In vivo MRS markers of response to chemotherapy in a relapsed non-Hodgkin's lymphoma xenograft

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Introduction

A multi-institutional study has recently demonstrated that ratios of the phosphomonoesters (PME), phosphoethanolamine plus phosphocholine, to NTP measured in ^{31}P MR spectra of human non-Hodgkin's lymphoma (NHL) before initiation of therapy can identify about 2/3 of the patients who will not exhibit a complete local clinical response.[1] These NMR studies have been largely limited to single studies performed prior to initiation of treatment and tumor sizes bigger than (3 cm)^3 because of the insensitiveness of ^{31}P NMR. We're developing more sensitive ^{1}H MR methods for detecting therapeutic response of NHL that can be translated into following treatment response of individual patients.

Methods

A diffuse large B-cell lymphoma, WSU-DLCL2 cell line[2], was obtained from Dr. Al-Katib's lab in the Wayne State University. The cell line was from a patient having relapsed large cell lymphoma.10^7 WSU-DLCL2 cells were subcutaneously implanted in the flanks of 6-8 week old SCID mice. When the tumor volume reached ~500 mm^3, CHOP chemotherapy was initiated; cyclophosphamide, 40 mg/kg i.v., day 1, hydroxydoxorubicin, 3.3 mg/kg i.v., day 1, oncovin (vincristine), 0.5 mg/kg i.v., day 1, and prednisone, 0.2 mg/kg *per os* for five days. The treatment was repeated every week for three cycles. *In vivo* MRS was performed before treatment and after each cycle of treatment using a Varian 9.4 T/8.9 cm vertical bore instrument and a home-built ^{1}H/^{31}P dual tuned slotted tube resonator. A selective multiple quantum coherence (SelMQC) sequence[3] was used for detecting lactate, a stimulated echo acquisition mode sequence (STEAM) for detecting total choline and lipid, and an image-selected *in vivo* spectroscopy (ISIS) sequence for detecting ^{31}P metabolites. High resolution *ex vivo* ^{1}H and ^{31}P spectra were obtained on a Bruker 9.4 T from perchloric acid extracts of the harvested tumors. To measure the proliferation rate, Ki67-staining was performed on tumor sections.

Results

After 3 cycles of weekly CHOP chemotherapy, tumor growth arrest was observed as the volume of these tumors remained stable (unchanged) while that of the untreated group increased twofold. The SelMQC sequence removes single-quantum (SQ) coherences of water and lipid selecting for the multiple-quantum (MQ) coherence of lactate (Fig. 1a). Along with the lactate peak at 1.3 ppm, another signal at 1.5 ppm was consistently observed, which was later assigned to be alanine as it has the same MQ coherence transfer pathway as lactate. The lactate/water decreased to 60% of the initial value after the 1st cycle of CHOP chemotherapy ($p<0.05$) (1 week), and further diminished to 25% after the 3rd cycle (3 week) (Fig. 1b). The choline containing metabolites measured from the STEAM sequence did not change with treatment (Fig. 1c). Lipids (CH_2 peak) slightly increased but there was no significant difference relative to the untreated group. Excellent resolution was observed in the ^{31}P spectra as resonances from phosphoethanolamine (PE), phosphocholine (PC) in the phosphomonoester region and glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC) in the phosphodiester region could be easily resolved (Fig. 1d). The phosphorous metabolite ratios and intracellular pH did not show significant change with treatment (Table 1). Extract NMR spectra showed a similar trend as in *in vivo* experiments (lactate 12.60±0.65 vs 9.40±0.70 μmol/gww (control vs treated); total choline, 3.44±0.24 vs 3.28±0.40 μmol/gww). Ki67-staining showed a substantially reduced proliferation rate in the CHOP-treated tumor relative to the untreated control.

Discussion

PME/NTP changes after treatment have been frequently observed in human lymphomas[4] and changes in total choline and lipids were observed during etoposide plus cyclophosphamide treatment of murine EL-4 lymphoma[5]. Although we did not see such PME or total choline changes, the rapid lactate change which correlated with the histologically proven decrease in proliferation rate indicates that lactate is the most sensitive and early MRS detectable therapeutic marker in WSU-DLCL2 lymphoma xenografts.

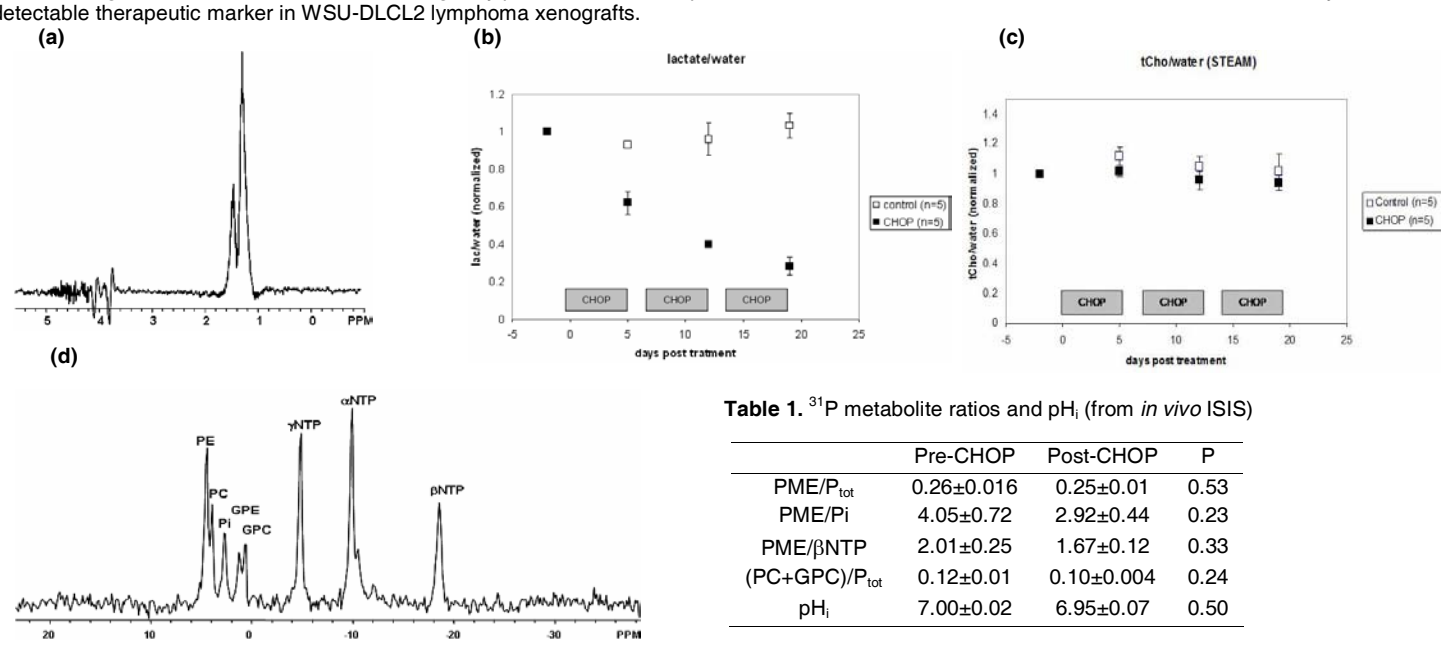

Table 1. ^{31}P metabolite ratios and pH_i (from *in vivo* ISIS)

	Pre-CHOP	Post-CHOP	P
PME/P_{tot}	0.26±0.016	0.25±0.01	0.53
PME/Pi	4.05±0.72	2.92±0.44	0.23
PME/βNTP	2.01±0.25	1.67±0.12	0.33
$(PC+GPC)/P_{tot}$	0.12±0.01	0.10±0.004	0.24
pH_i	7.00±0.02	6.95±0.07	0.50

Figure 1 (a) a SelMQC spectrum from a DLCL2 tumor, 2 min. (b) lactate/water vs time, (c) tCho/water vs time, (d) an ISIS spectrum from a DLCL2 tumor after one cycle of CHOP, 30 min.

Table 1 *Data are expressed as mean±SE. §n=5. ¶Data were measured before and after 1st cycle of CHOP.

References

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