Decreased choline levels detect response to Rituximab therapy in WSU-DLCL2 human diffuse large B-cell lymphoma xenografts

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Introduction

Previously we've reported ^{1}H and ^{31}P MRS studies of multicycle CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) treatment in WSU-DLCL2 human diffuse large B-cell lymphoma xenografts[1-2]. In that study, lactate was found to be an early indicator of therapeutic response for CHOP chemotherapy in this tumor model while tCho and PME:NTP did not. Rituximab is a monoclonal antibody approved by the FDA that is now included in the standard first line therapy for non-Hodgkin's lymphoma patient in combination with CHOP (R-CHOP). Addition of rituximab has been reported to prolong the overall survival rate of the non-Hodgkin's lymphoma patients compared to patients received CHOP alone. Monoclonal antibody therapy is a recent innovation in cancer therapy and rituximab was the first monoclonal antibody approved for use in the clinic. There has been no MRS study reported following monoclonal antibody in cells, animals or humans. The study presented here is the first MRS study following monoclonal antibody therapy.

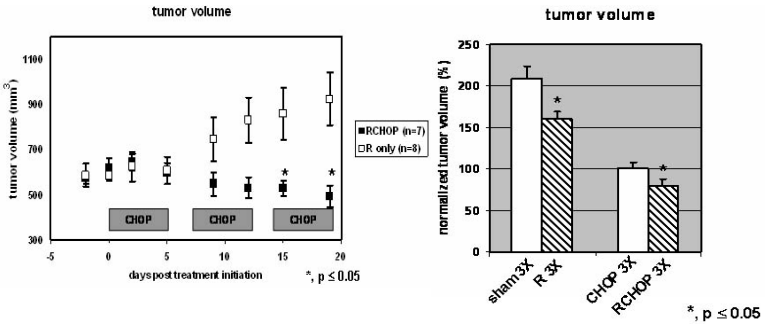
Methods

The diffuse large B-cell lymphoma, WSU-DLCL2 cell line[3], was obtained from Dr. Al-Katib's lab at Wayne State University. The cell line was isolated 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, rituximab alone or rituximab plus CHOP therapies were initiated; Rituximab was given 25 mg/kg i.p. at day 0. CHOP was given as cyclophosphamide, 40 mg/kg i.v., day 0, doxorubicin, 3.3 mg/kg i.v., day 0, vincristine, 0.5 mg/kg i.v., day 0, and prednisone, 0.2 mg/kg p.o., for five days. The treatment was repeated every week for three cycles. Tumor volumes were measured 2-3 times a week. *In vivo* MRS was performed before treatment and after the first and second cycles 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[4] was used for detecting lactate, and a stimulated echo acquisition mode sequence (STEAM) for detecting total choline. The Rituximab and R-CHOP treatment results were compared with our previous results on sham- and CHOP-treatments[2].

Results

The combination of R-CHOP induced tumor regression that was significantly different to the growth of tumors treated with R alone (Fig 1a). Moreover Rituximab induced tumor growth delay compared to sham-treatment (Fig 1b) or tumor regression when combined with CHOP compared to CHOP alone treatment (Fig 1b). After three cycles of treatment (day 19)., the differences were 20 % in either comparison in Fig 1b. Lactate decreases were observed to be significant in the R-CHOP treated group just after the 1st cycle of treatment ($p \leq 0.01$) but not in the R only treated group (Fig. 2a). However, when compared with sham-treated or CHOP-treated groups, rituximab was observed not to induce significant changes in lactate even after 2 cycles of treatment (Fig. 2b). tCho changed significantly both R-only and R-CHOP treatment groups after just the 1st cycle of treatment (Fig. 3a). Comparisons, of sham 2X vs R 2X and CHOP 2X vs R-CHOP 2X after 2 cycles of treatment showed that Rituximab induced significant differences in tCho:water ratios (Fig. 3b). Note that over the same time period the effect of Rituximab on the tumor volume was not significant.

Fig. 1 (a) **(b)**



Discussion

Rituximab induced a small but significant effect on the growth of WSU-DLCL2 tumors. This is consistent with a recent clinical study of 399 previously untreated elderly patients with diffuse large B-cell lymphoma, that reported a complete response in 76 % of patients for the CHOP plus rituximab treated group vs 63 % for CHOP alone, P=0.005 [5]. In the current study, tCho appears to be the most sensitive marker of rituximab-induced therapeutic effect and can be observed before changes in tumor volume.

Fig. 2 (a) **(b)** **Fig.3 (a)** **(b)**

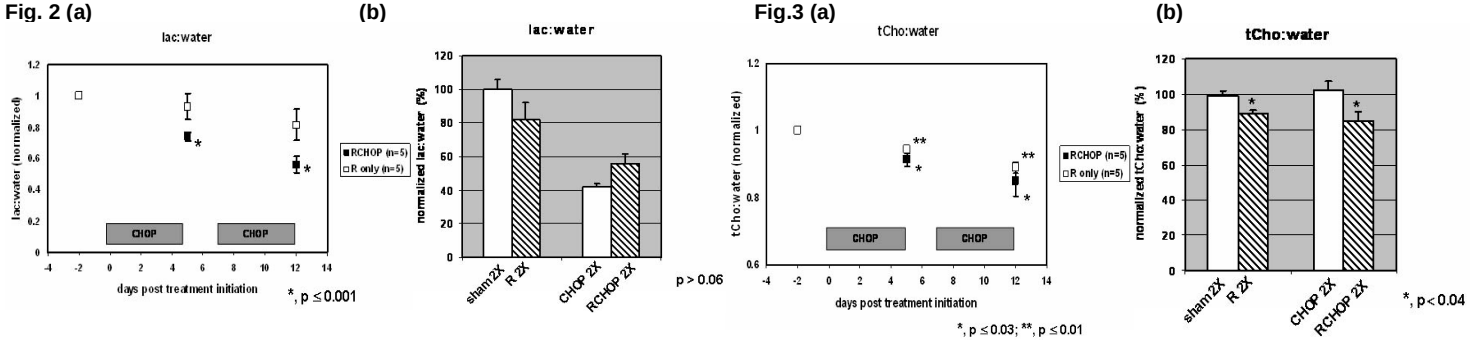


Figure 1 (a) Tumor volumes of the WSU-DLCL2 xenograft after R-CHOP and R-only treatments; *, comparison to the tumor volumes at day 0. (b) Comparisons of tumor volume for sham 3X vs R 3X , and CHOP 3X vs R-CHOP 3X. **Figure 2** (a) Lactate:water ratios from Sel-MQC spectra of WSU-DLCL2 after R-CHOP and R-only treatments. (b) Comparisons of lac:water ratios for sham 2X vs R 2X , and CHOP 2X vs R-CHOP 2X. **Figure 3** (a) tCho:water ratios from STEAM spectra of WSU-DLCL2 after R-CHOP and R-only treatments. (b) Comparisons of tCho:water ratio for sham 2X vs R 2X , and CHOP 2X vs R-CHOP 2X.

References

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