Introduction

5-FU\(^5\) (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-[\(^{18}\)F]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 inactivator 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-[\(^{18}\)F]FU from plasma into tumors (liver metastases and pancreatic tumor) of patients is low (\(K_t = 0.0033 \pm 0.0005\) ml plasma/ml tissue/min). In contrast, the initial (up to 10 min) clearance through catabolism in liver was high (\(K_t = 0.7313 \pm 0.092\) 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.

Materials and Methods

Study Design. The aims of the study were to determine the net clearance of 5-[\(^{18}\)F]FU from plasma into tissues and the magnitude of catabolite contribution to 5-[\(^{18}\)F]FU tissue PET data. This could be achieved by blocking catabolite formation in one arm of two PET scanning sessions, with the assumption that, other than catabolism, 5-[\(^{18}\)F]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/m\(^2\) of 5-FU containing tracer quantities of 5-[\(^{18}\)F]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-[\(^{18}\)F]FU signal after eniluracil administration (which contains no catabolites) was used to calculate the net clearance of 5-[\(^{18}\)F]FU into tumors and liver. The 5-[\(^{18}\)F]FU partitioning between plasma and tissues was also calculated and applied to 5-[\(^{18}\)F]FU studies in eniluracil-naı ¨ve 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-[\(^{18}\)F]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/m\(^2\) 5-FU containing tracer quantities of 5-[\(^{18}\)F]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 \(^{18}\)F-radiolabeled metabolites as previously described (3). The levels of stable compound associated with 5-FU only were obtained by normalizing the percentage of 5-[\(^{18}\)F]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).

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

\[
\text{Total tissue } \frac{t}{(t)} = \left[ \text{Plasma 5-FU} \right] \frac{t}{(t)} \otimes \text{IRF}_{5FU}(t)
\]
where \([\text{Tissue catabolites}] (t)\) and \([\text{Total tissue}] (t)\) denote the tissue concentration catabolites and total radiolabel in eniluracil-naïve patients, respectively, as a function of time.

Blood and plasma data were processed to generate a continuous plasma input function. Briefly, the fraction of 5-[\(^{18}\text{F}\)]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:

\[
[Tissue](t) = IRF(t) \otimes [Plasma](t) 
\]

where \(n\) is the number of identifiable kinetic components, \(\beta\) is constant such that \(\lambda < \beta < 1\) (\(\lambda\) 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 \([^{18}\text{F}]\)catabolites or 5-[\(^{18}\text{F}\)]FU/[\(^{18}\text{F}\)]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:

\[
IRF(t) = \sum_{i=1}^{n} \alpha_i e^{-\beta_i t} 
\]

\[
[Tissue](t) = K_i \int_0^t \{\text{Plasma}(t) \cdot [\text{Plasma}] \} dt + (V_o + V_p) \cdot [\text{Plasma}] \cdot (t) 
\]

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 \([\text{Tissue}] (t)/[\text{Plasma}] (t)\) versus \(\int_0^t \{\text{Plasma}(t)\} dt\) 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-[\(^{18}\text{F}\)]FU in tumors, \(K_i\), was determined using tissue 5-[\(^{18}\text{F}\)]FU data obtained after eniluracil treatment. The PET data in this case comprises of parent 5-[\(^{18}\text{F}\)]FU and \(^{18}\text{F}\)anabolites. A similar method was used to study the initial (up to 10 min) extraction and trapping of 5-[\(^{18}\text{F}\)]FU through catabolism by the liver. To do this, deconvolution methods were used to remove the contribution of 5-[\(^{18}\text{F}\)]FU plus \(^{18}\text{F}\)anabolites from the total liver signal and Patlak analysis performed on the resulting data using the 5-[\(^{18}\text{F}\)]FU input function.

**Statistics.** Summary statistics and statistical comparisons were generated using STATA (version 5.0; Stata Corporation, College Station, TX). Tissue

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**Table 1 Area under the plasma concentration versus time curve (AUC) for 5-FU at 1 mg/m²**

AUC was calculated from 0 to 4 h. Values were not extrapolated to infinity because of the long half-life of 5-FU in eniluracil-treated patients. 5-[\(^{18}\text{F}\)]FU levels, which were used to calculate total 5-FU concentrations, were below the limit of detection in plasma samples after 60 min in eniluracil-naïve patients.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>AUC(_{0-240}) min (-) eniluracil (ng/ml×min)</th>
<th>AUC(_{0-240}) min (-) eniluracil (ng/ml×min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>558.9</td>
<td>7288.7</td>
</tr>
<tr>
<td>2</td>
<td>406.3</td>
<td>9256.3</td>
</tr>
<tr>
<td>3</td>
<td>604.9</td>
<td>10939.0</td>
</tr>
<tr>
<td>4</td>
<td>519.5</td>
<td>8691.1</td>
</tr>
<tr>
<td>5</td>
<td>685.3</td>
<td>8658.2</td>
</tr>
<tr>
<td>Average</td>
<td>555.0</td>
<td>8958.3</td>
</tr>
<tr>
<td>SE</td>
<td>46.3</td>
<td>591.0</td>
</tr>
</tbody>
</table>

---

**Fig. 1. Metabolism of 5-FU.**

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**Fig. 2. Transabdominal 5-[\(^{18}\text{F}\)]FU PET images for patient 3 obtained without eniluracil (a) and after eniluracil (b) administration, showing liver, kidney, and liver metastases.
data (AUC, % [18 F]catabolites, % 5-[18 F]FU plus [18 F]anabolites, and $K_I$) were compared using the Mann-Whitney $U$ test. $P_{so} \leq 0.05$ were considered statistically significant.

Results

Magnitude of Catabolism. As previously reported, 5-[18 F]fluorouracil (5-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-derived radioactivity. 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$_{0-95}$ min [18 F]Total</th>
<th>AUC$_{0-95}$ min [18 F]Catabolites</th>
<th>AUC$_{0-95}$ min [18 F]FU</th>
<th>% [18 F]catabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[m$^2$/ml$^2$]s</td>
<td>[m$^2$/ml$^2$]s</td>
<td>[m$^2$/ml$^2$]s</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.175</td>
<td>0.118</td>
<td>0.024</td>
<td>80.0</td>
</tr>
<tr>
<td>2</td>
<td>0.261$^a$</td>
<td>0.232</td>
<td>0.032</td>
<td>86.4</td>
</tr>
<tr>
<td>3</td>
<td>0.233</td>
<td>0.323</td>
<td>0.030</td>
<td>90.8</td>
</tr>
<tr>
<td>4</td>
<td>0.220</td>
<td>0.422</td>
<td>0.028</td>
<td>93.3</td>
</tr>
<tr>
<td>5</td>
<td>0.280</td>
<td>0.159</td>
<td>0.054</td>
<td>66.1</td>
</tr>
<tr>
<td>6</td>
<td>0.335</td>
<td>0.188</td>
<td>0.038</td>
<td>80.0</td>
</tr>
<tr>
<td>7</td>
<td>0.228</td>
<td>0.202</td>
<td>0.030</td>
<td>84.9</td>
</tr>
<tr>
<td>Average</td>
<td>0.247</td>
<td>0.235</td>
<td>0.034</td>
<td>83.1</td>
</tr>
<tr>
<td>SE</td>
<td>0.019</td>
<td>0.039</td>
<td>0.004</td>
<td>3.4</td>
</tr>
</tbody>
</table>

$^a$ Data from individual tumors in patients with multiple tumors.
components. Similar results were obtained for normal liver. IRF
FU
values were then used to deconvolve tumor and liver radioactivity
curves into a \([^{18}F]catabolites\) (mainly \([^{18}F]FBAL\)) component and a
component comprising of parent 5-\([^{18}F]FU\) and \([^{18}F]anabolites\). Fig.
4
a
and 4
b
illustrates the results of such deconvolution for tumor and
liver, respectively. The AUC \(0 –95\) min for total \([^{18}F]\) radioactivity in
eniluracil-treated and -naïve patients, as well as AUC \(0 –95\) min for
5-\([^{18}F]FU\)/anabolites and proportion of \([^{18}F]catabolites\) in tumor and
normal liver regions are presented in Tables 2 and 3. There was a
statistically significant difference for AUC\(_{0 –95}\) 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-\([^{18}F]FU\)/anabolites. In Fig. 4
b
, the tissue levels of
\([^{18}F]catabolites\) seem to follow that of the plasma input function,
supporting the assertion that tumor catabolites are derived predomi-
nantly from circulating catabolites.

**Net Clearance of 5-\([^{18}F]FU\) in Tumors and Liver.** Patlak anal-
ysis (Eq. E) was used to evaluate the net clearance of 5-\([^{18}F]FU\) from
plasma into tumors. The presence of a linear phase of the curve (Fig.
5
a
) indicated that irreversible uptake of 5-\([^{18}F]FU\) occurred in tumors.
However, the magnitude of uptake was low (Table 4). As a compar-
ision, the \(K_I\) 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-\([^{18}F]FU\) through
catabolism by the liver was also determined. Fig. 5
b
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
\((K_I = 0.0036 \pm 0.0005\) 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 ex-
change. A general deconvolution technique was used to determine the
contribution of catabolite signal to total tissue signal. This was nec-

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**Table 3** Area under the liver 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(_{0–95}) min ([^{18}F])Total</th>
<th>AUC(_{0–95}) min ([^{18}F])anabolites</th>
<th>AUC(_{0–95}) min ([^{18}F]catabolites)</th>
<th>% [^{18}F]catabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.357</td>
<td>1.924</td>
<td>0.044</td>
<td>97.7</td>
</tr>
<tr>
<td>2</td>
<td>0.459</td>
<td>1.325</td>
<td>0.058</td>
<td>95.7</td>
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<td>3</td>
<td>0.338</td>
<td>1.262</td>
<td>0.041</td>
<td>96.8</td>
</tr>
<tr>
<td>4</td>
<td>0.292</td>
<td>1.146</td>
<td>0.035</td>
<td>96.9</td>
</tr>
<tr>
<td>5</td>
<td>0.369</td>
<td>1.103</td>
<td>0.070</td>
<td>93.6</td>
</tr>
<tr>
<td>Average</td>
<td>0.380</td>
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<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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![Diagram](https://via.placeholder.com/150)

**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-\([^{18}F]FU\)
and \([^{18}F]anabolites\) radioactivity were removed from the total liver
radioactivity data. The resulting tissue data comprising of catabolites
were fitted using plasma 5-\([^{18}F]FU\) as input function.
Table 4 Determination of the overall (0–90 min) 5-18F[FU] influx rate constant (K\textsubscript{I}) and plasma plus exchangeable volume (V\textsubscript{P} + V\textsubscript{E}) for tumor and initial K\textsubscript{I} of 5-18F[FU] extraction through catabolism by liver

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>K\textsubscript{I} of tumor (ml/ml tissue/min)</th>
<th>V\textsubscript{P} + V\textsubscript{E} of tumor (ml/ml tissue/min)</th>
<th>K\textsubscript{I} for liver (ml/ml tissue/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0050</td>
<td>0.393</td>
<td>1.186</td>
</tr>
<tr>
<td>2</td>
<td>0.0033</td>
<td>0.523</td>
<td>2.186</td>
</tr>
<tr>
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<td>0.0026</td>
<td>0.444</td>
<td>0.731</td>
</tr>
<tr>
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<td>0.0040</td>
<td>0.548</td>
<td>0.797</td>
</tr>
<tr>
<td>5</td>
<td>0.0042</td>
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<td>0.779</td>
</tr>
<tr>
<td>6</td>
<td>0.0051</td>
<td>0.920</td>
<td>0.392</td>
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<tr>
<td>7</td>
<td>0.0012</td>
<td>0.738</td>
<td>0.571</td>
</tr>
<tr>
<td>Average</td>
<td>0.0036</td>
<td>0.622</td>
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<td>0.0005</td>
<td>0.074</td>
<td>0.092</td>
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</tbody>
</table>

Acknowledgments

We thank Drs. Gavin Brown, Sajinder Luthra, and Frank Brady for radio-labeling of 5-18F[FU] and its automated synthesis; the metabolite analysis, radiopharmacy, and quality control staff at the Medical Research Council cyclotron unit, Hammersmith Hospital, London, United Kingdom, for their expert assistance.

References

22. Nagah, F. N., el Kouni, M. H., and Cha, S. Enzymes of uracil catabolism in normal liver is abundant in dihydropyrimidine dehydrogenase (22) and, hence, a major organ for the catabolism of 5-FU. The extraction of 5-18F[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\textsubscript{I} 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 5-18F[FU] catabolism in patients with gastrointestinal tumors. In addition we have been able to determine the net clearance of 5-18F[FU] by tumor and liver.

nesary because the PET signal does not differentiate between chemical species. In addition, the net rate constant for the unidirectional clear-
Extraction of 5-Fluorouracil by Tumor and Liver: A Noninvasive Positron Emission Tomography Study of Patients with Gastrointestinal Cancer


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