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Comparison of 5-fluorouracil anabolite levels after intravenous bolus and continuous infusion

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Abstract. The levels of 5-fluorouracil (5FU) and its anabolites in the serum, bile, pancreatic juice, liver, pancreas, and skeletal muscle of dogs were compared after single bolus administration and after continuous infusion. Six dogs had a bolus of 5FU (15 mg/kg) and were studied for 120 min. Five dogs had a continuous infusion of 5FU (30 mg/kg) and were studied for 24 h. After the bolus infusion, serum 5FU levels were initially high and then declined, whereas anabolite levels gradually increased over 45 min. Within 2 h, anabolite levels exceeded 5FU levels in tissues but were undetectable in bile and pancreatic juice. During the continuous infusion, anabolite levels in serum increased more rapidly than 5FU levels and remained significantly higher for 24 h. Anabolite levels also exceeded 5FU levels in bile, pancreatic juice, pancreas, and muscle but not in the liver. Continuous infusion of 5FU produced higher levels of the anabolites than did bolus infusion and maintained constant levels throughout the infusion period.

Key words: 5-Fluorouracil – Infusion – Tissue levels

Introduction

5-Fluorouracil (5FU) has been given alone or in combination with other agents to treat malignancies. A complete remission occurs in only a few patients with colon and breast cancer [3, 14]. The response rate is 20% in patients with metastatic colorectal carcinoma and 25% in those with advanced breast carcinoma [6, 10]. Most patients become refractory to chemotherapy in spite of an initial response.

The efficacy of 5FU treatment by i. v. bolus injection is limited by the short half-life in plasma, which varies from 8 to 20 min [15]. Since the uptake of 5FU by cells is proportional to plasma concentrations and antineoplastic activity occurs primarily during the S phase of the cell cycle, cytotoxicity would be limited to only a small number of cells. For most common adult carcinomas this proportion of cells is 5%–20% [11]. Studies on human colon carcinoma cells treated with 5FU and pulse-labeled with tritiated thymidine indicated that only 3% of the cells were affected by 5FU treatment [18]. Treatment is also limited by the dose and by toxicity to the bone marrow, the gastrointestinal tract, and, occasionally, the heart [8].

To optimize the antineoplastic response and minimize the systemic toxicity, 5FU has been given by continuous i. v. infusion to treat metastatic carcinoma of the breast and colorectum [10, 12]. Although prolonged i. v. infusion of 5FU appears to enhance the response rate in these patients, a significant number of patients nonetheless have either stable or progressive disease.

Ideally, if the serum concentration of 5FU could be maintained at levels that achieve tolerable toxic levels of active anabolites in tumors, the number of remissions would be expected to increase. Unfortunately, the measurement of 5FU levels in serum is a poor indicator of tissue anabolite levels and the clinical response [21]. In the present study, serum 5FU and anabolite levels were compared with tissue levels after either i. v. bolus or continuous infusion of 5FU.

Materials and methods

A total of 11 healthy mongrel dogs were obtained from Biomedical Associates (Friedenburg, Pa.). Animals were housed in a United States Department of Agriculture (USDA)-approved facility for at least 1 week before the experiment and were fed a standard laboratory canine diet.

Six dogs were fasted overnight. Anesthesia was induced with sodium phenobarbital (35 mg/kg, i. v.). Dogs were allowed to breath room air spontaneously via an endotracheal tube. A celiotomy was performed and the bile and pancreatic ducts were cannulated to collect secretions. A combination of secretin (1.8 units kg^{-1} h^{-1}) and the octapeptide of cholecystokinin (0.12 mg kg^{-1} h^{-1}) was infused through

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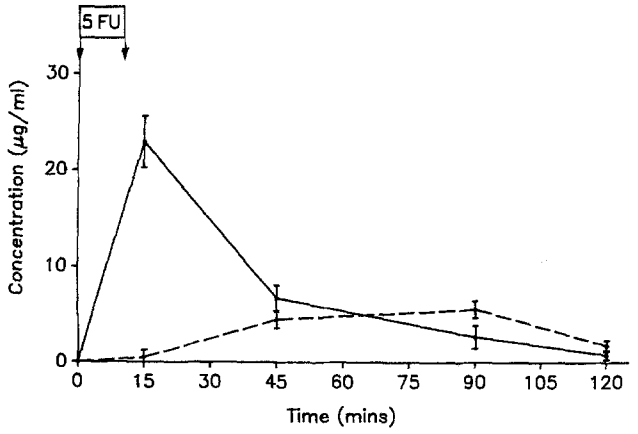

Fig. 1. Serum concentrations of 5FU (*solid line*) and 5FU anabolites (*dashed line*) measured after a 10-min i. v. bolus infusion of 5FU at 15 mg/kg. Samples were obtained at 0, 15, 45, 90, and 120 min after the beginning of the infusion. Data represent mean values ± SEM for 6 dogs

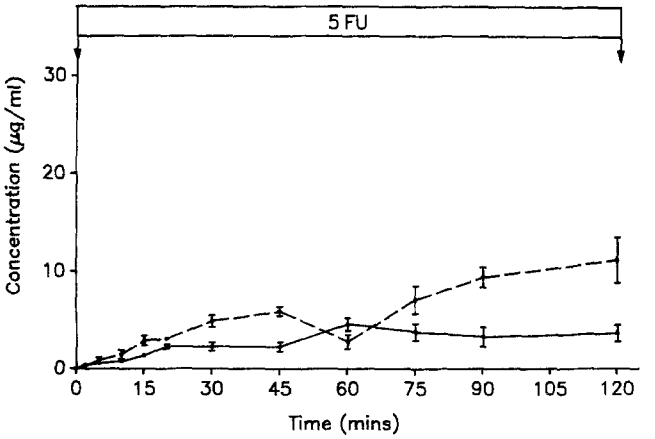

Fig. 2. Serum concentrations of 5FU (*solid line*) and 5FU anabolites (*dashed line*) measured during the initial 2 h of a 24-h continuous i. v. infusion of 5FU at 30 mg/kg. Samples were obtained at 0, 2, 5, 10, 15, 20, 30, 45, 60, 75, 90, and 120 min. Data represent mean values ± SEM for 5 dogs

an i. v. cannula in a foreleg vein to stimulate a submaximal flow of bile and pancreatic juice [2]. Another cannula was placed in a vein in the other foreleg for infusion of 5FU and for blood withdrawal. 5FU was given as a bolus infusion of 15 mg/kg over 10 min. This dose has been used clinically in the treatment of colorectal cancer [5]. At 0, 15, 45, 90, and 120 min, 2-ml samples of blood were obtained and discarded and then 3-ml samples were collected for assay. Samples of bile and pancreatic juice and wedge-biopsy specimens of liver, pancreas, and rectus abdominus muscle were obtained at 2 h.

In five conscious dogs, 5FU at 30 mg/kg was continuously infused for 24 h (20.8 μg kg^{-1} min^{-1}) into one foreleg vein and blood samples were withdrawn from the other foreleg. This dose corresponds to recent clinical recommendations [9]. Blood samples were collected at 0, 2, 5, 10, 15, 20, 30, 45, 60, 75, and 90 min and at 2, 4, 6, 12, 18, and 24 h. After 23 h animals were anesthetized, a celiotomy was performed, bile and pancreatic ducts were cannulated, and secretin plus octapeptide of cholecystokinin was infused. Samples of bile, pancreatic juice, liver, pancreas, and rectus abdominus muscle were obtained for assay at the end of the 24-h infusion.

Levels of 5FU and 5FU anabolites (fluorouracil nucleosides in serum, bile, and pancreatic juice; fluorouracil nucleosides and nucleotides in tissues) were determined by a method that has been reported elsewhere [19]. Studies using radioactively labeled 5FU have demonstrated that 5-fluorouridine triphosphate (5FUTP) is the predominant product and that the intracellular concentration of 5FU anabolites is greater than that of 5FU by a factor of 2–6 [1], which is similar to our results. From 150 to 300 mg of tissue or 0.2 ml of fluid samples was homogenized in a mixture of petroleum ether and *n*-propanol at pH 4.8 using a Polytron homogenizer to extract 5FU. The aqueous layer was washed twice with the ether-alcohol mixture and the extracted 5FU was measured. To determine the level of 5FU anabolites, first a tissue or fluid sample was hydrolyzed with hot perchloric acid and then 5FU was extracted as described above. The total amount of 5FU present after hydrolysis and ether-alcohol extraction consisted of the sum of the levels of 5FU and its anabolites. The amount of 5FU anabolites was calculated by subtracting the level of unreduced drug from the total amount of 5FU.

Mean values and SEM were calculated. The differences between the groups were determined by Student's *t*-test. By plotting the data obtained for each animal, product-moment correlations were calculated to determine the relationship of levels detected in serum to those measured in liver, pancreas, muscle, bile, and pancreatic juice as well as the relationships between levels in different tissues. A value of $P < 0.05$ indicated statistical significance.

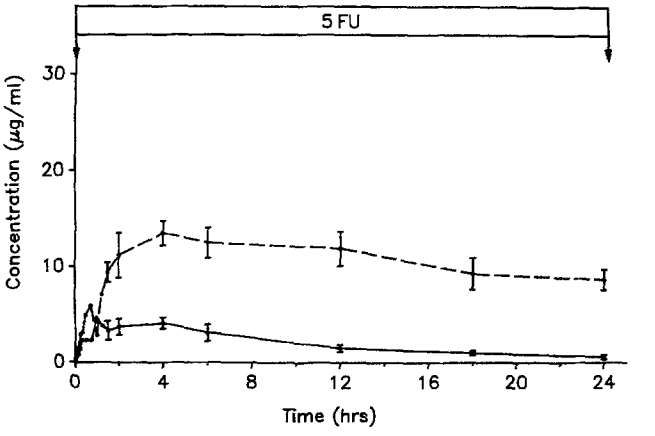

Fig. 3. Serum concentrations of 5FU (*solid line*) and 5FU anabolites (*dashed line*) measured during the 24-h continuous i. v. infusion of 5FU at 30 mg/kg. Samples were obtained at 0, 2, 5, 10, 15, 20, 30, 45, 60, 75, and 90 min and at 2, 4, 6, 12, 18, and 24 h. Data represent mean values ± SEM for 5 dogs

Results

At 15 min after the beginning of the bolus infusion of 5FU at a rate of 1.5 mg kg^{-1} min^{-1} for 10 min, 95% of the total 5FU measured in serum was the parent drug (Fig. 1). Levels of the anabolites rose gradually in serum, were similar at 45 and 90 min, and then declined.

Serum levels of 5FU and its anabolites were determined during a continuous infusion of 5FU at a rate of 0.021 mg kg^{-1} min^{-1} for 24 h. In Fig. 2 the concentrations are plotted for the first 2 h. Anabolite concentrations increased steadily and exceeded those of 5FU. Anabolite concentrations were initially similar to the levels achieved with the bolus infusion and then became higher by 90 min. 5FU levels rose gradually, stabilized from 1 to 4 h at 3 μg/ml, and then declined (Figs. 2, 3). Anabolite concentrations reached a peak of 13.5 μg/ml at 4 h and then declined at a rate of 0.22 μg ml^{-1} h^{-1} (Fig. 3). At 24 h the level of anabolites

Table 1. Serum, bile, pancreatic juice, and tissue levels of 5FU measured 2 h after an i.v. bolus and 24 h after the start of a continuous infusion

	Bolus		Continuous	
	5FU	Anabolites	5FU	Anabolites
Serum	0.8 ± 0.5	1.8 ± 0.6	0.6 ± 0.2	8.7 ± 1.1
Bile	<0.1	<0.1	1.3 ± 0.1	4.1 ± 0.9
Pancreatic juice	<0.1	0.4 ± 0.1	0.8 ± 0.1	3.5 ± 0.4
Liver	5.0 ± 1.7	5.9 ± 0.4	5.8 ± 1.0	5.2 ± 0.1
Pancreas	1.3 ± 0.8	3.6 ± 0.4	0.5 ± 0.1	7.7 ± 0.2
Muscle	0.6 ± 0.5	3.7 ± 2.1	0.4 ± 0	5.5 ± 0.5

Data represent mean values ± SEM and are expressed in μg/ml for fluids and in μg/g for tissues

was 67% of the peak value and the 5FU concentration had fallen to 10% of the peak value.

The levels of 5FU measured in serum, bile, pancreatic juice, liver, pancreas, and muscle after bolus or continuous infusion are compared in Table 1. The liver contained greater amounts of 5FU, but the values did not correlate with the serum levels. In serum the level of anabolites was about twice that of 5FU. None was detected in bile. Levels measured in pancreas and muscle were twice those detected in serum but did not correlate with serum levels. In liver the level of anabolites was similar to that of 5FU and also did not correlate with serum concentrations.

At 24 h after the beginning of the continuous 5FU infusion, serum 5FU levels were similar to those determined following the bolus dose (Table 1). Although the 24-h serum values were similar to 5FU levels in the pancreatic juice, pancreas, and muscle, there was no significant correlation with the serum or tissue values. As compared with the serum value, the levels of 5FU measured in liver and bile were 10 and 2 times greater, respectively.

The 24-h serum anabolite levels were twice the values determined in bile and pancreatic juice and had correlation coefficients of 0.773 and 0.803 ($P < 0.06$), respectively. Serum anabolite levels were also slightly higher than the levels measured in the liver, pancreas, and muscle and had correlation coefficients of 0.624, 0.756, and 0.775, respectively. A significant correlation did occur between anabolite levels detected in pancreas and muscle ($r = 0.943$, $P < 0.01$).

Discussion

The critical tissue levels of the anabolites of 5FU, 5-fluoro-2′-deoxyuridine monophosphate (5FdUMP) and 5FUTP, required for clinical efficacy are not known. Obtaining tumor samples during chemotherapy is difficult, and blood levels of 5FU do not accurately reflect tissue anabolite levels [5]. In the present study we confirmed the poor relationship of serum concentrations of 5FU to tissue levels of 5FU and its anabolites. At 2 h after the bolus infusion, serum 5FU levels were low, and no measurable amount was detected in bile or pancreatic juice. Although 5FU excretion from the liver and the pancreas is related to the serum concentration [20], serum levels fall rapidly after bolus administration. Biliary levels of 5FU after a bolus infusion of 750 mg/m^2 have been detected only during the 1st h [13].

Within cells, 5FU nucleotides bind to thymidylate synthetase and do not diffuse through the cell membranes [17]. Cellular levels of anabolites consist of both 5FU nucleosides and 5FU nucleotides, but serum levels of anabolites comprise only 5FU nucleosides. Cellular fluorinated nucleosides easily traverse the plasma membrane and diffuse into blood. In the present study, at 2 h after bolus infusion of 5FU, levels of 5FU anabolites exceeded 5FU levels in serum, whereas they were virtually undetectable in bile and pancreatic juice.

Continuous infusion of twice the dose of 5FU given over 24 h resulted in serum anabolite concentrations considerably greater than those of 5FU. These anabolite levels were higher than those measured following the bolus injection and remained higher for the duration of the study. At 24 h anabolite levels detected in the bile and pancreatic juice were 40%–50% of the serum values. Anabolite excretion in bile and pancreatic juice was related to the uptake of 5FU from serum by hepatocytes and acinar cells and the synthesis of nucleosides. Elimination of anabolites by the liver and pancreas was enhanced during continuous infusion of the drug and reflects the higher 5FU-nucleoside concentrations detected in these tissues.

Serum levels of 5FU measured after either bolus or continuous infusion did not reflect the content of fluorinated nucleosides and nucleotides determined in the liver, pancreas, and muscle. In contrast, serum levels of anabolites reflected the tissue content. With continuous infusion the 5FU-anabolite content detected in the liver, muscle, and pancreas was 60%, 62% and 89%, respectively, of the serum level. Correlation coefficients between serum levels of anabolites and tissue levels were positive but did not reach significance. The significant correlation noted between pancreas levels and muscle levels suggests similar anabolite kinetics in these tissues. Due to our inability to differentiate between ribonucleoside and deoxyribonucleoside metabolism in the present study, we could not determine the preferential metabolism of 5FU to FUTP or FdUMP. Other studies should be performed using methods that directly measure these metabolites to determine the effect of bolus and continuous infusion on the direction of 5FU anabolism.

The induction of anesthesia 1 h before the end of the continuous infusion caused no change in the serum 5FU or anabolite levels. Both the animals receiving continuous infusions and those given bolus injections were anesthetized during the tissue and fluid sampling. Although we have no reason to believe that the 5FU or anabolite levels were altered by anesthesia, such a possibility cannot be excluded. These data should therefore be extrapolated with reservation to the clinical situation in unanesthetized patients.

Continuous infusion of 5FU achieved higher and more prolonged serum and tissue levels of anabolites, suggesting that this is the preferred method of administration. Although the bolus and continuous infusions did not cause apparent lesions in the gastrointestinal epithelium, other studies using toxic doses of 5FU should be performed to

determine the relationship 5FU and its anabolites to damage to the mucosa, bone marrow, and skin. The therapeutic dose of 5FU has varied from 200 to 5000 mg/m^2 per day to achieve a clinical response. Severe complications occur in some patients, resulting in myocardiotoxicity, neurological dysfunction, and death [4, 7, 16]. Ideally, the dose of 5FU should be customized for each patient to optimize tumor necrosis while minimizing the severity of complications. Measuring serum 5FU-nucleoside levels should help achieve this goal.

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