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Biomedical Applications

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Note

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

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1-Hexylcarbamoyl-5-fluorouracil (HCFU), a recently discovered anticancer agent [1], was shown to have more favorable therapeutic ratios than its parent compound, 5-fluorouracil (FU), by oral administration. This compound, a masked form of FU [2], is metabolized in a mammalian body to give FU and other derivatives such as 1-ω-carboxypentylcarbamoyl-5-fluorouracil (CPEFU), and 1-ω-carboxypropylcarbamoyl-5-fluorouracil (CPRFU) [3]. Although HCFU and all its metabolites have an anticancer activity, the main activity is considered to reside in FU. The adverse side-effect of HCFU is suspected to be connected with CPEFU and/or CPRFU [4].

Detailed pharmacokinetic studies could provide the necessary information for the optimal drug administration schedule, which offers maximum therapeutic response with minimum toxicity. A rapid, sensitive method of assay is required for this purpose, since the microbiological method does not give enough accuracy. In our laboratory, high-performance liquid chromatography (HPLC) is used for monitoring the anticancer agents such as FU, 1-(tetrahydro-2-furanyl)-5-fluorouracil (FT) and mitomycin C. This paper describes the HPLC analysis of HCFU, FU, CPEFU and CPRFU in biomedical specimens.

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EXPERIMENTAL

Reagents

HCFU, CPEFU and CPRFU used in this investigation were kindly supplied by Mitsuiseiyaku (Tokyo, Japan) and FU by Kyowahakkokogyo (Tokyo, Japan). All solvents for HPLC and chemicals were certified grade and products of Wakojunyaku (Osaka, Japan).

HPLC instrumentation

A Waters Assoc. liquid chromatograph equipped with a Model 6000 solvent delivery system, a Model U6K universal injector, a Model 440 UV detector operated at 254 nm and a μBondapak C_{18}/Porasil (particle size, 8—10 μm; 300 × 3.9 mm I.D.) column was used. The flow-rate of the mobile phase was 1 ml/min, at a pressure of about 1500 p.s.i.

Eluent

Although a number of mobile phases were tested during the investigation, no single solvent system afforded simultaneous resolution of HCFU, its metabolite and endogenous components of serum and tissues. Solvent systems used for the separation and determination of HCFU and the metabolites are described in the Results section.

Biomedical sample preparation

Human serum for analysis was prepared in the usual way. Tumors or other tissues obtained at operation or biopsy were homogenized to a 20% suspension in distilled water at 0°, and centrifuged at 7000 *g* for 30 min. To 0.5 ml of serum or the homogenate supernatant, 0.1 ml of 1 *N* HCl and 4.0 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 100 μl of methanol for analysis by HPLC.

RESULTS AND DISCUSSION

Fig. 1a shows a chromatogram of extract of human serum obtained from a patient administered HCFU. Water—acetonitrile (70:30) was used as mobile phase. An extract of tissue homogenate gave a similar chromatogram under the same conditions. The metabolites were not separated with this solvent system. No interfering peak arose from endogenous serum or tumor tissue components. This solvent system was shown to be suitable for the determination of HCFU. Recovery of HCFU added to serum was 97.2 ± 1.0%.

HCFU, CPEFU and CPRFU were separated with a solvent system composed of water—tetrahydrofuran—acetonitrile (50:35:15), their retention times being 9, 4.5 and 3.5 min, respectively. The retention times of CPEFU and CPRFU were so close that their simultaneous determination in dilute biological samples would meet with difficulties. A better separation was achieved with the solvent system tetrahydrofuran—water (35:65). Fig. 1b is a chromatogram of human serum with this solvent system. The retention times of CPRFU and CPEFU were 6.4 and 10.0 min, respectively. This solvent system

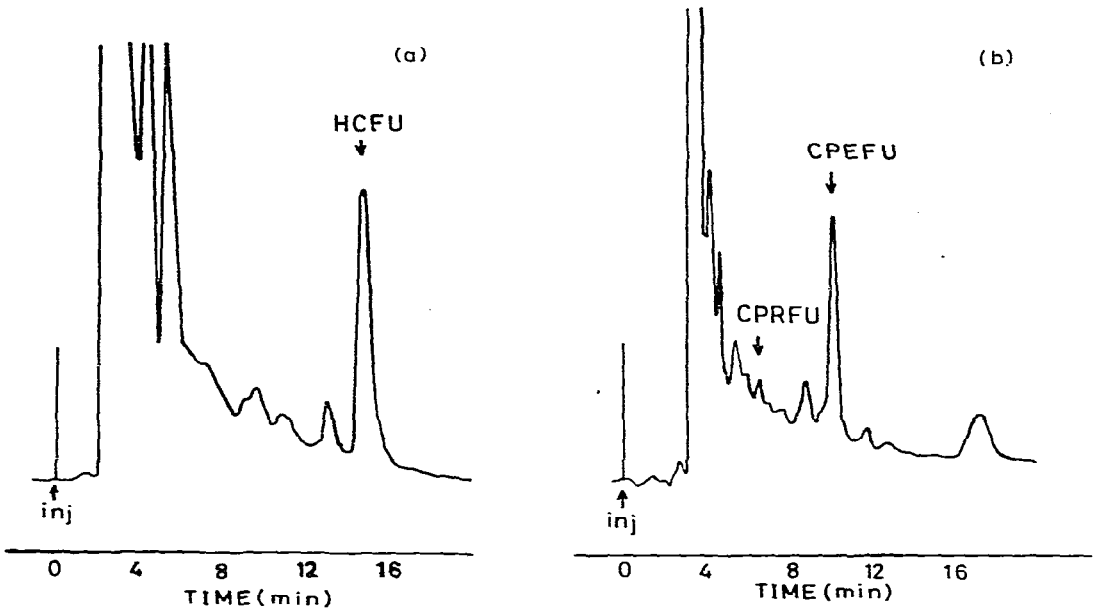

Fig. 1. Chromatograms of an extract of serum collected 3 h after the oral administration of 200 mg HCFU to an adult woman with cancer. The mobile phases are (a) water—acetonitrile (70:30) and (b) tetrahydrofuran—water (35:65). For HPLC conditions, see text.

proved suitable for the determination of CPRFU and CPEFU, but not of HCFU, since the latter compound has a long retention time. Recoveries of CPRFU and CPEFU added to serum were 79.4 ± 7.1 and 85.0 ± 6.2%, respectively.

Since FU is more hydrophilic than the other three compounds, it was not separated with the solvent system mentioned above. Use of a water—organic gradient method was not successful. FU was well separated from its derivatives and from the serum and tissue components with distilled water. Chromatograms of human serum and tissue homogenates of patients administered FU and HCFU are shown in Figs. 2 and 3. With water, a linear relationship was obtained between the peak height and the amount of FU injected into the chromatograph. Recoveries of FU added to serum and homogenate were 68.3 ± 4.9 and 54.2 ± 3.0%, respectively. The low recoveries come from the low lipophilicity of FU.

From the results described above the following solvent systems were used for the determinations; water—acetonitrile (70:30) for HCFU, water—tetrahydrofuran (65:35) for CPRFU and CPEFU, and water for FU. It was not too inconvenient to change the mobile phase during the HPLC study.

HCFU, CPEFU, and CPRFU were somewhat unstable in neutral and alkaline conditions, and gradually gave rise to FU. They were quite stable in 1 *N* HCl.

HCFU and its metabolites were dissolved in methanol at a concentration of 25 μg/ml. Appropriate dilution of these solutions with methanol was made to give the desired concentration. A 1-μl aliquot of the methanol solution was injected into the liquid chromatograph. Standard curves obtained by plotting the peak height against the amount of the substances injected were linear in

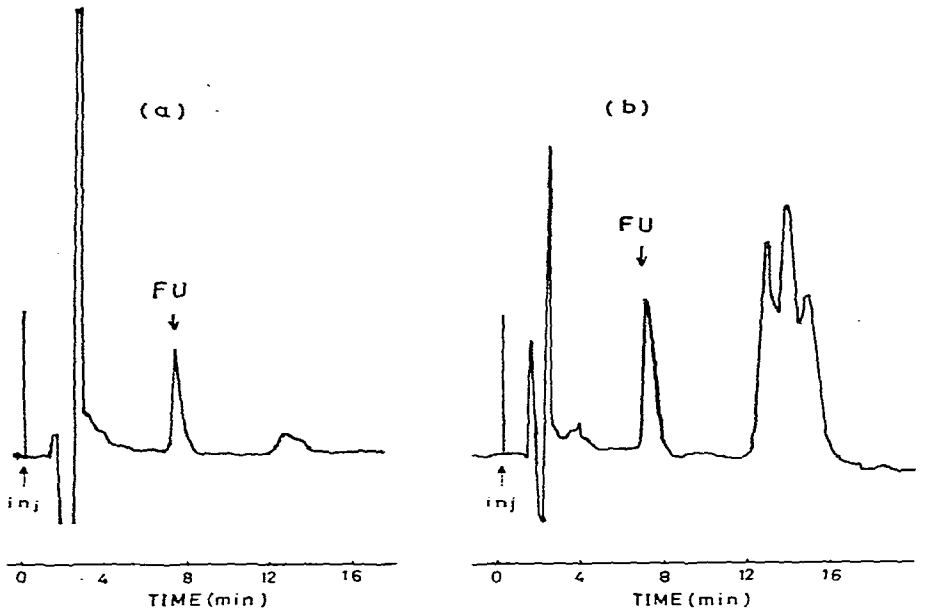

Fig. 2. Chromatograms of an extract of (a) serum and (b) tumor tissue homogenate collected 15 min after the selective arterial injection of 100 mg FU to an adult man with cancer. The mobile phase is distilled water. For HPLC conditions, see text.

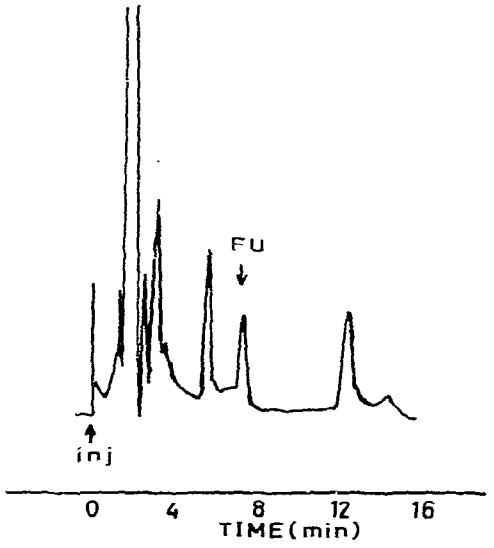

Fig. 3. Chromatogram of an extract of serum collected 3 h after the oral administration of 200 mg HCFU to an adult woman with cancer.

the range 0.5—25 ng. In the determination of the compounds in solutions containing 15 ng, standard deviations were 0.30, 0.66, 0.33, and 1.0 ng for FU, HCFU, CPRFU, and CPEFU, respectively.

Appropriate amounts of the substances were added to blank serum or homogenate. These spiked standards were carried through the procedure de-

scribed above and the peak heights plotted against the amounts of the spiked substances. The curves thus obtained were linear and comparison of the slopes with those obtained with the standard methanol solution gave recovery values for each substance described above. With this method, as little as 100 ng/ml serum and 250 ng/g tissue of HCFU and its metabolites were determined.

Along with the clinical studies, pharmacokinetic studies of the anticancer agent are in progress in this laboratory using the analytical method described. The present method is suited for drug monitoring studies in the therapeutic dose range (600—1800 mg/day). The results will be reported in the near future.

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