High-Pressure Liquid Chromatographic Determination of Ftorafur [1-(Tetrahydro-2-furanyl)-5-fluorouracil] and GLC–Mass Spectrometric Determination of 5-Fluorouracil and Uracil in Biological Materials after Oral Administration of Uracil plus Ftorafur

TERUYOSHI MARUNAKA *x, YUKIHIKO UMENO *, KOZO YOSHIDA *, MASAHIRO NAGAMACHI *, YOSHINORI MINAMI *, and SETSURO FUJII ‡

Received November 14, 1979, from the **Research Laboratory, Taiho Pharmaceutical Co., Ltd., Tokushima, 771-01, Japan,* and the ‡*Division of Regulation of Macromolecular Function, Institute for Protein Research, Osaka University, Osaka, Japan.* Accepted for publication May 19, 1980.

Abstract □ Oral adminstration of uracil plus ftorafur [1-(tetrahydro-2-furanyl)-5-fluorouracil] has a greater antitumor effect than that of ftorafur alone. A high-pressure liquid chromatographic method was developed for the determination of ftorafur in plasma and visceral tissues with a sensitivity of 0.025 μg/ml or g of wet weight. A combination of GLC–mass fragmentography and GLC–mass spectrometry with total-ion monitoring was developed for the specific, simultaneous determination of 5-fluorouracil, an active metabolite, and uracil as their trimethylsilylated derivatives. The detection limits for 5-fluorouracil and uracil in the first method were 0.001 μg/ml for plasma and 0.001–0.005 μg/ml for visceral tissues, and those in the second method were 0.2 μg/ml or g of wet weight. The precision and sensitivity of the assay appear to be satisfactory for determination of the levels of these compounds in plasma and visceral tissues.

Keyphrases □ Ftorafur—high-pressure liquid chromatographic analysis in biological materials □ 5-Fluorouracil—GLC–mass spectrometric analysis in biological materials □ Uracil—GLC–mass spectrometric analysis in biological materials □ High-pressure liquid chromatography—analysis, ftorafur in biological materials □ GLC–mass spectrometry—analysis, 5-fluorouracil and uracil in biological materials □ Antineoplastic agents—uracil plus ftorafur, high-pressure liquid chromatographic and GLC–mass spectrometric analysis in biological materials

5-Fluorouracil and its derivatives have been widely used in cancer chemotherapy. Ftorafur [1-(tetrahydro-2-furanyl)-5-fluorouracil], a masked form of 5-fluorouracil, is an effective antitumor agent. It can be given orally and has lower toxicity than 5-fluorouracil (1–3).

Fujii and coworkers (4–8) recently reported that oral administration of uracil plus ftorafur (4:1 mole/mole) was more effective than that of ftorafur or 5-fluorouracil alone and resulted in a higher level of 5-fluorouracil, an active substance, in tumor tissues than did administration of an equimolar amount of ftorafur or 5-fluorouracil.

For elucidation of the mechanism of action of uracil, it seemed important to establish a quantitative method for the determination of the concentrations of uracil, ftorafur, and 5-fluorouracil in the blood and visceral tissues after uracil plus ftorafur treatment. A method already was reported for the determination of ftorafur and 5-fluorouracil in plasma and visceral tissues after administration of ftorafur in studies on a new antitumor agent, 1,3-bis(tetrahydro-2-furanyl)-5-fluorouracil (9–11); ftorafur was determined by TLC scanning densitometry or high-pressure liquid chromatography (HPLC), and 5-fluorouracil was determined by a microbiological assay using a sensitive bacterial strain or GLC–mass fragmentography with selected-ion monitoring.

Several other methods also have been reported: separate microbiological assays of ftorafur and 5-fluorouracil in plasma and visceral tissues (12, 13), simultaneous HPLC analysis of ftorafur and 5-fluorouracil in plasma and urine using a reversed-phase column (14, 15), and assay of ftorafur by GLC and of 5-fluorouracil by GLC–mass fragmentography in plasma and urine (16, 17).

The present paper describes a precise and sensitive method for the assay of ftorafur, 5-fluorouracil, and uracil in plasma and visceral tissues. Ftorafur is separated from the other compounds by solvent extraction and analyzed by HPLC using an adsorption chromatographic system. 5-Fluorouracil and uracil are silylated and then analyzed by GLC–mass fragmentography. Uracil also is determined simultaneously by GLC–mass spectrometry with total-ion monitoring.

EXPERIMENTAL

Materials—Ftorafur was synthesized and purified (18). 5-Fluorouracil[1], uracil[1], [1,3-$^{15}N_2$]-5-fluorouracil[2] (95% enrichment), and [1,3-$^{15}N_2$]uracil[3] (95% enrichment) were used as received. *N,O*-Bis(trimethylsilyl)trifluoroacetamide[4] and pyridine[4] were derivatizing agents for silylation. Chloroform, methanol, ethanol, and ethyl acetate were liquid chromatographic reagent grade[5]. The other chemicals[5] were analytical reagent grade. Ethylene dichloride[5] was dehydrated and distilled before use.

The samples of blood and visceral tissues were from male AH-130 tumor-bearing rats (160–180 g, 6–7 weeks old).

HPLC Conditions—The liquid chromatograph[6] was equipped with a high-pressure injection valve[7] and a UV detector[8] operating at 254 nm. An adsorption chromatographic column[9] (25 cm × 6.2 mm i.d.) was used for separation with a mobile phase of ethylene dichloride–ethanol (24:1 v/v) at a flow rate of 1.7 ml/min. The column was maintained at room temperature. A gradientor[10] was used for controlling the mobile phase concentration. For quantitative calculations, a computer system[11] was employed.

GLC–Mass Fragmentographic and GLC–Mass Spectrometric Conditions—A mass spectrometer[12] with an electron-impact ion source connected to a gas chromatograph[13] was used.

The coiled glass column (1.0 m × 2.0 mm i.d.) of the gas chromatograph was packed with 3% OV-17 on Chromosorb W AW[14] (80–100 mesh) and

[1] Sigma Chemical Co., St. Louis, Mo.
[2] PCR Inc., Gainesville, Fla.
[3] Merck Sharp and Dohme Canada Ltd., Quebec, Canada.
[4] Pierce Chemical Co., Rockford, Ill.
[5] Wako Pure Chemical Co., Osaka, Japan.
[6] Model LC-2, Shimadzu, Kyoto, Japan.
[7] Model SIL-1A, Shimadzu, Kyoto, Japan.
[8] Model UVD-2, Shimadzu, Kyoto, Japan.
[9] Zorbax SIL (Du Pont), purchased from Shimadzu, Kyoto, Japan.
[10] Model GRE-2, Shimadzu, Kyoto, Japan.
[11] Model 1A Chromatopac, Shimadzu, Kyoto, Japan.
[12] Model JMS-D 300, JEOL, Tokyo, Japan.
[13] Model JGC-20KP, JEOL, Tokyo, Japan.
[14] Gaschro Kogyo Co., Tokyo, Japan.

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was conditioned at 280° for 24 hr. The injector, column, and ion source temperatures were 210, 150, and 230°, respectively. After 5-fluorouracil, uracil, and their internal standard had been detected, the temperature of the column was raised to 280° for 3 min to burn out the remaining materials and then was returned to the operational temperature before the next analysis. The carrier gas was helium, and the flow rate was 30 ml/min.

For mass fragmentography, the mass spectrometer was set at the following conditions: ionization energy, 70 ev; ionization current, 300 μamp; accelerating voltage, 3.0 kv; and ion multiplier voltage, 1.4 kv. The peaks of the molecular ions of the trimethylsilyl derivatives of 5-fluorouracil, uracil, and the respective internal standards ([1,3-$^{15}N_2$]-5-fluorouracil and [1,3-$^{15}N_2$]uracil), *m/e* 274, 256, 276, and 258, respectively, were selected for mass fragmentographic analysis. When necessary, uracil was simultaneously analyzed by GLC–mass spectrometry.

Analytical Procedure—Blood samples were collected in heparinized containers and centrifuged to separate the plasma. Visceral tissues were collected by the freeze-clamping technique and rapidly bled or perfused with cold physiological saline to remove blood. The plasma and visceral tissues then were frozen until analysis.

The plasma (0.5 ml) was diluted to 2.0 ml with distilled water containing 0.1 μg of [1,3-$^{15}N_2$]-5-fluorouracil and 0.5 μg of [1,3-$^{15}N_2$]uracil as internal standards. A sample of 0.5–1.0 g of visceral tissue was homogenized in an ice bath with physiological saline containing 0.1 μg of [1,3-$^{15}N_2$]-5-fluorouracil and 5.0 μg of [1,3-$^{15}N_2$]uracil and then centrifuged at 2000×*g* for 20 min; 1.0 ml of the resulting supernate was diluted to 2.0 ml with distilled water. The samples of plasma and visceral tissue homogenates were adjusted to pH 4.0 with 5 *N* HCl and shaken vigorously with 20 ml of chloroform at room temperature for 10 min.

This extraction was repeated again, and the combined chloroform extracts containing ftorafur were evaporated to dryness at <25°, transferred to a 10-ml test tube by washing with chloroform, and dried under nitrogen. The residue was dissolved in 100 μl of ethylene dichloride, and 20 μl of this solution was injected into the liquid chromatograph.

The aqueous layer containing 5-fluorouracil and uracil separated from the chloroform layer was neutralized with sodium hydroxide solution, adjusted to pH 6.0 with 0.2 ml of 0.5 *M* NaH_2PO_4, and centrifuged at 2000×*g* for 15 min to remove protein. The supernate was extracted with 40 ml of ethyl acetate by vigorous shaking for 20 min. The ethyl acetate layer was evaporated at 40°, transferred to a 1.0-ml reaction vial by washing with methanol, and dried under nitrogen at 40°. The residue was dried thoroughly over phosphorus pentoxide under reduced pressure and subjected to silylation at 70° for 20 min by addition of a freshly prepared solution of 100 μl of pyridine containing 20% *N,O*-bis(trimethylsilyl)-trifluoroacetamide. Then 1–3 μl of the solution was injected into the gas chromatograph–mass spectrometer.

Calibration Curves—Calibration curves were prepared by adding known amounts of ftorafur (20.0, 10.0, 5.0, 1.0, 0.5, 0.1, and 0.05 μg/ml), 5-fluorouracil (1.0, 0.5, 0.25, 0.10, 0.05, 0.01, and 0.005 μg/ml), and uracil (50.0, 25.0, 10.0, 5.0, 2.5, 1.0, 0.5, 0.25, 0.10, 0.05, 0.01, and 0.005 μg/ml) to plasma (1.0 ml) and organ homogenates (1.0 ml from 1.0 g of wet weight) and then analyzing by the same extraction procedure.

The calibration curve for the determination of ftorafur by HPLC was prepared by plotting the peak area against the concentration of ftorafur. Calibration curves for 5-fluorouracil and uracil analyzed by GLC–mass fragmentography and GLC–mass spectrometry were obtained by plotting the ratio of the peak heights of the respective trimethylsilyl derivatives to those of the trimethylsilyl derivatives of the internal standards, [1,3-$^{15}N_2$]-5-fluorouracil and [1,3-$^{15}N_2$]uracil, using GLC–mass fragmentography against the concentrations of these compounds. All of the calibration curves gave good results.

RESULTS AND DISCUSSION

The investigation of the simultaneous determination of ftorafur, 5-fluorouracil, and uracil by HPLC using a reversed-phase column and by GLC based on silylation or on-column methylation with a flame-ionization detector showed that a suitable HPLC retention time of each compound could not be obtained and that ftorafur and 5-fluorouracil were difficult to separate by GLC after silylation, while 5-fluorouracil and uracil were not separated by on-column methylation. Thus, reversed-phase HPLC and GLC were not suitable for the simultaneous assay of ftorafur, 5-fluorouracil, and uracil.

Two independent assay procedures were investigated next on the basis of the physicochemical properties of these compounds. Ftorafur could be recovered from aqueous solution with an organic solvent, and 5-fluorouracil and uracil remained in the aqueous layer. Therefore, extraction

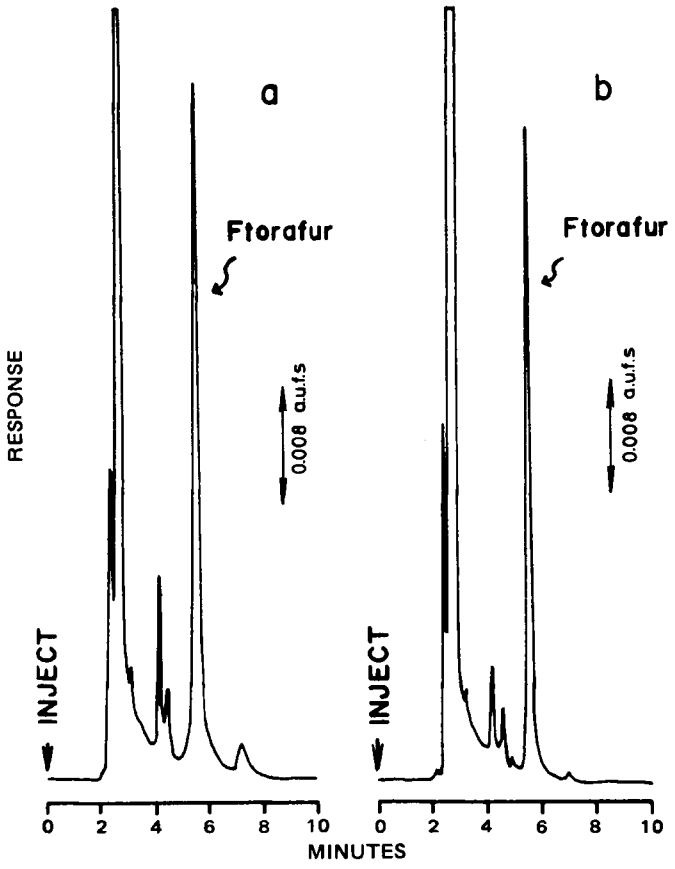

Figure 1—*High-pressure liquid chromatograms showing the separation of ftorafur extracted from plasma* (a) *and tumor tissue* (b) *of AH-130 tumor-bearing rats after addition at 5.0 μg/ml or g of wet weight. The conditions were: column, Zorbax SIL (25 cm × 6.2 mm i.d.); mobile phase, ethylene dichloride–ethanol (24:1 v/v); and flow rate, 1.7 ml/min (room temperature).*

with chloroform from an aqueous solution acidified with hydrochloric acid was employed for quantitative separation of ftorafur from 5-fluorouracil and uracil. Ftorafur then was determined by HPLC using an adsorption column, and 5-fluorouracil and uracil were analyzed by GLC–mass fragmentography and GLC–mass spectrometry.

A Zorbax SIL column was used for the separation of ftorafur extracted from chloroform. Ftorafur was well separated from biological constituents when a mixture of a nonpolar solvent (*e.g.*, chloroform, methylene chloride, ethylene dichloride, or *n*-hexane) containing 1–5% alcohol (*e.g.*,

Table I—Recoveries of Ftorafur, 5-Fluorouracil, and Uracil from Plasma and Visceral Tissues of AH-130 Tumor-Bearing Rats

Tissue	Recovery[a], %		
	Ftorafur	5-Fluorouracil	Uracil
Saline	100.7 ± 1.5	91.4 ± 3.2	84.1 ± 3.1
Plasma	100.3 ± 1.2	86.1 ± 3.7	81.5 ± 3.8
Heart	99.9 ± 1.4	72.0 ± 4.1	66.8 ± 4.3
Intestine	100.1 ± 1.2	76.8 ± 3.5	69.3 ± 3.2
Kidney	99.8 ± 1.6	67.5 ± 4.8	65.0 ± 3.7
Liver	99.6 ± 1.8	63.5 ± 5.1	62.4 ± 4.1
Lung	100.5 ± 1.3	71.0 ± 3.5	64.7 ± 4.9
Muscle	101.1 ± 1.3	79.0 ± 3.4	73.3 ± 3.3
Spleen	100.0 ± 1.8	71.0 ± 4.1	64.9 ± 4.2
Stomach	100.1 ± 1.4	77.0 ± 3.3	69.8 ± 3.2
Testis	99.9 ± 1.1	72.0 ± 3.5	63.2 ± 4.4
Thymus	99.7 ± 1.3	64.5 ± 4.3	61.4 ± 4.6
Tumor	100.4 ± 1.5	81.0 ± 3.3	76.3 ± 3.3

[a] Each value is the mean of five determinations for the particular amount added. Amounts added were: ftorafur, 20.0, 5.0, 0.5, 0.1, and 0.05 μg/ml or g of wet weight; 5-fluorouracil, 0.5, 0.1, 0.05, 0.01, and 0.005 μg/ml or g of wet weight; and uracil, 50.0, 25.0, 10.0, and 2.5 μg/g of wet weight and 0.5, 0.1, 0.05, 0.01, and 0.005 μg/ml for saline, plasma, and visceral tissues.

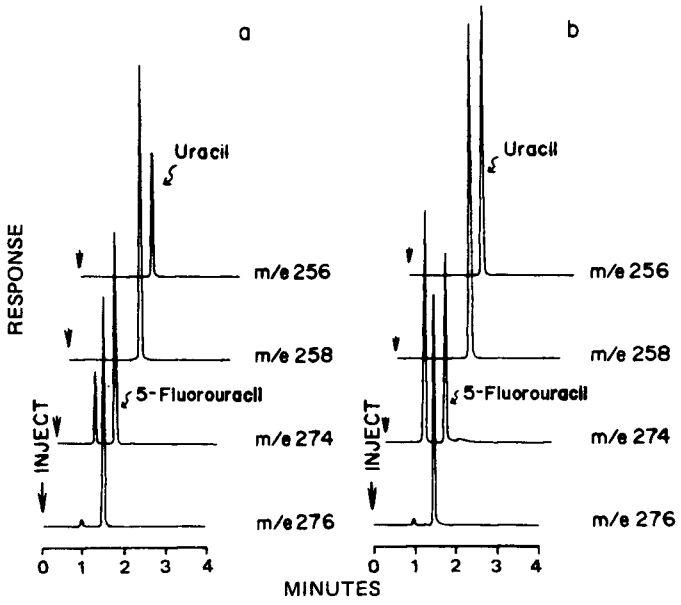

Figure 2—*Mass fragmentograms showing the separation of 5-fluorouracil, uracil, and the respective internal standards extracted from plasma* (a) *and tumor tissue* (b) *of AH-130 tumor-bearing rats. Results are for the trimethylsilylated derivatives.*

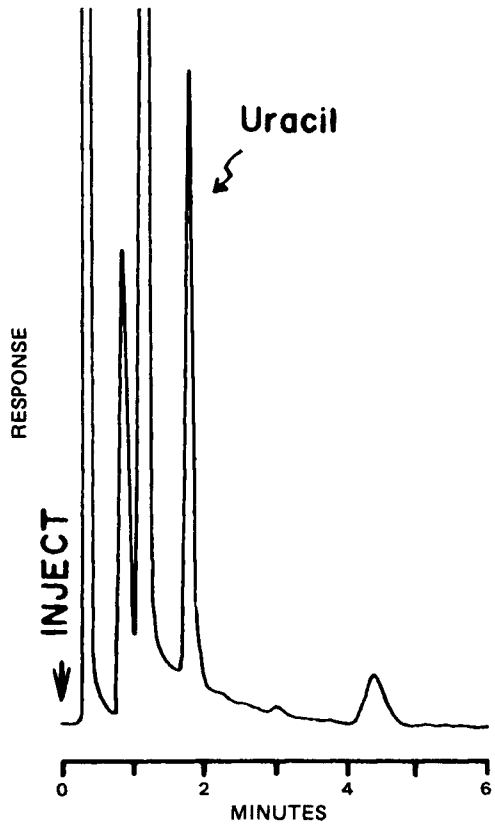

Figure 3—*Chromatogram by GLC–mass spectrometry showing the separation of uracil extracted from tumor tissue of AH-130 tumor-bearing rats. Results are for the trimethylsilyl derivatives.*

methanol, ethanol, or isopropanol) was the mobile phase. Of the solvent systems tested, ethylene dichloride–ethanol (24:1) was chosen as the mobile phase because it gave suitable chromatographic separation and analysis time.

The supernate of each organ homogenate must be extracted with chloroform; otherwise, the large amounts of lipid-soluble materials extracted with chloroform disturb the HPLC analysis.

Known amounts of ftorafur were added to plasma and homogenates of various organs of AH-130 tumor-bearing rats. The HPLC separation of ftorafur extracted from plasma and tumor tissue is shown in Fig. 1. The retention time of ftorafur was 6.3 min. As shown in Table I, the recoveries of ftorafur from plasma and each organ were quantitative, and the detection limit for ftorafur using this HPLC method was 0.025 μg/ml or g of wet weight for plasma and visceral tissues. The reproducibility was ±1.5–2.1%.

5-Fluorouracil and uracil have similar physicochemical properties. Thus, the addition of 0.5 *M* NaH_2PO_4 to the aqueous layer containing 5-fluorouracil and uracil and subsequent extraction with ethyl acetate as described previously (10, 11) resulted in good, constant recoveries.

The silylation procedure with *N,O*-bis(trimethylsilyl)trifluoroacetamide was sensitive, giving excellent GLC–mass fragmentographic and GLC–mass spectrometric separations of 5-fluorouracil and uracil extracted with ethyl acetate.

The internal standards, [1,3-$^{15}N_2$]-5-fluorouracil and [1,3-$^{15}N_2$]uracil, which are labeled with the stable isotope of nitrogen, were used for the simultaneous determination of 5-fluorouracil and uracil by the multiple-ion detection technique. The mass fragment ions detected for GLC–mass fragmentography were the molecular ion peaks (M^+) in the mass spectra of the trimethylsilyl derivatives of 5-fluorouracil and uracil since their base peaks ($M - CH_3$) were not separated clearly in some samples.

The best ion multiplier voltage of the mass spectrometer was 1.4 kv for the simultaneous determination of 5-fluorouracil and uracil by GLC–mass fragmentography. At this voltage, these compounds could be measured at concentrations of 0.001–2.5 μg/100 μl of silylating solvent. Under this condition, the concentrations of 5-fluorouracil could be analyzed in most samples, but the concentrations of uracil exceeded the upper detection limit. In particular, an extremely large difference was observed between the concentrations of 5-fluorouracil and uracil in tumor tissue having a large pool of uracil and those in the plasma collected soon after coadministration of uracil and ftorafur. In such samples, the two compounds could not be measured simultaneously at the ion multiplier voltage of 1.4 kv since the concentration of uracil was saturated. This problem could be overcome by two analytical runs at different values of the ion multiplier voltage: 5-fluorouracil was measured at an ion multiplier voltage of 1.4 kv, and uracil was measured at 1.2 kv. However, in this study, simultaneous detection by GLC–mass fragmentography and GLC–mass spectrometry was used, and conditions were developed for the simultaneous measurement of 5-fluorouracil and uracil in all kinds of samples.

Known amounts of 5-fluorouracil and uracil were added to plasma and homogenates of various organs of AH-130 tumor-bearing rats, and the samples were analyzed. The GLC–mass fragmentographic separation

Table II—Changes in the Concentration of Uracil Pooled in the Plasma, Liver, and Tumor of AH-130 Tumor-Bearing Rats by Incubation with Shaking at Various Temperatures

Incubation Conditions	Concentration of Uracil Detected[a]		
	Plasma, μg/ml	Tumor, μg/g	Liver, μg/g
Control	0.283 ± 0.067	32.793 ± 1.783	15.803 ± 0.998
−20 – −30°, 24 hr	0.282 ± 0.031	32.864 ± 1.345	15.834 ± 0.878
5°, 2 hr	0.283 ± 0.024	32.866 ± 1.567	15.821 ± 0.985
5°, 24 hr	0.283 ± 0.064	35.416 ± 1.857	16.751 ± 1.007
Room temperature, 12 hr	0.281 ± 0.058	57.654 ± 4.171	26.783 ± 3.110
Room temperature, 24 hr	0.276 ± 0.074	124.900 ± 7.895	56.406 ± 5.157
37°, 24 hr	0.255 ± 0.088	339.576 ± 23.694	140.078 ± 14.953

[a] Average of three determinations.

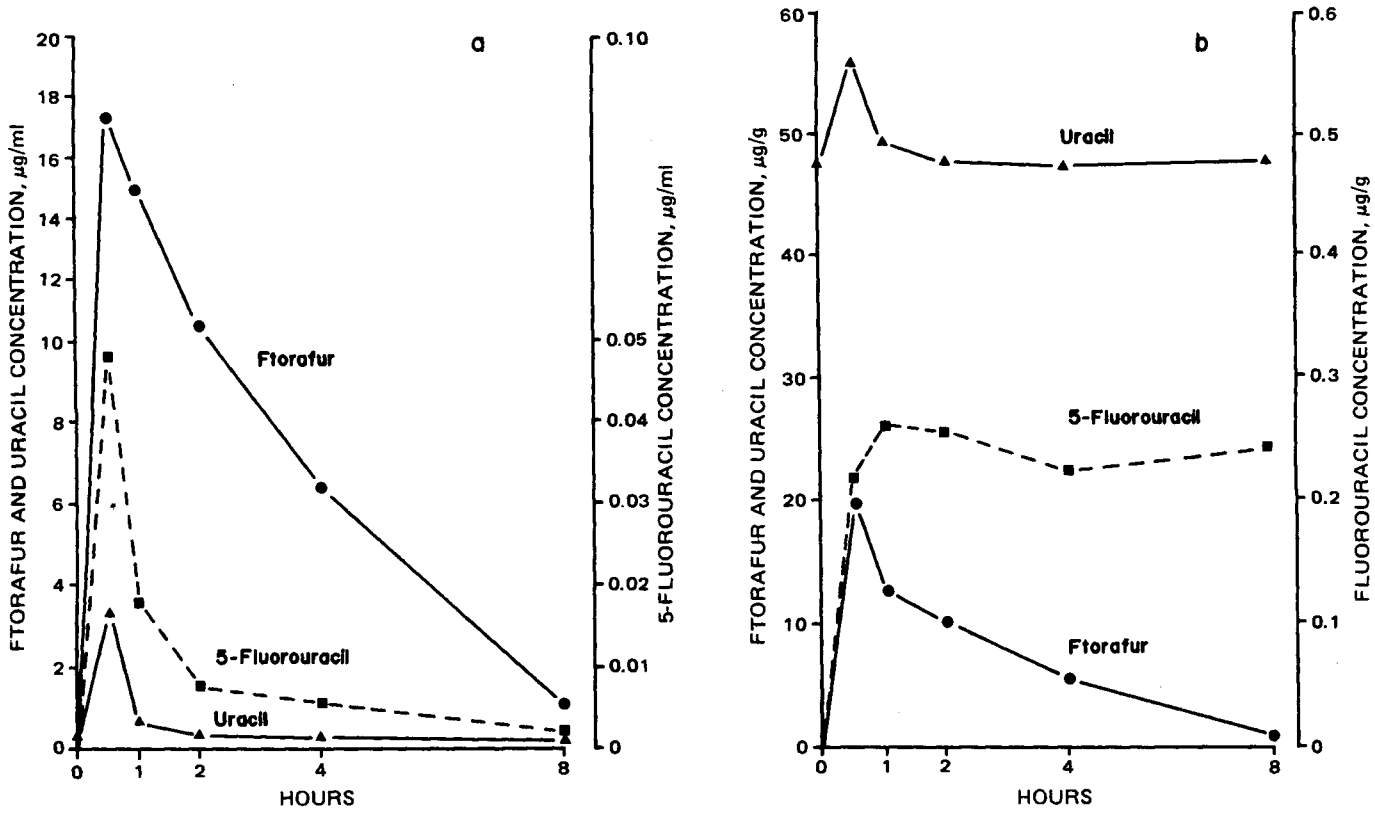

Figure 4—*Plasma levels* (a) *and tumor levels* (b) *of ftorafur, 5-fluorouracil, and uracil after a single oral dose of uracil (33.6 mg) plus ftorafur (5.0 mg) (4:1 mole/mole) to AH-130 tumor-bearing rats.*

of the trimethylsilyl derivatives of 5-fluorouracil, uracil, and their internal standard extracted from plasma and tumor tissue is shown in Fig. 2. The GLC–mass spectrometric separation of uracil extracted from tumor tissue is shown in Fig. 3. The retention times of 5-fluorouracil and uracil as their trimethylsilyl derivatives were 1.5 and 1.8 min, respectively. The recoveries from plasma were 86.1 ± 3.7% for 5-fluorouracil and 81.5 ± 3.8% for uracil, and those from visceral tissues were ~75% for 5-fluorouracil and ~70% for uracil. Since uracil forms pools naturally in all biological materials, the recovery of uracil was determined in a separate experiment by GLC–mass fragmentography and GLC–mass spectrometry utilizing [1,3-$^{15}N_2$]uracil and 5-chlorouracil (ion peak detected was *m/e* 275, M − CH_3) as internal standards.

The detection limits for 5-fluorouracil and uracil for GLC–mass fragmentography were 0.001 μg/ml for plasma and 0.001–0.005 μg/g of wet weight for visceral tissues; the detection limit for GLC–mass spectrometry was 0.2 μg/ml or g of wet weight. The reproducibility of this method was ±2.2–3.4%.

As reported previously (10, 11), the decomposition of ftorafur and 5-fluorouracil nucleosides or nucleotides had no significant effect on the assay of 5-fluorouracil. To examine the effect of decomposition of uracil nucleosides or nucleotides, authentic samples of these compounds were dissolved in physiological saline, plasma, and tissue homogenates of the liver and tumor and then analyzed for their uracil concentration. The results showed no significant differences between the values of the control and test samples and that decomposition of uracil nucleosides and nucleotides had no appreciable influence on the measurement of uracil with the present method.

The results obtained with the present method showed that the pools of uracil in various tissues were fairly high, as reported by Fujimoto *et al.* (19). Thus, the following experiments were conducted. First, changes in the uracil concentration were examined by incubation of plasma and tissue homogenates of the liver and tumor with shaking at various temperatures before measurement. As shown in Table II, the uracil concentration did not differ significantly from control values after incubation at various temperatures for plasma and at 5 or −20–−30° (frozen) for the liver and tumor. However, the uracil concentration increased with incubation at room temperature or 37° for the liver and tumor. This change in the uracil concentration probably is due mainly to the enzymatic decomposition of nucleosides and nucleotides.

Next, plasma and tissue homogenates of the tumor and liver were acidified with hydrochloric acid and extracted with chloroform, and the supernate of the aqueous layer was incubated with shaking at 37° for 24 hr. No significant difference between the concentration of uracil in the control and test samples was observed. Thus, as well as causing deproteinization, extraction with chloroform in hydrochloric acid inactivated enzymes that hydrolyzed the glycoside bonds of nucleosides and nucleotides.

On the basis of these observations, it was concluded that the present method, in which tissues were homogenized in an ice bath at a temperature below 5° and the supernate was acidified with hydrochloric acid and extracted with chloroform, did not involve any procedure that affects the determination of 5-fluorouracil and uracil.

The present method also can be applied to plasma and visceral tissues of other animals. The results obtained for the chromatographic separation, recoveries, and sensitivities were in good agreement with those obtained with AH-130 tumor-bearing rats. It is not ethically possible to obtain all kinds of human tissues; however, results with plasma and tumor tissue from humans gave similar results to those obtained with animals, indicating the applicability of the present method to human tissues.

Uracil plus ftorafur (4:1 mole/mole) was given orally to AH-130 tumor-bearing rats, and the time course of changes in the concentrations of ftorafur, 5-fluorouracil, and uracil in plasma and tumor tissue was measured by the present method (Fig. 4). Since the concentration of uracil represents the sum of the original amount and that resulting from uracil plus ftorafur treatment, individual differences in the original pools of uracil, especially in organs, sometimes were so large that they masked the time course of change in exogenous uracil after administration of uracil plus ftorafur. However, it should be possible to measure the time course using a labeled compound.

Because ftorafur and 5-fluorouracil are metabolized together with uracil, the present method for the simultaneous determination of ftorafur, 5-fluorouracil, and uracil in plasma and visceral tissues should be useful. Furthermore, this method will be helpful for basic and clinical pharmacological studies on uracil plus ftorafur and ftorafur alone.

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Use of Stable Isotopes in the Study of Pharmacokinetics of Drugs by Mass Fragmentography II: Detailed Examination of Pharmacokinetics of a Single Oral Dose of Phenytoin in Humans

SHIGEO BABA ˣ, TSUYOSHI GOROMARU, KAZUSHIGE YAMAZAKI, and YASUJI KASUYA

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Abstract □ To study the pharmacokinetics of phenytoin in detail, a mass fragmentographic method was applied for the precise, sensitive, and specific analysis of phenytoin, 5-(4-hydroxyphenyl)-5-phenylhydantoin, and 5-(4-hydroxyphenyl)-5-phenylhydantoin glucuronide in plasma and urine after administration of a single oral dose of phenytoin to two healthy volunteers. Salivary phenytoin concentrations also were measured. Phenytoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin were analyzed after the addition of deuterium-labeled internal standards and conversion to volatile methyl derivatives for mass fragmentographic analysis. The lower limit of detection was ~10 ng/ml. The simultaneous pharmacokinetic analysis of the plasma levels and urinary excretion data of phenytoin and its major metabolite, 5-(4-hydroxyphenyl)-5-phenylhydantoin, yielded detailed information about the pharmacokinetics of phenytoin.

Keyphrases □ Mass fragmentography—application to pharmacokinetic study of single oral phenytoin dose using stable isotope labeling, humans □ Phenytoin—pharmacokinetics, application of mass fragmentography after single oral dose using stable isotope labeling, humans □ Pharmacokinetics—phenytoin, application of mass fragmentography after single oral dose using stable isotope labeling, humans

GLC–mass spectrometry coupled with selected-ion monitoring is used increasingly in drug research because of its high sensitivity and specificity. In this technique, stable isotope-labeled carriers serve as ideal internal standards to correct for losses of the compound under study in the initial isolation procedures. The usefulness of mass fragmentography in conjunction with stable isotope labeling has been of recent interest in the measurement of trace amounts of substances in biological materials (1–3). This method was used in these laboratories for the metabolic study of drugs in animals and humans (4–6). The mass fragmentographic technique also has been applied for the sensitive, specific, and reliable determination of plasma testosterone levels in humans (7).

Pharmacokinetic studies represent one field in which the sensitivity and specificity of the mass fragmentographic technique offer an advantage. Sullivan and coworkers (8, 9) successfully used stable isotope-labeled drugs together with mass fragmentography to study steady-state pharmacokinetics. An investigation of the elimination kinetics of drugs was carried out in these laboratories by measuring the nanogram levels of a drug and its major metabolites in human urine using this technique (10).

The present paper describes the use of deuterium-labeled phenytoin (I) and its major metabolite, 5-(4-hydroxyphenyl)-5-phenylhydantoin (II), along with mass fragmentography to follow saliva, plasma, and urinary concentrations of I and II for a reasonable period after a single oral administration of I to humans. A detailed analysis and interpretation of the pharmacokinetic data of I from two healthy volunteers also are presented.

EXPERIMENTAL

Synthesis of Deuterated Internal Standards—*Pentadeuterophenytoin (I-*d_5*)*—Deuterobenzophenone (benzophenone-d_5) was prepared by refluxing a mixture of 9.50 g of hexadeuterobenzene[1] (99.5 atom % D) and 2.44 g of benzoyl chloride[2] for 5 hr with 2.70 g of anhydrous aluminum chloride. The I-d_5 then was synthesized from the benzophenone-d_5 according to the method described by Baty and Robinson (11).

[1] Merck.
[2] Wako Pure Chemical Industries.

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