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Relationship between *ABCB1* gene polymorphisms and severe neutropenia in patients with breast cancer treated with doxorubicin/cyclophosphamide chemotherapy

Midori Ikeda [a], Daiki Tsuji [a, *], Keisuke Yamamoto [b], Yong-Il Kim [c], Takashi Daimon [d], Yutaro Iwabe [a], Masahiro Hatori [a], Ryo Makuta [a], Hideki Hayashi [a], Kazuyuki Inoue [a], Hidenori Nakamichi [b], Mitsuru Shiokawa [b], Kunihiko Itoh [a]

[a] Department of Clinical Pharmacology and Genetics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan
[b] Department of Pharmacy, Seirei Hamamatsu General Hospital, Hamamatsu, Japan
[c] Department of Medical Oncology, Seirei Hamamatsu General Hospital, Hamamatsu, Japan
[d] Department of Biostatistics, Hyogo College of Medicine, Japan

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ABSTRACT

Chemotherapy-induced neutropenia is one of the major adverse events which results in the reduction of chemotherapy. Doxorubicin is a substrate of the adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) transporter; reportedly, *ABCB1* polymorphisms influence doxorubicin pharmacokinetics. We evaluated the association between chemotherapy-induced neutropenia and *ABCB1* polymorphisms in patients with breast cancer. We investigated 141 patients with breast cancer treated with doxorubicin and cyclophosphamide (AC) chemotherapy. Peripheral blood samples obtained from patients were genotyped for the *ABCB1* 2677G>T/A and 3435C>T polymorphisms. The genotypes were then investigated for their association with grade 3 or greater neutropenia, and further their risk factors were examined using a multivariate logistic regression. The proportion of patients with grade 3 or greater neutropenia was 85.7% in the homozygous variant group, and 80% and 58.6% in the heterozygous variant and GG genotype groups, respectively ($p = 0.021$). The multivariate logistic regression analysis revealed that the *ABCB1* 2677G>T/A polymorphism was a strong predictor of grade 3 or greater neutropenia (odds ratio: 3.76; 95% confidence interval: 1.44–9.81; $p = 0.007$). *ABCB1* polymorphisms may influence the extent of chemotherapy-induced neutropenia in AC combination-treated patients with breast cancer.

1. Introduction

Chemotherapy-induced neutropenia is an important dose-limiting toxicity of cytotoxic agents such as doxorubicin, and often necessitates dose reduction from the initial dose. Neutropenia, specifically febrile neutropenia, has a negative impact on the quality of life, increases morbidity and mortality rates, and elevates treatment costs [1,2]. Therefore, an adequate management of chemotherapy-induced neutropenia in patients with cancer is essential.

Anthracycline-based chemotherapy containing doxorubicin and cyclophosphamide (AC) is a chemotherapy regimen commonly used worldwide to treat early-stage breast cancer [3,4]. Doxorubicin is a cytotoxic chemotherapeutic agent belonging to the anthracycline family of antibiotics and a key drug for the treatment of breast cancer.

Doxorubicin, a nucleolar nonselective Class I anthracycline, is mainly exported by the adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) transporter [5].) Lal et al. [6] reported that single-nucleotide polymorphisms (SNPs) in the *ABCB1* gene alter doxorubicin pharmacokinetics. Furthermore, specific *ABCB1* genotypes significantly influence the behavior of doxorubicin in patients with breast cancer, resulting in significantly increased exposure levels and reduced clearance in patients with variant *ABCB1* alleles.

Recently, Bray et al. [7] reported that patient with breast cancer treated with AC chemotherapy and carrying the 2677A *ABCB1* allele demonstrated a significantly shorter time to progression and

* Corresponding author.
E-mail address: d-tsuji@u-shizuoka-ken.ac.jp (D. Tsuji).

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reduced overall survival. Therefore, it is important to evaluate the role of *ABCB1* polymorphisms to improve treatment outcomes and reduce adverse events.

Studies have shown that a higher proportion of Asian patients than Caucasian patients develop severe neutropenia [8–10]. Interethnic differences are increasingly recognized as underlying interindividual variations in drug responsiveness. Nevertheless, little is known about the pharmacogenetics in breast cancer treatment.

We evaluated the association between grade 3 or greater neutropenia and *ABCB1* polymorphisms in patients with breast cancer receiving AC combination chemotherapy. In addition, we have also attempted to identify risk factors predisposing these patients to severe neutropenia.

2. Patients and methods

2.1. Study design and patients

We conducted a retrospective genetic polymorphism association study. Japanese patients with breast cancer attending the Department of Medical Oncology at Seirei Hamamatsu General Hospital, Hamamatsu, Japan, were recruited between January 2009 and January 2014. Patients were eligible for inclusion in this study if they: had histologically confirmed breast cancer and planned to receive four cycles of the standard AC regimen (doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 on Day 1 of a 21-day cycle) as neoadjuvant or adjuvant chemotherapy; were aged ≥20 years at diagnosis; had an Eastern Cooperative Oncology Group performance status of 0–1; and possessed adequate baseline hematologic, hepatic, and renal functions [white blood cell (WBC) count: $\geq 3 \times 10^9$/L; platelet count: $\geq 100 \times 10^9$/L; aspartate aminotransferase (AST): <100 IU/L; alanine aminotransferase (ALT): <100 IU/L; total bilirubin: <2 mg/dL; and serum creatinine level: <1.5 mg/dL].

Written informed consent was obtained from all patients. This study was approved by the institutional review board at the Seirei Hamamatsu General Hospital and complied with the provisions of the *Declaration of Helsinki*.

2.2. Assessment

Hematologic toxicities were graded according to the *Common Terminology Criteria for Adverse Events*, version 4.0. As absolute neutrophil count (ANC) is the most important indicator and most readily quantifiable parameter of myelotoxicity, a clinical significantly severe chemotherapy induced myelotoxicity was defined as having grade 3 or greater neutropenia (ANC: $<1 \times 10^9$/L). The main evaluation parameter was the percentage of patients with grade 3 or greater neutropenia in any cycle. The neutropenia grade was based on the lowest recorded neutrophil count for a patient between the first day of chemotherapy and 3 weeks after the final dose.

Each patient was assessed for a number of baseline variables that potentially influence severe chemotherapy induced neutropenia, including age, body mass index (BMI), clinical stage, and baseline laboratory data. The cut-off value of laboratory data was chosen based on the median in this study. Patients with a BMI above 25 kg/m^2 were considered overweight; this cut-off was chosen in accordance with the universal BMI criteria developed by the World Health Organization [11].

2.3. ABCB1 genotyping

Genomic DNA was isolated from peripheral leukocytes using a QIAamp® DNA Blood Maxi Kit (Qiagen N.V., Limburg, The Netherlands) before the patients started the treatment. The *ABCB1* 2677G>T/A (Ala839Ser, Thr): rs20325282, and 3435C>T (synonymous SNP): rs1045642 variants were determined by polymerase chain reaction-restriction fragment length polymorphism analysis, as described elsewhere [12,13]. The 2677G>T/A SNP has three variants, G, T, and A, that occur at the same position, and patients were classified into three groups based on the presence of the G variant (GG; GT or GA: TT, TA or AA), in accordance with 2677G>T/A genotype expression studies [14–16].

2.4. Statistical analysis

Continuous variables are summarized as means ± standard deviations (SDs) and categorical variables as frequencies and proportions. All the continuous variables were checked for normality using the Shapiro–Wilk test. Normally and non-normally distributed variables were compared using analysis of variance and the Kruskal–Wallis test, respectively. Categorical variables were compared using the chi-square test. The genotypic distribution of each SNP was also examined for deviations from the Hardy–Weinberg equilibrium using the chi-square test. Risk factors associated with the occurrence of grade 3 or greater neutropenia in any cycle were examined using the chi-square test as univariate analysis and logistic regression as multivariate analysis. Risk factors having a p-value of <0.15 in the univariate analysis were included in the multivariate analysis. The results in the multivariate analysis are reported as odd ratios (ORs) with 95% confidence intervals (CIs) and p-values. All statistical tests were two-sided, and p-values of <0.05 were considered statistically significant. Statistical analyses were performed using PASW Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Patient characteristics

One hundred forty-one patients with breast cancer were included in this study. The baseline demographic and clinical characteristics of study participants classified by *ABCB1* genotype are shown in Table 1. Baseline characteristics were well balanced between each *ABCB1* genotype group. All patients were female and received a combination of doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 as adjuvant or neoadjuvant treatment. Twenty-nine patients (20.6%) received the treatment as neoadjuvant chemotherapy. The proportions of estrogen receptor-, progesterone receptor-, and human epidermal growth factor receptor type 2-positive patients were 63.8%, 51.8%, and 37.6%, respectively.

The frequencies of the *ABCB1* 2677 GG, GT, GA, TT, TA, and AA genotypes were 20.6%, 36.9%, 12.8%, 17.7%, 7.8%, and 4.3%, respectively. The frequencies of the *ABCB1* 3435 CC, CT, and TT genotypes were 35.5%, 44.7%, and 19.9%, respectively. The observed genotype frequencies were consistent with the Hardy–Weinberg equilibrium for the *ABCB1* 2677G>T/A and 3435C>T polymorphisms.

3.2. Association between the incidence of grade 3 and greater neutropenia with ABCB1 genotypes

In the entire study group, the proportion of patients who experienced grade 3 or greater neutropenia was 77.3%. Thus, the worst grade of neutropenia was observed within the first cycle. Therefore, all patients received a standard dose of AC combination chemotherapy in the first cycle. Table 2 shows the incidence of neutropenia of grade 3 or greater classified by *ABCB1* genotype. The proportion of grade 3 or greater neutropenia and the *ABCB1*