Quantitation of 5-Fluorouracil Catabolism in Human Liver in Vivo by Three-Dimensional Localized 19F Magnetic Resonance Spectroscopy

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ABSTRACT

The development of clinical applications of 19F magnetic resonance (MR) spectroscopy of 5-fluorouracil (5-FU) has been limited by the inability to localize 19F spectra to specific regions of interest, making it difficult to quantify drug and metabolite concentrations accurately. To develop methodology for quantitation, we studied the liver of patients receiving rapid bolus i.v. injections of 5-FU. In serial studies, 5-FU disappeared from the liver within 17–26 min, and its catabolite, α-fluoro-β-alanine (FBAL), rose to a plateau after 40 min. A high peak level of fluoro-ureido-propionic acid preceded that of FBAL in only one patient, and dihydrofluorouracil was never observed. During the plateau, we obtained MRI-directed 19F MR spectra localized in three dimensions using three-dimensional chemical shift imaging. The spin-lattice relaxation time of FBAL in liver, measured using a variable nutation angle method, was 1.6 ± 0.2 s (mean ± SD; n = 5). The concentration of FBAL at 60 ± 10 min after injection was 1.0 ± 0.2 mM in liver (mean ± SD; n = 7). This amount represents ~20% of the injected dose and 1.4 times the initial hepatic 5-FU concentration. Our approach may permit one to obtain molar concentrations of fluoropyrimidine metabolites simultaneously in hepatic cancers and surrounding liver, and it helps expand pharmacokinetic modeling of fluoropyrimidine catabolism.

INTRODUCTION

19F MRS studies have indicated that NMR-detectable intratumoral uptake of 5-FU correlates with a high probability of response of an individual patient’s cancer to treatment with 5-FU (1, 2). 19F MRS also permits examination of the rate of catabolism of 5-FU within the liver (3, 4, 5). However, development of clinical applications of 19F MRS of 5-FU has been hampered by two problems. The first is the inability to localize 19F spectra to specific regions of interest. This is particularly a problem when studying liver metastases, because one must be able to distinguish 19F signals in cancer from those in liver. The second problem is the inability to quantitate drug and metabolite concentrations. Quantitation requires accurate localization of the 19F signals to defined regions of interest, knowledge of the radiofrequency excitation pulse angles within the regions of interest, and knowledge of the T1 of the 19F signals. To develop a method for molar quantitation, we studied the livers of patients receiving rapid bolus i.v. injections of 5-FU in which the catabolite FBAL reaches a plateau after 40 min and gives a relatively stable 19F signal for about 30 min. During the FBAL plateau, we obtained MRI-directed 19F MR spectra localized in three dimensions (6) and measured the T1 of FBAL by using the variable nutation angle method (7, 8) with adiabatic pulses (9, 10). We report the use of this approach to quantitate 5-FU catabolites in the livers of patients receiving rapid bolus i.v. injections of the drug.

MATERIALS AND METHODS

The 12 patients participating in this study received rapid (1-min) i.v. bolus injections of 5-FU at doses ranging from 320 to 600 mg/m² given as adjuvant treatment following surgery for colorectal or breast cancer. These patients did not have known liver metastases based on contrast-enhanced computed tomographic or MRI studies. Quantitation was performed in seven patients, and FBAL T1s were obtained in five. Studies were performed in a 1.5-tesla Siemens Magnetom clinical imager and spectrometer. A 16-cm-diameter circular surface coil was tuned doubly to 19F and 1H and packaged in plexiglass to permit flexibility in its placement over the anterolateral trunks of supine subjects. The same coil was used for MRI, shimming of the magnetic field on the 1H signal, and 19F MRS. Fast-scan, gradient-echo MR images (TR, 310 ms; echo time, 15 ms) in

Received 7/10/95; revised 9/20/95; accepted 10/20/95.

1 This work was supported in part by NIH Grants CA58632, CA54339, and CA41078.

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3 The abbreviations used are: MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance; MRI, MR imaging; 1H, proton; 5-FU, 5-fluorouracil; CSI, chemical shift imaging; FBAL, α-fluoro-β-alanine; T1, spin-lattice relaxation time; ppm, parts/million; S/N, signal/noise; TR, repetition time; FUPA, fluoro-ureido-propionic acid; B1, magnetic field of the antenna.
three dimensions were used to confirm the optimal coil position over the liver and to guide voxel shifting in postprocessing to optimize the voxel positions relative to the regions of interest. Shimming was performed initially by manual adjustment of lower- and higher-order shim currents and more recently by automatic adjustment of these currents using an autoshimming algorithm based on three-dimensional 'H CSI (11, 12). The line width at half height of the water resonance of the nonlocalized 'H spectrum was typically 20–40 Hz. To examine the kinetics of hepatic 5-FU loss and FBAL accumulation, nonlocalized spectra were obtained every min during and after 5-FU injection for ~60 min.

Localization of 19F MRS in three dimensions was obtained using CSI (6). Data sets were acquired in 8.5 min with an 8 x 8 x 8 matrix of 64-cm³ voxels, using a 250-μs rectangular excitation pulse, 2 kHz spectral width, a TR of 1 s, and one average per phase-encoding step. The carrier frequency was set between the 5-FU and FBAL peaks. A 4-cm³ reference sample of 1.3 mM trifluoroacetic acid solution was placed at the center of the coil. A 58° nutation angle was used at the center of a region of interest within the liver 6–10 cm below the center. 5-FU was injected i.v. as a bolus over 1 min, and the first 19F acquisition began immediately. Six or seven serial three-dimensional CSI MRS studies with acquisitions of one average over 8.5 min each were obtained for 50–60 min. The 5-FU peak, which appeared in the spectra right after the 5-FU bolus injection, was assigned to 0 ppm on the frequency axis. The first catabolite, dihydro-FU, would be expected to appear at -33 ppm; the next, FUPA, expected to appear at -19 ppm (13).

RESULTS AND DISCUSSION

Quantitation was performed using the phantom replacement method (14, 15). A 2-liter spherical phantom containing 5.8 mM 5-FU was placed under the coil. The relative position of the coil to the surface of the standard phantom was similar to the relative position of the coil to the surface of the liver in vivo, as shown in Fig. 1. By using a standard phantom of this size and shape placed in this position relative to the coil, we minimize errors in quantitation due to the point spread effect (16, 17). The point spread effect, inherent in phase encoding used to localize signals in CSI, causes variations in signal intensity in one voxel due to (positive or negative) signals from other voxels. Because the standard phantom has a geometry within the sensitive region of the coil similar to that of the liver (Fig. 1), and because the same three dimensional CSI MRS parameters with the same voxel size (64 cm³) used in vivo were applied to this phantom, the phantom and liver are subject to similar point spread effects. Peak integrals were determined using New Methods Research 1 software to fit Gaussian line shapes to the data. The accuracy and reproducibility of peak integration of a low S/N, isolated peak was evaluated on simulated spectra. A low S/N (S/N = 4) peak showed no difference in accuracy compared with a high S/N peak (S/N = 25) but had higher variation (coefficient of variance, 12 versus 5%). The absolute concentration of the FBAL in the liver was calculated by the equation:

$$C_t = C_p \times \frac{[I_p(I_{ref}(p) \times B_{si}(p) \times SF)]}{[I_p(I_{ref}(p) \times B_{si}(p) \times SF)]}$$

where $C_t$ and $C_p$ are the concentrations in the liver and standard phantom, respectively; $I_p$ and $I_{ref}$ are the localized signal integrals from the liver and standard phantom, respectively; $B_{si}$ and $I_{ref}$ are the signal integrals of the trifluoroacetic acid reference, which were used to correct for different coil loadings by subjects and the phantom, respectively; $B_{si}$ and $B_{si}$ are the B1 fields at the center of the selected voxel in the liver and standard phantom, respectively; $B_t$ is proportional to $1/(1 + (a/r)^2)$, where $a$ is the distance between the coil and the center of the voxel of interest, and $r$ is the coil radius; and $SF_t$ and $SF_p$ are the saturation factors of FBAL in the liver study and 5-FU in the standard phantom study, respectively. This is calculated by the equation:

$$SF = [1 - e^{-TR/T1}] \times \sin \theta[1 - \cos \theta \times e^{-TR/T2}]$$

where $\theta$ is the nutation angle.
(3, 4, 5, 19). In a second subject (Fig. 3B), who received a 600-mg/m² i.v. bolus of 5-FU, only FBAL could be resolved. In serial localized three-dimensional CSI studies in five patients, 5-FU disappeared within 17–26 min, and FBAL appeared after 10 min to reach a plateau at 40–60 min. The accumulation of FBAL concentration versus time in these five patients is summarized in Fig. 3C. The time constant ($\tau$) of FBAL accumulation was 27 min. The $\tau$ value was calculated using a three-parameter ($A$, $B$, and $\tau$), least squares fit of signals to $S = A + B \times e^{-\frac{t}{\tau}}$, where $t$ is the time following 5-FU infusion.

The time resolution of the CSI-localized data (8.5 min) precludes determination of the kinetics of 5-FU elimination. However, the kinetics of 5-FU elimination as well as FBAL accumulation can be determined from nonlocalized spectra, as shown in Fig. 4. In
four studies, 5-FU peaked at 1–2 min and then decreased. Although it was evident in all four studies that the 5-FU decaying signal followed more than a single exponential function, a time constant for the later-decaying fraction could not be obtained reliably, because the later 5-FU signals were too close to the noise. Therefore, we report an apparent time constant of 5-FU elimination from the initial decay (over 15–20 min), realizing that it is a slight overestimate. This time constant, which is derived from a two-parameter (A and τ), least squares fit of signals to \( S = A \times e^{-\tau s} \), is \( 10 \pm 3 \) min. FBAL increased with a time constant, derived from a three-parameter, least squares fit, of \( 24 \pm 5 \) min to reach a plateau after about 40 min.

The kinetics of intrahepatic 5-FU loss and FBAL accumulation (Figs. 3C and 4) is qualitatively similar to that reported previously using \(^{19}\)F MRS in vivo (3, 4, 5, 19). It is interesting to note that the time of hepatic FBAL accumulation to a quasi plateau and the initial time course of 5-FU elimination are similar to those measured in plasma following rapid bolus i.v. injection, in which FBAL plateaued over 45–120 min, and 5-FU decreased with a \( t_{\frac{1}{2}} \) of 13 min (20). Following the plateau of FBAL in plasma, it is eliminated very slowly, with a \( t_{\frac{1}{2}} \) of 33 h (20). In a few patients studied with \(^{19}\)F MRS for long times, hepatic FBAL began to decline slowly after a similar plateau (5, 19, 21).

Fig. 5A illustrates the measurement of the \( T_1 \) of the \(^{19}\)F signal of FBAL obtained with nutation angles between 20 and 90°. The corresponding plots of \( I(\theta)/\sin(\theta) \) against \( I(\theta)/\tan(\theta) \) in Fig. 5B show a linear correlation of 0.95 to give a \( T_1 \) of 1.4 s. In five patients, the \( T_1 \) of FBAL in liver was \( 1.6 \pm 0.2 \) s (mean \( \pm \) SD).

The concentrations of the catabolite FBAL (or, in one case, FUPA and FBAL) at 60 ± 10 min after bolus 5-FU injection are shown in Table 1. The average was 1.0 ± 0.2 (SD) mM in liver. If we assume an average liver size of 1.5 liters, then the mean amount of FBAL at the plateau is derived from about 20% of the injected 5-FU dose. These results are similar to those obtained in rat (22) and human (19) liver studies. The former reported an FBAL concentra-

![Fig. 2](https://example.com/f2.png)
Fig. 3  Series of in vivo $^{19}$F MR three-dimensional CSI data sets from a 64-cm$^3$ voxel in the liver of patients 2 (A) and 3 (B) following bolus i.v. 5-FU injection at time 0. The times following 5-FU infusion are indicated. These times represent the midpoints of the 8.5-min three-dimensional CSI acquisitions. C, summary of data from five patients. The concentration was calculated by Equation A. The curve was derived using a three-parameter ($A$, $B$, and 7), least squares fit of signals to $S = A + B \times e^{-x/T_2}$, where $t$ is the time following 5-FU infusion, $x$, 5-FU; $\circ$, FBAL.

A summary of the concentration of about 2 mmol/kg liver 1 h after a 50-mg/kg 5-FU infusion (22). The latter obtained an FBAL concentration of 1.30 ± 0.33 mmol/kg liver tissue using a 15-cm surface coil in 17 patients who received i.v. bolus 5-FU doses varying from 750 to 2000 mg (19). This result was obtained without precise localization of the $^{19}$F signal and with an assumed value for $T_1$ of 2.4 s, which is longer than the $T_1$ of 1.6 s we measured and would lead to an overestimate of the concentration by about 15%.

By combining the molar quantitation of FBAL at its quasi plateau with the kinetic information illustrated in Fig. 4, we can estimate that the peak hepatic tissue concentration
of 5-FU at 1–2 min after rapid bolus i.v. injection averaged 0.7 (range, 0.5–1.0) mM in liver. This peak hepatic 5-FU occurs at the time expected for the peak plasma concentration (20, 23, 24). However, most of the observed 5-FU is not within the plasma; with a peak plasma concentration following similar i.v. injections of similar 5-FU doses of <1 (range, 0.3–1) mM (20, 23, 24) and an intrahepatic blood volume of 20–30% (25), no more than 0.15 mM 5-FU in liver could have been within the plasma.

We have demonstrated the feasibility of MRI-directed, three-dimensional CSI-localized 19F MRS of 5-FU and its catabolites in vivo in the livers of human subjects. Our approach may permit one to obtain molar concentrations of fluoropyrimidine metabolites simultaneously in hepatic cancers and the surrounding liver, and it helps expand pharmacokinetic modeling of fluoropyrimidine catabolism.

**Table 1** Hepatic FBAL concentrations following bolus i.v. 5-FU injection, calculated using Equation A

<table>
<thead>
<tr>
<th>Patient</th>
<th>5-FU dose (mg/m²)</th>
<th>Time after the ending of the 5-FU infusion (min)</th>
<th>Concentration (mM in liver)</th>
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<tr>
<td>1</td>
<td>450</td>
<td>48</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>54</td>
<td>0.8</td>
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<tr>
<td>3</td>
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<td>53</td>
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</tr>
<tr>
<td>4</td>
<td>450</td>
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<td>1.2</td>
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<tr>
<td>5</td>
<td>600</td>
<td>54</td>
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<tr>
<td>6</td>
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<td>74</td>
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</tr>
<tr>
<td>7</td>
<td>320</td>
<td>61</td>
<td>1.0</td>
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</table>

Mean ± SD 500 ± 110 910 ± 160 60 ± 10 1.0 ± 0.2

**ACKNOWLEDGMENTS**

We thank Radka Stoyanova, M.S., for assistance in processing the data in Fig. 4; Chris Elsasser for assistance in coil construction; and James Gallo, Ph.D., for reviewing the manuscript.

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