Advances in Brief

Extraction of 5-Fluorouracil by Tumor and Liver: A Noninvasive Positron Emission Tomography Study of Patients with Gastrointestinal Cancer

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Abstract

Tumor and normal tissue pharmacokinetics of 5-Fluorouracil (5-FU) in patients can be determined with positron emission tomography scanning. However, the data obtained are of limited value because of the inability to distinguish catabolites (inactive species) from parent 5-FU and anabolites (cytotoxic species). In this paper, we have blocked 5-FU catabolism in one arm of a paired study with eniluracil, an inhibitor of dihydropyrimidine dehydrogenase, enabling catabolite correction and calculation of tissue pharmacokinetic parameters to be achieved. Using this novel approach, we report for the first time that the net clearance of 5-[18F]FU from plasma into tumors (liver metastases and pancreatic tumor) of patients is low (KI = 0.0033 ± 0.0005 ml plasma/ml tissue/min). In contrast, the initial (up to 10 min) clearance through catabolism in liver was high (KI = 0.7313 ± 0.092 ml plasma/ml tissue/min). In the absence of eniluracil, catabolites in tumors accounted for 83% of total tumor exposure (range, 66 –91%), whereas catabolites in liver accounted for 96% of total liver exposure (range, 94 –98%). This study provides definitive evidence that the cytotoxicity of 5-FU in patients with gastrointestinal cancer could be compromised by its intrinsically low uptake by tumors, as well as decreased systemic availability through hepatic catabolism.

Introduction

5-FU1 (Fig. 1) is the most widely used chemotherapeutic agent for the systemic therapy of gastrointestinal cancers. Response rates from 5-FU monotherapy are <14% in the treatment of this cancer (1). Thus, knowledge about the pharmacology of the compound in patients will help in strategies for improving response. Of particular importance is information on the pharmacokinetics of 5-FU, which has mostly been derived from plasma data. Plasma pharmacokinetics cannot predict tissue behavior because of the nonlinear metabolism (2), thus, there is the need for alternative strategies for obtaining such information. Noninvasive imaging of tissue pharmacokinetics with NMRS and PET allows parameters of drug localization and metabolism to be estimated in tissues of interest (3–6).

Previously we have performed PET studies to assess the pharmacokinetics of 5-[18F]FU in humans (3, 6, 7). The pharmacokinetic information derived from these previous studies was, however, limited because of the inability of PET to distinguish between labeled chemical forms of a compound, e.g., parent drug, anabolites, and catabolites. Attempts have been made to use compartmental modeling to evaluate the contribution of the various chemical forms. For instance, Kissel et al. (8) used a 6-compartment model (formulated from a 12-compartment model) to analyze PET 5-FU pharmacokinetics. In this manuscript we have blocked catabolism of 5-FU in one arm of a paired study with eniluracil, an inhibitor of dihydropyrimidine dehydrogenase, to obtain “pure” 5-FU (plus anabolite) tissue signal. This has enabled us to calculate rate constants for net clearance from plasma into tumor and liver, as well as the extent of metabolism in the absence of eniluracil. This approach has less inherent assumptions compared with the compartmental approach.

Materials and Methods

Study Design. The aims of the study were to determine the net clearance of 5-[18F]FU from plasma into tissues and the magnitude of catabolite contribution to 5-[18F]FU tissue PET data. This could be achieved by blocking catabolite formation in one arm of two PET scanning sessions, with the fundamental assumption that, other than catabolism, 5-[18F]FU behaves similarly before and after inhibition of catabolism. Five liver metastases and one primary pancreatic tumor from four patients with gastrointestinal cancer were evaluated. Patients received 1 mg/m2 of 5-FU containing tracer quantities of 5-[18F]FU before and on the third day after a course of eniluracil (20 mg twice daily for 4 days; Ref. 3). Eniluracil inactivates dihydropyrimidine dehydrogenase, the first enzyme involved in the catabolism of 5-FU (9–11). The tissue 5-[18F]FU signal after eniluracil administration (which contains no catabolites) was used to calculate the net clearance of 5-[18F]FU into tumors and liver. The 5-[18F]FU partitioning between plasma and tissues was also calculated and applied to 5-[18F]FU studies in eniluracil-naïve patients to evaluate the magnitude of catabolism. Eligibility criteria for this study have been described previously (3).

PET Imaging. The imaging procedure used here has been reported previously (3). Briefly, 5-[18F]FU was synthesized as described by Brown et al. (12). With the aid of computed tomography images, the central plane to be imaged was localized by a radiographic simulator. PET scanning was performed after a single i.v. injection of 1 mg/m2 5-FU containing tracer quantities of 5-[18F]FU over a period of 30 –60 s. All PET scans were performed on an ECAT 931-08/12 scanner (CTI/Siemens, Knoxville, TN), which allows simultaneous data acquisition to form 15 transaxial planes spaced 6.75 mm apart (axial field of view 10.8 cm). PET data were acquired simultaneously with arterial blood sampling to enable comparison of tissue radioactivity with plasma input. In addition to continuous blood sampling, discrete samples were also obtained for high-performance liquid chromatography analysis of parent and 18F-radiolabeled metabolites as previously described (3). The levels of stable compound associated with 5-FU only were obtained by normalizing the percentage of 5-[18F]FU to total radioactivity of the sample and specific radioactivity of the injected dose solution. Regions of interest on tumor and liver were manually defined with the aid of X-ray computed tomography and the image-analysis software, Analyze (Mayo Clinic, Rochester, MN).4

Catabolite Correction. A general deconvolution technique (13, 14) was used to determine the magnitude of catabolism to the 5-[18F]FU tissue radioactivity data. The first step involved derivation of the relationship between plasma 5-[18F]FU levels and tissue response (5-[18F]FU plus [18F]anabolites), referred to as the 5-[18F]FU unit impulse response function (IRF5F). Given that eniluracil completely inhibits 5-[18F]FU catabolism (10, 15), the following equation holds when 5-[18F]FU is injected after eniluracil treatment: [Total tissue,](t) = [Plasma 5-FU](t) ⊗ IRF5F(t)

4 Kinetic data presented here have been corrected for decay of radioactivity and normalized to injected activity/body surface area.

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3 The abbreviations used are: 5-FU, 5-fluorouracil; NMRS, nuclear magnetic resonance spectroscopy; PET, positron emission tomography; FBAL, α-fluoro-β-alanine, IRF, unit impulse response function; Ki, rate constant for net clearance of radiolabel from plasma to tissue; AUC, area under the radioactive concentration versus time curve.
where \([\text{Tissue catabolites}]\) and \([\text{Plasma}]\) denote the concentration of total radiolabel in tissue and of 5-[18 F]FU in plasma, respectively, as a function of time. Blood and plasma data were processed to generate a continuous plasma input function. Briefly, the fraction of 5-[18 F]FU in discrete plasma samples was fitted to a sigmoid function with 1/\(y\) weighting in the absence of eniluracil and an exponential-to-straight line function in the presence of eniluracil. The partitioning of radioactivity between whole blood and plasma was fitted to a linear function. These functions were then used to derive the plasma input function from the continuous blood radioactivity data. Deconvolution of tissue data were achieved by a spectral analysis algorithm previously described by Cunningham and Jones (13), where the plasma input function is convolved with the IRF expressed as a sum of exponential terms:

\[
[Tissue](t) = I RF(t) \otimes [Plasma](t) \tag{D}
\]

where \(n\) is the number of identifiable kinetic components, \(\beta\) is constant such that \(\lambda < \beta, < 1 \lambda = \text{decay constant of }^{18}\text{F}; 0.00632\), and \(\alpha\) is the intensity of the kinetic component at \(\beta\). Data were expressed as AUC and as proportions of [18 F]catabolites or 5-[18 F]FU/[18 F]anabolites at the end of PET scanning.

**Determination of the Net Unidirectional Clearance of Radiolabel.** A noncompartmental approach, Patlak analysis (18), was used to determine the net unidirectional clearance of radiolabel from plasma into tissue. A major advantage of using this model is that it is independent of the number of reversible components (18). Assuming that there is a single source for the radiotracer, the plasma, the following equation holds:

\[
[Tissue](t) = K_i \int_0^t \frac{[\text{Plasma}]\delta t + (V_o + V_p) \cdot [\text{Plasma}](t)}{[\text{Plasma}] + [\text{Plasma}](t)} \tag{F}
\]

where \(K_i\) is the net clearance from plasma into tissues (also referred to as the net unidirectional influx constant or the net irreversible uptake rate constant), \(V_o\) is the steady-state space of the exchangeable region and \(V_p\) is the plasma volume within the tissue. A plot of \([Tissue](t)/[Plasma](t)\) versus \(\int_0^t [\text{Plasma}]\delta t/[\text{Plasma}](t)\) gives a slope equal to \(K_i\). If there is unidirectional uptake, a linear phase of the curve is discernible.

The net clearance of 5-[18 F]FU in tumors, \(K_i\), was determined using tissue 5-[18 F]FU data obtained after eniluracil treatment. The PET data in this case comprises of parent 5-[18 F]FU and [18 F]anabolites. A similar method was used to study the initial (up to 10 min) extraction and trapping of 5-[18 F]FU through catabolism by the liver. To do this, deconvolution methods were used to remove the contribution of 5-[18 F]FU plus [18 F]anabolites from the total liver signal and Patlak analysis performed on the resulting data using the 5-[18 F]FU input function.
data (AUC, % $^{18}$F)catabolites, % $^{18}$F FU plus $^{18}$F anabolites, and $K_I$) were compared using the Mann-Whitney $U$ test. $P$ sof $\leq 0.05$ were considered statistically significant.

**Results**

**Magnitude of Catabolism.** As previously reported, $^{5-}[^{18}$F$]$FU is rapidly catabolized in the absence of eniluracil, with $^{18}$F FBAL being the predominant plasma catabolite (3). Eniluracil produced a 16-fold increase in plasma 5-FU AUC 0–240 min (Table 1). Evidence that eniluracil inactivated tissue dihydropyrimidine dehydrogenase activity was demonstrated by the marked decrease in $^{5-}[^{18}$F$]$FU-derived $^{18}$F radioactivity in normal liver. Fig. 2a and 2b are typical transabdominal PET $^{5-}[^{18}$F$]$FU images acquired before and after eniluracil treatment. The high signal intensity in normal liver was attributed to catabolism and retention of $^{18}$F FBAL (3, 8, 11, 17). Eniluracil decreased liver exposure to $^{5-}[^{18}$F$]$FU. Fig. 3a and 3b shows representative plasma input function and tissue response, respectively, for $^{5-}[^{18}$F$]$FU in eniluracil-treated patients. $IRF_{FU}$ was calculated from these curves using Eq. A. Fig. 3c and 3d shows corresponding $IRF_{FU}$ displayed as a curve and as a spectrum of kinetic components. Most of the kinetic components were reversible.

**Table 2.** Area under the tumor $5-[^{18}$F$]$FU-derived activity versus time curves for $5-[^{18}$F$]$FU normalized to body surface area (AUC) in the presence and absence of eniluracil, and the percentage of $[^{18}$F$]$catabolites in the latter

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>AUC$<em>{0-95}$ AUC$</em>{0-95}$</th>
<th>AUC$<em>{0-95}$ AUC$</em>{0-95}$</th>
<th>AUC$<em>{0-95}$ AUC$</em>{0-95}$</th>
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<tr>
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<td>$[^{18}$F$]$Total</td>
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<td>$[^{18}$F$]$Total</td>
<td>$[^{18}$F$]$Total</td>
</tr>
<tr>
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<td>0.175</td>
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<td>0.024</td>
</tr>
<tr>
<td>2</td>
<td>0.261$^a$</td>
<td>0.232</td>
<td>0.032</td>
</tr>
<tr>
<td>3</td>
<td>0.233</td>
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<td>0.422</td>
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</tr>
<tr>
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<tr>
<td>6</td>
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<td>0.188</td>
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<tr>
<td>Average</td>
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<td>0.202</td>
<td>0.030</td>
</tr>
<tr>
<td>2</td>
<td>0.247</td>
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<td>SE</td>
<td>0.019</td>
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$^a$ Data from individual tumors in patients with multiple tumors.
components. Similar results were obtained for normal liver. IRF and FU values were then used to deconvolve tumor and liver radioactivity curves into a [18F]catabolites (mainly [18F]FBAL) component and a component comprising of parent 5-[18F]FU and [18F]anabolites. Fig. 4a and 4b illustrates the results of such deconvolution for tumor and liver, respectively. The AUC 0–95 min for total 18F radioactivity in eniluracil-treated and -naïve patients, as well as AUC 0–95 min for 5-[18F]FU/anabolites and proportion of [18F]catabolites in tumor and normal liver regions are presented in Tables 2 and 3. There was a statistically significant difference for AUC0–95 min between eniluracil-treated and eniluracil-naïve patients for both tumor and liver (P < 0.05). Eniluracil produced a 7-fold increase in tumor and liver 5-[18F]FU/anabolites AUC0–60 min. In Fig. 4b, the tissue levels of [18F]catabolites seem to follow that of the plasma input function, supporting the assertion that tumor catabolites are derived predominantly from circulating catabolites.

**Net Clearance of 5-[18F]FU in Tumors and Liver.** Patlak analysis (Eq. E) was used to evaluate the net clearance of 5-[18F]FU from plasma into tumors. The presence of a linear phase of the curve (Fig. 5a) indicated that irreversible uptake of 5-[18F]FU occurred in tumors. However, the magnitude of uptake was low (Table 4). As a comparison, the KI for fluorodeoxyglucose in a tumor with a standardized uptake value of 12 was 8-fold higher (0.0295 ml plasma/ml tissue/min). The initial (up to 10 min) extraction of 5-[18F]FU through catabolism by the liver was also determined. Fig. 5b shows a typical plot, and data are summarized in Table 4.

**Discussion**

5-FU is cytotoxic to tumor cells in vitro (2). However, its efficacy in patients is only modest, as evidenced by response rates of <14% when used as a single agent (1). We have provided very strong evidence in this paper to suggest that the low efficacy could be attributable, in part, to the low net clearance of 5-FU by tumors (KI5 = 0.0036 ± 0.0005 ml plasma/ml tissue/min). This finding was made possible by our ability to block 5-FU catabolism with eniluracil and the use of mathematical models to describe plasma-tissue exchange. A general deconvolution technique was used to determine the contribution of catabolite signal to total tissue signal. This was nec-

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>AUC0–95 min [18F]Total</th>
<th>AUC0–95 min [18F]Total</th>
<th>AUC0–95 min 5-[18F]FU</th>
<th>% [18F]catabolites</th>
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<tr>
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<td>97.7</td>
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</tr>
<tr>
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<td>0.338</td>
<td>1.262</td>
<td>0.041</td>
<td>96.8</td>
</tr>
<tr>
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<td>0.292</td>
<td>1.146</td>
<td>0.035</td>
<td>96.9</td>
</tr>
<tr>
<td>5</td>
<td>0.318</td>
<td>1.131</td>
<td>0.048</td>
<td>95.8</td>
</tr>
<tr>
<td>Average</td>
<td>0.380</td>
<td>1.333</td>
<td>0.052</td>
<td>96.0</td>
</tr>
<tr>
<td>SE</td>
<td>0.031</td>
<td>0.108</td>
<td>0.005</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Data from one of two regions of interest in normal liver.

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**Fig. 5.** Results of Patlak analysis for patient 1. a, tumor data and line of best fit. Data from studies in eniluracil-treated patients were used for this analysis. b, liver data and line of best fit. Parent 5-[18F]FU and [18F]anabolites radioactivity were removed from the total liver radioactivity data. The resulting tissue data comprising of catabolites were fitted using plasma 5-[18F]FU as input function.
essary because the PET signal does not differentiate between chemical species. In addition, the net rate constant for the unidirectional clear- ance of radiolabel from plasma into tissue ($K_t$) was calculated. This parameter describes the steady-state irreversible uptake (flux) of the radiolabel from plasma into tissues through rate-limiting biological processes such as phosphorylation, metabolite trapping, and ligand-receptor binding. The total flux of drug into tissues can be calculated by multiplying plasma concentrations by this flux constant. $K_t$ may differ between tumors, and the ability to calculate interpatient differences in tissue clearance is key to the individualization of therapy.

The intertumor variability in magnitude of $K_t$ observed here (range, 0.0012–0.0050) could be due to: (a) differences in intracellular versus extracellular pH gradient (19); and (b) differences in rate of anabolism of 5-[18 F]FU (2, 20). Both processes are rate limiting and can influence tumor response. Shani and Wolf (21) were first to study the biodistribution of 5-[18 F]FU in mice, where a direct relationship between response and tumor levels of 5-[18 F]FU was observed. In that study, average tumor uptake of radioactivity/g of tissue after injection of 5-[18 F]FU was found to be low (~2% in mouse tumors and ~0.8% in rat tumors; Ref. 21). Subsequently, Presant et al. (4) showed by NMRS that increased intratumor 5-FU half-life was associated with response in tumors of patients. In their studies, 8 of 9 patients who showed trapping (defined as 5-FU half-life of ≥20 min) had partial response compared with only 2 of 25 patients whose tumors did not trap the drug. No fluorinated nucleotides were detected in that study. Unlike the work of Presant et al. (4), our data will reflect changes in both tumor 5-[18 F]FU half-life and anabolism. We did not have a large enough patient population to assess the effect of $K_t$ on response.

Liver is abundant in dihydropyrimidine dehydrogenase (22) and, hence, a major organ for the catabolism of 5-FU. The initial extraction of 5-[18 F]FU from plasma into liver was found to be 205-fold higher than the extraction by tumors. This high initial uptake will explain the high proportion (96%) of catabolites in the liver. $K_t$ was variable between patients (range, 0.392–1.186). This could be attributable to the differences in dihydropyrimidine dehydrogenase expression and activities between patients (23). Liver is not the only source of the catabolic enzyme. Peripheral blood mononuclear cells and kidneys also possess high levels of the enzyme (22). We hypothesized that tumor catabolites were derived predominantly from plasma. This assumption was supported by the similarities in time versus radioactivity profiles for catabolites in plasma and tumors. The observed proportion of catabolites in tumors (83%) is consistent with the high catabolic activity of liver. FBAL catabolites have been detected in tumors by NMRS (20) and shown to be derived mainly from plasma (8, 17, 24).

In conclusion we have used a novel methodology to determine the magnitude of 5-[18 F]FU catabolism in patients with gastrointestinal tumors. In addition, we have been able to determine the net clearance of 5-[18 F]FU by tumor and liver.

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References

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