Pharmacokinetics of 5-Fluorouracil following Different Routes of Intrahepatic Administration in the Canine Model

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ABSTRACT

In an effort to achieve high concentrations of 5-fluorouracil (5-FUra) in the hepatic circulation while minimizing systemic exposure, several routes of intrahepatic administration were compared in the canine model. To ascertain these data, 5-FUra (30 mg/kg) was given as a bolus into either a systemic vein (femoral vein), hepatic artery, hepatic artery distal to its ligation after hepatic dearterialization, or through the portal vein. Three dogs were studied for each route with concomitant blood samples taken from the inferior vena cava and hepatic vein at 1, 2, 3, 5, 10, 15, 30, and 60 min after injection. 5-FUra levels were determined in plasma by high-pressure liquid chromatography. Blood flow in the portal vein and hepatic artery was measured by an electromagnetic flowmeter. The data were best described by a multicompartmental model including the measured flows. Hepatic components of the model were separate arterial and portal compartments, with elimination from each described by linear kinetics. Analysis of the results indicated that the highest hepatic levels with the least systemic exposure, as indicated by drug levels in hepatic and peripheral vein, were realized following hepatic artery administration distal to its ligation after hepatic dearterialization.

INTRODUCTION

The pharmacokinetics of 5-FUra2 has been studied, using different routes of administration in humans and animals (6, 8, 9, 14, 15). Among the routes studied have been i.v., via portal vein, via hepatic artery, p.o., and i.p. The intent of several of these investigators has been to delineate the role of route of administration in increasing the local delivery to the liver, while minimizing the amount of drug in the systemic circulation. In addition to the above routes which have been well studied, another potential route of entry would be the distal hepatic artery after its ligation, and transection of hepatic ligaments (hepatic dearterialization) which may have clinical and pharmacokinetic relevance. It has been shown clinically that disruption of the hepatic arterial blood supply produces massive tumor necrosis, while reducing the recirculation integral, making the injection into the distal portion of the ligated artery highly advantageous (7, 12). However, there is a paucity of kinetic data available to evaluate this route of administration versus the others.

The aim of the present study is to compare systemic and hepatic vein levels of 5-FUra following bolus injection of a comparable mg/kg dose via the hepatic artery, hepatic artery distal to its ligation after hepatic dearterialization, portal vein, or systemic vein in the canine model, to determine the route that gives the highest hepatic levels with the least amount of systemic exposure.

MATERIALS AND METHODS

Chemicals. Pure 5-fluorocytosine, used as the internal standard, was purchased from Sigma Chemical Co., St. Louis, MO, while 5-FUra was obtained from Hoffmann-La Roche Inc., Nutley, NJ. All other chemicals and reagents were of analytical grade or better.

Assay Procedure. Plasma samples were analyzed by high-pressure liquid chromatography (5). The high-pressure liquid chromatography system consisted of a C18 Bondapak column, 30 cm x 3.9 mm, preceded by a 5-cm x 4-mm 10-μm particle-size Co-Pell ODS (Whatman, Inc., Clifton, NJ) guard column, and a Waters Model 600 A solvent delivery system equipped with a Model 666 injector. A Laboratory Data Control 1201 variable-wavelength detector operated at 267 nm was used for detection of 5-FUra.

Animals. Twelve mongrel dogs, 3 for each route of administration, were used. The animals weighed between 11.4 and 18.1 kg. They were deprived of food but had free access to water for 24 hr before the experiment. General anesthesia was induced by i.v. Nembutal (30 mg/kg), which was supplemented as necessary. The animals were intubated with ventilation provided by a respirator. The laparotomy was performed via a midline abdominal incision. The portal vein, hepatic artery, and hepatic vein were isolated. Catheters were introduced into the femoral and hepatic veins, as well as hepatic artery or portal vein, for either the administration of the drug or the sampling. An electromagnetic flowmeter was calibrated and placed around the isolated portal vein and hepatic artery for blood flow measurement. Three measurements of flow were taken at the beginning of the study, after an interval of 5 min, to allow the establishment of circulatory steady state. For animals that were dearterialized, the hepatic artery was ligated, hepatic ligaments were transected, and a cannula was placed in the distal segment of the artery with blood flow measured in this segment. 5-FUra, a 30-mg/kg bolus, was given by one of the following routes: systemic vein, hepatic artery, hepatic artery distal to its ligation, or portal vein. Blood samples, 2.0 ml, were taken simultaneously from the femoral and hepatic veins at 1, 2, 3, 4, 5, 10, 15, 30, and 60 min.

Pharmacokinetic Data Analysis. The model developed to fit the canine plasma volume and drug levels was based on analysis of the average levels for the 3 dogs comprising a treatment group. The proposed model presented in Chart 1 includes flow-dependent clearance of 5-FUra by the liver, and linear elimination via the kidney. Incorporated into the model is the direct transfer of arterial flow into a mixing venous compartment that also receives portal flow. A similar mixing of arterial and portal hepatic flows has been previously reported in dogs (13, 16). The tissue-blood partition coefficient was assumed to be unity in this analysis, based on the report of Collins et al. (6).

Model physiological parameters not measured were either obtained from published estimates or were estimated from data simulation based upon the physiological constraints reported in the literature (2, 9). These values are presented in Table 1. The CONSAM computer program was used for model analysis. It estimated the parameters by a minimum variance approach. This measures each residual term in the minimized...
function inversely weighted by the variance of the measurement error. The differential equations used to describe the model are:

\[
\frac{dC_{S}}{dt} = (Q_{m} + Q_{w}) CL_{v} + Q_{C_{l}} - (Q_{m} + k_{u})C_{s}
\]

\[
\frac{dC_{L}}{dt} = Q_{m}C_{s} - (k_{2} + Q_{m})CL_{v}
\]

\[
\frac{dC_{V}}{dt} = Q_{m}C_{s} - (k_{2} + k_{3})CL_{v}
\]

\[
\frac{dC_{t}}{dt} = Q_{t}C_{s} - Q_{C_{t}}
\]

RESULTS

The mean plasma levels of 5-FUra in hepatic and systemic vein samples were expressed as a fraction of the dose per ml in Charts 2 and 3. Initially, the hepatic vein levels of 5-FUra were always higher than the corresponding systemic vein levels of the drug. These high levels were seen consistently for the first 10 to 15 min after the bolus dose of 5-FUra. Later, an equilibrium in these 2 levels of drug was observed, with subsequent levels in hepatic vein being lower than the levels in systemic vein. The highest magnitude of the difference was noted for intraarterial route after dearterialization.

The characteristic convex behavior of nonlinear elimination of 5-FUra after i.v. bolus injection was not readily apparent in the log concentration-time curves.

The arterial, portal, and systemic administration of 5-FUra was simulated according to the proposed model. The difference in simulated dose/ml 5-FUra fraction levels in the hepatic artery and hepatic vein after intraarterial administration, with and without dearterialization, is shown in Chart 4. A very high level of 5-FUra was achieved in hepatic circulation after dearterialization, as compared to the other routes of administration.

The parameter values for elimination of 5-FUra from the hepatic arterial and portal compartments were lowest for dearterialized animals, as compared to the values in the other 3 groups of animals, indicating a maximum exposure of 5-FUra to the hepatic tissue via this route (Table 2).

Fractional catabolic rate and extraction of 5-FUra from hepatic arterial and portal compartments for dearterialized and nonearterialized groups of animals are presented in Table 3. Fractional catabolic rate values indicate the prolongation of the turnover time by 6-fold for the hepatic arterial compartment, and 2-fold for the portal compartment in the dearterialized group. Thus, it is additionally suggested that very high levels of 5-FUra are maintained in dearterialized animals. Based upon computer analysis of the data and the high level of 5-FUra in the hepatic vein immediately following dearterialization, it is estimated that a 90% decrease in arterial metabolic capacity of 5-FUra in the liver occurred, while only a 17% decrease occurred in portal metabolic capacity of the liver.

The area under the best-fit plasma level-time curve values for the systemic and the hepatic vein are given in Table 4. Although high levels of 5-FUra in the hepatic vein were noted in the dearterialized group of animals, the systemic vein: hepatic vein level ratio was the lowest (5.7) for this group of animals, as compared to the other 3 groups. This result further suggests that more 5-FUra was present in hepatic circulation, with a comparatively smaller amount being in the systemic circulation in dearterialized animals.

DISCUSSION

The primary goal of regional chemotherapy drug delivery to hepatic tumors is to establish a high level of drug in hepatic tissue. The mean plasma levels of 5-FUra in hepatic and systemic vein samples were expressed as a fraction of the dose per ml in Charts 2 and 3. Initially, the hepatic vein levels of 5-FUra were always higher than the corresponding systemic vein levels of the drug. These high levels were seen consistently for the first 10 to 15 min after the bolus dose of 5-FUra. Later, an equilibrium in these 2 levels of drug was observed, with subsequent levels in hepatic vein being lower than the levels in systemic vein. The highest magnitude of the difference was noted for intraarterial route after dearterialization.

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5-FUra Pharmacokinetics

Chart 2. Hepatic vein level data, mean ± S.D. (n = 3), for 5-FUra following administration of 30 mg/kg to dogs via either the hepatic artery (HA), portal vein (PV), or hepatic artery after dearterialization (HA/D), or systemic route (SYS).


Chart 4. Simulated concentrations of 5-FUra in the hepatic artery (ART) and hepatic vein following administration of drug into the hepatic artery, with and without dearterialization (DA).

circulation and the least in systemic circulation, thus achieving a more favorable therapeutic index. In the past, it has been demonstrated that metastatic and primary hepatic tumors obtain blood supply from the hepatic artery (3). Currently, many reports have shown the therapeutic advantage of this route of administration (10).

The effect of regional chemotherapy drugs on tumors occurs during the first passage of the drug through the area supplied by the infused artery (7). This is enhanced if the drug exhibits high clearance by the hepatic tissue and a low integral of recirculation (8). Based upon the reported pharmacokinetic and clinical studies, 5-FUra and 5-fluoro-2′-deoxyuridine are the most commonly used drugs for this mode of administration (6, 8, 10).

However, the major limitation in the pharmacokinetic studies of 5-FUra has been its apparent dose dependency in humans (6, 9). Consequently, this factor imposes limitations on the delivery in regard to the dose and rate of infusion. The primary reason for this is the effect of the dose on the area under the plasma curves. Moreover, the drug concentration areas under the curve become a complex function of the clearance, the Michaelis
can be adequately described by linear kinetics in dogs. Addition-
ally, the estimated hepatic clearance of 337 ml/min from our
first order of elimination of the drug from all compartments.

The current dosage in dogs did not exhibit the characteristic
constant (Km), maximal turnover rate (Vmax), and the initial dose
(4).

However, the pharmacokinetic data collected in this study at
the current dosage in dogs did not exhibit the characteristic
curvature of the drug concentration curve as predicted by non-
linear kinetics. Therefore, we utilized a physiological model with
the first order of elimination of the drug from all compartments.

Thus, indeed for the plasma levels of 5-FUra between the 0.01
and 0.5 fraction of the dose/ml (0.3 to 460.0 µg/ml), the data
can be adequately described by linear kinetics in dogs. Addition-
ally, the estimated hepatic clearance of 337 ml/min from our
fitted data for the 3 groups of nondearterialized animals was well
within the range of 431.1 ± 146.9 (S.D.) ml/min reported by
Gustavsson et al. (9) in dogs at a total daily dose of 15 mg/kg.

The primary effect of hepatic dearterialization followed by
intraarterial administration of 5-FUra, compared with other meth-
ods of administration, was the persistent high level of 5-FUra in
hepatic arterial and venous compartments. All the parameters
were affected by dearterialization. The most notable among them
was the extremely low ratio of systemic plasma 5-FUra concen-
tration to hepatic plasma 5-FUra concentration.

The other important effect of dearterialization, compared to
the other modes of administration, was on the turnover time
of 5-FUra by the liver. It clearly exhibited a consistent decrease in
hepatic elimination of 5-FUra following dearterialization. All pa-
rameters of the liver were affected by the decreased arterial
flow; most notable was fractional catabolic rate and hepatic
clearance, which decreased 83 and 92%, respectively, for the
liver arterial compartment. As a result of the decreased hepatic
elimination, the ratio of systemic to hepatic vein area under the
curve for the 4 routes of administration clearly showed that hepatic
dearterialization gave the best results.

However, one must be cautious in interpretation of these
results in regard to the dearterialized group. The drug was
injected immediately after ligation of the hepatic artery, the time at
which anoxic injury to the liver cell is maximal. This observation
of the temporary anoxic damage to the hepatic cells, resulting in
rise of hepatic enzymes with return to normal values in 1 to 2
weeks, is well known (1,11). This phenomenon has implications
on our pharmacokinetic study in dearterialized animals, since the
portal vein provides the only oxygen supply to the liver, and the
study was done in the first 3 hr after the anoxic injury to the
hepatic parenchyma. The decreased fractional catabolic rate and
low clearance of 5-FUra could be due to temporary anoxic
damage to hepatic cells, and if the same studies would have
been repeated 1 to 2 weeks after dearterialization, we might
have found different values, and perhaps a normal catabolic rate
and extraction of the drug. The studies in this regard are now in
progress in patients who have had this procedure, and are
receiving intraarterial infusion chemotherapy.

A few other limitations in interpretation of the results in this
study should be brought forth.

In the dearterialized group of animals, in addition to acute
anoxic injury to the hepatic parenchyma, the theoretical possi-
bility of development of collateral circulation through the hepatic
ligaments and bare area of liver does exist. This could give
fallacious results with regard to arterial blood flow, despite the
ligation of hepatic artery. However, we routinely transected the
left and right triangular hepatic ligaments to gain access to
hepatic veins. Furthermore, we did not come across any major
vessels in these ligaments; however, capillary circulation could
not be evaluated at the time of the experiment.

We did not study drug distribution in the hepatic circulation by
radionuclide-labeled albumin to assure the even distribution of
drug throughout the hepatic parenchyma. In a clinical situation

### Table 2

<table>
<thead>
<tr>
<th>Liver volume, arterial (liters)</th>
<th>Extrarehepatic tissue volume (liters)</th>
<th>H(1) (min⁻¹)</th>
<th>K(1) (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic artery, portal vein, or systemic vein injection</td>
<td>0.03 ± 0.01</td>
<td>1.48 ± 0.12</td>
<td>6.0 ± 2.3</td>
</tr>
<tr>
<td>Hepatic artery injection after dearterialization</td>
<td>0.03 ± 0.01</td>
<td>1.48 ± 0.12</td>
<td>4.0 ± 0.09</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Fractional catabolic rate (min⁻¹)</th>
<th>Hepatic extraction</th>
<th>Hepatic clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial hepatic</td>
<td>Normal: 8.18, Dearterialized: 1.40</td>
<td>0.56, 0.27</td>
<td>189.01, 14.79</td>
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<tr>
<td>Portal hepatic</td>
<td>Normal: 1.41, Dearterialized: 0.75</td>
<td>0.37, 0.29</td>
<td>149.00, 81.69</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Sampled compartment</th>
<th>AUC (µg/ml × min)²</th>
<th>SYS/HV ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic artery</td>
<td>SYS</td>
<td>1002.5 ± 1283.15</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>HV</td>
<td>133.6 ± 46.6</td>
<td></td>
</tr>
<tr>
<td>Portal vein</td>
<td>SYS</td>
<td>2641.1 ± 5810.2</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>HV</td>
<td>371.3 ± 11.1</td>
<td></td>
</tr>
<tr>
<td>Hepatic artery of dearterialized animals</td>
<td>SYS</td>
<td>3517.8 ± 562.8</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>HV</td>
<td>615.9 ± 252.5</td>
<td></td>
</tr>
<tr>
<td>Femoral vein</td>
<td>SYS</td>
<td>4024.4 ± 2736.5</td>
<td>13.87</td>
</tr>
<tr>
<td></td>
<td>HV</td>
<td>290.1 ± 72.55</td>
<td></td>
</tr>
</tbody>
</table>

- Table 3: Values of fractional catabolic rate, hepatic extraction, and hepatic clearance from hepatic arterial and portal compartments after bolus dose of 5-FUra (30 mg/kg) in normal (nondearterialized) and dearterialized animals.

- Table 4: Area under the best-fit systemic and hepatic vein plasma level time curves following bolus administration of 30 mg/kg of 5-FUra by 4 different routes, and the ratio of areas under curve.

### Notes

- AUC, area under the curve; SYS, systemic vein; HV, hepatic vein.
- Mean ± S.D. of 3 determinations.

- *A* Calculated by inversion of A matrix and obtaining reciprocal of mean residence time for that compartment.
- **B** Calculated by

\[
CL_h = \frac{Q_h}{1 - E}
\]

assuming 100% hepatic metabolic conversion of 5-FUra.

- Mean ± S.D. of 3 determinations.
this is done by \textsuperscript{99m}Tc-labeled human serum albumin perfusion scans, and also by injecting fluorescein dye into the hepatic artery, followed by UV visualization, to confirm that the entire parenchyma of liver shows the dye uptake. The former method is advisable and is often done in follow-up studies.

Recently, most of the 5-FUra or 5-fluoro-2'-deoxyuridine infusions for hepatic cancer are administered with continuous infusion rather than bolus injection. In our study we did the bolus injection of 5-FUra as the first set of experiments, which allowed us to establish the most appropriate model, since oftentimes a more complex input may confound data analysis. We are now completing the second set of experiments with continuous infusion of 5-FUra so that more resemblance to the clinical situation could be achieved. Additionally, we would be able to compare and contrast, pharmacokinetically, the difference between the 2 methods of administration.

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