Metabolic Characterization of Human Soft Tissue Sarcomas in Vivo and in Vitro
Using Proton-decoupled Phosphorus Magnetic Resonance Spectroscopy


ABSTRACT

We applied 1H-decoupling and nuclear Overhauser enhancement to obtain well-resolved 31P magnetic resonance spectra accurately localized to 20 soft tissue sarcomas in vivo, using three-dimensional chemical shift imaging. Fifteen spectra had large phosphomonoester signals (21% of total phosphorus) that contained high amounts of phosphoethanolamine (compared to those of phosphocholine) but no signals from glycerophosphoethanolamine, and glycero phosphocholine was detected in only four cases. Prominent nucleoside triphosphates (52% of phosphorus) and low inorganic phosphate (10% of phosphorus) indicated that a large fraction of these 15 sarcomas contained viable cells, and this impression was confirmed histologically in 13 of the sarcomas. High-resolution in vitro 31P spectra of extracts of surgical specimens of four of the sarcomas studied in vivo and six additional sarcomas confirmed the in vivo assignments of metabolites and revealed considerable inter- and intratumoral variations of metabolite concentrations associated with histological variations in the relative amounts of cells and of matrix materials or spontaneous necrosis. Seven sarcomas, all high grade with pleomorphic or round cells rather than spindle cells, contained an unidentified phosphodiester signal in vivo; its absence in the extract spectra indicates that it may be from an abnormally mobile membrane component. We have documented a means to obtain new information about in vivo metabolism in human sarcomas and to provide a basis on which to examine the uses of 31P magnetic resonance spectroscopy in the clinical management of sarcomas.

INTRODUCTION

Observations that indicate the importance of aspects of energy metabolism and phospholipid metabolism in malignant behavior, treatment sensitivity, and resistance both in experimental models (1, 2) and in patients (3–7) have stimulated interest in the biochemistry of sarcomas. An attractive way to obtain information about the metabolism of sarcomas in vivo is to use 31P MRS. The 31P MR spectrum has signals from phospholipid metabolites, NTPs, and other energy-related metabolites, and it provides a means to measure intracellular pH. The 31P MR spectra of approximately 100 human soft tissue sarcomas in vivo have been reported (1, 3–6, 8–16). In general, these spectra had relatively strong signal intensities in the PME and PDE regions and an intracellular pH (determined from the position of the P, signal on the frequency axis) of approximately 7.25. However, most of the reported spectra were incompletely localized to the sarcomas: (a) many were heavily contaminated with signals from muscle; (b) many had insufficient resolution to clearly distinguish overlapping PME, P, and PDE signals; and (c) none had sufficient resolution to distinguish the major components within the PME and PDE regions.

Two factors contribute to the poor resolution of metabolites in the 31P MR spectra: (a) the inhomogeneity of the magnetic field within the region of interest. The adequacy of magnetic field homogeneity depends upon the extent of the efforts devoted to shimming procedures; and (b) the broadening of the 31P signal peaks by coupling between magnetic fields of 31P nuclei and those of neighboring 1H. This effect may be reduced by radio frequency irradiation of 1H during the acquisition of 31P signals, a technique referred to as 1H-decoupling. In addition, the irradiation of 1H between acquisitions can increase some of the 31P signal intensities by a process called NOE enhancement.

We have recently applied the combination of 1H-decoupling and full NOE enhancement in vivo to 31P MRS studies of brain, calf muscle, liver, and non-Hodgkin’s lymphomas (17–20). We report here the use of this technique, in conjunction with the means to optimize the magnetic field homogeneity automatically within the region of interest (autoshimming; Ref. 21) to improve the resolution within the PME and PDE regions of the spectrum in patients with sarcomas. We used MRI-directed, three-dimensional CSI to accurately localize 31P MR spectra to the regions of interest (22). To permit application of these techniques in various anatomic sites, we constructed dual-tuned (31P and 1H) surface coil arrangements. This approach enabled us to obtain more information about the in vivo metabolic characteristics of soft tissue sarcomas than has heretofore been available.

Soft tissue sarcomas are heterogeneous histologically. Many contain large amounts of matrix materials of a fibroid, lipid, or myxoid character, and many undergo spontaneous focal necrosis. These factors can reduce the fraction of viable cells within a region studied by MRS and therefore reduce the metabolite signals relative to the noise in the spectrum. This could account in part for the considerable variations in quality reported among in vivo 31P spectra in human sarcomas (1, 3–6, 8–16). To examine this issue, we determined the histopathological characteristics of the sarcomas that were studied in vivo before surgery, and we obtained high-field 31P MR spectra of the water-soluble extracts of surgical specimens of soft tissue sarcomas, some of which were also studied in vivo. This approach enabled us to confirm the in vivo assignments of metabolites to observed signals, to obtain molar concentrations of the metabolites, and to examine the aspects of inter- and intratumoral heterogeneity.

PATIENTS AND METHODS

Patient Population. Eligibility for the in vivo 31P MRS study required a biopsy-proven diagnosis of soft-tissue sarcoma, a tumor mass of approximately 3-cm diameter or larger located within 10 cm of the surface of the body, an absence of the standard contraindications to MRI, and signed informed consent as approved by the Institutional Review Board. Of 20 patients, 16 were newly diagnosed, 3 were recurrent after prior treatment, and 1 was resistant to chemotherapy. From four of these patients and six additional patients, portions of surgical specimens were obtained and extracted for in vitro 31P MRS study.

Received 4/17/96; accepted 4/24/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by NIH Grants CA56960, CA54339, and CA41078 and by Siemens Medical Systems (Iselin, NJ).

2 To whom requests for reprints should be addressed, at Department of Nuclear Magnetic Resonance and Medical Spectroscopy, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

3 The abbreviations used are: MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance; MRI, magnetic resonance imaging; PME, phosphomonoester; PDE, phosphodiesters; NTP, nucleoside triphosphate; PC, phosphocreatine; PEth, phosphoethanolamine; PChol, phosphocholine; GPEth, glycerophosphoethanolamine; GPChol, glycerophosphocholine; NOE, nuclear Overhauser effect; CSI, chemical shift imaging; NDP, nucleoside diphosphate; MR, magnetic resonance; RIF-1, radiation-induced fibrosarcoma.
Characteristics of the patients and their sarcomas are summarized in Table 1, where they are lettered a-z and referred to as such throughout the manuscript.

Histopathological Analysis. Surgical specimens were obtained in 23 of the 26 cases; most of these were from resections performed after the 31P MRS study. In one additional case (case k), only a needle aspiration biopsy was obtained. In another case (case g), the recurrent tumor was not biopsied, and the original resection was several years old. In case m, the resection (which showed >95% necrosis) was performed 3 months after the patient received neoadjuvant chemotherapy, and the original biopsy was not available from an outside institution. The categorization of each sarcoma followed current WHO typing (23). The grade was determined based on the tumor differentiation, mitosis count, and extent of necrosis (24). The predominant cell type was designated as spindle, pleomorphic, round, or giant cell. The relative amounts of cells and noncellular materials were noted, as was the variability of this feature within the tumor. The extent and nature of matrix (myxoid, fibroid-collagen, chondroid, and so forth) and the nature and extent of necrosis (none, focal, or extensive) were noted.

Procedures for in Vivo 31P MRS. The procedures for in vivo 31P MRS were described recently in detail (20), so only a brief description will be given here. In vivo studies were performed at 1.5 Tesla in a Siemens Magnetom clinical image/spectrometer (Siemens AG, Erlangen, Germany). The surface coils constructed to receive NMR signals from tumors in various anatomic sites in vivo included a 12- or 15-cm diameter, single-turn, 31P coil of copper tubing underlying a 22 X 14-cm butterfly 1H coil of 1-cm-wide copper foil. The patient was positioned and made comfortable on the instrument table, the coil assembly was placed over the region of interest, and the coils were tuned and isolated at the 31P and 1H frequencies. 1H MR images of 10-mm thickness were obtained in nine slices in each of three dimensions and were used to localize signals in CSI (29). The NTPs (adenosine-, cytosine-, guanosine-, and UTPs) are nonspecifically designated NTP because they occur at the same frequency and cannot be distinguished in vivo.

In Vivo MRS Data Processing. In vivo MRS data were processed on a Sparc Station 2 (Sun Microsystems) computer, using programs created in our laboratory (17, 26). Sagittal, axial, and coronal MR images were created and overlaid with 8x8 grids indicating the 31P MRS voxel positions. The CSI spectra were plotted in 8 x 8 voxel arrays in each of three dimensions. The spectra from one or more voxels within the regions of interest were extracted and processed using NMR1 software (New Methods Research, Syracuse, NY). Peak areas were estimated by fitting to Gaussian lineshapes. Metabolite peak signal intensities were expressed as fractions of the total phosphorus signal. The pH was determined from the position of P relative to a-NTP and applying the Henderson-Hasselbalch equation (27). Peak assignments were based on the positions of known metabolites in high-resolution NMR spectra (28) and were confirmed by high-field 31P NMR studies of the extracts of sarcoma specimens. The frequency scale was expressed in parts/million (ppm) and was set by placing the center of the a-NTP peak at ~10.0 ppm; this peak was selected because its position is not affected by pH within the range of physiological values (27), and because it is present in all cases. PCr occurs as a contaminant from muscle either directly because of muscle within the voxel or indirectly as a "bleed" artifact associated with the phase-encoding used to localize signals in CSI (29). The NTPs (adenosine-, cytosine-, guanosine-, and UTPs) are nonspecifically designated NTP because they occur at the same frequency and cannot be distinguished in vivo.

In Vivo 31P MRS of Surgical Specimens. Between one and three biopsies (0.3-3.5 g) were obtained during surgery and were freeze-clamped within 10 min of removal of the tumor from the patient. Locations of the biopsies were selected to represent solid tumor, and evident necrotic regions were avoided.

Table 1 Characteristics of patients and histopathological review

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Site</th>
<th>Clinical status</th>
<th>MRS studies</th>
<th>Type</th>
<th>Grade</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>44</td>
<td>F</td>
<td>Thigh</td>
<td>New</td>
<td>in vivo</td>
<td>MFH</td>
<td>High</td>
<td>Cellular (P), focal necrosis</td>
</tr>
<tr>
<td>b</td>
<td>48</td>
<td>M</td>
<td>Thigh</td>
<td>Rec</td>
<td>+</td>
<td>Liposar</td>
<td>High</td>
<td>Cellular (Ro), myxoid matrix</td>
</tr>
<tr>
<td>c</td>
<td>67</td>
<td>F</td>
<td>Calf</td>
<td>New</td>
<td>+</td>
<td>Leiomyo</td>
<td>High</td>
<td>Cellular (Sp)</td>
</tr>
<tr>
<td>d</td>
<td>80</td>
<td>F</td>
<td>Calf</td>
<td>Rec</td>
<td>+</td>
<td>Leiomyo</td>
<td>High</td>
<td>Cellular (Sp)</td>
</tr>
<tr>
<td>e</td>
<td>65</td>
<td>F</td>
<td>Thigh</td>
<td>New</td>
<td>+</td>
<td>Leiomyo</td>
<td>High</td>
<td>Cellular (Sp)</td>
</tr>
<tr>
<td>f</td>
<td>73</td>
<td>F</td>
<td>Ankle</td>
<td>New</td>
<td>+</td>
<td>MFH</td>
<td>High</td>
<td>Cellular (Gn, Sp)</td>
</tr>
<tr>
<td>g</td>
<td>34</td>
<td>M</td>
<td>Paraspinal</td>
<td>Res</td>
<td>+</td>
<td>Undiff</td>
<td>High</td>
<td>Not available</td>
</tr>
<tr>
<td>h</td>
<td>21</td>
<td>F</td>
<td>Shoulder</td>
<td>New</td>
<td>+</td>
<td>Ewing's</td>
<td>High</td>
<td>Cellular (Ro)</td>
</tr>
<tr>
<td>i</td>
<td>75</td>
<td>F</td>
<td>Pelvis</td>
<td>New</td>
<td>+</td>
<td>Leiomyo</td>
<td>High</td>
<td>Cellular (P), focal fibrosis</td>
</tr>
<tr>
<td>j</td>
<td>63</td>
<td>F</td>
<td>Ankle</td>
<td>New</td>
<td>+</td>
<td>MFH</td>
<td>Interm</td>
<td>Cellular (P)</td>
</tr>
<tr>
<td>k</td>
<td>54</td>
<td>M</td>
<td>Sacrum</td>
<td>New</td>
<td>+</td>
<td>Chordoma</td>
<td>High</td>
<td>Variable: cells (P) and matrix</td>
</tr>
<tr>
<td>l</td>
<td>73</td>
<td>M</td>
<td>Thigh</td>
<td>New</td>
<td>+</td>
<td>Fibrosar</td>
<td>Low</td>
<td>Fibrous matrix, cells (Sp), focal hyaline</td>
</tr>
<tr>
<td>m</td>
<td>54</td>
<td>M</td>
<td>Arm</td>
<td>New</td>
<td>+</td>
<td>MFH</td>
<td>High</td>
<td>Not available</td>
</tr>
<tr>
<td>n</td>
<td>64</td>
<td>F</td>
<td>Thigh</td>
<td>New</td>
<td>+</td>
<td>Rhado</td>
<td>High</td>
<td>Cellular (P, Sp), muscle, focal necrosis</td>
</tr>
<tr>
<td>o</td>
<td>46</td>
<td>M</td>
<td>Thigh</td>
<td>New</td>
<td>+</td>
<td>MFH</td>
<td>High</td>
<td>Extensive necrosis, cells (P)</td>
</tr>
<tr>
<td>p</td>
<td>80</td>
<td>F</td>
<td>Shoulder</td>
<td>Rec</td>
<td>+</td>
<td>Liposar</td>
<td>Low</td>
<td>Mature fat, focal necrosis</td>
</tr>
<tr>
<td>q</td>
<td>72</td>
<td>F</td>
<td>Thigh</td>
<td>New</td>
<td>+</td>
<td>Liposar</td>
<td>Interm</td>
<td>Cells (P), fibrosis, focal hyaline</td>
</tr>
<tr>
<td>r</td>
<td>60</td>
<td>F</td>
<td>Flank</td>
<td>Rec</td>
<td>+</td>
<td>Liposar</td>
<td>Interm</td>
<td>Cells (P), fibrosis, focal hyaline</td>
</tr>
<tr>
<td>s</td>
<td>44</td>
<td>M</td>
<td>Arm</td>
<td>New</td>
<td>+</td>
<td>Rhado</td>
<td>High</td>
<td>Cystic with rim of sarcoma cells (myxoblast)</td>
</tr>
<tr>
<td>t</td>
<td>69</td>
<td>M</td>
<td>Thigh</td>
<td>New</td>
<td>+</td>
<td>Liposar</td>
<td>High</td>
<td>Variable: cells (Gn, Sp), myxoid, focal necrosis</td>
</tr>
<tr>
<td>u</td>
<td>65</td>
<td>F</td>
<td>Abdomen</td>
<td>Rec</td>
<td>+</td>
<td>Leiomyo</td>
<td>High</td>
<td>Extensive necrosis</td>
</tr>
<tr>
<td>v</td>
<td>77</td>
<td>F</td>
<td>Abdomen</td>
<td>New</td>
<td>+</td>
<td>Leiomyo</td>
<td>Interm</td>
<td>Cellular (Sp)</td>
</tr>
<tr>
<td>w</td>
<td>67</td>
<td>F</td>
<td>Retroper</td>
<td>New</td>
<td>+</td>
<td>Leiomyo</td>
<td>High</td>
<td>Cellular (Sp)</td>
</tr>
<tr>
<td>x</td>
<td>52</td>
<td>F</td>
<td>Thigh</td>
<td>New</td>
<td>+</td>
<td>MFH</td>
<td>High</td>
<td>Extensive necrosis</td>
</tr>
<tr>
<td>y</td>
<td>34</td>
<td>M</td>
<td>Thigh</td>
<td>Rec</td>
<td>+</td>
<td>NF</td>
<td>Low</td>
<td>Cellular (Sp)</td>
</tr>
<tr>
<td>z</td>
<td>54</td>
<td>M</td>
<td>Retroper</td>
<td>Rec</td>
<td>+</td>
<td>Liposar</td>
<td>Low</td>
<td>Mature fat</td>
</tr>
</tbody>
</table>

---

* Rec, recurrent; Res, resistant to chemotherapy.
* MFH, malignant fibrous histiocytoma; NF, neurofibrosarcoma; Liposar, liposarcoma; Leiomyo, leiomyosarcoma; undiff, undifferentiated; Fibrosar, fibrosarcoma; Rhado, rhabdomyosarcoma; Ewing’s, Ewing’s sarcoma.
* Gn, giant cells; Pl, pleomorphic cells; Ro, round cells; Sp, spindle cells; variable, variations from region to region.
* Needle aspiration biopsy.

2965

Downloaded from cancerres.aacrjournals.org on January 23, 2018. © 1996 American Association for Cancer Research.
The tissue was stored at —70°C until extraction. The tissue was extracted with the dual-phase extraction method of Tyagi et al. (30), modified for use with frozen tissue specimens. The frozen tissue was powdered with a Bio-Pulverizer™ (BioSpec Products, Bartlesville, OK) cooled in liquid nitrogen. The powder was homogenized with a Tissue-tearer™ (BioSpec Products) in 10 v/w of ice-cold methanol containing 0.4 mM phenylphosphonic acid as the quantitation standard. An equal amount of ice-cold chloroform was added, followed by homogenization, the addition of an equal amount of ice-cold distilled water, and re-homogenization. The phases were separated by centrifugation at 1000 × g for 10—60 min at 4°C, and the tissue residue was re-extracted as described above (except that the methanol did not contain the standard). The water phases were combined and dried on a rotating evaporator. The dried residue was redissolved in 6—9 ml of water, the pH was adjusted to 6.3, and the solution was freeze-dried. The freeze-dried residue was taken up in 400 μl D2O and 1.3 ml of buffer solution (88 mM N-[2-hydroxyethyl]-piperazine-N'-3-propane-sulfonic acid; Sigma Chemical Co.) containing 44 mM trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (Aldrich Chemical Co.). The pH was adjusted to 8.0. The buffer prevented the pH changes (of up to 0.5 pH units) that would otherwise have occurred during the data acquisition.

$^3$P NMR spectra were obtained at 162 MHz on a 9.4-Tesla Bruker AM 400 spectrometer (Bruker, Karlsruhe, Germany) at 25°C. A 16 K free-induction decay was acquired in 1.02 s using a 45 degree pulse (8.6 μs), broadband composite-pulse $^1$H-decoupling, and a total repetition time of 2.7 s. Several acquisitions with 1000 or 2500 transients were accumulated. The total number of transients varied between 1000 and 25000, depending on the size of the sample and the signal intensity. The free-induction decays were multiplied with a Lorentz-Gaussian function (Bruker parameters: LB, —¿ 1; GB, 0.01), were zero filled to 32 k, and were Fourier-transformed. After baseline correction, the resonances of the metabolites of interest were quantified by fitting Gaussian lines, using WIN-NMR software (Bruker). The resonance areas obtained were recalculated to yield μmol metabolite/g tissue. The chemical shifts were referenced to that of GPChol at 0.49 ppm.

RESULTS

The influence of 1H-decoupling and NOE enhancement on the in vivo $^3$P MR spectrum is observed by comparing nonlocalized coupled and decoupled spectra from a thigh containing a malignant fibrous histiocytoma (case a; Fig. 1). Two separate components in each of the PME and PDE regions are resolved, and the signal intensities of many of the peaks (especially PME, NTP, and PCR) are increased, resulting in an increased signal:noise ratio. The method by which we obtained MRI-directed $^3$P MR spectra localized in three dimensions using CSI is illustrated in Fig. 2. The benefit obtained by localizing with three-dimensional CSI is evident when one compares the localized spectrum in Fig. 2C to the nonlocalized spectra from the same tumor (Fig. 1). In the localized spectrum, there is a nearly complete elimination of the intensity of the PCR signal from surrounding muscle.

The spectrum localized to the sarcoma in Fig. 2C has strong signals from the PMEs (PEth and PChol) and PDE signals at 0.5 and —¿0.3 ppm but no detectable signal from GPEth at 1.0 ppm. The nonlocalized $^1$H-decoupled spectrum in Fig. 1 contains the same two PDE signals. The stronger signal near 0.5 ppm is at the position of GPChol, but the weaker signal at —¿0.3 ppm is unidentified. In the spectrum localized to the sarcoma in Fig. 2C, the unidentified signal is stronger (hence from the sarcoma), whereas the GPChol signal is weaker, as...
Fig. 3. 31P MR spectrum from the water-soluble extract of a portion of the surgical specimen of a leiomyosarcoma (case d): 1, α-glycerol phosphate; 2, unassigned PME; 3, IMP/GMP; 4, AMP; 5, unassigned PME; 6, α-phosphate of NDP-hexoses and NDP-e (e.g., NAD); 7, CDP-choline; 8, β-phosphate of NDP-hexoses. Inset, the in vivo 31P MR spectrum obtained before surgery. The signal cutoff (top) is PCr from muscle contaminating the voxel. U, an unidentified PDE signal at ~0.3 ppm, which did not appear in the extract spectrum.

Fig. 4. 1H-decoupled, NOE-enhanced 31P NMR spectra from each of 16 sarcomas. Spectra are lettered according to the case designations in Table 1 and were plotted so that the height of the dominant PME peak is the same in all spectra (except those with high PCr). In processing, the spectra were line-broadened by a 3–6-Hz Gaussian filter.

expected (18), from muscle. The strong signals from the NTPs lead us to infer that a significant part of this sarcoma is composed of metabolically viable cells and is therefore relatively well perfused.

The in vitro 31P spectrum of the water-soluble extract of a leiomyosarcoma (case d) is shown in Fig. 3, and the in vivo 31P spectrum obtained from this sarcoma before surgery is shown in the inset. The in vivo spectrum is quite similar to the spectrum from a malignant fibrous histiocytoma shown in Fig. 2C: the relative signal intensities of the PMEs, the P1, and the NTPs are essentially the same. However, the PDE regions differ in that the spectrum in the inset in Fig. 3 has a smaller signal from the unknown metabolite at ~0.3 ppm and a higher signal at the position expected for GPChol. The in vitro spectrum confirms the in vivo metabolite assignments. It shows that the highest signals within the PME region are from PEth and PChol, that GPChol is present as observed in vivo, and that the unknown PDE metabolite observed in vivo does not appear in the extract spectrum. The relative signal intensities in the extract spectrum differ from the in vivo ones in part because of the different spin-lattice relaxation times at different magnetic fields, and because of the different degrees of signal saturation caused by the shorter repetition time used in vivo (1 s) than in vitro (2.7 s).

We have obtained 1H-decoupled, NOE-enhanced 31P MR spectra localized to each of 20 soft tissue sarcomas in vivo. Sixteen spectra contained 31P signals and were evaluable. These are displayed in Fig. 4. Eleven spectra (a-k) were of high quality, with high signal:noise ratios and good or excellent resolution of adjacent peaks. Nine of the sarcomas from which these spectra were obtained were examined histologically, and all nine were predominantly or entirely cellular (Table 1). Five spectra (l-p) were of poorer quality but were adequate to quantitate the PME, P1, and, in some cases, the unidentified mobile metabolite.
PDE (asterisk) peak intensities. These spectra were from sarcomas that contained large amounts of matrix or necrosis relative to cells (Table 1). Four spectra (data not shown in Fig. 4) contained little or no signals above the noise level. In three of these spectra, the explanation for the lack of signals was evident when the tumors were surgically resected (Table 1). One (case q) was a low grade liposarcoma composed of mature fat and fibrous tissue with only scattered cells. The second (case r) contained a large fraction of fibrotic matrix and hyalinized areas relative to the fraction of cells. The third (case s) contained predominantly a large cystic region that in the MRI was not distinguished from a rim of viable tumor found at surgical resection. In retrospect, this tumor had undergone extensive partial resection at the time it was biopsied. In the fourth case (t), subsequent surgical resection showed large regions of myxoid matrix and focal necrosis.

With the exception of p, the 16 spectra displayed in Fig. 4 have two major features in common: (a) prominent signal intensities in the PME region, with the signal intensity of PEth (4.2 ppm) greater than that of PChol (3.8 ppm); and (b) prominent NTP signals. The PDE region is more variable among these spectra. Seven spectra (a, b, i, m, n, o, and p) contain a prominent signal (asterisk) at approximately -0.3 ppm. Four spectra (d, e, f, and i) have signals at the position expected for GPChol (0.49 ppm).

The metabolic characteristics of soft tissue sarcomas derived from the 15 spectra (from cases a-o) in Fig. 4 are summarized in Table 2. The results are expressed as fractions of total 31P signal after excluding the contaminating signal from muscle PCr in those cases in which it was present. The mean PME signal intensity is 21% of the total phosphorus signal, and in cases in which PChol as well as PEth is resolved, the PEth:PChol peak height ratio is 2.1. The mean signal in the PDE region is 17% of the total but has the greatest variability in accord with the impression gained in the presentation of the cases in Figs. 2 and 3. The mean P\textsubscript{i} signal is 10% of the total and occurs at positions corresponding to a mean pH of 7.26. The NTPs account for the remaining 52% of the total signal.

In vitro 31P spectra from the extracts of surgical specimens of four leiomyosarcomas are shown in Fig. 5. These include the case (d) in which an in vivo spectrum (d) was also obtained (Fig. 3). From a qualitative standpoint, these spectra, as well as those (data not shown) from a malignant fibrous histiocytoma (case x) and a neurofibrosarcoma (case y), have features in common with one another and with the in vivo spectra. These features include prominent PME signals with a higher concentration of PEth than PChol, very low concentrations of GPEth, variable concentrations of GPChol (but in all cases lower than that of PChol), and the presence of NTPs. The spectra from extracts of four liposarcomas, on the other hand, had much more variable features (Fig. 6). Two spectra (b and r) did have greater concentrations of PEth than PChol as well as high concentrations of NTPs, like the in vivo spectra (Fig. 4) and the extract spectra from leiomyosarcomas (Fig. 5). These two liposarcomas, however, differed by having very high concentrations of GPChol. In case r, the in vivo spectrum (r)
they appeared solid and viable, whereas the in vivo spectra obtained from 27-cc voxels are from signals averaged over heterogeneous regions within the tumors; and (d) the ability to quantitate the in vivo metabolite signals only by reference to the total phosphorus signal or to one another; actual concentrations of the metabolites could vary considerably among the tumors. The extract spectra give the opportunity to examine the extent of inter- and intratumoral heterogeneity both qualitatively and quantitatively because molar concentrations are obtained. Concentrations of the major metabolites, summarized in Table 3, reveal a marked heterogeneity among tumors. Even among leiomyosarcomas, which qualitatively have similar spectra (Fig. 5), the concentration ranges span an order of magnitude. The total metabolite concentrations varied from 0.3–6.1 μmol/g tissue and the NTP + NDP from 0–1.5 μmol/g tissue. The tissue metabolite concentrations are primarily a function of cellularity; all five surgical specimens with above-median total metabolite concentrations were entirely or predominantly cellular (case d, r, v, w, and y in Table 1); whereas all five with below-median total metabolite concentrations were not. Cases u and x had extensive necrosis, and case z was predominantly mature fat. Case p varied from region to region and also had a very low signal:noise ratio in vivo (Fig. 4). In case b, the low metabolite concentrations seem to contradict the in vivo study that produced a high-quality spectrum (Fig. 4). The histological examination provided an explanation for this discrepancy (Table 1). Case b was a recurrent myxoid liposarcoma with transformation to a high-grade, round cell liposarcoma; some regions were predominantly myxoid matrix, some were entirely cellular, and some were mixed. The specimen used to obtain the extract was from a myxoid region, whereas the in vivo spectrum contains signals averaged over a large part of the tumor.

Data indicating the extent of intratumoral heterogeneity are summarized in Table 4. Each of two samples from cases u and v were quite similar. In the other cases, metabolite concentrations varied by a factor of two or more. The heterogeneity of metabolite concentrations evident in the data in Tables 3 and 4 was not simply due to variable cellularity because different metabolites were affected to different degrees. For example, there was no correlation at all between the concentrations of the NDP-hexoses and those of NTP + NDP among the 10 cases (Table 3).

An unidentified PDE signal at ~0.3 ppm was evident in seven in vivo spectra (a, b, i, m, n, o, and p in Fig. 4). All of the sarcomas containing this signal were high-grade. Histological examination was available in six cases: five had pleomorphic cells, and one was in transformation to a round cell liposarcoma (Table 1). This PDE signal was not observed in any of the spectra obtained from extracts of surgical specimens (e.g., Fig. 3).

The signals from NDP-hexoses were obtained in the extract spectra in most (8 of 10) cases. As indicated in Fig. 3, their α-phosphate resonances appear around ~10.5 to ~11 ppm, and their β-phosphate

### Table 3 Concentrations of major metabolites (μmol/g) in soft tissue sarcomas

<table>
<thead>
<tr>
<th>Case</th>
<th>Dx</th>
<th>PEth</th>
<th>PC Chol</th>
<th>GP Eth</th>
<th>GPC Chol</th>
<th>CDP-chol</th>
<th>IMP/GMP</th>
<th>AMP</th>
<th>NTP + NDP</th>
<th>NAD</th>
<th>NDP-hex 4</th>
<th>NDP-hex 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>Leio</td>
<td>1.14</td>
<td>0.47</td>
<td>0.06</td>
<td>0.36</td>
<td>0.08</td>
<td>0.27</td>
<td>0.04</td>
<td>1.05</td>
<td>0.11</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>u</td>
<td>Leio</td>
<td>0.17</td>
<td>0.03</td>
<td>0.02</td>
<td>0.06</td>
<td>0.01</td>
<td>0.07</td>
<td>0.10</td>
<td>0.25</td>
<td>0.05</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>v</td>
<td>Leio</td>
<td>2.22</td>
<td>0.62</td>
<td>0.02</td>
<td>0.05</td>
<td>0.07</td>
<td>0.77</td>
<td>0.07</td>
<td>1.45</td>
<td>0.29</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>w</td>
<td>Leio</td>
<td>1.91</td>
<td>0.37</td>
<td>0.08</td>
<td>0.13</td>
<td>0.07</td>
<td>0.47</td>
<td>0.04</td>
<td>0.47</td>
<td>0.19</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>b</td>
<td>Lipo</td>
<td>0.14</td>
<td>0.04</td>
<td>0.04</td>
<td>0.33</td>
<td>bd</td>
<td>0.19</td>
<td>0.01</td>
<td>0.54</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
</tr>
<tr>
<td>p</td>
<td>Lipo</td>
<td>bd</td>
<td>bd</td>
<td>0.24</td>
<td>0.82</td>
<td>0.04</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
<td>0.11</td>
<td>0.16</td>
<td>0.31</td>
</tr>
<tr>
<td>r</td>
<td>Lipo</td>
<td>1.01</td>
<td>0.09</td>
<td>0.27</td>
<td>0.62</td>
<td>0.10</td>
<td>0.18</td>
<td>0.08</td>
<td>1.14</td>
<td>0.27</td>
<td>0.16</td>
<td>0.28</td>
</tr>
<tr>
<td>z</td>
<td>Lipo</td>
<td>0.06</td>
<td>0.02</td>
<td>0.02</td>
<td>0.15</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
<td>bd</td>
</tr>
<tr>
<td>x</td>
<td>MFH</td>
<td>0.18</td>
<td>0.06</td>
<td>0.06</td>
<td>0.22</td>
<td>bd</td>
<td>0.02</td>
<td>0.02</td>
<td>0.43</td>
<td>0.10</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>y</td>
<td>NF</td>
<td>1.20</td>
<td>0.09</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
<td>0.32</td>
<td>0.10</td>
<td>0.69</td>
<td>0.10</td>
<td>0.08</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* Dx, diagnosis; Leio, leiomyosarcoma; Lipo, liposarcoma; MFH, malignant fibrous histiocytoma; NF, neurofibrosarcoma.

* bd, below detection limit.
resonances appear around \(-12\) to \(-13\) ppm. Although five different NDP-hexoses were found, the two labeled NDP-hexose 4 and NDP-hexose 5 were detected most frequently, were present in the highest concentrations, and (from their positions) are tentatively assigned to UDP-N-acetyl-galactosamine and UDP-N-acetylgalactosamine, respectively (31). Their concentrations, which were included in Tables 3 and 4, did not correlate with any particular tissue type or histological characteristic.

**DISCUSSION**

By implementing \(^1\)H-decoupling and NOE enhancement in conjunction with dual-tuned surface coils, adequate shimming to obtain good homogeneity of the magnetic field within regions of interest, and accurate localization of \(^{31}\)P MR spectra to regions of interest using three-dimensional CSI, we have been able to obtain more information about the in vivo metabolic characteristics of soft tissue sarcomas than has heretofore been available. We confirm the high PME signal intensity and the slightly alkaline pH reported previously in sarcomas (1, 3–6, 8–15). In addition, we show that the PME region is dominated by PEth over PChol, and that the PDE region contains no detectable GPEth and only occasionally detectable GPChol. That GPEth would be detected in \(^1\)H-decoupled \(^{31}\)P MR spectra if it were present in significant quantities has been demonstrated in the brain (17) and liver (19). The absence of detectable GPEth and PEth was observed by Bachert et al. (15) in a malignant fibrous histiocytoma in the only previously reported study of a human sarcoma in vivo using \(^1\)H-decoupled \(^{31}\)P MRS. Redmond et al. (4) found the same characteristics in an extract of surgical tissue from one patient with a malignant fibrous histiocytoma, and, interestingly, Evanochko et al. (2) observed almost exactly the same spectrum in an extract of a murine RIF-1 sarcoma. We have confirmed the essence of these anecdotal observations in our high-resolution 3D MR studies of tissue extracts from 10 sarcomas, 4 of which were studied in vivo. Several sarcomas had in their in vivo spectra a prominent mobile PE signal from an unidentified metabolite. Because it did not appear in the water-soluble extracts of sarcoma surgical specimens, it may be associated with either an abnormally mobile phospholipid moiety or, conceivably, the fragments of nucleic acids. The in vitro studies revealed marked variations among sarcomas in the actual concentrations of metabolites. To a great extent, this correlated with the relative amounts of cells and of noncellular regions (matrix or necrosis) within the tumor.

By using appropriate surface coil designs, we have managed to study sarcomas in several different anatomic sites. Nevertheless, the application of \(^{31}\)P MRS remains limited by the inability to access sarcomas deeper than 10–12 cm from the surface and the difficulty in studying sarcomas smaller than approximately 3-cm diameter. In most cases, it is necessary to acquire four averages of the CSI dataset to ensure high enough signal relative to noise to obtain spectra of the quality typical of those shown in Fig. 4. The variations in spectral quality among evaluable spectra are likely to be determined primarily by variations in the relative amounts of malignant cells and matrix or necrosis within them. Some sarcomas (4 of 20 in this series) have so much matrix or so much spontaneous necrosis that they do not contain enough viable cells to produce an evaluable spectrum. The histological heterogeneity that characterizes many sarcomas is associated with metabolic heterogeneity, as we have documented in cases in which two or three specimens could be obtained from the same surgical specimen.

The prominent NTP signals in vivo in 14 of our cases suggest that part of the solid fraction of many sarcomas in vivo contains viable cells with well-preserved energy metabolism. The alkaline intracellular pH (mean 7.26) in sarcomas might also be taken as an indication of well-preserved energy metabolism. However, a similar pH measured by \(^{31}\)P MRS seems to be typical of many other types of human cancers, some of which are expected to be uniformly well perfused (e.g., lymphomas; Ref. 20), and some of which are not (32). Although the pH is slightly acidic relative to the plasma pH (7.4), it is more alkaline than the pH measured using \(^{31}\)P MRS in muscle (7.10) and in the brain (7.05). This observation in cancers contradicted expectations that cancer cells might be more acidic because of the production of lactate from excessive glycolysis, and its possible mechanism and significance have been discussed and debated by a number of authors (11, 33–36).

Our observations are relevant to the development of experimental sarcoma models because our results indicate that some experimental models have phospholipid metabolite patterns that differ from those of sarcomas in vivo in patients. For example, although RIF-1 tumors growing in the mouse had \(^{31}\)P MR spectra remarkably similar to those we observed in patients (including a predominance of PEth over PChol; Ref. 2), RIF-1 cells in culture contained mostly PChol (2). This situation arises partly because the levels of PEth and PChol in cultured cells can be markedly affected by the concentrations of choline and ethanolamine in the medium as well as by its pH (31, 37–40). We believe that our observations should help guide the development of experimental models that have metabolic characteristics similar to those that occur in vivo in patients.

The mechanisms underlying the pattern of phospholipid metabolites we observed in sarcomas are unknown. The general pattern, however, is very similar to that observed using \(^1\)H-decoupled \(^{31}\)P MRS in non-Hodgkin’s lymphomas (20) and in a variety of cancer surgical specimens studied by high-field \(^{31}\)P NMR spectroscopy (2, 41–46). Based on reports of similar patterns in experimental cancer models (47, 48), we have hypothesized that the high concentration of PEth relative to PChol, occurring in the presence of a low concentration of GPEth, is in part a manifestation of a sustained activation of a phosphatidylethanolamine-specific phospholipase D (20).

Shinkwin et al. (14) suggested that the amounts of PME and PDE...
relative to NTP in $^{31}$P MRS might distinguish low and high grade soft tissue sarcomas. This result was not statistically significant, however, and the study was limited by poor spectral resolution. Improvements in acquisition of $^{31}$P MR spectra, as outlined in this report, should permit a rigorous test of the ability of MRS to grade sarcomas according to their metabolic characteristics. Because the histological grade remains one of the most reliable (49, 50) potential markers of biological or clinical aggressiveness of a soft tissue sarcoma, it would be interesting to explore the ability of MRS to provide metabolic markers that correlate with histological grade. Our results to date do not indicate different metabolic features in $^{31}$P MRS among different grades of sarcomas or among non-Hodgkin’s lymphomas (20), except that the unidentified PDE signal occurred only in high-grade sarcomas.

The significance of the presence of NDP-hexoses in sarcomas is uncertain. UDP-hexoses play a role in the synthesis of glycoproteins, glycolipids, and proteoglycans. UDP-N-acetylgalactosaminе and UDP-N-acetylgalactosamine, in particular, are involved in the anabolism of proteoglycans that are constituents of the extracellular matrix. It would not be surprising, therefore, that NDP-hexoses are present in sarcoma cells that have a mesenchymal origin, although we found no evident correlation with histological features.

Characteristics in $^{31}$P MR spectra may correlate with the sensitivity or resistance of an individual patient’s sarcoma to treatment. Koutcher et al. (3) found a higher PME:PDE ratio in the baseline spectra of three sarcomas that responded to chemotherapy than in three sarcomas that did not. Sostman et al. (5) found that the mean pH (7.30) of 10 soft tissue sarcomas that responded to treatment with neoadjuvant radiotherapy and hyperthermia was higher than the mean pH (7.16) of the 10 sarcomas that did not. Sijens et al. (6) found that an early decrease in PME correlated with response (as measured by the fraction of necrosis at surgery performed 2 months later) in 11 extremity sarcomas treated with TNF-α + melphanal via isolated limb perfusion, and that an early decrease in PDE correlated with the extent of measurable shrinkage of the sarcoma. Other investigators (3, 4, 9, 51) obtained baseline and follow-up $^{31}$P MR spectra in 11 sarcomas treated with chemotherapy: all 7 sarcomas that eventually responded had a decrease in the PME signal intensity in follow-up spectra obtained within 2 weeks after initiation of treatment, whereas none of the 4 that failed to respond to treatment did so. On the other hand, an increased PME:NTP ratio after the first hyperthermia treatment correlated with the eventual response of soft tissue sarcomas to radiation and hyperthermia as measured by the fraction of necrosis in the surgical specimen (1). These reports suggest that early metabolic changes occur consistently in sarcomas destined to respond to treatment, but that the particular metabolic event may depend upon the nature of the treatment. This phenomenon could be useful in the clinical management of patients. Because over 50% of patients with advanced, recurrent, or metastatic soft tissue sarcomas fail to respond to chemotherapy, and only 10% have complete responses (52), the ability to identify patients whose sarcomas are going to be resistant to treatment is likely to be cost-effective. The technique we report here should permit a more rigorous test of the hypothesis that metabolic changes predict sensitivity of a sarcoma to a particular treatment. Using this technique, we have recently shown that early treatment-induced decrease in PDEs specifically correlates with the eventual response of non-Hodgkin’s lymphomas to treatment (53). We believe that the study reported here provides a good technical basis on which to examine potential clinical uses of $^{31}$P MRS in the management of soft tissue sarcomas as well.
Metabolic Characterization of Human Soft Tissue Sarcomas \textit{in Vivo} and \textit{in Vitro} Using Proton-decoupled Phosphorus Magnetic Resonance Spectroscopy

Chun-Wei Li, Annette C. Kuesel, Kristin A. Padavic-Shaller, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/13/2964

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/56/13/2964. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.