Pharmacological Evaluation of Experimental Isolated Liver Perfusion and Hepatic Artery Infusion with 5-Fluorouracil


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ABSTRACT

The intention of this study was to estimate the pharmacological advantage of a clinically applicable method of isolated liver perfusion (ILP) over hepatic artery infusion (HAI) administering various doses of 5-fluorouracil (FUra). FUra concentrations were measured using high-performance liquid chromatography in liver tissue (pigs and rats), hepatic tumor tissue (rats), and in the systemic circulation (pigs) following ILP and HAI.

Forty-two pigs and 36 rats were subjected to either ILP or HAI with 20, 40 or 80 mg of FUra/kg of body weight. ILP resulted in significantly increased FUra concentrations in the liver as compared with the results with HAI in rats and pigs. Median areas under the concentration-time curve in liver tissue were 122.7 μmol·g⁻¹·min and 59.9 μmol·g⁻¹·min (40-mg/kg dose-group) and 236.3 μmol·g⁻¹·min and 45.1 μmol·g⁻¹·min (80 mg/kg) for ILP and HAI, respectively in pigs (both P < 0.05). Systemic plasma areas under the curve were significantly lower for ILP compared with HAI in 40- and 80-mg/kg dose-groups with 2.2 μmol·ml⁻¹·min and 9.2 μmol·ml⁻¹·min (40 mg/kg; P < 0.01) and 6.8 μmol·ml⁻¹·min and 43.2 μmol·ml⁻¹·min (80 mg/kg; P < 0.01) for ILP and HAI-treated pigs, respectively. In hepatic tumor tissue a dose-dependent increase of mean FUra concentration was found for ILP-treated rats (P < 0.05). No significant differences were observed in median FUra concentrations in tumor tissue between ILP- and HAI-treated rats (0.66 μmol·g⁻¹ and 0.63 μmol·g⁻¹ for ILP- and HAI-treated groups with 80 mg/kg; P > 0.05). The mean FUra concentration tumor/liver ratio was 0.26.

In order to clarify the metabolic fate of high-dose FUra, five rats were subjected to ILP with 150 mg of FUra/kg, and hepatic tumor extracts excised at t = 0 min, t = 5 min, and t = 15 min after infusion were analyzed using ¹⁸F nuclear magnetic resonance. Catabolite α-fluoro-β-alanine appeared rapidly at t = 5 min and t = 15 min in liver tissue. Significant amounts of the presumed active nucleotides were not detected in tumor tissue. We conclude that ILP is a means to improve selectivity of administration of antitumor agents to the liver, as compared with HAI. The pharmacological advantage of ILP over HAI administering equivalent doses that might be required to achieve effective antitumor activity, was not demonstrated in tumor tissue, because of a large differential between liver tissue extraction and tumor tissue extraction of FUra, which was influenced by the mode of administration.

INTRODUCTION

The liver is the dominant or solitary site of dissemination primarily in colorectal cancer patients (1). These patients could benefit from aggressive local treatment. For the majority of these patients, the fluoropyrimidines are the only established therapeutic option (2). Systemic FUra may result in a 20% tumor response rate, and drug resistance is the common factor in limiting the effectiveness of the antitumor agent.

There is substantial evidence, mainly in experimental systems, that fluoropyrimidines, like most other antitumor agents, have a more or less steep relationship between dose and response (3–5). In order to exploit the observed dose-response relationship, while maintaining tolerable systemic drug levels, hepatic artery infusion of fluoropyrimidines has been used to treat colorectal hepatic metastases. The rationale of this approach rests on the observation of a high hepatic extraction of fluoropyrimidines, thus allowing dose escalation (6, 7). Furthermore, the blood supply of established hepatic metastases is thought to rely entirely on the hepatic artery (8). The pharmacological advantage of administering fluoropyrimidines by HAI as compared with systemic administration was calculated by measuring drug levels in hepatic arterial and venous blood in patients. A 50- to 400-fold and a 2- to 6-fold increase of local drug delivery was predicted for short-term and long-term HAI, respectively, as compared with systemic administration of fluoropyrimidines (6, 9, 10). However clinical data on the therapeutic advantage of HAI over systemic administration of fluoropyrimidines were not conclusive. A number of trials, of which the majority were not randomized, comparing HAI versus systemic administration of fluoropyrimidines demonstrated significantly increased response rates for the HAI group, however, without improved survival rates (11–14).

Obviously, the dose-response relationship of colorectal hepatic metastases for fluoropyrimidines is not very steep, at least, not within the 2- to 3-fold higher dose range as allowed with HAI (11, 12). To obtain insight into time periods and doses that might be required to achieve effective antitumor activity, a number of studies have been done in cultured human colonic tumor cells. Resistance was overcome by dose intensification (15), and a logarithmic decrease in tumor cell survival was observed with 1-h exposure to up to 20- to 40-fold higher FUra concentrations than clinically achievable (16). Obviously, extrapolation of these doses to humans was not possible, because of dose-limiting systemic (or local) toxicity with current modes of administration.

ILP has been suggested as a way of circumventing dose-limiting systemic toxicity and promoting selective exposure of the target organ to higher antitumor doses than otherwise possible. However, successful clinical experience with ILP is limited. Various methods of ILP have been attempted, but the encountered technical difficulties resulted often in incomplete isolation and subsequent systemic leakage of perfusate (17, 18). A recent report on clinical ILP showed a partial response in 6 of 12 patients (19) treated with low doses of FUra. Systemic leakage of perfusate was not monitored. Perioperative mortality was 20% in this series (19).

We developed a reliable and technically feasible technique of ILP with a sensitive method for continuous leakage detection (20). This ILP method should facilitate delivery of higher doses of antitumor agents to the liver higher than is possible by...
conventional modes of administration. Our method can easily be adapted for clinical application. However, ILP requires a complicated operation, with associated morbidity and possibly mortality. The precise pharmacological advantage of ILP has not been determined yet. The aim of this study was to examine local and systemic FUra concentrations following ILP and to compare the results with those obtained with HAI, currently the best method of regional chemotherapy (10, 11). This study has been performed in pigs, as they have a hepatic physiology similar to that of humans (21).

Failure to control human solid tumors has been attributed to inability to achieve effective tumoricidal concentrations of antitumor agents within the tumor mass (22, 23). We investigated this in tumor-bearing rats by measuring FUra concentrations within the hepatic tumors. An insight into actual tumor uptake of FUra has been impeded by difficulties in obtaining tumor biopsies in patients and by the lack of sensitive and specific assays for drug measurements in tissues. An adapted HPLC assay and 19F NMR allowed measuring of FUra in tissues and biofluids of interest. Tumor tissue concentrations of FUra were determined after the administration of various dosages of FUra using either ILP or HAI, and the results were compared.

MATERIALS AND METHODS

Chemicals

FUra and pure 2-thiouracil (used as internal standard for analytical chemistry) were kindly supplied by Hoffmann-La Roche, Mijdrecht, The Netherlands. All other chemicals and reagents were obtained from standard chemical sources and were of analytical grade.

Animals and Operative Procedures

**Pig Study**

Yorkshire pigs weighing 23 to 31 kg were used. General anesthesia was maintained with an O2/N2O (40/60) mixture with 0.5% halothane (Fluothane). Ventilation was facilitated by a muscle relaxant (Pavulon) and an opiate (Fentanyl). Central venous and arterial cannulas (in the internal jugular vein and the carotid artery, respectively) were introduced for monitoring infusions and sampling procedures.

ILP in Pigs (Fig. 1). The method of ILP and a continuous leakage detection system are described previously (20). Briefly, the pancreatic-duodenal artery is cannulated with the tip up to the hepatic artery. A double lumen shunt is introduced in the intrahepatic part of the vena cava and secured below and above the liver. The inner lumen of this intracaval shunt (length, 18 cm) allows undisturbed caval blood flow to the right heart.

A temporary portacaval shunt is created to avoid splanchic blood pooling. The outer lumen of the shunt permits collection of the hepatic venous outflow and is connected to an extracorporeal perfusion circuit. By clamping the celiac axis and the gastric artery, vascular isolation is achieved. The perfusion circuit consists of a pressure- and flow-guided coronary pump, an oxygenator, a heat exchanger, and a scintillation photomultiplier detector. The perfusate was a mixture of 500 ml of Haemaccel and 300 ml of blood, with a final hematocrit of 15 to 20%. The temperature of the perfusate was kept at 36.5 ± 1°C. Flow was maintained at 200 ml-min⁻¹. Leakage from the isolated circuit was below 1% (detection threshold) of the perfusate volume as assessed by measuring labeled red blood cells in isolated and systemic circulation (20). In vivo isolated perfusion with FUra was performed during 60 min.

HAI in Pigs. The pancreatic-duodenal artery was cannulated with the tip of the catheter up to the hepatic artery. The gastric branches of the hepatic artery and the common hepatic artery were temporarily clamped, and the FUra dose was infused in 10 min.

**Rat Study**

Inbred WAG/Rij rats (TNO, Rijswijk, The Netherlands) weighing 300 to 350 g were used for this study. The selected tumor line (CC 531) was a dimethylhydrazine-induced, moderately differentiated carcinoma of the colon, which is marginally sensitive to FUra when implanted s.c. in the flank site (24). Multiple hepatic tumors were evoked by injecting a suspension of 5 × 10⁶ viable tumor cells into a proximal branch of the portal vein. Six wk after inoculation 80% of the rats showed 2 to 4 tumor nodules (total weight, about 2 g). All experiments were performed at passages 9 to 12 of the tumor.

ILP in Rats (Fig. 1). We developed a modified in vivo method of isolated liver perfusion, details of which were described elsewhere (25). Briefly, the pyloric vein was cannulated, as well as the intraportal caval vein. The supraportal and infraportal caval veins were temporarily ligated, as well as the infradiaphragmatic aorta, the distal portal vein and the common hepatic artery. The perfusion circuit consisted of a low-flow roller pump (Watson Marlow, de Jong B. V., Rotterdam, The Netherlands), an adapted oxygenator (from a kidney perfusion set; Gambro, Breda, The Netherlands), and a specially constructed heat exchanger fitted with polyvinylchloride tubing. The recirculating system was primed with 30 ml of Haemaccel and added heparin (50 units), and the pH was kept between 7.3 and 7.4 using bicarbonate. The perfusate was infused into the pyloric branch of the portal vein at a flow rate of 25 ml-min⁻¹. The hepatic venous outflow was collected by an intracaval cannula and was returned to the oxygenator by gravity feed. The perfusate was gassed during perfusion with a mixture of O₂/CO₂ (95/5) at a flow rate of 50 ml-min⁻¹. The perfusate temperature was kept at 36.5 ± 1°C. The final hemoglobin content of the perfusate was 0.6 mmol-liter⁻¹. In vivo perfusion was carried out for 25 min.

HAI in Rats. HAI was performed via the cannulated duodenal branch of the common hepatic artery, and the latter was temporarily occluded for 5 min during infusion to prevent retrograde flow into the aorta.
Study Design

In Pigs

FUra concentrations were determined in liver tissue, bile, and systemic plasma following ILP or HAI and also in perfusate following ILP. Forty-two pigs were assigned to either ILP or HAI administering 20, 40, or 80 mg of FUra/kg and, within these dose groups, 7, 6, and 10 pigs (ILP) and 7, 5, and 7 pigs (HAI), respectively.

Samples from liver tissue (0.5 g), bile (2 ml), and systemic blood (4 ml) were collected at 15, 30, 45, and 60 min (blood and bile also at 75, 90, and 120 min) after the start of ILP or after the end of HAI. With ILP, an additional blood sample was collected at 70 min.

In Rats

FUra concentrations were measured in liver and tumor tissue following ILP and HAI. Thirty-six rats with hepatic tumors were assigned to 20, 40, or 80 mg of FUra/kg: 7, 8, and 6 rats (ILP) and 5, 6, and 4 rats (HAI), respectively, for these dose groups. In rats, wedge biopsies (250 mg) were excised from liver and tumor at 5 and 15 min after initiating the perfusion (ILP) or after the end of the infusion (HAI).

The relatively large amount of rat liver and tumor tissue needed for (250 mg) were excised from liver and tumor at 5 and 15 min after initiating the perfusion (ILP) or after the end of the infusion (HAI). The relatively large amount of rat liver and tumor tissue needed for analysis and technical difficulties in controlling leakage from the biopsied liver allowed only two sampling times. FUra metabolism was studied to clarify the fate of high-dose FUra. Five rats with hepatic tumors were subjected to HAI of 150 mg of FUra/kg (the MTD with ILP in rats).4 At t = 0 and 5 and 15 min after HAI, liver and tumor samples (500 mg) were excised and used for 19F NMR analysis.

Analysis

HPLC Assays for FUra in Biofluids, Perfusate, and Tissue Biopsies

Blood samples were cooled in an ice bath and centrifuged as soon as possible. Plasma was separated and stored, as well as bile samples, at −20°C prior to analysis. Plasma and bile samples were thawed, and 250 µl of plasma or bile were adjusted to pH 6.0 by adding 250 µl of Tris buffer (0.5 M) and extracted with 7 ml of freshly distilled ethylacetate by vortexing for 60 s. After separation by centrifugation for 5 min at 1000 × g, 6 ml of the organic phase were evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 100 µl of the mobile phase. Liver or tumor samples were minced, and 250 µg were suspended in 2 ml of a 0.05 M Borax buffer (pH 8.7) by vortexing for 20 s. Subsequently the sample was homogenized for 5 min. The homogenate was poured onto a waterpretreated ultrafilter (Amicon Centroflo) and centrifuged at 40°C for 60 min at 2000 × g. Aliquots of 10 to 100 µl of the ultrafiltrate were injected into the HPLC system. The HPLC system consisted of a MOS hypersil (5 µm) column (100 x 3 mm), packed according to a method described previously (26). A Kontron Model 410 solvent delivery system was equipped with a Kontron Model MSI 660 autosampler. The mobile phase consisted of 2% methanol in Tris buffer (pH 8; 0.05 M) containing 0.5 mm citrulate. The effluent was monitored at 266 nm using an UV/vis detector. The limits of determination for FUra, based on a 3:1 signal-to-noise ratio, were 76.4 nmol-m¹ for body fluids, the coefficients of variation for three successive determinations were 3%, and the recovery ranged between 70 and 75% (27). The limit of determination of FUra concentration in liver and tumor tissue was 0.14 µmol·g⁻¹.

19F NMR Assays

For tissue pretreatment, 500 mg of liver or tumor tissue were suspended in 2 ml of cold 4% (v/v) perchloric acid using a PT 10–35 polytron (Kinematica, Krienz-Luzern, Switzerland) twice at 6-s maximum speed immediately after excision. Two ml of cold 0.05 M phosphate buffer (pH 4.0) were added to the suspension. Subsequently, the suspension was centrifuged at 3000 rpm for 5 min. The supernatant was removed and frozen at −169°C (pH 7.0). The solution was thawed, dried under a gentle stream of air, and stored at −20°C. The powder was dissolved in 400 µl of MilliQ water (MilliQ water purification system; Millipore, Bedford, MA) and 50 µl of D₂O, for lock, just prior to 19F NMR analysis. 19F NMR spectra were recorded at 282.4 MHz on a Bruker Model WM-300 (Bruker, Bremen, Germany) in the fluor mode, connected with an Aspect-2000 computer. The resonance positions were referenced to an external standard CF₃COOH resonance peak. For liver and tumor tissue samples, the instrumental settings were established as follows: probe temperature, 25°C; sweep width 50 kHz; 45° pulse, recycling time, 0.964 s; number of scans, 1000; computer resolution 6.104 Hz/point; and line broadening caused by exponential multiplication, 50 Hz. Chemical shifts (ppm) were assigned to the various FUra metabolites according to the report of Malet-Martino et al. (28).

Pharmacokinetic Evaluation

Rats

The pharmacokinetic parameters were AUC, calculated using the trapezoidal rule, with extrapolation to infinity, and Cmax, which was determined from the raw data. The target organ-related advantage (Rd) was calculated for ILP over HAI according to the formula

\[ R_d = \frac{\text{AUC}_{\text{system}(ILP)}}{\text{AUC}_{\text{system}(HAI)}} \]

Statistical Evaluation

Data were computerized to facilitate statistical analysis. The Mann-Whitney test was used for comparisons between two treatment groups, and the Kruskal-Wallis test was used for comparisons of more than two groups. P < 0.01 was considered as minimal level of significance.

RESULTS

Pigs. The liver tissue concentration-time curves shown in Fig. 2 demonstrated that approximately 4 times higher maximal FUra concentrations were achieved with ILP as compared with HAI; the (median) maximal FUra concentrations were 8.44 µmol·g⁻¹ and 2.0 µmol·g⁻¹ for ILP- and HAI-treated animals, respectively (P < 0.01). With ILP, a small systemic release of FUra from the liver compartment was measured after reestablishing the normal circulation (Fig. 2A). In systemic plasma, the (median) maximal FUra concentrations were 2.07 µmol·ml⁻¹ and 0.84 µmol·ml⁻¹ with ILP (Fig. 2A) and HAI (Fig. 2B) in the highest dose groups, respectively (P < 0.01).

AUCs of FUra in liver tissue, systemic plasma, and bile relating to ILP and HAI with 20, 40, or 80 mg/kg in pigs are depicted in Fig. 3. Median AUCs of FUra in liver tissue were 65 µmol·g⁻¹·min, 122.7 µmol·g⁻¹·min, and 236.3 µmol·g⁻¹·min for ILP, and 68.6 µmol·g⁻¹·min, 59.45 µmol·g⁻¹·min, and 45.1 µmol·g⁻¹·min for HAI with 20, 40, and 80 mg/kg, respectively (Fig. 3A). The dose increment with HAI did not result in higher FUra concentrations in the liver, contrary to ILP. AUCs in liver tissue were significantly higher for the ILP-
treated animals as compared with HAI in the 40- and 80-mg FUra/kg groups (P = 0.01 and P < 0.01, respectively).

Median AUCs of FUra in systemic plasma were 1.2 μmol·ml⁻¹·min, 2.2 μmol·ml⁻¹·min, and 6.8 μmol·ml⁻¹·min for ILP and 7.6 μmol·ml⁻¹·min, 9.2 μmol·ml⁻¹·min, and 43.2 μmol·ml⁻¹·min for HAI with 20, 40, and 80 mg/kg (Fig. 3B). In systemic plasma, much smaller AUCs were observed in the ILP-treated animals with 40 and 80 mg of FUra/kg as compared with the results with HAI (P < 0.01 for both comparisons). In systemic plasma, ratios of AUC (ILP)/AUC (HAI) were 0.2 and 0.15 for the 40-mg/kg and 80-mg/kg groups, respectively.

Based on the AUCs of FUra in liver tissue and in systemic plasma, the target organ-related advantage Rₜ (ILP/HAI) was 1 (20-mg/kg dose group), 11 (40 mg/kg), and 24.7 (80 mg/kg) for ILP.

Median AUCs of FUra in bile were 16.8 μmol·ml⁻¹·min, 19.2 μmol·ml⁻²·min, and 47.3 μmol·ml⁻¹·min for ILP and 4.1 μmol·ml⁻¹·min, 14.5 μmol·ml⁻¹·min, and 26.1 μmol·ml⁻¹·min for HAI with 20, 40, and 80 mg/kg-treated pigs (Fig. 3C). No significant differences were found between the dose groups subjected to either ILP or HAI in bile. With ILP administration, a dose-dependent increase in AUC was observed, but not with HAI.

Median FUra concentrations in perfusate were 2.0 μmol·ml⁻¹, 5.3 μmol·ml⁻¹, and 9.4 μmol·ml⁻¹ with ILP administering 20 mg of FUra/kg, 40 mg/kg, and 80 mg/kg, respectively.

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Fig. 2. Time-concentration curves of median FUra values (μmol·ml⁻¹ or μmol·g⁻¹) following isolated liver perfusion (A) and hepatic artery infusion (B) with 20 mg/kg, 40 mg/kg, or 80 mg/kg in pigs (n = 42). Symbols indicate FUra concentrations in liver tissue per dose group: 20 mg/kg (●), 40 mg/kg (×), and 80 mg/kg (○); and FUra concentrations in systemic plasma per dose group: 20 mg/kg (■), 40 mg/kg (+), and 80 mg/kg (•).

Fig. 3. AUCs of FUra versus dose (20 mg/kg, 40 mg/kg, and 80 mg/kg) with ILP and HAI in pigs (n = 42): A, in liver tissue (μmol of FUra·g⁻¹·min); B, in systemic plasma (μmol of FUra·ml⁻¹·min); C, in bile (μmol of FUra·ml⁻¹·min). Bars, median values.
Rats. The mean Fura concentrations in liver and tumor tissue following ILP and HAI with 20, 40, and 80 mg/kg in rats are depicted in Fig. 4. Median values for the mean Fura concentrations in liver tissue were 0.3 μmol·g⁻¹, 1.1 μmol·g⁻¹, and 4.7 μmol·g⁻¹ for ILP and 0.6 μmol·g⁻¹, 0.3 μmol·g⁻¹, and 2.05 μmol·g⁻¹ for HAI with 20, 40, and 80 mg of Fura/kg, respectively (Fig. 4A). In the 80-mg/kg group, significantly higher Fura concentrations were found in liver tissue for the ILP-treated animals (P < 0.05), a trend similar to the results observed in pigs.

Median Fura concentrations in tumor tissue were 0.11 μmol·g⁻¹, 0.29 μmol·g⁻¹, and 0.66 μmol·g⁻¹ for ILP and 0.22 μmol·g⁻¹, and 0.63 μmol·g⁻¹ for HAI with 20, 40, and 80 mg/kg, respectively (Fig. 4B). The Fura concentrations in tumor tissue are approximately 0.26-fold those in liver tissue: Rₐ is 0.29 for HAI and 0.24 for ILP.

No significant differences were found in Fura concentrations in tumor tissue between ILP- and HAI-treated animals in the three dose groups. Fura concentrations in tumor tissue increased as a function of dose with ILP.

19F NMR Data. The fate of Fura and metabolites was investigated following the administration of the MTD with ILP in five rats using 19F NMR and rendered similar results in all experiments. Fura concentrations in tumor tissue remained high after treatment, with no significant amounts of metabolites being formed during the sampling interval. (Typical results are represented in Fig. 5.) In liver tissue, the rapid appearance of the catabolite FBAL was noted, as well as small amounts of fluorinated nucleotides/nucleosides.

DISCUSSION

The aim of this study has been to delineate the role of ILP in increasing local drug concentrations, while minimizing systemic drug exposure, in order to estimate the possible advantage of an experimental method of ILP above HAI. HAI is currently the purported best mode of regional chemotherapy in the treatment of hepatic metastases. Despite a large range, ILP administration of the drug resulted in significantly higher Fura concentrations in liver tissue of animals in the higher dose groups (rats and pigs). In pigs, AUC did not increase in liver tissue when the drug was administered by HAI. This indicated that drug levels by HAI administration exceeded the liver’s capacity for uptake. High dose rates with fluoropyrimidines as well as high flow rates with short-term HAI, as used in this study, are reported to exceed the hepatic capacities for Fura uptake (6, 9, 29). Therefore, our pharmacokinetic data on HAI may not reflect the pharmacological advantage of continuous HAI, currently the most frequently used method, administering low doses (below hepatic saturation threshold) during prolonged periods of time (29). The hepatic saturation of Fura uptake with HAI was associated with dose-dependent increases of Fura concentrations in bile and in systemic plasma. ILP resulted in significantly lower systemic drug levels in the higher dose groups, as compared with HAI. The data suggested that, to achieve similar systemic drug levels, 5 times the HAI dose can be administered with ILP. Indeed, our toxicity study showed a MTD of 160 mg of Fura/kg for ILP (with 4 of 5 pigs surviving) and a MTD of 40 mg/kg for HAI (also 4 of 5 pigs surviving) (31), and systemic toxicity was dose limiting.

Based on the AUCs in liver tissue and in the systemic circulation in pigs, the pharmacological advantage of ILP over HAI with 80 mg/kg was 24.7. However, the value of this ratio may be limited, since this advantage of ILP was not apparent in hepatic tumors in rats. No significant differences were found in Fura concentrations in tumor tissue between ILP and HAI administering similar doses. Obviously, the mode and route of administration influence the Fura distribution pattern, as was shown for patients (32) and for animals (33). Fura concentrations in tumor tissue were much lower than in liver tissue (tumor/liver ratio, approximately 0.26), reflecting a differential in Fura extraction and/or in perfusion. Well-known mecha-
nisms of drug resistance, which might be responsible for the relatively low (parent) drug concentrations in tumor tissue, include (a) a defective cell membrane transport, (b) an increased drug degradation, and (c) an abnormal tumor vasculature (34).

No data are currently available on in situ tumor cell drug uptake. Only a few studies refer to uptake of fluoropyrimidines in tumorous and liver tissues (35–38). One clinical study addressed FdUrd concentrations in tumor and in liver following short-term HAI (36). The mean FdUrd concentration tumor/dressed FdUrd concentrations in tumor and in liver following drug degradation, and (c) an abnormal tumor vasculature (34). Of value in estimating the pharmacological advantage of a given mode of administration.

The question of whether rapid intracellular degradation of FUra might be responsible for the low tumor tissue concentrations of FUra was answered in our hepatic metastases model using 19F NMR. No catabolites were detected in tumor tissue (Fig. 4), and this finding is in accordance with the previously described reduced capability of tumor cells to catabolize FUra (39, 40).

In our isolated rat liver perfusion system, the perfusate was infused into the portal vein. The hepatic artery might be preferable as the afferent route of the perfusion circuit, since established hepatic metastases are reported to depend mainly on arterial blood supply (8). Also defective anabolism to active metabolites has been recognized as a mechanism of drug resistance, and an important question is whether high FUra concentrations within the tumor would give rise to high concentrations of cytotoxic metabolites of FUra. FUra is thought to exert its principal mechanism of cytotoxicity through (a) its anabolic conversion to FdUMP, which binds to thymidylate synthetase and thereby inhibits de novo synthesis of dTMP and, hence, DNA synthesis (41), and (b) its conversion to FUTP, which is incorporated into RNA, thereby inhibiting RNA maturation and function (42). The major metabolic pathway is rapid degradation, primarily in the liver, to the presumed inactive catabolic products, FUPA and FBAL (43–45), and DHFU, which may also have cytotoxic properties itself (46). 19F NMR analysis of rats treated with the MTD of FUra (with ILP) failed to demonstrate anabolites in tumor tissue. This finding may also be due to slow conversion to these metabolites (47) or the high detection threshold of 19F NMR (48, 49).

In liver tissue, a rapid appearance of FBAL was noted (at t = 5 min and t = 15 min), consistent with earlier reports on FBAL being the main catabolite (48, 50). However, no significant amounts of DHFU and FUPA were detected in the liver tissue samples, pointing at the transient properties of these metabolites (48). Nucleosides and nucleotides were detected only in our liver tissue samples, which may be related to the hepatotoxicity frequently observed with continuous HAI.

In conclusion, ILP is a method to improve selective administration of antitumor agents to the liver while maintaining very low systemic drug concentrations. In our experiments of rats, no pharmacological advantage was demonstrated for ILP as compared with HAI at the tumor site, when administering similar doses of FUra. However, the low systemic drug concentrations with ILP may allow delivery of higher nontoxic FUra doses to tumorous tissue. Also, the portal vein may be a less preferable route for effective tumor perfusion as compared with the hepatic artery. Further studies are needed to clarify whether ILP via the hepatic artery and the portal vein with the MTD of FUra would increase drug levels and its relation to anabolism, as well as the pharmacodynamic properties of high-dose FUra.

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