**In Vivo** Measurements of Intratumoral Metabolism, Modulation, and Pharmacokinetics of 5-Fluorouracil, Using $^{19}$F Nuclear Magnetic Resonance Spectroscopy

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**ABSTRACT**

In vivo $^{19}$F nuclear magnetic resonance spectroscopy was used to monitor and measure 5-fluorouracil and some of its cytotoxic anabolites directly in rats bearing the Walker 256 adenocarcinoma by using a 4.7-T horizontal bore magnet. A two-compartment subsystem model was used to estimate intratumoral transfer parameters. The apparent rate of formation of the nucleosides/tides in the tumor, $K_{in}$, changed following methotrexate pretreatment, from a value of 6.4 ± 2.4 to 15.5 ± 5.0. These values were statistically significant to $P < 0.01$. This 2-compartment model was validated by excising the tumors and measuring the $^{19}$F content of the acid soluble and the RNA fractions. The increase of the mean $K_{in}$ value (2.4) estimated in vivo correlated favorably with the increase of the levels of the fluorinated nucleosides/nucleotides (2.2) observed by in vitro analysis. The in vitro measurements also revealed that the increase in the fluorinated nucleosides was accompanied with a similar increase in 5-fluorouracil incorporation into the RNA fraction, which suggests that increases or decreases in the relative intensity of the fluorinated nucleoside/nucleotide signals observed in vivo could be indicative of similar changes of the nuclear magnetic resonance invisible fluorinated RNA.

The present study documents that it is now possible to estimate the pharmacokinetic behavior of 5-fluorouracil and of its active metabolites at its target site, tumor tissue, by using noninvasive measurements. Such measurements may provide useful means of assessing, during treatment, the possible effect of 5-fluorouracil on the tumor, and its response to treatment, both when used by itself as well as a function of biochemical modulation.

**INTRODUCTION**

Cancer chemotherapy is often thought to be limited by the uptake and activation of the anticancer drug by the tumor, but there has not been a noninvasive way of assessing this in living systems, whether in animals or in clinical practice.

In vivo NMRS is a new technique (1, 2) that allows and makes it possible to detect changes in the body chemistry at the site of disease. Because such noninvasive studies do not cause perturbations of the living system, they can capture the fine elements of biochemical and pharmacological processes in a specific individual (human or animal). Because a living system can be monitored sequentially for at least several hours, it serves as its own control and may provide, from a single individual, data that previously would require a significantly larger number of individuals. In addition, data from a single living system are devoid of interindividual variations, thereby making information generated individual specific (3).

The first report that used $^{19}$F NMRS to study drug metabolism, in vivo, was the report of Stevens et al. (4) on the metabolism of 5-fluorouracil in the liver and tumors of mice. This was followed by the first human study, reported by our group (5), where $^{19}$F NMR spectra were collected from the livers of cancer patients being treated with 5-fluorouracil. To our knowledge, no study has been published to unfold the pharmacokinetics or 5-fluorouracil (or for that matter, of any other anticancer drug) in tumors, using data acquired by these noninvasive methods, followed by a compartmental model pharmacokinetic analysis.

Although 5-fluorouracil represents one of the more effective agents in the treatment of certain carcinomas, e.g., gastrointestinal, pancreas, breast, head and neck, ovary and prostate (6-8), it only produces objective tumor regressions in approximately 20% of treated patients when used as a single agent; in spite of this poor response, 5-fluorouracil remains a major component of frequently used chemotherapy regimens, in combination chemotherapy, or as a part of multimodality therapy. Among the reasons for the continued, and even increased use of 5-fluorouracil, are the absence of cross-resistance with other active agents, as well as the lack of a better chemotherapeutic drug for the treatment of digestive tract tumors. Because of significant interpatient variations, it is also possible that some of the low response may be due to our lack of knowledge of the optimized dosage regimen required by a given patient. This dosage regimen may be affected further when drugs that modulate the biotransformation and activation of 5-fluorouracil are used concurrently. MTX, which among other effects, increases the intracellular concentration of 5-phosphoribosylpyrophosphate, has been used concurrently with 5-fluorouracil so as to enhance the activation of this drug to some of its nucleotides (especially 5-fluorouridine monophosphate), which would result in enhanced formation of those molecules through which 5-fluorouracil appears to exercise its cytotoxic action: fluorinated RNA, fluorodeoxyuridine monophosphate bound to thymidylate synthase, and fluorinated DNA.

One of the questions that was to be addressed in the present study was whether MTX did, indeed, have a detectable effect, in vivo, on the metabolism of 5-fluorouracil, and whether a pharmacokinetic approach using noninvasive data would allow an estimation of such effects. If this were to be proven, then methods that allow monitoring of the intratumoral metabolism of 5-fluorouracil could provide information on the possible effectiveness of the drug, and on whether a given modulator would further enhance the response to chemotherapy with 5-fluorouracil.

**MATERIALS AND METHODS**

5-Fluorouracil (50 mg/ml), was obtained from Solo Pak Laboratories, Franklin Park, IL; methotrexate (25 mg/ml), was from Quad Pharmaceuticals, Inc., Indianapolis, IN. Fluoro-$\beta$-alanine (2-fluoro-$\beta$-amino-propionic acid) was from Koch-Light Laboratories, Ltd.
Colnbrook, Buckinghamshire, England; 5-fluorouridine was from Sigma Chemical Co., St Louis, MO; RPMI medium 1640 and fetal serum albumin, were from Gibco Laboratories, Grand Island, NY; Ketaset (ketamine hydrochloride) 100 mg/ml, was from Veterinary Products, Bristol Laboratories, Bristol-Myers Co., Syracuse, NY; Rompun (xylazine) 20 mg/ml, was from Haver Mobay Corporation, Shawnee, KS. All other chemicals and solvents were analytical reagents or high-performance liquid chromatography grade.

Tumorous Rats. The Walker 256 carcinosarcoma cells were stored in freezing medium containing RPMI 1640, fetal calf serum, and dimethyl sulfoxide (7:2:1) at -180°C. The tumor cell line is maintained by serial transplantation into rats. To implant, cells were thawed, washed with saline, and their viability, was ascertained by using the trypan blue exclusion test. Tumor cells (1 × 10⁷) were suspended in RPMI medium and injected sc into the right forelimb of male Sprague-Dawley rats, 200–250 g. This site of inoculation was chosen to avoid any contribution of signals from bladder or urine. Tumors were normally grown for 10–14 days and were used for NMRS when equal to or greater than the diameter of the surface coil used.

In Vivo ¹⁹F NMRS. ¹⁹F spectra were obtained at 188 MHz by using a 4.7-T 33-cm horizontal bore magnet (General Electric CSI) at the Huntington Research Institute. A surface coil of 2.2 cm in diameter was used as a receiver and transmitter. With this coil a 90-degree flip angle at R/2 distance from the center of the coil corresponded to a 15-μs pulse. Spectra were recorded under conditions of pulse spacing and flip angle which provided the highest sensitivity of detection of ¹⁹F for this coil and magnet: 1028 transients (FIDs) were collected by using a 10-μs pulse width, 400-msec repetition time, and a spectral width of 5500 KHz; 10 min were required for each set of spectra. A microcell containing 40 μmol of fluorine as DFB was permanently attached to the surface coil at 1.0 cm from the center of the coil, to provide an external reference standard and chemical shift reference. Each set of 1028 FIDs were base line corrected, trapezoidally multiplied, and fourier transformed. The signal intensities were normalized to that of the external reference standard (DFB).

In vivo animal studies were performed in the following manner: general anesthesia was induced in a Sprague-Dawley rat, 200–250 g, with xylazine (5 mg/kg) and ketamine hydrochloride (50 mg/kg) injected i.m. An i.v. line was implanted into the tail vein to allow for drug administration. The anesthetized rat was placed in a custom designed holder in such a manner that the tumor is positioned exactly over the surface coil. Following proper shimming and tuning, 150 mg/kg of FUra were injected as a bolus (2 min), and serial data acquisitions were collected for at least 2–3 h, sometimes for a longer period. Anesthesia was maintained by hourly readministration of the xylazine/ketamine mixture. Some of the rats were sacrificed at the end of data acquisition by a lethal dose of phenobarbital, the tumor tissues were thawed in 1 volume of ice-cold 0.6 N HClO₄, neutralized with 2 N KOH, then lyophylized and reconstituted with distilled water for analysis by NMR.

In Vivo ¹⁹F NMRS. ¹⁹F NMR spectra of the ASF and the RNA fractions were collected by using the IBM-Brucker WP-270 FT NMR spectrometer operating at 254 MHz. Calibrated reference standards of FUra, FUrtd and, FBAL were used to allow the quantitation of the metabolite concentration in the excised tumor tissues. Spin-lattice relaxation times (T₁) for FUra and its metabolites were measured by using the inversion recovery sequence. A pulse width of 9.5 μs, which reflects the Ernst angle, has been used for all the experimental runs. In order to attain maximum signal to noise ratio a 1-s pulse repetition rate was used. Because this is obviously not long enough to allow full relaxation of the products present, correction factors (15 versus 1 s) were estimated for FUrtd, FUra, and FBAL: 1.168, 1.531, and 1.680, respectively. Absolute metabolite level determinations required the use of an external standard. A 2-mm capillary tube was filled with 40 μl of a 10.15E-3 M solution of 1,2-difluorobenzene in benzene (which corresponds to 800 nmol of fluorine) and 30 μl of a 2.8E-3 M solution of chromium acetyl acetone (a relaxation agent), also in benzene. This external reference standard was calibrated against known and exact amount of solutions of FUra, FUrtd and, FBAL containing 100, 250, 500, 1000, 1750 nmol, respectively, of each compound. The calibrated capillary was used for the quantitation of the metabolite concentration of the excised tumor tissues or the acid extracts.

Pharmacokinetic Modeling. Based on the available knowledge of the metabolism of FUra and the nature of the data that can be obtained, a series of models of increasing complexity were formulated (Fig. 1). Because of the complex nature of the metabolism of FUra, related metabolites that could not be detected separately were lumped into single compartments. Thus, the fluorinated nucleosides and nucleo-

**Fig. 1. Schematic representation of various compartmental subsystem models for analyzing the biodistribution and the metabolism of 5-fluorouracil in mammalian tumors.**

Model 1A, 3-compartment model, with lumped compartmental transfer constants; Model 1B, a simplified, 2-compartment variant of Model 1A, allowing for the estimation of the transfer rate constant K₂ as an elimination rate constant K₂. Model 2A, 3-compartment model with explicit expression of the rate constant that represents the hydrolysis of the high molecular weight anabolites; Model 2A2, a simplified, 2-compartment variant of Model 2A, allowing for the estimation of the transfer rate constant K₂ as an elimination rate constant K₂. Model 2B, 3-compartment model with explicit expression of the rate constant that represents the hydrolysis of the high molecular weight anabolites; Model 2C, 3-compartment model with explicit expression of the rate constants of both the formation as well as the hydrolysis of the high molecular weight anabolites and of the nucleosides/nucleotides.
tides, including any FUra, fluorodeoxyuridine, fluorodeoxyuridine di-phosphate; fluorodeoxy triphosphate, fluorouridine monophosphate, etc., have been included in the FNUC compartment, whereas all FUra that would have been incorporated into RNA, DNA, and thymidylate synthase was included into the HMWA compartment. Each model was subject to a structural identifiability analysis (12), and only those models that were structurally identifiable were used in this study. The ADAPT-II program (13) was used for parameter estimation and simulation. The assumption was made that more than 95% of the FUra that would enter the tumor did so in the first, or at most, the first few passages, allowing the estimation of the input function, using the "initial condition" calculation in the ADAPT-II program. The least squares estimates of the parameters of each of the structurally identifiable models were evaluated by the minimal Akaike information criterion estimation (14) to determine the best model that can explain the experimental data.

RESULTS

A typical spectra acquired in vivo from a rat receiving FUra alone is shown in Fig. 2, and from a rat that had been predosed with MTX 5 h previously is shown in Fig. 3. Data acquisition started at the time of the bolus injection of FUra. Each spectral line represents the accumulation over a 10-min period of acquisitions. The DFB reference peak appears at 28 ppm downfield from FUra, the FNUC peak at 4–5 ppm downfield from the FUra peak, and the FBAL signal at 19 ppm upfield from FUra. No signals were detected from the other low molecular metabolites of FUra, such as 2-fluoro-octapropionic acid, at 18 ppm upfield from FUra, or 5,6-dihydrofluorouracil, at 32 ppm upfield from FUra.

Qualitatively, it can be seen that tumors receiving FUra alone metabolized the drug to FNUC at a slower rate; FNUC started to be detectable 30 min postdrug administration. By 90 min, the peak intensities of FUra and FNUC appeared to be similar, while FBAL began to be detectable 100 min after drug administration.

Pretreatment with MTX appears to result in a significant change in the pattern of metabolism of FUra in the tumor: the FNUC signals appeared in the first 10 min and their intensity appeared to increase at a faster rate than in the rats treated with FUra alone, with a concomitant faster decrease in the intensity of the FUra signals.

Subsystem Model 1A (Fig. 1), which is one of the simplest compartmental models to describe the biochemical events in the tumor following the administration of FUra, is not structurally identifiable when the output functions available were those of the time course of FUra and FNUC in the tumor. Fig. 1, Model 1B, is structurally identifiable under these conditions and was used for the analysis and the comparison of the transfer microconstants of the treatment groups; although it is overparameterized for certain estimates in each treatment group, as indicated by minimal Akaike information criterion estimation analysis (14), it is the simplest model that can be used to estimate the time course of FUra and its metabolites in the tumor. $K_{01}$ represents the apparent rate of elimination of FUra from the tumor (including its anabolism to FBAL), $K_{21}$ is the apparent composite rate of formation of FNUC from FUra (no estimation of the forward and reverse rate constants appeared possible at this time), and $K_{20}$ is the apparent rate of disappearance of FNUC to products other than FUra, hence, its apparent rate of incorporation into RNA, DNA, and thymidylate synthase.

A typical result of estimations performed with the data from 3 rats that had been administered FUra, and been predosed with no MTX or with MTX 5 and 22 h prior to FUra administration, is given in Fig. 4. The estimation summaries of the parameters from these 3 individual rats are listed in Tables 1, 2, and 3. The mean value distribution of the microconstants under different treatments are summarized in Table 4. An F test revealed a statistically significant difference at the 1% level ($P < 0.01$) in the mean $K_{21}$ between the rats treated with FUra alone and those that had received MTX treatment 5 h before, and at the 2% level when comparing FUra alone to MTX 22 h before.

The estimates of $K_{01}$, which represent the catabolism and/or the excretion of FUra from the tumor, are significant only for those animals treated with FUra alone; estimation of $K_{01}$ for those rats that received either the MTX pretreatment schedule gave a very low value that is statistically different at $P < 0.001$ for FUra versus MTX 5 h before, and at $P < 0.02$ for FUra versus MTX 22 h before.

The estimates of $K_{20}$, which represent the apparent further anabolism of the FNUC, presumably to the HMWA, shows a significant increase when MTX is added as a pretreatment. The F test reveals a statistically significant difference between FUra alone and either of the MTX pretreatments ($P < 0.01$). There is no statistically significant difference between any of the mean

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rate constants ($K_{01}$, $K_{02}$, or $K_{21}$) for the two groups of rats pretreated with MTX.

In addition to these statistical comparisons between two groups of animals at a time, a Scheffé test was performed among all three groups. The differences in $K_{01}$ and $K_{02}$ were statistically different at $P < 0.05$ among all three dosage groups, but for $K_{21}$ only between MTX pretreated at 5 h and FUra alone, and again not between the two groups pretreated with MTX at 5 and 22 h.

Validation studies of this model were performed by sacrificing several rats at the end of 2 h of in vivo $^{19}$F NMRS data collection, extracting their tumors, and measuring the amount of $^{19}$F-containing products in the ASF and the RNA fractions. The products detected (FUra, FNUC, and FBAL) were the same products as those observed in the in vivo experiments. Tables 5 and 6 summarize the results from these in vitro experiments. The only fluorinated products detected in the hydrolysates of the RNA fraction were the FNUC (both as nucleosides and nucleotides). For tabulation purposes, the amounts of the fluorinated nucleosides and nucleotides have been summed as FNUC, inasmuch as the in vivo measurements preclude a clear separation between these two sets of fluorinated compounds. Analysis of the precipitate containing the DNA and the protein fractions failed to reveal any detectable (<0.1 mC) $^{19}$F signals. These data also revealed that in rats pretreated with MTX (5 h), the amount of FNUC in the ASF, as well as the amount of fluorinated products in the RNA fraction, had doubled.

**DISCUSSION**

The first critical and essential step for any drug to exhibit its antitumor activity is that it penetrates into the target tissue. While most authors have assumed that the uptake of FUra by

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**Table 1 Weighted least-squares estimation summary of rat FU12989**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV (%)</th>
<th>Confidence interval (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{21}$</td>
<td>0.89E-02</td>
<td>16.1</td>
<td>0.590E-02 – 0.119E-01</td>
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<tr>
<td>$K_{01}$</td>
<td>0.14E-01</td>
<td>18.1</td>
<td>0.838E-02 – 0.187E-01</td>
</tr>
<tr>
<td>$K_{02}$</td>
<td>0.66E-07</td>
<td>0.5E+07</td>
<td>-0.662E-02 – 0.662E-02</td>
</tr>
</tbody>
</table>

* CV, coefficient of variation.

**Table 2 Weighted least-squares estimation summary of rat A32190**

<table>
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<th>Parameter</th>
<th>Estimate</th>
<th>CV (%)</th>
<th>Confidence interval (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{21}$</td>
<td>0.19E-01</td>
<td>8.9</td>
<td>0.158E-02 – 0.239E-01</td>
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<tr>
<td>$K_{01}$</td>
<td>0.99E-05</td>
<td>0.2E+5</td>
<td>-0.506E-02 – 0.508E-02</td>
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<tr>
<td>$K_{02}$</td>
<td>0.11E-02</td>
<td>123.6</td>
<td>-0.195E-02 – 0.418E-02</td>
</tr>
</tbody>
</table>

* CV, coefficient of variation.

**Table 3 Weighted least-squares estimation summary of rat r11790mtxfu**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV (%)</th>
<th>Confidence interval (95%)</th>
</tr>
</thead>
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<tr>
<td>$K_{21}$</td>
<td>0.14E-02</td>
<td>4.6</td>
<td>0.123E-01 – 0.150E-01</td>
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<td>$K_{01}$</td>
<td>0.87E-02</td>
<td>18.8</td>
<td>0.528E-02 – 0.122E-01</td>
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<tr>
<td>$K_{02}$</td>
<td>0.48E-03</td>
<td>162.6</td>
<td>-0.114E-02 – 0.210E-02</td>
</tr>
</tbody>
</table>

* CV, coefficient of variation.

Fig. 4. Pharmacokinetic analysis, using Model 1B, of the normalized peak intensities obtained by noninvasive $^{19}$F NMRS of 5-fluorouracil (FU) (C) and FNUC (O) from the tumor of Sprague-Dawley rats bearing the Walker 256 carcinosarcoma, following administration of 150 mg/kg of FUra. The solid curves were estimated using the ADAPT-II program (13). Fig. 4A is from rat fu112989, which had received only a bolus dose of FUra; Fig. 4B is from rat A32190, which had received a dose of 40 mg/kg of MTX 5 h prior to the administration of a bolus of 150 mg/kg of FUra; Fig. 4C is from rat r11790mtxfu, which had received a dose of 40 mg/kg of MTX 22 h prior to the administration of a bolus of 150 mg/kg of FUra.
tumor cells occurs by passive diffusion (15), some have suggested facilitated diffusion (16, 17) or even active transport (18). The present study supports a previous suggestion (19, 20) that FUra uptake is probably due to one of the latter two mechanisms. Au et al. (21) had reported at $t_0$, of 15.3 min for FUra in rat plasma. The present work has shown that the $t_0$ of FUra in the tumor ranged from 42.2 to 59.4 min, which is significantly larger than what might be expected if FUra would diffuse freely across the cell membrane, and that the highest level of FUra detected in all tumors studied was achieved in the first set of spectra, and then decreased at a much slower rate than the FUra of the blood. These findings support the notion of either active transport or facilitated diffusion as the mechanism for the cellular uptake of FUra in tumors.

The next step for FUra to be able to exert its cytotoxic effect is its conversion to its anabolites; these are formed intracellularly and remained trapped within the cell. Hence, the amount and the rate of formation of these anabolites are critical determinants of the potency, duration, and therapeutic effectiveness of FUra. While in cell cultures cellular sensitivity to FUra has been shown to be related to different rates of intracellular drug metabolism rather than to variation of cellular uptake of the parent drug (22), both processes are likely to be significant in vivo. The appearance of FNUC, which can be measured in the Walker 256 tumor model in the rat (23, 24), but not in human tumors (19, 20), has been postulated as a potential indicator of tumor response (25). The present study provides further support to this prediction, by documenting that MTX, whose mechanism is to promote the formation of the FNUC, does indeed have such an effect. Also of significant interest is the observation that the mean value for $K_{10}$ (the rate of the first step in the formation of cytotoxic anabolites, as estimated from the in vivo data) was at least doubled (2.4 times higher), a value that agrees very closely with the increase in FNUC measured by in vitro analysis (2.2 times higher). Of interest is a somewhat related study of FUra metabolism in CD8F1 murine mammary tumor in mice (26), where a similar increase was observed in the formation of the FNUC following predosing with MTX. The results of the in vitro measurements validate the compartment model estimates, and suggest the usefulness of such a pharmacokinetic analysis in predicting tumor response.

Comparing the two MTX pretreatment schedules, and while the in vivo spectra suggest that the 5-h pretreatment appears to cause a more significant enhancement on the rate and extent of anabolism of FUra in the tumors than the 22-h pretreatment, an $F$ test of the pharmacokinetic parameters of the two treatment groups indicated that the difference is not statistically different ($P > 0.05$). Thus, more studies are needed, using NMRS, to evaluate and determine the optimum modulation regimen to be used.

A more detailed examination of the results reported in Table 4 (the values of the parameters estimated from Model 1B) reveals that when MTX stimulates the rate of the conversion of FUra to FNUC, it decreases or totally suppresses the exit of FUra from the tumor and/or its conversion to its catabolites.

Although it would be of interest to obtain in vivo information on the balance of catabolism versus anabolism of FUra in the tumor, our data suggest that the variable amounts of FBAL detected in these rat tumors did not fit any specific pattern. Prior et al. (27) had shown that FBAL was present in rat tumors after i.v. administration of FBAL to tumorous rats; thus, FBAL might be taken up from the plasma, and not necessarily be formed by intratumoral metabolism. Because the catabolism of FUra is practically an irreversible step, the compartmental models used in this study did not consider FBAL as a separate compartment, but included all catabolic processes in the elimination compartment of FUra to FNUC, it decreases or totally suppresses the exit of FUra from the tumor and/or its conversion to its catabolites.

The estimated rate of formation of FNUC was slower than the estimated rate of disappearance of FUra in control tumorous rats, suggesting that a measurable amount of FUra is eliminated from the tumor without contributing to the formation of cytotoxic products. The significant decrease of the elimination rate constant ($K_{t0}$) from the FUra compartment in the tumor. Based on the observations of Prior et al., and our observations in this work, we can suggest that measurements of FBAL in the tumor, or the FNUC/FBAL ratio, do not appear to have a prognostic value in determining the possible effectiveness of FUra.

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proaches when attempting to understand the uptake of a drug into tissues and organs, as the time course of drug concentration in blood does not usually reflect or provide a meaningful indicator of the dynamics of drug accumulation and washout in tissues. Similarly, Hull et al. (29) had reported that when FUrA and its metabolites were monitored in plasma and urine, no significant differences could be detected between responders and nonresponders of those patients that had received FUrA alone and those that had received FUrA following pretreatment with MTX, and suggested that measurements of FUrA and its anabolites in tumor tissue appeared to be essential to the development of strategies for improving therapeutic regimens or to provide an early prognosis. The present work illustrates how noninvasive measurements, and the use of the data they provide to pharmacokinetic modeling, permit a much more accurate description of the time course of the drug in the target tissue.

However, and contrary to other approaches to pharmacokinetic modeling that use parameters and rate constants obtained from other systems, we have developed an approach where the only data to be used are those generated in the specific living system that is under study. Furthermore, we have been focusing on a subsystem approach (30), and attempted to determine whether a simple subsystem model of FUrA could estimate the rate of FUrA uptake, retention, and metabolism in tumor tissues.

The goodness of fit of Model 1B was evaluated by consideration of the randomness of scatter, weighted sum of squared residuals, and the Akaike information criterion. Other models, which did not entail changes in the number of products but only in that of the parameters to be estimated, did not significantly reduce the sum of squares, and did not appear to provide information beyond what has been supplied by model 1B. The relative validity of model 1B was documented further by the in vitro measurements, which suggested that there was a reasonable correlation between the estimated values for the pool of the HMWA (not detectable by noninvasive measurements), and those actually measured following tumor excision. One further measure of the correlation between the model estimates and the metabolites measured is given by the increase in the mean value for $k_2$ (the rate of formation of the cytotoxic anabolites) when comparing the FUrA alone versus MTX 5-h pretreatment; while this rate constant increased 2.4-fold, the increase of the FNUC was 2.2-fold.

Interanimal differences in the values of the parameters between experiments in the same treatment groups are consistent with expected interindividual variations and differences in the size and location of the tumors, which also affect the different sensitivity profiles detected by surface coils.

In conclusion, we have documented once again that NMRS can be used to generate, noninvasively, critical information from the tumor such as drug uptake and drug activation, both of which are data which could predict therapeutic efficacy; that such noninvasive measurements of a drug in tumor tissue, augmented by a pharmacokinetic analysis of the intratumoral fate of a drug at its target site, may provide key information about the kinetics of both the parent drug and its potentially active metabolites, information which may be required for proper prediction of how much drug is needed to effect tumor cell kill; and finally, that such studies provide useful means of studying and correlating the effects and responses of FUrA directly in the tumor as a function of biochemical modulation, dosage, or treatment schedule. We believe that the extension of such studies to humans will make available sufficient new information which could allow the development of an optimal dosage regimen or more effective combination chemotherapies for individual patients.

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