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QUANTITATIVE DETERMINATION OF 1-HEXYLCARBAMOYL-5-FLUOROURACIL AND ITS METABOLITES IN MAN*

OSAMU NAKAJIMA*, YOHKO YOSHIDA, TAKAKO ISODA, YUHZO TAKEMASA, YUKIO IMAMURA and YOSHIYUKI KOYAMA

Clinical Research Institute and Department of Medicine, National Medical Center Hospital, Toyama-cho 1, Shinjuku-ku, Tokyo, 162 (Japan)

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SUMMARY

A high-performance liquid chromatographic (HPLC) method for the quantitative determination of 1-hexylcarbamoyl-5-fluorouracil (HCFU) and its metabolites using μBondapak C_{18} and μPorasil has been developed. Two mobile phases containing PIC-B7 (consisting of acetic acid and 1-heptanesulphonic acid) were used for the separation, and good separations were obtained. With methanol—water (56:44) as the mobile phase, the separation of HCFU and its three metabolites was achieved within 4 min. With methanol—water (32:68) a new metabolite, 1-ω-carboxymethylcarbamoyl-5-fluorouracil, was revealed in human plasma. The recovery of each substance was 80% or greater and the sensitivity was at the nanograms per millilitre level. The coefficient of variation was less than 3.6% for each component.

INTRODUCTION

The antineoplastic activity of 1-hexylcarbamoyl-5-fluorouracil (HCFU) against various kinds of experimental tumours has been thoroughly examined by Kobari et al. [1] and Hoshi et al. [2], who found that HCFU is active against rapidly growing tumours and early or advanced slowly growing tumors. The metabolic pathway of HCFU in animals has been examined with ^{14}C-labelled HCFU [1]. However, the metabolic pathway of this drug in man has not been elucidated because the isolation and quantitative determination of HCFU and its metabolites have not been established. This paper describes a high-performance liquid chromatographic (HPLC) method for the quantitative determination of HCFU and its four metabolites in human plasma.

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EXPERIMENTAL AND RESULTS

Blood samples were obtained from patients who had been administered HCFU orally. Procedures for the extraction of HCFU and its metabolites are summarized in Fig. 1. The HPLC apparatus was obtained from Waters Assoc. (Milford, MA, U.S.A.). A stainless-steel column (300 × 3.9 mm I.D.) was filled with μBondapak C_{18} (8—10 μm) (Waters) and a pre-column (30 × 3.9 mm I.D.) filled with μPorasil (8—10 μm) (Waters) was used. The elution pattern was detected at 254 nm.

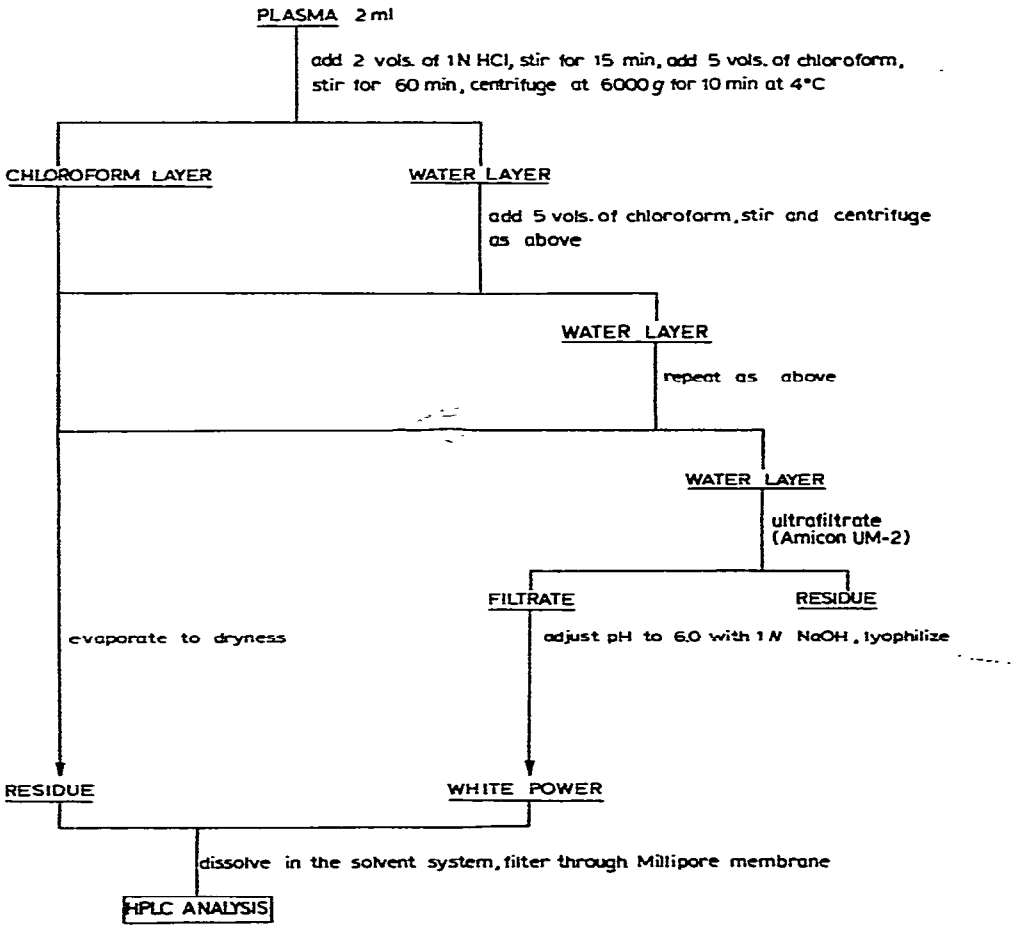

Fig. 1. Procedure for extraction of HCFU and its metabolites from human plasma.

All solutions used contained PIC-B7 (Waters), which consisted of acetic acid and 1-heptanesulphonic acid. One ampoule of PIC-B7 was dissolved in 1 l of water or methanol to give concentrations of 0.005 *M* acetic acid and 0.005 *M* 1-heptanesulphonic acid.

Authentic samples of HCFU, 1-ω-carboxymethylcarbamoyl-5-fluorouracil (CMEFU), 1-ω-carboxypropylcarbamoyl-5-fluorouracil (CPRFU), 1-ω-carboxy-

pentyl carbamoyl-5-fluorouracil (CPEFU) and 5-fluorouracil (5-FU) were kindly donated by Mitsui Pharmaceutical (Tokyo, Japan). The water used was distilled and deionized just before use with a Milli-R/Q water purifier. Methanol of chromatographic grade was obtained from Wako (Osaka, Japan).

Separation of authentic samples

Authentic samples were dissolved in methanol at a concentration of 0.12 μg/ml. A 20-μl aliquot of the solution was injected through the universal injector of the apparatus. When elution was performed with water—methanol (44:56), 5-FU, CMEFU, CRPFU, CPEFU and HCFU were separated and eluted with elution times of 1.6, 1.7, 1.8, 2.6 and 4.0 min, respectively. However, the separation of 5-FU and CMEFU was not satisfactory. The mobile phase was changed to methanol—water (32:68), and then the separation of 5-FU and CMEFU was adequate but the elution time was prolonged, as shown in Fig. 2, with HCFU being eluted after 17 min and CPEFU, CPRFU, CMEFU and 5-FU at 7.5, 3.2, 1.8 and 1.6 min, respectively.

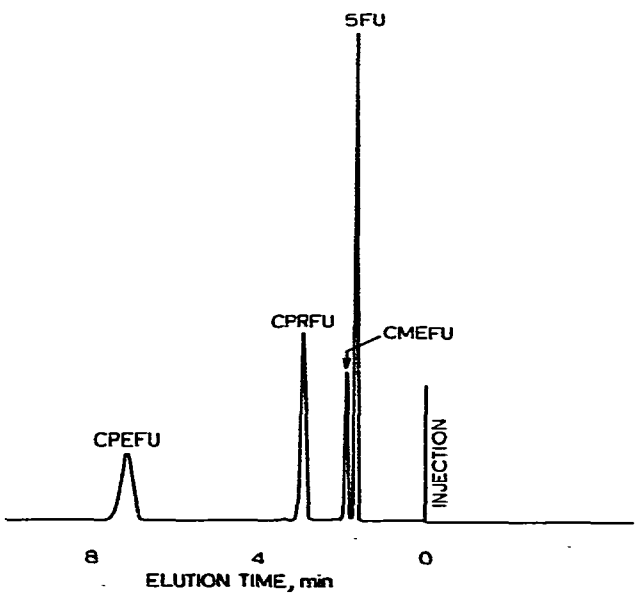

Fig. 2. Chromatogram of authentic HCFU and its metabolites. Mobile phase: methanol—water (32:68).

Calibration graphs for HCFU and its four metabolites

Calibration graphs for the determination of HCFU and its metabolites CPEFU, CPRFU, CMEFU and 5-FU were constructed from the results for authentic samples. The calibration graphs for the determination of HCFU, CPEFU, CPRFU, CMEFU and 5-FU can be expressed by the equations $y=1.958x-0.011$, $y=8.129x-0.284$, $y=4.112x-0.118$, $y=7.891x-0.654$ and $y=22.514x+0.140$, respectively. In addition, the r values for these equations were calculated to be 0.9985, 0.9962, 0.9990, 0.9882 and 0.9998, respectively.

Recovery of authentic samples from pooled plasma

The five authentic samples were dissolved in 2 ml of pooled plasma to give a final concentration of 0.12 μg/ml and then the samples were extracted as outlined in Fig. 1. The elution pattern obtained with methanol—water (32:68) as the mobile phase is shown in Fig. 3. The elution pattern of human pooled

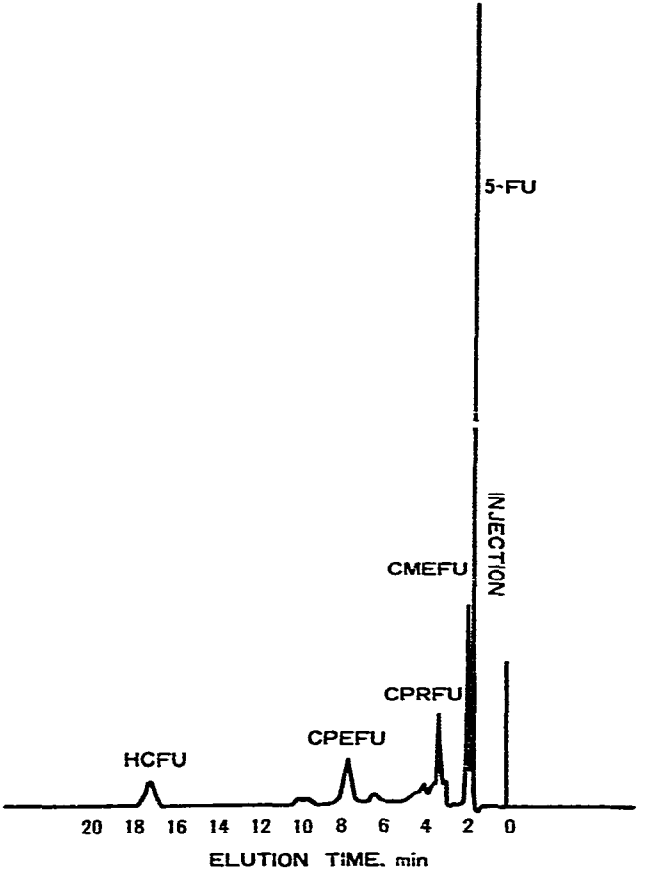

Fig. 3. Chromatogram of authentic samples of HCFU and its four metabolites extracted from human plasma. HCFU and the metabolites were dissolved in 2 ml of pooled plasma and stirred well, then extracted as shown in Fig. 1.

plasma used as a control did not exhibit any interfering peaks (Fig. 4). The recovery was 82 ± 1.0% for HCFU and 85 ± 1.0% for other metabolites. When different concentrations, ranging from 0 to 50 ng/ml, of the authentic compounds were injected into the HPLC apparatus the graphs of peak area against concentration showed good linearity up to 50 ng/ml in each instance. The coefficients of variation ($n = 5$) for HCFU, CPEFU, CPRFU, CMEFU and 5-FU were 3.2, 3.4, 3.4, 3.3 and 3.6%, respectively. In addition, the sensitivity for each component was 1 ng/ml.

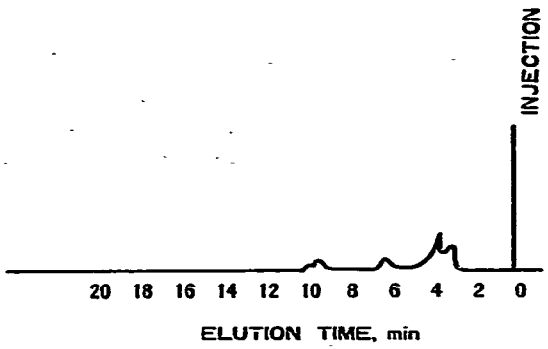

Fig. 4. Chromatogram of human plasma as control: 2 ml of pooled plasma were extracted as shown in Fig. 1 and the residues obtained were dissolved in methanol—water (56:44).

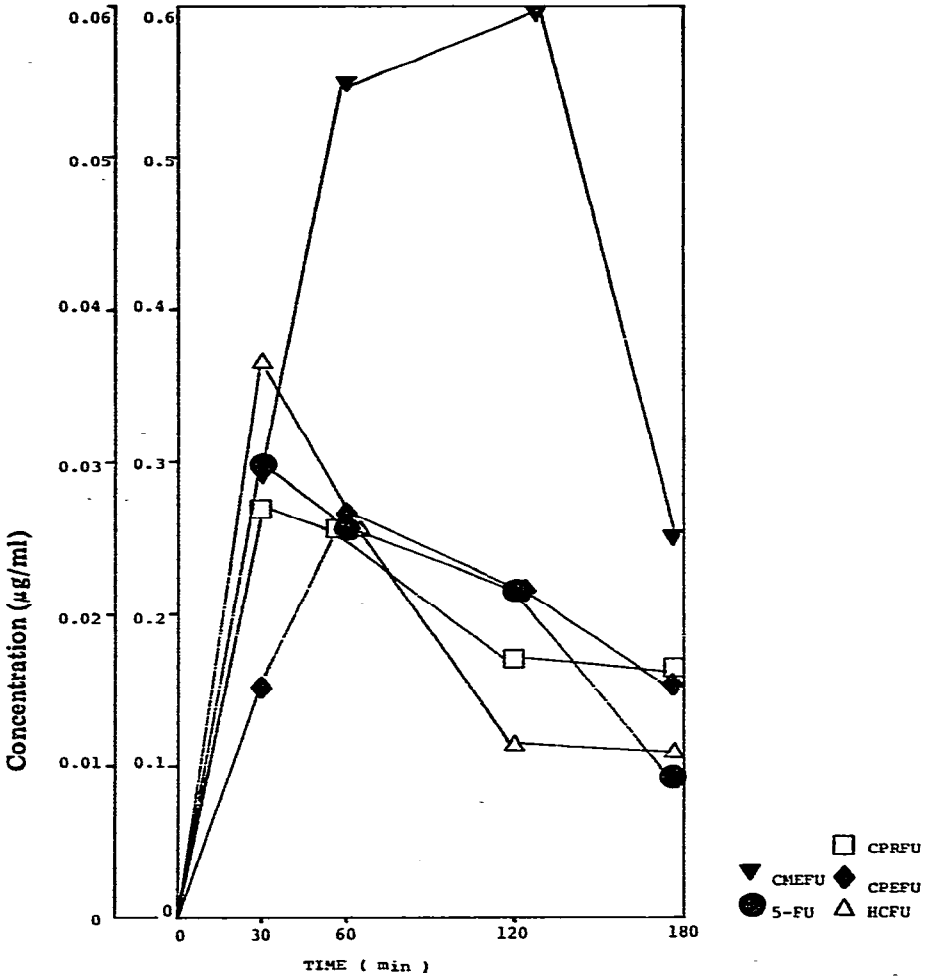

Fig. 5. Time course of HCFU and its four metabolites in a human patient. HCFU (300 mg) has been administered to a patient orally. Blood samples were taken from the cubital vein and the plasma was separated and extracted as shown in Fig. 1.

Concentration of HCFU and its metabolites in plasma

A patient was administered 300 mg of HCFU granules orally and then 5-ml blood samples were collected from the cubital vein after different times with 0.1 ml of heparin as shown in Fig. 5. The time course of HCFU showed a maximal value after 30 min. CPRFU and 5-FU also reached maximal concentrations after 30 min. However, CPEFU and CMEFU exhibited maximal concentrations 120 min after oral administration of HCFU.

DISCUSSION

Recently, many masked compounds of 5-FU have been synthesized, but there have been few papers [3] concerned with the pharmacokinetics of these drugs because of the difficulty of assaying them. We therefore tried to determine the concentrations of HCFU and its metabolites in human plasma.

The results on the experiments using authentic samples showed that μBondapak C_{18} is suitable for separating these compounds and exhibited good recovery and accuracy. Two mobile phases were examined for HPLC. With methanol—water (56:44) the separation of 5-FU and CMEFU was unsatisfactory but HCFU and its metabolites were eluted within 4 min. On the other hand, when methanol—water (32:68) was used, the separation of 5-FU and CMEFU was satisfactory, but the elution time of HCFU was prolonged to 17 min. Kono et al. [4] found that HCFU was eluted after approximately 15 min when water—acetonitrile (70:30) was used as the mobile phase. However, with tetrahydrofuran—water used as the mobile phase a longer retention time was found [4]. During the present experiments, PIC-B7 was added to the methanol—water [5, 6], which led to good separations of HCFU and its metabolites, as shown in Figs. 2 and 3.

In rats, HCFU is metabolized into 5-FU through two or three intermediates [1]. However, CMEFU has not been detected in the plasma of rat and it was therefore assumed that CMEFU is not an intermediate metabolite in the metabolic pathway of HCFU in humans also. However, in three out of five cases, CMEFU was detected in high-performance liquid chromatograms [7].

The time course of HCFU, CPEFU, CPRFU, CMEFU and 5-FU is shown in Fig. 5. The coefficient of variation of the determination of the concentration of each component was calculated from five experiments, and ranged between 3.2 and 3.6%, i.e., the accuracy was good.

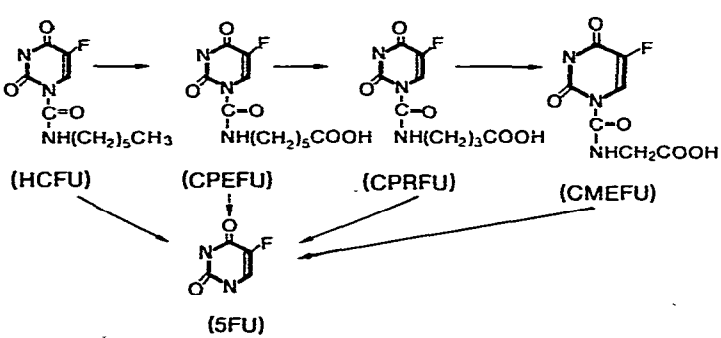

Fig. 6. Proposed metabolic pathway of HCFU in humans.

In conclusion, this system appears to be satisfactory for the quantitative determination of HCFU and its four metabolites in humans. Because it exhibits good recovery and has sufficient sensitivity it can be used to study the pharmacokinetics of HCFU. In addition, a new metabolite, CMEFU, has been detected using methanol—water (32:68) as the mobile phase, and a metabolic pathway of HCFU in humans is proposed in Fig. 6.

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