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Note

Quantitative determination of 1,3-bis(tetrahydro-2-furanyl)-5-fluoro-2,4-pyrimidinedione and its metabolites in visceral tissues by high-performance liquid chromatography and gas chromatography–mass fragmentography

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A method for assay of 1,3-bis(tetrahydro-2-furanyl)-5-fluoro-2,4-pyrimidinedione (FD-1), which is a new derivative of 5-fluoro-2,4-pyrimidinedione (5-FU) and an effective antitumour agent[1], and its metabolites in visceral tissues has been reported which involves thin-layer chromatography, scanning densitometry and microbiological assay (bioassay)[2]. However, in bioassay the antibacterial potency of 5-FU is greatly influenced by constituents of visceral tissues and this method is thus unreliable, particularly at lower 5-FU concentrations. We have estimated the levels of these compounds in plasma[3]: FD-1, 1- and 3-(tetrahydro-2-furanyl)-5-fluoro-2,4-pyrimidinedione(1-FT, 3-FT) were separated from 5-FU by extraction, and determined by high-performance liquid chromatography (HPLC): 5-FU was measured by gas chromatography–mass fragmentography (GC–MF).

MATERIALS AND METHODS

FD-1, 1-FT and 3-FT were synthesized and purified in our laboratory[4,5]. 5-FU was obtained from Sigma (St. Louis, Mo., U.S.A.). [1,3-$^{15}N_2$]5-FU (95% enrichment) was from PCR (Gainesville, Fla., U.S.A.). N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine for the silylating solvent were purchased from Pierce (Rockford, Ill., U.S.A.). The other chemicals used were liquid chromatographic grade materials. The tissues used in this study were obtained from AH-130 bearing rats.

A LC-1 liquid chromatograph (Shimadzu–DuPont, Kyoto, Japan) and a JMS D-300 mass spectrometer (electron impact) (JEOL, Tokyo, Japan) connected with a JEOL JGC-20KP gas chromatograph were used. The conditions for HPLC and GC–MF were as described previously[3] except for the following: ionization energy, 70 eV; ionization current, 300 μA; acceleration voltage, 3.0 kV; ion multiplier voltage, 1.4 kV; and use of a coiled glass column (1 m × 2 mm I.D.). The peaks of molecular ions of the silylated derivatives of 5-FU and [1,3-$^{15}N_2$]-5-FU, of *m/e* 274 and 276, respectively, were selected for GC–MF analysis.

Samples of 0.5–1.0 g of each organ were homogenized in an ice-bath in 2–3 volumes physiological saline containing 0.1 μg of [1,3-$^{15}N_2$]-5-FU as an internal

standard and then centrifuged at 2000 × *g* for 20 min at less than 5°. A 1.0-ml volume of the supernatant solution was treated in the same way as plasma samples[3], except that for silylation a freshly prepared solution of 100 μl pyridine containing 25% BSTFA was added to the residue extracted with ethyl acetate, and the solution was kept at 70° for 10 min to allow silylation.

RESULTS AND DISCUSSION

It was essential to use the supernatant of the homogenate rather than the whole homogenate because otherwise the chloroform layer contained too much lipo-soluble material, mainly fat, for HPLC. The stable isotopically labelled [1,3-$^{15}N_2$]-5-FU was chosen as an internal standard for multiple ion detection in GC–MF instead of thymine used in plasma samples, because thymine is a constituent of visceral tissues. The fragment ions detected were the molecular ion peaks because the basic ion peaks ($M-CH_3$) were not clearly separated in some samples.

The HPLC separation of FD-1, 1-FT and 3-FT extracted from tumour tissue is shown in Fig. 1. The retention times of FD-1, 1-FT and 3-FT were 4.5, 6.9 and 10.5 min, respectively, and the recoveries of the compounds from each organ were quantitative. The detection limit by this GC–MF method were 0.001 μg/g wet weight for FD-1 and 0.025 μg/g wet weight for 1-FT and 3-FT. The GC–MF separation of 5-FU extracted from tumour tissue is shown in Fig. 2. The retention time of the silylated derivative of 5-FU was 1.5 min. The recovery from each organ was *ca.* 70–75%, although it varied slightly in different organs. The detection limit of 5-FU in this GC–MF method also varied slightly in different organs and samples in the range of 0.001–0.005 μg/g wet weight. The HPLC and GC–MF separation patterns of each organ were similar.

The present method can be used for measuring the low concentrations of FD-1 and 1-FT and its metabolites in visceral tissues after administration of FD-1 and 1-FT.

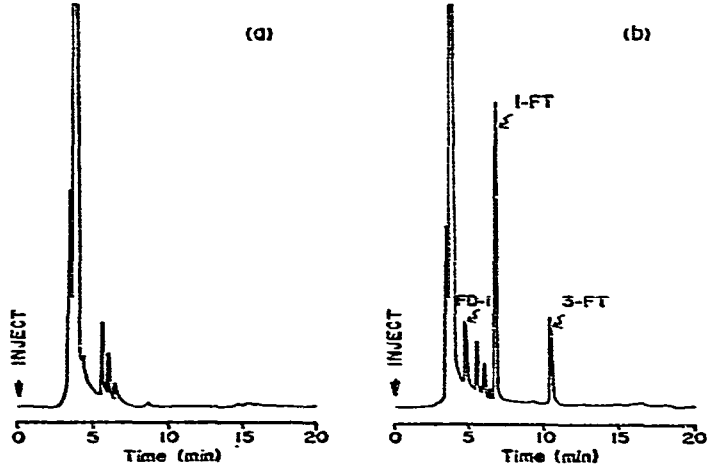

Fig. 1. Liquid chromatograms showing the separation of (a) control and (b) FD-1, 1-FT and 3-FT extracted from tumour tissue of AH-130 bearing rats.

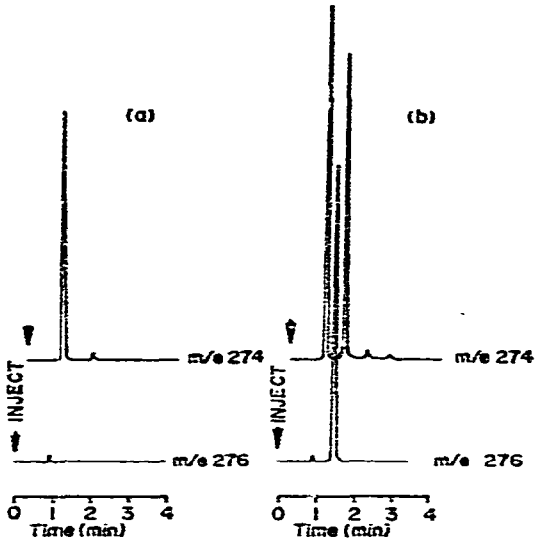

Fig. 2. Mass fragmentograms showing the separation of (a) control and (b) 5-FU and [1,3-$^{15}N_2$]-5-FU (internal standard) extracted from tumour tissue of AH-130 bearing rats. Results are for silylated derivatives.

REFERENCES

1 N. Unemi, S. Takeda ,K. Kitazato, M. Kajiwara and S. Fujii, *Chemotherapy (Japan)*, 26 (1978) 200.
2 Y. Kawaguchi, Y. Nakamura, T. Sato, S. Takeda, T. Marunaka and S. Fujii, *Yakugaku Zasshi*, 98 (1978) 525.
3 T. Marunaka and Y. Umeno, *J. Chromatogr.*, 157 (1978) 321.
4 M. Yasumoto, I. Yamawaki, T. Marunaka and S. Hashimoto, *J. Med. Chem.*, 21 (1978) 738.
5 M. Yasumoto, J. Yamashita and S. Hashimoto, *Yakugaku Zasshi*, 98 (1978) 1151.