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Note

Rapid determination of 1-(2-tetrahydrofuryl)-5-fluorouracil in human blood by high-pressure liquid chromatography

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1-(2-Tetrahydrofuryl)-5-fluorouracil (FT-207) and its major metabolite, 5-fluorouracil (5-FU), are chemotherapeutic agents frequently used in the treatment of various cancers. The analysis of 5-FU in plasma has been achieved using low-pressure column chromatography on Sephadex G-10 [1] and also by gas—liquid chromatography [2]. Fujita et al. [3] have already determined the levels of both FT-207 and 5-FU in circulating blood using a biological assay. The bioassay is probably the most commonly used technique for measuring the concentration of FT-207 in blood. Since interference by other antibiotic drugs limits the specificity of this test, we investigated the utility, sensitivity and specificity of a high-pressure liquid chromatographic (HPLC) technique for measuring FT-207 levels in human blood.

EXPERIMENTAL

All analyses were performed using a high-pressure liquid chromatograph from Waters Assoc. (Milford, Mass., U.S.A.). An M 6000A pump and a U6K injector were coupled to a μBondapak C_{18} (particle size, 8—10 μm column 300 × 4 mm I.D.). FT-207 was quantified by measuring absorbancy at 280 nm with a Waters Assoc. UV detector (Model 440) and recorded on a 10-mV full-scale National pen recorder VP-6521W. Blood concentrations of FT-207 were determined from peak-height ratios with a known amount of 6-mercaptopurine (6-MP) added as internal standard.

FT-207 was added in vitro to 0.4 ml human whole blood in amounts suitable for preparing a standard curve. After standing at room temperature for 30 min, haemolysis was completed by adding 0.5 ml water; 9 ml acetonitrile were then added to the mixtures. These were shaken vigorously for 1 min by hand and centrifuged for 5 min at 500 *g*. Seven ml of the upper phase

were transferred by pipette to another test-tube. The solvent was evaporated to dryness with a rotary evaporator at 40°. The residue was redissolved in 0.5 ml methanol and added to 0.05 ml 6-MP (0.4 mg/ml in methanol). Samples were filtered through Fluoropore filters (0.45 μm, Type FT-045, Sumitomo, Osaka, Japan), and 20-μl aliquots of the filtrate containing 0.06—1.68 μg of FT-207 were injected onto the column and eluted with 10 % aqueous methanol at a flow-rate of 2 ml/min.

For measuring FT-207 levels in blood, FT-207 was given intravenously to three cancer patients who had normal liver functions in a single dose of 16 mg per kg body weight. Blood samples were collected at 1, 3, 5, 12 and 24 h after the drug was injected. The FT-207 in 0.5 ml blood was extracted as described above and injected onto the column.

FT-207 and 6-MP were obtained from Taiho Pharmaceuticals (Tokyo, Japan) and Takeda Chemicals (Osaka, Japan) respectively. Methanol, acetonitrile and water were purchased from Wako (Osaka, Japan). All were liquid-chromatographic reagent grade.

RESULTS AND DISCUSSION

High-pressure liquid chromatograms of untreated human blood as a control and of FT-207 added to blood are shown in Fig. 1A and B. FT-207 eluted as a sharp symmetrical peak with a retention time of 7.8 min. An internal standard added to reduce the chance of pipetting errors did not interfere with the separation of this drug.

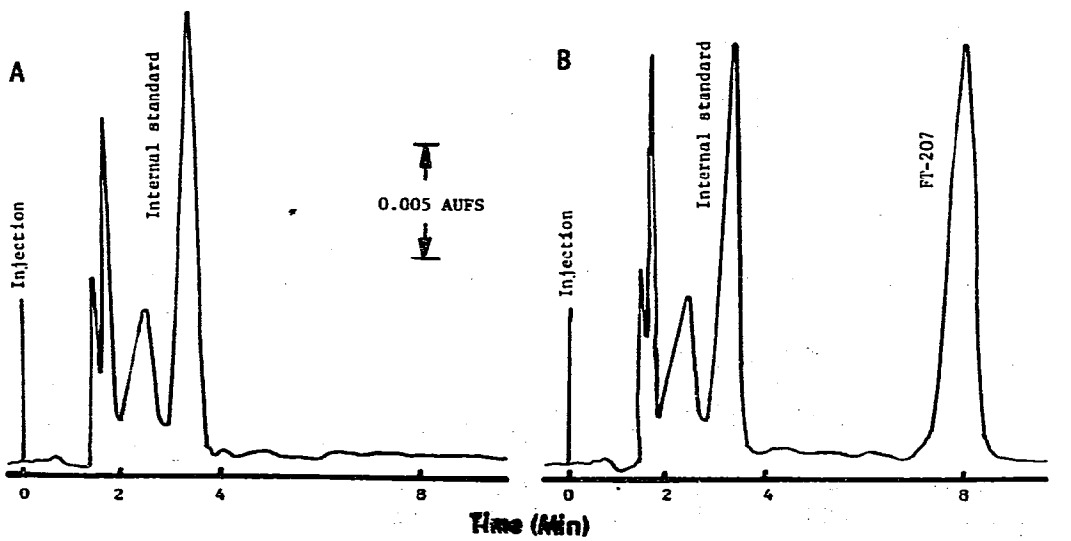

Fig.1. High-pressure liquid chromatograms of (A) human blood alone as a control, and (B) FT-207 (20 μg) added to 0.4 ml blood. Extraction procedures are described in Experimental.

The standard curve constructed from known amounts of FT-207 added to human blood is linear between at least 0.1 and 1.7 μg for FT-207. Quantitative results obtained by this procedure are given in Table I. The recovery of FT-207 extracted from whole blood and serum is approximately 100 %.

FT-207 levels in blood from three cancer patients with normal hepatic functions were determined by this technique following a single intravenous dose of FT-207 (16 mg per kg body weight). Levels were maintained at approx. 20 μg/ml up to 5 h after administration and then gradually decreased (Table II). 5-FU was similarly extracted and injected onto the column, as described for FT-207. A satisfactory recovery was obtained in the range 0.1—1.0 μg 5-FU. The method is not suitable for 5-FU, however, because 5-FU concentrations in the blood following a therapeutic dose of FT-207 or 5-FU rapidly [4] decreased below the limits of sensitivity of the method. Since it was found that blood concentrations of FT-207 at the 0.1 μg/ml level could be specifically measured in less than 15 min after a simple extraction process, this method may be useful in clinical pharmacology for detecting the efficacy and side-effects of FT-207.

TABLE I

PERCENTAGE RECOVERY OF FT-207 EXTRACTED FROM WHOLE BLOOD AND SERUM

Sample	FT-207 (μg)		Recovery (%)
	Added	Recovered (mean ± S.E.)*	
Whole blood	100	99.3 ± 2.4	99.3
	75	76.9 ± 4.1	102.5
	50	48.2 ± 1.5	96.4
Serum	50	49.9 ± 4.3	99.8

*$n = 3$.

TABLE II

BLOOD LEVELS OF FT-207 IN CANCER PATIENTS WITH NORMAL HEPATIC FUNCTIONS

Patients	FT-207 (μg/ml blood)				
	Time after administration (h)				
	1	3	5	12	24
Rectal cancer	26.8	23.0	17.4	10.5	5.6
Lung cancer	30.6	23.6	21.2	11.0	5.8
Ovarian cancer	28.6	24.2	16.3	12.7	2.5
Mean	28.7	23.6	18.3	11.4	4.6
S.E.	1.1	0.3	1.5	0.7	1.1

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