 days	2.4	2.2	8.4	8.0	8.0	11.4	0.48	0.39	0.83	1.53	0.55
2	142	1.4	2.1	3.3	8.0	2.9	11.6	4.75	0.00	1.52	1.21	1.25
3	113	1.6	2.0	5.3	8.0	5.7	9.9	1.36	0.50	1.08	1.28	0.84
4	86	1.7	2.2	5.9	8.0	6.1	11.0	2.13	0.28	1.20	1.01	1.19
5	43	1.6	2.3	5.2	8.0	7.2	13.0	1.63	0.28	0.84	1.39	0.60
6	118	1.1	2.2	2.7	8.0	3.1	12.2	3.76	0.56	0.47	0.95	0.50
7	24	2.9	2.7	7.2	8.0	8.2	10.2	3.97	0.27	1.12	1.17	0.96
8	3	2.7	1.9	6.6	8.0	8.1	11.7	1.24	0.41	1.00	1.26	0.79
9	36	1.9	2.1	6.4	8.0	5.2	10.4	7.19	0.36	1.40	1.15	1.21
10	41	2.0	2.1	4.5	8.0	4.5	10.0	5.83	0.00	1.08	0.84	1.29
11	108	1.9	2.8	5.3	8.0	5.0	10.9	5.47	0.00	1.38	1.26	1.09
12	3	2.7	2.2	9.3	8.0	12.4	11.7	2.41	0.00	1.38	1.10	1.25
Mean (SD)		2.0 (0.6)	2.2 (0.3)	5.8 (1.8)	8.0 (0.0)	6.3 (2.5)	11.2 (0.9)	3.13 (2.20)	0.26 (0.20)	1.06 (0.33)	1.17 (0.19)	0.92 (0.31)

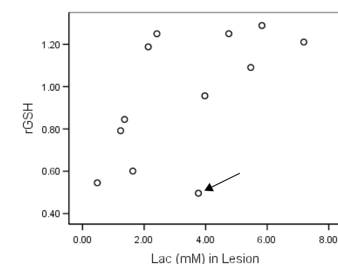

Fig. 2. *Scatterplot of rGSH vs. Lac in lesion.*

References: 1. Zimmermann C, et. al., Eur Neurol 2004;51(3):157-161. 2. Terpstra M, et. al., MRM 2003;50:19-23
3. An L, et. al., ISMRM 2008;16:1640.