ABSTRACT

Development of biological and clinical uses of in vivo 31P magnetic resonance spectroscopy has been hampered by poor anatomic localization of spectra and poor resolution of overlapping signals within phospholipid and phosphodiester regions of the spectrum. We applied 1H-decoupling and nuclear Overhauser enhancement to improve resolution of 31P magnetic resonance spectra accurately localized to 21 non-Hodgkin's lymphomas (NHL) by using three-dimensional chemical shift imaging. All 21 spectra had large phosphomonoester signals (26% of total phosphorus) that contained high amounts of phosphoethanolamine relative to phosphocholine. There were no signals from glycerophosphoethanolamine or glycerophosphocholine but only a broad signal from membrane phospholipids in the phosphodiester region (20% of phosphorus). Prominent nucleoside triphosphates (47% of phosphorus) and low inorganic phosphate (7% of phosphorus) indicate well-perfused tissue with viable cells. Mean intracellular pH was 7.23. These characteristics were similar in all grades and stages of NHL. By analogy with recently reported studies in cell lines in vitro, we hypothesize that the pattern of phospholipid metabolites observed in NHL in vivo is partly a manifestation of sustained activation of phospholipase C or D. The techniques we implemented permitted us to obtain more information about in vivo metabolism of NHL than has heretofore been available. This information is important for the establishment of appropriate experimental models and provides a basis from which to examine potential clinical uses of 31P magnetic resonance spectroscopy.

INTRODUCTION

NMR spectroscopy (MRS) provides the ability to examine some aspects of metabolism in vivo in a noninvasive manner. A 31P NMR spectrum contains information about the amounts of phospholipid metabolites, NTP, and energy-related metabolites, as well as a means to measure intracellular pH. 31P NMR spectra of more than 200 human cancers in vivo have been reported, and results were reviewed recently (1). Cancers of a variety of tissue types typically had strong signal intensities in the PME and PDE regions and little or no signals from PCr. The mean pH, determined from the position of the P1 signal on the frequency axis, was approximately 7.25.

Studies in human cancer cell lines and transplanted murine tumors indicate that the nature and concentrations of PME and PDE phospholipid metabolites are related to cell proliferation and tumor growth (1), tumor cell death (2), and treatment sensitivity and resistance (3-5). However, the specific results of these studies have often been contradictory, and the concentrations of phospholipid metabolites are modulated by experimental conditions (6-12), making the relevance of experimental models to human cancers unclear. Therefore, it is important to know which metabolic characteristics occur in vivo in human cancers, not only to determine how closely metabolism in experimental models is analogous to metabolism in cancers in patients in clinical settings, but to pave the way for studies of clinical uses of MRS (1).

The development of potential biological and clinical uses of in vivo 31P MRS has been hampered by a number of technical limitations: (a) poor anatomic localization of the NMR signals results in contamination of the spectra by signals from tissues surrounding the region of interest. This situation is a problem particularly for cancers occurring within organs, such as liver, spleen, and brain, that contain large amounts of PMEs and PDEs. Poor localization introduces uncertainty in pH measurements because the position of P1 may be taken relative to a metabolite (e.g., PCr) that is not actually contained within the cancer; and (b) poor resolution of overlapping signals makes it impossible to distinguish individual components within the PME region (e.g., PEth and PChol) and PDE region (e.g., GPEth and GPChol) or to distinguish GPEth and GPChol from immobile PDEs within phospholipids. One reason for the poor resolution of these metabolites is broadening of the 31P signals by coupling between the magnetic fields of 31P nuclei and those of nearby protons. This broadening may be eliminated by radiofrequency irradiation of protons during acquisition of the 31P signal with the use of a 1H-decoupling technique, which is now feasible for use in vivo in human subjects (13). In addition, irradiation of protons between acquisitions can increase the 31P signal by NOE enhancement.

The combination of 1H-decoupling and full NOE enhancement in vivo was recently implemented in 31P MRS studies of brain (14). We report the use of this technique, in conjunction with means to optimize the magnetic field homogeneity within the region of interest (shimming), to improve resolution within the PME and PDE regions of the spectrum in patients with NHL. We used MRI-directed, three-dimensional CSI to accurately localize 31P NMR spectra to regions of interest (15, 16). To permit application of these techniques in various anatomic sites, we constructed dual-tuned (31P, 1H) surface coil arrangements. This approach enabled us to obtain more information about the in vivo metabolic characteristics of NHL than has heretofore been available. The results provide stronger bases for the establishment of appropriate experimental models of NHL and for the study of potential clinical uses of 31P MRS in this disease.

MATERIALS AND METHODS

Patient Population. Eligibility for study required a biopsy-proven diagnosis of NHL, a lymphoma-containing lymph node or mass of approximately 3-cm diameter or larger located within 10 cm of the surface of the body, absence of the standard contraindications to MRI, and signed informed consent as approved by the Institutional Review Board. One patient (case f) was unable to tolerate the full 1.5-h study, but a high-quality nonlocalized spectrum was
obtained and is included in this report. Of 21 patients, 13 were newly diagnosed and previously untreated, and 8 were recurrent after previous treatment. Characteristics of the patients and their lymphomas are summarized in Table 1.

Dual-tuned Surface Coils. Surface coils were constructed to receive NMR signals from lymphomas in superficial lymph node, anterior mediastinum, abdomen, and pelvis. They included a 12- or 15-cm-diameter circular single-turn 31P coil of copper tubing underlying a 22 x 14 cm butterfly 1H coil of 1-cm-wide copper foil. The 1H coil has two 11 x 14 cm rectangular elements in a figure-eight configuration with a common central conductor. It operates in a counterrotating fashion with currents moving in the same direction through the central conductor. To access spleen or liver, a 15-cm-diameter circular 31P coil was combined with a 22 x 18 cm rectangular 1H coil. The 1H coil was curved on a 30-cm radius to permit apposition of the coil assembly to the side of the trunk overlying the liver or spleen to optimize the region amenable to MRI and 1H decoupling. Each 31P coil and its companion 1H coil were packaged together in a Flexiglass frame. The 31P coil, tuned to 25.7 MHz, was used to excite the 31P nuclei and to receive NMR signals from them. The 1H coil, tuned to 63.6 MHz, was used to perform MRI in three dimensions, shrink the static magnetic field to optimize its homogeneity in the region of interest, and conduct 1H decoupling and NOE enhancement of the 31P NMR spectra.

Hardware and Decoupling. Studies were performed at a static magnetic field strength of 1.5 Tesla in a Siemens Magnetom clinical image/spectrometer (Siemens AG, Erlangen, Germany). An additional radiofrequency channel, described elsewhere (14), was constructed to provide excitation of the 1H surface coil. Broadband 1H decoupling was accomplished with the use of Waltz-4 modulation centered 1 ppm downfield from the water proton at 16 W power for 256 ms during acquisition of 31P. NOE enhancement was accomplished by delivering continuous-wave, low-level power (2 W) for 744 ms between acquisitions. The total transmitted power during a 1H-decoupled, NOE-enhanced acquisition was 5.6 W. The transmitter line attenuation was 1 decibel, and the loaded Q value was 0.5 that of the unloaded value such that the power delivered by the coil was 2.2 W. The calculated average SAR was 1 W/kg within the sensitive volume of the 22 x 14 cm coil and 1.2 W/kg within the sensitive volume of the curved 22 x 18 cm coil. The actual average tissue SAR was less than the calculated SAR because the coils were placed 2.1 cm from the surface of the skin.

Procedures for 31P MRS. 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. MR images of 10-mm thickness were obtained in nine slices in each of three dimensions with the use of a gradient-echo technique (17) with a repetition time of 310 ms and an echo time of 15 ms. The images were used to define and, when necessary, adjust the coil position relative to the lymphomatous mass and, later, to guide registration of the CSI voxels with regions of interest. The magnetic field was then shimmed on the proton signal to optimize local static magnetic field homogeneity. This was initially performed by manual adjustment of lower- and higher-order shim currents and later by automatic adjustment of these currents with the use of a recently developed autoshimming algorithm based on three-dimensional 1H CSI (18, 19). Shimming typically resulted in nonlocalized water proton peak line widths at a half-height of between 20 and 40 Hz. Approximately 10 min was devoted to shimming, because it is necessary to achieve adequate homogeneity of the static magnetic field within the region of interest to realize the benefit (i.e., improved metabolite peak resolution) of 1H decoupling of the 31P spectra.

The 31P transmitter voltage was optimized for a 45° pulse angle within the center of the lymphoma to be studied with the use of the known magnetic field plot of the coil, along with a triphenylphosphate reference signal from a small sample placed near the center of the 31P coil. The carrier frequency was set between the γ and α-NTP peaks. Nonlocalized 31P NMR spectra were obtained from free induction decays with and without 1H decoupling and NOE enhancement with the use of a rectangular 250-μs pulse, 512 points, and 64 acquisitions with 1-s repetition times. The nonlocalized free induction decays were obtained after the same delay (0.8 ms) required for phase-encoding gradients in the CSI acquisitions to permit use of the nonlocalized spectrum to obtain phase-correction parameters applicable to the CSI data sets. Three-dimensional CSI was then performed in an 8 x 8 x 8 array of cubic voxels of 16 to 64 ml (usually 27 ml), depending on the size of the mass. A 45° rectangular 250-μs excitation pulse was followed by 0.8-ms triangular phase-encoding gradients applied simultaneously in three dimensions and then acquire the data in 8 x 8 x 8 grids indicating the 31P MRS voxel positions. The CSI data were Fourier transformed in the three spatial dimensions and in the time dimension and were phase corrected with the use of parameters obtained in the processing of the nonlocalized spectrum. The CSI spectra were plotted in 8 x 8 arrays in each of three dimensions. On the basis of examination of the spectra and the corresponding images, the data analysis was repeated; the voxels were shifted to center them on regions of interest. The CSI data from one or more voxels within regions of interest were extracted and processed with the use of NMR software (New Methods Research, Syracuse, NY). A filter was applied to give 3–6-Hz line broadening. The baseline was straightened with the use of the NMRI semiautomatic baseline deconvolution software. Peak areas were estimated by fittng to Gaussian line shapes; Gaussian functions were chosen because, in general, they gave a smaller residual error in the fitting than did Lorentzian line shapes. Metabolite peak signal intensities were expressed as fractions of the total phosphorus signal. The pH was determined from the position of Pγ relative to αNTP and by applying a Henderson-Hasselbalch relation (20).

Assignment of Metabolites to Peaks in the 31P NMR Spectrum. Peak assignments are based on the positions of known metabolites in high-resolution NMR spectra (21). The frequency scale was expressed in ppm and was set by placing the center of the α-NTP peak at ~10 ppm; this peak was selected because its position is not affected by the pH within the range of physiological values (20) and because it is present in all cases. Peaks visible in vivo 1H-decoupled, NOE-enhanced 31P NMR spectra are illustrated in the CSI-located magnet. Signals from regions of interest in the liver, spleen, and, later, to guide registration of the CSI voxels with regions of interest. The CSI data were processed on a Sparc Station 2 (Sun Microsystems, Mountain View, CA) computer with the use of programs created in our laboratory (14, 16). The coupled and decoupled nonlocalized free induction decays were Fourier transformed, phase corrected, and printed.

RESULTS

The procedure by which we obtain MRI-directed 31P NMR spectra localized in three dimensions with CSI is illustrated by the study in the low grade NHL in the right inguinal region in patient a (Fig. 2). 1H decoupling and NOE enhancement have two major effects on the 31P
within liver (A) and spleen (B) of normal human subjects. U, an unidentified PDE signal.

NMR spectrum (Fig. 3): (a) the separate components of the PME region become resolved; and (b) the signal intensities of the PME, PCr, and NTP peaks are increased, resulting in an increased signal:noise ratio. We assign the components of the PME region to PEth and PChol based on their positions on the ppm axis and their documentation in $^{31}$P NMR studies of extracts of biopsy specimens of lymphomas (29). The small amount of PCr in the lymphoma spectrum is contamination, resulting primarily from the bleeding of signals from muscle in adjacent voxels. There is only a broad signal in the PDE region, and there are no signals from GPEth, GPChol, or other mobile PDEs. That these PDEs would be observed in the $^1$H-decoupled $^{31}$P NMR spectrum were they present is shown in the spectrum of normal liver in Fig. 1A.

$^1$H-decoupled, NOE-enhanced $^{31}$P NMR spectra from adjacent voxels within enlarged femoral lymph nodes containing a low-grade NHL in patient b are shown in Fig. 4. These spectra are remarkably similar to one another, compatible with a uniformity of the metabolic characteristics within this lymphoma. These characteristics, a high ratio of PEth to PChol within the PME region, a broad component only within the PDE region, and prominent NTPs, are quite similar to those in the lymphoma in patient a shown in Figs. 2 and 3.

$^1$H-decoupled, NOE-enhanced $^{31}$P NMR spectra localized to each of the 21 NHLs are shown in Fig. 5, where they are lettered according to Table 1. Spectra a–r are from lymphomas in tissues or organs other than spleen, and spectra s–u are from spleens infiltrated with lymphoma. The spectra are scaled so that the peak in the PME region has the same height in all spectra, because this was the tallest peak in all but two spectra. The spectra vary in intensities of signal relative to noise and in the resolution of one peak from an adjacent peak. Spectra a–l, s, and t have moderate-to-high signal:noise ratios and good peak resolution. Spectra p, q, r, and u have the lowest signal:noise ratio and poorest peak resolution. Spectra p and q were obtained from lymph nodes in the neck early in the study, when we did not have an optimal coil size to access them, and, for spectrum q, only two acquisitions could be obtained; spectrum r was from a deep mesenteric lymphoma that was poorly defined anatomically; and spectrum u was from a splenic lymphoma that had produced a large central necrotic region. These factors, as well as our inability to shim the magnetic field well in these four cases, may account for their low signal:noise ratio and poor resolution. In addition, spectra p and q were both from high-grade NHLs; these are more likely than are intermediate- or low-grade NHLs to have necrotic foci (30), which can result in a low signal:noise ratio because of a small fraction of viable cells within the tumor.

An index of the accuracy of localization of the spectra to the lymphomas is obtained by examining the intensity of the contaminating signal from PCr, because most lymphomas are adjacent to muscle (e.g., Fig. 2). The mean intensity of PCr was only 3.8 ± 3.9% (SD) of the total phosphorus signal. The highest PCr intensities, 6–11% of total phosphorus, occurred in case f, in which only a nonlocalized spectrum was obtained, and in cases in which it was not possible to avoid some muscle or brain within the voxel localized to a small lymphomatous lesion (cases d, m, n, and r) or to a mass of lymph nodes interspersed with muscle (case p). However, the presence of PCr did not affect ability to interpret the spectra. Moreover, because the amounts of Pi and ATP in spectra from muscle are each approximately one-fifth that of PCr (e.g., Fig. 2C), virtually all of the Pi and NTP in the lymphoma spectra are not contaminants from muscle but are contained within the lymphomas.

The 21 spectra (Fig. 5) show several features in common: (a) all have high signal intensities in the PME region dominated by PEth at 4.2 ppm; (b) most have a broad PDE signal, but only a few have a resolvable sharp peak within the PDE region; and (c) most have prominent NTP signals. The individual spectra in Fig. 5 are grouped according to the nature of the PME region, and their letter designations correspond to those in Table 1. Cases a–i have sufficient resolution of PMEs to permit quantitation of both the peak integrals and the peak heights of both PEth and PChol. In cases j–l, PChol appears only as a notch on the upfield shoulder of PEth, and it is possible to quantify only the peak heights of both PEth and PChol. In cases m–r, PME is too broad to resolve PChol from PEth, although in cases m–q the peak occurs at the position of PEth between 4.1 and 4.3 ppm. In case r only is it impossible to determine the dominant component of PME. In the three remaining cases (s–u), which are lymphomas infiltrating the spleen, PChol, if present, cannot be detected because of the 2-P peak of DPG at 3.8 ppm. However, a high signal compatible with PEth is evident in all three cases and is higher relative to the DPG signals than in normal spleen (Fig. 1B). Therefore, PEth is the dominant phospholipid metabolite within the PME region in at least 20 of the 21 NHL we studied.

Four of the spectra in Fig. 5 (c, g, i, and s) contain sharp peaks within the PDE region that may be above the noise level. However, only case i had a sharp signal near 0.5 ppm, which could be GPChol, and only case s had a signal near 1 ppm, which could be GPEth. Because $^1$H decoupling and NOE enhancement readily bring out GPChol and GPEth signals when these metabolites are present, as in normal liver (Fig. 1A) and brain (14), we conclude that NHLs contain low concentrations of GPEth and GPChol.

The metabolic characteristics of NHL derived from the 21 spectra in Fig. 5 are summarized in Tables 2 and 3. In the 18 NHLs in tissues
Fig. 2. MRI-directed, $^{31}$P MRS study in patient a. A, a coronal MR image shows enlarged lymph nodes in the right inguinal region. The 8 x 8 array of 3 x 3 x 3 cm CSI voxels overlies the image. B, an axial MR image through the highlighted region in the coronal image in A. The highlighted region in B indicates the CSI voxels that contain the spectra shown in C. C, $^1$H-decoupled, NOE-enhanced $^{31}$P NMR spectra from the voxels highlighted in B. The spectrum with the square borders was accurately localized to the enlarged lymph nodes by voxel shifting the data sets. Signals in the other voxels, which are in muscle, are dominated by the high PCr peak.

Other than spleen (Table 2), PME is 26% of the total phosphorus signal, and, in cases in which PChol as well as PEth is resolved, the PEth/PChol ratio is 2.9. The PDE region contains 20% of the signal; the broadness of most of the PDE signal indicates that it comes from relatively immobile membrane phospholipids. $P_i$ is 7% of the signal and occurs at positions corresponding to a mean pH of 7.23. The NTPs account for the remaining 47% of the total signal. The percentages of the PME, PDE, $P_i$, and NTP signals in the lymphomas infiltrating spleen (Table 3) are similar to those in other organs but slightly modified by the presence of signals from 2,3-DPG.

It should be emphasized that some of the data in Tables 2 and 3, expressed as a fraction of the total phosphorus signal, may not appear to match the expectation based on a visual inspection of the spectra in Fig. 5. Factors that could contribute to this impression include the way in which the spectra are scaled for display in Fig. 5 and the variable widths of the peaks relative to their heights. In 5 cases ($a$, $c$, $h$, $j$, and $s$), the PDE region in the spectrum (Fig. 5) may appear to be smaller than the PDE fraction in Tables 2 and 3. This is a result of the very broad nature of the PDE signal in these cases. The broad PDE signal is most susceptible to variations in the baseline straightening process; thus, it is most susceptible to error in the peak intensity fitting routine.

To obtain some idea about the extent of this error, we examined inter- and intraobserver variations in spectral processing by using NMR1. Two observers independently analyzed nine cases selected to represent spectra of a range of quality ($d$, $e$, $h$, $i$, $l$, $o$, $q$, $t$, and $u$). The median interobserver variation in the PDE signal intensity was 8.3% ($n = 9$), which resulted in differences in the PDE peak intensity, expressed as a fraction of the total phosphorus signal, ranging from $-0.02$ to $+0.04$ (median, $0.00$). The median intraobserver variation was 4.6% ($n = 5$), which resulted in differences in the PDE peak intensity, expressed as a fraction of the total phosphorus signal, ranging from $-0.01$ to $+0.06$ (median, $0.01$). These variations are remarkably small and support the conclusion that the fitting routine is robust and reproducible.

**DISCUSSION**

$^1$H decoupling and NOE enhancement, implemented 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 spectra to regions of interest with the use of CSI, have enabled us to overcome the technical limitations mentioned...
above, broaden the scope of \( ^{31}P \) MRS investigations, and obtain more information about the in vivo metabolic characteristics of lymphomas than has heretofore been available. A uniformly high PME signal intensity and a slightly alkaline pH confirm previous observations in NHL (29, 31–38). However, \(^1H\) decoupling and NOE enhancement permitted us to show that the PME region is dominated by PEth and that the PDE region rarely contains GPEth or GPChol, but instead contains only a broad phospholipid component.

The benefits obtained from \(^1H\) decoupling are illustrated by our observations in spleens infiltrated with lymphoma. Although an elevated PME has been reported in lymphomatous spleens, it was not possible to clearly resolve signals from PMEs, DPG, and Pj (34). Our techniques, which permit us to resolve DPG from other signals, enabled us to show that spleens infiltrated with NHL have an abnormally high PEth signal. Our observations also suggest that the rather high pH of 7.6 reported in normal spleens (34) may have been based on the 3-P peak of DPG rather than the Pj peak.

Although we have managed to study NHL in a variety of anatomic sites, the application of \( ^{31}P \) MRS remains limited by our inability to access lymphomas deeper than 10–12 cm from the surface and by difficulty studying lymphomas smaller than approximately 3-cm diameter. Unless the lymphoma is large and near the surface, it is necessary to acquire four averages of the CSI data set to ensure a high enough signal relative to noise to obtain spectra of the quality that is typical of those shown in Fig. 5. To realize the benefit of \(^1H\) decoupling, it is necessary to be able to shim the magnetic field adequately to obtain sufficiently narrow peaks to resolve individual PME and PDE components. This is now achievable in most cases in a short time with the use of an automatic shimming routine based on \(^1H\) CSI (18, 19). The information available in the \( ^{31}P \) MR spectrum might be increased by the ability to express the metabolite concentrations in absolute rather than relative terms. To obtain absolute concentrations requires knowledge of the spin-lattice relaxation times of the metabolite signals, a correction for magnetic field inhomogeneity in the sensitive region of the surface coil, a correction for NOE effects, and a correction for the intervoxel signal contamination (point-spread effect) inherent in phase encoding used to localize spectra with CSI. Models have been developed recently to account for these effects (28), and we are applying them to \( ^{31}P \) MRS studies in lymphomas.

The predominance of PEth over PChol in the PME region was observed in high resolution \( ^{31}P \) MRS studies of extracts of biopsy specimens of lymphomas (29). Although not mentioned in that report, the spectrum included in it contained very low amounts of GPEth or GPChol, in agreement with our results. Our observation of the absence of detectable mobile PDEs was also made in a malignant fibrous histiocytoma in the only previously reported study of a human cancer in vivo with the use of \( ^1H\)-decoupled \( ^{31}P \) MRS (39).

The prominent NTP signals suggest that NHLs in vivo generally have well-preserved energy metabolism and, therefore, are presumably well perfused. Moreover, they are relatively uniform in their metabolic characteristics: the coefficients of variation of the larger peak signal intensities of NTPs and PMEs and of intracellular pH are not much larger than those in \( ^{31}P \) MRS studies of tissues in normal subjects (40–43). We observed a trend toward a higher fraction of NTP and lower fractions of Pj and PME when comparing low-, intermediate-, and high-grade NHLs; however, we have only three high-grade cases, and these trends were not significant as determined by an ANOVA statistic. These observations are compatible with the relatively homogeneous nature of most NHLs in MRI (44) and with their tendency to be uniformly cellular histologically (45). Some high-grade NHLs do have foci of necrosis large enough to affect their MRI appearance (30). Two of the four spectra with low signal:noise ratios (\( p \) and \( q \)) were from high-grade NHLs, but they had relatively low Pj and relatively high NTP signals and, as mentioned in the "Results," technical limitations in these two cases may account for the low signal:noise ratios in their spectra.

Although the alkaline, predominantly intracellular, pH in NHLs may be an indication of good vascular perfusion, it is slightly acid relative to plasma pH (7.4). Moreover, a pH of approximately 7.25 measured by \( ^{31}P \) MRS appears to be typical of many other types of
human cancers, some of which are not expected to be as uniformly well perfused (1). This observation appeared to contradict expectations that cancer cells should be very acidic because of production of lactate from excessive glycolysis, and its significance has been discussed and debated in a number of reviews (1, 46-49). It is conceivable that cancer cells readily extrude or buffer H+. It is also conceivable that intracellular alkalinization is related to sustained signal transduction, perhaps along with stimulation of exchange of other ions such as sodium or bicarbonate (50, 51). These concepts were supported by the report of alkalinization occurring during mitogenic transformation of lymphocytes (52). However, subsequent studies have not reproduced that observation (53, 54) and have indicated that such intracellular alkalinization was an artifact occurring under nonphysiological conditions when bicarbonate is absent from the medium (55). At present, one can only speculate on the mechanism and significance of the observed pH in NHL and other cancers.

Our observations indicate that some experimental lymphoma models have phospholipid metabolite patterns which differ from those of NHL in vivo in clinical settings. For example, PEth was the dominant PME in some human lymphoid malignancy cell lines (2, 7, 56, 57), but PChol was the dominant PME in others (2, 58, 59). 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 (6-10), and may or may not resemble those occurring in...
vivo. For instance, RIF-1 tumors in culture contained PChol, whereas the same tumor growing in the mouse contained primarily PEth (11); and some human tumors in culture had PEth:PChol ratios similar to those of the same tumors xenografted in mice, whereas others did not (12). Therefore, our observations should help guide the development of experimental models (cell lines, xenografts, and transplanted animal tumors) that have metabolic characteristics analogous to those that occur in vivo in patients in clinical settings.

The mechanisms underlying the pattern of phospholipid metabolites we observed in NHL are unknown. To facilitate discussion, a diagram of the major pathways of metabolism of choline and ethanolamine is shown in Fig. 6, and key enzymes are indicated by bold numbers. The steady-state concentrations of PEth and PChol are determined by a number of factors including: the availability to the cells of ethanolamine and choline; the activities of their respective kinases (1,1'), cytidylyltransferases (2,2'), and phosphotransferases (3,3') in the formation of membrane phospholipids; the activities of various transferases (7, 7', 11, 12); and the activities of phospholipases (4, 9, 10) in the catabolism of membrane phospholipids. The $^{31}P$ NMR spectra of normal human peripheral blood lymphocytes (57, 60–62) are similar to those we observed in NHL. Lymphocytes have a higher activity of CTP:cholinephosphate cytidylyltransferase than of choline kinase, which may account for their low concentrations of PChol (61). When mitogenically stimulated, they accumulated increased levels of GPChol and GPEth (61, 62), compatible with the activity of CTP:ethanolamine phosphate cytidylyltransferase (2.7.7.15); 3, CDP-choline:1,2-diacylglycerol cholinephosphotransferase (2.7.8.2); 4, phospholipases A2 (phosphatidylcholine 1- and 2-acylhydrolases, 3.1.1.32 and 3.1.1.4); 5, 2-lysophosphatidylcholine acylhydrolase (3.1.1.5); 6, sn-glycerol-3-phosphocholine phosphohydrolase (3.1.4.38); 7, acyltransferases; 8, acyl-CoA:1- and 2-acyl-sn-glycerol-3-phosphocholine O-acyltransferases (2.3.1.23 and 2.3.1.62); 9, phospholipase C (phosphatidylinositol phospholipase D, 3.1.4.3); 10, phospholipase D (phosphatidylcholine phospholipid hydrolase, 3.1.4.4); 11, phosphatidylethanolamine-cholesterol- transferase; 12, Sadenosyl-l-methionine:phosphatidylethanolamine N-methyltransferase and phosphatidyl-N-methyl ethanolamine N-methyltransferase (2.1.1.17 and 2.1.1.71). Separate enzymes involved in ethanolamine metabolism but corresponding to those in choline metabolism are marked by a prime; those not primed are presumably the same for choline and ethanolamine pathways, and it is possible that 1' is the same as 1. For additional details, see Ref. 9.

Table 2 Metabolic characteristics of NHL derived from the $^{31}P$ spectra in Fig. 5

<table>
<thead>
<tr>
<th>Patient</th>
<th>PME</th>
<th>P'</th>
<th>PDE</th>
<th>NTP</th>
<th>PEth/PChol</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0.25</td>
<td>0.06</td>
<td>0.28</td>
<td>0.41</td>
<td>2.3</td>
<td>7.14</td>
</tr>
<tr>
<td>b</td>
<td>0.29</td>
<td>0.12</td>
<td>0.16</td>
<td>0.43</td>
<td>3.2</td>
<td>7.14</td>
</tr>
<tr>
<td>c</td>
<td>0.20</td>
<td>0.05</td>
<td>0.32</td>
<td>0.43</td>
<td>1.4</td>
<td>7.17</td>
</tr>
<tr>
<td>d</td>
<td>0.21</td>
<td>0.07</td>
<td>0.03</td>
<td>0.69</td>
<td>3.0</td>
<td>7.31</td>
</tr>
<tr>
<td>e</td>
<td>0.31</td>
<td>0.06</td>
<td>0.14</td>
<td>0.49</td>
<td>3.1</td>
<td>7.14</td>
</tr>
<tr>
<td>f</td>
<td>0.26</td>
<td>0.09</td>
<td>0.14</td>
<td>0.37</td>
<td>1.3</td>
<td>7.10</td>
</tr>
<tr>
<td>g</td>
<td>0.26</td>
<td>0.09</td>
<td>0.14</td>
<td>0.37</td>
<td>4.1</td>
<td>7.24</td>
</tr>
<tr>
<td>h</td>
<td>0.22</td>
<td>0.08</td>
<td>0.25</td>
<td>0.45</td>
<td>2.8</td>
<td>7.16</td>
</tr>
<tr>
<td>i</td>
<td>0.19</td>
<td>0.07</td>
<td>0.13</td>
<td>0.61</td>
<td>3.6</td>
<td>7.31</td>
</tr>
<tr>
<td>j</td>
<td>0.20</td>
<td>0.05</td>
<td>0.23</td>
<td>0.52</td>
<td>3.2</td>
<td>7.10</td>
</tr>
<tr>
<td>k</td>
<td>0.36</td>
<td>0.05</td>
<td>0.15</td>
<td>0.44</td>
<td>4.1</td>
<td>7.21</td>
</tr>
<tr>
<td>l</td>
<td>0.38</td>
<td>0.06</td>
<td>0.10</td>
<td>0.46</td>
<td>7.45</td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>0.31</td>
<td>0.02</td>
<td>0.19</td>
<td>0.48</td>
<td>NR</td>
<td>7.31</td>
</tr>
<tr>
<td>n</td>
<td>0.18</td>
<td>0.05</td>
<td>0.13</td>
<td>0.34</td>
<td>0.33</td>
<td>7.17</td>
</tr>
<tr>
<td>o</td>
<td>0.34</td>
<td>0.10</td>
<td>0.11</td>
<td>0.45</td>
<td>NR</td>
<td>7.35</td>
</tr>
<tr>
<td>p</td>
<td>0.23</td>
<td>0.03</td>
<td>0.18</td>
<td>0.56</td>
<td>NR</td>
<td>7.40</td>
</tr>
<tr>
<td>q</td>
<td>0.26</td>
<td>0.05</td>
<td>0.30</td>
<td>0.39</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.27</td>
<td>0.00</td>
<td>0.19</td>
<td>0.54</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.26</td>
<td>0.07</td>
<td>0.20</td>
<td>0.47</td>
<td>2.9</td>
<td>7.23</td>
</tr>
<tr>
<td>SD</td>
<td>0.06</td>
<td>0.04</td>
<td>0.09</td>
<td>0.09</td>
<td>1.0</td>
<td>0.11</td>
</tr>
<tr>
<td>CV</td>
<td>23%</td>
<td>27%</td>
<td>45%</td>
<td>19%</td>
<td>34%</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

* Metabolite signal intensities are expressed as fractions of total phosphorus signal in each spectrum. PEeth/PChol ratio is derived from peak heights; it is designated NR when the PME signal is too broad to resolve PChol from PEth. pH is predominantly intracellular pH determined from the position of P¡ relative to a-NTP; in cases in which P¡ was not well resolved, pH was not determined. CV, coefficient of variation.

Table 3 Metabolic characteristics of NHLs infiltrating spleen

<table>
<thead>
<tr>
<th>Patient</th>
<th>PME+2P-DPG</th>
<th>P' +3P-DPG</th>
<th>PDE</th>
<th>NTP</th>
<th>PEth/PChol</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>s</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
<td>0.34</td>
<td>NR</td>
<td>7.24</td>
</tr>
<tr>
<td>t</td>
<td>0.23</td>
<td>0.10</td>
<td>0.29</td>
<td>0.38</td>
<td>NR</td>
<td>7.14</td>
</tr>
<tr>
<td>u</td>
<td>0.18</td>
<td>0.12</td>
<td>0.23</td>
<td>0.47</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.21</td>
<td>0.14</td>
<td>0.25</td>
<td>0.40</td>
<td>7.19</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.03</td>
<td>0.06</td>
<td>0.04</td>
<td>0.07</td>
<td>9.07</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>14%</td>
<td>43%</td>
<td>16%</td>
<td>18%</td>
<td>3%</td>
<td></td>
</tr>
</tbody>
</table>

* Metabolite signal intensities and pH are derived as described in Table 2. CV, coefficient of variation.

In summary, our study provides a good technical basis from which to rigorously test the hypothesis, derived from scattered observations in the literature (1), that an early treatment-induced decrease in PME predicts rigorously test the hypothesis, derived from scattered observations in the literature (1), that an early treatment-induced decrease in PME predicts
sensitivity of a cancer to that particular treatment, and we can determine the specific PME components involved in that process.

Acknowledgments

We thank Tamela Greenberg, Libo He, Connie Scutti, and Ravi Srivinasa for technical assistance and Annette C. Kuesel for critical review of the manuscript.

References


Metabolic Characterization of Human Non-Hodgkin's Lymphomas *in Vivo* with the Use of Proton-decoupled Phosphorus Magnetic Resonance Spectroscopy


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/55/15/3286

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/55/15/3286.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.