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, 15) 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-FUra 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-FUra 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 them having liver and/or brain metastasis, were included in this study. The patients were randomly recruited from a Phase 1 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 μg/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 g/sq m) after the end of the infusion. The blood samples were then centrifuged for 15 min at 2400 x g. The plasma was adjusted to a pH value of approximately 7, frozen, and stored at -20 °C 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-methylxanthine 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 including the conversion of 5'-dFUrd to 5-FUra and the reduction of 5-FUra to 5-FUraH₂ is proposed in humans.
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RESULTS

The chemical decomposition of 5'-dFURd during analysis and contamination of the 5'-dFURd dosage form with its metabolites was insignificant (data not shown) so the observed 5-FUra and 5-FUraH2 concentrations could represent only the 5'-dFURd metabolism. Chart 1 shows plasma concentrations of 5'-dFURd 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'-dFURd 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'-dFURd, 5-FUra, and 5-FUraH2 from plasma in Patient 3 following administration of 5'-dFURd, 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

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Chart 1. Disappearance of 5'-dFURd and its metabolites 5-FUra and 5-FUraH2 from the plasma in Patient 1 after a 25-min infusion of 5'-dFURd, 1 g/sq m (A) and in Patient 2 after a 35-min infusion of 5'-dFURd, 2 g/sq m (B). Zero time refers to the end of the infusion.
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'-dFUrd 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 new 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-FUra 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'-dFUrd 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 5-FUraH₂ formation after administration of 5'-dFUrd in humans. However, further studies are needed to compare the

![](chart1.png)

**Chart 1. Proposed metabolic pathway of 5'-dFUrd in humans.**

**Table 1. Dose and pharmacokinetic data of 5'-dFUrd in plasma**

<table>
<thead>
<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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<td>1</td>
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<td>30</td>
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* t₁/₂, apparent elimination half-life; AUC, area under plasma level-time by trapezoidal rule; CL, total plasmatic clearance.
rate and the amount of 5-FUraH₂ produced in vivo after 5'-dFUrd or 5-FUra administration.

These data appear to be fundamental in the design of a nonlinear model for the kinetics of 5'-dFUrd 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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