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Note

Determination of two new metabolites of 1-hexylcarbamoyl-5-fluorouracil in biomedical specimens by high-performance liquid chromatography

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A recently discovered anticancer agent, 1-hexylcarbamoyl-5-fluorouracil (HCFU) [1], has more favorable therapeutic ratios than its parent compound, 5-fluorouracil (FU), by oral administration [2]. A study of the metabolic fate of HCFU showed that the major metabolites in the mammalian body were FU, 1-(carboxypentylcarbamoyl)-5-fluorouracil (CPEFU), and 1-(carboxypropylcarbamoyl)-5-fluorouracil (CPRFU) [3]. The structural formulae of these compounds are shown in Fig. 1. We have reported the high-performance liquid chromatographic (HPLC) determination of HCFU and its three metabolites in biomedical specimens [4].

In the analysis of sera of patients administered HCFU, we noted in the chromatograms a small, but well-defined peak, which does not arise from endogenous serum components. It is concluded that the peaks can be attributed to two hitherto unknown metabolites, 1-(5′-oxohexylcarbamoyl)-5-fluorouracil (OHCFU) and 1-(5′-hydroxyhexylcarbamoyl)-5-fluorouracil (HHCFU). The present paper is concerned with the HPLC analysis of the two new metabolites of HCFU.

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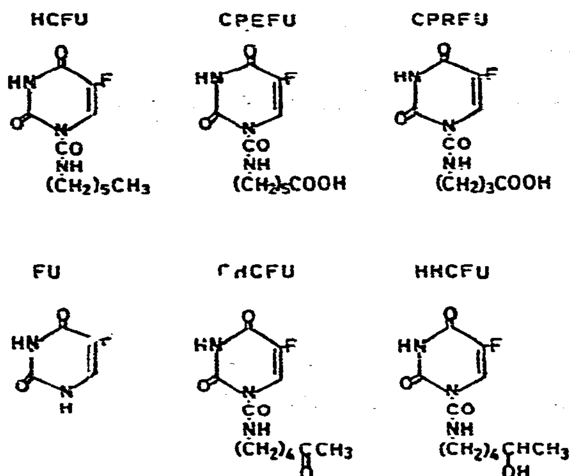

Fig. 1. Structures of HCFU and its metabolites.

EXPERIMENTAL

Materials

OHCFU, HHCFU, CPEFU and CPRFU were synthesized in the Laboratory of Mitsui Pharmaceutical (Tokyo, Japan), and are the generous gift from that company. The methods of synthesis and analytical data will be reported in the near future. Chemicals and the solvents for HPLC were of certified grade and products of Wako (Osaka, Japan).

HPLC instrumentation

A Waters Assoc. liquid chromatograph equipped with a Model 6000 solvent delivery system, a Model U6K universal injector, and a Model 440 UV detector, operated at 254 nm, and a μBondapak C_{18}/Porasil (particle size, 8–10 μm; 300 mm × 3.9 mm I.D.) column were used. The flow-rate of the mobile phase was 1 ml/min, at a pressure of about 1800 p.s.i.

Method

Standard solutions were prepared in analytical grade methanol so as to contain OHCFU and HHCFU at 0.5–10.0 ng/μl, and CPEFU and CPRFU at 1.0–25 ng/μl. Samples were injected into the chromatograph in a volume of exactly 1.0 μl using a 10-μl Hamilton syringe.

Human serum was collected from patients administered HCFU. HCFU-free biomedical samples were obtained either from a healthy adult man or from patients who had not been administered HCFU.

To 1.0 ml serum, 0.1 ml of 1 *N* HCl and 8 ml of ethyl acetate were added and the sample was extracted with vigorous shaking. The organic layer was separated by centrifugation and evaporated to dryness using a water-bath at 30° and a water-pump vacuum. The residue was dissolved in 50–200 μl of methanol for the HPLC analysis.

RESULTS AND DISCUSSION

Fig. 2 is a chromatogram of an extract of serum obtained from a patient administrered HCFU. Water—tetrahydrofuran (65:35) was used as the mobile phase. CPRFU and CPEFU were detected under these conditions [4]. On the chromatogram, an unassigned peak is seen between the peaks of CPRFU and CPEFU. The corresponding peak was absent in HCFU-free sera. The peak was likely to be due to a new metabolite.

The typical metabolic pathways in mammals for an alkyl group in a foreign compound are ω-, ω—1-, and β-oxidation. CPEFU is an ω-oxidation product of HCFU, and CPRFU is a β-oxidation product of CPEFU. An ω—1-oxidation product has not been found among the metabolites of HCFU.

The unassigned peak was suspected to be related to an ω—1-oxidation product. The probable ω—1-oxidation products are OHCFU and HHCFU.

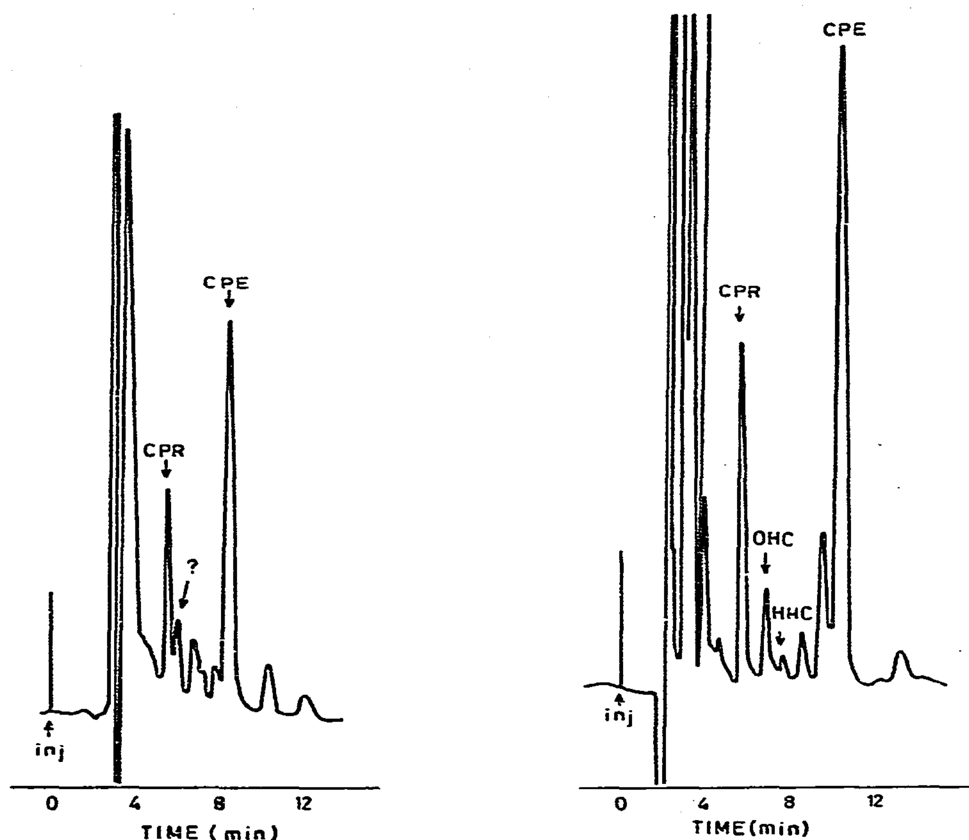

Fig. 2. Chromatogram of an extract of serum collected 3 h after the oral administration of 200 mg of HCFU to an adult man with cancer. The mobile phase is water—tetrahydrofuran (65:35).

Fig. 3. Chromatogram of an extract of serum collected 0.5 h after the oral administration of 200 mg of HCFU to an adult man with cancer. The mobile phase is water—tetrahydrofuran (75:25).

Therefore, OHCFU and HHCFU were synthesized. A methanolic solution of OHCFU, HHCFU, CPRFU and CPEFU was submitted to HPLC. With water—tetrahydrofuran (65:35) as the solvent system, the peaks of OHCFU and HHCFU partially overlapped, and had the same retention time as the unassigned peak of the serum. With water—tetrahydrofuran (75:25) as the solvent system, OHCFU and HHCFU were well separated from each other and from CPRFU and CPEFU. With the latter solvent system, four well-separated peaks with retention times of 5.85, 6.45, 6.75, and 10.15 min were obtained from the serum (Fig. 3). The peaks with retention times of 5.85 and 10.15 are attributed to CPRFU and CPEFU, respectively. Addition of OHCFU to the serum enhanced the peak at 6.45 min, whereas addition of HHCFU intensified the peak at 6.75 min. These results indicate that the peaks with retention times of 6.45 and 6.75 min in the chromatograms of the serum can be attributed to OHCFU and HHCFU, respectively.

Standard methanolic solutions of CPRFU, CPEFU, OHCFU and HHCFU were diluted with methanol to give the desired concentration. An aliquot of each solution was injected into the liquid chromatograph. Standard curves obtained by plotting the peak heights against the amounts of the substances injected were linear in the range 0.5—25 ng; 0.5 ng of each metabolite can be

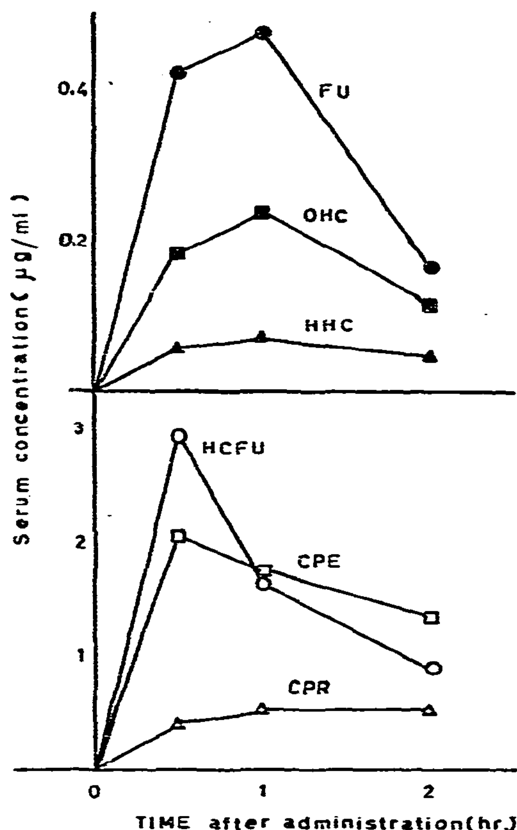

Fig. 4. Concentrations of HCFU and its metabolites in the serum of an adult man with cancer administered 200 mg of HCFU orally.

estimated using the available detector. In the determination of the substances in solutions containing 10 ng, standard deviations were 0.57, 0.55, 0.58, and 0.54 ng for OHCFU, HHCFU, CPRFU, and CPEFU, respectively. Appropriate amounts of these substances were added to HCFU-free serum and these spiked standards were carried through the procedure. Plots of the peak heights against the amounts of spiked metabolites gave straight lines, and comparison of the slopes with those obtained with the standard methanolic solutions afforded the recoveries, which were 101.2 ± 7.7% and 100.0 ± 5.3% for OHCFU and HHCFU, respectively. The recoveries of CPRFU and CPEFU were the same as those reported using the water—tetrahydrofuran (65:35) solvent system [4].

The present method permits the accurate determination of OHCFU, HHCFU, CPRFU and CPEFU in biological specimens in concentrations as low as 50 ng/ml, and is suited for monitoring the drug in therapeutic doses (600 mg/day). Fig. 4 is an example of the time—concentration curves in the serum of a patient administered 200 mg of HCFU orally. OHCFU and HHCFU seem to be minor metabolites and were not detected in the urine of patients administered HCFU.

These pharmacokinetic measurements of HCFU and its metabolites in tumor-bearing patients are continuing. The correlation of therapeutic effects to concentration in body fluids and tissues is under investigation.

ACKNOWLEDGEMENT

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