Journal of Chromatography, 206 (1981) 407–409
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,468

Note

Determination of 5-fluoro-2′-deoxyuridine[6-^{3}H] as an impurity in 5-fluorouracil[6-^{3}H] by high-performance liquid chromatography

JAMES L. DAY*·*, JONATHAN MAYBAUM and WOLFGANG SADÉE

School of Pharmacy, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 (U.S.A.)

(Received October 17th, 1980)

The antimetabolite, 5-fluorouracil, has been shown to be an effective antitumor agent against various types of carcinomas[1] and studies of its metabolism have been quite numerous[2,3]. We have been investigating the metabolism of 5-fluorouracil in the wild-type mouse T-cell lymphosarcoma line S-49[4]. Previous studies indicated that the concentration of 5-fluorouracil which inhibited the growth 50% (EC_{50}) of the S-49 cells in continuous culture was approximately 0.8 μM. When attempting to reproduce some experiments using 5-fluorouracil[6-^{3}H] obtained from a different commercial source than originally used, an EC_{50} of 0.08 μM was observed. An investigation was then undertaken to determine the cause for this sudden ten-fold increase in cytotoxicity. The 5-fluorouracil[6-^{3}H] preparation (sample A) was analysed using high-performance liquid chromatography (HPLC) and the radioactivity profile determined. An impurity seen at a retention volume of 76 ml was found to be 5-fluoro-2′-deoxyuridine[6-^{3}H].

EXPERIMENTAL

Instrumentation

HPLC was performed using an ALC/GLC 244 chromatograph equipped with a U6K injector, dual wavelength detector (254 nm, 280 nm) and a Houston Omniscribe dual pen 10-mV recorder (Waters Assoc., Milford, MA, U.S.A.). The column was prepacked stainless steel (250 × 10 mm) containing 10 μm LiChrosorb RP-18 obtained from Altex (Berkley CA, U.S.A.). The mobile phase was 1% acetonitrile in distilled water. It was filtered (filter apparatus 47 mm, 5.0 μm pore size; Millipore, Bedford, MA, U.S.A.) and then deaerated prior to use by the brief application of a vacuum. The temperature was ambient and the solvent flow-rate was 4.0 ml/min. The detector was set at a sensitivity of 0.5 a.u.f.s. and the chart speed of the recorder was 30 cm/h. The effluent from the HPLC system was collected using a Model 2112 Redirac fraction collector (LKB, Stockholm, Sweden). Radioactivity was determined

* Present address: School of Pharmacy, Florida A & M University, Tallahassee, FL 32307, U.S.A.

0021-9673/81/0000–0000/$02.50 © 1981 Elsevier Scientific Publishing Company

with a Mark III liquid scintillation system (Searle Analytic, Des Plaines, IL, U.S.A.) set for a 2-min counting period. Using the above conditions, retention volumes for 5-fluorouracil, 5-fluorouridine and 5-fluoro-2'-deoxyuridine were found to be 24, 52 and 76 ml, respectively.

Chemicals

5-Fluorouracil[6-^{3}H] (sample A), batch 30 (2.1 Ci/mmol) was obtained from Amersham (Arlington Heights, IL, U.S.A.); 5-fluorouracil[6-^{3}H] (sample B) (20 Ci/mmol) from Moravek Biochemicals (City of Industry, CA, U.S.A.); and Aquasol® from New England Nuclear (Boston, MA, U.S.A.). 5-Fluorouracil, 5-fluorouridine and 5-fluoro-2'-deoxyuridine were supplied by Nutritional Biochemical Co. (Cleveland, OH, U.S.A.).

Procedure

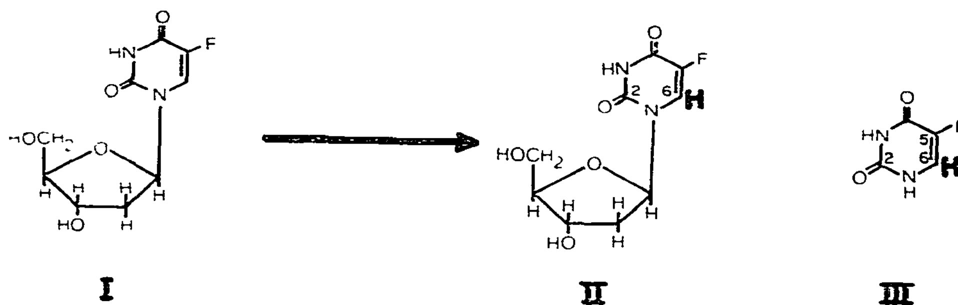
5-Fluorouracil[6-^{3}H] (1 μl) from either sample A or sample B was added to a vial containing 1000 μl of distilled water. To the vial was added a control mixture (50 μl) of cold 5-fluorouracil (0.4 mg/ml), 5-fluorouridine (2 mg/ml) and 5-fluoro-2'-deoxyuridine (2 mg/ml). After thoroughly mixing, a 200-μl sample was then injected into the HPLC system and fractions were collected at 1-min intervals. After placing 1 ml of a collected fraction in a scintillator vial and adding 10 ml of Aquasol®, the radioactivity of each fraction was determined. A radioactivity profile for sample A and sample B was then constructed. The remainder of the effluent in collected fractions 19 and 20 from the analysis of sample A were combined and the solvent removed by lyophilization. To the dry residue in a 2-ml ampoule was added 200 μl of 6 *M* HCl. The ampoule was sealed and then heated at 105°C for a period of 15 min. The contents of the ampoule were taken to dryness under a gentle stream of nitrogen at 60°C, reconstituted in 500 μl of distilled water and then injected into the HPLC system. Fractions were collected at 1-min intervals and the radioactivity profile determined as before.

RESULTS

When the HPLC radioactivity profiles of 5-fluorouracil[6-^{3}H] from the two different commercial sources were compared, it was found that sample A contained an impurity at a retention volume of 76 ml which was not present in sample B. Since this retention volume corresponded to 5-fluoro-2'-deoxyuridine, a sample of the impurity was subjected to acid hydrolysis. Previous studies had shown that 5-fluorouridine is stable whereas 5-fluoro-2'-deoxyuridine is unstable to 6 *M* HCl at 105°C. The HPLC analysis of the acid hydrolysis product gave a profile showing that almost all of the radioactivity was located at a retention volume of 24 ml corresponding to 5-fluorouracil[6-^{3}H].

DISCUSSION

The procedure[5] for the preparation of 5-fluorouracil[6-^{3}H] (sample A) was by the base catalysed tritium exchange reaction with 5-fluoro-2'-deoxyuridine (I).



Two major products are obtained from this reaction; 5-fluoro-2′-deoxyuridine[6-^{3}H] (II) and 5-fluorouracil[6-^{3}H] (III). These are separated by preparative paper chromatography to give two commercially available products from one chemical process. The 0.5–1% contamination of 5-fluorouracil[6-^{3}H] with 5-fluoro-2′-deoxyuridine-[6-^{3}H] is within the limits of the stated purity for this product (sample A).

In most drug metabolism studies the presence of radiochemical impurities at the level of 1 or 2% are frequently of little consequence[6]. However, when these impurities have a biological activity which is many times greater than the labeled drug, they present a very serious problem. An example of such a problem is presented in this paper. Previous cell culture studies using the S-49 cell line had shown that the cytotoxicity of 5-fluoro-2′-deoxyuridine is approximately 1000-fold greater than 5-fluorouracil. The presence of only a small amount of 5-fluoro-2′-deoxyuridine as an impurity will therefore have a great effect on the cytotoxicity of a sample of 5-fluorouracil when using a sensitive cell line as the S-49.

In summary, we would like to caution researchers using 5-fluorouracil[6-^{3}H] that it could contain 5-fluoro-2′-deoxyuridine[6-^{3}H]. Any effort made before starting a study with this drug would be well worth the time and effort in order to demonstrate the absence of this radiochemical impurity.

ACKNOWLEDGEMENT

Supported in part by USPHA grant GM-16496 from the National Institutes of General Medical Sciences and a HEW-MARC fellowship.

REFERENCES

1 A. Clarysse and G. Mathe, in G. S. Avery (Editor), *Drug Treatment*, Adis Press, Sydney, 1976, Ch. XXIII, p. 717.
2 W. L. Washtien and D. V. Santi, *Cancer Res.*, 39 (1979) 3397.
3 C. Heidelberger, in A. C. Sartorelli and D. G. Johns (Editors), *Handbook of Experimental Pharmacology, Vol. 38, Fluorinated Pyrimidines and Their Nucleosides, Part. 2*, Springer, New York, 1974, p. 193.
4 B. Ullman, A. Cohen and D. W. Martin, Jr., *Cell*, 9 (1976) 205.
5 N. Short, Techical Services, personal communication.
6 R. E. Ober, *J. Label. Compounds*, 2 (1966) 203.