Kinetics and Metabolism of a New Fluoropyrimidine, 5'-Deoxy-5-fluorouridine, in Humans

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ABSTRACT

5'-Deoxy-5-fluorouridine (5'-dFUrd) is a new antineoplastic agent which possesses a higher therapeutic index in several experimental tumors compared to other fluoropyrimidines. During a Phase I trial, 5'-dFUrd, 1 to 15 g/sq m/week, was administered to patients as a 25- to 35-min i.v. infusion. Plasma kinetics and metabolism of 5'-dFUrd were investigated. The unmetabolized drug was measured by a high-performance liquid chromatography assay. 5-Fluorouracil and 5,6-dihydrofluorouracil, the two detected plasma metabolites, were quantitated by a gas chromatography-mass spectrometry methodology with a detection limit of 0.07 μM for both metabolites.

The disposition of 5'-dFUrd in humans at therapeutic doses followed a nonlinear kinetic process. Plasma concentrations of 5-fluorouracil generated in vivo represented approximately 6% of 5'-dFUrd concentrations and the 5-fluorouracil half-life ranged from 8.8 to 27.1 min. High plasma values of 5, 6-dihydrofluorouracil (14.5 to 30 μM) were observed in patients, indicating the importance of this pathway in humans.

INTRODUCTION

Since the synthesis of 5-FUra3 and the demonstration of its cytotoxicity were indicated in 1957 (12, 15), a number of fluorinated pyrimidine analogues have been synthesized for trial as anticancer drugs (8). 5'-dFUrd is a relatively new fluoropyrimidine derivative synthesized in 1976 by Cook et al. (11). 5'-dFUrd has been reported to have antineoplastic activity against several experimental tumors and dimethylbenzanthracene-induced skin carcinomas (1, 5, 16, 17). 5'-dFUrd also exhibits a higher therapeutic index (2, 5, 17) and is far less immunosuppressive than other fluorinated pyrimidines (1, 2, 20). It is thought that 5'-dFUrd acts as a prodrug of 5-FUra, releasing 5-FUra within the cell through the action of uridine phosphorylase (1, 23). However, the rate and duration of 5-FUra production (i.e., plasma levels) have not been evaluated in humans. We recently reported the necessity to determine 5-FUraH2 levels after 5-FUra administration in humans (13) because of the importance of the catabolic pathway (a) to design a more complete pharmacokinetic model of 5-FUra in humans than those described in the literature (6, 10) and (b) to set up more effective dosage regimens as suggested by various authors (9, 18, 21). In addition, the knowledge of 5-FUraH2 formation after 5'-dFUrd administration might provide an important means of understanding the metabolism in vivo and hence the antineoplastic activity of this new fluoropyrimidine derivative.

In this paper, we report kinetic and metabolic studies of 5'-dFUrd during a Phase I clinical trial. A metabolic pathway including the conversion of 5'-dFUrd to 5-FUra and the reduction of 5-FUra to 5-FUraH2 is proposed in humans.

MATERIALS AND METHODS

Drugs and Chemicals. 5'-dFUrd, 5-FUra, 5-FUraH2, and 5-BrUra were generously supplied by Hoffmann-LaRoche AG, Basel, Switzerland. All other biochemicals were of analytical grade and were purchased from Sigma Chemical Co., St. Louis, Mo.; Fluka AG, Buchs, Switzerland; and Carlo Erba, Milan, Italy.

Patient Protocol. Three patients with pancreatic or colon carcinomas, 2 of whom having liver and/or brain metastasis, were included in this study. The patients were randomly recruited from a Phase I clinical trial with 5'-dFUrd. 5'-dFUrd was provided in sterile vials containing 1 g of lyophilized drug. This was dissolved in sterile water with a maximum final concentration of 100 mg/ml. The prescribed dose was then infused i.v. at a constant rate over 25 to 35 min. Dosage escalation between 1 and 15 g/sq m/week, the maximum tolerated dose, was carried out according to a modified Fibonacci scheme (14). A transient neutropenia was noticed in the patient who received 15 g/sq m/week. No patient received chemotherapy or radiotherapy during the last 4 weeks prior to the study. Informed consent was obtained on all patients for participation in this study.

Blood Sampling. Venous blood samples were drawn at specified times into 10-ml oxalated tubes over 4 hr for the low doses (<2 g/sq m) and over 6 hr for the high doses (2-15 g/sq m) after the end of the infusion. The blood samples were then centrifuged for 15 min at 2400 × g. The plasma was adjusted to a pH value of approximately 7, frozen, and stored at −20° until analysis. A pretreatment plasma sample was also processed for each patient to verify the absence of interferences by endogenous compounds with the chromatographic procedures.

Analytical Methods. Unmetabolized 5'-dFUrd in plasma was assayed by a reversed-phase high-performance liquid chromatography method as described previously (22). After addition of 3-methylkanthine as an internal standard and precipitation of proteins by methanol/acetic acid 0.3 M, 5'-dFUrd was extracted with 20 ml of diethyl ether/isopropyl alcohol (8/2). Recovery of parent drug from normal plasma to which 0.2 μM to 10 μM 5'-dFUrd had been added was approximately 95%. Following evaporation of the organic phase to dryness under N2, the residue was resuspended in 50 to 100 μl of water. Ten to 25-μl aliquots were analyzed on a Hewlett-Packard Model 1084 B high-performance liquid chromatograph equipped with automatic injector, variable wavelength spectrophotometer, and chromatograph terminal (Hewlett-Packard 79850 ALC). All analyses were performed on a 5-μm (125 x 4 mm) Lichrosorb RP-18 (Merck, Darmstadt, Germany). Elution was carried out isocratically at 1 ml/min with water/methanol/
acetonitrile (97/1.5/1.5). Column temperature was maintained at 25°C, and absorbance was monitored at 269 nm. Under these conditions, retention times of 3-methylxanthine and 5′-dFUr were 6.72 and 12.34 min, respectively. The limit of sensitivity of this technique was about 0.2 μM.

After administration of 5′-dFUr, the 2 plasma metabolites, 5-FUra and 5-FUraH₂, were measured simultaneously by a new GC/MS method (4). Following addition of 5-BrUra as internal standard to 0.1 to 0.8 ml of plasma, pH was adjusted precisely to 6.8 to 6.9 with 0.05 N HCl and/or 0.05 N NaOH; 5-FUra and 5-FUraH₂ were then extracted with 20 ml of diethyl ether/isopropyl alcohol (8/2). After evaporation of the organic phase to dryness at 40°C under N₂, the residue was suspended in 400 μl of N,N-dimethylacetamide, 80 μl of 24% tetramethylammonium hydroxide in methanol, and 180 μl of pentafluoropropionic acid. After storage for 30 min at room temperature and 15 min in a drying oven at 80°C, the N,N-dipentylated derivatives were reextracted into 3 ml of diethyl ether. The resulting extract was concentrated to dryness at 40°C under N₂, and the residue was dissolved in 100 μl of methyl alcohol. Portions of 1 to 3 μl were then injected into the GC/MS system. All analyses were performed in EI or CI mode on a Hewlett-Packard Model 5980 or 5980 GC/MS interfaced to a Hewlett-Packard 5934 A data system. Gas chromatography separations were accomplished using a glass column (2.5 m x 3 mm inner diameter) packed with 3% OV 275 (Alltech Associates, Inc., Deerfield, Ill.) on Chromosorb WHP (80 to 100 mesh; Spiral Co., Dijon, France). The oven was programmed at 210°C. The injector, ion source, and GC/MS interface (jet separator) were 250, 200, and 260°C, respectively. Helium was used as carrier gas (flow rate, 30 ml/min). The mass spectrometer was operated under the following conditions: emission current, 300 μA; electron energy, 70 eV for EI and 150 eV for CI. In CI mode, methane was the reagent gas (ion source pressure, 1 torr). Quantitation of 5-FUra and 5-FUraH₂ in kinetic studies were made by selected ion monitoring. The data system was programmed to record the ions m/z 253 (5-FUra), m/z 203 (5-FUraH₂), and m/z 261 (5-BrUra) in EI mode and ions m/z 273 (5-FUraH₂) and m/z 331 (5-BrUra) with the CI system. EI mode permitted the simultaneous measurement of 5-FUra and 5-FUraH₂ with a detection limit of 0.07 and 0.6 μM, respectively. CI was used where smaller amounts of 5-FUraH₂ were observed in plasma patients (the sensitivity of this assay was 0.07 μM). For both systems, the percentage of recovery for 5-FUra and 5-FUraH₂ over the range of the concentrations expected in vivo was approximately 70.

Pharmacokinetic Analysis. Elimination half-lives of the 5′-dFUr and 5-FUra were obtained by linear regression analysis of the kinetics terminal points (least-squares method) (13). For the calculation of the area under the curve (AUC), the trapezoidal rule on all experimental points with extrapolation to infinity was applied. The total plasmatic clearance (CL) of 5′-dFUr was assessed according to the relationship:

\[
CL = \frac{Dose}{AUC}
\]

RESULTS

The chemical decomposition of 5′-dFUr during analysis and contamination of the 5′-dFUr dosage form with its metabolites was insignificant (data not shown) so the observed 5-FUra and 5-FUraH₂ concentrations could represent only the 5′-dFUr metabolism. Chart 1 shows plasma concentrations of 5′-dFUr and its plasma metabolites 5-FUra and 5-FUraH₂ after i.v. infusion of 1 and 2 g/sq m for 25 to 35 min in Patients 1 and 2, respectively. A rapid decrease of 5′-dFUr plasma levels was observed within the first 2 to 3 hr. 5-FUra and 5-FUraH₂ in plasma were at measurable concentrations within 1 hr in these patients. The levels of 5-FUra approximated 29 to 43 μM at the end of the infusion and 0.38 to 0.53 μM after 1 hr. The apparent elimination half-life of 5-FUra was 8.8 and 10.3 min. 5-FUraH₂ plasma concentrations at the same time periods were between 15 and 4 μM with a peak level of 20 to 24 μM at 10 min. Chart 2 illustrates the disappearance of 5′-dFUr, 5-FUra, and 5-FUraH₂ from plasma in Patient 3 following administration of 5′-dFUr, 15 g/sq m, over 27 min. At this dose, the kinetics of the parent drug shows a convex log plasma concentration-time curve consistent with a nonlinear or saturable clearance process. The 5-FUra concentration reached a maximum level of 175 μM following therapy and decreased to 0.5 μM after 6 hr. A similar convex behavior for 5-FUra on the log concentration-time graph was observed. This disappearance curve differs...
J-P. Sommadossi et al.

Table 1

<table>
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<tr>
<th>Patient</th>
<th>Dose (g/sq m/wk)</th>
<th>Infusion time (min)</th>
<th>AUC (μM × hr)</th>
<th>CL (ml/min)</th>
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*a t1/2, apparent elimination half-life; AUC, area under plasma level-time by trapezoidal rule; CL, total plasmatic clearance.

markedly from previous kinetic studies after 5'-FUra administration (10). A possible explanation is that this phenomenon might reflect the saturability of both enzymatic processes, uridine phosphorylase and dihydrouracil dehydrogenase, concomitant with the saturation of another elimination route for the 5'-dFUrd. The 5'-FUra elimination half-life was calculated as 27.1 min. In contrast, 5'-FUraH2 plasma concentrations remained at a constant level of 22.7 ± 2.6 μM (mean ± coefficient of variation of the initial 12 points) by the end of the infusion to 5 hr and subsequently declined. The 5'-dFUrd dose with infusion time and pharmacokinetic parameters is summarized in Table 1.

DISCUSSION

5'-dFUrd, a fluoropyrimidine derivative, has recently been shown to have activity against several tumors (1, 5, 16, 17). Pharmacokinetic and metabolic studies were undertaken in humans based on a postulated metabolic pathway which included the conversion of 5'-dFUrd into 5'-FUra and the reduction of 5'-Fu to 5'-FUraH2 (Chart 3). The high-performance liquid chromatography assay for 5'-dFUrd was specific for unmetabolized drug and had a sensitivity limit of 0.2 μM (22). The new highly specific and sensitive GC/MS technique represents a significant advance over previous similar 5-FUra assays, allowing the simultaneous measurement of 5'-FUra and 5'-FUraH2 after administration of 5'-dFUrd (4). The sensitivity of both methods permitted the quantitation of 5'-dFUrd and its metabolites 5'-FUra and 5'-FUraH2 easily in all postinfusion samples obtained from patients treated in this trial.

The disappearance of the parent drug approximated first-order kinetics for low doses between 1 and 2 g/sq m. In contrast, at the maximum nontoxic dose, 15 g/sq m, the 5'-dFUrd concentration profile can be described by a zero-order process over 1 hr followed by a first-order process, consistent with a nonlinear model for this drug. Furthermore, the significant increase in apparent half-life of 5'-dFUrd and a greater than proportional increase in area under the drug concentration-time curve (Table 1) as doses were escalated during this Phase I trial confirmed the dose-dependent relationship. The renal excretion of 5'-dFUrd, another factor that might account for this dose dependency, is currently being investigated.

Previous studies which examined the mechanism of action of this fluoropyrimidine derivative demonstrated that metabolism occurred in vitro and in vivo with formation of 5'-FUra (1). In this study, at low doses of 5'-dFUrd (1 and 2 g/sq m), 5'-FUra plasma levels never exceeded 43 μM, and the highest value (175 μM) was observed after i.v. infusion of 5'-dFUrd, 15 g/sq m. These 5'-FUra concentrations in plasma, compared to those obtained after 5'-FUra administration at therapeutic doses, led to the proposal that the conversion of 5'-dFUrd to 5'-FUra in humans is not complete.

Some reports on 5'-FUra pharmacokinetics have suggested that the dose dependency was related to the conversion of 5'-FUrd to 5'-FUraH2 (3, 6, 10, 19). High values of 5'-FUraH2 (from 14.5 to 30 μM) were seen in all patients treated in this clinical trial. The 5'-FUraH2 plasma levels were never proportional to the administered doses of 5'-dFUrd or to 5'-FUra formed and even remained relatively constant as plasma concentrations of the parent drug and 5'-FUra increased. Therefore, these data indicate the saturation of a metabolic or transport process in the formation after administration of 5'-dFUrd in humans. However, further studies are needed to compare the
rate and the amount of 5-FUraH₂ produced in vivo after 5'-dFUr or 5-FUra administration.

These data appear to be fundamental in the design of a nonlinear model for the kinetics of 5'-dFUr and its metabolites 5-FUra and 5-FUraH₂ to provide rational design of regimens for this new antineoplastic agent.

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REFERENCES

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