
Jutta Kissel, Gunnar Brix, Matthias E. Bellemann, Ludwig G. Strauss, Antonia Dimitrakopoulou-Strauss, Rüdiger Port, Uwe Haberkorn, and Walter J. Lorenz

Forschungsschwerpunkt Radiologische Diagnostik und Therapie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

ABSTRACT

The purpose of our study was to develop a pharmacokinetic model to quantify the intracellular 5-fluorouracil (5-FU) concentration in liver metastases, which is expected to be closely correlated to therapy response. In addition, the influence of the biomodulator folinic acid on the action of 5-FU in the metastases was investigated.

After i.v. application of 5-FU labeled with the positron emitter fluorine-18 (5-[^18]F]FU), the kinetics of the regional 5-[^18]F]FU/uptake was measured dynamically with positron emission tomography over 120 min in 14 patients with a total of 27 liver metastases from colorectal adenocarcinoma. Activity-time curves were evaluated in the metastases, the normal liver tissue, as well as in the aorta and analyzed by a six-compartment model. The catabolic breakdown of 5-FU to α-fluoro-β-alanine (FBAL) in the normal liver tissue was modeled to separate the catabolites from the cytostatic agent 5-[^18]F]FU and the active 5-[^18]F]fluoroorthouracil nucleotides.

With our model, all measured activity-time courses could be described adequately with only small interindividual variations in parameters connected with liver and blood. Extrahepatic clearance of 5-FU was estimated as 0.66 ± 0.33 liters/min, whereas the hepatic clearance was 0.52 ± 0.25 liters/min. The Michaelis-Menten parameters describing the nonlinear conversion of 5-FU to FBAL were \( K_m = 11.3 \pm 6.4 \) μmol and \( V_{max} = 147.1 \pm 130.7 \) μmol/min. The maximum FBAL concentration in the liver was reached between 35 and 65 min after i.v. 5-FU infusion. The most sensitive parameters for therapy monitoring were \( k_{in} \) and \( k_{out} \), which characterize the transport in and out of the intracellular volume of the metastases, respectively. Tumor response can only be expected if \( k_{in} \) is high and \( k_{out} \) is low ("trapping"). These criteria were met by 6 of the 27 metastases, which were identical to those with high values for the area under the intracellular 5-FU concentration curve (AUC\(_{IC}^{0\rightarrow t}\)). The parameters \( k_{in} \) and \( k_{out} \) were also used to investigate the influence of the biomodulating agent folinic acid on drug effect. Five of the six metastases that showed trapping belonged to patients who received folinic acid. With the exception of one patient, however, all patients who received folinic acid had multiple metastases, of which only one was able to trap 5-FU. Because patient response can only be expected when all metastases trap 5-FU, folinic acid showed no effect on the overall clinical response.

With the quantitative modeling approach used, trapping of 5-FU can be assessed noninvasively and on an individual basis. This makes it possible to adjust the dose for each individual patient to optimize the treatment schedule.

INTRODUCTION

The pyrimidine analogue 5-FU\(^2\) is one of the most widely used cytostatic agents for the treatment of hepatic metastases from colorectal carcinoma (1, 2). The cytotoxic effect of the drug is believed to occur by: (a) incorporation of the 5-FU anabolite 5-fluoro-UTP into RNA with subsequently delayed maturation of rRNA; and (b) interference with DNA synthesis by inhibition of the enzyme thymidylate synthetase via the 5-FU anabolite FdUMP (3). The competitive catabolic pathway causes rapid breakdown of 5-FU to the low cytotoxic amino acid derivative FBAL and thus reduces the bioavailability of the drug. The rate-limiting step in this pathway is catalyzed by the enzyme dihydrouracil dehydrogenase that can be found in high amounts mainly in normal liver cells (3).

Despite the fact that 5-FU has been in clinical use for almost 40 years, it is still not possible to predict tumor response in individual patients. Therefore, the direct and noninvasive assessment of the transport and metabolism of the administered drug in various organs of the body and particularly in the tumor is necessary to make more accurate prognosis for cancer patients. Advances in modern imaging techniques have resulted in a noninvasive methodology that can be used to obtain pharmacokinetic and metabolic data from deep tissues. Information about fluorinated anticancer drugs like 5-FU can be obtained noninvasively with PET using fluorine-18 labeled 5-FU (5-[^18]F]FU; Refs. 4–6) as well as with MRS (7–12) or metabolic MR imaging (13, 14) employing the spin-1/2 nucleus fluorine-19.

The metabolic pathways of 5-FU have been analyzed biochemically and by high resolution MRS studies (15–22). However, the application of MRS techniques to observe the pharmacokinetics and metabolism of 5-FU in human subjects in vivo is seriously hampered by the low physical sensitivity of the MRS approach, which is lower than that of the radionuclide method by a factor of 10\(^{7–10}\). Because of this fact, it is not possible to detect 18F MRS signals from spatially well-resolved tissue regions with a sufficiently high temporal resolution. Moreover, an absolute quantitation of drug concentration in various tissues using 18F MRS is very difficult. Thus, the question of whether the uptake of 5-FU takes place in liver metastases or in surrounding healthy liver tissue cannot be answered by MRS; therefore, the hypothesis of intratumoral retention ("trapping") of 5-FU cannot be verified directly. This assumption is based on the observation that the detection of a 5-FU component with a half-life of 20–130 min in the liver, which is significantly longer than the half-life of 5-FU in the blood (5–15 min), correlates directly with tumor response to 5-FU chemotherapy (10, 11).

Using dynamic PET of 5-[^18]F]FU, on the other hand, the transport and the metabolism of the antineoplastic agent can be observed in various organs and tumors in animals and patients with a high spatial and temporal resolution (4–6, 14). Another advantage of PET compared to MRS is its ability to measure absolute tissue concentrations, after having used efficient scatter and attenuation correction techniques as well as an optimized iterative reconstruction algorithm. The apparent disadvantage that PET always detects the total concentration of 18F, regardless of the molecule the label belongs to, can be overcome by using a suitable pharmacokinetic model that makes it possible to distinguish the 5-FU signal from signals of its catabolites (usually the main catabolite FBAL) and anabolites. Such a model may not only be useful for quantifying transport and metabolism rates in tissues but also for predicting the clinical response in individual patients.
patients and for investigating the influence of biomodulating agents on drug effect.

It was the aim of the present study to: (a) formulate a pharmacokinetic model for 5-FU; and (b) apply it to dynamic 5-FU PET data of patients with liver metastases from colorectal cancer to obtain information on the distribution and metabolism of 5-FU in normal and neoplastic tissue.

MATERIALS AND METHODS

Patients. A total of 14 patients (8 males, ages 51–76 years; 6 females, ages 43–66 years) with liver metastases from colorectal adenocarcinoma were examined retrospectively. Additional patient data are given in Table 1. The metastases were diagnosed prior to the PET examination by X-ray computed tomography. Only those metastases with a diameter larger than 2 cm and visible in at least two adjacent PET slices were used for further evaluation. For eight patients, the standard therapeutic protocol included a 12-mm infusion of 500 mg/m²/24 h 5-FU (Fluoroblastin; Farmitalia, Freiburg, Germany) for 5 days every 3 weeks. Six patients received a 30-mm infusion of 375 mg/m²/24 h 5-FU together with an i.v. bolus of 200 mg/m² folinic acid (Leucovorin; Lederle International, Wayne, NJ). Folic acid is believed to enhance the effect of 5-FU due to a stabilization of the thymidylate synthetase/FdUMP complex, thus improving the inhibition of DNA synthesis in tumor cells. To exclude potential effects of chemotherapy on the PET results, patients were examined with PET at the first day of the first cycle of chemotherapy (n = 13) or, in one case, 1 week after the last 5-FU application (n = 1; Ref. 5).

In seven patients, tumor response was documented by serial volumetric computed tomography scanning according to WHO criteria: partial response, 50% reduction in tumor size sustained for at least 3 months (“+” in Table 1); stable disease, less than 25% reduction and less than 25% increase (“—” in Table 1); progressive disease, more than 25% increase in tumor size (also “—”) in Table 1).

PET Measurements and Data Postprocessing. Fluorine-18 labeled 5-FU was prepared by direct fluorination of uracil in acetic acid using [18F]F₂ diluted in neon (23). Typically, 17.5 mg of [18F]F₂ was obtained with a specific activity of 11 Ci/mmol and a radiochemical purity higher than 99% as determined by preparative high performance liquid chromatography. [18F]F₂ (299–869 MBq) was given together with 500–650 mg (dependent on the body weight) unlabeled 5-FU in a 12-mm (n = 8) or a 30-mm (n = 6) i.v. infusion using an infusion pump (PERFUSOR secura; Braun Melsungen Co., Melsungen, Germany).

The radiotracer measurements were carried out using a whole-body PET scanner (PC 2048–7WB; Scanditronix, Uppsala, Sweden) with a two-ring detector system of alternating Bi₄Ge₃O₁₂ and Gd₂SiO₅ crystals. Each ring has a diameter of 107 cm and contains 256 Bi₄Ge₃O₁₂/Gd₂SiO₅ detector pairs (crystal size = 6 × 20 × 30 mm²) each coupled to one photomultiplier. The system allows the simultaneous acquisition of three slices, i.e., two direct planes (slice thickness, 11 mm) and one cross plane (slice thickness, 8 mm). The emission scans were measured dynamically for a total acquisition time of about 2 h, starting with 5-[18F]FU infusion: 12 2-mm frames, 7 5-mm frames, and 6 10-mm frames. The measured sinograms were corrected for scattered radiation (24) and attenuation (25). Transmission data for attenuation correction were acquired over 18 min prior to the 5-[18F]FU application.

Images were iteratively reconstructed using a maximum-likelihood algorithm (26) with successive overrelaxation for accelerated convergence (27). The discretization of the field-of-view was 256 × 256 pixels each with a size of 2 × 2 mm². To minimize partial volume effects, the in-plane resolution of the images was improved by the implementation of the line-spread function of the PET scanner into the process of iterative image reconstruction by a simple convolution step during forward projection (28). This resulted in an almost constant spatial resolution of about 4.5 mm over the examined field-of-view without a perceptible decrease in the signal-to-noise ratio. With this algorithm, 50 iteration steps were sufficient to accurately quantify regional [18F] activity concentrations (quantification error, ≤2%).

For a quantitative analysis of the activity-time data, ROI were defined to represent metastases, normal liver parenchyma, and aorta. Tissue concentrations of fluorinated drug and/or metabolites were calculated by computing the mean of all pixels in each ROI and correcting the data for radionuclide decay.

The definition of the ROI for the aorta was hampered by its low contrast to the background, which made it impossible to detect the aorta in the reconstructed PET images. Therefore, the sum of all time frames was computed, which allowed delineation of the aorta. Another problem, related to the definition of the ROI over the aorta, was caused by the small size of the vessel as compared to the resolution of the scanner. Consequently, partial volume effects had to be considered. In addition, patient movement can result in displacement of the aorta relative to the ROI defined on the sum image. To take these problems into account, we calculated the upper quartile of all pixels in the ROI instead of the mean to represent the actual activity in the aorta. This allowed the definition of larger ROIs, which reduced the probability that due to patient movement, the ROI did not completely coincide with the aorta. The 75% percentile was chosen as a compromise between smoothness of the activity-time courses and activity recovery.

To compare the modeling approach (see below) with the semiquantitative PET analysis method usually used in clinical routine, the standardized uptake value:

$$SUV = \frac{\text{Tissue concentration (kBq/g)}}{\text{Injected dose (kBq)/body weight (g)}}$$

was calculated from the last frame of each dynamic PET measurement (i.e., about 2 h after injection). This point in time was chosen because it should be the best determinant of intratumoral retention of 5-FU that can be obtained by this approach (5).

Pharmacokinetic Model. The transport and metabolization of 5-FU in the human body can be conceptualized by the complex compartmental model shown in Fig. 1. Separate compartments for 5-FU and FBAL are used in the liver, the aorta, and the metastases. The liver is composed of an intracellular (IC) and an intravascular (IV) fraction, the metastases of an intracellular and an extracelluar, i.e., interstitial and intravascular, fraction. In addition to renal elimination of 5-FU and FBAL out of the plasma, peripheral compartments are

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Body weight (kg)</th>
<th>No. of metastases</th>
<th>5-FU dose (mg)</th>
<th>Duration of infusion (min)</th>
<th>Folinic acid</th>
<th>Therapy response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>54</td>
<td>73</td>
<td>2</td>
<td>650</td>
<td>30</td>
<td>Yes</td>
<td>NA*</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>72</td>
<td>72</td>
<td>2</td>
<td>650</td>
<td>30</td>
<td>Yes</td>
<td>NA*</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>75</td>
<td>65</td>
<td>2</td>
<td>600</td>
<td>30</td>
<td>Yes</td>
<td>NA*</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>56</td>
<td>75</td>
<td>3</td>
<td>500</td>
<td>12</td>
<td>No</td>
<td>NA*</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>50</td>
<td>67</td>
<td>1</td>
<td>500</td>
<td>30</td>
<td>Yes</td>
<td>NA*</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>51</td>
<td>82</td>
<td>2</td>
<td>650</td>
<td>30</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>58</td>
<td>59</td>
<td>2</td>
<td>500</td>
<td>12</td>
<td>No</td>
<td>NA*</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>50</td>
<td>57</td>
<td>3</td>
<td>500</td>
<td>12</td>
<td>No</td>
<td>NA*</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>48</td>
<td>67</td>
<td>1</td>
<td>500</td>
<td>12</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>43</td>
<td>55</td>
<td>3</td>
<td>500</td>
<td>12</td>
<td>No</td>
<td>NA*</td>
</tr>
<tr>
<td>11</td>
<td>Male</td>
<td>69</td>
<td>67</td>
<td>2</td>
<td>500</td>
<td>12</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>Female</td>
<td>56</td>
<td>70</td>
<td>1</td>
<td>600</td>
<td>30</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Female</td>
<td>65</td>
<td>50</td>
<td>1</td>
<td>500</td>
<td>30</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>Female</td>
<td>63</td>
<td>71</td>
<td>2</td>
<td>500</td>
<td>12</td>
<td>No</td>
<td>–</td>
</tr>
</tbody>
</table>

*NA, not available.
used to describe the distribution of both substances into slowly exchanging tissues.

Based on this model, the activity concentrations measured with PET in the aorta \( (C_{\text{aorta}}) \), the liver \( (C_{\text{liver}}) \), and the metastases \( (C_{\text{metastas}}) \) can be described by the following weighted sums of activity concentrations in the compartments defined in Fig. 1:

\[
C_{\text{aorta}} = (1-f_{\text{H}}) \cdot (C_{\text{plasma}}^{\text{a}} + C_{\text{plasma}}^{\text{FBAL}}) + f_{\text{H}} \cdot (C_{\text{RBC}}^{\text{a}} + C_{\text{RBC}}^{\text{FBAL}}) \quad (A1)
\]

\[
C_{\text{liver}} = (1-f_{\text{IC}}) \cdot C_{\text{aorta}} + f_{\text{IC}} \cdot (C_{\text{IC}}^{\text{a}} + C_{\text{IC}}^{\text{FBAL}}) \quad (A2)
\]

\[
C_{\text{metastas}} = (1-f_{\text{IC}}) \cdot f_{\text{IC}} \cdot (C_{\text{IC}}^{\text{metastas}}) + f_{\text{IC}} \cdot C_{\text{metastas}} \quad (A3)
\]

with \( f_{\text{H}} \) indicating the hematocrit, \( \text{RBC} \) the RBCs, and \( f_{\text{IC}} \) the intracellular fraction of the liver volume. \( f_{\text{IC}}^{\text{metastas}} \) stands for the intracellular fraction of the tumor volume, and \( f_{\text{IC}}^{\text{RBC}} \) stands for the relative blood volume of the tumor.

The complexity of the model is caused by the fact that PET measures the total concentration of \(^{18}\text{F}\), regardless of the compartment and the molecule the label belongs to. To reduce the number of parameters and thus to enable their quantitation, the model was simplified based on the following considerations (the following items correspond to the labels in Fig. 1):

(1) As shown by metabolic MR imaging studies in animals, the formation of FBAL in extracellular spaces as well as the distribution of FBAL into the extravascular space of the body after its formation in the liver can be neglected (13, 14).

(2) FBAL, as a charged particle, does not penetrate into RBCs, and one may assume that there is no binding of 5-FU and FBAL to plasma proteins (29, 30). The rate constants describing the exchange of 5-FU between plasma and RBCs have been obtained from animal studies, where the time course of the plasma and whole-blood concentrations have been analyzed separately. \( k_{12} \), describing the influx of 5-FU into the RBCs, was calculated to be 0.19 min\(^{-1}\); \( k_{21} \), describing the outflow out of the RBCs, was 0.31 min\(^{-1}\).

(3) The concentration of FBAL in liver parenchyma is much higher than its concentration in the plasma. Therefore, the transport of FBAL from the plasma back into the intracellular space of the liver can be neglected.

(4) Because the elimination of 5-FU out of the plasma can be described as a monoexponential function (31), the peripheral compartment was discarded. The validity of this approach was confirmed by our observation that the peripheral compartment, if taken into consideration, could not be identified from the measured PET data. Instead of the peripheral compartment, another nonhepatic route of elimination was considered in addition to renal elimination. Both routes can be described by one parameter, which comprises the total extrahepatic elimination.

(5) For all patients, the parameter describing the transport of 5-FU from the intracellular space of the liver back to the plasma was fitted to nearly zero, suggesting that this process is negligible. To stabilize the estimation of the other parameters, we used a model configuration in which this parameter was set to zero.

(6) We assumed that the exchange of 5-FU between plasma and interstitium in the metastases is so fast that both compartments can be taken together with a time course following the one of the plasma curve. This was justified by the analysis of a model configuration, which was not based on assumptions about the time course of the interstitial curve. Comparisons of the fits and the Schwartz criterion for both models, which combines the number of parameters in the model and the number of observations with the goodness of the fit, revealed that our assumption seemed to be reasonable. On the other hand, the intravascular contribution of FBAL (which cannot be found in the interstitial space) to the metastases signal was neglected due to the small plasma volume of the metastases.

Taking these considerations into account, we obtained the simplified six-compartment model shown in Fig. 2. From this figure, a system of coupled differential equations describing the concentrations in the different compartments can be derived. The measured activity concentrations are brought into relation to these concentrations by the equations:

\[
C_{\text{aorta}} = (1-f_{\text{H}}) \cdot (C_{\text{plasma}}^{\text{a}} + C_{\text{plasma}}^{\text{FBAL}}) + f_{\text{H}} \cdot C_{\text{RBC}}^{\text{a}} \quad (B1)
\]

\[
C_{\text{liver}} = (1-f_{\text{IC}}) \cdot C_{\text{aorta}} + f_{\text{IC}} \cdot (C_{\text{IC}}^{\text{a}} + C_{\text{IC}}^{\text{FBAL}}) \quad (B2)
\]

\[
C_{\text{metastas}} = (1-f_{\text{IC}}) \cdot f_{\text{IC}} \cdot C_{\text{IC}}^{\text{metastas}} + f_{\text{IC}} \cdot C_{\text{metastas}} \quad (B3)
\]

According to these equations and to Fig. 2, the following 14 parameters are needed to describe the model: \( V_{\text{f}}, V_{\text{v}}, V_{\text{liver}}, k_{\text{f}}, k_{\text{r}}, k_{\text{FBAL}}, k_{\text{IC}}^{\text{a}}, k_{\text{IC}}^{\text{RBC}}, k_{\text{IC}}^{\text{metastas}}, f_{\text{IC}}^{\text{a}}, f_{\text{IC}}^{\text{RBC}}, f_{\text{IC}}^{\text{metastas}}, f_{\text{R}}, \) as well as the parameters describing the saturable conversion of 5-FU to FBAL by a Michaelis-Menten kinetics (32, 33): \( V_{\text{max}} \) and \( K_{\text{m}} \).

Based on further considerations, we were able to enter the following parameters as constants into the model: the plasma volume \( V_{\text{p}} \), assumed to be 4.3% of the body weight (34) and \( V_{\text{liver}} \) (in liters) was calculated for each patient according to the formula (35-37):

\[
V_{\text{liver}} = (0.00733 \times w^{0.423} \times h^{0.725} - 0.59)/0.81 \quad (C)
\]

where \( w \) and \( h \) are the individual patient weight (in kilograms) and height (in centimeters), respectively. Moreover, \( f_{\text{IC}}^{\text{a}} \) was set to 0.7 and \( f_{\text{f}} \) to 0.47. As a consequence, the parameters that remained to be estimated from the three measured activity-time curves were reduced to 10.

The actual modeling process was performed in two steps. In the first step, we only considered the activity-time curves measured in the aorta as well as in...
the liver and calculated all parameters relevant for Eqs. B1 and B2 (i.e., $V_1$, $k_{12}$, $k_{21}$, $k_{13}$, $k_{45}$, $V_{max}$, and $K_m$). By this approach, we were able to isolate the plasma curve of 5-FU that served as arterial input function for the metastases. Using this input function, the remaining three parameters ($k_{in}$, $k_{out}$, and $f^{IC}_{met}$) were estimated in a second modeling step. The separation of the systemic part of the model from the part describing the behavior of 5-FU in the metastases is justified by the fact that the presence of the metastases hardly influences the systemic parameters.

For further evaluations, the area under the FBAL curve in the liver integrated from zero to infinity ($AUC_{FBAL}$) was used as a measure of the catabolic activity of the liver. In addition, the time of the maximum of the FBAL curve ($t_{max}$) was estimated to allow comparisons with results from MRS studies.

The area under the intracellular 5-FU curve of the metastases ($AUC_{met}$) representing the amount of free and trapped 5-[$^{18}$F]FU plus [$^{18}$F]-containing anabolites was calculated as a parameter characterizing the uptake of 5-FU in the tumors. Here, the integration extended from zero to 24 h, because the patients received a 5-FU dose every 24 h on 5 consecutive days, and we were interested in the amount of 5-FU in the metastases resulting from a single administration.

Kinetic model fitting was performed with MKMODEL (version 5.0; Biosoft, Cambridge, UK), an extended least squares modeling program package that runs on personal computers under MS-DOS.

**Statistical Evaluations.** The correlation of $t_{max}$ with the duration of the infusion was tested with a linear regression analysis. Pearson product moment correlation was used to test the correlation between $AUC_{FBAL}$ and $AUC_{met}$ as well as between $AUC_{met}$ and SUV$_{meta}$. The influence of folinic acid on the $k_{in}$ and $k_{out}$ values was examined using a Mann-Whitney rank sum test. All statistical evaluations were performed with the program package SigmaStat (version 2.0; Jandel Scientific Software, Erkrath, Germany).

**RESULTS**

With the formulated model, we were able to describe activity-time courses measured in the aorta, the liver, and the metastases of all patients. Fig. 3 shows a typical PET image (patient no. 7) acquired 27 min after injection of 5-[$^{18}$F]FU. Two metastases can be well delineated as regions with a low [$^{18}$F] uptake. The measured and fitted data for this patient are given in Fig. 4. The estimated 5-FU and FBAL concentration-time courses in the liver (Fig. 4a) and the aorta (Fig. 4b) are plotted as well as the extracellular and intracellular concentration-time curves in the ventromedial metastasis (Fig. 4c).

The estimated parameters describing the systemic part of the model and the calculated $AUC_{FBAL}$ and $t_{max}$ values are summarized for all patients in Table 2, together with the corresponding mean values and SD. A dependence of $t_{max}$ on the duration of the infusion was not found ($r = 0.147, P = 0.617$). The data in Table 2 showed only small interindividual variations; for example, $V_1$ varied by a factor of 2.5.

The parameters describing the 5-FU kinetics in the metastases are presented in Table 3. The data show much greater variations; the parameter $k_{in}$ which describes the transport of 5-FU from the extracellular to the intracellular volume of the metastases, ranged from 0.048 to 0.854. Thus, the maximum and minimum values differed by a factor of 18. The parameter $k_{out}$ which describes the transport of 5-FU from the intracellular back to the extracellular volume, ranged from 0 to 0.014. The table also summarizes the intracellular fraction of the metastases $f^{IC}_{met}$, as well as the estimated $AUC_{met}$ and SUV$_{meta}$ values. The mean $f^{IC}_{met}$ value was found to be 0.66 ± 0.17, suggesting that in the metastases, the intracellular volume accounts for about two-thirds of the whole tissue volume.

Fig. 5 depicts a plot of $AUC_{met}$ versus $AUC_{FBAL}$. No correlation between the catabolic activity of the liver and the amount of 5-FU in the tumor was found ($r = -0.212, P = 0.289$). This statement is also confirmed by the observation that within one patient, there can be one metastasis that is able to trap 5-FU and another one which does not (Fig. 6), although both have the same input function and the same underlying liver activity. On the other hand, a correlation with $r = 0.63 (P < 0.01)$ was found between SUV$_{meta}$ and $AUC_{met}$ (Fig. 7).

Fig. 8 presents a two-dimensional plot of the parameters $k_{in}$ and $k_{out}$ where high $k_{in}$ values indicate a high transport of 5-FU into the metastatic cells and small $k_{out}$ values suggest high retention of 5-FU in the cells. The metastases that are thought to respond to 5-FU therapy are those with high $k_{in}$ and low $k_{out}$ values.

Metastases in patients who received folinic acid are marked as black symbols in Fig. 8. Four of five metastases with small $k_{out}$ values belong to patients who received folinic acid, whereas only 6 of the 22 metastases with high $k_{out}$ values belong to patients who received folinic acid. However, no statistically significant difference in the $k_{out}$ values between patients with and without folinic acid was found ($P = 0.730$). For the $k_{in}$ values, the differences in the mean values among the treatment groups were greater than would be expected by chance ($P < 0.01$). Because overall clinical response, by definition, involves a reaction of every metastasis in a given cancer patient, it cannot be maintained that folinic acid had a significant influence on therapy response in this group of patients.
PHARMACOKINETIC ANALYSIS OF 5-\[^{18}\text{F}]\text{FUOROURACIL}

**DISCUSSION**

Since its introduction by Heidelberger et al. (1) in 1957, 5-FU has found extensive use in clinical oncology, especially for the treatment of metastatic colorectal cancer. Based on a literature survey, however, Kemeny (2) reported an average response rate for hepatic metastases of only 23%. Due to high interindividual variations, the prediction of the individual response probability or the identification of those patients who are likely to respond to therapy is extremely difficult. New imaging techniques like PET with 5-\[^{18}\text{F}]\text{FU}

Two large liver metastases can be well delineated as regions with a low 5-\[^{18}\text{F}]\text{FU}

Fig. 3. Reconstructed PET image of patient no. 7 (compare Table 1) taken 27 min after injection of 5-\[^{18}\text{F}]\text{FU}

A monoeponential function in our model, we obtained a half-life of 5.0 ± 2.5 min. Because we neglected a possible redistribution of 5-FU from the peripheral compartment, this value is likely to be slightly underestimated. From in vivo MRS measurements, Presant et al. reported the half-life of 5-FU in the plasma to be between 5 and 15 min (11). Based on plasma data after i.v. bolus administration, Heggie et al. (38) reported a 5-FU half-life of 12.9 ± 7.3 min. For the mean elimination of FBAL out of the liver, Port et al. (39) reported a value of \(k_{s5} = 0.056 \text{ min}^{-1}\), whereas a value of 0.063 ± 0.018 \text{ min}^{-1}

The Michaelis-Menten parameters \(V_{\text{max}}\) and \(K_m\) in our study were \(K_m = 11.3 \pm 6.4 \mu\text{mol}\) and \(V_{\text{max}} = 147.1 \pm 130.7 \mu\text{mol/min}\). Wagner et al. (42) reported values of \(K_m = 10.9 \mu\text{mol}\) and \(V_{\text{max}} = 2.02 \mu\text{mol/kg/min}\). Assuming an average body weight of 70 kg, our value for \(V_{\text{max}}\) comes close to the latter. Port et al. (39) reported a value of \(V_{\text{max}} = 121 \mu\text{mol/min}\).

The correctness of the separation of 5-FU and FBAL in the liver provided by our model is confirmed by comparisons with MRS data. Using surface-coil MRS without further spatial localization, Port et al. (39) and Schlemmer et al. (Ref. 12; both using an i.v. 5-FU infusion of 10 min duration) reported \(t_{\text{max}}\) values of 30–60 min and 49 ± 11 min, respectively. We obtained a value of \(t_{\text{max}} = 43 ± 12\) min (Table 2). The independence of \(t_{\text{max}}\) on the duration of the infusion observed in our study may be explained by the fact that the conversion step of 5-FU to FBAL is already saturated at the 5-FU dose administered.

The estimated hepatic clearance of 0.52 ± 0.25 liters/min is similar to the values measured by Ensminger et al. (Ref. 40; 0.24–0.45 liters/min) after peripheral i.v. infusion. Our assumption that the transport of 5-FU back from the liver cells to the plasma is negligible

3419
A similar effect might explain why we could not identify a peripheral compartment connected with the plasma compartment. The relatively high value of extrahepatic clearance with respect to the hepatic clearance (0.66 ± 0.33 liters/min versus 0.52 ± 0.25 liters/min) is in agreement with these considerations. The extrahepatic clearance may also incorporate to some extent an extrahepatic catabolism of 5-FU to FBAL, because the enzyme dihydrouracil dehydrogenase, although found in high amounts only in normal liver cells, is quite ubiquitous, being present in peripheral blood mononuclear cells, gastrointestinal tissues, and even tumor cells (41). As discussed above [model assumption (I)], the extrahepatic formation of FBAL is neglected in our model.

The fact that it is possible to separate the total $^{18}$F pool in the liver and the aorta into its 5-FU and FBAL components by a pharmacokinetic model suggests that, at least in the case of 5-FU investigations, the missing chemical specificity of the PET approach is no obstacle for pharmacokinetic studies. The advantages of the good spatial and temporal resolution of PET as compared to MRS measurements can be exploited and make it the method of choice for pharmacokinetic analyses. The volume parameters estimated from the model give absolute values (in liters) as compared to the relative parameters obtained by MRS measurements (39).

Thus, with PET quantitative information can be gained on the volume of 5-FU distribution or the fraction of the intracellular volume in the metastases.

**Patient Study.** As shown in the patient study, the model presented provides additional information that cannot be obtained by a conventional semiquantitative analysis procedure, such as the assessment of the SUV.

Although there is a correlation between the SUVmeta and the $AUC^{\text{SUV}}_{\text{5-FU,metast}}$, the same SUVmeta of 1.8, for example, corresponds for patient no. 10 to $AUC^{\text{SUV}}_{\text{5-FU,metast}} = 38.4$ mmol-min, whereas for patient no. 11 to $AUC^{\text{SUV}}_{\text{5-FU,metast}} = 155.9$ mmol-min/l (Table 3). This discrepancy, which might influence the therapeutic procedure, is explained by the fact that the SUVmeta takes into consideration only one point at the end of the investigation, whereas the $AUC^{\text{SUV}}_{\text{5-FU,metast}}$ reflects the total time course of the measured data plus its extrapolation to longer times. To predict therapy outcome, we therefore suggest to consider the $AUC^{\text{SUV}}_{\text{5-FU,metast}}$ as a prognostic parameter.

According to our data (Fig. 7), a threshold value of 50 mmol-min/liter might be adequate to distinguish metastases that trap 5-FU from those that do not trap it. In our opinion, however, the most sensitive parameters for describing trapping are $k_{in}$ and $k_{uar}$, which characterize the transport in and out of the metastases, respect-

---

### Table 2. Pharmacokinetic parameters for liver and blood

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>$V_{d1}$ (liters)</th>
<th>$CL_{d1}^{5-FU}$ (mL/min)</th>
<th>$CL_{d1}^{5-FU}$ (mL/min)</th>
<th>$CL_{d1}^{5-FU}$ (mL/min)</th>
<th>$CL_{d1}^{FBAL}$ (mL/min)</th>
<th>$AUC_{\text{FBAL}}^{\text{SUV}}$ (mmol/min/liter)</th>
<th>$I_{\text{max}}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.13</td>
<td>0.94</td>
<td>0.42</td>
<td>0.13</td>
<td>40.1</td>
<td>0.28</td>
<td>33.9</td>
</tr>
<tr>
<td>2</td>
<td>5.88</td>
<td>0.81</td>
<td>0.40</td>
<td>1.88</td>
<td>17.8</td>
<td>0.11</td>
<td>15.3</td>
</tr>
<tr>
<td>3</td>
<td>7.32</td>
<td>0.67</td>
<td>0.78</td>
<td>36.4</td>
<td>1.4</td>
<td>10.7</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>10.97</td>
<td>0.61</td>
<td>0.35</td>
<td>14.4</td>
<td>0.11</td>
<td>33.9</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>6.59</td>
<td>0.60</td>
<td>0.60</td>
<td>13.9</td>
<td>0.10</td>
<td>10.7</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>6.06</td>
<td>1.21</td>
<td>0.82</td>
<td>20.4</td>
<td>0.22</td>
<td>27.1</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>7.38</td>
<td>0.41</td>
<td>0.16</td>
<td>19.5</td>
<td>0.09</td>
<td>95.6</td>
<td>57</td>
</tr>
<tr>
<td>8</td>
<td>7.18</td>
<td>0.33</td>
<td>0.24</td>
<td>13.8</td>
<td>0.08</td>
<td>12.6</td>
<td>47</td>
</tr>
<tr>
<td>9</td>
<td>14.48</td>
<td>0.99</td>
<td>0.57</td>
<td>22.5</td>
<td>0.12</td>
<td>15.3</td>
<td>73</td>
</tr>
<tr>
<td>10</td>
<td>9.53</td>
<td>0.35</td>
<td>0.93</td>
<td>26.8</td>
<td>0.16</td>
<td>45.4</td>
<td>57</td>
</tr>
<tr>
<td>11</td>
<td>13.07</td>
<td>1.27</td>
<td>0.26</td>
<td>7.6</td>
<td>0.20</td>
<td>14.7</td>
<td>47</td>
</tr>
<tr>
<td>12</td>
<td>8.13</td>
<td>0.29</td>
<td>0.89</td>
<td>14.6</td>
<td>0.13</td>
<td>16.7</td>
<td>47</td>
</tr>
<tr>
<td>13</td>
<td>6.93</td>
<td>0.34</td>
<td>0.33</td>
<td>11.1</td>
<td>0.05</td>
<td>76.1</td>
<td>47</td>
</tr>
<tr>
<td>14</td>
<td>8.39</td>
<td>0.38</td>
<td>0.44</td>
<td>13.4</td>
<td>0.22</td>
<td>20.7</td>
<td>65</td>
</tr>
<tr>
<td>Mean</td>
<td>8.57</td>
<td>0.66</td>
<td>0.52</td>
<td>19.1</td>
<td>0.14</td>
<td>33.2</td>
<td>43</td>
</tr>
<tr>
<td>±SD</td>
<td>2.50</td>
<td>0.33</td>
<td>0.25</td>
<td>9.3</td>
<td>0.06</td>
<td>25.2</td>
<td>11</td>
</tr>
</tbody>
</table>

---

**Fig. 4.** Concentration-time curves measured in the liver (a), in the blood (b), and in the ventromedial metastasis (c) of the patient shown in Fig. 3. In addition to the measured and fitted data (solid lines), the estimated 5-FU and FBAL concentration-time curves in the liver and the aorta, as well as the intra- and extracellular 5-FU curves in the metastasis, are plotted (dotted lines). For the liver, the 5-FU as well as the FBAL curve comprise both the intra- and the extracellular compartment.
Table 3  Pharmacokinetic parameters concerning the metastases

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Metastasis no.</th>
<th>(k_{in}) (min(^{-1}))</th>
<th>(k_{out}) (10(^{-5}) min(^{-1}))</th>
<th>(f_{IC})</th>
<th>(AUC_{meta,IC}^{5-FU}) (mmol/min/liter)</th>
<th>(SUV_{meta}^{5-FU})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.85</td>
<td>0.12</td>
<td>0.60</td>
<td>778.3</td>
<td>9.7</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.26</td>
<td>0.00</td>
<td>0.76</td>
<td>15.8</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.21</td>
<td>0.17</td>
<td>0.76</td>
<td>9.0</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.23</td>
<td>0.99</td>
<td>0.57</td>
<td>17.6</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0.20</td>
<td>0.10</td>
<td>0.68</td>
<td>275.2</td>
<td>2.9</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0.21</td>
<td>0.17</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0.17</td>
<td>0.11</td>
<td>0.76</td>
<td>9.0</td>
<td>1.4</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>0.15</td>
<td>0.00</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>0.13</td>
<td>0.09</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>0.09</td>
<td>0.07</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>0.07</td>
<td>0.05</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>0.05</td>
<td>0.03</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>0.03</td>
<td>0.02</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>0.01</td>
<td>0.01</td>
<td>0.76</td>
<td>12.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Mean</td>
<td>0.26</td>
<td>0.09</td>
<td>0.02</td>
<td>0.68</td>
<td>133.1</td>
<td>2.8</td>
</tr>
<tr>
<td>±SD</td>
<td>0.22</td>
<td>0.04</td>
<td>0.01</td>
<td>0.17</td>
<td>278.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Tumor response can only be expected if the transport in the metastases is high, while the outflow is low (trapping). Three of the metastases shown in Fig. 8 meet this criterion very well, two have small \(k_{out}\) values (below 0.002 min\(^{-1}\)) with medium \(k_{in}\) values, and one has a relatively low \(k_{out}\) value (0.005 min\(^{-1}\)) in combination with a relatively high \(k_{in}\) value (0.42 min\(^{-1}\)). These six metastases are those with high \(AUC_{meta,IC}^{5-FU}\) values (see the squares in Fig. 8).

Based on the \(k_{in}\) and \(k_{out}\) parameters, one cannot only hope to predict tumor response but, in the cases where there is no response, the reason why 5-FU is absent in the tumor might also be more exactly defined; it could be due to a reduced influx or the lack of trapping ability. For those seven patients for whom therapy outcome is available, our predictions are in accordance with the observations (compare Table 1 with Table 3). In a future prospective study, the validity of the correlation will be studied. It should be mentioned, however, that the retention of 5-FU in tumor cells as quantified by our model, although important, is not the sole determinant of 5-FU response. The degree of thymidylate synthase inhibition, the relative amount of thymidylate synthase enzyme, as well as the amount of active metabolites formed are also key factors. It may be expected, therefore, that a better determinant of tumor responsiveness would be the intratumoral retention of 5-FU at later points in time. An extension of the PET measurements out

Fig. 5. Two-dimensional plot of the area under the intracellular 5-FU curve in the metastasis (\(AUC_{meta,IC}^{5-FU}\)) versus the area under the FBAL curve in the liver (\(AUC_{FBAL}^{5-FU}\)). There is no correlation between the two quantities (\(r = -0.212, P = 0.289\)).

Fig. 6. Concentration-time curves of the two metastases of patient no. 10 (compare Table 1). The double peak in the extracellular 5-FU curves \(5-FU_{EC}\) results from a short interruption of the infusion due to a change of the infusion pump. Metastasis no. 2 shows a trapping effect (a), whereas no trapping can be observed for metastasis no. 1 (b).

Fig. 7. Comparison of the \(AUC_{meta,IC}^{5-FU}\) with the SUV data. For better resolution in the lower range of the \(AUC_{meta,IC}^{5-FU}\) values, all \(AUC_{meta,IC}^{5-FU}\) values ≥80 mmol/min/liter were set equal to 80 mmol/min/liter. The true values are summarized in Table 2. A threshold value of 50 mmol/min/liter (indicated as a dotted line) may be adequate to distinguish trapping from nontrapping metastases. According to the statistical evaluation, there is a good correlation between the two values \((r = 0.63, P < 0.01)\).
to longer times, however, is hampered due to the short half-life of the positron emitter fluorine-18 of about 110 min.

According to our results, there was no influence of folinic acid on the effect of 5-FU as far as the clinical response is concerned. However, treatment schedules with longer times, however, is hampered due to the short half-life of fluorine-18 of about 110 min.

Another result, the missing correlation between the catabolic activity of the liver and the amount of 5-FU within the tumor cells, suggests that a higher concentration of unmetabolized 5-FU in plasma is not necessarily related to an increase of 5-FU uptake in the metastases. This statement questions the role of biomodulators that reduce the catabolic activity of the liver to increase the amount of 5-FU available for the metastases.

Conclusions. With the pharmacokinetic model presented, it is possible to estimate the intracellular 5-FU concentration in liver metastases without having to take arterial blood samples in short time intervals and to separate the 5-FU metabolites by high-performance liquid chromatography analysis. Therefore, tumor trapping of 5-FU can be assessed noninvasively and on an individual basis. This makes it possible to adjust the dose and timing for each individual patient to optimize the treatment schedule. Furthermore, the effects of biomodulators can be studied.

REFERENCES

Pharmacokinetic Analysis of $5-[^{18}F]$Fluorouracil Tissue Concentrations Measured with Positron Emission Tomography in Patients with Liver Metastases from Colorectal Adenocarcinoma

Jutta Kissel, Gunnar Brix, Matthias E. Bellemann, et al.