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Note

High-pressure liquid chromatographic analysis of ftorafur and its metabolites in biological fluids

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Ftorafur (FT)**, the 1-tetrahydrofuran-2-yl derivative of 5-fluorouracil, is effective in the treatment of breast and gastrointestinal malignancies[1,2]. Although FT has a qualitatively similar antitumor activity as 5-FU, it is markedly less myelosuppressive[1]. The biological activity of FT may be due to the slow metabolic release of 5-FU[3,4]; its toxicity in fact simulates that of 5-FU given by slow constant infusion.

The cytotoxicity of 5-FU is contingent upon the stepwise conversion of 5-FU finally to FdUMP, which blocks DNA synthesis by competitive inhibition of thymidylate synthetase. There are several pathways by which FdUMP is formed from 5-FU. First, 5-FU is converted to FUR, followed by phosphorylation to FUMP, then to FUDP; reduction of FUDP by ribonucleotide reductase affords FdUDP, which is rapidly dephosphorylated to FdUMP. Secondly, FUMP may be formed directly from 5-FU and phosphoribosylpyrophosphate. Additionally, 5-FU may be converted to FUdR, which is subsequently phosphorylated by thymidine kinase to FdUMP[5].

To study the clinical pharmacology of FT, it is necessary to be able to separate efficiently and quantitate many of the intermediate metabolites of 5-FU. This communication describes the use of high-pressure liquid chromatography (HPLC) in achieving these goals.

MATERIALS AND METHODS

A Waters Assoc. ALC Model 202/401 liquid chromatograph equipped with a variable-wavelength ultraviolet detector (Schoeffel, Westwood, N.J., U.S.A.) operating at 270 nm was used for the analyses. The separations were achieved utilizing a Waters Assoc. μBondapak/C_{18} column (30 cm × 4.0 mm I.D.). The mobile phase was filtered (MF-Millipore filter HWAP 04700, pore size 0.45 μm, Millipore, Bedford, Mass., U.S.A.), deionized water and the flow-rate was 2.0 ml/min. FT, 5-FU, FUdR, FUR, and [2-^{14}C]-FT (46.5 μCi/mg) were obtained from the Drug Development

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** Abbreviations: FT = ftorafur; 5-FU = 5-fluorouracil; FdUMP = 5-fluoro-2′-deoxyuridine-5′-monophosphate; FUR = 5-fluorouridine; FUMP = 5-fluorouridine-5′-monophosphate; FUDP = 5-fluorouridine-5′-diphosphate; FdUDP = 5-fluoro-2′-deoxyuridine-5′-diphosphate; FUdR = 5-fluoro-2′-deoxyuridine.

Branch, Drug Research and Development, National Cancer Institute, Bethesda, Md., U.S.A.; FdUMP was obtained from Terra-Marine Bioresearch (La Jolla, Calif., U.S.A.).

Biological samples were obtained from patients receiving FT at doses of 4 or 5 g/m^2. Urine samples (5 ml) were lyophilized, dissolved in water (1 ml), and chromatographed on Dowex 1-X8 formate resin (200–400 mesh, 20 × 1 cm I.D. column) using formic acid buffer as eluent[6]. The UV-absorbing fractions eluting with 0.05 *M* formic acid were combined, lyophilized, and reconstituted in 0.7 ml of water. Samples of 10 μl were injected into the instrument.

Plasma samples were ultrafiltered (Model 12 stirred ultrafiltration cell, Diaflow Type PM 30 ultrafiltration membrane from Amicon, Lexington, Mass., U.S.A.); 50-μl samples were analyzed.

Peak areas were determined by a Columbia Scientific Model CSI 38 integrator. Standard curves were prepared by plotting peak area against drug concentration and were linear for both plasma and urine.

For radiochemical analysis, ultrafiltered plasma samples (200 μl) were applied to Whatman No. 1 paper and the chromatograph developed with ethyl acetate–formic acid–water (65:5:5). The paper was cut into 2-cm strips, placed in vials containing 11 ml PCS (Amersham/Searle, Arlington Heights, Ill., U.S.A.), and radioactivity was determined by liquid scintillation counting (Packard Tri-Carb Model 3755). The degree of quenching was determined by comparison with an automatic external standard.

RESULTS AND DISCUSSION

The reversed-phase chromatographic separation of an aqueous solution of FdUMP, 5-FU, FUR, FUdR, and FT was achieved at room temperature using water as eluent (Fig. 1). The retention times were: FdUMP, 1.45 min ($K' = 0.32$); 5-FU, 3.29 min ($K' = 1.99$); FUR, 7.34 min ($K' = 5.67$); FUdR, 10.9 min ($K' = 8.91$); FT,

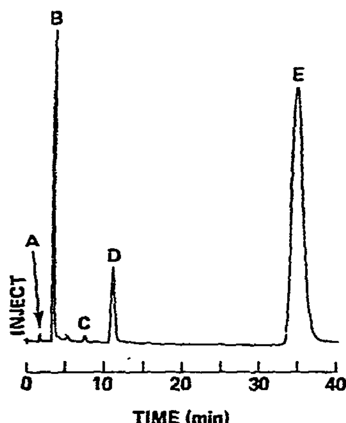

Fig. 1. Separation of FdUMP (A), 5-FU (B), FUR (C), FUdR (D), and FT (E), by high-pressure reversed-phase chromatography. Operating conditions: column, μBondapak/C_{18}; mobile phase, water; flow-rate, 2 ml/min; detector, UV, 270 nm, 0.1 a.u.f.s.

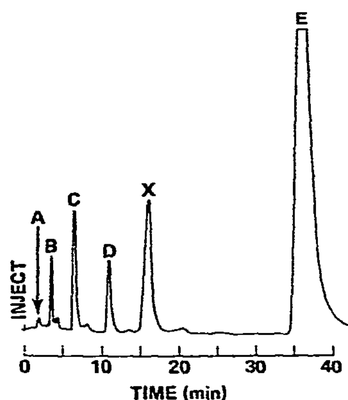

Fig. 2. Elution profile for urine sample collected from patient receiving 4 g/m^2 of FT. A = FdUMP; B = 5-FU; C = FUR; D = FUdR; E = FT; X = unknown metabolite. The operating conditions are the same as in Fig. 1.

35.4 min ($K' = 31.2$). These values inversely correlated with the polarities of the compounds.

The elution profile of a urine sample from a patient receiving 4 g/m^2 of FT is shown in Fig. 2. The sample, which was collected 4 h after drug administration, was pre-chromatographed on Dowex 1-X8 formate resin. 5-FU, FUR, FUdR, and FT were identified and quantitated. Additionally, an unknown metabolite (X) with a retention time of 16 min was observed. The presence of a UV-absorbing chromophore and the decreased retention time (increased polarity) of X relative to FT indicate that X most likely arises from an alteration of the tetrahydrofuran ring. Mass spectral analysis of this compound is under way.

The analysis of plasma samples from patients receiving FT indicated only FT and 5-FU in sufficient concentration to be identified and quantitated (Fig. 3). A comparison of FT plasma concentrations determined by HPLC and radiochromatography is shown in Fig. 4. Samples from seven patients administered 5 g/m^2 of FT

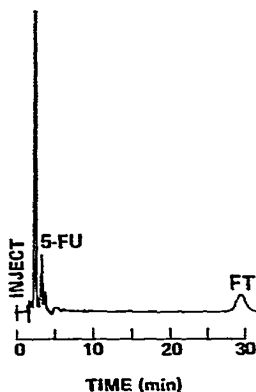

Fig. 3. Elution profile for a plasma sample collected from a patient receiving 5 g/m^2 of FT. The operating conditions are the same as Fig. 1.

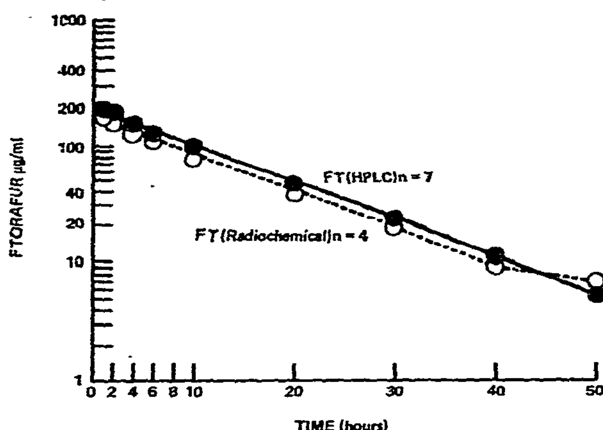

Fig. 4. Comparison of plasma clearance of FT determined by HPLC and radiochromatography.

were studied by HPLC; four of these patients received radiolabelled FT. Good agreement between the two analytical methods was observed, although the radiochemical assay generally gave lower results.

Thus, the HPLC method is sensitive and specific for the determination of FT, 5-FU, and 5-FU anabolites in biological fluids. This technique is being applied to the study of the clinical pharmacology of FT.

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