RESEARCH ARTICLE

Prevalence of *CTR1* and *ERCC1* Polymorphisms and Response of Biliary Tract Cancer to Gemcitabine-Platinum Chemotherapy

Skolchart Pongmaneratanakul[1], Suebpong Tanasanvimon[2], Thitima Pengsuparp[1]*, Nutthada Areepium[1]

Abstract

Purpose: Biliary tract cancer (BTC)is an aggressive disease with a poor prognosis. Most patients are diagnosed at an advanced stage for which curative surgery is not possible and gemcitabine-platinum chemotherapy is the treatment of choice for advanced cases. Several studies had focused on biomarkers to predict response from platinum drugs in lung cancer, but information is limited for BTC. In this study, two single nucleotide polymorphisms (SNPs) in the copper transporter (CTR1) and excision repair cross-complementary group 1 (ERCC1) genes were investigated as predictive biomarkers of objective response to gemcitabine-platinum. **Methods:** This cohort study aimed to assess any associations of genetic polymorphisms of these proteins active in drug pathway with treatment response in advanced BTC patients. Twenty six patients were enrolled. DNA was extracted from peripheral blood and genetic polymorphisms were assessed by Taqman allelic discrimination assay. Response was evaluated according to RECIST version 1.1. Results: For the *CTR1* polymorphism, GT was the most common genotype (61.5%) followed by GG (34.6%), and TT (3.8%). For the ERCC1 polymorphism, only 2 genotypes were found, CC and CT at 57.7% and 42.3%, respectively. Genetic polymorphisms were not found to be singly associated with response. However, when the 2 genetic polymorphisms were combined, GG/CC showed a higher response rate than the others (p=0.018, Fisher's Exact Test). **Conclusion:** This is the first study to show an association between *CTR1* and *ERCC1* polymorphisms and response to gemcitabine-platinum in advanced BTC patients. These polymorphisms might be used as biomarkers to predict response in such cases in the future.

Keywords: Biliary tract cancer- platinum- *CTR1- ERCC1*- treatment response

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Introduction

Biliary tract cancer (BTC) is a heterogeneous group of cancer in biliary tract which consists of cholangiocarcinoma (CCA), both intrahepatic and extrahepatic, and gallbladder cancer (GBC) (Park et al., 2015). Even CCA and GBC are differentiated by epidemiology, and clinical presentation, they are studied together because of rarity, pattern of progression, and anatomical relation (Zhu et al., 2010). The global incidence of BTC are low, but not in the Northeast of Thailand where the highest rate of CCA are found. The study in 1988 showed the rates of CCA in Thailand as 89.2 and 35.5 per 100,000 population in males and females, respectively, and the rates remain high (Vatanasapt et al., 1990). BTC is an aggressive disease with poor prognosis. The overall median survival in Thai CCA patients mostly received only supportive care was 4 months and 2-year survival was only 8.1% (Luvira et al., 2016). The only curative treatment is surgical resection, but most of patients are diagnosed at advanced stage when surgical resection is not possible (Rizvi and Gores, 2013). Therefore, chemotherapy treatment is usually suggested to patients with advanced stage BTC to extent patients' survival (Park et al., 2015).

Gemcitabine, a pyrimidine analogue, is a standard treatment of pancreatic cancer and is introduced to treat BTC. The efficacy of gemcitabine is synergized by the other cytostatic agent, like antitumor platinum drug (Valle et al., 2010). The study of Valle and team in 2010 showed the higher overall survival in BTC patients treated with gemcitabine-cisplatin than patients treated with gemcitabine alone (11.7 months vs. 8.7 months, p>0.001)(Valle et al., 2010). Base on this result, the combination of gemcitabine-cisplatin is recommended as the standard of care for advanced BTC. In BTC patients with contraindication to cisplatin such as kidney insufficiency, cisplatin was replaced by oxaliplatin (Andre et al., 2008) or carboplatin (Williams et al., 2010) which had comparable efficacy.

Even gemcitabine-cisplatin counts as the standard treatment for advanced BTC, the response and overall survival is still low (Ramirez-Merino et al., 2013). Some researchers believed that single nucleotide polymorphisms (SNPs) of gene encoded key proteins in drug pathway are related with clinical outcomes. They hypothesized that those SNP may provide different amount of proteins

*[1]Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, [2]Medical Oncology Unit, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. *For Correspondence: Thitima.pe@pharm.chula.ac.th*

or alter protein functions as a result in different drug response. Furthermore, SNP may be used to predict patient's response to chemotherapy and to select for the most effective chemotherapy regimens for each patients.

Copper transporter (*CTR1*) plays a role in platinum influx as demonstrated in in vitro and in vivo studies in mouse model (Holzer and Howell, 2006). There is only one study of association between *CTR1* polymorphism (rs12686377) and response to platinum-based regimens in non-small cell lung cancer (NSCLC) patients. The result showed that TT genotype carriers were more resistant to platinum-based therapy than carriers of the other genotypes (Xu et al., 2012).

Excision repair cross-complementation (*ERCC1*) protein is a rate-limiting protein in nucleotide excision repair which is responsible for detection and removal of damaged DNA after formed complex with platinum (Rose et al., 2014). Wei and colleague performed a meta-analysis from NSCLC patients treated by platinum-based chemotherapy and found a relationship between response rate and polymorphism of *ERCC1* gene (rs11615) (Wei et al., 2011). In contrast, Yu and team performed a similar meta-analysis and showed no association between *ERCC1* polymorphism and platinum-based treatment response (Yu et al., 2012).

Until now, there have been no studies demonstrated the relationship between *CTR1* and *ERCC1* polymorphisms and treatment response in BTC patients treated by gemcitabine-platinum. The aim of this study was to present the prevalence of *CTR1* and *ERCC1* polymorphisms in Thai patients and relationship between polymorphisms and treatment response in BTC patients.

Materials and Methods

Patients

This cohort study was conducted from January 2016 to June 2016. Twenty six patients with pathologically confirmed advanced BTC treated at King Chulalongkorn Memorial Hospital (Bangkok, Thailand) were recruited. The characteristics of the subjects were summarized in Table 1. Among the 26 patients, 9 (34.6%) were male and 17 (65.4%) were female with the median age of 61.5 (range, 44-89 years). More than half (53.8%) of patients presented with metastatic stage. Twenty two (84.6%) patients were cholangiocarcinoma and 4 (15.4%) patients were gallbladder cancer. Most of patients (80.8%) had Eastern Cooperative Oncology Group (ECOG) performance status of 1.The total cycles of chemotherapy administration ranged from 1 to 8 (median 4) cycles. The combined chemotherapeutic agents with gemcitabine were cisplatin in 20 (76.9%) cases and carboplatin in 6 (23.1%) cases. The Institutional Review Board of Faculty of Medicine, Chulalongkorn University approved this study (COA No. 154/2016) and all patients signed informed consents in compliance with Declaration of Helsinki before blood samples were drawn.

Methods

Peripheral venous blood was collected from all patients and genomic DNA was extracted according to the manufacturer's protocol using QIAamp Blood Mini kit (Qiagen, Germany). In this study, the genetic polymorphisms of *CTR1* and *ERCC1* of 26 patients were determined by the Taqman allelic discrimination assay. The primers and probes for assay are commercial available through Applied Biosystems Inc, USA. Polymerase chain reaction and fluorescent measurements were performed in StepOnePLus Real time PCR systems (Applied Biosystems Inc, USA). According to the results of DNA sequencing for the SNP of *CTR1* gene, there were three possible genotypes (GG, GT, and TT). For the SNP of *ERCC1* gene, there were CC, CT, and TT. The efficacy of chemotherapy was evaluated with a computed tomography scan after three or four cycles and recorded in accordance with RECIST version 1.1 as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Those patients with CR or PR were called responders and patients with SD or PR were called non-responders. In addition, patients with CR, PR, or SD were grouped as tumor control (TC).

Statistical data analysis

Statistical analysis was performed using SPSS 22.0 software package. Patients' characteristics were reported as frequencies and percentage. The observed number and estimated frequencies of SNPs in *CTR1* and *ERCC1* genes were tested for Hardy-Weinberg equilibrium using Chi square ($\chi 2$) test. The differences of response among patients with different genotypes were statistically analyzed by chi-square test or Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Results

Distribution of genotypes

Twenty six of DNA samples were used to determine SNPs of *CTR1* and *ERCC1* genes. GG homozygous variant of *CTR1* was presented in 9 (34.6%) patients, GT heterozygous variant was presented in 16 (61.5%) patients, and TT homozygous variant was presented in 1 (3.8%) patients. CC homozygous variant of *ERCC1* polymorphism was found in 15 (57.7%) patients, CT heterozygous variant was found in 11 (42.3%) patients, and TT homozygous variant was not found in this study. There were no significant difference between the observed and estimated frequencies of each genotype (p=0.0668 and p=0.1713, respectively). The data was shown in Table 3. Distribution of genotypes did not depend on clinical factor such as sex, age, extent of disease, primary tumor site, and performance status. The data was not shown.

Response to chemotherapy

All of 26 patients were assessed for treatment response. There were no case of complete response from gemcitabine and platinum chemotherapy. Tumor control (PR+SD) was observed in 19 (73.1%) patients, of which PR and SD respectively occurred in 2 (7.7%) and 17 (65.4%) patients. Seven (26.9%) patients had progression of disease during treatment. Basic clinical characteristics including sex, age, extent of disease, primary tumor site, ECOG performance status, and chemotherapeutic

Table 1. Comparison on the Baseline Characteristic of Responders and Non-Responders [n (%)]

Characteristics	N=26	Responders (PR) n= 2	Non-responders (SD+PD) n= 24	P-value (Fisher's exact test)
Gender				
Male	9 (34.6)	1 (11.1)	8 (88.9)	1.000
Female	17 (65.4)	1 (5.9)	16 (94.1)	
Age (year)				
Median (range)	61.5 (44-89)			
< 65 Year	14 (53.8)	0 (0.0)	14 (100.0)	0.203
≥ 65 Year	12 (46.2)	2 (16.7)	10 (83.3)	
Extent of disease				
Locally advanced	7 (26.9)	0 (0.0)	7 (100.0)	0.395*
Metastatic	14 (53.8)	2 (14.3)	12 (85.7)	
Mixed	5 (19.2)	0 (0.0)	5 (100.0)	
Primary tumor site				
Cholangiocarcinoma	22 (84.6)	2 (9.1)	20 (90.9)	1.000
Gallbladder	4 (15.4)	0 (0.0)	4 (100.0)	
Performance status (ECOG)				
0	4 (15.4)	0 (0.0)	4 (100.0)	0.773*
1	21 (80.8)	2 (9.5)	19 (90.5)	
2	1 (3.8)	0 (0.0)	1 (100.0)	
Chemotherapeutic Regimens				
Gemcitabine/Cisplatin	20 (76.9)	1 (5.0)	19 (95.0)	0.415
Gemcitabine/Carboplatin	6 (23.1)	1 (16.7)	5 (83.3)	

*-Chi-square test

regimens were not significantly different between responders and non-responders or tumor control group and progressive disease. The data was shown in Table 1 and Table 2.

From previous study, TT genotype of *CTR1* polymorphism showed more resistant than the other genotypes (Xu et al., 2012). In this study, *CTR1* polymorphism was divided in GG genotypes and GT+TT genotypes according to presenting of T allele. There was no relationship between *CTR1* polymorphism and response (p=0.111, Fisher's exact test) as well as tumor control (p=0.188, Fisher's exact test). The data was not shown. For *ERCC1* polymorphism, there was no TT genotype in our study. The response and tumor control were compared between CC carriers and CT carriers. However, we found no different between these two groups (p=0.492 and p=0.407, Fisher's exact test, respectively). The data was not shown.

To observe the combined effect of genotypes of both genes, carriers were classified by the potential of treatment response. Four patient had GG genotype of *CTR1* polymorphism and CC genotype of *ERCC1* polymorphism (GG/CC), while the others showed GG/CT, GT/CC, GT/CT, TT/CT, and TT/CC. Combination of GG/TT, GT/TT and TT/TT were not found in this study. The response rate of GG/CC carriers was significantly higher than the carriers of the other genotypes (p=0.018, Fisher's exact test). However, we found no relationship between genetic combination and tumor control (p=1.000, Fisher's exact test). The data was shown in Table 4.

For further analysis, we reclassified patients into GG/CC + GT/CC groups and the others. However, no significant difference in response rate and tumor control rate between the two groups (p=0.483 and p=0.665, respectively). The data was not shown.

Discussion

Biliary tract cancer, especially cholangiocarcinoma, is most found in the Northeast of Thailand. Biliary tract cancer had poor prognosis and most of patients had advanced stage when diagnosed. Gemcitabine-cisplatin regimen in Valle and team study provided longest median overall survival and was suggested as the standard treatment in advanced biliary tract cancer since 2010 (Valle et al., 2010). However, the data from previous studies showed the wide range of overall survival and response rate (Park et al., 2015). In the aggressive disease like cancer, researchers attempt to find the biomarker to predict and maximize the treatment response.

Single nucleotide polymorphisms of genes that coding proteins in pathway of platinum drug were chosen to study its effect on treatment outcome. Copper transporter is responsible for platinum influx into cell and affect intracellular level of drug. There is only one study of *CTR1* polymorphism and response to platinum-based regimen in

Table 2. Comparison on the Baseline Characteristic of Tumor Control Group and Progressive Disease Group [n (%)]

Characteristics	Tumor control (PR+SD) n= 19	Progressive disease (PD) n= 7	P-value (Fisher's exact test)
Gender			
Male	7 (77.8)	2 (22.2)	1.000
Female	12 (70.6)	5 (29.4)	
Age (year)			
Median (range)			
< 65 Year	10 (71.4)	4 (28.6)	1.000
≥ 65 Year	9 (75.0)	3 (25.0)	
Extent of disease			
Locally advanced	4 (57.1)	3 (42.9)	0.290*
Metastatic	12 (85.7)	2 (14.3)	
Mixed	3 (60.0)	2 (40.0)	
Primary tumor site			
Cholangiocarcinoma	16 (72.7)	6 (27.3)	1.000
Gallbladder	3 (75.0)	1 (25.0)	
Performance status (ECOG)			
0	3 (75.0)	1 (25.0)	0.817*
1	15 (71.4)	6 (28.6)	
2	1 (100.0)	0 (0.0)	
Chemotherapeutic Regimens			
Gemcitabine/Cisplatin	13 (65.0)	7 (35.0)	0.146
Gemcitabine/Carboplatin	6 (100.0)	0 (0.0)	

*-Chi-square test

Table 3. Observed Number and Estimated Frequencies of CTR1 and ERCC1 Polymorphisms [n (%)]

	Observed	Estimated	
CTR1			
GG	9 (34.6)	10.7	$\chi2$=1.9769
GT	16 (61.5)	12.6	P=0.0668
TT	1 (3.8)	3.7	
ERCC1			
CC	15 (57.7)	16.2	$\chi2$=1.8715
CT	11 (42.3)	8.7	P=0.1713
TT	0 (0.0)	1.2	

Table 4. Combination of ERCC1 and CTR1 Polymorphisms and Both Response Rate and Tumor Control [n (%)]

N=26	Responders (CR+PR)	Non-resonders (SD+PD)	P-value (Fisher's exact test)
GG/CC	2 (50.0)	2 (50.0)	0.018
The others	0 (0.0)	22 (100.0)	
	Tumor control (PR+SD)	Progressive disease (PD)	
GG/CC	3 (75.0)	1 (25.0)	1.000
The others	16 (72.7)	6 (27.3)	

NSCLC patients. Excision repair cross-complementation group 1 play a role in detection and removal of damage DNA by platinum drug. Even there are systemic reviews about effect of *ERCC1* polymorphism on treatment response in NSCLC patients, the result are controversial and more studies are needed. There is no study of those genetic polymorphisms in biliary tract cancer patients treated by gemcitabine-platinum regimen.

In term of prevalence, GG genotype of *CTR1* polymorphism which provide more drug sensitivity was found in 34.6 % of patient, while GT and TT was found in 61.5% and 3.8% of patients, respectively. Our result was not consistent with the study of Kumpiro in Thai NSCLC patients which showed GG genotype is the most commonly found as 62.5% (Kumpiro et al., 2016). In early study in Chinese NSCLC patients showed that GT was the most found and counted as 51.4% of patients which were closer to our result (Xu et al., 2012). The discrepancy may be explained by the number of patients in the study. The study with larger sample may reveal more obvious proportion of genotypes. For *ERCC1* polymorphism, we assumed that CC genotype was linked with better response to platinum regimen. CC genotype was found as 57.7% which slightly higher than the prevalence (45.2%-49.45%) in recent previous studies (Gao et al., 2014; Lv et al., 2014; Zhao et al., 2014). The proportion of genotype may change if number of patients was increased.

The results showed no case of complete response and partial response was found in 2 cases. We found no association between SNPs and treatment outcomes. The reason could be explained by different type of tumors and response. Previous studies which recruited NSCLC patients had higher response rate and had small gap of difference in the number of responders and non-responders. In this study, BTC patients which were mostly in advanced stage had low response rate and most of patients had stable disease as best response.

We observed the effect of combined polymorphisms and found that GG/CC genotype was associated with response rate. Half of patients with GG/CC genotype had PR while all of patients with the other genotypes had SD or PD. The mechanism could be explained by synergistic function of the two proteins on platinum level. Even there is no explanation about the effect of *CTR1* polymorphism on copper transporter protein, we believed that GG genotype may expressed copper transporter that had more permeability to platinum drug and increased platinum level in cell. For *ERCC1* polymorphism, previous study showed significant increase in *ERCC1* protein expression in CT or TT carriers and it was associated with significantly lower response to platinum drug (Chang et al., 2009). Those two mechanisms lead to higher intracellular platinum level resulting in tumor cell sensitization.

To our knowledge, this is the first prospective cohort study that demonstrate the association between SNP of *CTR1* and *ERCC1* genes and the response to chemotherapy treatment in BTC. BTC patients who were unresectable should be selected to gemcitabine-platinum by types of both *CTR1* and *ERCC1* polymorphisms.

The limitation of our study is small sample size due to rarity of disease/ condition of patients and using single study site. The result from small sample may not represent for the whole population. For rare disease like BTC, we believe that number of patients is enough to show the association trend like previous studies by Keawbubpa (Kaewbubpa et al., 2016) who studied about *ERCC1* polymorphism. The study with larger sample size and long-term outcome is needed to confirm our results.

Conflict of interest statement

The authors declared no conflict of interest.

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