Effects of temperature and time on cholines observed by HRMAS ^{1}H MRS of rat brain and human brain tumor samples

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Introduction

HRMAS ^{1}H MRS of tumor biopsies provides well-resolved spectra for detailed biochemical analysis. However, during surgical removal of a tumor biopsy there can be a period of ischemia prior to the sample being snap frozen. Long acquisition times are also required for 2D HRMAS sequences, such as TOCSY, used particularly to analyse the choline peaks [choline (Cho), glycerophosphocholine (GPC) and phosphocholine (PCh)] and study differences in phospholipid metabolism [1]. In this study we investigate the effects of temperature and time of ischemia on the choline compound concentrations in normal rat brain under well-controlled conditions of initial ischemia and also on human brain tumor biopsy samples.

Methods

Normal rodent brain. To assess the biochemical changes occurring at 4 °C after a minimal period of ischemia, brain tissue was obtained from rat brain after liquid N_2 funnel freezing. Frozen brain tissue samples (n = 4) were subsequently transferred to the HRMAS probe with minimal warming and spectra acquired at 12 min intervals for 4 hours at 4 °C. To mimic the situation of tissue ischemia during open surgery a rat brain was rapidly removed post-sacrifice and left on the bench at 20 °C. Sagittal brain slices of ~2 mm thick were removed and snap frozen in liquid N_2 at 30 min intervals over 3 hours for subsequent HRMAS. *Human brain tumors*. Biopsy samples of 6 astrocytoma grade II (AS2) and 6 glioblastomas (GBM) were obtained from consenting patients during routine surgical resection of their tumor and then snap frozen in liquid N_2. Each tumor biopsy sample underwent a 3 hour acquisition protocol (involving 1D & 2D acquisitions) with presaturation spectra acquired at the beginning and end of each experiment to determine metabolite changes. *HRMAS ^{1}H MRS.* CPMG or presaturation spectra were acquired on a 600 MHz Bruker Avance spectrometer using an HRMAS probe spun at 5000 Hz at 4 °C. 10 - 15mg of frozen tissue was placed in 50 µl inserts, over ice to minimize warming, and the remaining space filled with D_2O. All spectra were analysed using LCModel and quantified using the tissue water signal as reference, assuming a water concentration of 44 M [2]. Acetate (Act), Cho, Creatine (Cr), GPC, Lactate (Lac), N-acetyl aspartate (NAA) and PCh were individually quantified in all samples.

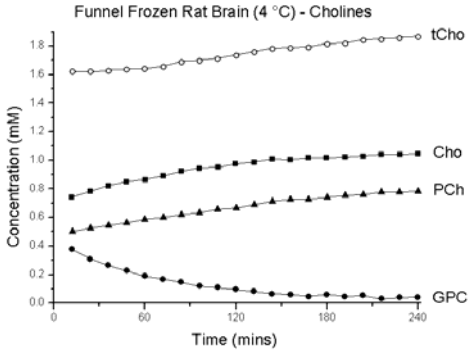

Figure 1

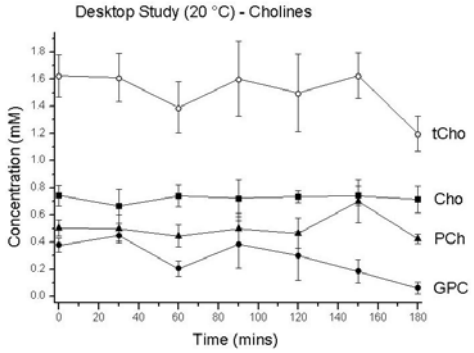

Figure 2

Table 1

	Δ concentration (mM)		
	AS2	**GBM**	**Rat**
Cho	0.19±0.10	0.10±0.10	0.28±0.05
GPC	-0.31±0.16	-0.19±0.09	-0.32±0.04
PCh	0.16±0.04	0.00±0.06	0.24±0.03
tCho	0.05±0.10	-0.09±0.06	0.19±0.03

Results

The funnel frozen brain study shows that at 4 °C there is an exponential decrease in GPC with corresponding increase in Cho and an approximate linear increase in PCh (Figure 1). The 4 °C study also showed that Cr, Lac, tCho and tNAA all show a slight increase in concentration over the 4 hours. The 20 °C ischemia study shows more stability over time for most metabolites, but the cholines show a similar pattern to 4 °C, with a decrease in GPC but a much reduced increase in Cho and PCh (Figure 2). Lac showed an increase of ~5 times the concentration measured at time 0 within the first 30 mins. The human tumor biopsy data also showed a similar pattern of changes in cholines (Cho, GPC, PCH and tCho) to the 4 °C rat brain results, with Table 1 giving the changes in concentration between the beginning and end of each experiment (mean ± SD).

Discussion

The observed changes in choline levels for all three experiments agree with phospholipid metabolism [1] for which GPC is a highly labile compound. Summation of the concentrations of the 3 choline compounds (Cho, GPC and PCh) in AS2 and rat brain shows a slight elevation over time. Although this is not observed in GBM spectra, due to the reduced cellular density of these tumors by necrosis the reliability of the quantification is also reduced. In general, a delay in the time to freezing of the tissue up to ~2 hr did not significantly alter the metabolite concentrations, with the exception of lactate, whose levels increase 5-fold immediately after tissue removal. But after ~3 hr of ischaemia at 20 °C there were changes (Figure 2). For tissue maintained at 4 °C there were significant changes in the individual choline compounds over 3 - 4 hours and increased tCho, Cr, Lac and tNAA. It is possible that over such an extended time tissue damage occurs due to the spinning releasing previously NMR-invisible metabolites.

In conclusion, when studying phospholipid metabolism of tumors by HRMAS, caution must be used when interpreting the results to account for these time and temperature effects.

References

[1] Podo F. *NMR Biomed* **27**; 1-12; 1999. [2] Opstad KS *et al. Proc ISMRM*, **14**, 2531; 2006.

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