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Dihydropyrimidine Dehydrogenase Activity in Cancer Patients

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Dihydropyrimidine dehydrogenase (DPD) is the major catabolic enzyme of pyrimidines and fluoropyrimidines. The clinical course of 2 patients with suspected DPD deficiency is described. Both patients had significantly delayed clearance of fluorouracil (5-FU), elevated plasma uracil concentrations, and subsequent lethal toxicity. The prevalence of DPD deficiency in the general population is unknown, but given the large number of cancer patients treated with 5-FU, it may be of great clinical significance. Lymphocytes have been previously shown to be a useful marker of systemic DPD activity. Because DPD activity has not been previously reported in a large population of cancer patients using 5-FU as the substrate, we determined DPD activity in lymphocytes from 66 patients with cancer. DPD activity was determined by a sensitive high performance liquid chromatography method. The mean DPD activity (S.D.) in 66 patients with head and neck cancer was 0.189 (0.071) nmol/min/mg protein with wide interpatient variability (range 0.058–0.357). DPD activity was not correlated to age ($r = -0.164$, $P = 0.188$). The mean DPD activity in men [0.192 (0.074)] was not significantly different from that in women [0.172 (0.057); t-test $P = 0.418$]. Likewise, there was no statistical difference in DPD activity in patients who had not received prior chemotherapy [0.195 (0.066)] to patients receiving one or more cycles of chemotherapy [0.186 (0.074); t-test $P = 0.638$].
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INTRODUCTION

FLUOROURACIL (5-FU) is one of the most widely used and active anticancer drugs in treating digestive, breast, and head and neck cancer. Recent advances in basic and clinical research have enhanced the therapeutic efficacy of 5-FU with synergistic drug combinations (cisplatin, interferon) and methods of biochemical modulation (folinic acid, dipyridamole) [1].

While much research has focused towards correlating the complex anabolism of 5-FU with cytotoxicity [2], relatively little research has concentrated on discerning the contribution of 5-FU catabolism to its cytotoxicity. The extent of catabolism of 5-FU influences the availability of 5-FU for anabolic conversion to cytotoxic nucleotides [3]. In addition, 5-FU systemic exposure (5-FU plasma area under the concentration–time

curve) has been correlated with various indices of patient toxicity [4, 5].

Dihydropyrimidine dehydrogenase (DPD) is the initial and perhaps rate-limiting enzyme in the catabolism of uracil, thymine and 5-FU [6]. More than 80% of an administered 5-FU dose is eliminated by this route with less than 20% excreted as the parent drug in the urine [6]. Several children [7–9] have been identified with an inborn deficiency of DPD manifesting as neurological and developmental disorders. Three recent reports of cancer patients with pyrimidinaemia who developed severe toxicities following administration of 5-FU were suggestive of a metabolic defect in pyrimidine and fluoropyrimidine catabolism [10–12]. No abnormalities were suggestive of this defect in the patients' past medical histories. Enzymatic studies performed in lymphocytes from two of these patients demonstrated no detectable DPD activity [11, 12]. Lymphocytes may serve as a surrogate marker of systemic DPD activity. DPD activity in lymphocytes was recently correlated to systemic 5-FU pharmacokinetics [13].

The activity of many enzymes responsible for the metabolism of xenobiotics is under genetic control such that a fixed percentage of individuals either lack or have diminished enzyme activity [14]. In such cases, the enzyme activity is described as being polymorphically distributed. In the absence of important catabolic enzymes, patients often develop severe, drug-induced toxicities. The metabolism of the anticancer drugs amonafide (*N*-acetyltransferase) and azathioprine (thiopurine methyltransferase) is polymorphically distributed [15, 16].

Because of the large number of cancer patients receiving 5-FU therapy, the DPD genetic polymorphism may represent a pharmacogenetic syndrome of significant clinical importance. The prevalence of DPD deficiency in the general population is currently unknown. Because DPD is the key catabolic enzyme of 5-FU, it is also important to observe if certain host factors (i.e. age, sex, prior chemotherapy) influence DPD activity and contribute to altered 5-FU disposition and subsequent toxicity.

We describe the clinical course of 2 patients with suspected DPD deficiency who had significantly reduced plasma clearance of 5-FU, elevated plasma uracil concentrations, and lethal toxicity after 5-FU administration. In addition, we present data concerning DPD activity in lymphocytes from a population of patients with head and neck cancer.

PATIENTS AND METHODS

Patient A. A 65-year-old female with pancreatic carcinoma presented for her first course of chemotherapy. Prior cancer therapy had consisted of both surgery and radiation therapy. At the time of admission, all haematological, hepatic, and renal function tests were within normal limits. 5-FU, 1 g/day, was initiated on day 1 by continuous intravenous infusion for 5 days. By day 5, the patient had developed moderate mucositis (grade II) which worsened to severe mucositis (grade IV) by day 7. In addition, the patient complained of increased fatigue. Because of the suspicion of altered 5-FU elimination, monitoring of 5-FU plasma concentrations was initiated on day 9 (Fig. 1). The elimination half-life of 5-FU was calculated to be 3465 min (normal individuals <15 min) [1]. Plasma uracil concentrations were also determined at this time (1.164 μg/ml, patient controls 0–0.09 μg/ml). Because of the significantly delayed clearance of 5-FU from plasma and the development of grade IV haematological toxicity, the patient was hemodialysed for 3 h in an effort to increase the clearance of 5-FU from plasma. 6 h after dialysis, plasma concentrations had rebounded to concentrations greater than pretreatment concentrations (Fig. 1). On day 12, uridine rescue was administered at 2 g/m²/h by intravenous infusion for 3 h, the administration interrupted for 3 h and this pattern of administration repeated for a total of 72 h. Despite the above therapy and supportive care, the patient died on day 25 due to haemorrhagic complications.

Patient B. A 37-year-old male with head and neck cancer (T4N0) presented for his first course of cisplatin/5-FU therapy. Haematological, hepatic, and renal tests were within normal limits. On day 1, cisplatin, 97 mg/m², was administered without complication.

On day 2, 5-FU, 970 mg/m²/day, was administered by continuous intravenous infusion for a planned duration of 5 days. The plasma uracil concentration was determined on day 2 (0.115 μg/ml, patient controls 0–0.09 μg/ml). Plasma pharmacokinetic monitoring of 5-FU was initiated at the start of 5-FU therapy. The elimination half-life and clearance of 5-FU were 540 min and 99.3 ml/min/m² (normal values <15 min and approximately 2000 ml/min/m², respectively) [1]. The plasma area under curve (AUC) of 5-FU at 48 h was noted to be extremely elevated (229 000 ng/ml × h, Fig. 2). Previous reports from our laboratory [4] have determined a significantly increased risk of toxicity with a 5-FU AUC at 48 h > 15 000 ng/ml × h. The 5-FU infusion was terminated. Charcoal haemoperfusion, initiated on day 4 for 12 h, was effective in increasing the clearance of 5-FU from plasma (Fig. 2). On day 4, uridine rescue was administered as described for patient A. The patient developed grade IV mucositis and grade IV haematological toxicity on day 5. Despite the above therapy and supportive care the patient died on day 10 due to complications of an infection.

Patient population and treatment protocol

Informed consent was obtained from all patients prior to study entry. Lymphocytes were collected from 66 patients with

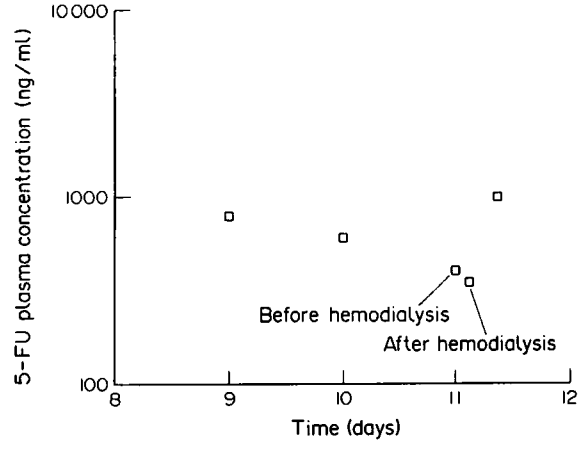

Fig. 1. Plot of 5-FU plasma concentration–time profile for Patient A.

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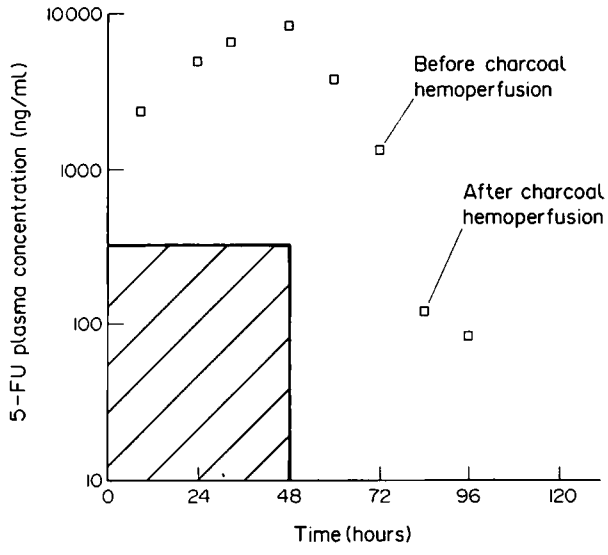

Fig. 2. Plot of 5-FU plasma concentration–time profile for patient B. Shaded area represents the mid-cycle 5-FU AUC (5-FU AUC_{48} h) associated with significantly less patient toxicity.

squamous carcinoma of the head and neck (56 males, 10 females). The median age was 61-years-old (range 42–87). The chemotherapeutic regimen consisted of cisplatin, 100 mg/m^2, on day 1 (intravenous infusion) followed by 5-FU, 1000 mg/m^2/day, administered on days 2–6 by continuous intravenous infusion. Lymphocytes were collected from 19 patients who had received no prior chemotherapy and 47 patients who had received $\geq$1 cycle of cisplatin/5-FU therapy.

Collection of lymphocytes

Approximately 20 ml of blood were collected from each patient in heparinised tubes on day 0. In patients who had received prior cisplatin/5-FU therapy, lymphocytes were collected at least 21 days after receiving this therapy. The blood was transferred into a 50-ml tube and mixed with 15 ml of RPMI 1640 cell culture media (Whittaker Bioproducts Inc., U.S.A.). Because of the previously reported circadian variability of DPD activity in lymphocytes [13], blood samples were collected between 8 a.m. and 11 a.m. to minimise circadian variability. This mixture was layered onto 15 ml of Histopaque (Sigma Chemical Co.) and centrifuged for 30 min at 400 *g*. The mononuclear cell layer was transferred to another centrifuge tube, washed twice with phosphate buffered saline, and contaminating red blood cells were hypotonically lysed. The lymphocytes were suspended in 35 mmol/l sodium phosphate buffer (pH 7.5), freeze–thawed three times, and placed in an ice bath and sonicated for 10 s (5 times total at 30 s intervals) to lyse the cellular membranes. The cells were centrifuged for 30 min at 20 000 *g* at 4°C. The supernatant was kept for the determination of DPD activity.

Determination of DPD activity

The supernatant was assayed by a modification [13] of a method originally described by Marsh and Perry [17]. After optimisation of assay conditions, 0.05–0.1 mg of cytosolic protein, 250 μmol/l NADPH, 2.5 mmol/l $MgCl_2$, and 20 μmol/l of [^{14}C]5-FU (2.04 GBq/mmole, Amersham, France) in 35 mmol/l sodium phosphate buffer, pH 7.5, were incubated for 60 min at 37°C in a total volume of 0.125 ml. At the end of the incubation, the reaction was stopped by the addition of an equal volume of ice-cold ethanol. The samples were refrigerated at −20°C for 30 min then centrifuged (400 *g*, 5 min) to remove precipitated proteins. An aliquot of the supernatant was analysed for the presence of 5-FU catabolites by a high performance liquid chromatography (HPLC) method [18] with the output monitored by a radioactive flow detector (Flo-one, Radiomatic Instruments, Inc., U.S.A.). Cytosolic protein concentrations were determined by the dye-binding method (Bio-Rad S.A., France) using bovine gamma globulin as the protein standard. As noted by other investigators [19] using crude cytosolic extracts of lymphocytes to determine DPD activity, the only 5-FU catabolite quantitated was dihydrofluorouracil (FUH_2). Thus enzyme activity was expressed as nmoles of FUH_2/min/mg protein. This method for determining DPD activity was both very sensitive and reproducible (limit of sensitivity approximately 0.010 nmol product and intra- and interday coefficients of variations <7%).

Sample collection (plasma 5-FU samples)

In Patient A, no standard sampling procedure was performed during the collection of plasma 5-FU samples. Blood (10 ml) was collected in heparinised tubes on days 9, 10, and 11 following the initiation of 5-FU therapy.

In Patient B, blood sampling was performed as described previously [4]. Briefly, blood samples (10 ml) were collected in heparinised tubes twice daily (8 a.m. and 5 p.m.) during 5-FU administration. In Patients A and B, blood samples were immediately placed on ice, transported to the laboratory, and centrifuged (10 min, 2500 r.p.m). Plasma was stored (−20°C) until analysed (within 1–3 days).

Patient controls (plasma uracil concentrations)

In 50 patients with head and neck cancer (median age 64-years-old, range 23–83; 45 males and 5 females), approximately 10 ml of blood was collected in a heparinised tube the day prior (day 0) to receiving cisplatin therapy. These patients were not the same as those from which lymphocytes were collected. Plasma uracil concentrations were determined as described below and a normal plasma uracil range was determined from these results.

Assay methodology

Both plasma uracil and 5-FU concentrations were determined by a previously published HPLC method [20]. The intra- and interday coefficients of variation for uracil and 5-FU were <10%. Limits of sensitivity for uracil and 5-FU were 10 and 8 ng/ml, respectively.

Pharmacokinetic analysis

The area under the concentration–time curve (AUC) for 5-FU was determined by the logarithmic trapezoidal method [21]. The elimination rate constant was determined by log-linear least squares regression of the plasma concentration time points in the terminal phase of the plasma disposition curve. The elimination rate constant was used to extrapolate the area from the last measured concentration to infinity. The elimination half-life was calculated by dividing the elimination rate constant into the natural logarithm of 2. Systemic clearance was calculated by dividing the total dose administered by the AUC [22].

Statistical analysis

The mean, S.D., and range of DPD activity in lymphocytes were determined. Sex-related differences in DPD activity were

assessed by the two-sample t-test. Differences in the DPD activity in untreated patients and those previously treated with chemotherapy were assessed by the two-sample t-test. Correlations between age and DPD activity were assessed by simple linear regression analysis. The Statgraphics statistical package was used for all statistical analysis (Statistical Graphics Corp., U.S.A.). The *a priori* level of significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

As observed in the 3 previous cases of suspected or confirmed DPD deficiency [10–12] in cancer patients, the past medical history of our 2 patients was not suggestive of this metabolic defect. The 3 previous cases were in breast cancer patients receiving cyclophosphamide, methotrexate and fluorouracil (CMF) therapy. 2 of these patients [10, 11] received two or more cycles of CMF therapy before DPD deficiency was suspected. In contrast, our 2 patients (pancreatic cancer and head and neck cancer) developed severe and subsequent lethal toxicity after their first cycle of 5-FU therapy. DPD activity was not determined in our 2 patients with severe 5-FU toxicity to confirm DPD deficiency (as done by Diasio *et al.* [11]). Although lack of enzymatic activity would have confirmed DPD deficiency in our patients, DPD deficiency is strongly suspected based on the significantly altered 5-FU clearance, the presence of elevated plasma uracil concentrations, and lethal toxicity (Table 1).

Although familial studies were not performed in our 2 patients, familial studies in the 2 previous cases of DPD deficiency in cancer patients suggested an autosomal recessive pattern of inheritance for this trait [10, 11]. Based on the number of patients who have recently been identified (including another recent case report [23]) with this deficiency the prevalence of this defect may be higher than previously suspected. Given the potential severity of this deficiency, large population studies are necessary to determine the prevalence of DPD deficiency.

As to date, the detection of this enzyme deficiency in cancer patients has occurred after the development of severe toxicities following the administration of 5-FU. Elevations of plasma or urinary pyrimidines have been the only significant laboratory findings in all 4 patients identified with suspected or confirmed DPD deficiency (Table 1). Thus, screening urine or plasma samples for elevations in concentrations of pyrimidines may be useful in identifying individuals with this metabolic defect. We routinely screen patients for plasma uracil concentrations prior to the patients receiving 5-FU. Patients with an elevated plasma uracil concentration are subsequently tested for *in vitro* DPD activity in lymphocytes. Given the increasing number of patients

Table 1

Reference	5-FU clearance* (ml/min/m²)	5-FU* $t_{\frac{1}{2}}$ (min)	Plasma uracil concentration (μg/ml)
10	N/A	N/A	0.736
11	70	159	1.510
This study			
Patient A	N/A	3780	1.164
Patient B	99.3	540	0.115

*Normal values for 5-FU clearance and elimination half-life are approximately 2000 ml/min/m² and <15 min, respectively.

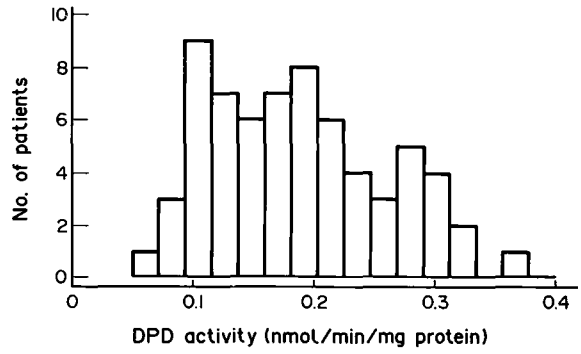

Fig. 3. Frequency distribution of DPD activity in peripheral lymphocytes from 66 patients with head and neck cancer.

treated with 5-FU, a simple test to identify patients with diminished or deficient DPD activity is needed.

Various treatment strategies to reduce or prevent 5-FU toxicity in our 2 patients were unsuccessful. Haemodialysis, although used briefly, was ineffective in reducing plasma concentrations of 5-FU. Plasma concentrations of 5-FU rebounded to concentrations exceeding those prior to haemodialysis. However, charcoal haemoperfusion was very effective in reducing plasma concentrations of 5-FU (elimination half-life of 210 min during charcoal haemoperfusion). No rebound phenomenon was observed after stopping charcoal haemoperfusion. Uridine administration was ineffective in both patients in reversing the cytotoxicity of 5-FU when administered at the dose, schedule, and time after the start of 5-FU therapy as previously mentioned. The optimal dose and administration schedule of uridine to rescue patients from 5-FU toxicity are still under investigation [1].

The mean DPD activity for the entire patient population was [mean (S.D.)] 0.1888 (0.0714) nmol/min/mg protein which is similar to values reported by other investigators [13, 19]. An approximately 6-fold range of DPD activity was observed (range 0.0576–0.3574). The range and distribution of DPD activity in lymphocytes from a large population of cancer patients have not been previously reported. Tuchman *et al.* [24] observed a 3-fold range of DPD activity in lymphocytes using thymine as the substrate. A wide range of interpatient DPD activity has been reported (using 5-FU as the substrate) for several other human tissues [25]. The frequency distribution of DPD activity in lymphocytes is shown in Fig. 3. The mean (S.D.) DPD activity in males and females was 0.1918 (0.0737) and 0.1718 (0.0568) nmol/min/mg protein, respectively. The DPD activity in females was lower than that in males but this did not approach statistical significance ($P = 0.418$). Although the activities of certain enzymes responsible for drug metabolism are perceived to be influenced by advanced age, age was not correlated to DPD activity in our patient population ($r = -0.164$, $P = 0.188$). There was no significant difference noted in DPD activity from untreated patients and those previously treated [19 patients, 0.195 (0.066) vs. 47 patients, 0.186 (0.074) nmol/min/mg protein, $P = 0.638$].

It is generally believed that DPD activity in the liver is responsible for the majority of 5-FU catabolism although extrahepatic tissues contribute to its catabolism [6]. The greatest DPD activity in human tissues has been observed in liver and lymphocytes with other tissues having less activity [19]. DPD activity in lymphocytes may reflect the activity observed in hepatic tissue. In an elegant study, Harris *et al.* [13] demon-

strated that circadian variability of 5-FU plasma concentrations was correlated to circadian fluctuations in DPD activity. Furthermore they demonstrated a significant correlation existed between DPD activity in lymphocytes and 5-FU plasma concentrations in human cancer patients. Thus DPD activity in lymphocytes may serve as a biochemical marker for identifying individuals at risk for altered clearance of 5-FU and treatment-related toxicities (mucositis, myelosuppression). Studies in a large population of cancer patients are currently ongoing to confirm their preliminary findings.

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