Noninvasive Real-Time Monitoring of Intracellular Cancer Cell Metabolism and Response to Lonidamine Treatment Using Diffusion Weighted Proton Magnetic Resonance Spectroscopy

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ABSTRACT

We have used diffusion-weighted proton magnetic resonance spectroscopy (DWMRS) to noninvasively selectively observe only the intracellular metabolites of breast cancer and melanoma cell lines in vitro in real time. Breast cancer cell lines representing different stages in breast cancer progression were chosen for study. Intracellular biochemical profiles of six cell lines perfused in alginate beads were obtained. Spectral differences between groups of cell lines, including choline, lactate, and threonine peaks, were investigated. We also monitored response to the antineoplastic agent, lonidamine (LND), as a function of time and drug concentration in perfused cancer cells. Previous studies reported that this drug induced intracellular acidification and lactate accumulation. Diffusion weighted proton spectra demonstrated a 2- to 9-fold increase in the intracellular lactate signal as a response to LND treatment in several cancer cell lines. These results are consistent with the hypothesis that the principal mechanism of LND in some cancer cells is marked inhibition of lactate transport. Moreover, we have shown that there is a factor of two to three between the response of melanoma cells and that of most types of breast cancer cells. The higher sensitivity of the melanoma cells, as predicted by proton DWMRS, was correlated with changes in water-suppressed magnetic resonance spectra and confirmed by a biological assay. This study demonstrates the feasibility of using DWMRS for monitoring intracellular metabolism and for studying the effects and mechanisms of action of anticancer drugs. We believe that this method can be used for noninvasive clinical applications, such as the differentiation between benign and malignant tissue, real-time monitoring of response to therapy, dose response, and toxicity effects.

INTRODUCTION

Development of effective therapies against malignant diseases and monitoring their effect in a noninvasive manner is one of the most important challenges to biomedical research. MRS is a very useful experimental approach to this end because it enables noninvasive continuous monitoring of biochemical processes.

31P and 13C nuclei have been used in the majority of MRS studies of the metabolism of perfused cells (1). Proton is the most sensitive MR nucleus. It is intrinsically 14 times more sensitive than 31P, thus enabling shorter acquisition times and higher spatial resolution in vivo. Moreover, proton MRS offers the advantage that numerous natural biological compounds, as well as drugs and their metabolites, can be detected. However, the presence of signals originating from extracellular compounds and the immense water signal, as well as many overlapping proton signals, has hindered the application of proton MRS in studies of biological samples. Some proton MRS studies have been reported in vivo (2–4); however, these have not yet attained translation to routine clinical application. In this work, we present the first application of DW proton MRS (5) to selectively observe only the intracellular metabolites of several cell lines in vitro. We anticipate that this method will significantly improve the application of proton MRS for in vivo and clinical applications. This method is based on differences in motional properties of the components attributable to the higher intracellular protein concentration and to the consequent restriction of molecular motion within cells. Intracellular components have a lower ADC than extracellular components and free water. We address the signal overlap problem by working with a high magnetic field strength (600 MHz), which results in greater chemical shift dispersion and by using curve fitting for the data analysis.

Many breast cancer tumors appear to be initially responsive to endocrine manipulation, and/or are sensitive to cytotoxic chemotherapy, but later progress to a more malignant phenotype, characterized by invasive/metastatic foci that are resistant to both endocrine manipulation and chemotherapeutic intervention (6). To study the progression from estrogen-dependent growth to estrogen-independent and antiestrogen-resistant tumors, a series of MCF7 variants has been isolated, which together represent the most critical stages in breast cancer progression (6–8). The cell lines studied in this work are all variants that have been selected and extensively characterized, and exhibit specific phenotypic changes that reflect critical characteristics of the progression in therapy from the hormone-sensitive to -insensitive phenotype. The characteristics of the cells chosen for study are detailed in Table 1 (8).

Developing new, effective anticancer drugs and optimizing the use of presently available drugs requires understanding of their mechanism of action. LND is an anticancer drug derived from indazole-3-carboxylic acid, which was first introduced as an antipsoriatic and embryotoxic agent (9). Clinical studies have shown that a wide range of cancer types respond to the drug (10). Previous studies, based on measurements of the extracellular milieu alone (9, 11), suggested that LND inhibits glycolysis, thus inhibiting production of lactate (the end product of glycolysis). Previous 31P and 13C MRS studies (12, 13) concluded that LND induced intracellular acidification and lactate accumulation. These results supported the hypothesis that the main effects of LND are not through inhibition of glycolysis, but are based on profound intracellular acidification and inhibition of lactate efflux. This proposed mechanism led us to choose LND as an example for these studies. Observing the intracellular lactate signal with proton DWMRS enabled us to study the mechanism of action of this anticancer drug directly. Moreover, it enabled us to determine the metabolic response to therapy of different types of cancer cells.

In this study, we have investigated potential biochemical differences among human breast cancer cell lines and a murine melanoma
cell line by comparison of their intracellular proton spectra. We have also investigated the effects of LND on the metabolism of several of these cell lines, as a function of time and drug concentration. To confirm the drug sensitivity of these cells, as detected by the DW-MR method, we correlated it with other standard MRS and with non-MR studies.

MATERIALS AND METHODS

Cell Culture. MDA-MB231, MDA-MB435, and MCF7 human breast cancer cells were routinely maintained in DMEM and supplemented with penicillin-streptomyecin, l-glutamine (2 mM), and 5% FCS. MCF7/MIIICells, an estrogen-independent variant of MCF7 cells, and MCF7/LCC2 cells, an antiestrogen-resistant variant of MCF7/MIIICells, were maintained in DMEM without phenol red (which has an estrogenic-like activity; Ref. 14) and supplemented with penicillin-streptomyecin, l-glutamine (2 mM), and 5% charcoal-stripped calf serum. F10–9 murine melanoma cells were routinely maintained in DMEM and supplemented with penicillin-streptomyecin, l-glutamine (2 mM), and 10% FCS. All cell cultures were maintained at 37°C in a humidified 5% CO2/95% air atmosphere incubator.

Cell Preparation for Proton MRS Studies. Cells were grown in flasks to 90% confluence, harvested with 0.05% trypsin, centrifuged at 4°C at 1000 g for 5 min, and washed twice with the growth medium. Cell pellet (0.5–0.6 ml containing ~5 × 10^7 cells) was mixed with an equal volume of liquid alginate (2%) in PBS. Beads were prepared by dripping the mixture from a capillary into a calcium chloride solution. This caused immediate gelling, and the microcapsules were then washed three times with fresh medium. The capsules were kept at 4°C for a few hours and then perfused with growth medium at 37°C for at least 30 min before the experiment started.

LND Treatment. LND experiments were performed with low glucose DMEM (5.5 mM glucose), similar to glucose physiological concentration. LND was dissolved in DMSO and PEG200 (1 g of LND/10 ml DMSO/25 ml PEG) before adding to the perfusion solution; the final LND concentration used in the perfusion solution was 200 μg/ml unless otherwise mentioned. In control experiments, we used the same volume of DMSO and PEG as in the LND solutions.

Evaluation of Cellular Response to LND Treatment with a Cell Counting Method. Cells were grown in flasks to 90% confluence, and a pellet of 10^7 cells was harvested as described above. The cells were resuspended in 2 ml of the growth medium with the addition of LND at two concentrations: 400 and 600 μg/ml LND was dissolved in DMSO and PEG200 before it was mixed in the medium as described above. In control experiments, we used the same volume of DMSO and PEG as in the LND solutions. The cell suspension was incubated for 2 h during which the suspension was mixed every 10 min. The cells were rinsed twice with PBS and then resuspended in 2 ml of PBS. To count the percentage of dead cells, 20 μl of cell suspension were mixed with 20 μl of trypan blue (0.2%) and placed for counting under the microscope.

Cell Perfusion. The perfusion system used was described previously (15). The inflow tube was 0.5 mm inner diameter, and was placed near the bottom of a Wilmad 8-mm MR tube. The outflow was directed into openings in a plastic insert, and then into an outflow tube. The gel beads, which filled the MR tube, were concentrated at the bottom of the tube by the plastic insert. Perfusion rate (~0.9 ml/min) was maintained using a peristaltic pump. Before the experiment, the system was washed with 70% ethanol and then with the growth medium. The cells were perfused continuously with appropriate fresh media at 37°C. The medium was bubbled gently with a mixture of 5% CO2/95% O2 to ensure sufficient oxygenation and to maintain a physiological pH.

DW MR Method. Stejskal and Tanner (16) showed that application of a pair of pulsed magnetic field gradients sensitizes spin echo MR experiments to diffusion. Tanner (17) subsequently showed that the stimulated echo obtained in a three 90° rf pulse experiment is useful when strong diffusion weighting is required with minimal loss of echo time. To further suppress the water signal, a CHESS sequence was added, which consists of a selective 90° rf pulse followed by gradient dephasing, before the first and third rf pulses (18). A schematic representation of the pulsed gradient stimulated echo sequence used in this study is shown in Fig. 1.

In the pulsed gradient method, the normalized intensity of the water signal is given by:

$$I/I_0 = \exp[ -\gamma^2 B^2 g/3 (\Delta - \delta/3) D ] = \exp[ -bd ]$$  \hspace{1cm} (A)

where, I and I0 are signal intensities in the presence and absence of diffusion sensitizing gradients respectively, γ is the gyromagnetic ratio of the nuclei, g and δ are gradient strength and duration, respectively, (Δ - δ/3) is the effective diffusion time, D is the molecular diffusion coefficient, and b is the diffusion weighting factor, which is expressed in units of s/cm². By varying g, δ, and/or Δ, a diffusion curve can be obtained (5), and from the echo amplitude dependence on b, one can calculate the ADCs (Eq. A). In biological systems, there are usually several spin populations with different ADCs, so that the signal attenuation is not monoexponential. In the simplest case of a two-compartiment system (intra- and extracellular), with very slow exchange between them, the attenuation of the water signal should be a biexponential function of b. The lower D value originates from the intracellular molecules, and the higher D value arises from the sum of the intra- and extracellular molecules.

Presaturation MR Method. In the case of non-DW proton MR spectroscopy, water suppression is essential to observe the much smaller metabolite signals. A common approach is to presaturate the water signal using a selective inversion pulse followed by a delay chosen so that the water is passing through a null when the localization part of the sequence is started. We used this sequence to obtain water-suppressed spectra that represent a convolution of intra- and extracellular metabolites. In subsequent reference to water-suppressed spectra, we mean spectra obtained using the presaturation method.

MR Experiments. The experiments were carried out on a Bruker 600 DMX spectrometer, using a 1H8mm probe with one gradient in the z direction with a maximum intensity of 50 gauss/cm. Proton DW and water-suppressed MR spectra were continuously recorded in an alternating manner. All experiments were performed at 37°C. Intracellular spectra were acquired with echo time TE = 11 ms, repetition time TR = 2.2 s, diffusion gradient intensity of 17.5 gauss/cm, duration of 5 ms, and diffusion time of 127 ms. For the water suppression CHESS sequence, we used 8-ms single lobe sinc rf pulses.

Data Analysis. For well-resolved peaks, quantification of the spectra was obtained by integrating over the peak of interest (after a baseline correction), using the program MestRe-C, version 1.5.1 (written by Departamento de Quimica Organica, Facultad de Quimica, Universidad de Santiago de Com-

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Table 1 Characteristics of cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>ER</th>
<th>Estrogen dependencea</th>
<th>Estrogen-responsiveb</th>
<th>TAM-responsive</th>
<th>ICT 182, 780-responsive</th>
<th>Metastases</th>
</tr>
</thead>
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<tr>
<td>MCF7</td>
<td>+</td>
<td>Dependent</td>
<td>Responsive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>No</td>
</tr>
<tr>
<td>MIIIC</td>
<td>+</td>
<td>Independent</td>
<td>Responsive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Yes</td>
</tr>
<tr>
<td>LCC2</td>
<td>+</td>
<td>Independent</td>
<td>Responsive</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>ND</td>
</tr>
<tr>
<td>MDA-MB231</td>
<td>–</td>
<td>Independent</td>
<td>Unresponsive</td>
<td>Resistant</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>MDA-MB435</td>
<td>–</td>
<td>Independent</td>
<td>Unresponsive</td>
<td>Resistant</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

a Requirement for E2 to form tumors in nude mice.

b Responsive to E2 by inducing specific genes/mutagenesis.

ND, no data.
postela, Santiago, Spain) on a Pentium III personal computer. In more complicated cases, such as the lactate and threonine peaks, the region of interest was fitted to a function consisting of a sum of gaussian curves (again, after a baseline correction), using the program Physics Analysis Workstation (PAW version 2.09/18, CERN Program Library Q121, Cern, Geneva 1995) on a Pentium III personal computer. Resonance assignments are based on the abundant literature of extracts and suspensions of different tumor cell lines and other tissues (1, 19). Some assignments were confirmed by perfusing with high concentrations of specific metabolites and observing the rise in the corresponding intra/extracellular peaks.

RESULTS

Differentiation between Intra- and Extracellular Signals via DWMRS. The logarithmic normalized water signal intensity as a function of the squared gradient intensity for cells embedded in alginate beads is shown as the open triangles in Fig. 2 (5). The gradient duration and separation time were kept fixed. Therefore, the squared gradient intensity is proportional to the DW factor \( b \) (Eq. A). The curve is clearly biexponential, where the slow component represents the intracellular region. To prove that the slow component is indeed intracellular, we repeated the experiment with beads and medium only (see filled triangles in Fig. 2), and no slow component was found. An additional test was performed by changing the extracellular medium to a buffer without any metabolites (PBS). The intracellular spectrum did not change (Fig. 3A). By contrast, some of the peaks in the water-suppressed spectrum (consisting of both intra- and extracellular molecules) decreased or vanished (Fig. 3B). The latter are correlated with the peaks in the extracellular spectrum, representing the metabolic profile of the perfusion medium (Fig. 3C).

Signal:Noise Ratio. Comparing DWMRS with the water suppression approach (Fig. 3, A and B) shows a significantly greater noise level in the DWMRS spectra (Fig. 3A), although both measurements were acquired alternately with the same number of scans. This results from several factors. The stimulated echo sequence used in the DWMRS method loses a factor of 2 in signal relative to the spin echo (Eq. A). The slow slope represents the intracellular component. Therefore, the signal/noise ratio of regions b and a is respectively (Fig. 4). The ratio between the integrated signal intensities of the intracellular species (the slow component in Fig. 2). Finally, the intra- and extracellular metabolites have a higher absolute concentration than the intracellular alone.

Comparison of DWMR Cell Spectra. We measured intracellular proton spectra of five human breast cancer cell lines (see Table 1; Ref. 8) and one mouse melanoma cell line, using the DWMRS method. The intracellular spectra were obtained by choosing a gradient value in the region of the slow slope of the diffusion curve (Fig. 2), where the MR signal obtained originates from slow moving, i.e., intracellular, molecules. Between three and seven spectra were recorded for each cell line, at least 1 month apart. Fig. 4 shows an example of one spectrum taken for each cell line. By looking at the shapes of these spectra, it seems reasonable to divide them into three groups: the first group consists of the top three spectra, MCF7, MDA-MB231, and MDA-MB435; the second group consists of the next two spectra, MCF7/LCC2 and MCF7/MII; and the third is the melanoma cell line. The differences between the cell lines are described in terms of ratios between areas under peaks at given regions. We define the choline, lactate/threonine, and leucine/soleucine regions as regions a, b, and c, respectively (Fig. 4). The ratio between the integrated signal intensities of regions b and a is >1.0 in the first group, <1.0 in the second group, and close to 1.0 in the third. The same is true for the ratio between regions b and c. The lactate/threonine region contains several overlapping peaks. The area under the lactate (b1) and threonine (b2) subpeaks was calculated using curve fitting. It can be seen that in the first group of cells, the threonine subpeak dominates the lactate/threonine region, whereas in the second group, the lactate dominates. The melanoma cell line has similar contributions from both metabolites. The calculated ratios discussed above are displayed in Fig. 5. The ratios presented in the figure were calculated per spectrum and then averaged over several measurements per cell line. The error bars are the SDs of these measurements. Fig. 5, A–D shows a ratio of quantitative manner the qualitative difference seen in Fig. 4. Fig. 5, A–C shows the average ratios a:b, c:b, and a:c. Fig. 5D shows the ratio of...
b2:b1. In all plots of Fig. 5, the dashed line represents the average value of the first group of cell lines: MCF7, MDA-MB231, and MDA-MB435. The dotted line represents the average value of the second group: MCF7/LCC2 and MCF7/MIII. The lines demonstrate the difference in the metabolite ratios for the two groups of cell lines. Fig. 5, A–C suggests that the difference between the two groups of cell lines originates from the lactate/threonine region (region b). To determine which metabolite it is that contributes to this difference, we calculated the ratio of the subpeaks in this region, the lactate signal (b1) and the threonine signal (b2), to the choline (a) and leucine/isoleucine (c) regions, and to each other. These calculated ratios (Fig. 5, E–H) suggests that the main component that contributes to the difference between the cell groups is threonine.

Ischemia. The effect of ischemia on the metabolism of MDA-MB435 breast cancer cells was simulated by stopping the perfusion for a certain period of time. The first spectrum was taken while perfusing at a flow rate of 0.9 ml/min. The next three spectra were taken while the perfusion was off, 5 min apart. The perfusion was turned back on, and the last three spectra were acquired. The main response to ischemic stress observed using proton DWMRS was a rise in the intracellular lactate signal. Fig. 6 shows the lactate signal intensity (calculated using curve fitting and normalized to the choline signal) as function of time. It can be seen that the lactate rise, after stopping the perfusion, was steeper than the recovery, after perfusion was resumed. It can also be seen that about 50 min after the perfusion was turned back on, the lactate signal restored to its original value. The rise and the recovery of the lactate signal were fitted to an exponential function: \( p + qe^{rt} \). The \( \chi^2 \) of the fit was 0.1.

Drug Response, Comparison between Cell Lines. The effect of the antineoplastic agent LND on the metabolism of perfused cancer cells, MCF7, MDA-MB231 and MCF7/MIII human breast cancer cells, and F10–9 murine melanoma cells, was studied by DWMRS. LND was dissolved in the perfusion medium as described in the “Materials and Methods” section.

DW spectra demonstrated a 2- to 9-fold increase in the intracellular lactate signal as response to LND treatment (Figs. 7 and 8). A moderate decrease of the lactate signal in the perfusate was also observed in the water-suppressed spectra of the effluent solution. No change was detected in the lactate signal while perfusing with the control medium. Similar results were obtained with all four cancer cell lines.

The response of F10–9 to LND was significantly stronger than that of the breast cancer cell lines (Fig. 8), suggesting increased sensitivity to the drug. To test this hypothesis, we compared the DW spectra (intracellular only) of the cells with the water-suppressed spectra.
The peaks chosen for comparison were the DMSO and the choline peaks. DMSO was dissolved in the perfusion medium in a known concentration and crossed the membrane freely. Choline was present in the perfusion medium only in insignificant quantities; therefore, the choline signal in the water-suppressed spectra represented the intracellular choline only, whereas the DMSO water-suppressed signal represented the sum of the intra- and extracellular signals. We have compared the intracellular choline: DMSO peak ratio before and after LND treatment and found that this ratio did not change. Therefore, a decrease of the choline:DMSO peak ratio in the water-suppressed spectra in response to LND treatment implies a decrease in the total number of intact cells, or in other words, an indication of cell death. The change in the choline:DMSO peaks for three cell lines, MCF7, MDA-MB231, and F10–9, is shown in Fig. 9. The open triangles represent the DW data, serving as a control, and the filled triangles represent the water-suppressed spectra. The melanoma water-suppressed choline:DMSO ratio was significantly decreased, whereas the breast cancer cells showed no significant change.

To confirm the higher sensitivity of the murine melanoma cells to LND treatment, three cell lines were tested via a biological assay (described in the “Materials and Methods” section): MCF7, MDA-MB231, and F10–9. The cells were treated for 2 h at two LND concentrations: 400 and 600 µg/ml. Control experiments were performed with medium containing DMSO and PEG200 in the same volume in the LND experiments. The percentage of dead cells in the LND treated samples relative to control is presented in Fig. 10. It can be seen that F10–9 cells show the highest sensitivity to LND treatment.

**Drug Response, Concentration Study.** To test the effect of LND concentration on the metabolism of cancer cell lines, we perfused F10–9 murine melanoma cells with increasing concentrations of LND. Spectra were acquired 30 min after each LND increase. Fig. 11 shows the intensity of the lactate peak (arbitrary units) as a function of LND concentration (in µg per ml). It is shown that the response to the drug increases up to a certain level where is plateaus. This can be
and a C values, for details). It has been shown previously using 31 P MRS (8) that phenotypic changes associated with malignant progression in breast cancer (6, 21). The authors conclude that this may reflect a fundamental difference in how the intermediate phenotype of the ER-positive phenotype. The higher sensitivity of the melanoma cells, as predicted by Ben-Horin et al. (12). Moreover, we have shown that there is a factor of 2–3 between the response (the increase in intracellular lactate) of F10–9 melanoma cells and that of breast cancer cells (Fig. 8). The higher sensitivity of the melanoma cells, as predicted by proton DWMR, was correlated with a decrease in the choline/DMSO peak in the water-suppressed MR spectra (Fig. 9) and confirmed by a biological assay (Fig. 10).

Proton DWMR was also used to monitor the intracellular meta-

DISCUSSION

We applied the method outlined by van Zijl et al. (5) and showed that we are able to obtain a complete intra- and extracellular signal separation by using proton DWMR on perfused cells with a 600-MHz MR spectrometer. We used the method to compare spectra of different cancer cell lines and to monitor their response to therapy and stress.

The development of estrogen independence, antitrogen resistance, and increased metastatic potential are among the most important phenotypic changes associated with malignant progression in breast cancer (6, 21). It has been shown previously using 31P MRS (8) that relative to the parental MCF7 cells, the baseline spectra for both MCF7/MI  and MCF7/LCC2 cells exhibit ~50% higher UDPG levels. These differences are associated with perturbations in the glucose/glycoprotein/proteoglycan metabolism and with the acquisition of an intermediate estrogen-independent and estrogen-responsive/ER-positive phenotype. The authors conclude that this may reflect a fundamental difference in how the intermediate phenotype of the MCF7/MI  and MCF7/LCC2 cells use glucose metabolites relative to ER-positive, E2-responsive cells (e.g., MCF7). We also observed qualitative differences in the proton DWMR spectra between two equivalent groups of breast cancer cell lines (Figs. 4 and 5). However, the differences we observed after final analysis were mainly related to the threonine signal.

Some of the average differences in the calculated ratios between the two groups of breast cancer cell lines were very large. For example, the ratio of the lactate region:choline region shown in Fig. 5A was about an order of magnitude larger in the MCF7, MDA-MB231, and MDA-MB435 group than in the MCF7/MI, MCF7/LCC2 group. Still, the metabolic profile of cells can be easily affected by growth conditions and preparations for MRS studies (22, 23). As a result, we also observed deviations between different measurements of the same cell line, leading to large SDs of the average calculated ratios (Fig. 5).

Therefore, we conclude that for the purpose of differentiating between cell lines, we were able to show the general trend, but not to prove that there is a statistically significant difference between cell lines in vitro. However, non-DW proton MRS studies in vivo have shown significant differences between peak ratios of different stages of malignancies in the brain (24–26). Moreover, in a comparison between in vitro and in vivo proton MR spectra of squamous cell carcinoma, it was observed that the ratio of choline:creatine in vivo was consistently larger than the in vitro ratio (27). These results suggest that DWMRs might be a better tool for in vivo studies than for in vitro studies in this respect.

In contrast to the comparison of cellular spectra, the cell response to LND treatment, as measured by proton DWMR, is reproducible and statistically significant. All of the cell lines tested responded to LND treatment by a marked increase of the intracellular lactate signal and a moderate decrease in the extracellular lactate signal. Thus, real-time monitoring of intracellular metabolic response to LND treatment was consistent with the LND mechanism of action as described by Ben-Horin et al. (12). Moreover, we have shown that there is a factor of 2–3 between the response (the increase in intracellular lactate) of F10–9 melanoma cells and that of breast cancer cells (Fig. 8). The higher sensitivity of the melanoma cells, as predicted by proton DWMR, was correlated with a decrease in the choline/DMSO peak in the water-suppressed MR spectra (Fig. 9) and confirmed by a biological assay (Fig. 10).

Proton DWMR was also used to monitor the intracellular meta-

Fig. 11. LND concentration dependence. The increase of the intracellular lactate signal (normalized to the choline signal) of F10–9 murine melanoma cells is plotted as a function of LND concentration. The data were fitted to a sigmoidal function. See text for details.
bolic response to the ischemic stress induced by stopping the perfusion for a limited period of time, and to monitor the metabolic recovery after the perfusion was turned back on. The exponential behavior, in response to ischemic stress, was previously shown by Knop et al. (28) for ATP and Pi in phosphorus MR spectra of perfused cells at 25°C. The authors were not able to repeat the measurement at 37°C because the response at higher temperature is faster and there are too few data points to adequately fit an exponential function. This is probably attributable to the long acquisition times needed in phosphorus MRS (each spectrum was obtained in 22 min). This example demonstrates the advantage of using proton MRS in terms of time resolution. The proton DW spectra (8 scans) were obtained in 40 s each (Fig. 6). The short acquisition time enabled us to estimate the time dependence of the response to ischemic stress at 37°C with good temporal resolution.

This study demonstrates the feasibility of using DW proton MRS for monitoring intracellular metabolism noninvasively and in real time. We have shown that this method can be used for following the metabolic response of cells to stress as a function of time and to follow their recovery. We have also shown that DWMRS is a very useful tool to study the effects and mechanism of action of an antineoplastic drug on perfused cells, as a function of time and drug concentration. Finally, we have shown that DWMRS was able to predict that one type of cell is more sensitive to a certain antineoplastic treatment than others. In comparison with other MR techniques, the proton DWMRS method has distinct advantages in that it is noninvasive (no contrast agents used), information can be obtained for proton-containing molecules (most drugs and metabolites), the MR signal is strong (allowing shorter acquisition times and better spatial resolution than 31P MRS), and complete intra- and extracellular separation of signals is attainable (compared to standard proton MRS).

We anticipate that DWMRS will provide a significant improvement over standard proton MRS, especially in the case of cancer. Because of the heterogeneous nature of tumors, standard proton MRS averages over different types of tissues, such as viable tissue, necrosis, and edema. The extracellular volume fraction in tumors has been shown to vary between 30% in viable tissue to 90–100% in necrotic areas (29–31). By using DWMRS to suppress the signals from nonviable extracellular matter, enhanced sensitivity for the viable tissue can be obtained. The results presented in this work demonstrate the potential of using DWMRS for noninvasive in vivo and clinical applications, such as real-time differentiation between benign and malignant tissue, detection of residual malignancy after treatment (surgery, laser ablation, cryogenic ablation, radiation), monitoring of cellular metabolic response to therapy, dose response studies, and investigation of metabolic effects in tissues susceptible to drug toxicity. However, applying the DWMRS method clinically will not be a trivial task. There are several problems that have to be overcome, such as the fact that magnetic field gradients on clinical MRI machines are normally limited in intensity. Therefore, to obtain high b values it will be necessary to use longer gradient duration times and longer diffusion times, allowing more exchange between intra- and extracellular species (32, 33). Still, the exchange between intra- and extracellular metabolites is slower than that of water, thus the separation between intra- and extracellular metabolites should be better than that of water. Other technical problems concerning clinical DWMRS are that the gradients might not have strong enough shielding, the magnetic field homogeneity in vivo might be problematic, and DWMRS is sensitive to motion artifacts. We are presently carrying out in vivo DWMR spectroscopy and imaging animal studies and intend to extend to clinical studies in the near future.

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