Drug Related Genetic Polymorphisms Affecting Adverse Reactions to Methotrexate, Vinblastine, Doxorubicin and Cisplatin in Patients With Urothelial Cancer

Norihiko Tsuchiya, Takamitsu Inoue, Shintaro Narita, Teruaki Kumazawa, Mitsuru Saito, Takashi Obara, Hiroshi Tsuruta, Yohei Horikawa, Takeshi Yuasa, Shigeru Satoh and Tomonori Habuchi*

From the Department of Urology, Akita University School of Medicine, Akita, Japan

Purpose: There is considerable interindividual diversity in the development of adverse reactions during chemotherapy for cancers. This diversity is suggested to be attributable to differences in the disposition of chemotherapeutic agents, which is modified by genetic polymorphisms. In this study we evaluated the possible association of polymorphisms of genes involved in the metabolism, detoxification and transport of the agents with adverse reactions to methotrexate, vinblastine, doxorubicin and cisplatin therapy.

Materials and Methods: A total of 40 patients with urothelial cancer who received methotrexate, vinblastine, doxorubicin and cisplatin or high dose methotrexate, vinblastine, doxorubicin and cisplatin chemotherapy between 1996 and 2005 at Akita University Medical Center were included in this study. Four genetic polymorphisms (*ABCB1*, *GSTP1*, *CYP3A5* and *MTHFR*) and clinical parameters were included in the analysis to determine whether there was any association with the grade of adverse reactions at the first cycle and the worst grade of each adverse reaction throughout the chemotherapy period.

Results: On multivariate analysis the *CYP3A5 A6986G* genotype **3/*3* (OR 8.205, 95% CI 1.616–41.667, p = 0.011) and smaller number of treatment cycles (OR 0.156, 95% CI 0.037–0.659, p = 0.011) were independent factors for leukocytopenia (grade 3 or greater) throughout the period of chemotherapy. The mean white blood cell count nadir in patients with genotype **3/*3* was significantly lower than that in those with the **1* allele (1,542 ± 903 vs 2,431 ± 973/mm^3, p = 0.009).

Conclusions: The *A6986G* polymorphism of *CYP3A5*, which is involved in the metabolism of vinblastine and doxorubicin, might be a genetic predictor of the severity of leukocytopenia induced by chemotherapy with methotrexate, vinblastine, doxorubicin and cisplatin.

Key Words: urothelium; neoplasms; antineoplastic agents; adverse effects; polymorphism, genetic

Systemic chemotherapy has been accepted as a treatment option for patients with advanced or metastatic urothelial cancer. Among various chemotherapeutic regimens MVAC has been the standard and most widely used for neoadjuvant, adjuvant or therapeutic intent.[1] Although the dose of each chemotherapeutic agent is determined on the basis of body surface area, considerable interindividual diversity in therapeutic effects and AR severity is observed during chemotherapy. Identification of the factors that lead to these differences is one of the key aims of research to provide individualized cancer therapy.

The individual diversity in therapeutic effect may be due to the biological characteristics of the cancer itself and the innate properties of the patient as the cancer host. On the other hand, the difference in the severity of ARs is suggested to be attributable to differences in the disposition of the chemotherapeutic agents. These individual variations have been partially explained by genetic polymorphisms. The SNP is the most common source of genetic polymorphism, and SNPs are frequently used as markers of susceptibility, progression and prognosis in the study of cancer. It has been reported that for each anticancer agent there are specific molecules responsible for membrane transport, metabolism and detoxification of the agent, and some of these molecules show functional polymorphisms.[2] Recent studies have demonstrated that enzymes involved in the metabolic pathways of anticancer agents are associated with the development of chemotherapy induced ARs or tumor responses.[3,4]

Each agent constituting MVAC has specific molecules that affect its disposition. The gene *ABCB1*, also known as *MDR1*, encodes p-glycoprotein, an adenosine triphosphate dependent efflux pump on the cellular membrane which is expressed at high levels in some cancers and is associated with drug resistance. With regard to MVAC it is known that vinblastine, doxorubicin and cisplatin are substrates of p-glycoprotein. The gene *ABCB1* has a synonymous SNP, *C3435T*, which is reportedly associated with the blood concentrations of several drugs.[5] Glutathione S-transferase pi, encoded by *GSTP1*, is an enzyme that has an important role in the detoxification of hydrophobic compounds such as doxorubicin and cisplatin. The *GSTP1* polymorphism Ile105Val is associated with reduced en-

Submitted for publication April 8, 2008.
Study received institutional review board approval.
* Correspondence: Department of Urology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan (telephone: +81-18-884-6156; FAX: +81-18-836-2619; e-mail: thabuchi@doc.med.akita-u.ac.jp).

DOI:10.1016/j.juro.2008.08.035

zyme activity, and better survival was reported in patients with colorectal cancer with the Val/Val genotype after 5-fluorouracil/oxaliplatin chemotherapy.[6] CYP3A, of which CYP3A4 and CYP3A5 are abundant isoforms, has an important role in the oxidative, peroxidative and reductive metabolism of at least 50% of all drugs including vinblastine and doxorubicin.[7] The *CYP3A5* gene has an important SNP, *A6986G*, the *A* and *G* alleles of which are designated **1* and **3*, respectively. This polymorphism reportedly has a marked influence on the pharmacokinetics of various drugs.[8] MTHFR is an enzyme important for maintaining folate pools and may affect sensitivity to methotrexate. The *MTHFR* gene has a polymorphism, *C677T*, the *T* allele of which correlates with reduced enzyme activity and increased thermolability.[9]

We hypothesized that the severity of ARs in patients undergoing chemotherapy is affected by genetic polymorphisms, as determinants of individual diversity, in addition to clinical factors. In this study the possible association of polymorphisms that modify the metabolism and/or disposition of anticancer agents with adverse reactions to MVAC was evaluated retrospectively.

PATIENTS AND METHODS

Patients

A total of 40 patients with urinary tract cancers who received combination chemotherapy with MVAC or HD-MVAC between 1996 and 2005 at Akita University Medical Center participated in this study. The relative inclusion criteria for chemotherapy at our institution were 1) TCC of the urinary tract (cT3-4a, cN1 or greater for neoadjuvant therapy; pT3 or greater or positive lymph node involvement for adjuvant therapy; metastatic, recurrent or locally invasive cancer not suited to surgery for therapeutic chemotherapy), 2) Eastern Cooperative Oncology Group performance status 0–1, 3) white blood cells 4,000/mm^3, 4) platelets 100,000/mm^3 or greater and 5) serum creatinine 1.5 or more times the upper limit of normal.

The classic MVAC and HD-MVAC regimens were performed as previously reported.[1,10] Briefly classic MVAC consisted of 28-day cycles of 30 mg/m^2 i.v. methotrexate on days 1, 15 and 22; 3 mg/m^2 i.v. vinblastine on days 2, 15 and 22; 30 mg/m^2 i.v. doxorubicin on day 2 and 70 mg/m^2 cisplatin on day 2. The HD-MVAC regimen consisted of 14-day cycles of 30 mg/m^2 i.v. methotrexate on day 1; 3 mg/m^2 i.v. vinblastine on day 2; 30 mg/m^2 i.v. doxorubicin on day 2; 70 mg/m^2cisplatin on day 2 and subcutaneous G-CSF on days 4 to 10. In our study the dose of G-CSF in HD-MVAC was reduced to 100 to 150 μg per body per day and then increased to 300 μl per body per day, as necessary, because the standard dose of G-CSF is beyond the permissible limit of national insurance coverage in Japan. Although cisplatin was reduced to 70% of the standard dose in the presence of renal impairment (Ccr less than 50 ml per minute) the doses of other chemotherapeutic agents were not reduced. In this study 11 patients (27.5%) had a Ccr of less than 50 ml per minute. On days 2 to 4, 5-hydroxytryptamine receptor antagonists were administered intravenously. Steroids were not routinely used as antiemetics. The patients were monitored for complete blood counts and serum biochemistry values at least every week and more frequently when any abnormalities were observed or expected. All chemotherapy regimens were administered in the hospital. For the purpose of neoadjuvant therapy 2 cycles of either regimen were scheduled before radical cystectomy or radical nephroureterectomy, whereas 2 cycles of classic MVAC or 3 cycles of HD-MVAC were administered after surgery as adjuvant therapy. The first cycle of adjuvant chemotherapy was administered 3 to 4 weeks after surgery. Chemotherapy for metastatic or recurrent cancers was continued until persistent disease progression was evident, there was unacceptable toxicity or the patient refused further chemotherapy.

Analysis of Possible Association Between Genetic Polymorphisms and ARs to MVAC

Blood samples were collected from each patient and DNA was extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany). Patients were genotyped for polymorphisms of 4 genes *ABCB1*, *GSTP1*, *CYP3A5* and *MTHFR*. SNPs were determined by the PCR-RFLP method. The site of polymorphism in each gene as well as the primer sequences and restriction enzymes used for genotyping are summarized in the Appendix. For the *CYP3A5 A6986G* polymorphism the *A* and *G* alleles were designated **1* and **3*, respectively. The validity of the PCR-RFLP analysis was confirmed by the direct sequencing of several PCR products showing each genotype using a Dye Terminator Sequencing Kit version 1.0 (Applied Biosystems, Foster City, California) and an ABI PRISM® 310 Genetic Analyzer.

Clinical parameters (age, MVAC or HD-MVAC regimen, total number of cycles, intent of chemotherapy, pretreatment 24-hour Ccr, WBC, hemoglobin and platelets) and genetic parameters (genotypes for *ABCB1*, *GSTP1*, *CYP3A5* and *MTHFR*) were analyzed to evaluate the association with AR grade. The AR grades for leukocytopenia, thrombocytopenia, anemia, anorexia, vomiting and diarrhea at the first cycle and the worst grade of each AR throughout the chemotherapy period were evaluated according to the Common Terminology Criteria for Adverse Events v3.0. Information on these ARs was obtained from medical charts. This study was approved by the institutional review board (the Ethical Committee) of Akita University School of Medicine. Written informed consent for the use of DNA and clinical information were obtained from all patients participating in this study.

Statistical Analysis

The data are expressed as mean ± SD. We defined severe toxicity of the chemotherapy as grade 3 or greater for leukocytopenia and grade 2 or greater for other ARs. The OR and 95% CI for dichotomized grades of AR (ie grades 0–2 vs grades 3–4 for leukocytopenia and grades 0–1 vs grades 2–4 for other ARs) in each genotype group were determined by multiple logistic regression analysis. Each continuous independent variable was dichotomized at the median value and genotypes of each polymorphism were dichotomized according to previous studies. Univariate analyses included age, type of regimen, intent of chemotherapy, pretreatment 24-hour Ccr and the genotypes of 4 polymorphisms. Pretreatment WBC, hemoglobin and platelets were added only in the analysis that assessed the association with grades of leukocytopenia, anemia and thrombocytopenia, respectively. The total number of cy-