MRS assessment of lactate in dedifferentiated liposarcoma models treated with chemotherapy

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Introduction: Well-differentiated/undifferentiated liposarcomas occur mainly in the abdomen, and surgery is the mainstay of treatment. However, the local recurrence rate is high even if margins are negative and de-differentiation is predictive of poor outcome. The lack of effective chemotherapy of de-differentiated liposarcoma (DDLs) leaves non-surgical candidates with few options. Recent investigations have revealed genes abnormally expressed in DDLs which are potential therapeutic targets (1,2). For example, CEBPα, which is a transcription factor involved in adipocyte differentiation, is under-expressed in DDLs. Administration of the SN-38 prodrg CPT11 (irinotecan) caused increased CEBPα expression and growth delay in DDLS cells and xenografts (3). A non-invasive marker reflecting the effect of such agents could be quite valuable in pre-clinical drug evaluations. Furthermore, in the clinic, an early marker of response/non-response could permit the physician to discontinue ineffective treatment without delay. Lactate, an end-product of glycolysis has the potential to be a biomarker of prognosis and treatment effect. The goal of the current study was to assess the change in lactate levels in a human DDLS tumor xenograft (DDLS BWH) implanted in mice in response to CPT-11. A second cohort was treated with doxorubicin (Adriamycin), an anthracycline used to treat sarcomas.

Methods: Overview: 35 BWH tumors were grown in the flanks of SCID mice and monitored until the volume was approximately 100 mm3. For tumor growth delay studies, tumor volume was monitored in 7 control, 5 Adria-treated, and 5 CPT-11-treated mice (V= (π/6) * L * W2 * D). MRI/MRSi was performed serially at day 1 (baseline), day 3 and day 6 in 7 control, 6 Adria-treated, and 5 CPT-11 treated mice. The mice were treated twice per week (Adria 0.9 mg/kg i.p., CPT-11 100mg/kg i.p.) with the first treatment given immediately after the baseline MRS scan. MR Experiments: Mice were anesthetized with isoflurane during the MR experiments on a Bruker 4.7-T Biospec Spectrometer. Two home built 2-turn solenoid coils with diameters of 10 mm and 14 mm were used. T2-weighted sagittal MR images were collected (slice thickness = 1 mm, number of slices=10, FOV = 24 mm, TR = 3000ms, TE = 40 ms, matrix= 512 X 256, number of acquisitions = 4). Lactate detection was performed using the Selective Multiple Quantum Coherence (Sel-MQC) editing sequence (4,5). Lactate spectra were obtained from a 5-mm thick center slice with TR = 2 sec, number of excitations =512, 1024 data points, and spectral width of 2510 Hz. Peak fitting was performed in Matlab (Natick, MA). Qualitation of lactate was performed using the phantom substitution technique.

Histology: Selected animals were injected with Pimonidazole hydrochloride (PIMO) (hypoxprobe-1, HPI) and Hoechst 33342 (Sigma-Aldrich) at 60 and 40 mg/kg, respectively. These agents were dissolved in PBS and administered via the tail vein at 1 hour before sacrifice (PIMO) and 5 minutes pre-sacrifice (Hoechst 33342). Tumors were excised, embedded in cutting medium (OCT 4583, Sakura Finetek), snap-frozen and stored at −80°C. 10μm thick frozen sections were obtained using a cryostat microtome (Microm International GmbH). Sections were fixed in 4% paraformaldehyde then blocked in Superblock-PBS (Pierce, USA) followed by hematoxylin/eosin staining. Data Analysis: The lactate peak area was normalized by the slice volume and compared to the lactate content of a phantom section of known volume and concentration. Results: The non-treated tumors grew more rapidly than the CPT-11-treated (Fig 1A). Adria had little effect on tumor growth. On day 3, the CPT11 treated group had lower lactate than the control and Adria groups (P = 0.001 for CPT11 vs control, P = 0.0006 for CPT11 vs Adria). At day 6, the CPT-11 treated tumors still had significantly lower lactate concentrations than the control and Adria groups, although lactate had decreased in these groups compared to baseline (P <0.0001 for CPT11 vs. control, P < 0.0001 for CPT11 vs Adria). Histologic and IHC analysis (Fig 2) showed regions of reduced cell density, reduced vascularity (Hoechst) and hypoxia (PIMO) in the growing control and Adria-treated tumors at 1 week. In contrast, in the growth-halted CPT-11-treated tumors, the tissue appeared fairly homogeneous, well vascularized and free of hypoxia. The trend toward reduced lactate concentration at 1 week in the Adria and control groups is probably due to reduced cell density and/or the development of necrosis as seen on histology.