CASE REPORT / OLGU SUNUMU

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Effects of Cytochrome P450 (CYP) 2C19 Genetic Polymorphisms on Voriconazole Serum Levels: A Report of Two Cases

Cytochrome P450 (CYP) 2C19 Genetik Polimorfizmlerinin Vorikonazol Serum Düzeyleri Üzerindeki Etkileri: İki Olgu Sunumu

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Abstract

Voriconazole (VCZ), a triazole antifungal, is the first-line drug used in the treatment of invasive aspergillosis. Voriconazole is primarily metabolized by the enzyme cytochrome P450 (CYP) 2C19. Genetic polymorphisms of *CYP2C19* have been reported to be associated with variability in VCZ pharmacokinetics that may lead to a decrease in its efficacy. In this report, we describe two patients who were treated by VCZ for presumed invasive pulmonary aspergillosis, who had *CYP2C19*1/*17* genotypes and whose target serum levels could not be reached despite the appropriate dose of VCZ given. Trough serum levels of VCZ were measured on the 5th day of VCZ treatment which was reported as 0.2 mg/l for the first patient, and 0.1 mg/l for the second patient. We suspected that the patients may be an ultrarapid or rapid metabolizer of VCZ, and genotyping for *CYP2C19* was performed. The *CYP2C19*1/*17* genotype was detected in two of the patients which predicted phenotype of rapid metabolizer for *CYP2C19* activity. To the best of our knowledge, these patients are the first cases reported to have very low plasma levels of VCZ associated with *CYP2C19* genetic variant alleles in Turkey. *CYP2C19* genotyping may potentially improve the safety and efficacy of VCZ treatment.
Keywords: Voriconazole, polymorphism, cytochrome P450, invasive fungal disease

Öz

Vorikonazol (VCZ), invaziv aspergilloz tedavisinde ilk tercih olan triazol türevi bir antifungal ilaçtır. Vorikonazol esas olarak sitokrom P450 (CYP) 2C19 enzimi ile metabolize olmaktadır. *CYP2C19* enziminde olan genetik polimorfizmler VCZ'nin antifungal etkisinde azalmaya yol açabilecek farmakokinetik değişkenliklerle ilişkili olabilmektedir. Bu olgu raporunda, uygun VCZ dozuna rağmen hedef serum seviyelerine ulaşılamayan *CYP2C19*1/*17* genotipi olan, invaziv pulmoner aspergilloz tanısıyla tedavi verilen iki hasta tanımlanmıştır. Serum vadi VCZ düzeyleri VCZ tedavisinin beşinci gününde ölçülmüştür; ilk hastada 0,2 mg/l, ikinci hastada ise 0,1 mg/l olarak belirlenmiştir. Hastaların hızlı veya çok hızlı metabolize edici genotipe sahip olabileceğinden şüphelenilmesi üzerine *CYP2C19* için genotipleme yapılmıştır. Hastaların *CYP2C19*1/*17* genotipine sahip olduğu, *CYP2C19* aktivitesi için muhtemelen hızlı metabolize edici fenotipe sahip olduğu düşünülmüştür. Bildiğimiz kadarıyla, bu hastalar Türkiye'de *CYP2C19* genetik varyantlarıyla ilişkili çok düşük VCZ plazma düzeylerine sahip olduğu bildirilen ilk olgulardır. *CYP2C19* genotiplendirmesi VCZ tedavisinde etkinliğin ve güvenliliğin artmasını sağlayabilir.
Anahtar Kelimeler: Vorikonazol, polimorfizm, sitokrom P450, invaziv fungal hastalık

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Introduction

Voriconazole (VCZ), a triazole antifungal, is the first-line drug used in the treatment of invasive aspergillosis (IA). As VCZ has a narrow therapeutic range, achieving therapeutic VCZ concentrations is a challenge due to its nonlinear pharmacokinetics and inter-patient variability. The pharmacokinetic variability depends on several factors, such as body weight of the patient, food intake, and drug-drug interactions[1]. The successful treatment outcomes were correlated with the plasma trough concentrations between 1.5 and 5.5 mg/l[2], whereas lower VCZ plasma levels may lead to treatment failure, and higher levels may lead to drug toxicity[3]. Therapeutic drug monitoring (TDM) is recommended to ensure efficacy and safety with VCZ treatment[2].

Voriconazole is mainly metabolized by *CYP2C19*. Other CYP enzymes, such as *CYP2C9* and *CYP3A4*, play a minor role in biotransformation. Genetic polymorphisms of these enzymes, especially of *CYP2C19*, may have an important impact on VCZ pharmacokinetics and variability of the plasma concentrations despite weight-based dosing schemes. The activity of *CYP2C19* is related to various genetic polymorphisms. *CYP2C19*1* is the wild-type allele associated with normal function. *CYP2C19*2* and *CYP2C19*3* alleles are related to decreased activity, while the *CYP2C19*17* allele is associated with enhanced *CYP2C19* activity with very low VCZ levels in plasma[3]. In a previous study with a Turkish population, the frequencies of *CYP2C19*17* and *CYP2C19*2* alleles were determined as 24% and 10%, respectively[4].

Case Report

This case report is of two patients with *CYP2C19*1/*17* genotypes, whose target serum levels could not be attained despite administration of the appropriate VCZ dose. The first patient was a 55-year-old male with gastric cancer who received his last chemotherapy (folinic acid, fluorouracil, oxaliplatin) one month prior. The patient was consulted for progressive pulmonary consolidation on chest computed tomography (CT) with *Aspergillus fumigatus* growth in deep tracheal aspirate culture and a serum galactomannan index of 5.4 (cut-off: 0.5). He was treated with VCZ for presumed invasive pulmonary aspergillosis. The VCZ dose was driven by the actual body weight. He received intravenous (i.v.) VCZ at a loading dose of 360 mg twice, followed by a maintenance dose of 240 mg twice daily.

Voriconazole trough serum concentration was ordered on the fifth day of VCZ therapy. Therapeutic drug monitoring was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays with the EUREKA® kit (limit of detection: 0.01 mg/l) (https://www.eurekakit.com/en/)[5]. The blood sample was obtained 30 minutes before the next VCZ dose was given, which yielded a level of 0.2 mg/l. The patient did not receive any drugs that might have interfered with the VCZ levels. The VCZ dose was increased to 6 mg/kg, which required the administration of 360 mg i.v. every 12 hours. On the 7th day of VCZ treatment, the drug level was 0.8 mg/ml, which is out of therapeutic concentration; however, there were only two days after the dosing schedule was changed, it may not have reached its steady-state target yet. Hence, we continued the treatment with VCZ. Serum levels that were measured at the 10th and 11th days of the treatment were within the therapeutic concentration range as 4.7 mg/l and 4 mg/l, respectively. The treatment was continued with the same VCZ dose. The patient passed away with meropenem-resistant *Pseudomonas aeruginosa* bacteremia on the 18th day of VCZ treatment. Therefore, we were unable to evaluate VCZ treatment response.

The second patient was a 39-year-old female with non-Hodgkin's lymphoma who was treated with VCZ for the suspicion of IA with persistent febrile neutropenia after cytotoxic chemotherapy (etoposide, vincristine, doxorubicin, cyclophosphamide, methylprednisolone). The fever persisted with broad-spectrum antibiotics despite negative blood cultures. Although chest and paranasal CT were reported as unremarkable, Aspergillus galactomannan (serum galactomannan index: 1.1) was positive and VCZ was started as a preemptive therapy. The VCZ dose was driven by the actual body weight. She received i.v VCZ at a loading dose of 300 mg twice followed by a maintenance dose of 200 mg twice daily. Voriconazole trough serum level yielded 0 mg/l on the 5th day of the treatment which was obtained 30 minutes before the next VCZ dose was given. The patient did not receive any drugs that might have interfered with the VCZ levels. Voriconazole measurement was repeated, which

Table 1. Primer sequences and enzymes were used to determine cytochrome P450 2C19 (CYP2C19) polymorphisms and restriction fragments in the polymerase chain reaction-restriction fragment length polymorphism method

Genetic polymorphism	Primer sequence	Endonuclease	Cleavage pattern (bp)
*CYP2C19*17* –3402C>T rs12248560	F[a]: 5′-AATAAAGATGACCTTGATCTGG-3′	*MnlI*	280 + 224 (WT)[c]
	R[b]: 5′-GTCTCCTGAAGTGTCTGTAC-3′		504
*CYP2C19*2* rs4244285	F[a]: 5′-CAGAGCTTGGCATATTGTATC-3′	*SmaI*	212 + 109 (WT)[c]
	R[b]: 5′-GTAAACACACAACTAGTCAATG-3′		321

[a]Forward primer sequence, [b]Reverse primer sequence, [c]Wild type

was 0.1 mg/l. Antifungal treatment was changed to liposomal amphotericin-B (5 mg/kg/day) which was ceased upon recovery from neutropenia.

The low VCZ levels that were detected in these patients raised a concern of *CYP2C19* polymorphism, and genotyping for *CYP2C19* was performed by polymerase chain reaction-based restriction fragment length polymorphism, as described by Gumus et al.[4] with minor modifications (Table 1). The genotypes for *CYP2C19*2* and *CYP2C19*17* variants were investigated. We did not genotype the patients' genes for the *CYP2C19*3* allele, since its frequency is very low in Caucasians, including the Turkish population[6].

The genotype of *CYP2C19* was *CYP2C19*1/*17* in our patients which predicted rapid metabolizer phenotype according to the clinical pharmacogenetics implementation consortium guidelines[3]. The *CYP2C19*2* allele was not detected.

Discussion

The effects of *CYP2C19* genetic variants on VCZ pharmacokinetics has been previously investigated in several studies. The *CYP2C19*17* allele is associated with rapid and ultrarapid metabolizer phenotypes[7]. This polymorphism was detected more frequently in European and African populations (18-27% and 10-26%) than in Asian populations (0.15-0.44%) [8]. In contrast, the *CYP2C19*2* loss of function allele was more frequently observed in Asian populations (~25-30%) than in European populations (~15%)[9]. A previous study in the Turkish population revealed that the frequencies of *CYP2C19*1/*17* and *CYP2C19*2/*17* genotypes were 30.3% and 5.3%, respectively[4]. Therefore, we investigated *CYP2C19*17* and *CYP2C19*2* alleles in our patients.

Mason et al.[10] suggested that proactive testing of *CYP2C19* genotypes in patients who receive VCZ prophylaxis may be more cost effective. In their simulated model, increasing the initial VCZ dose for the patients with *CYP2C19*17* alleles (i.e., *CYP2C19*1/*17* or *CYP2C19*17/*17*) was related to a decrease in the incidence of fungal diseases and overall treatment costs. Recently, an initial VCZ dose, which was 1.5 times higher than the standard dose with TDM, was recommended by the Dutch pharmacogenetics working group for patients who were defined as *CYP2C19* ultrarapid metabolizers[11].

The published experience on TDM of VCZ is limited to one center in Turkey. The results of 26 patients who had TDM of VCZ were reviewed retrospectively. Voriconazole concentration was <1 mg/l in seven patients. Six out of seven patients were <16 years[12]. These findings were followed by a prospective study from the same center. They investigated *CYP2C19* polymorphisms in 11 patients with different hematological malignancies who were at high risk for IA. The *CYP2C19*2/*17* genotype was detected in six patients, which resulted in subtherapeutic VCZ levels in one patient[13]. It takes five to seven days to have VCZ steady levels. This is a critical period in a severely ill patient. Detection of *CYP2C19* genotypes in high-risk patients for IA may improve the dosing schedules without losing time. About 10 days were required to reach target drug concentrations in one of our patients, and antifungal treatment was changed to a more expensive alternative in the second patient.

Conclusion

The presented cases underline the impact of availability of *CYP2C19* genotyping and TDM as a routine test in hospitals to improve the safety and efficacy of VCZ treatment.

Ethics

Informed Consent: Informed consent was taken from both patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.T., E.K., Concept: G.M., M.Ö.B., M.A., Design: G.M., M.A., Data Collection or Processing: Z.T., E.K., O.D., M.İ., Analysis or Interpretation: M.A., M.Ö.B., Literature Search: Z.T., E.K., O.D., M.İ., Writing: Z.T., E.K., G.M.

Conflict of Interest: Gökhan Metan has received honoraria for speaking at symposia and lectures organized by Gilead; Merck, Sharp, and Dohme (MSD); and Pfizer. He has received fees from Pfizer and MSD for advisory board meetings. He has received travel grants from MSD, Pfizer, and Gilead to participate in conferences. Murat Akova has received honoraria for lectures and research support, both paid to his institution, from Pfizer, MSD, and Gilead. The other authors have no conflicts of interest relevant to this article.

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