The Effect of *Cytidine* Deaminase Polymorphisms on Hematotoxicity in Thai Cancer Patients Treated with Gemcitabine-based Chemotherapy

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Background and Objective: Gemcitabine (dFdC) is a high efficacy chemotherapy that used in pancreatic cancer, non-small cell lung cancer (NSCLC), ovarian cancer, bladder cancer and cholangiocarcinoma patients. However, clinical outcome and adverse effect depend on variation of gemcitabine pharmacokinetics. However, 90% of gemcitabine metabolized to inactive form by cytidine deaminase enzyme (CDA). Patients who carried*CDA*2* and *CDA+435C>T* have high CDA activity and decrease risk of hematotoxicity. Previous report showed patients who carried *CDA1/*2* and **2/*2* mutant allele decrease risk of neutropenia and thrombocytopenia from gemcitabine more than wildtype group (**1/*1*). Moreover,NSCLC patients who carried *CDA+435CT* or *TT* genotype have lower gemcitabine response rate than wild type. This study aimed to determine correlation between *CDA* polymorphisms and hematotoxicity in Thai cancer patients who treated with gemcitabine-based chemotherapy.

Methods: Seventy patients who treated with gemcitabine-based chemotherapy were enrolled in this study. *CDA* genotype analysis was performed by Real-time PCR technique with specific TaqMan® probe. Severity of hematotoxicity was evaluated by National Cancer Institute Common Toxicity Criteria (CTCAE) version 4.0 guideline. Severity of hematotoxicity was determined during the first 3 months after treatment. Correlation between *CDA* polymorphism and hematotoxicity were assessed by Binary Logistic regression test in SPSS statistic software version 17.0 (SPSS Inc., Chicago, USA)

Results: Patients who carried *CDA*1/*2* and *CDA+435CT/TT* polymorphisms tended to decrease risk of neutropenia. However,correlation between *CDA* polymorphisms and hematotoxicity not statistically significant.

Conclusion: *CDA*1/*2* and *CDA+435CT/TT* SNP tended to decrease risk of hematotoxicity but no statistic significant because of small sample size.

Keywords: cytidine deaminase, *CDA* polymorphisms, gemcitabine, hematotoxicity

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Proceeding

Introduction

Gemcitabine (2', 2'-difluorodeoxycytidine, dFdC) is an anticancer drug for various cancers such as non-small cell lung cancer (NSCLC), pancreatic cancer, breast cancer, bladder cancer and cholangiocarcinoma. When dFdC was uptake into cell, it is phosphorylated by three enzymes deoxycytidine kinase (CDK), cytidine monophosphate kinase 1(CMPK1) and nucleosidediphosphate kinase, repectively to its active triphosphate form[2]. It is deoxycytidine analog that replace cytidine nucleotide (C) to inhibit DNA synthesis. Drug response and adverse effect depended on

individual pharmacokinetic of patients. However, 90% of dFdC was metabolized to inactive form (2',2'-difluorodeoxyuridine, dFdU) by cytidine deaminase enzyme (CDA)[3]. Recent studies reported that pharmacogenetic variations of dFdC metabolism effected gemcitabine efficacy. Single nucleotide polymorphism of *CDA* gene at 79 A> C(K27Q; rs2072671) or *CDA*2* correlated with CDA high activity[1]. Patients who carried *CDA*2* (**1/*2* and **2/*2*) mutant allele have high CDA activity and decrease risk of neutropenia and thrombocytopenia from gemcitabine when compared to wild type (**1/*1*) (P = 0.006 and 0.03 respectively)[5]. Non-small cell lung cancer (NSCLC) patients who carried *CDA*2* had lower gemcitabine response rate than wild type group (37.7% versus 13.8%; p = 0.006)[6].

In addition,polymorphism of *CDA+435C > T* (Thr145Thr; rs1048977) effected on CDA activity. People who carried*CDA+435TT* genotype have high expression of *CDA* in peripheral mononuclear blood cells[7]. Moreover, NSCLC patients who carried *CDA+435CC* genotype had highest gemcitabine response rate than *CDA+435CT/TT* genotype (72% vs 28% respectively, p = 0.026)[4].

Hematotoxicity is common adverse effected from gemcitabine. The correlation between *CDA* polymorphism and hematotoxicityhas not been reported in Thai cancer patients. This study expected *CDA* polymorphism analysis can predict risk of hematotoxicity from gemcitabine. To determine correlation between *CDA* polymorphisms and hematotoxicity in Thai cancer patients who treated with gemcitabine-based chemotherapy.

Proceeding

Methods

This study was multicenter study including Srinagarind Hospital, KhonKaen Hospital and Udonthani Cancer Hospital, Thailand. The research protocol was approved by Ethic Committee for Human Research of each site (Reference No. HE581266, KE58074 and 10/2557 respectively). Written informed consent was obtained from all patients.

Seventy cancer patients who treated with gemcitabine-based chemotherapy were enrolled in this study. Inclusion criteria were patients age > 18 years, Eastern Cooperative Oncology Group (ECOG) performance status grade 0-2 and normal liver and kidney function. Hematotoxicity were evaluated by National Cancer Institute Common Toxicity Criteria (CTCAE) version 4.0 guideline, during first 3 months of therapy.

Three milliliter of venous blood sample was collected in EDTA-containing vacutainer tubes from each patient. Blood sample were collected before starting day 1 treatment of a cycle. Then, blood samples were centrifuged at 2,500 rpm for 10 min. and collected only plasma and buffy coat. Genomic DNAs extraction was isolated from buffy coat with the QIAamp® DNA blood extraction kit (Qiagen, Hilden, Germany). The *CDA*2* and *CDA+435 C>T* polymorphism were verified by Real-time PCR technique with specific TaqMan® probe. Genotype analyses were performed by using the Light-Cycler 480 technology (Roche Diagnostics, Meylan, France).

Correlation between *CDA* polymorhisms and hematotoxicity were assessed by Logistic regression test. This study analyzed by SPSS statistic software version 17.0 (SPSS Inc., Chicago, USA).

Result

All of the patient's baseline characteristics were showed in table 1. Seventy Thai cancer patients (40 men, 30 women) were enrolled in this study. The mean age was 58 ± 10.5 years (range, 25-79 years).Majority of the patients were cholangiocarcinoma (48.6%) and NSCLC (38.6%). Most of the patients are advance stage of cancers (stage IV) 92.5%.

The prevalence of *CDA*1/*1* and **1/*2* genotype were 81.4% and 18.6% respectively. No homozygous mutant of *CDA*2/*2* was found. *CDA+435CC*, *CT* and *TT* genotype were 62.9%, 32.6% and 4.3% respectively (Table 2).

Patients who carried *CDA*1/*2*, *CDA+435CT/TT* genotype had lower neutropenia and anemia than wild type but no statistical significance (table 3). Moreover *CDA*1/*2* or *CDA +435CT/TT* patients had lower incidence of delay/skip chemotherapy and blood transfusion, but non statistical significance.

Table 1 Patients characteristics

Characteristic	Patients N (%)
Gender	
Men	40 (57.1)30
Women	(42.9)
Cancer type	
Cholangiocarcinoma	34 (48.6)
NSCLC	27 (38.6)
other	9 (12.8)
Staging	
Stage	65 (92.7)
IVother	5 (7.1)
Chemotherapy regimen	
Gemcitabine	39 (55.7)
-CisplatinGemcitabine	28 (40)
-CarboplatinGemcitabine	3 (4.3)
Hematotoxicity	
Leukopenia (WBC ≤ 3,000 cell/mm3)	27 (38.6)
Neutropenia (ANC ≤1,500 cell/mm3)	38 (54.3)
Anemia (Hbd" 12 g/dl)	45 (64.3)
Thrombocytopenia (Platelet ≤100,000 cell/mm3)	17 (24.3)
Treatments for adverse effect	
Blood transfusion	27 (38.5)
Delay/skip chemotherapy	31 (44.3)

Discussion

CDA is involved in the salvage pathway of pyrimidine and play a key role in detoxify gemcitabine[8]. Previous studies reported *CDA* polymorphisms related to gemcitabine metabolism and toxicity[4,5]. Hematotoxicity is the most common adverse effect of gemcitabine.

Table 2 The distributions of *CDA* polymorphisms in Thai patients.

Genotype	Patients No. (%)
***CDA*2,79A> C (K27Q)**	57 (81.4)
**1/*1*	13 (18.6)
**1/*2*	
***CDA+435 C>T*(Thr145Thr)**	44 (62.9)
CC	23 (32.6)
CT	3 (4.3)
TT	

Previous study showed CDA polymorphism correlated hematotoxicity.

This study is the first report of correlation between *CDA* polymorphism and hematotoxicity in Thailand. Previous studies reported patients who carried *CDA*1/*2* and *CDA+435CT/TT* genotype had high CDA activity and decreased risk of hematotoxicity. We found that patients who carried *CDA*1/*2*and *CDA+435CT/TT* genotypehad lower risk of neutropenia, anemia and adverse effect treatment (blood transfusion and delay/skip chemotherapy) than wild type. However, this study showed no statistically significant of association between *CDA* variations with toxicity in this population because of small sample size. The correlation not statistic significant because in this study we not founded *CDA*2/*2* genotype or founded a few *CDA+435TT* genotype for compare to wild type. Therefore, enhance sample size may need further investigation for this potential correlation.

Table 3 Hematotoxicity and genotype (N = 70)

Toxicity	Genotype N (%)		P-value	OR (95% CI)
***CDA*2,79A> C (K27Q)**	**1/*1* (N = 57)	**1/*2* (N = 13)		
Leukopenia (WBC≤3,000 cell/mm^3)	22 (38.6)	5 (38.5)	0.900	0.924 (0.27-3.18)
Neutropenia (ANC≤1500 cell/mm^3)	32 (56.1)	6 (46.2)	0.255	0.488 (0.14-1.68)
Anemia (Hb≤ 12 g/dl)	37 (64.9)	8 (61.5)	0.599	1.414 (0.39-5.15)
Thrombocytopenia (Plt≤100,000 cell/mm^3)	13 (22.8)	4 (30.8)	0.455	1.667 (0.437-6.358)
delay/skip chemotherapy	27 (47.4)	4 (30.8)	0.566	0.677 (0.18-2.56)
blood transfusion	23 (40.4)	4 (30.8)	0.524	0.657 (0.18-2.39)
***CDA+435 C>T*(Thr145Thr)**	CC (N = 44)	CT/TT(N = 26)		
Leukopenia (WBC≤3,000 cell/mm^3)	17 (38.6)	10 (38.5)	0.988	0.993 (0.37-2.69)
Neutropenia (ANC≤1500 cell/mm^3)	26 (59.1)	11 (42.3)	0.295	0.593 (0.22-1.58)
Anemia (Hb≤ 12 g/dl)	30 (68.2)	15 (57.7)	0.622	0.779 (0.29-2.10)
Thrombocytopenia (Plt≤ 100,000 cell/mm^3)	9 (20.5)	8 (30.8)	0.334	1.728 (0.57-5.24)
delay/skip chemotherapy	22 (50)	9 (34.6)	0.167	0.487 (0.18-1.35)
blood transfusion	17 (38.6)	10 (38.5)	0.988	0.993 (0.37-2.69)

Conclusion

Patients who carried *CDA*1/*2* and *CDA+435CT/TT* genotypemay decrease risk of neutropenia, anemia and adverse effect treatment (blood transfusion and delay/skip chemotherapy). However, we need more sample size for further investigation.

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