Correlation between Dihydropyrimidine Dehydrogenase Activity in Peripheral Mononuclear Cells and Systemic Clearance of Fluorouracil in Cancer Patients

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ABSTRACT

Dihydropyrimidine dehydrogenase (DPD) is the initial key enzyme in the catabolism of 5-fluorouracil (5-FU). We measured DPD activity in lymphocytes from 57 consecutive head and neck cancer patients while simultaneously monitoring 5-FU pharmacokinetics during 5-day, continuous infusion (1000 mg/m²/day) 5-FU therapy (82 cycles in total). The mean value for DPD activity was 0.186 ± 0.068 (SD) nmol/min/mg of protein (range, 0.058 to 0.357). The mean value for 5-FU clearance was 2522.6 ± 684.2 ml/min/m² (range, 1052 to 4029). A significant linear correlation was observed between DPD activity and 5-FU clearance (r = 0.716, P < 0.0001). DPD activity was poorly correlated to plasma uracil concentrations (r = -0.260, P = 0.0215). Likewise, plasma uracil concentrations were poorly correlated to 5-FU clearance (r = -0.214, P = 0.0595). In patients evaluated for more than one cycle (n = 18), there was large intrapatient variability in both DPD activity and 5-FU clearance. No significant difference was noted between cycles for DPD activity or 5-FU clearance (Kruskal-Wallis test). Monitoring DPD activity in lymphocytes may be useful in identifying patients at risk for altered 5-FU disposition.

INTRODUCTION

5-FU4 is commonly used in the treatment of digestive, breast, and head and neck cancer. Its mechanism of cytotoxicity is complex, requiring conversion of 5-FU into cytotoxic nucleotides. Inhibition of thymidylate synthase by 5-fluoro-2′-deoxyuridine 5′-monophosphate is regarded as the main mechanism of cytotoxicity for 5-FU, although incorporation of fluorouridine 5-triphosphate into RNA and 5-fluoro-2′-deoxyuridine 5′-triphosphate into DNA may contribute to its cytotoxic effects (1). The clinical efficacy of 5-FU continues to be enhanced through discoveries of synergistic drug combinations and methods of biochemical modulation (2).

Significant interpatient variability in the pharmacokinetics of 5-FU, including the systemic clearance and AUC, has been observed following continuous infusion of 5-FU in cancer patients (2). Intrapatient variability in the systemic clearance and systemic exposure (i.e., the AUC) of 5-FU has been demonstrated to be clinically significant. Several studies have reported correlations between various 5-FU pharmacokinetic parameters and indices of patient toxicity (3-7). A previously reported relationship between the 5-FU AUC and toxicity (both hematological and nonhematological) (3) was recently prospectively validated (4). Other studies have identified significant correlations between the 5-FU AUC (5) and steady-state concentrations (6, 7) and hematological and/or nonhematological toxicity. Indicative of these relationships, the therapeutic index of 5-FU can be significantly improved by adjusting 5-FU doses based on plasma pharmacokinetic data (4).

Although diminished 5-FU systemic clearance has been reported in certain disease states (8), there are no precise means (e.g., hepatic function tests, etc.) other than pharmacokinetic monitoring of plasma 5-FU concentrations which can identify patients at risk for altered 5-FU disposition and subsequent toxicity.

DPD is the initial enzyme in the catabolism of 5-FU (9). The majority of an administered 5-FU dose (>80%) is catabolized by DPD, with the remaining unchanged drug excreted in the urine. The majority of DPD is reported to be in the liver, but since 5-FU systemic clearance significantly exceeds liver blood flow, DPD activity in other tissues contributes to metabolism (9). In vitro studies have demonstrated that hepatic tissue and lymphocytes have the greatest DPD activity (10). A wide interpatient range of DPD activity has been reported for a number of human tissues (11). The clinical importance of DPD in the catabolism of 5-FU has recently been demonstrated with the identification of five cancer patients with suspected or proven DPD deficiency (12-15). After receiving 5-FU, these patients had significantly delayed 5-FU systemic clearance and developed life-threatening toxicities (lethal toxicity in two patients).

Plasma and urinary pyrimidines (uracil, thymine) are significantly elevated in individuals with DPD deficiency (12, 13), but their utility as a marker for identifying patients at risk for altered 5-FU disposition has not been evaluated. Recently, Harris et al. (16) reported a correlation between DPD activity in lymphocytes and plasma 5-FU concentrations, thus suggesting that DPD activity in lymphocytes may serve as a biochemical marker of 5-FU pharmacokinetics. Thus, DPD activity in lymphocytes could be useful for identifying individuals at risk for altered 5-FU disposition.

The purpose of this study was to evaluate in a large population of patients treated by 5-FU (a) the relation between DPD activity in human lymphocytes and systemic clearance of 5-FU, (b) the relation between plasma uracil concentrations and 5-FU systemic clearance, and (c) the relation between DPD activity and plasma uracil concentrations.

MATERIALS AND METHODS

Materials. All chemicals were obtained from Sigma Chemical Co. (St. Quentin Fallavier, France) and were of the highest purity available. Dihydrofluorouracil was kindly provided by Roche Laboratories (Nevilly, France). 14C-labeled 5-FU (55 mCi/mmol) was obtained from Amersham (Amersham, United Kingdom). Histopaque (Ficoll and sodium diatrizoate) was purchased from Sigma. RPMI 1640 cell culture medium was purchased from Whittaker Bioproducts (Fontenay sous Bois, France).

Patients and Treatment Regimen. From January to July 1991, 57 previously untreated consecutive patients (50 males and 7 females) with...
histologically confirmed carcinoma of the head and neck were entered into the study. Informed consent was obtained from each patient prior to study entry. The median age of the patients was 61 yr (range, 42 to 87 yr). Prior to receiving chemotherapy, hepatic, renal, and hematological function tests were performed for all patients to ensure normal organ function (i.e., hemoglobin ≥ 10 g/dl, WBC ≥ 3,000/µl, platelets ≥ 100,000/µl for hematological function; bilirubin ≤ 1.5 mg/dl for hepatic function; serum creatinine ≤ 1.3 mg/dl for renal function). Each patient received a chemotherapy regimen consisting of cisplatin, 100 mg/m², on Day 1 (i.v. infusion) followed by 5-FU, 1000 mg/m²/24 h, administered on Days 2 to 6 by continuous infusion with a controlled flow pump. 5-FU doses were adjusted at midcycle (48 h) based on our previous finding that the therapeutic index of 5-FU can be significantly improved by adjusting 5-FU doses based on the 5-FU AUC midcycle (4). The interval between cycles was 3 wk. The total number of cycles analyzed for all patients was 82. Eighteen patients were followed for more than one cycle (mean, 2.4 cycles; range, 2 to 4).

Plasma Uracil and 5-FU Samples. Ten ml of blood were collected in a heparinized tube at 8 a.m. on Day 0 for the determination of plasma uracil concentrations. For 5-FU plasma samples, approximately 10 ml of blood were collected in heparinized tubes at 8 a.m. and 5 p.m. during each day of 5-FU administration. The samples were immediately centrifuged (10 min, 250 × g), and the plasma was stored at −20°C until analyzed.

Collection of Lymphocytes. Because of the previously reported circadian variability of DPD activity (16) in human peripheral lymphocytes, blood samples were collected between 8 a.m. and 11 a.m. to minimize the influence of circadian variability. In patients who had received prior cycles of cisplatin/5-FU therapy, lymphocytes were collected at least 21 days after receiving this therapy. Approximately 20 ml of blood were collected in heparinized tubes, transferred into a 50-ml tube, and mixed with 15 ml of RPMI cell culture medium (Whittaker Bioproducts, Inc.). The RPMI/blood mixture was carefully layered on 15 ml of Ficoll and sodium diatrizoate (Histopaque; Sigma Chemical Co.). After centrifugation (30 min, 400 × g), the lymphocytes were removed and washed twice with phosphate-buffered saline. Contaminating RBC were hypotonically lysed. The lymphocytes were suspended in 35 mm sodium phosphate buffer, freeze-thawed 3 times, and then sonicated on ice (5 times for 10-s intervals). This solution was centrifuged for 30 min (20,000 × g, 4°C), and the supernatant was kept on ice until assayed (within 15 min) for DPD activity.

Determination of DPD Activity. The supernatant was assayed for DPD activity by a previously reported method (16). A reaction mixture was prepared in 35 mm sodium phosphate buffer, pH 7.5, consisting of 2.5 mm MgCl₂, 250 µM NADPH, 20 µM 14C-labeled 5-FU (55 mCi/mmoll (Amersham), and 0.05 to 0.1 mg of cytosolic protein (total volume of reaction mixture, 125 µl). The samples were incubated for 1 h at 37°C. Preliminary studies indicated the reaction was linear up to 2 h. The reaction was terminated by the addition of an equal volume of ice-cold ethanol and immediately stored at −20°C for 30 min. The samples were centrifuged (400 × g, 5 min) to remove precipitated proteins, and the supernatant was analyzed for the presence of 5-FU metabolites by a previously reported high-pressure liquid chromatographic method (17). Output was monitored by a radioactive flow monitor (Flow-One, Flocel, Tampa, FL) using a 4.5:1 scintillation fluid:mobile phase ratio. Cytosolic protein concentrations were determined by the dye-binding method (Bio-Rad S.A., Ivry sur Seine, France) using bovine γ-globulin as the protein standard. As observed by other investigators (10), dihydrofluorouracil is the only product formed when determining DPD activity from crude cytosolic extracts of lymphocytes. Enzyme activity was expressed as nmol of dihydrofluorouracil/min/mg of protein. This method for determining DPD activity was both sensitive (limit of sensitivity, 0.010 nmol of product) and reproducible (intra- and interday coefficients of variation, <10%).

Determination of Uracil and 5-FU Plasma Concentrations. Both uracil and 5-FU plasma concentrations were simultaneously determined by a high-performance liquid chromatographic method (18). The intra- and interday coefficients of variation for uracil and 5-FU were <10%. The percentage of recoveries for 5-FU and uracil was 74 and 75%, respectively. Limits of sensitivity for uracil and 5-FU were 10 ng/ml. Concentrations below the limit of sensitivity were recorded as zero.

Pharmacokinetic Analysis. The 5-FU AUC was determined by the logarithmic trapezoidal method from time 0 until 48 h (19). 5-FU systemic clearance was calculated by dividing the dose administered during 48 h by the AUC₀⁻⁴₈ h (20).

Statistical Analysis. The distribution of values for DPD activity, uracil concentration, and 5-FU clearance was tested for normality by χ² analysis. In patients having more than one determination of DPD activity, intracycle differences in DPD activity were assessed by the Kruskal-Wallis test. Intracycle differences in 5-FU clearance were determined in a similar manner. Correlations between DPD activity and 5-FU clearance, between DPD activity and plasma uracil concentrations, and between plasma uracil concentrations and 5-FU clearance were assessed by simple linear regression analysis. The Statgraphics statistical package was used for all statistical calculations (Statistical Graphics Corp., Rockville, MA). The a priori level of significance was set at P < 0.05.

RESULTS

The values for DPD activity were normally distributed. The mean DPD concentration was 0.186 ± 0.068 nmol/min/mg of protein (n = 82). There was significant interpatient variability in DPD activity (range, 0.058 to 0.357 nmol/min/mg of protein). In 18 patients, DPD activity was determined for 2 or more cycles of 5-FU. The intrapatient variability in DPD activity between cycles was 1.03- to 2.36-fold in 18 patients with DPD activity determined for more than one cycle. The evolution of DPD activity and 5-FU clearance between therapy cycles.
in 5 patients (DPD and 5-FU determined for >2 cycles) is shown (Fig. 1). There was no statistical difference in DPD activity from cycle to cycle in patients having more than one determination of DPD activity ($P = 0.7019$).

The mean 5-FU systemic clearance for our patient population was $2522.6 \pm 684.2 \text{ ml/min/m}^2$ ($n = 82$). As observed with DPD activity, there was a wide range of interpatient variability in 5-FU systemic clearance (range, 1052 to 4029 ml/min/m$^2$). Likewise, significant intrapatient variability in 5-FU clearance was observed. The intrapatient variability in 5-FU clearance between therapy cycles was 1.10- to 2.75-fold in 18 patients with 5-FU clearance determined for more than one cycle. As seen with DPD activity, there was no statistical difference in 5-FU clearance between cycles in the patients having more than one determination of 5-FU clearance ($P = 0.9359$). The median plasma uracil concentration was 24.0 ng/ml ($n = 78$; range, 0 to 65).

A significant linear relationship (Fig. 2) was observed between DPD activity in lymphocytes and 5-FU systemic clearance ($r = 0.716$, $P < 0.0001$). DPD activity and plasma uracil concentrations (Fig. 3) were poorly correlated ($r = -0.260$, $P = 0.0215$). Likewise, plasma uracil concentrations and 5-FU systemic clearance (Fig. 4) were poorly correlated ($r = -0.214$, $P = 0.0595$).

**DISCUSSION**

In the present study, many precautions were taken to limit the sources of interpatient variability. All patients had the same tumor localization and a comparable stage of disease. All patients were previously untreated. Additionally, all patients received the same cisplatin/5-FU treatment protocol. Harris et al. have reported a more than 3-fold range of DPD activity (within 24 h) because of circadian variability (16). Since it was not practical to collect lymphocytes at several time intervals, we attempted to minimize circadian variability by collecting the lymphocytes within a set time interval (and at a time corresponding to plasma 5-FU sampling) for all patients.

We observed a significant relationship between pretreatment DPD activity in lymphocytes and 5-FU systemic clearance, thus extending the findings of Harris et al. (16) that DPD activity in lymphocytes reflects variations in 5-FU concentrations during 5-day continuous infusions. The mean DPD activity reported in our study is similar to that reported by other investigators (10). Likewise, the approximately 6-fold range of DPD activity in lymphocytes is similar to that previously reported (16) using 5-FU as the DPD substrate but greater than the range reported by Tuchman et al. (3-fold range) using radiolabeled thymine (21). In addition, we demonstrated that there was substantial intrapatient variability in DPD activity (between different chemotherapy cycles), which has not been previously reported. In patients evaluated for more than one chemotherapy cycle, no significant difference was noted in DPD activity between various cycles.

The mean and interpatient range of 5-FU systemic clearance in our study is similar to that reported by several investigators (2). In most patients having more than one determination of DPD activity (Fig. 1), an increase/decrease in DPD activity (within a patient) resulted in a corresponding change in 5-FU clearance. These results imply that intrapatient variability in 5-FU clearance is related to corresponding changes in DPD activity in lymphocytes which reflect those occurring in the whole organism. It is necessary to identify what factors (nutritional, metabolic) may influence DPD activity, thus altering 5-FU clearance. Nutritional status has been shown to affect 5-FU clearance. Nutritional status has been shown to affect 5-FU clearance. Nutritional status has been shown to affect 5-FU clearance.
rather than detecting subtle changes in enzyme activity in patients with normal DPD activity. Since the 5-FU dose at midcycle was adjusted according to the AUCo-48h, it was not possible to assess correlations between DPD activity and patient toxicity. As previously discussed, several pharmacodynamic studies have reported significant correlations between 5-FU systemic pharmacokinetics (i.e., AUC, Cm) and patient toxicity (3–7). Furthermore in patients with suspected or proven DPD deficiency, all patients developed life-threatening toxicity following 5-FU administration (12–15). Thus, it is probable that a relationship exists between DPD activity and patient toxicity. However, this needs to be confirmed prospectively by comparing pretreatment DPD activity and pharmacodynamic parameters (mucosities, leukopenia) in 5-FU-treated patients.

The use of peripheral blood cells as markers of anticancer drug metabolism and toxicity has been previously reported. Lennard et al. (24) have reported an inverse relationship between RBC 6-thioguanine nucleotide concentrations and thiorurine methyltransferase in children treated by 6-mercaptopurine (24). These results indicated that those children with lower thiorurine methyltransferase activities were more susceptible to 6-mercaptopurine-induced myelosuppression (24). Based on the findings in this report and the previous findings of others (16), DPD activity in lymphocytes appears to reflect the systemic pharmacokinetics of 5-FU. It is suggested that monitoring of DPD activity in lymphocytes may be beneficial in identifying patients at risk for altered 5-FU disposition.

REFERENCES

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