Journal of Chromatography, 151 (1978) 237–240

CHROM. 10,616

Note

High-performance liquid chromatographic analysis of 5-fluorouracil in plasma

JORDAN L. COHEN* and RICHARD E. BROWN

University of Southern California, School of Pharmacy, 1985 Zonal Avenue, Los Angeles, Calif. 90033 (U.S.A.)

Received September 30th, 1977)

5-Fluorouracil (FU) remains an important antineoplastic agent, more than 20 years after its introduction into clinical medicine[1–3]. It is used primarily for the treatment of solid tumours of the breast, colon and rectum, and for disseminated disease, mainly in combination with other chemotherepeutic agents designed to be synergistic in cytotoxicity while minimizing serious side-effects and toxicities[4]. Although FU exerts its anticancer activity following metabolic activation to 5-fluorodeoxyuridine monophosphate (FdUMP) intracellularly, the measurement of free FU plasma concentrations remains the most reasonable clinical pharmacological approach for studying individual variation in metabolism and response.

Several analytical methods have been reported for FU in plasma. We have reported[5] a gas chromatographic procedure, following silylation of FU, that is sensitive to 0.3 μg/ml in plasma and that is applicable to disposition studies following typical bolus dose administration. Others workers[6–8] haved described various gas chromatography–mass spectrometry methods with lower limits of sensitivity (1–25 ng/ml). These have been used mainly to detect FU at considerable time after bolus doses or prolonged intravenous infusion. Retention time data for ftorafur, FU and several metabolites have been reported for a reversed-phase high-performance liquid chromatographic (HPLC) system, but no analytical details were given[9]. In the present paper we report a rapid HPLC assay for FU using a strong anion-exchange column sensitive to 100 ng/ml in plasma.

MATERIALS AND METHODS

A Waters ALC Model 202 liquid chromatograph (Milford, Mass., U.S.A.) equipped with a UK-6 injector and a Model 440 UV detector was used for the analyses. Chromatography was performed on a 30 cm × 2.0 mm I.D. Aminex A-25 strong anion-exchange column (Bio-Rad, Richmond, Calif., U.S.A.), with a mean particle diameter of 17.5 ± 2 μm packed at 3000 p.s.i., at a flow-rate of 1.0 ml/min. The mobile phase was 0.3 *M* acetate buffer (pH 4.5) at a flow-rate of 0.7 ml/min, and the column was heated to 30° by a regulated water jacket. The mobile phase was degassed by sonicating for 30 min, and the detector wavelength was 254 nm.

* To whom correspondence should be addressed.

FU was obtained from the Drug Development Branch of the National Cancer Institute (Bethesda, Md., U.S.A.) and 5-chlorouracil (CU), used as the internal standard, was obtained from K & K Laboratories (Irvine, Calif., U.S.A.). Distilled water was purified by passing it through a reverse osmosis four-filter system (Millipore, Bedford, Mass., U.S.A.). All other reagents and chemicals were of analytical reagent grade.

Plasma samples were either from patients receiving 15 mg/kg bolus doses weekly or from normal human pools. The analytical procedure involved the addition of 10 μg of CU and 1.0 ml of saturated ammonium sulphate to a 1.0-ml plasma sample, followed by rapid shaking for 30 min with 6 ml of ether–*n*-propanol (80:20). The organic layer was removed and evaporated under a stream of filtered air at 60°. The residue was reconstituted in 100 μl of a 0.005 *M* K_2HPO_4 solution (pH 9.8) and 5–25 μl were injected. Quantitative determination in the 100–300 ng/ml range required purification on a 3-cm Sephadex LH-20 (Pharmacia, Piscataway, N.J., U.S.A.) column. FU and CU were eluted with 0.005 *M* K_2HPO_4 (pH 9.8), which was then evaporated and reconstituted as above. Peak height ratios (FU/CU) were used to produce standard curves from spiked pooled plasma and aqueous samples.

RESULTS AND DISCUSSION

Typical chromatograms for a patient sample and a plasma blank are shown in Fig. 1. Retention times for FU and CU were 8.0 and 17.0 min, respectively, under the conditions described. Standard curves from spiked pooled plasma were found to be linear from 0.5 to 40.0 μg/ml (Fig. 2), and the lower limit of sensitivity employing the Sephadex LH-20 column clean-up and a 20-μl injection volume was 100 ng/ml. Typical variation in the analysis of duplicate specimens was found to be of the order of 5–10%, and a 1.0 μg/ml plasma standard had a relative standard deviation of 7.5% when run six times on separate occasions.

Because the high polarity of FU requires a highly polar organic solvent that remains immiscible with the aqueous phase for extraction, the clean-up of samples by

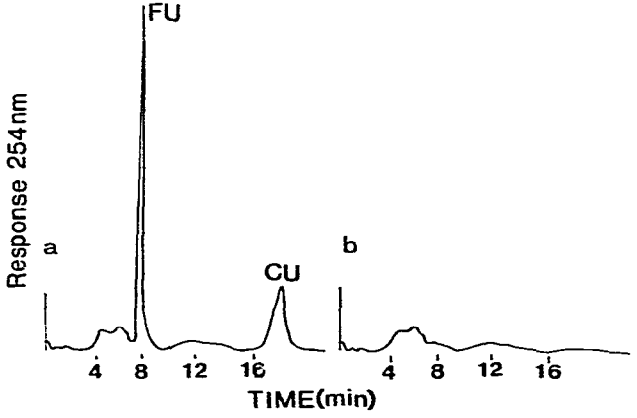

Fig. 1. High-performance liquid chromatograms from (a) a patient receiving a 15 mg/kg bolus intravenous dose of FU and (b) from an extracted patient blank.

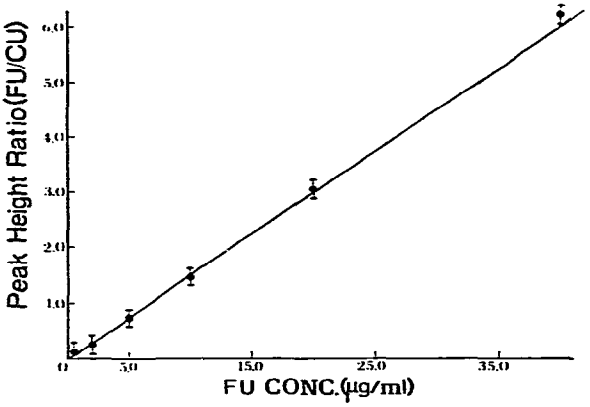

Fig. 2. Standard curve obtained from pooled plasma samples spiked with FU.

extraction is difficult and tedious. Although the chromatogram is generally free from interference at the point of FU elution, the expanded scale required for the detection of concentrations of less than 1 μg/ml causes the FU peak to appear as a shoulder on the interference peak, eluting at *ca.* 6.8 min. The Sephadex column clean-up procedure, although somewhat more time-consuming, eliminates this interference and allows quantitation of levels down to 100 ng/ml. Relative to unextracted standard curves the extraction procedure is *ca.* 80% and 94% efficient for FU and CU, respectively, whereas Sephadex chromatography results in the loss of an additional 10% of each component. pH control was critical for the separation, with lower pH markedly decreasing the retention time and higher pH causing peak broadening. The mildly elevated temperature dramatically sharpened the FU peak to allow increased sensitivity.

The method is somewhat more sensitive than the gas chromatographic procedure previously reported[5]. It is suitable for the routine determination of FU following intravenous or oral bolus dose therapy, and in the support of clinical pharmacology studies of FU up to its sensitivity limitations. Sensitivity could perhaps be

TABLE I

COMPARISON OF GAS AND LIQUID CHROMATOGRAPHIC ANALYSIS OF FLUOROURACIL PLASMA TIME COURSE

The gas chromatographic procedure was a slight modification of that used by Cohen and Brennan[5].

Time (min) *	*FU concentration (μg/ml)*	
	*GLC***	*HPLC***
0	—	—
5	46.8	52.5
10	30.7	32.3
15	28.9	26.5
60	3.3	4.4
90	0.2	0.5

* Time following 1.0 g (15 mg/kg) bolus intravenous dose.

** Each concentration represents an average of two determinations.

improved two-fold if a continuously variable UV detector were available to allow monitoring at 266 nm, the λ_{max} of FU[10]. Analysis by both methods of samples from patients following 15 mg/mg doses produced comparable results (Table I).

ACKNOWLEDGEMENT

This work was supported by grant CA-14089 from the National Cancer Institute, National Institute of Health, U.S. Public Health Service.

REFERENCES

1 C. Heidelberger, N. K. Chaudhuri, P. Dannenberg, D. Moorsen, L. Griesbach, R. Duchinsky, R. J. Schnitzer, E. Pleven and T. Scheiner, *Nature (London)*, 179 (1957) 663.
2 A. R. Curreri, F. J. Ansfield, F. A. McIver, H. A. Waisman and C. Heidelberger, *Cancer Res.*, 18 (1958) 478.
3 R. B. Livingston and S. K. Carter, *Single Agents in Cancer Chemotherapy*, Plenum Publ., New York, 1970, pp. 195–226.
4 C. Heidelberger, in J. F. Holland and E. Frei (Editors), *Cancer Medicine*, Lea and Febiger, Philadelphia, Pa., 1973, pp. 768–791.
5 J. L. Cohen and P. B. Brennan, *J. Pharm. Sci.*, 62 (1973) 572.
6 C. Finn and W. Sadee, *Cancer Chemother. Rep.*, 59 (1975) 279.
7 B. L. Hillcoat, M. Kawai, P. B. McCullock, J. Rosenfeld and C. K. O. Williams, *Brit. J. Clin. Pharmacol.*, 3 (1976) 135.
8 J. P. Horwitz, E. B. Hills, V. C. Godefroi, I. A. O'Leary, M. Burke, P. Andrzejewski and W. Brukwinski, *Proc. Amer. Assoc. Cancer Res.*, 18 (1977) 20.
9 J. A. Benvenuto, K. Lu and T. L. Loo, *J. Chromatogr.*, 134 (1977) 219.
10 B. C. Rudy and B. Z. Senkowski, in K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. 2, Academic Press, New York. 1972, pp. 225–226.