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ORIGINAL ARTICLE

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Dihydropyrimidine dehydrogenase deficiency in an Indian population

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Abstract *Background*: Dihydropyrimidine dehydrogenase (DPD) deficiency is prevalent in 3–5% of the Caucasian population; however, the frequency of this pharmacogenetic syndrome in the Indian population and other racial and ethnic groups remains to be elucidated. *Patients and methods*: We describe an Indian patient who presented to clinic for the treatment of gastric adenocarcinoma with 5-flurouracil (5-FU) therapy who subsequently was diagnosed with DPD deficiency by using the peripheral blood mononuclear cell (PBMC) DPD radioassay. This observation prompted us to examine the data generated from healthy (cancer-free) Indian subjects who were enrolled in a large population study to determine the sensitivity and specificity of the uracil breath test (UraBT) in the detection of DPD deficiency. Thirteen Indian subjects performed the UraBT. UraBT results were confirmed by PBMC DPD radioassay. *Results*: The Indian cancer patient demonstrated reduced DPD activity (0.11 nmol/min/mg protein) and severe 5-FU toxicities commonly associated with DPD deficiency. Of the 13 Indian subjects [ten men and three women; mean age, 26 years (range: 21–31 years)] enrolled in the UraBT, 12 Indian subjects demonstrated UraBT breath profiles and PBMC DPD activity within the normal range; one Indian subject demonstrated a reduced breath profile and partial DPD deficiency. *Conclusions*: DPD deficiency is a pharmacogenetic syndrome which is also present in the Indian population. If undiagnosed, the DPD deficiency can lead to death. Future epidemiological studies would be helpful to determine the prevalence of DPD deficiency among racial and ethnic groups, allowing for the optimization of 5-FU chemotherapy.

Keywords Dihydropyrimidine dehydrogenase (DPD) enzyme · 5-Fluoruracil (5-FU) · Fluoropyrimidine · DPD deficiency · Indian · Race

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Introduction

5-Flurouracil (5-FU) and its derivatives (e.g., capecitabine) are widely prescribed in the management of gastro-intestinal cancers. Despite widespread use, approximately 31–34% of cancer patients develop severe 5-FU related toxicities [1]. In approximately 61% of these cases, the etiology of 5-FU related toxicities has been linked to reduced activity in the dihydropyrimidine dehydrogenase (DPD) enzyme [2]. As the initial and rate-limiting enzyme of the pyrimidine catabolic pathway, DPD degrading thymine, uracil, and the anticancer drug 5-FU to dihydrothymine, dihydrouracil, and 5-fluoro-dihydrouracil, respectively [3, 4]. Pharmacokinetic studies have suggested reduced DPD activity (DPD deficiency) may reduce 5-FU catabolism resulting in a clinically dangerous increase in 5-FU half-life and severity of 5-FU related toxicities [5, 6].

DPD deficiency is a pharmacogenetic syndrome which manifests primarily as severe life-threatening toxicity subsequent to administration of standard doses of 5-FU [7, 8]. Symptoms frequently observed following the administration of 5-FU include mucositis, granulocytopenia, neuropathy, and death [2, 8, 9]. The prevalence of this autosommal codominently inherited pharmacogenetic syndrome is approximately 3–5% in the Caucasian population and 8% in the African-American population [10–13]. However, the prevalence in the Indian population has not been determined. We report on an Indian patient with DPD deficiency who developed 5-FU toxicity in the course of his treatment

for gastric adenocarcinoma. A literature search suggests that this is the first description of DPD deficiency in an Indian cancer patient. The observation prompted us to further evaluate peripheral blood mononuclear cell (PBMC) DPD activity and uracil breath test (UraBT) profiles from Indian subjects who were enrolled in a large population study to examine correlation among DPD enzyme activity and the UraBT [14, 15].

Patients and methods

Case report of an Indian cancer patient with dihydropyrimidine dehydrogenase deficiency

A 59-year-old Indian male underwent a distal subtotal gastrectomy with a Bill Roth (B) II reconstruction on 11/17/01. Six of seven lymph nodes were tumor positive with a tumor score of T3N1M0. On February 6, 2002 the patient was started on an adjuvant chemotherapy/radiotherapy based on the McDonald Study for gastric cancer [16]. After completing the initial 5 days of 5-FU/leucovorin therapy for cycle 1, the patient was seen in the oncology clinic with complaints of, diarrhea, mouth sores and ulcers associated with bleeding upon minor trauma, and multiple areas of bruising around the area of his central line. His diarrhea was described as loose watery stools, without blood or mucous, with an average of five bowel (range 4–7) movements per day (grade 2). The mucositis had made him unable to tolerate oral intake, causing him to lose eight pounds. He reported significant bruising at the sight of his central line, but denied active bleeding. The patient denied fevers, night sweats, or any other bruising. He also denied peripheral neuropathy, insomnia, and loss of consciousness. He did admit to some dizziness. The physical examination was remarkable for severe mucositis apparent as confluent ulcers encroaching his lips, with notable thrush. The ulcers bled easily upon touching (grade 3). He had a 4–5-cm area of bruising around his porta catheter on the left side of chest with small petechiae around it, without active bleeding (grade 2). Neurologically, the patient's cranial nerves, motor and sensory functions were intact. His laboratory workup including a complete blood count, fluid balance profile, and liver function tests were within normal limits.

Given the patient's presentation of severe mucositis, diarrhea, and bruising after 1 week of 5-FU chemotherapy, DPD deficiency was a major diagnostic concern. DPD activity was evaluated by radioassay using PBMC as previously described [14]. The patient was given cisplatin 60 mg/m^2 with concurrent radiation from 3/4/02 to 4/5/02, which he tolerated well.

Unfortunately, in July of 2002, the patient developed a metastatic small bowel obstruction which was deemed inoperable. After having a discussion with his family, the patient opted for hospice care. The patient died shortly thereafter.

Pilot study for detection of dihydropyrimidine dehydrogenase deficiency in the Indian population

Uracil breath test subjects

Thirteen Indian subjects [ten men and three women; mean age, 26 years (range: 21–31 years)] were recruited as a part of a larger population study at the University of Alabama at Birmingham. Following an explanation of procedures, informed consent was obtained from all subjects prior to initiation of this IRB approved protocol. To be eligible for this study, healthy subjects were at least 19 years old, cancer-free, and had no history of metabolic or respiratory disease.

Rapid oral UraBT

The UraBT principle and methodology is described in greater elsewhere [15]. To minimize variation resulting from a circadian rhythm in DPD activity [17], the UraBT protocol started at approximately 8:00 a.m. Fasting subjects were weighed and an aqueous solution containing 6 mg/kg 2-^{13}C-uracil (Cambridge Isotope Laboratories Inc., Andover, MA) was formulated. Subjects donated three baseline breath samples into 1.2-l bags (Otsuka Pharmaceuticals, Tokushima, Japan) prior to administration of the non-radioactive oral solution. Twenty-one post-dose breath samples were collected into 100-ml breath bags (Otsuka Pharmaceuticals, Tokushima, Japan) during the 180-min period immediately following ingestion. Post-dose breath samples were collected every 5 min for the first 30 min and every 10 min thereafter. The concentration of $^{13}CO_2$ in breath, reported in delta over baseline (DOB) notation, was determined by infrared spectrophotometry (Meretek, Lafayette, CO; [18]). These data were graphed [DOB (y axis) versus time (x axis)] and C_{max}, T_{max}, and DOB_{50} ($^{13}CO_2$ concentration in breath 50 min following 2-^{13}C-uracil ingestion) were determined by inspection. The percentage dose of 2-^{13}C-uracil, recovered in the breath as $^{13}CO_2$ (PDR), was calculated as described elsewhere [19]. Subjects were considered to be DPD deficient by UraBT when their $DOB_{50} < 128.9$ DOB [15].

Peripheral blood mononuclear cell DPD radioassay

UraBT results were confirmed by DPD radioassay, which is described in greater detail elsewhere [14, 20]. To minimize variation resulting from a circadian rhythm in DPD activity [17], 60 cc of whole blood was drawn from a peripheral vein into heparinized vacutainers at approximately 12:00 p.m. PBMCs were isolated by separation on a ficoll gradient. These cells were washed three times with PBS and lyzed by sonication in an ice bath. The lyzed cells were then centrifuged to remove cellular debris and the cytosol was collected. The protein

concentration of the cytosol was quantitated by a Bradford assay [21].

Two-hundred-fifty micrograms of cytosolic protein was added to a reaction mixture containing NADPH and [6-^{14}C]-5-FU. The reaction mixture was incubated at 37°C for 30 min. One-hundred-twenty-five microliters of aliquots of the reaction mixture were removed every 5 min during the incubation period and added to an equal volume of ice-cold ethanol to terminate the reaction. This mixture was incubated overnight at −80°C, thawed, and then filtered prior to HPLC analysis. Reversed-phase HPLC was used to separate [6-^{14}C]-5-FU from its catabolite, [6-^{14}C]-5-FUH$_2$. The amount of [6-^{14}C]-5-FUH$_2$ formed at each time point was quantified and then graphed against time as described elsewhere [14, 20]. From these data, the formation rate of [6-^{14}C]-5-FUH$_2$ was calculated. DPD enzyme activity was determined by standardizing the formation rate of [6-^{14}C]-5-FUH$_2$ to the amount protein used in the reaction mixture (i.e., nmol/min/mg protein). Based upon previous population studies by our laboratory, subjects were considered to have DPD enzyme activity within the normal range when their fresh PBMC DPD activity was ≥0.182 nmol/min/mg protein, partially deficient when their fresh PBMC DPD activity was <0.182 nmol/min/mg protein but ≥0.10 nmol/min/mg protein, and profoundly DPD deficient when their fresh PBMC DPD activity was <0.10 nmol/min/mg protein [14, 20].

*Genotypic screening for the DPYD*2A sequence variant by denaturing high performance liquid chromatography*

The *DPYD* gene of DPD deficient patients and subjects was evaluated for the *DPYD*2A* sequence variation by using a previously described denaturing high performance liquid chromatography (DHPLC) method [22].

Results

Case report: quantification of DPD activity from an Indian patient treated with 5-FU

The fresh PBMC DPD activity was 0.11 nmol/min/mg protein, demonstrating partial DPD deficiency.

Pilot study: identification of DPD deficiency in an Indian subject by rapid UraBT

Breath profiles from 12 Indian subjects with DPD activity in the normal range and one Indian subject with partial DPD deficiency are shown in Fig. 1. From this study population, we identified a second partially DPD deficient Indian subject. The Indian subject with partial DPD deficiency demonstrated lower UraBT values compared to Indian controls with DPD activity within the normal range. Specifically, this partially DPD deficient Indian subject demonstrated a lower C_{max}, PDR, and DOB_{50} than Indian subjects with DPD activity within the normal range (Table 1). The partially DPD deficient Indian also demonstrated an increased T_{max} compared to Indian subjects with normal DPD activity (Table 1).

Genotypic screening of the DPD deficient cancer patient and volunteer

DHPLC analysis demonstrated that *DPYD*2A* was not present in the *DPYD* gene from either the DPD deficient Indian cancer patient or the DPD deficient Indian volunteer.

Discussion

5-FU and its derivatives are widely prescribed to treat epithelial cancers [23]. Although 5-FU is generally well tolerated at standard doses, approximately 40–60% of cancer patients that develop severe, life-threatening 5-FU toxicities are DPD deficient [24, 25]. The presented Indian patient demonstrated toxicities consistent with 5-FU toxicity. Specifically, we observed mucositis, thrush, and bruising around his central line. He also had complained of diarrhea. The patient's Indian ethnicity was particularly noteworthy, as the given the incidence of DPD deficiency in this population group is not known. We confirmed partial DPD deficiency following measurement of PBMC DPD activity by radioassay.

Most phenotypic assays that are currently available to detect this pharmacogenetic syndrome, including the PBMC radioassay, are too labor-and-time intensive to be routinely used to screen cancer patients prior to 5-FU administration [15]. Recently, we developed and validated an oral UraBT which may potentially be used as a screening method to rapidly detect DPD deficiency in cancer patients prior to 5-FU administration [15]. This in vivo assay utilizes 2-^{13}C-uracil, which has a similar substrate affinity for the DPD enzyme as 5-FU [26]. As the 2-^{13}C-uracil substrate is degraded by DPD and other enzymes of the pyrimidine catabolic pathway, the ^{13}C probe is released as $^{13}CO_2$ [15]. The $^{13}CO_2$ present in breath can then be quantified by infrared spectrophotometry [15]. Previously, we demonstrated DPD deficient individuals have an impaired ability to catabolize the 2-^{13}C-uracil, which results in altered $^{13}CO_2$ breath profiles (e.g., significantly lower C_{max}, PDR, and DOB_{50}, and increased T_{max}; reference [15]).

Several genotypic methods have been described to rapidly examine the *DPYD* gene for sequence variations; however, the clinical relevance and application of genotyping will continue to be limited as long as the genotype/phenotype relationship remains unclear. To date, the more than 30 sequence variations in the *DPYD* gene have been described [27]. Yet, only *DPYD*2A* and

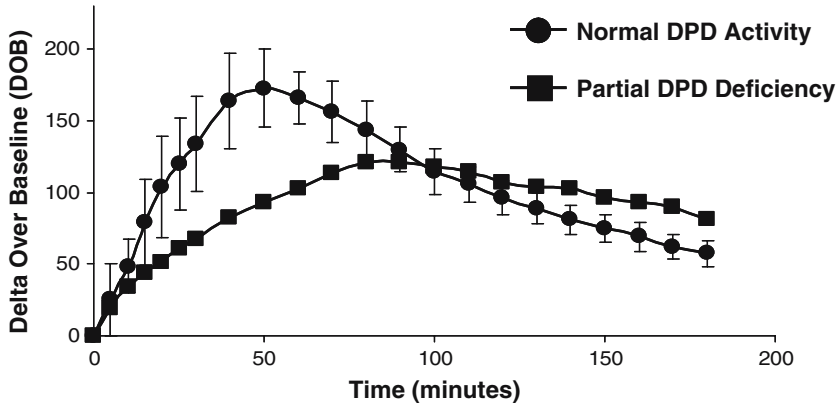

Fig. 1 The UraBT breath profiles from 12 Indian subjects (mean ± SD) with DPD activity in the normal range (*filled circles*) and one partially DPD deficient Indian subject are shown (*filled squares*). All cancer-free subjects ingested a 6 mg/kg solution of 2-^{13}C-uracil. Post-dose breath samples were collected for 180 min after substrate ingestion and the amount of $^{13}CO_2$ in breath [expressed as delta over baseline (*DOB*)] was determined for each time point

*DPYD**13 have been consistently associated with DPD deficiency [28]. Genotypic screening for *DPYD**2*A* may benefit only a limited number of cancer patients due to the low frequency of this variation in the general population (0.94% frequency in the Caucasian population [29]). Furthermore, effect of genetic and epigenetic events on DPD enzyme activity is not well understood. Recently, our laboratory observed aberrant methylation in the *DPYD* promoter of several DPD deficient individuals who demonstrated no sequence variations in their *DPYD* gene [30]. Alternatively, another laboratory has proposed allelic regulation of the *DPYD* gene as a potential mechanism to explain the normal DPD enzyme activity observed from a *DPYD**2*A* heterozygous individual [31]. In the current study, we screened the *DPYD* gene from the DPD deficient Indian cancer patient and the DPD deficient Indian volunteer for the *DPYD**2*A* sequence variation. *DPYD**2*A* was not detected in the *DPYD* gene from either individual. Additional genotypic studies of the *DPYD* gene from these individuals continue to be performed by our laboratory.

Several ethnic studies to phenotypically and/or genotypically evaluate DPD have been conducted in African (Eqyptian, Kenyan, and Ghanaian), Asian (Japanese, Korean, Indian, Pakastani, and Sri Lankan) and European Caucasian (British, German, and Dutch) populations [32–38]. As a part of a larger population study, we examined 13 cancer-free Indians for DPD deficiency using a novel, rapid, and non-invasive UraBT. Indian subjects with DPD activity in the normal range demonstrated UraBT values (C_{max}, T_{max}, DOB_{50}, and PDR) similar to those observed from normal subjects who participated in earlier studies to develop and validate the UraBT [15]. These earlier volunteers were primarily Caucasian or African American. Alternatively, the partially DPD deficient Indian subject demonstrated altered $^{13}CO_2$ breath levels and UraBT indices similar to those previously observed from other partially DPD deficient subjects [15]. Although Indians ($N = 43$) were included in a previous examination of the distribution of DPD activity in Southwest Asians [33], a large population study to examine the frequency of DPD deficiency in Indians is warranted and is now feasible by utilization of UraBT. Furthermore, additional studies of the Indian population should be undertaken to establish whether the genetic basis of DPD deficiency is unique and evaluate the allelic frequency of activity-reducing *DPYD* variants in this population.

Another scintillating feature of this patient with DPD deficiency was the absence of myelosuppression. Indeed, DPD-deficient cancer patients are at risk for developing severe myelosuppression. Raida and colleagues have

Table 1 Uracil breath test and DPD radioassay indices from 13 Indians

Volunteers[a]	*N*	Activity (nmol/min/mg)[b]	DOB_{50} (DOB)[c]	T_{max} (min)[d]	C_{max} (DOB)[c]	Percentage of ^{13}C-uracil dose recovered in breath (%)[c]
Indians with normal DPD activity	12	0.29 ± 0.05	172.4 ± 27.3	50.8 ± 10.8	181.3 ± 24.1	55.1 ± 7.0
Indians with partial DPD deficiency	1	0.17	93.3	80	121.0	47.9

[a]Thirteen cancer-free Indian subjects were examined by 2-^{13}C-uracil breath test and DPD radioassay as described in Patients and Methods
[b]The peripheral blood mononuclear cell DPD activity is shown. DPD activity within the normal range was observed for 12 Indian subjects. One Indian subject demonstrated partial DPD deficiency (data are shown as mean ± SD)
[c]Lower 2-^{13}C-uracil breath test indices (DOB_{50}, C_{max}, percentage of ^{13}C-uracil recovered in breath) were observed from the partially DPD deficient Indian subject compared to those from Indian subjects with normal DPD activity (data are shown as mean ± SD)
[d]A higher 2-^{13}C-uracil breath test T_{max} was observed from the partially deficient Indian subject compared to Indians with normal DPD activity (data are shown as mean ± SD)

examined the relationship between myelosuppression and DPD deficiency in a population ($n = 25$) of cancer patients with severe 5-FU toxicity (grades 3–4) [29]. Six of these cancer patients demonstrated either heterozygosity ($n = 5$) or homozygosity ($n = 1$) for the *DPYD*2A* sequence variation, which is commonly associated with reduced DPD enzyme activity. All six patients demonstrated grade 4 myelosuppression. Our laboratory has also examined the relationship between DPD activity and 5-FU toxicity [24]. We observed that approximately 73% of partially DPD deficient cancer patients (16/22) and 40% of profoundly DPD deficient patients (4/10) experienced moderate to severe granulocytopenia. Interestingly, myelosuppression has also been observed in cancer patients who were administered the DPD inactivator and eniluracil [39].

Conclusions

In summary, we describe DPD deficiency in two Indians: one cancer patient and one healthy volunteer. DPD deficiency is an important pharmacogenetic syndrome to be aware of when considering 5-FU chemotherapy administration. Several 5-FU related toxicities commonly associated with DPD deficiency include mucositis, granulocytopenia, diarrhea, and neuropathy. If undiagnosed, DPD deficiency can lead to death. Treatment consists of stopping 5-FU and is otherwise largely supportive. Further study would be helpful to determine the epidemiological incidence of DPD deficiency among ethnic and racial groups to determine "at risk" populations. This issue is of paramount importance, as 5-FU remains the third most commonly used chemotherapy worldwide.

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