5-Fluorouracil Concentrations in Rat Plasma, Parotid Saliva, and Bile and Protein Binding in Rat Plasma

LEE A. CELIO, G. JOHN DiGREGORIO ˣ, EILEEN RUCH
JOSEPH N. PACE, and ANTHONY J. PIRAINO

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Abstract □ The pharmacokinetics of 5-fluorouracil were studied over a 60-min period in rats that received 12.5, 25.0, and 50.0 mg/kg iv. The plasma concentration–time relationship and the detectability in bile and parotid saliva (a route of elimination heretofore given little or no attention) were examined. Protein binding of 5-fluorouracil at concentrations chosen to approximate those found in plasma was determined by equilibrium dialysis. Bile–plasma and parotid saliva–plasma concentration ratios were calculated. 5-Fluorouracil concentrations were quantitated by high-performance liquid chromatography. Plasma concentrations at all doses studied appeared to rapidly decline. The half-life, however, at the 50.0-mg/kg dose (27 min) was significantly longer ($p < 0.025$) than the corresponding half-life at the 25.0-mg/kg dose (22 min). This may be attributed to an easily saturable hepatic degradation. Although an observed decline in bile–plasma and parotid saliva–plasma concentration ratios at higher doses may represent saturation of these excretary routes, the small amounts of 5-fluorouracil detected in bile and parotid saliva probably contribute negligibly to the elimination of the total drug equivalents administered. Parotid saliva–plasma concentration ratios were not useful in predicting plasma protein binding as determined by equilibrium dialysis. Excretion of intravenously administered 5-fluorouracil in saliva, however, exposes the upper GI tract to this agent and may play a part in causing stomatitis in patients receiving the drug by this route.

Keyphrases □ 5-Fluorouracil—concentrations in rat plasma, bile, and parotid saliva as determined by high-performance liquid chromatography, protein binding, pharmacokinetics □ Chemotherapeutic agents—5-fluorouracil, concentration in rat plasma, bile, and parotid saliva, protein binding, pharmacokinetics *in vivo* □ Pharmacokinetics—of the chemotherapeutic agent 5-fluorouracil in rats, concentrations in plasma, bile, and parotid saliva

5-Fluorouracil, an antineoplastic agent which inhibits the synthesis of DNA (1), is extensively used in the treatment of neoplasms of the breast and GI tract (2), with a trend toward utilizing it in combination with several chemotherapeutic agents in complex schedules for disseminated disease (3–5). Information concerning the concentration–time course in plasma and body excretions may be helpful in establishing dose schedules with a pharmacological rationale. In this work a simple, sensitive, and specific method, high-performance liquid chromatography (HPLC), was used to study the decline of 5-fluorouracil concentration in plasma and its excretion in bile and parotid saliva, a route of excretion heretofore given little or no attention. Bile–plasma and parotid saliva–plasma concentration ratios were calculated and the significance of biliary and parotid saliva excretion as routes of elimination were evaluated. The extent to which 5-fluorouracil is bound to rat plasma proteins, at a concentration chosen to approximate amounts detected in rat plasma, was measured by equilibrium dialysis. The calculated percentage of free drug in plasma was determined from the parotid saliva–plasma concentration ratios and compared with the calculated percentage of free drug determined by equilibrium dialysis.

Table I—Concentration of 5-Fluorouracil in Rat Plasma Following Intravenous Administration

Minutes Postdose	Concentration in Plasma, μg/ml[a]		
	12.5-mg/kg Dose[b]	25.0-mg/kg Dose[b]	50.0-mg/kg Dose[c]
5	9.9 ± 0.9	33.6 ± 1.7	52.8 ± 3.8
10	8.2 ± 1.2	21.6 ± 2.1	45.5 ± 4.7
15	6.0 ± 0.6	15.1 ± 1.3	34.2 ± 3.7
30	3.5 ± 0.8	9.0 ± 1.7	23.0 ± 1.9
60	1.6 ± 0.2	5.9 ± 1.4	11.7 ± 1.0

[a] Data expressed as mean ± *SE*. [b] $n = 5$. [c] $n = 6$.

EXPERIMENTAL

Material—All solvents and reagents were analytical grade. A commercial preparation of 5-fluorouracil[1] (injectable form) and 5-fluorocytosine[1] (powder form) were utilized.

Apparatus—The liquid chromatograph[2] was fitted with a loop injector and fixed-wavelength UV detector (254 nm). A 1-mV recorder[3] was utilized and the detector was attenuated at 0.20 AUFS. A prepacked, 300 × 3.9-mm (i.d.) reverse-phase μ-Bondapak C_{18} analytical column[4] was run at ambient temperature. One liter of 5% glacial acetic acid was added to 27 mg of heptane sulfonic acid for the mobile phase. The mixture was pumped isocratically through the column at a flow rate of 1.5 ml/min.

Animal Experiment—Male Wistar rats, 220–270 g, were anesthetized and prepared for drug administration and parotid saliva collection by the method described by Piraino, DiGregorio, and Ruch (6). In addition, the bile duct was surgically exposed and cannulated with polyethylene tubing[5]. Blood samples (0.5 ml) were collected in heparinized tubes at 0, 5, 10, 15, 30, and 60 min after the administration of 5-fluorouracil through the femoral vein. Parotid saliva and bile were collected over 0–15, 15–30, and 30–60 min postdose.

Equilibrium Dialysis—One-milliliter samples of rat plasma containing 60.0 or 12.5 μg of 5-fluorouracil were placed into bags formed from standard dialysis tubing[6]. The bags were tied and put in 50-ml culture tubes containing 10 ml of 0.067 *M* phosphate buffer and spiked to yield a solution of 100% of the 5-fluorouracil concentration that was placed in the plasma compartment. The tubes were sealed tightly and agitated in a shaker-water bath maintained at 37° for 4–12 hr.

Extractions—Extractions were done using a modified method of Christophides *et al.* (7). Plasma (200 μl), bile (100 μl), parotid saliva (50–100 μl), or dialysis tubing was added to 0.5 ml of saturated sodium sulfate solution and 50 μl of sodium acetate (200 g/liter). 5-Fluorocytosine, the internal standard, was dissolved in distilled water to yield a solution of 1.00 μg/ml, and 100 μl was added to the sample before extraction. The sample was then shaken with 5 ml of a solution of ether and *n*-propyl alcohol (4:1 v/v) for 5 min. After centrifugation the aqueous phase was removed and discarded. The organic phase was evaporated under a steady stream of air in a water bath maintained at 55°. The residue was redissolved in 20–50 μl of distilled water, and a 5–10 μl aliquot was injected into the liquid chromatograph. Quantitation was performed using the method of internal standards.

Calculations—Linear regression analysis of the serum level data from each animal after logarithmic transformation of the serum concentration data was used to calculate half-lives, volumes of distribution, and total body clearances. Statistical significance was determined at $p < 0.05$ using two-sided variance analysis to determine the significance of differences between the groups studied.

[1] Roche Laboratories, Nutley, N.J.
[2] Mode 200; Waters Associates, Milford, Mass.
[3] Perkin-Elmer Corp., Norwalk, Conn.
[4] Waters Associates, Milford, Mass.
[5] PE 50; Clay Adams, Parsippany, N.J.
[6] Fisher Chemical Co., King of Prussia, Pa.

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Table II—Concentration of 5-Fluorouracil in Rat Bile Following Intravenous Administration

Minutes Postdose	Concentration in Bile, μg/ml[a]		
	12.5-mg/kg Dose[b]	25.0-mg/kg Dose[b]	50.0-mg/kg Dose[c]
15	6.1 ± 0.9	17.3 ± 2.2	28.5 ± 2.9
30	3.8 ± 0.3	8.2 ± 1.5	11.6 ± 2.1
60	2.9 ± 0.4	4.4 ± 0.7	7.3 ± 1.1

[a] Data expressed as mean ± *SE*. [b] $n = 5$. [c] $n = 6$.

RESULTS AND DISCUSSION

The separation and identification of 5-fluorouracil was suitable for monitoring plasma, bile, and parotid saliva concentrations of the drug. The retention time for 5-fluorouracil was 2.0 min; the internal standard, 5-fluorocytosine, had a retention time of 4 min. The separation was reproducible and sensitive for extracted plasma, bile, and parotid saliva. Calibration curves for spiked plasma, bile, and parotid saliva were linear when the injected sample contained 0.05–5.0 μg of 5-fluorouracil, corresponding to concentrations over a range of 0.25–25 μg/ml. Extraction recoveries were 65% for 5-fluorouracil.

Following surgery, parotid saliva and bile flow rates did not differ among experimental groups over the range of doses administered. The mean (±*SE*) plasma, bile, and parotid saliva 5-fluorouracil concentrations in rats receiving 12.5, 25.0, and 50.0 mg/kg iv are presented in Tables I–III. The plasma half-lives (±*SE*) for the 12.5-, 25.0-, and 50.0-mg/kg doses were 22 ± 2, 25 ± 5, and 27 ± 3 min, respectively. The half-life at the 50.0-mg/kg dose was significantly longer ($p < 0.025$) than the corresponding half-life at the 12.5-mg/kg dose. Total body clearances (±*SE*) were 2.4 ± 0.2, 1.6 ± 0.2, and 1.4 ± 0.1 liters/kg/hr in rats receiving 12.5, 25.0, and 50.0 mg/kg iv of 5-fluorouracil, respectively. Volumes of distribution (±*SE*) were 1.23 ± 0.11, 0.89 ± 0.10, and 0.94 ± 0.10 liters/kg. While volumes of distribution did not change significantly over the range of doses studied, the total body clearance of 5-fluorouracil decreased significantly ($p < 0.001$) in animals that received 50.0 mg/kg compared with the group which received 12.5 mg/kg. The mean (±*SE*) bile–plasma and parotid saliva–plasma concentration ratios were calculated and are presented in Tables IV and V. Compared with bile–plasma concentration ratios at 60 min for a dose of 50.0 mg/kg, bile–plasma concentration ratios were approximately two times higher with the administration of 25.0 mg/kg and four times higher with the administration of 12.5 mg/kg (Table IV). The parotid saliva–plasma concentration ratios also tended to decrease at higher doses (Table V). The free plasma 5-fluorouracil concentrations predicted using the parotid saliva–plasma concentration ratios 60 min following the intravenous administration of 5-fluorouracil were 49, 36, and 24% for the 12.5-, 25.0-, and 50.0-mg/kg doses, respectively.

When determined by equilibrium dialysis, however, the extent to which 5-fluorouracil is bound to rat plasma at 18.7 ± 2.0 and 65.7 ± 0.9 μg/ml, was <10%. The percentage of drug calculated to be free from protein binding was >90% for the aforementioned concentrations. The percent protein-bound drug did not vary significantly between 0.40 and 12-hr incubation when the drug was added to both sides of the system. Ninety-eight percent of the measured concentration of 5-fluorouracil added to the dialysis system was accounted for by drug assayed from both sides of the dialysis system after equilibrium. No 5-fluorouracil binding to the dialysis membrane was detected.

5-Fluorouracil, an antimetabolite, is an antineoplastic agent extensively used for a variety of solid neoplasms and has toxicities that include bone marrow suppression and damage to the GI tract (8). Stomatitis and esophagopharyngitis are common side effects. In the past, pharmacokinetic studies using rats have shown saturation of degradative metabolism at high doses, which is presumed to correlate with the clinical observation that increasing doses of 5-fluorouracil results in unproportional increases in toxicity (9, 10). This investigation examined the plasma concentration–time relationship and the detectability and excretion in parotid saliva and bile of intravenously administered 5-fluorouracil in rats. The excretion of 5-fluorouracil in body fluids, in addition to the plasma–concentration time course, may be useful in predicting the mechanisms of clinically encountered toxicities.

In this work 5-fluorouracil plasma concentrations dropped rapidly after intravenous administration. This is consistent with the hypothesis that only a small amount of pharmacologically active drug remains in the tissues, while most of the dose administered is rapidly eliminated from the body (11). As reported previously, the plasma half-life of 5-fluorouracil was found to increase at higher doses (9). While differences in plasma half-life were not striking, the half-life at the 50.0-mg/kg dose appeared significantly ($p < 0.05$) prolonged compared with the corresponding half-life at the 12.5-mg/kg dose.

5-Fluorouracil is metabolized primarily via the liver by an easily saturable pathway (9, 10). Although bile flow rates were unchanged, the proportion of 5-fluorouracil excreted in bile, as reflected by the bile–plasma concentration ratio at a dose of 50.0 mg/kg, was approximately one-fourth and one-half of that at the 12.5- and 25.0-mg/kg doses, respectively. A decline in the bile–plasma concentration ratio at higher doses is consistent with a saturable hepatic excretory process. The relative decrease in biliary excretion probably does not contribute greatly to a longer plasma half-life at higher doses, since 5-fluorouracil is mainly eliminated by urinary excretion (12). In general, low molecular weight compounds are not eliminated primarily *via* bile even in rats with ligated renal pedicles (13). A longer half-life at higher doses remains best explained by saturation of the degradative metabolism. The dependency of half-life on dose can not be attributed to changes in the volumes of distribution since these tended to decrease at higher doses. Total body clearances, however, of 5-fluorouracil decreased at higher doses consistent with a saturable elimination. Ongoing clinical investigations are assessing how alterations in urinary excretion of 5-fluorouracil and its metabolites can account for some of the variation observed in response to therapeutic doses (14).

Although urinary and biliary excretion have been previously studied, heretofore little or no information has been available concerning the detectability and excretion of 5-fluorouracil in saliva. This work demonstrates that this chemotherapeutic agent is excreted in detectable amounts in rat parotid saliva after intravenous administration. The concentration of 5-fluorouracil in parotid saliva is low and probably contributes negligibly to the total drug equivalents eliminated from the body. The calculated percentage of free 5-fluorouracil in plasma predicted by the parotid saliva–plasma concentration ratios consistently yielded values too low to reflect the percentage of free drug in plasma as determined by equilibrium dialysis. Parotid salivary excretions of 5-fluorouracil may be affected by back-diffusion, ion trapping, alterations in blood supply, and damage of the parenchyma of the gland. It is hypothesized that cytological changes in the salivary glands of mice following sublethal doses of 5-fluorouracil may impair the discharge of secretory materials (15). Furthermore, excretion in the saliva exposes the mucosa of the upper GI tract to 5-fluorouracil after intravenous administration. This may correlate with the clinical observation that intravenously administered 5-fluorouracil has been found to cause stomatitis (11). Structural changes similar to those found in the parotid glands of mice have been described in the pancreas (16) of Brunner's glands (17) of rats. 5-Fluorouracil may affect these metabolically active organs by incorpo-

Table III—Concentration of 5-Fluorouracil in Rat Parotid Saliva Following Intravenous Administration

Minutes Postdose	Concentration in Parotid Saliva, μg/ml[a]		
	12.5-mg/kg Dose[b]	25.0-mg/kg Dose[b]	50.0-mg/kg Dose[c]
15	3.0 ± 0.7	7.3 ± 0.3	7.6 ± 0.6
30	1.6 ± 0.1	3.9 ± 0.4	5.9 ± 0.5
60	0.7 ± 0.1	1.7 ± 0.4	2.8 ± 0.7

[a] Data expressed as mean ± *SE*. [b] $n = 5$. [c] $n = 6$.

Table IV—Bile–Plasma Concentration Ratios Following Intravenous Adminstration of 5-Fluorouracil to Rats

Minutes Postdose	Concentration Ratio[a]		
	12.5-mg/kg Dose[b]	25.0-mg/kg Dose[b]	50.0-mg/kg Dose[c]
15	1.03 ± 0.12	1.18 ± 0.18	0.84 ± 0.04
30	1.28 ± 0.24	0.95 ± 0.17	0.54 ± 0.12
60	1.80 ± 0.22	1.00 ± 0.39	0.64 ± 0.09

[a] Data expressed as mean ± *SE*. [b] $n = 5$. [c] $n = 6$.

Table V—Saliva–Plasma Concentration Ratios Following Intravenous Administration of 5-Fluorouracil to Rats

Minutes Postdose	Concentration Ratios[a]		
	12.5-mg/kg Dose[b]	25.0-mg/kg Dose[b]	50.0-mg/kg Dose[c]
15	0.50 ± 0.10	0.50 ± 0.05	0.24 ± 0.04
30	0.56 ± 0.13	0.49 ± 0.10	0.27 ± 0.04
60	0.49 ± 0.08	0.36 ± 0.05	0.24 ± 0.04

[a] Data expressed as mean ± *SE*. [b] $n = 5$. [c] $n = 6$.

rating into RNA, since it has been reported to inhibit protein synthesis in mammals by this mechanism (16).

In the past rat studies have been used to develop optimal scheduling programs and predict mechanisms of toxicity of 5-fluorouracil (9, 18). Findings of these investigations are presumed to correlate with clinical observations. In this work, concentrations of 5-fluorouracil in the excreted fluids of rats were used to supplement information derived from the plasma concentration time course. This work has demonstrated that unmetabolized 5-fluorouracil is excreted in detectable amounts in rat bile and parotid saliva. Biliary excretion appears to occur *via* a saturable process. Excretion of 5-fluorouracil in parotid saliva exposes the upper GI tract to this agent, even when administered intravenously. These routes of excretion appear to contribute negligibly to the total drug equivalents eliminated from the body.

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Liquid Membrane Phenomenon in Reserpine Action

S. B. BHISE, P. R. MARWADI, S. S. MATHUR, and R. C. SRIVASTAVA ˣ

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Abstract □ Reserpine was shown to generate a liquid membrane. Transport of adrenaline, noradrenaline, dopamine, 5-hydroxytryptamine, glutamic acid, and γ-aminobutyric acid in the presence of the reserpine liquid membrane was studied. The data indicate that the phenomenon of liquid membrane formation is likely to play a role in the mechanism of reserpine action.

Keyphrases □ Reserpine—liquid membrane phenomenon, biogenic amines, neurotransmitter amino acids, surface activity □ Liquid membrane phenomenon—reserpine action, biogenic amines, neurotransmitter amino acids, surface activity

Surface activity is exhibited by a wide variety of biologically active agents (1). The fact that surface activity may play a role in the mechanism of action of some drugs is evident from the correlations obtained (2) between surface activity and biological effects. Previous researchers (3) have concluded that in the case of psychotropic drugs, surface activity is the primary factor which determines their potency and not the specific chemical structure.

According to a previous hypothesis (4), surface-active agents, when added to water or aqueous solutions, generate liquid membranes which completely cover the interface at concentrations equal to the critical micelle concentration of the surfactant. It is, therefore, logical to expect that the liquid membranes generated by surface-active drugs may play a role in the mechanism of their action. Studies on haloperidol, a surface-active neuroleptic drug, were recently undertaken (5), and it was shown that the liquid membrane generated by haloperidol contributes significantly to the mechanism of its action.

To establish the role of liquid membrane phenomena in the mechanism of action of surface-active drugs, it is necessary to conduct studies, on structurally dissimilar drugs. Reserpine, a drug structurally different from haloperidol, is discussed in the present report. Existence of a liquid membrane generated by reserpine was demonstrated and data on the transport of biogenic amines and relevant neurotransmitter amino acids, through the liquid membrane generated by reserpine, were obtained.

EXPERIMENTAL

Materials—Reserpine[1], dopamine chlorhydrate[2], adrenaline hydrogen tartrate[2], L-noradrenaline[3], 5-hydroxytryptamine creatinine sulfate[4], L-glutamic acid[5], γ-aminobutyric acid[5], and distilled water (glass-distilled once from potassium permanganate) were used.

Methods—The critical micelle concentration (CMC) of aqueous reserpine was determined from the variation of surface tension with concentration. The surface tensions were measured using a tensiometer[6]. To prepare aqueous solutions of reserpine, the necessary volume of an ethanolic solution of known concentration of the drug was added to the aqueous phase with constant stirring. Since the aqueous solution of reserpine always contained some alcohol, in no case >1%, the blanks used also contained the same amount of alcohol in water. The CMC value of aqueous reserpine was found to be 1.6×10^{-6} *M*.

The all-glass cell described earlier (5, 6) was used for transport studies. A cellulose nitrate millipore filter[7], which acted as a support for the liquid

[1] BP-USP Roussel UCLAF, Paris.
[2] Loba Chemie.
[3] Fluka A.G.
[4] Koch-Light Laboratories Ltd.
[5] BDH.
[6] Fisher Surface Tensiomat Model 21.
[7] Sortorious Cat. No. 11307 of thickness 1×10^{-4} m and area 5.373×10^{-5} m^2.

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