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

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ABSTRACT

The development of clinical applications of $^{19}$F magnetic resonance (MR) spectroscopy of 5-fluorouracil (5-FU) has been limited by the inability to localize $^{19}$F spectra to specific regions of interest, making it difficult to quantitate 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, $\alpha$-fluoro-$\beta$-alanine (FBAL), rose to reach 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 $^{19}$F 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 $1.6 \pm 0.2$ s (mean $\pm$ SD; $n = 5$). The concentration of FBAL at 60 $\pm$ 10 min after injection was $1.0 \pm 0.2$ mM in liver (mean $\pm$ SD; $n = 7$). This amount represents $\sim 20\%$ 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

$^{19}$F 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). $^{19}$F MRS also permits examination of the rate of catabolism of 5-FU within the liver (3, 4, 5). However, development of clinical applications of $^{19}$F MRS of 5-FU has been hampered by two problems. The first is the inability to localize $^{19}$F spectra to specific regions of interest. This is particularly a problem when studying liver metastases, because one must be able to distinguish $^{19}$F 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 $^{19}$F signals to defined regions of interest, knowledge of the radiofrequency excitation pulse angles within the regions of interest, and knowledge of the $T_1$ of the $^{19}$F 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 $^{19}$F signal for about 30 min. During the FBAL plateau, we obtained MRI-directed $^{19}$F MR spectra localized in three dimensions (6) and measured the $T_1$ 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 computer tomographic or MRI studies. Quantitation was performed in seven patients, and FBAL $T_1$ 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 $^{19}$F and $^1$H 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 $^1$H signal, and $^{19}$F MRS. Fast-scan, gradient-echo MR images (TR, 310 ms; echo time, 15 ms) in

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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 1H CSI (11, 12). The line width at half height of the water resonance of the nonlocalized 1H 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 m 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 beneath the coil. 5-FU was injected i.v. as a bolus over 1 min, and the first 19F acquisition begun 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, would be expected at ~17 ppm; and the last, FBAL, would be expected to appear at ~19 ppm (13).

The T₁ of FBAL was measured during the stable plateau of hepatic FBAL by using the variable nutation angle method (7, 8) with the phase-cycled, B₁-independent rotation, adiabatic pulse (9, 10). Due to the low S/N ratio and long time requirement for the three-dimensional CSI experiment in the in vivo study, nonlocalized spectra were collected for the T₁ measurement. The appropriateness of using the nonlocalized data to measure the T₁ values was validated by comparing it with the inversion-recovery method on the 5-FU phantom (T₁, 4.2 s by the variable nutation angle method and 4.6 s by the inversion-recovery method). By varying the nutation angle systematically, a plot of I(θ)/sin(θ) versus I(θ)/tan(θ) for a single T₁ will provide a straight line with the slope of e⁻⁻TR/T₁, where I(θ) is the signal intensity, and θ is the applied nutation angle. The T₁ is obtained by a linear regression analysis of the data. The T₁ measurement was begun when the FBAL signal reached a plateau at about 40 min after the 5-FU bolus injection. Spectra were obtained with nutation angles varied over the range 10–90° at a TR of 1 s, with 128 acquisitions in each measurement. Seven dummy prescans were applied before each measurement to establish a steady state magnetization. The total T₁ measurement experiment took about 20 min. To prevent systematic errors caused by an increase or decrease of the FBAL signal during the T₁ measurement, the sequence of nutation angles was scrambled. At the end of the measurements, the first measurement was repeated, using the same nutation angle. This showed that the FBAL signal intensity did not change significantly during this period (average, 5%).

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 thecoil 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:

\[ C = \frac{I_p(I_{ref}(p) \times B_{1p}(p) \times SF_p)}{S_F} \]

where \( C \), \( I_p \), and \( I_{ref}(p) \) are the concentrations in the liver and standard phantom, respectively; \( I_p \) and \( I_{ref}(p) \) are the localized signal integrals from the liver and standard phantom, respectively; \( B_{1p}(p) \) and \( B_{1ref}(p) \) are the B₁ fields at the center of the selected voxel in the liver and standard phantom, respectively; \( B_1 \) is proportional to 1/[1 + (a/2r)²], where \( a \) is the distance between the coil and the center of the voxel of interest, and \( r \) is the coil radius; and \( S_F \) and \( S_p \) 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:

\[ SF = \left[ 1 - e^{-TR/T_1} \right] \times \sin \left( \theta - \cos \theta \times e^{-TR/T_1} \right) \]

where \( \theta \) is the nutation angle.

RESULTS AND DISCUSSION

Fig. 2 illustrates the MRI-directed, three-dimensional CSI localized 19F MRS study. Fig. 2 is an axial image with overlying grids, which show the positions of the MRS voxels in this slice. Fig. 2 shows the 19F spectra from nine voxels. In these spectra, obtained 16 min after bolus 5-FU injection, 5-FU had been eliminated, and only FBAL was detected in the liver.

Serial localized 19F data sets from the liver of one subject following bolus i.v. injection of 5-FU at a dose of 450 mg/m² are shown in Fig. 3A. In this subject, 5-FU disappeared rapidly. The first catabolite to appear was FUPA, followed by FBAL. Although the formation of the intermediate catabolite FUPA has been observed in the mouse liver using 19F MRS (18), it has not been resolved from FBAL in vivo in human subjects previously.
Fig. 1  Experimental setup for quantitative measurements of three-dimensional CSI-localized $^{19}$F MR spectra in the human liver and standard phantom. A, axial MR image from a patient with the grid overlaid on it to show the positions of the 4 x 4 x 4-cm$^3$ voxels. B, axial MR image from the standard phantom with the same size voxel of the grid as in A. The coil positions are also shown, their centers indicated by white dots in both panels. The position of the coil relative to the surface of the standard phantom was similar to the position of the coil relative to the surface of the liver in vivo. The $^{19}$F signals from the highlighted voxels are used to calculate the hepatic FBAL concentration by Equation A.

(3, 4, 5, 19). In a second subject (Fig. 3B), who received a 600-mg/m$^2$ 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 $S = A + B \times e^{-\tau t}$, 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.
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Fig. 2 $^{19}$F three-dimensional CSI collected 16 min after 5-FU bolus injection in patient 1. An axial MR image with the grid overlaid on it to show the positions of 16 of the MRS voxels (4 x 4 x 4 cm$^3$) in this slice and spectra from 9 of these voxels is shown.

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 $\tau$), least squares fit of signals to $S = A \times e^{-\alpha t}$, is 10 ± 3 min. FBAL increased with a time constant, derived from a three-parameter, least squares fit, of 24 ± 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 ± 0.2 s (mean ± 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. 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 $\tau$), least squares fit of signals to $S = A + B \times e^{-\tau t}$, where $t$ is the time following 5-FU infusion, $\times$, 5-FU; $\circ$, FBAL.

Interpretation 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...
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Fig. 4 19F MR signal intensities of 5-FU and FBAL versus time after start of the rapid bolus i.v. injection of 5-FU in two patients. Each point is the sum of signals obtained every second for 1 min. The signal intensities were obtained from nonlocalized spectra by applying principal component analysis to the entire data set (26). The 5-FU elimination curve is derived from a two-parameter (A and τ) least squares fit of signals to $S = A \times e^{-t/\tau}$, and the FBAL accumulation curve was derived using a three-parameter (A, B, and τ), least squares fit of signals to $S = A + B \times e^{-t/\tau}$, where $t$ is the time following 5-FU infusion.

Fig. 5 Example of $T_1$ measurement with the variable nutation angle method. A, FBAL signal from the liver obtained with 8 different nutation angles by phase-cycled, $B_1$-independent rotation, adiabatic pulses. B, plot of $I(\theta) / \sin(\theta)$ against $l(\theta) / \tan(\theta)$, where $I(\theta)$ is the signal intensity and $\theta$ is the applied nutation angle. Linear correlation is 0.95, and $T_1$ is 1.39 s (95% confidence limits for the fitting, 1.04–1.92 s).

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

<table>
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<th>Patient</th>
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<th>Timea (min)</th>
<th>Concentration (mM in liver)</th>
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Table 1. Mean ± SD 500 ± 110 910 ± 160 60 ± 10 1.0 ± 0.2

aTime after the ending of the 5-FU infusion.

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