RESEARCH ARTICLE

Impact of the Copper Transporter Protein 1 (*CTR1*) Polymorphism on Adverse Events among Advanced Non-Small Cell Lung Cancer Patients Treated with Carboplatin-Gemcitabine Regimen

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Abstract

Background: Platinum-based regimens are effective treatments for advanced non-small cell lung cancer (NSCLC), but the five-year survival rate is still less than 20%. One possible factor appears to be resistance involving polymorphisms in the *CTR1* gene which plays an importance role in accumulation of platinum in the cytoplasm. Purpose: To establish both prevalence of *CTR1* polymorphism and its impact on treatment related toxicity in Thai advanced NSCLC patients. Materials and Methods: Thirty-two advanced NSCLC participants received carboplatin and gemcitabine during January to June 2016 at King Chulalongkorn Memorial Hospital (KCMH) were recruited for analysis of the *CTR1* rs12686377 genotype. These participants were planning to be treated with platinum-based chemotherapy for at least two cycles. Results: Allele frequency of *CTR1* polymorphism G→T was found to be 25%. The results showed that genetic polymorphism at *CTR1* rs12686377 was associated with emesis side effects (P = 0.020) and neuropathic symptoms (P = 0.010). In addition, hematologic side effects in terms of anemia also tended to be related to this polymorphism. Conclusions: This is the first study suggesting that polymorphism at *CTR1* rs12686377 may be associated with toxicity from platinum-based regimens. Therefore, it could be a factor to aid in treatment decision-making.

Keywords: Advanced NSCLC - platinum resistance - *CTR1* - adverse events

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Introduction

The platinum-based regimen is an effective treatment for advanced non-small cell lung cancer (NSCLC) (Network, 2016). Platinum-doublet therapy shows significant beneficial outcomes such as prolong survival, improve clinical symptoms, and improve quality of life (QOL) (Rinaldi et al., 2006). Although platinum-based therapy show several benefits, but the five-year survival rate still less than 20% (Rose et al., 2014) . Moreover, platinum drugs are related to several adverse effects such as nephrotoxicity, bone marrow suppression and neurotoxicity (Rabik and Dolan, 2007). Recent publications reported that the response and resistance of platinum agents involved in at least three different pathways which are accumulation of platinums in cytoplasm, intracellular detoxification of platinums, and capacity of DNA repair. Intracellar accumulation of platinum appears to be important source of drug resistance (Amable, 2016). Both yeast and mammalian cells which had higher accumulation of platinum agents were more sensitive to the drug.

Many evidences indicated that alteration of copper transporter protein 1 (*CTR1*) which is the major plasma membrane transporter responsible for platinum uptake, was associated with platinum sensitivity and toxicity (Safaei and Howell, 2005). Elevated intracellular cisplatin concentration was likely to associate with the up regulation of *CTR1* (Kommuguri et al., 2012) . Moreover, Kim and colleague presented that early stage NSCLC patients who had *CTR1* expression had reduced tumor response ($P<0.0001$) (Kim et al., 2014). Genetic polymorphisms of *CTR1* also effect to platinum treatment response. Advanced NSCLC patients with heterozygous variant (GT) at rs12686377 had increased platinum resistance ($P<0.05$), whereas a AG halotype had longer survival ($P<0.05$) (Xu et al., 2012a). In term of toxicity, patients with C-allele at rs10981694 apparently represented more sensitivity to otoxicity ($P<0.05$) (Xu et al., 2012b).

To date, there is no data about prevalence of *CTR1* polymorphism in Thai lung cancer patients who must be treated with platinum-base regimen, therefore, we decided

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to find out both prevalence of *CTR1* polymorphism and its impact on the treatment related toxicities in Thai advanced NSCLC patients.

Materials and Methods

Participant

This present study protocol was approved by Institutional Review Board, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, IRB number 598/58. All patients signed in the informed consent form, which performed in accordance with the ethical standard laid down in 1964 Declaration of Helsinki. The study is prospective cohort study, which enrolled 32 participants with advanced NSCLC received platinum-based regimen which was carboplatin and gemcitabine during January to June 2016 at the oncology department, King Chulalongkorn Memorial Hospital (KCMH).

Participants were included in this study according to these criteria: (1) Age more than 18 years old; (2) were diagnosed with histologically or cytologically documented advanced NSCLC stages of IIIB and IV disease; (3) were in Eastern Cooperative Oncology Group (ECOG, PS) status 0–2; (4) were planning to be treated with platinum–based chemotherapy for at least two cycles. The exclusion criteria for this study were following: (1) Participants who were pregnant or lactating; (2) Participants who had baseline estimated creatinine clearance rate (CrCl) below 30 ml/min that calculation by using the Cockcroft and Gault equation. (3) Participants who were received surgery or radiation for lung cancer treatments, or any chemotherapy regimen other than platinum-contained agents; (4) Participants who disagreed to sign informed consent.

Method

After participants signed informed consent, their blood samples were collected and baseline characteristic information (e.g., age, sex, weight, performance status (PS), histological types and TMN stage) were recorded. For smoking history and symptoms related to adverse events (AEs), participants were interviewed by investigator. As regarding laboratory tests, there were including complete blood count (CBC) and renal function tests. In term of CBC, there were collected follow as: Red blood cell (RBC), Hemoglobin (Hgb), Hematocrit (Hct), White blood cell (WBC), neutrophils, lymphocyte, monocyte and eosinophil. With regarding renal function tests, serum creatinine, blood urea nitrogen (BUN) and proteinuria were collected.

Blood sampling were processed for DNA extraction and genotyping. For the isolated of DNA from peripheral blood lymphocytes, Qiagen blood mini kit (Qiagen, German) were using by the manufacture's protocol. Analysis genotypes of *CTR1* rs12686377 were performed by using real time polymerase chain reaction restriction (qPCR): Taqman® assay. Each cycle of chemotherapy, participants were evaluated for AEs according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Statistical analysis

Statistical analysis was performed by using the computer software SPSS version 22 (SPSS. Co., Ltd., Bangkok, Thailand). As for Demographic data, the baseline characteristics were shown as mean ± standard deviation (SD) for continuous variables and number of participants (%) for category variables. SNPs tested obeyed Hardy-Wienberg equilibrium. Each AEs from participants were compared among genotypes by using Chi square test.

Results

Participant characteristics

There were 32 participants included in this study. The number of male to female participant was nearly equal. Seventeen (53.1%) of participants were male and 15 (46.9%) were female, their mean age 63.41 ± 10.49 years. Almost all of participants (27 patients or 84.4%) had clinical stage IV. With regard to histology, nearly all of them (28 patients or 87.5%) had adenocarcinoma. In term of smoking status, a smaller proportion was never smoked (14 or 43.8%) whereas both ever and second hand smoke were 18 or 56.2%. For family history, there were more patients with no known cancer family (21 patients or 65.5%) than who with present cancer in family history (11 patients or 34.4%). All of participants were in good

Table 1. Participant Characteristics

Total number of participants	32	
Age (mean ± SD)	63.41 ± 10.49 (30, 84)	
Characteristic	N	%
Gender		
Male	17	53.1
Female	15	46.9
Clinical Stage		
IIIB	5	15.6
IV	27	84.4
Histology		
Adenocarcinoma	28	87.5
Squamous cell carcinoma	3	9.4
Unspecified	1	3.1
Smoking Status		
Ever	9	28.1
Never	14	43.8
Second hand smoke	9	28.1
Family History		
No	21	65.6
Yes	11	34.4
Performance Status		
ECOG score = 0	4	12.5
ECOG score = 1	28	87.5
CTR1 genotype		
Wild type (GG)	20	62.5
Heterozygous variant (GT)	8	25
Homozygous variant (TT)	4	12.5
allele frequency		
allele G	48	75
allele T	16	25

Table 2. Association between Polymorphism of *CTR1* and Adverse Events

Toxicities	No	Yes	P value
Emesis			0.020*
GG (%)	2 (11.1)	16 (88.9)	
GT (%)	5 (62.5)	3 (37.5)	
TT (%)	2 (50)	2 (50)	
Neuropathy			0.010*
GG (%)	18 (100)	0 (0)	
GT (%)	5 (62.5)	3 (37.5)	
TT (%)	4 (100)	0 (0)	
Weight loss			0.329
GG (%)	15(83.3)	3 (16.7)	
GT (%)	8 (100)	0 (0)	
TT (%)	4 (100)	0 (0)	
Anemia			0.051
GG (%)	2 (11.1)	16 (88.9)	
GT (%)	0 (0)	8 (100)	
TT (%)	2 (50)	2 (50)	
Thrombocytopenia			0.543
GG (%)	10 (55.6)	8 (44.4)	
GT (%)	4 (50)	4 (50)	
TT (%)	1 (25)	3 (75)	
Neutropenia			0.956
GG (%)	8 (44.4)	10 (55.6)	
GT (%)	4 (50)	4 (50)	
TT (%)	2 (50)	2 (40)	

performance status represented by the ECOG score 0 and 1. When analysis polymorphism at *CTR1* rs12686377 was performed, allele frequency of *CTR1* polymorphism G→T was found at 25%. Genotypic distribution was found that the frequency of wild type (GG), heterozygous variant (GT) and homozygous variant (TT) were 62.5%, 25% and 12.5%, respectively.

Polymorphism of CTR1 rs12686377 and adverse events

Our findings indicated that when considering polymorphisms at *CTR1* rs12686377 it could be seen that was negatively correlation with weight loss, thrombocytopenia, and neutropenia. On the contrary, emesis side effect was found to be associated with genetic polymorphism with statistical significance (P = 0.020). Similar to neuropathic symptom which was statistically significantly related to polymorphisms at *CTR1* rs12686377 (P = 0.010). In addition, hematologic side effects in term of anemia also tended to be related with the polymorphism.

Discussion

This study was aimed to find out both prevalence of *CTR1* polymorphism and its impact on the treatment related toxicities in Thai advanced NSCLC patients. We found that allele frequency of polymorphism G→T were 25%. Whereas previous study Xu and colleague shown allele frequency of polymorphism G→T in Chinese NSCLC patients were 46.63%, but our study was smaller population than previous study. (Xu et al., 2012a).

The results showed that the polymorphisms of *CTR1* gene at rs12686377 appeared to associate with adverse events from platinum-based regimen such as emesis, neuropathy and anemia. AEs in this study were classified into one to four grading scale according to CTCAE criteria. Based on metabolic pathway of platinum agents, it could be explained that toxicities related to higher intracellular accumulation of platinum agents. Previous studies explained the relationship between *CTR1* expression and intracellular concentration of platinums. In *Saccharomyces cereviae* which up regulation of *CTR1* revealed enhancing uptake and accumulation of cisplatin (Kommuguri et al., 2012). Indeed, in NSCLC patients with *CTR1* expression who receiving neo-adjuvant platinum-based chemotherapy, their tissue platinum concentration were correlated with tumor response (Kim et al., 2014). Furthermore, later studies reported that polymorphism at *CTR1* gene seemingly affect to modulate concentration of platinum accumulation. Xu and colleague noted that SNPs in *CTR1* at rs7851395 and rs12686377 had a significant correlation with clinical outcomes (Xu et al., 2012a). Therefore, these previous studies could be describing that polymorphism at rs12686377 contributed to regulate platinum intracellular concentration, which wild type of polymorphism were appeared to have higher concentration than variants. In term of emesis, participants with GG genotype were likely to have more emetic symptoms than other genotypes. Likewise, participants with GG genotype was tend to have higher risk of anemia.

In contrast, Xu and colleague found that severe ototoxicity was associated with patient who had C allele in *CTR1* polymorphism at rs10981694 A>C (Xu et al., 2012b). Similar to this study, neuropathy seemed to be associated with participants who had GT genotype. The dorsal root ganglia of the spinal cord at central nervous system are hazard by platinum agents. Neuropathy is characterized by decreased sensory nerve conduction velocity, possibly by acting as a calcium channel blocker.

One strength of the study resulting from prospective research in advanced NSCLC who received carboplatin plus gemcitabine, but the participants of this study relatively small as well as the treatment responses also pending. However, it is recommended that further study with larger sample size should be conducted.

In conclusion, *CTR1* rs12686377 polymorphism was associated with some adverse events in advanced NSCLC patients treated with platinum-based regimen. The similar relationship could be found in treatment response as well. Therefore, genetic polymorphism is one of factors to consider in treatment selection.

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