Use of Phosphorous-31 Nuclear Magnetic Resonance Spectroscopy to Determine Safe Timing of Chemotherapy after Hepatic Resection

David A. Kooby, Kristen L. Zakian, Surya N. Challa, Cornelia Matei, Henrik Petrowsky, Hyok-Hee Yoo, Jason A. Koutcher, and Yuman Fong


ABSTRACT

Liver resection induces accelerated growth of residual hepatic and metastases. Adjuvant chemotherapy may improve outcome if administered early after resection but may prove lethal if initiated prior to completion of DNA synthesis in regenerating liver. This study investigates phosphorous-31 nuclear magnetic resonance (31P-NMR) as a noninvasive tool for measuring energy changes reflective of hepatic DNA synthesis and for predicting safe timing of chemotherapy after 70% hepatectomy. To evaluate metabolic changes in regenerating liver, quantitative three-dimensional 31P-NMR was performed, using the technique of chemical shift imaging at various time points after 70% hepatectomy in adult male Fischer rats. Animals receiving a course of 2-deoxy-5-fluorouridine (FUDR; 100 mg/kg, i.p. four times per day × 5), initiated at the time of operation, were also evaluated to observe the effects of chemotherapy on liver regeneration. Forty-eight hours after resection, hepatic nucleoside triphosphate (NTP), which reflects ATP content, fell 37% (P < 0.03) in animals undergoing hepatectomy alone. By contrast, animals receiving FUDR after hepatectomy demonstrated a mitigated NTP response, with a drop of only 17% (P = not significant), suggesting that interruption of DNA synthesis leads to a reduced consumption of ATP. Direct measures of DNA synthesis and nuclear proliferation were correlated with NMR findings. [3H]Thymidine incorporation and Ki67 immunohistochemistry were performed on liver samples from rats undergoing 70% hepatectomy with and without FUDR. Both [3H]thymidine incorporation and Ki67 expression were inhibited significantly at 48 h in animals receiving hepatectomy and FUDR, compared with those not treated with FUDR. To determine whether NMR changes could be used to identify safe timing of chemotherapy after hepatectomy, rats were treated with a 5-day course of FUDR initiated either prior to or after NMR changes normalized. Animals treated with FUDR at the point of NTP normalization (72 h) showed significantly improved survival over those that began treatment at operation (75% versus 17%; P = 0.0005, log rank test). FUDR inhibits hepatic DNA synthesis and influences mortality if administered too early after hepatectomy. Chemical shift imaging is a noninvasive tool that can identify metabolic changes coinciding with DNA synthesis and nuclear proliferation after hepatectomy. 31P-NMR may be useful for determining safe timing of chemotherapy after liver resection.

INTRODUCTION

In the United States, ~50,000 patients will develop hepatic metastases from primary colorectal cancer each year (1). Currently, hepatic resection provides the only opportunity for cure in this group of patients; however, two-thirds of those who undergo resection develop recurrent disease (2). The most common site of recurrence is in the remaining liver (3), suggesting that the unresected portion often harbors undetectable microscopic foci of disease. Furthermore, several animal models have demonstrated that partial hepatectomy actually accelerates growth of residual microscopic disease (4–6). The benefits of adjuvant chemotherapy for primary colorectal cancer (7, 8) and its role after hepatic resection for metastatic disease (9) have been demonstrated. Most oncologists, however, are reluctant to initiate therapy until 4 weeks after liver resection, because of concerns that cytotoxic agents will interfere with the process of DNA synthesis in the regenerating liver. These concerns prevail, despite experimental evidence obtained in animal studies, which demonstrate the peak of hepatic DNA synthesis to occur before 72 h after 70% hepatectomy (10, 11).

DNA synthesis in the regenerating human liver is difficult to measure, because methods used to obtain this information in animal models are invasive. Furthermore, medications and co-morbidities such as cirrhosis and hepatitis may alter the timing and extent of the regenerative process (12, 13). 31P-NMR spectroscopy has been used to assess energy metabolism after partial hepatectomy in animal studies, although previous studies have required laparotomy for placement of the surface coil (14, 15). Three-dimensional 31P CSI is a noninvasive 31P-NMR technique that can be used to measure levels of high-energy phosphate compounds in situ (16). This study investigates the application of 31P-NMR using the CSI technique to measure energy changes in rat livers after standard 70% hepatectomy. It evaluates the effects of chemotherapy on liver regeneration through 31P-NMR and correlates these changes with direct measurements of hepatic DNA synthesis and nuclear proliferation. Finally, it examines whether 31P-NMR can be used as a marker of liver regeneration to determine safe timing of chemotherapy after hepatic resection.

MATERIALS AND METHODS

Partial Hepatectomy

All animal work was performed under the guidelines approved by the Memorial Sloan-Kettering Institutional Animal Care and Use Committee. Adult male Fischer rats (Charles River, Wilmington, MA), weighing between 280 and 350 g, were housed in pathogen-free quarters in the animal facility. The animals were maintained in a 12-h day/night cycle and provided access to rat chow (PMI Mills, St. Louis, MO) and water ad libitum until the time of operation, at which point animals were pair-fed to the poorest eaters. Partial (70%) hepatectomy was performed similar to the method described previously (17). Briefly, animals were anesthetized with pentobarbital sodium (50 mg/kg; Wyeth Laboratories, Inc., Philadelphia, PA) by i.p. injection. Under sterile conditions, laparotomy was performed through a midline incision. Left and median hepatic lobes were identified, ligated with 3-0 silk suture (Ethicon, Inc., Somerville, NJ), and removed. Abdominal closure was performed in two layers with 4-0 nylon suture (Ethicon, Inc.), and all animals received 3 ml of fluid resuscitation (0.9% NaCl by i.p. injection) at the end of the procedure. All sham-operated animals underwent similar surgical exposure and gentle manipulation of the left and median hepatic lobes to control for surgical stress. All operations were performed between the hours of 10 a.m. and noon to prevent effects of diurnal mitotic variation (18).

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2 To whom requests for reprints should be addressed, at Department of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021.

3 The abbreviations used are: 31P-NMR, phosphorous-31 nuclear magnetic resonance; 5-FU, 5-fluorouracil; CSI, chemical shift imaging; FUDR, 2-deoxy-5-fluorouridine; MRI, magnetic resonance imaging; NTP, nucleoside triphosphate; PC, phosphocholine; PE, phosphoethanolamine; PME, phosphoethanolamine; qd, four times per day.
**31P-NMR Spectroscopy**

A total of 61 rats were used to observe the 31P-NMR spectral changes associated with partial hepatectomy and chemotherapy as summarized in Table 1. Seventeen animals underwent 70% hepatectomy; 11 animals underwent sham-laparotomy and began a course of FUDR (100 mg/kg, i.p. qd × 5; Roche Laboratories, Nutley, NJ); and 16 rats underwent 70% hepatectomy and received FUDR therapy as described. At 48, 72, and 96 h after operation, five or six animals from each group were subjected to 31P-NMR (at 96 h, four animals undergoing hepatectomy and FUDR were evaluated, and no sham-laparotomy FUDR controls were examined). Six additional animals subjected to hepatectomy underwent 31P-NMR at 120 h. To obtain baseline metabolite levels, five animals undergoing sham-laparotomy and six nonoperated controls were analyzed.

Localized 31P-NMR was used to monitor the individual and combined effects of chemotherapy (FUDR) and 70% hepatectomy on liver energy metabolism. Studies were performed on a 4.7 Tesla Bruker-CSI spectrometer with a 33-cm horizontal bore as described previously (19). Animals were anesthetized with isoflurane inhalation and placed prone with the abdomen positioned over a 31-mm diameter, two-turn phosphorus surface coil. For quantification, a 65-µl sphere containing methylene diphosphoric acid diluted in water (50%), and HCl (50%) was located at the center of the coil. The entire 31P平台 was positioned inside a proton-tuned birdcage resonator, which was used to obtain T$_1$-weighted, cross-sectional, anatomical scout images of the animal (TR = 500 ms; TE = 10 ms; averages = 4; slice thickness = 3 mm; slice separation = 1 mm; FOV = 72 mm). Obtaining 31P-NMR data by the technique of CSI permits noninvasive localization of spectra to the liver and coregistration of the spectral grids with the MR images (16). Through this relationship, in vivo quantitative information can be obtained in situ without performing laparotomy for placement of a 31P coil directly on the organ’s surface.

The CSI pulse sequence consisted of a hard pulse, phase-encoding, and acquisition, using 10–18 averages with a TR of 0.5 s. The field of view was either 72 or 64 mm, depending on the size of the liver, and an 8 × 8 × 8 matrix was encoded. Total scan time ranged from 43 to 80 min. After corrections for saturation, flip angle, and receive coil sensitivity, single voxel spectra (voxel was encoded. Total scan time ranged from 43 to 80 min. After corrections for

**Ki67 Immunohistochemistry.** Ki67 immunohistochemistry was performed on liver specimens from 30 pair-fed Fischer rats at various time points after 70% hepatectomy. Half the rats ($n = 15$) received a course of FUDR (100 mg/kg i.p., qd × 5) at operation to control for injection volume of 0.9% NaCl, as control. At each time point (24, 48, 72, 96, and 120 h) after hepatectomy, three rats from each treatment group were sacrificed, and their livers were harvested and fixed in 4% paraformaldehyde (Sigma Chemical, St. Louis, MO). Tissues were subsequently transferred to 70% ethanol, embedded in paraffin blocks, and stored at 4°C until staining. Paraffin blocks were sectioned and boiled in 0.1 M citric acid (pH 6.0), in a microwave oven for 10 min to unmask tissue antigens. Sections were incubated at 4°C overnight with mouse monoclonal anti-mouse Ki67 antibody (NCL-Ki67-MM1; Novocastra Laboratories, Newcastle upon Tyne, United Kingdom) diluted 1:200 in PBS. Specimens were incubated with biotinylated antimouse secondary antibody (Histomouse-Sp Kit; Zymed Laboratories, San Francisco, CA), followed by peroxidase-conjugated streptavidin (Zymed) and, finally, a chromagen (ABC)-substrate mixture (Zymed). Ki67-positive cells were counted by computer-assisted image analysis of specimens from three animals/group and three slides/animal, using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Results were determined as the percentage of positive (red/brown) nuclei at ×200. Livers from three additional naïve animals served as baseline controls.

**Survival Study**

This study was performed to determine whether hepatic energy changes identified by 31P-NMR might be useful to guide administration of chemotherapy after partial hepatectomy. Sixty adult male Fischer rats were divided into five groups of 12 animals. Thirty-six rats underwent 70% hepatectomy and were treated with a 5-day course of an equal volume of 0.9% NaCl initiated at operation to control for injection trauma. Twelve rats underwent 70% hepatectomy and were treated with a 5-day course of an equal volume of 0.9% NaCl initiated at operation to control for injection trauma. Twelve sham-operated animals received a 5-day course of FUDR

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**Table 1. In vivo comparison of phosphorous metabolites in adult Fischer rat livers as measured using 31P-NMR by CSI**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>NTP (nmol)</th>
<th>P (nmol)</th>
<th>P/NTP</th>
<th>PME (pmol)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>7.46 ± 0.43</td>
<td>5.11 ± 0.60</td>
<td>0.69 ± 0.06</td>
<td>6.01 ± 0.92</td>
<td>7.36 ± 0.02</td>
</tr>
<tr>
<td>Sham-laparotomy</td>
<td>5</td>
<td>6.60 ± 0.99</td>
<td>6.08 ± 1.25</td>
<td>0.89 ± 0.13</td>
<td>6.12 ± 0.52</td>
<td>7.33 ± 0.02</td>
</tr>
<tr>
<td>48 h hepatectomy</td>
<td>5</td>
<td>4.17 ± 0.38</td>
<td>6.22 ± 1.25</td>
<td>1.37 ± 0.26</td>
<td>5.22 ± 1.76</td>
<td>7.29 ± 0.03</td>
</tr>
<tr>
<td>48 h sham-laparotomy + FUDR</td>
<td>6</td>
<td>5.93 ± 0.42</td>
<td>5.68 ± 0.97</td>
<td>0.95 ± 0.13</td>
<td>5.24 ± 0.84</td>
<td>7.36 ± 0.02</td>
</tr>
<tr>
<td>48 h hepatectomy + FUDR</td>
<td>6</td>
<td>5.49 ± 0.64</td>
<td>6.43 ± 1.07</td>
<td>1.17 ± 0.13</td>
<td>8.13 ± 1.33</td>
<td>7.34 ± 0.01</td>
</tr>
<tr>
<td>72 h hepatectomy</td>
<td>6</td>
<td>5.60 ± 0.87</td>
<td>5.65 ± 1.18</td>
<td>0.87 ± 0.14</td>
<td>5.69 ± 1.10</td>
<td>7.33 ± 0.02</td>
</tr>
<tr>
<td>72 h sham-laparotomy + FUDR</td>
<td>5</td>
<td>7.22 ± 1.03</td>
<td>6.24 ± 1.39</td>
<td>0.87 ± 0.18</td>
<td>5.85 ± 0.77</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>72 h hepatectomy + FUDR</td>
<td>6</td>
<td>5.65 ± 1.06</td>
<td>6.36 ± 1.36</td>
<td>1.14 ± 0.23</td>
<td>7.66 ± 2.57</td>
<td>7.43 ± 0.02</td>
</tr>
<tr>
<td>96 h hepatectomy</td>
<td>6</td>
<td>6.24 ± 0.11</td>
<td>5.87 ± 1.33</td>
<td>0.95 ± 0.22</td>
<td>5.55 ± 1.87</td>
<td>7.33 ± 0.02</td>
</tr>
<tr>
<td>96 h sham-laparotomy + FUDR</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>96 h hepatectomy + FUDR</td>
<td>4</td>
<td>5.88 ± 0.64</td>
<td>6.72 ± 1.24</td>
<td>1.13 ± 0.36</td>
<td>5.81 ± 1.71</td>
<td>7.39 ± 0.04</td>
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<tr>
<td>120 h hepatectomy</td>
<td>6</td>
<td>6.24 ± 0.87</td>
<td>5.10 ± 0.88</td>
<td>0.83 ± 0.17</td>
<td>6.76 ± 1.21</td>
<td>7.38 ± 0.02</td>
</tr>
</tbody>
</table>

*P < 0.05, as compared with sham-laparotomy pair-fed controls.

*NA, this group was not evaluated.*
initiated at operation to control for the effects of chemotherapy alone. All injections were administered i.p., and all animals were pair-fed to poorest eaters. Survival was recorded until 40 days postoperatively.

Statistics

Comparisons of 31P-NMR metabolite levels, [3H]thymidine incorporation, and Ki67 expression were performed using Student’s t test (two-tailed). Significance was defined by a $P < 0.05$ (two-sided). Survival analyses were performed by the Kaplan-Meier method (22). Log-rank testing was used to determine significance of survival curves (23, 24).

RESULTS

31P-NMR Spectroscopy

31P-NMR was performed on 61 adult male Fischer rats to identify changes in hepatic high-energy phosphate levels associated with liver regeneration. Additionally, animals undergoing 70% hepatectomy and simultaneous administration of FUDR were evaluated by 31P-NMR to detect alterations in liver metabolites induced by this agent and further characterize and validate concerns regarding administration of chemotherapy during active DNA synthesis. FUDR was selected for this study because this fluorinated pyrimidine is known to interfere with DNA synthesis, and it is the drug of choice for therapy of gastrointestinal liver metastases.

Fig. 1 illustrates results obtained by 31P-NMR. This is a representative T1-weighted, cross-sectional MRI through the liver of a Fischer rat lying in the prone position (spinal cord is seen near the top center of the image). Superimposed on the MRI is the corresponding 31P-NMR spectral grid. Each CSI voxel measures 9 × 9 × 9 mm for a volume of 729 mm$^3$. The highlighted voxel represents a spectrum from liver parenchyma only. The various peaks correspond to phosphorous metabolite levels present in the region of interest.

Our analysis included absolute quantitation of levels of NTPs, $P_i$, PMEs, and pH at various time points after partial hepatectomy in animals both with and without simultaneous administration of FUDR therapy. Table 1 shows summary data for this set of experiments. All results represent the average of five or six studies performed in different animals and expressed in mmol/l ± SD. The quantitation procedure did not correct for point spread function (25). In phantom studies, we have estimated that the point spread function results in a 30% overestimation of metabolite concentrations.

A significant drop in hepatic NTP (37%; $P < 0.05$) was observed 48 h after 70% hepatectomy, when compared with sham-laparotomy pair-fed controls (Fig. 2, A and B). NTP levels recovered by 72 h and normalized completely by 96 h. $P_i$ levels did not change significantly; however, the hepatic $P_i$:NTP ratio increased in hepatectomized animals at the 48-h time point (1.37 ± 0.26; $P < 0.05$). Animals that underwent 70% hepatectomy and simultaneous FUDR therapy demonstrated a mitigated NTP response at 48 h compared with hepatectomized animals that did not receive chemotherapy (Figs. 2D and 3). NTP levels in this group were depleted only 17% ($P \neq$ not significant). Both $P_i$ levels and $P_i$:NTP ratios did not change significantly in this group either.

Compared with sham-laparotomy controls, PME metabolites levels did not change significantly in animals that underwent 70% hepatectomy alone or in those that had sham-laparotomy and simultaneous FUDR therapy. By contrast, significantly elevated PME levels were observed in the group of animals that had 70% hepatectomy and simultaneous FUDR therapy at 48 h after operation (Table 1). No significant pH fluctuations were observed in any of the groups evaluated.

Measurements of DNA Synthesis and Nuclear Proliferation

[3H]Thymidine Incorporation. [3H]Thymidine incorporation was measured in liver samples from animals treated both with and without simultaneous FUDR therapy, at various time points after 70% hepatectomy. Liver samples from both sham-operated and sham-operated FUDR-treated animals demonstrated minimal [3H]thymidine incorporation. Peak uptake in hepatectomized animals occurred 48 h after operation (Fig. 4). Tissue from hepatectomized animals that did not receive FUDR therapy demonstrated 41% greater [3H]thymidine in-
suggest that FUDR significantly inhibits the regenerative process in the posthepatectomy liver at the nuclear level. Furthermore, the timing of antigen expression correlates with results obtained by $^{31}$P-NMR and $[^3H]$thymidine incorporation.

Survival Study

This survival study was undertaken to evaluate $^{31}$P-NMR as a tool for identifying metabolic changes in regenerating liver useful for timing administration of chemotherapy after partial hepatectomy. Hepatectomized animals were treated with “immediate FUDR” ($n = 12$), “immediate saline” ($n = 12$), “early FUDR” ($n = 12$) or “late FUDR” ($n = 12$). Twelve animals underwent sham-laparotomy and “immediate FUDR” therapy as additional controls. The hepa
tomized group that received “immediate FUDR” demonstrated the highest mortality, with a cumulative survival of only 17% by day 12 posthepatectomy. In this group, FUDR therapy was initiated prior to the peak of NTP depletion, as measured using $^{31}$P-NMR, and DNA synthesis and nuclear proliferation, as measured by $[^3H]$thymidine incorporation and Ki67 antigen immunostaining, respectively. The “early FUDR” hepatectomy group showed significantly improved survival (75%) over the “immediate FUDR” group (Fig. 6; $P = 0.0005$). This group began FUDR treatment 72 h after hepatectomy, just after the recovery of NTP, as observed by $^{31}$P-NMR. Excellent survival was observed in the sham-laparotomy FUDR-treated animals (100%), the hepatectomy “early saline” animals (83%), and the hepatectomy “late FUDR” group (92%). All animals that survived more than a week beyond completion of chemotherapy gained weight appropriately and survived long term.

DISCUSSION

Hepatic resection is potentially curative for patients with primary hepatocellular cancer (26) and isolated liver metastases from colorectal malignancies (2). Many of these patients, however, will develop hepatic recurrence despite appropriate patient selection and sound operative techni
eque because of undetected microscopic disease already present at the time of resection. Furthermore, after hepatectomy these micrometastases are subject to the surge of growth factors associated with the normal process of liver regeneration and may be stimulated to grow at an accelerated rate (4, 5, 27). Recent clinical evidence demonstrates that administration of adjuvant chemotherapy can improve results over sur-

corporation at 48 h than did tissue from animals that did receive chemotherapy (85 versus 50 cpm/μg DNA; $P < 0.05$). An overall reduction in the process of DNA synthesis was observed at all measured time points when FUDR was given. These results suggest that FUDR significantly inhibits DNA synthesis in regenerating liver.

Ki67 Immunohistochemistry. Ki67 immunohistochemical staining of paraffin-fixed liver tissue was performed to measure changes in hepatocellular nuclear proliferation after partial hepatectomy in animals treated both with and without simultaneous FUDR therapy. Ki67 antigen expression was negative in nonhepatectomized controls and in liver sections obtained 24 h after hepatic resection. Expression peaked 48 h after partial hepatectomy, fell at 72 h, and returned to baseline by 96 h (Fig. 5). Significantly lower expression was observed in FUDR treated animal livers at 48 h compared with sections from animals that did not receive chemotherapy (46%; $P < 0.05$). These data further
A
c
B

Fig. 5. Comparison of Ki67 expression after 70% hepatectomy in adult Fischer rats, both with and without simultaneous FUDR therapy. A, two photomicrographs (×200) showing hepatic Ki67 expression 48 h after operation in a hepatectomized rat (left panel) and a hepatectomized rat treated with FUDR (right panel). B, chart demonstrating percentage of nuclei that are Ki67 positive over time in untreated, hepatectomized animals (■) and in hepatectomized animals treated with FUDR (△). *, P < 0.05.

Numerous liver-directed applications for NMR spectroscopy are being explored as more sophisticated systems are developed. NMR is being used experimentally to evaluate hepatic function in disease (30, 31) and after transplantation (32), trace therapeutic metabolites (33–35), and evaluate response to therapy for various disease processes (36). Several studies have used NMR to evaluate spectral changes during the process of liver regeneration. Early efforts used proton NMR to measure lipid changes as determined through T1 and T2 relaxation times (37–40). Further insight was provided by studies using 31P-NMR, which permits in vivo, whole-organ relative-quantification of phospholipid and phosphoenergetic alterations. Campbell et al. (15) documented a drop in hepatic NTP levels with a concomitant rise in P1:NTP 48 h after 70% hepatectomy in rats. They reasoned that in the absence of necrosis, these changes reflect ATP hydrolysis associated with the energy-requiring process of hepatic DNA synthesis. Another study by Farghali et al. (14) compared 31P-NMR spectral changes in vivo in regenerating livers of Sprague Dawley rats with in vitro spectra obtained from perchloric acid extracts of corresponding liver tissue. They reported a relative correlation between ATP levels measured by both methods, suggesting that 31P-NMR provides accurate biochemical quantification of phosphate compound metabolism in this system.

The current study adds to the previous work by correlating 31P-NMR spectra in regenerating liver to direct measures of hepatic DNA synthesis and nuclear proliferation. It also investigates the effects of chemotherapy on the process of DNA synthesis and 31P metabolism, when administered at the time of resection. Most importantly, the present study documents such spectral changes to be potentially useful in directing administration of adjuvant chemotherapy after partial hepatectomy.

Prior to implementation of CSI, it was usually the practice to expose the liver for direct placement of the surface coil to avoid contamination of spectra by signals from surrounding tissue (14, 15, 41, 42). Therefore, previous 31P-NMR studies of liver regeneration were actually invasive. Furthermore, hepatic 31P-NMR changes have been associated with sham-laparotomy alone (40); thus, the process of operating on the animal to place the surface coil on the liver remnant potentially alters results. In the current study, spectra are obtained without performing a second operation. The 31P coil remains external to the animal, and these data are superimposed on a T1-weighted proton MR image in three dimensions, as described previously (16); thus, the results are not influenced by an additional laparotomy.

In most NMR studies, metabolite ratios, rather than absolute values, are used to demonstrate changes occurring in tissues. It can be difficult, however, to ascertain which metabolite is having a greater affect on the change in ratio. Use of an external standard to quantify each NMR peak provides a more specific and robust method for assessing changes in individual metabolites. This technique requires knowledge of the B1 profile the coil, tissue T1 values, and careful calibration; however, it enables us to report individual metabolite changes with confidence.

The hepatic phosphoenergetic changes we detected support the observations of Campbell et al. (15). We observed a significant depletion in hepatic NTP levels with an associated rise in the P1:NTP ratio 48 h after partial hepatectomy, which nearly recovered by 72 h. Because the NTP change was significant and the P1 change was not, it is apparent that the NTP depletion provided a greater contribution to...

Fig. 6. Results of survival study. Hepatectomized adult Fischer rats treated with “early FUDR” (initiated 72 h after hepatectomy, solid line) versus “immediate FUDR” (initiated at hepatectomy, broken line). P = 0.0005.
the $P_{i}$:NTP rise. These $^{31}$P-NMR changes correlated with direct measures of hepatic DNA synthesis and proliferation as determined by incorporation of $[3H]$thymidine and expression of the nuclear antigen Ki67. Previous studies have examined the effects of fluoropyrimidines on liver regeneration (43, 44). In our study, introduction of a course of FUDR therapy at the time of operation partially inhibited the energy-requiring process of DNA synthesis in the regenerating liver, as determined by $[3H]$thymidine incorporation and Ki67 immunohistochemistry. Corresponding hepatic NTP levels and $P_{i}$:NTP ratios were affected in these animals as well. The significant NTP depletion observed at 48 h in hepatectomized animals was not witnessed in the hepatectomized group treated with FUDR, presumably because less ATP was being used for de novo DNA synthesis. These observations support the premise that $^{31}$P-NMR can detect energy fluctuations reflexive of DNA synthesis and hepatocyte proliferation.

Numerous studies have evaluated the process of liver regeneration in animal models using varied techniques such as $[3H]$thymidine incorporation and labeling of various antigens expressed in proliferating cells such as Ki67, proliferating cell nuclear antigen, and bromodeoxyuridine (11, 45–47). Some variation in results is observed depending on the model being examined and the percentage of liver resected, but these methods are reliable and reproducible. $[3H]$Thymidine is a radiolabeled nucleotide that incorporates into DNA as it is being synthesized; therefore, it is intimately related to S-phase. The Ki67 antigen is expressed in all phases of the cell cycle except G0 and early G1 (45); therefore, Ki67 expression may persist despite absence of $[3H]$thymidine incorporation. This basic distinction in the information provided by these techniques can account for some inconsistency in the data at the various time points. The information obtained by $[3H]$thymidine incorporation is a better representation of DNA synthesis and probably correlates more closely to the changes in hepatic NTP levels observed by $^{31}$P-NMR.

In addition to evaluating phosphoenergetic changes, $^{31}$P-NMR provides information on phospholipid precursor levels. In our study, the total PME concentration did not change significantly after hepatectomy alone. This peak is comprised of PC, PE, and sugar phosphates. Some in vivo studies have reported elevated PME ratios in the days after partial hepatectomy (14, 48), whereas others have not (41). All of these studies were performed by surgically exposing the liver for direct application of the surface coil, which may confound results, as mentioned previously. In vitro studies have shown that PE is elevated after partial hepatectomy (14, 41, 48); however, PE may comprise only a small fraction of total PMEs, making the change difficult to detect in vivo. Additionally, PC levels have been shown to be dependent on diet (41), and although PE levels may be elevated, total PME concentration may be unchanged or even reduced based on the drop in PC associated with pair-feeding and decreased food intake in the postoperative period.

Interestingly, a significantly elevation of PME was observed in our studies at 48 h after hepatectomy, when a course of FUDR therapy was administered. Previous in vivo NMR studies of the effects of antineoplastic treatment on tumor metabolism have noted changes in both PE and PC levels. Specifically, PE:PC was noted to increase after administration of both chemotherapy and radiation (49–52). 5-Fluorouracil has been shown to induce a 70% increase in PE in a mammary carcinoma, with a minimal (nonsignificant) decrease in PC (51). These studies, and others have interpreted these changes to be attributable to decreased cellular proliferation or increased cell death. Thus, the increased PME observed in the FUDR-treated hepatocellularized animals may reflect chemotherapy-induced damage to the regenerating liver. Proton-decoupled phosphorus-31 CSI could be used to clarify findings in the regenerating liver by improving resolution of PE and PC peaks, as well as by increasing signal-to-noise ratio. Perhaps the most interesting and clinically relevant observation is the significant improvement in survival seen in animals in which FUDR therapy is initiated 72 h after partial hepatectomy. Hepatic NTP levels, $[3H]$thymidine incorporation, and Ki67 expression all normalize substantially by this time point. These data suggest that the current practice of waiting 4 weeks after hepatic resection to initiate adjuvant chemotherapy may be unnecessary, and that $^{31}$P-NMR can be used to identify hepatic high-energy phosphate changes reflective of DNA synthesis during the process of liver regeneration. It must be emphasized that we continue to caution against early use of chemotherapy immediately after NTP normalization. Clinical trials will be needed to determine how soon after NTP normalization adjuvant chemotherapy can safely be administered. Clearly though, if a patient has prolonged depression of NTP levels, chemotherapy should not be administered. Hepatic $^{31}$P-MR-spectroscopy may also be used to monitor toxicity of adjuvant chemotherapy after liver resection.

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REFERENCES


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