

LETTER

Long-Term Survival on Capecitabine in Two Gemcitabine Refractory Pancreatic Cancer Patients. Is there a Pharmacogenetic Explanation?

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Summary

Context Capecitabine has shown efficacy in treatment of metastatic pancreatic cancer. Several researchers have identified thymidine phosphorylase, dihydropyrimidine dehydrogenase, or their ratio as indicators of response to capecitabine in various cancers.

Case report We report two patients with metastatic pancreatic carcinoma who had long-term survivals on capecitabine after gemcitabine failure. These two cases prompted us to measure thymidine phosphorylase and dihydropyrimidine dehydrogenase levels to facilitate discourses regarding their relationship with efficacy of capecitabine. We also describe a novel method of measuring thymidine phosphorylase level from serum without an invasive tissue biopsy. One patient is alive as of today, with improved performance status, 50 months after capecitabine was started. CA 19-9 and CT scans remained stable during 57 cycles. Her thymidine phosphorylase level was 1.77 compared to a control level of 1.00. Dihydropyrimidine dehydrogenase level was 4.14 compared to a control level of 1.00. Their ratio was 0.43. The other patient was alive on capecitabine for 24 months. His performance status, bilirubin, AST, and ALT improved on capecitabine. CT scans and CA 19-9 remained stable during this period. He had thymidine phosphorylase level of 5.56,

dihydropyrimidine dehydrogenase level of 2.74, and their ratio of 2.03.

Conclusion Capecitabine resulted in long term survivals in two patients with metastatic pancreatic cancer after gemcitabine failure. The use of capecitabine as second-line treatment in metastatic pancreatic cancer should be further explored along with the role of thymidine phosphorylase and dihydropyrimidine dehydrogenase levels in its activity. A non-invasive method of thymidine phosphorylase measurement we described should be validated in larger trials.

Introduction

Each year, approximately 32,000 new patients are diagnosed with pancreatic cancer in United State. The incidence has been increasing in United States since 1930's. Prognosis of pancreatic carcinoma is extremely poor. Approximately 31,000 patients in United States die from pancreatic carcinoma each year, making it the 4th leading cause of cancer related death in US [1]. Poor prognosis had been attributed to inability to diagnose while tumor is resectable, and its propensity towards early vascular dissemination and spread to regional lymph nodes. In advanced pancreatic cancer, gemcitabine is the only agent with proven benefit [2, 3, 4]. Capecitabine is an oral fluorouracil that has

shown promising activity in chemo-naïve metastatic pancreatic carcinoma [5]. Earlier pharmacogenomic studies by our laboratory and others demonstrated that response to 5-FU depends on intra-tumor levels of dihydropyrimidine dehydrogenase (DPD) and thymidine synthase [6, 7, 8, 9, 10, 11]. High expression of DPD increases 5-FU catabolism (leading to inactivation and elimination) while low levels lead to decreased 5-FU clearance and increased anabolism (cytotoxic pathway). Similarly, the importance of thymidine synthase as a target of 5-FU has been underscored by studies demonstrating that incomplete inhibition of thymidine synthase in the tumor results in reduced chemotherapy effect [12, 13, 14]. Role of increased tumor levels of DPD and/or thymidine synthase as a mechanism of resistance has been demonstrated in patients treated with 5-FU [15, 16, 17]. Similar studies evaluating the efficacy of capecitabine have demonstrated that intra-tumor levels of TP and DPD are good indicators of tumor response. Thymidine phosphorylase (TP) is an enzyme that catalyzes the mutual transformation of the pyrimidine nucleosides thymidine and thymine in nucleic acid metabolism. TP is also an enzyme that converts the 5-FU-based anticancer drugs, 5'-deoxy-5-fluorouridine (5'-DFUR) and its derivative, capecitabine, into 5-FU, and it is therefore a limiting factor of the anti-tumor effects of these anticancer drugs. Capecitabine is a pro-drug that is converted to 5'-DFUR in the liver and tumor tissue, and is the first oral fluoropyrimidine-based anticancer drug that the American FDA has approved as first-line chemotherapy for the indication of metastatic colon cancer [18]. Since it is reported that TP is the same protein as platelet-derived endothelial cell growth factor (PD-ECGF), it is considered as a factor involved in tumor angiogenesis and reflects the biological characteristics of cancer [19]. Reports also show that high TP in stomach cancer and colon cancer is associated with a bad prognosis [20, 21], and that the 5-FU/leucovorin drug regimen is ineffective in colon cancer [22] suggesting that cases of

colon cancer with a high TP should be treated with 5'-DFUR and capecitabine [23].

Based on the metabolic pathway of capecitabine illustrated above, it seems intuitive that increased TP would result in higher intra-tumor 5-FU level thereby enhancing anti-tumor activity of capecitabine. This preferential activation of capecitabine to 5-FU was demonstrated in colorectal tumor by Shuller J *et al.* [24]. Tsukamoto *et al.* presented *in vitro* data suggesting that high DPD expression results in lower intra-tumor 5-FU levels through increased degradation [25].

An *in vitro* study suggested the importance of the ratio of TP and DPD (TP/DPD) in predicting activity of capecitabine against cancer cells. Ishikawa *et al.* showed that capecitabine can be effective in tumors expressing low TP if DPD expression was low as well. Conversely, capecitabine was not as effective as it was expected to be in tumors with high TP levels if tumors had high DPD levels [26]. Other recent studies suggested correlation between high TP/DPD ratio and anti-cancer activity of 5'-DFUR in human cancer xenograft models [27, 28]. A recent study using 5'-DFUR as adjuvant chemotherapy in colorectal cancer showed that patients with high TP/DPD ratio had better disease-free survival which supported the hypothesis that 5-FU is better activated with high TP levels and degraded to a lesser degree with low DPD levels thereby giving cancer cells maximum exposure its anticancer activity [27].

Currently, there are three main ways of measuring TP and DPD: i) immunohistochemistry; ii) a transcription-polymerase chain reaction method; iii) an enzyme-activity assay. However, these methods all require obtaining tumor samples from patients which involve invasive biopsies [23].

We report two patients with metastatic pancreatic carcinoma who had long-term survivals on capecitabine after gemcitabine therapy failure. These two encouraging cases prompted us to measure TP and DPD levels to facilitate further discourse regarding their possible roles in activity of capecitabine

against metastatic pancreatic cancer. We also describe a novel method of measuring TP level from serum without an invasive tissue biopsy.

Patients

Patient 1

A 61-year-old female was diagnosed with a stage IV small cell pancreatic cancer in September 2001. Her disease progressed after three cycles of first-line therapy including etoposide at 125 mg/m² on days 1-3, paclitaxel at 175 mg/m² on day 4, and carboplatin at an area under the curve of 5 on day 4. Her therapy was then switched to single agent gemcitabine 1,000 mg/m² i.v. over 30 minutes with three weeks on and one week off schedule until her disease progressed after 9 cycles. In September 2002, capecitabine single agent was started at 1,500 mg/m² twice daily with two weeks on and one week off schedule. She tolerated capecitabine very well during cycle 1, therefore, the dose was escalated to 1 800 mg/m² twice daily with two weeks on and one week off schedule, and subsequently to 2,000 mg/m² during cycle 3. More than 4 years after the initiation of the capecitabine and after 57 cycles (171 weeks), this patient is alive with Eastern Cooperative Oncology Group (ECOG) performance status of 1, improved from 2 from the time prior to the treatment. Her disease has not progressed on capecitabine; evidenced by stable CA 19-9 levels (Figure 1) and serial CT scans which revealed a minimal radiological evidence of disease progression. She continues to be treated with capecitabine at 1,500 mg/m² twice daily with no significant toxicity. Her dose was reduced due to second appearance of hand-foot syndrome. She was given the option of ‘chemotherapy holiday’ which she declined.

Patient 2

A 64-year-old male was diagnosed with stage IV pancreatic adenocarcinoma with liver metastasis in 2003. After progression of his disease on 6-month treatment with gemcitabine (1,000 mg/m²), he was started at

a reduced dose of capecitabine 750 mg/m² daily with two weeks on and one week off schedule starting November 2003 due to elevated liver enzymes secondary to liver metastases: total bilirubin 4.63 mg/dL (reference range: 0.2-1.3 mg/dL), AST 117 IU/L (reference range: 5-35 IU/L), and ALT 121 IU/L (reference range: 7-56 IU/L). An ultrasound was performed to evaluate hyperbilirubinemia and no biliary dilatation was found. The patient tolerated the first cycle of capecitabine and showed response evidenced by improvement of liver function test values which were significantly elevated prior to the treatment due to extensive liver metastasis: total bilirubin level (1.0 mg/dL), AST (46 IU/L) and ALT (37 IU/L). Subsequently the dose of capecitabine was gradually escalated to 1,000 mg/m² twice daily two weeks on and one week off. During 16 cycles (48 weeks) of capecitabine therapy, the patient’s ECOG performance status improved from 2 to 1, and serial CT scans and CA 19-9 levels indicated stable disease. In May 2005, capecitabine was discontinued due to worsening of his performance status, and the patient was transferred to a hospice due to failure to thrive.

Methods

Isolation of Total RNA from Whole Blood

Approximately 2.5 mL of human whole blood was drawn into PAXgene® Blood RNA tubes (Qiagen Inc., Valencia, CA, USA). Tubes

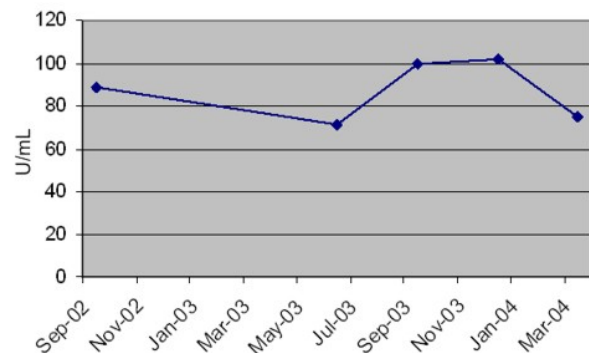


Figure 1. Serial CA 19-9 levels of the Patient 1 during capecitabine therapy. Stable CA 19-9 level during treatment correlated with this patient’s clinical response to capecitabine. (CA 19-9 reference range: 0-36 U/mL).

were incubated for 48 hours at 4°C for maximum cell lysis and RNA yield. Total RNA was then isolated using the PAXgene® Blood RNA Kit (Qiagen Inc., Valencia, CA, US) as per manufacturer’s instructions. RNA was eluted in 40 µL of RNase-free water and stored at -80°C. All sample concentrations were calculated spectrophotometrically at A260 and diluted to a final concentration of 20 ng/µL in RNase-free water containing 12.5 ng/µL of total yeast RNA (Ambion®, Austin, TX, USA) as a carrier.

Real-Time Quantitative PCR

Expression levels were determined using an ABI PRISM® 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) as previously described by our laboratory [29, 30]. The real-time quantitative PCR primers were as follows: human TP forward (5’ TCC TGC GGA CGG AAT CC-3’), reverse (5’ TGA GAA TGG AGG CTG TGA TGA G-3’), and fluorophore-labeled probe (FAM - CAG CCA GAG ATG TGA CAG CCA CCG T - TAMRA). The sequence for the primers and probes for human DPD, and S9 ribosomal have been previously described [31, 32]. Expression levels were calculated using the relative standard curve method [29, 30]. All reactions were run in triplicate and standard curves with correlation coefficients falling below 0.98 were repeated. Control reactions confirmed that no amplification occurred when yeast total RNA was used as a template. Similar results were observed when no-template-control reactions were performed.

Table 1. Thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) expression levels and their ratios in two metastatic pancreatic patients who achieved long-term survivals on capecitabine.

Sample	TP expression	DPD expression	TP/DPD ratio
Patient 1	1.77	4.14	0.43
Patient 2	5.56	2.74	2.03
Reference value ^a	1.00	1.00	1.00

^a A value of 1.00 is assumed to be the value of patients without cancer diagnosis.

Results

Expression levels were calculated relative to total RNA isolated from the blood of a study participant who was not diagnosed with pancreatic cancer. TP levels of Patients 1 and 2 were, respectively 1.77 and 5.56 compared to 1.00 which is assumed to be the TP level in blood from patients without cancer diagnosis. DPD levels were 4.14 for Patient 1 and 2.74 for Patient 2 compared to the reference value of 1.00. TP/DPD ratio of the Patient 1 was 0.43 compared to the reference value of 1.00 of patients without cancer diagnosis. The Patient 2 had the ratio of 2.03 (Table 1).

Discussion

Pancreatic cancer has extremely poor prognosis with few treatment options of proven benefits. Median survival is 3 to 6 months for patients with metastatic pancreatic cancer [1]. Gemcitabine has been the only chemotherapy proven to have modest activity against pancreatic cancer [2, 3, 4]. Numerous ongoing studies are in progress to develop new agents and to optimize treatment strategies.

Capecitabine is an oral 5-FU pro-drug designed to reduce burden of intravenous infusion, decrease toxicity, and increase efficacy by selectively delivering active cytotoxic agent to tumor cells. Capecitabine is absorbed through intestine in inactive form and is metabolized to the active compound 5-FU in three step-wise fashion. TP is a rate limiting enzyme in the final step that produces 5-FU. 5-FU then is further activated by TP in tumor tissue and form a cytotoxic compound [18].

TP is known to be expressed in tumor cells in a greater number compare to adjacent normal tissue allowing capecitabine’s selective activity against tumor cells. TP activity level is approximately 4 times higher in colorectal tumor compare to activity in healthy tissue which may explain capecitabine’s anti-cancer activity and further supports its selective activation in various solid tumors [20, 21, 28]. 5-FU in tumor cells is degraded by an enzyme DPD into an inactive compound. Deficiency in DPD level has been associated with

increased toxicity of 5-FU [30, 31, 32, 33]. On the other hand, increased DPD level has been associated with decreased anti-tumor activity of capecitabine even in cancers with high TP expressions [26].

Preclinical and clinical studies suggested that a high TP/DPD ratio can predict high positive response rates of various solid tumors to capecitabine. There is evidence suggesting that gastric and colorectal cancer patients with high TP/DPD ratio results in increased disease free survival benefits with 5 FU based agents such as capecitabine [26, 28].

Capecitabine has been shown to benefit pancreatic cancer patients. A phase II trial of capecitabine in advanced or metastatic pancreatic cancer showed that capecitabine resulted in objective response and clinical response with a favorable toxicity profile [5]. Based on this trial, capecitabine is being investigated in settings of advanced and metastatic pancreatic carcinoma as first line treatment as well as second line therapy after gemcitabine failure. Two patients we discussed had metastatic pancreatic cancers, and they both had remarkable long term survivals on capecitabine after having progressed on gemcitabine: one patient with 4-year, and the other with 2-year, survival. In these cases, capecitabine not only resulted in survivals far-exceeding median survival of metastatic pancreatic cancer, but also offered improved quality of life to both patients indicated by improved performance status. We measured TP and DPD levels from these patients' peripheral blood and noted that both patients had high TP levels. One patient had substantially high TP/DPD ratio.

While it is difficult to extrapolate pharmacogenetic explanation based on two-patient-experience and the TP measurement method that is not validated, it is possible that their unusual clinical responses from capecitabine are associated with the aforementioned hypothesis that high TP level and TP/DPD ratios enhance anti-tumor activity of capecitabine. If one can prove this hypothesis in a large scale clinical trial, clinicians in the future will be able to pre-select patients who will have good responses

to capecitabine with minimum toxicity based upon TP and DPD levels.

One of the limitations of investigating the relationship between TP and DPD levels and tumor responses to capecitabine is the invasive tissue biopsies that are currently required to measure TP and DPD levels. Development of a non-invasive yet accurate method will make TP and DPD measurements more available to a larger patient population hence facilitating further studies that will identify pharmacogenetic significance of TP and DPD levels in pancreatic cancer treatment with capecitabine.

There is a validated method of measuring DPD level via peripheral blood [33]. However, at present time, we do not have proven method to measure TP level without tissue biopsy. In this paper, we present use of a novel non-invasive method to measure TP levels from peripheral blood samples of two patients with metastatic pancreatic cancer who had remarkable responses to capecitabine. This method needs to be validated in a large clinical trial.

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Abbreviations 5-FU: 5-fluorouracil; 5'-DFUR: 5'-deoxy-5-fluorouridine; CAP: capecitabine; DPD: dihydropyrimidine dehydrogenase; ECOG Eastern Cooperative Oncology Group; PD-ECGF platelet-derived endothelial cell growth factor; TP: thymidine phosphorylase

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