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ORIGINAL ARTICLE

Benefit of uridine triacetate (*Vistogard*) in rescuing severe 5-fluorouracil toxicity in patients with dihydropyrimidine dehydrogenase (*DPYD*) deficiency

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Abstract
Background 5-Fluorouracil (5-FU), an analog of uracil, is one of the most commonly used chemotherapeutic agents and like other agents has a narrow therapeutic index limited by toxicity. Compared to previous attempts, uridine triacetate (Vistogard) has shown to increase the potential efficacy of 5-FU by allowing administering a higher dose and decreasing the toxicity. Recently, Vistogard received orphan drug designation from the FDA as an antidote in the treatment of 5-FU poisoning and from the European Medicines Agency as a treatment for 5-FU overdose. However, no data have been published to date in humans who were rescued by this agent following severe toxicity associated with 5-FU due to dihydropyrimidine dehydrogenase (*DPYD*) deficiency, the enzyme which is responsible for the elimination of approximately 80 % of the administered dose of 5-FU.
Patients and methods We identified two patients with advanced pancreatic cancer who were referred to us for testing of *DPYD* status following severe toxicity associated with 5-FU administered at a dose of 1400 mg/m^2 weekly bolus high-dose 5-FU followed by oral uridine triacetate as a part of a clinical trail. One patient developed grade 3 thrombocytopenia and grade 3 skin rash that resolved with discontinuation of 5-FU and supportive care, while second patient developed grade 4 thrombocytopenia, grade 3 coagulopathy and grade 3 neurological toxicity with a fatal outcome. *DPYD* status was evaluated as we have previously published.
Results The first patient was found to have an abnormally low *DPYD* activity of 0.087-nmol/min/mg protein by radioisotopic assay (reference normal range 0.182–0.688 nmol/min/mg protein). Because of pancytopenia, *DPYD* enzyme activity could not be assessed in patient 2; genotypic analysis of *DPYD* during autopsy revealed the presence of the heterozygous mutation, IVS14+1 G>A, *DPYD**2A, now recognized as the most common cause of *DPYD* deficiency.
Conclusion These two patients present the first two cases of *DPYD* deficiency that had either delay in severe toxicity or recovered from severe toxicity as they received oral Vistogard as a part of the conical trial. Toxicity was delayed in both patients by a mean of 3.5 weeks (range 3–4 weeks), indicating that Vistogard might be able to delay 5-FU toxicity despite higher doses than standard bolus dose of 5-FU used in gastrointestinal malignancies and the appearance of a potentially less toxic adverse effect of 5-FU at an unusual site (cutaneous) in one patient. The role of uridine triacetate with 5-FU in *DPYD*-deficient patients needs further investigation.

Keywords 5-Fluorouracil · Fluoropyrimidines · PN401 · Uridine · *DPYD* gene

Introduction

5-Fluorouracil (5-FU), an analog of uracil, is one of the most commonly used chemotherapeutic agents and constitutes the mainstay of chemotherapy for most gastrointestinal tumors [1]. The cytotoxicity of 5-FU is thought to be secondary to: inhibition of thymidylate synthase,

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principally via the actions of its metabolite, fluorodeoxyuridine monophosphate; and synthesis of defective RNA as a result of incorporation of a second metabolite, fluorouridine triphosphate (FUTP), into RNA [2]. The most common 5-FU toxicities include neutropenia, mucositis, diarrhea and hand-foot syndrome, with the latter two predominating when 5-FU is administered as a continuous intravenous infusion [3]. Like other conventional cytotoxic antineoplastic agents, 5-FU has a relatively narrow therapeutic index. Its toxicity often limits the dose that can be administered, limiting its overall therapeutic usefulness. Furthermore, since the late 1980s, there have been multiple case reports demonstrating unanticipated fatal and near fatal toxicities in patients with deficiency of dihydropyrimidine dehydrogenase (*DPYD*) [4–8], and the enzyme is responsible for the elimination of approximately 80 % of the administered dose of 5-FU.

Because both the antitumor effects and the systemic toxicities associated with 5-FU are related to its metabolite FUTP, uridine has been examined for the potential reduction of toxicity. Uridine is a naturally occurring pyrimidine nucleoside that augments cellular UTP pools and competes with FUTP for incorporation into the host RNA of hematopoietic progenitor and gastrointestinal mucosal cells, thereby attenuating 5-FU/FUTP toxicity in normal tissues [9–13]. The administration of uridine after 5-FU chemotherapy not only allows for the antitumor effect, but also for the "rescue" of normal host cells. In mouse models, administration of uridine following 5-FU selectively reduced toxicity to normal tissues, permitting substantial 5-FU dose escalation and increasing overall efficacy and antitumor activity of 5-FU [9–13]. Preclinical and clinical studies have revealed that sustained uridine concentrations of at least 50 μmol/l are required to confer protection to normal tissues from the toxic effects of 5-FU/FUTP [11]. Differences in uptake and utilization of uridine by tumor and normal tissues underlie the ability of uridine to reduce toxicity of 5-FU without proportionally reducing antitumor activity [3]. Both hematopoietic and gastrointestinal mucosal progenitors efficiently incorporate exogenous uridine (via the "salvage pathway"), whereas most other tissues, including malignant tumors, favor the de novo pathway of uridine nucleotide biosynthesis, in which free uridine is not an intermediate [3]. Thus, exogenous uridine is more effective at competing with FUTP for incorporation into host RNA in normal tissues versus all solid tumors tested to date in murine systems. Although uridine has also been demonstrated to protect against 5-FU toxicity in humans, its low oral bioavailability, toxicity including fever and phlebitis, and the requirement for central venous access for parenteral administration limit its clinical utility [14–17].

Uridine triacetate [(2′,3′,5′-tri-*O*-acetyluridine] (*Vistogard*, Wellstat Therapeutics Corporation, Gaithersburg, MD, USA), an orally active prodrug of uridine, has made a more effective uridine administration technique possible. Uridine triacetate is efficiently absorbed from the gastrointestinal tract and deacetylated by nonspecific esterases, yielding uridine and acetate. In contrast to oral uridine, uridine triacetate is not a substrate for the catabolic enzyme uridine phosphorylase and does not require pyrimidine transporter for absorption. Consequently, administration of uridine triacetate results in substantially more bioavailable uridine than does oral administration of uridine itself. Using uridine triacetate, it has been possible to increase the therapeutic index of 5-FU in BALB/c mice bearing advanced transplants of Colon 26 adenocarcinoma [18]. Furthermore, in clinical trials, it was possible to increase the dose of 5-FU, resulting in a significant increase in antitumor activity without increased toxicity [19]. Wellstat Therapeutics has conducted an open-label, randomized, controlled, phase III trial (NCT00024427) comparing vistonuridine in combination with high-dose 5-FU versus gemcitabine alone in patients with advanced pancreatic cancer. Results have yet to be announced. In a poster presentation at the annual meeting of the American Society of Clinical Oncology in 2010, researchers revealed that 37 cases of 5-FU overexposure were successfully treated with uridine triacetate. Even more impressively, one patient who accidentally received a 5-FU dose 10 times the recommended level survived the overdose [20].

On December 11, 2015, FDA approved Vistogard to treat patients following an overdose of 5-FU or capecitabine or in patients exhibiting early-onset, severe or life-threatening toxicity affecting the cardiac or central nervous system, and/or early-onset, unusually severe adverse reactions (e.g., gastrointestinal toxicity and/or neutropenia) within 96 h following the end of 5-FU or capecitabine administration. Vistogard® received orphan drug designation from the FDA as an antidote in the treatment of 5-FU poisoning and from the European Medicines Agency (EMA) as a treatment for 5-FU overdose. Wellstat Therapeutics developed Vistogard® and BTG will market, sell and distribute the drug for this indication in the USA.

Though it is speculated that few patients with severe toxicity associated with 5-FU or capecitabine might have abnormalities of *DPYD* or other enzymes, such human data have not been presented or published yet. However, a report about the benefit of treatment with uridine triacetate reduced 5-FU toxicity and mortality in *DPYD*-inhibited mice has been presented [21]. In this article, we report toxicity in two patients who were treated with high-dose 5-FU and uridine triacetate, and were subsequently demonstrated to be *DPYD* deficient. This probably constitutes the first

data on the effect of Vistogard on *DPYD*-associated severe toxicity following administration of 5-FU in humans.

Patients and methods

We identified two adult patients who received high-dose bolus 5-FU with uridine triacetate for the treatment of their cancer at the submission of plasma or tissue samples for testing of *DPYD* enzyme. Based on the clinical information, both patients were treated on a clinical trial NCT00024427. This was a randomized, open-label, multicenter study. Patients were randomized to one of two treatment arms. Arm I: Patients received high-dose fluorouracil (5-FU) IV over 30 min once weekly on weeks 1–3 followed by 1 week of rest. After each dose of 5-FU, patients received oral triacetyluridine every 8 h for a total of 8 doses. Courses were repeated every 4 weeks in the absence of disease progression or unacceptable toxicity. Arm II: Patients received gemcitabine IV over 30 min once weekly on weeks 1–7 followed by 1 week of rest (course 1). Subsequent courses were given on weeks 1–3. Courses were repeated every 4 weeks in the absence of disease progression or unacceptable toxicity. Patients were followed for survival. Two patients from our center were identified from *DPYD* data who developed severe untoward toxicities associated with 5-FU while being treated on this study as detailed below.

Patient 1

This patient was a 67-year-old African-American female with histologically proven metastatic pancreatic adenocarcinoma. Her past medical history was remarkable only for hypertension. Her medications included furosemide, captopril and a multivitamin. She denied previous smoking or alcohol abuse. The initial physical examination was notable for an ECOG performance status of 1 and a normal abdominal examination. Initial laboratory evaluation revealed a white blood cell count of 6.46×10^3, hemoglobin 11.5 g/dl, platelet count 200×10^3 and CA19-9 of 2 U/ml. She tolerated two treatments of high-dose 5-FU (2750 mg in cycle one, followed by a reduced dose of 1950 mg for cycle 2 due to a decrease in hemoglobin to 9.7 g/dl (grade 2) and platelets to 71×10^3 (grade 2)) with uridine triacetate administration. After cycle 3, week 4, however, she developed grade 3 thrombocytopenia (platelet count 59×10^3), grade 3 periorbital edema, grade 1 mucositis and a grade 3 desquamative rash over her face, trunk and forearms. She was admitted to the hospital for management, and blood was collected to measure *DPYD* activity. High-dose 5-FU therapy was discontinued with resolution of the rash, edema, mucositis and thrombocytopenia after 3 weeks. Management of rash included intravenous steroids and antihistamines.

Patient 2

This patient was a 75-year-old Caucasian male with histologically confirmed metastatic pancreatic adenocarcinoma. His past medical history was remarkable for peripheral vascular disease requiring arterial bypass surgery of the right lower extremity 5 years prior. His medications included oxycontin, warfarin, alprazolam and zolpidem. He had smoked one pack of cigarettes daily for over 50 years. The initial physical examination was notable for an ECOG performance status of 1, right upper quadrant tenderness and hepatomegaly. Initial laboratory evaluation revealed a white blood cell count of 11.1×10^3, hemoglobin 10.2 g/dl, platelet count 270×10^3, creatinine 1.4 mg/dl, aspartate transaminase (AST) 64 U/l, alanine transaminase (ALT) 30 U/l, total bilirubin 0.6 mg/dl, alkaline phosphatase 299 U/l, CA19-9 of 5743 U/ml and normal electrolytes.

The patient tolerated two treatments with high-dose 5-FU (2550 mg weekly) along with uridine triacetate without significant toxicity, except grade 1 nausea, grade 2 anemia and grade 1 thrombocytopenia. On the third week, however, he presented with generalized weakness, altered mental status, deterioration of his performance status to 3 and pancytopenia [white blood count 2.2×10^3, grade 2; hemoglobin 8.6 g/dl, grade 2; platelet count 14×10^3, grade 4; international normalized ratio (INR) 3.4, grade 3]. Other laboratory abnormalities included grade 2 creatinine elevation (2.4 mg/dl), grade 1 weight loss and grade 3 anorexia. He was admitted to the Oncology Unit for transfusion of red blood cells and platelets. In addition, the patient underwent an extensive workup to rule out an underlying infection, disseminated intravascular coagulation and gastrointestinal bleeding. His mental status continued to decline to grade 3, and on hospital day four, he died.

Assay for *DPYD* enzyme activity

Sixty milliliters of blood was drawn from the patient's peripheral vein between 9 a.m. and 12 p.m. to minimize variation resulting from the previously reported circadian rhythm in *DPYD* enzyme activity [22]. *DPYD* activity in peripheral blood mononuclear cells (PBMC) was determined using a previously described radioassay [23]. Individuals with PBMC *DPYD* activity <0.18 nmol/min/mg protein were considered to be *DPYD* deficient [23].

DPYD genotyping

Screening and genotypic analysis of homozygous and heterozygous, known and unknown sequence variants, of the

DPYD gene were performed using DHPLC as previously described [24]. All *DPYD* sequence variants identified by DHPLC were confirmed by DNA sequencing using a dideoxynucleotide chain termination method (Big Dye Kit; Applied Biosystems, Foster City, CA, USA) and capillary electrophoresis on an ABI 310 Automated DNA Sequencer (Applied Biosystems).

Results

Patient 1 was found to have an abnormally low *DPYD* activity of 0.087 nmol/min/mg protein by radioisotopic assay (reference normal range 0.182–0.688 nmol/min/mg protein). Standard treatment with gemcitabine was instituted as outpatient therapy.

Because of pancytopenia, *DPYD* enzyme activity could not be assessed for patient 2. After obtaining informed consent from his wife after his death, an autopsy was performed. Postmortem examination was remarkable for moderately to poorly differentiated adenocarcinoma of the pancreatic body and tail with extensive metastatic spread to the liver, diaphragm and regional lymph nodes. Gastrointestinal examination revealed a perforated duodenal ulcer. Central nervous system examination revealed lacunar infarction, hypertensive small vessel disease and remote focal hemorrhage of the basis pontis. Postmortem blood cultures were positive for *Candida albicans,* as well as tissue culture from the duodenal ulcer. Genotypic analysis of the *DPYD* gene revealed the presence of the heterozygous mutation, IVS14+1 G>A, *DPYD**2A, now recognized as the most common cause of *DPYD* deficiency [25].

Discussion

At the 2016 Gastrointestinal Cancers Symposium in San Francisco, CA, we presented data in 135 patients overdosed with 5-FU or capecitabine or exhibiting early onset of severe toxicities, possibly due to *DPYD* deficiency, other genetic variants, or other factors resulting in excessive susceptibility to 5-FU or capecitabine [26]. The patients were treated with a single course of 10 g of Vistogard given orally every 6 h for a total of 20 doses. The results showed that 96 % of patients treated with Vistogard recovered fully within 30 days of receiving treatment with Vistogard; 38 % of overdose patients resumed chemotherapy within 30 days. The most common toxicities associated with Vistogard were mild, including vomiting, nausea and diarrhea. However, no data are yet available on *DPYD* status and use of Vistogard as an antidote to date. Therefore, our patients probably present the first human report on its benefit in this pharmacogentic syndrome. However, it is expected as the data mature from the sponsor such data will be published in near future.

While most patients tolerate 5-FU reasonably well, a number of patients develop severe, and sometimes life-threatening, toxicity after standard doses of 5-FU [4]. Studies have demonstrated that many of these patients are *DPYD* deficient. In patients with severe *DPYD* enzyme deficiency, even a small dose or a very short administration of 5-FU could lead to a marked surge in plasma 5-FU concentration (Cmax), leading to increased 5-FU anabolism in susceptible tissues, e.g., the gastrointestinal tract, hair and bone marrow. Syndrome of *DPYD* deficiency manifests as diarrhea, stomatitis, mucositis and neurotoxicity and in some cases death [4, 5]. These individuals are at significant risk if they develop cancer and are given 5-FU. This is a true pharmacogenetic syndrome, with symptoms being unrecognizable until exposure to the drug.

Triacetyl uridine was developed so that patients could receive higher doses of 5-FU to bring toxicity down to an acceptable level, comparable to that of standard-dose 5-FU, to achieve a pharmacological gain in terms of a better response. It is, therefore, no surprise that toxicity perhaps comparable to that seen with standard-dose 5-FU in a *DPYD*-deficient patient was observed in this situation. But it is noteworthy that the toxicity was delayed in both patients by a mean of 3.5 weeks (range 3–4 weeks), indicating that Vistogard might be able to delay 5-FU toxicity despite higher doses than standard bolus dose of 5-FU used in gastrointestinal malignancies. A second scintillating feature is the appearance of a potentially less toxic adverse effect of 5-FU at an unusual site (cutaneous) in one patient.

Options for *DPYD*-deficient patients are limited, usually requiring discontinuation of 5-FU. The clinical feasibility of 5-FU dose escalation with uridine triacetate has been demonstrated in phase I studies [19, 27]. In the study at Memorial Sloan Kettering, oral uridine triacetate was well tolerated and total doses of 6 g every 6 h yield sustained levels of uridine in the target range of 50 μmol/l [19]. The maximum tolerated dose of 5-FU with uridine triacetate rescue was 1000 mg/m^2 and the recommended dose for phase II trials was 800 mg/m^2 given weekly for 6 weeks with dose escalation. 5-FU at doses of 800 mg/m^2 for 6 weeks was well tolerated without significant toxicity when given with uridine triacetate rescue [19]. The study carried out at the Institute for Drug Development, Cancer Therapy and Research Center and the University of Texas Health Science Center (San Antonio, TX, USA) showed that treatment with oral uridine triacetate beginning 8 h after 5-FU administration was well tolerated and resulted in sustained plasma uridine concentrations above therapeutically relevant levels [28]. The recommended 5-FU dosage

Table 1 Summary about administration and indications of Vistogard

Indications	Vistogard® is indicated for the emergency treatment of adult and pediatric patients: 1. Following a fluorouracil or capecitabine overdose regardless of the presence of symptoms, or 2. Who exhibit early-onset, severe or life-threatening toxicity affecting the cardiac or central nervous system, and/or 3. Early-onset, unusually severe adverse reactions (e.g., gastrointestinal toxicity and/or neutropenia) within 96 h following the end of fluorouracil or capecitabine administration
Caution	Vistogard is not recommended for the nonemergent treatment of adverse reactions associated with fluorouracil or capecitabine because it may diminish the efficacy of these drugs
Time period	Safety and efficacy of Vistogard® initiated more than 96 h following the end of fluorouracil or capecitabine have not been established
Adverse effects	Vomiting (10 %) Nausea (5 %) Diarrhea (3 %)
Administration	Ten grams of Vistogard orally every 6 h for a total of 20 doses

Please see full prescribing information

for phase II evaluations was 1250 mg/m^2/week for 3 weeks every 4 weeks with the intensified uridine triacetate dose schedule. At this dose, systemic exposure to 5-FU as measured by area under the curve was fivefold higher than that observed after administration of a conventional 5-FU bolus. However, no data on the effect of uridine triacetate in *DPYD*-deficient patients exist to our knowledge.

Our patients were treated as a part of a randomized phase III clinical study as data were derived from https://clinicaltrials.gov/ct2/show/NCT00024427. The treatment consisted of high-dose 5-FU per week × 3, with Vistogard administered orally versus gemcitabine. The data are pending from the study at present [29]. Animal experiments with ethynyluracil in our laboratory have shown that uridine triacetate can protect against 5-FU toxicity in the setting of *DPYD* deficiency, suggesting that uridine triacetate may be very helpful for patients at risk of toxicity of standard doses of 5-FU due to *DPYD* deficiency.

Partial deficiency of *DPYD* can be associated with adverse drug reactions, including death, following the administration of standard doses of 5-FU [30]. The mutation IVS14+1 G>A, *DPYD**2A, is the most common mutation associated with *DPYD* deficiency. A G>A base change at the splice recognition sequence of intron 14 leads to exon skipping and results in a 165-bp deletion in *DPYD* mRNA [31]. Ezzeldin et al. [24] by genotypic analysis of the *DPYD* gene demonstrated that a homozygous genotype results in complete deficiency while a heterozygous genotype results in partial deficiency of *DPYD*.

In the current study, two patients with at least partial *DPYD* deficiency developed severe toxicities from high-dose 5-FU despite being treated with uridine triacetate. Although uridine triacetate provided protection against 5-FU-related toxicity in most studied cases, *DPYD*-deficient patients may remain more susceptible to the side effects of 5-FU. In the single death that we report, the post-mortem examination was significant for a perforated duodenal ulcer and candidemia that we believe were related to 5-FU-associated severe mucositis and prolonged neutropenia, respectively.

Conclusion

Vistogard® is the first and only antidote to overdose and early-onset, severe or life-threatening toxicities from chemotherapy drugs 5-fluorouracil (5-FU) or capecitabine, an orally administered prodrug of 5-FU. Vistogard® was approved on December 11, 2015, following a priority review by the United States Food and Drug Administration (FDA) (Table 1). Therefore, it is expected that *DPYD*-deficient patients who have received 5-FU should also benefit from treatment with uridine triacetate if the deficiency is identified early enough after 5-FU dosing. Therapeutic monitoring of 5-FU during or after infusions could permit rapid detection of 5-FU overexposure due to *DPYD* deficiency, enabling the use of uridine triacetate as an antidote in *DPYD*-deficient patients.

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