Pharmacokinetics of Oral and Intravenous Fluorouracil in Humans

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Abstract □ The pharmacokinetics of fluorouracil were examined after single 250-mg iv doses and 500-mg oral doses to female patients with breast cancer. In five patients who received intravenous fluorouracil, the mean peak plasma level of unchanged drug was 13.4 μg/ml, the elimination half-life was 6.3 min, and the plasma clearance was 1410 ml/min. The last value is similar to the hepatic blood flow. In six patients who received oral fluorouracil, the mean peak value of unchanged drug in plasma, which occurred within 20 min of dosing, was 8.3 μg/ml, and the fluorouracil elimination half-life was 7.2 min. The overall bioavailability of oral fluorouracil as unchanged drug was 28%, and the variation in plasma drug levels between individuals was similar following oral and intravenous doses. The data provide additional evidence of saturable hepatic metabolism of fluorouracil during the first pass.

Keyphrases □ Fluorouracil—comparison of oral and intravenous doses pharmacokinetics, human plasma □ Pharmacokinetics—fluorouracil levels in human plasma following intravenous and oral doses □ Antineoplastic activity—fluorouracil, pharmacokinetics, human plasma

The pharmacokinetics of fluorouracil following intravenous administration to humans have been characterized (1–4). The drug distributes into an apparent body volume equivalent to 25% of the body weight and is cleared rapidly from the circulation, principally due to metabolism, with a half-life of 5–15 min. The high plasma clearance of fluorouracil suggests that considerable first-pass metabolism occurs following oral administration (5).

Reports on the availability of orally dosed fluorouracil to the general circulation are conflicting. Cohen *et al.* (1) reported 50 and 80% absorption in two patients, while other investigators reported much lower absorption (6). A recent study suggested that the availability of fluorouracil increases with increasing dose (2), which may be due to saturation of the initial degradative metabolic step in the liver (7).

The circulating levels of fluorouracil in patients receiving relatively low single doses of fluorouracil by the intravenous and oral routes were examined. The description of these data and comparisons with previous reports are the subjects of this paper.

EXPERIMENTAL

Subjects and Protocols—The subjects were 11 female patients undergoing therapy for breast carcinoma, postmastectomy, as outpatients at the Selly Oak Hospital, Birmingham, England. Characteristics of the subjects and diagnostic information are given in Table I. The subjects were ambulatory and were taking solid food. They were being treated under the conditions of a multicenter trial to examine the benefits of cyclic adjuvant chemotherapy in both node-positive and node-negative operable breast cancer. Node-positive patients received fluorouracil by intravenous injection; node-negative patients were dosed orally.

The medication received by the two groups of subjects is summarized in Table II. Since each subject received either oral or intravenous fluorouracil, it was not possible to examine fluorouracil kinetics after oral and intravenous doses in the same person.

All fluorouracil doses were administered at the hospital in the presence of the attending physician. Intravenous doses of 250 mg of fluorouracil were administered in 5 ml of water for injection into a forearm vein over 30 sec. Oral doses of 500 mg of fluorouracil were administered in 10 ml of water for injection followed immediately by 90 ml of orange juice. No dietary restrictions were applied. Since subjects had to travel from their homes to the hospital to receive the fluorouracil doses, an interval of at least 1 hr elapsed between eating and dosing.

Blood samples (10 ml) were taken from a forearm vein in the arm, not used for drug administration in the intravenous study, and were placed in heparinized tubes. Plasma was promptly separated and stored at −20° until it was assayed.

Assay—Fluorouracil was extracted from plasma by the method described previously (3). The high-pressure liquid chromatographic (HPLC) system consisted of a 25-cm × 4-mm (10-μm particle size) column[1] preceded by a 5-cm × 4-mm (10-μm particle size) guard column[2], a constant-volume pump[3], and a variable-wavelength detector[4] set at 265 nm. The solvent was 10^{-2} *M* phosphate buffer (pH 5.5) at a flow rate of 2 ml/min.

Uridine was used as the internal standard since it had a shorter retention time in this system than thymidine (3). Dried residues from extracted plasma were redissolved in 200 μl of 5% methanol in water, and 50 μl of this solution was injected into the chromatograph. Fluorouracil was measured by the peak height ratio method against uridine. Using this procedure, the retention volumes for fluorouracil and uridine were 11 and 21 ml, respectively, and the time required to analyze each sample was reduced from 30 (3) to 11 min. The method had similar sensitivity and reproducibility for fluorouracil to those reported previously (3). The lower limit of detection was 0.1 μg/ml, and neither commonly occurring nucleosides nor drugs other than fluorouracil being taken by the subjects interfered with the chromatographic peaks of fluorouracil or uridine.

All pharmacokinetic parameters were obtained by standard graphical procedures (9). All rate constants were calculated by linear regression of the natural logarithm of the fluorouracil concentration against time. In the intravenous studies, the value of C_0 was corrected for the 30-sec fluorouracil injection time. In the oral studies, it was not possible to obtain a value for the absorption rate constant for Patient MD because of the absence of data during the absorption phase of the plasma fluorouracil profile.

Fluorouracil standard[5] and uridine[6] were used as received. All other chemicals and reagents were of the highest grade available and were used as received.

RESULTS

Individual plasma levels of fluorouracil obtained from the intravenous and oral doses are given in Table III, and the mean values from the two dosage forms are summarized in Fig. 1. The results of the pharmacokinetic analysis are described in Table IV.

Following intravenous dosing, plasma fluorouracil levels at the 5-min sampling varied from 6.4 to 29.5 μg/ml with a mean value of 13.4 μg/ml. Drug levels declined monoexponentially between 5 and 45 min and had returned to baseline values by 60 min. The mean half-life of fluorouracil in plasma was 6.3 min, the mean distribution volume was 0.2 liter/kg, and the plasma clearance was 1410 ml/min.

Following oral dosing, peak plasma levels of fluorouracil ranged from 4.4 to 14.3 μg/ml with a mean value of 8.3 μg/ml and occurred 10–20 min postdosing. The mean half-life of drug elimination was 7.2 min, statis-

[1] Whatman Partisil PXS ODS-2 column.
[2] Waters RP-8 Lichrosorb.
[3] Waters model M6000.
[4] Pye Unicam model LC3.
[5] Lot 578103, Hoffmann-La Roche Laboratories, Nutley, N.J.
[6] Analytical reagent, Sigma Chemical Co., St. Louis, Mo.

0022-3549/80/1200-1428$01.00/0

Table I—Subject Statistics and Clinical and Diagnostic Data

Subject	Age, years	Weight, kg	Height, cm	White Blood Cells, $\times 10^3/mm^3$	Red Blood Cells, $\times 10^6/mm^3$	Platelets, $\times 10^3/mm^3$	Hemoglobin, g %	Albumin, g %	Serum Glutamic Oxalacetic Transaminase, units/liter	Alkaline Phosphatase, K.A. units[a]
					Intravenous					
MG	44	74	168	3.7	4.4	254	12.9	4.0	29	9
MF	44	77	178	4.2	4.7	187	13.6	4.1	17	4
FT	53	70	166	5.7	4.7	152	14.7	4.8	20	7
ID	52	66	154	5.6	4.0	382	12.4	4.7	21	6
ME	61	56	168	5.1	4.9	220	13.6	—[b]	—	—
					Oral					
EG	52	80	163	5.8	3.9	282	13.1	4.6	19	14
AM	55	90	157	6.2	4.4	174	13.7	4.1	10	9
DT	57	55	160	5.7	4.5	167	13.6	4.2	17	7
ET	62	57	155	6.1	3.8	227	12.2	4.4	15	6
RO	50	62	164	6.5	4.1	187	12.4	3.9	13	3
MD	59	66	158	4.9	4.6	254	13.5	4.4	13	4

[a] King–Armstrong units (8). [b] Not determined.

Table II—Medication Received on the Day Preceding and on the Day of Fluorouracil Administration

Dosage Group	Medication	Time of Medication, hr
Intravenous, 250 mg, five patients	Day preceding study	
	20 mg of prochlorperazine maleate po	1030
	20 mg of prochlorperazine maleate po	1330
	50 mg of doxorubicin iv	1430
	1 mg of vincristine iv	1430
	250 mg of cyclophosphamide iv	2000
	150 mg of methotrexate iv[a]	2000
	20 mg of prochlorperazine maleate po	2015
	Day of study	
	20 mg of prochlorperazine maleate po	0845
	250 mg of fluorouracil iv	0900
	15 mg of leucovorin iv	0930
	15 mg of leucovorin po	1700
	15 mg of leucovorin po	2300
Oral, 500 mg, six patients	Day of study	
	500 mg of fluorouracil po with 10 mg of chlorambucil po and 25 mg of methotrexate po[b,c]	0900

[a] Methotrexate administered as a 12-hr infusion in 1 liter of normal saline, starting at 2000 hr. [b] Chlorambucil and methotrexate were taken by the subjects at their home after blood sampling procedures were completed. [c] On the following day, subjects received additional oral doses of 500 mg of fluorouracil and 10 mg of chlorambucil.

Table III—Plasma Levels of Fluorouracil following Intravenous and Oral Doses

Subject	Dose, mg/kg	Plasma Fluorouracil, μg/ml									
		0 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	90 min	120 min
						Intravenous					
MG	3.4	0.0	6.4	8.3	—[a]	1.9	0.3	0.2	ND[b]	—	—
MF	3.3	0.0	13.8	3.4	—	2.6	1.2	0.3	ND	—	—
FT	3.6	0.0	29.5	17.8	—	5.7	0.5	0.1	ND	—	—
ID	3.8	0.0	8.1	3.2	1.9	0.9	0.4	0.1	ND	—	—
ME	4.5	0.0	9.2	4.5	3.1	2.3	0.8	0.2	0.1	—	—
Mean	3.7	0.0	13.4	7.4	2.5	2.7	0.6	0.2	0.0	—	—
SD	0.5	0.0	9.4	6.1	—	1.8	0.4	0.1		—	—
						Oral					
EG	6.3	0.0	—	1.2	—	5.0	2.7	0.6	0.1	—	—
AM	5.6	0.0	—	3.1	4.4	2.9	0.6	0.2	0.1	—	—
DT	9.1	0.0	—	2.8	—	8.9	2.1	0.3	0.2	ND	ND
ET	8.8	0.0	—	8.7	10.7	12.2	5.5	0.6	0.1	ND	ND
RO	8.1	0.0	—	3.4	5.0	4.6	1.8	0.4	0.2	0.1	ND
MD	7.6	0.0	—	14.3	11.2	10.9	6.4	1.6	0.9	0.1	0.1
Mean	7.6	0.0	—	5.6	7.8	7.4	3.2	0.6	0.3	0.1	0.0
SD	1.4	0.0	—	4.9	3.6	3.8	2.3	0.5	0.3	0.1	—

[a] Not determined. [b] Not detectable.

tically indistinguishable from the equivalent value after intravenous dosing, while the absorption half-time was 3.4 min. This value was obtained by conventional curve-stripping procedures assuming first-order absorption and elimination (9) and indicates fast absorption of fluorouracil under the conditions employed in this study. The absorption phase was essentially complete within 20 min of dosing.

The ratio of the mean areas under the plasma fluorouracil curves from the oral and intravenous doses, corrected for the individual elimination rate constants (Table IV), provides a measure of the efficiency with which fluorouracil is absorbed from the oral dose as unchanged drug (10). The

Table IV—Pharmacokinetic Values Obtained from Analysis of Plasma Fluorouracil Data

Subject	Pharmacokinetic Parameters[a] k_a, min^{-1}	$t_{1/2a}$, min	k_{el}, min^{-1}	$t_{1/2el}$, min	C_0, μg/ml	C_{max}, μg/ml	t_{max}, min	AUC, (μg min)/ml	$AUCk_{el}$, μg/ml	V, liter/kg	Vk_{el}, ml/min
					Intravenous						
MG	—[b]	—	0.11	6.3	19.7	—	—	173	19	0.17	1384
MF	—	—	0.10	6.9	17.1	—	—	170	17	0.19	1463
FT	—	—	0.13	5.3	68.7	—	—	545	71	0.051	464
ID	—	—	0.13	5.3	16.0	—	—	122	16	0.23	1973
ME	—	—	0.09	7.7	12.7	—	—	141	13	0.35	1764
Mean	—	—	0.11	6.3	26.8	—	—	230	27	0.20	1410
SD	—	—	0.02	1.0	23.5	—	—	177	25	0.11	579
					Oral						
EG	0.20	3.5	0.09	7.7	—	5.0	20	121	11	—	—
AM	0.23	3.0	0.09	7.7	—	4.4	15	80	7	—	—
DT	0.19	3.6	0.07	9.9	—	8.9	20	152	11	—	—
ET	0.20	3.5	0.12	5.8	—	12.2	20	290	35	—	—
RO	0.22	3.2	0.09	7.7	—	5.0	15	121	11	—	—
MD	—	—	0.06	11.6	—	14.3	10	287	17	—	—
Mean	0.21	3.4	0.09	8.4	—	8.3	17	175	15	—	—
SD	0.02	0.3	0.02	2.0	—	4.2	4	91	10	—	—

[a] The notations are: k_a, first-order rate constant for drug absorption; $t_{1/2a}$, half-time for absorption; k_{el}, first-order rate constant for drug elimination; $t_{1/2el}$, half-life of elimination; C_0, concentration at time zero obtained by extrapolation of plasma fluorouracil levels after intravenous dosing; C_{max}, maximum concentration of fluorouracil in plasma after oral dosing; t_{max}, time at which C_{max} occurs; AUC, area under fluorouracil concentration *versus* time curve in plasma calculated by the trapezoidal rule; $AUCk_{el}$, area corrected for variance in k_{el}; V, apparent distribution volume of fluorouracil in the body obtained from V = dose/C_0; and Vk_{el}, plasma clearance of fluorouracil. [b] Not determined.

mean corrected area from the oral dose of 500 mg is 15 μg/ml, while that from the intravenous dose is 27 μg/ml. Correction for dose size yields a ratio of 0.28, indicating an average fluorouracil availability of 28% from the oral dose.

DISCUSSION

The plasma levels of fluorouracil obtained from the intravenous dose in this study are comparable to those obtained previously (1–3). The mean distribution volume for fluorouracil of 0.2 liter/kg and the plasma clearance of 1410 ml/min are consistent with previously reported values of 0.35 liter/kg and 1441 ml/min obtained in three patients (2) and of 0.25 liter/kg and 1265 ml/min obtained in eight patients (3).

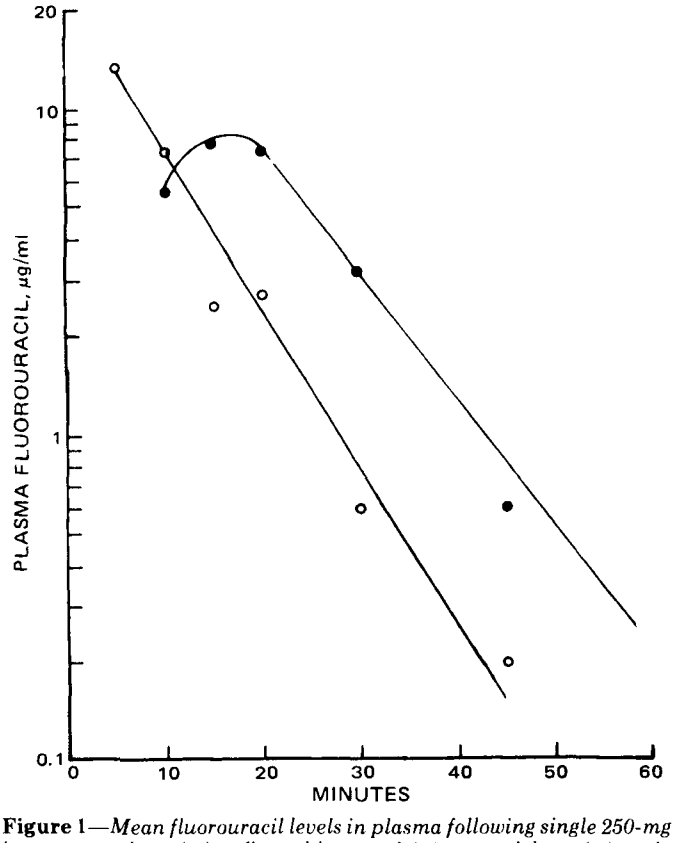

Figure 1—*Mean fluorouracil levels in plasma following single 250-mg intravenous doses (○) to five subjects and 500-mg oral doses (●) to six subjects.*

Although the overnight methotrexate infusion in these patients was terminated 1 hr before the fluorouracil dose, circulating levels of methotrexate still would be present at the time of fluorouracil dosing and could have affected the circulating levels of fluorouracil. However, due to the relatively low dose of methotrexate and the low binding of both compounds to plasma proteins (2, 11), direct interaction between these drugs in plasma, which might influence fluorouracil distribution, is unlikely.

Previous reports suggested that absorption of fluorouracil from oral dosage forms is extremely variable (6, 7). Clarkson *et al.* (12) reported a two- to 10-fold reduction in the initial plasma fluorouracil levels in patients after oral doses compared to equivalent intravenous doses. Finn and Sadée (13) also obtained variable plasma levels of fluorouracil after oral administration to three patients with colon cancer, with peak drug levels varying between 0.8 and 60 μg/ml. However, in two patients, the circulating levels of fluorouracil were prolonged relative to those after intravenous dosing. Other studies demonstrated efficient absorption of oral fluorouracil (1, 14), which has been associated with high and prolonged drug levels in plasma and bile. High fluorouracil levels in bile after oral doses may be particularly advantageous in treating hepatic metastases and tumors of the biliary tree (14).

Cohen *et al.* (1) suggested that the nature of fluorouracil absorption may depend on the vehicle with which the drug is administered but also may be influenced by the degree of hepatic involvement. Their data suggested that patients with hepatic tumor or metastases achieve lower but more prolonged circulating levels of fluorouracil than patients with no hepatic involvement. This theory was supported partially by Finn and Sadée (13), who obtained prolonged plasma levels of fluorouracil in patients with hepatic involvement, and by Garrett *et al.* (2), who obtained a similar disappearance rate for fluorouracil from plasma after oral and intravenous doses to patients with relatively normal liver function. In the latter study, fluorouracil absorption as unchanged drug was more efficient from a 1000-mg oral dose than from a 500-mg dose in two individuals, suggesting saturable metabolism of fluorouracil during the first pass (5).

The results obtained in the present study are consistent with the hypotheses (1, 2). Absorption of fluorouracil from the oral doses to subjects who had no discernible hepatic involvement was efficient and rapid. Although the clinical protocol prevented intravenous and oral doses being given to the same subjects, thus preventing direct comparisons within an individual, the plasma data nevertheless indicate that the oral dose was ~28% available as unchanged drug. This value is intermediate between the values of 1–15% reported by Garrett *et al.* (2) and of 50–80% reported by Cohen *et al.* (1) and provides further evidence that hepatic clearance of fluorouracil, which is similar to hepatic blood flow after parenteral doses (10), is highly saturable during the first pass.

Furthermore, the data obtained in this study indicate that circulating levels of oral fluorouracil may be as reproducible between individuals as

those obtained from intravenous doses. The apparent inconsistency between this observation and some earlier reports may be explained by the relatively homogeneous patient population used in this study and the heterogeneous (at least regarding the degree of hepatic involvement) patient group (1) or the very small numbers of subjects (2, 13) used previously. Considerable variability in circulating levels of fluorouracil also was reported following intravenous doses to male and female patients (4).

While it is recognized that the clinical effectiveness of fluorouracil is a complex function of the anabolic metabolism of fluorouracil and the biochemistry of cell death and cell resistance, the present results indicate that oral fluorouracil in this patient population is absorbed with reproducible efficiency, resulting in no greater variation in circulating drug levels than that following intravenous doses.

This observation may have important implications in fluorouracil treatment of patients with breast cancer, particularly in view of the relationship that exists between the patient response rate and circulating levels of fluorouracil (15).

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ACKNOWLEDGMENTS

The authors thank Mr. Michael Morrison for permission to carry out this study in his ward at Selly Oak Hospital, the staff nurses at the Selly Oak Hospital for their help in blood sampling procedures, and Ms. Barbara Munday, Medical School, University of Birmingham, England, for setting up the chromatography procedures.

NOTES

Structural Elucidation of Adducts Formed by Ninhydrin with Indoles and Thiourea by ^{13}C-NMR Spectroscopy

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Abstract □ Condensation products were prepared by the reaction of ninhydrin with indole and 2,5-dimethylindole. The structures of these 1:1 adducts were assigned as 3-(2-hydroxy-2-indane-1,3-dionyl)indole and 3-(2-hydroxy-2-indane-1,3-dionyl)-2,5-dimethylindole, respectively, on the basis of spectral data including ^{13}C-NMR evidence. ^{13}C-NMR also was used to confirm the structure of a thiourea–ninhydrin adduct as a substituted thioindeno[1,2-*d*]imidazole-2,8-dione.

Keyphrases □ Ninhydrin—condensation products with indoles and thiourea, structural elucidation by NMR spectroscopy □ Heterocyclic adducts—cyclic ureides, condensation products of ninhydrin with indoles and thiourea, structural elucidation by NMR spectroscopy □ NMR spectroscopy—structural elucidation of condensation products of ninhydrin with indoles and thiourea

Interest in the heterocyclic adducts formed between ninhydrin (triketohydrindene hydrate, I) and aromatic amines (1, 2) and enamines (3) led to the investigation of the reaction products of this reagent with certain aromatic compounds whose structure might contain an enamine moiety. The structures of indole and 1-naphthylamine could be visualized as such; the N-1, C-2, and C-3 positions of indole and the NH_2, C-1, and C-2 positions of 1-naphthylamine possess the enamine triad (4). It was found that 1-naphthylamine forms a stable pentacyclic adduct with I, whose mode of cyclization has not been definitely established (5).

BACKGROUND

The ready formation of a 1:1 adduct between I and indole was first reported by Tomita and Fukagawa (6). However, the structure assigned by these investigators involved attack of the C-2 position of indole at the masked, central carbonyl group of ninhydrin to give II. This is in contrast to the expected reactivity of the C-3 position in indole (7, 8).

The reaction between I and indole was repeated by Roth and Kok (9); a stable, yellow adduct (1:1) was obtained with the same melting point as reported previously (6). These investigators (9) reported this adduct

0022-3549/80/1200-1431$01.00/0