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Determination of posaconazole concentration with LC–MS/MS in adult patients with hematologic malignancy

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ABSTRACT

Background: Posaconazole has an important role in the prophylaxis of invasive fungal infections (IFIs), however oral suspension formulation is associated with variable bioavailability. The relationship between posaconazole concentrations achieved with the oral suspension and the IFI occurrence were analyzed along with demographic and clinical covariates (mucositis, diarrhea, liver enzymes, co-medications, and food intake).
Methods: One hundred twenty-two adult patients with AML/MDS undergoing remission induction chemotherapy were enrolled. They received posaconazole as prophylaxis and 557 posaconazole measurements were performed with a validated LC–MS/MS method.
Results: The median (range) posaconazole concentration (ng/ml) on days 2, 3, 7, 14, and 21 was 271 (43–493), 564 (101–1461), 713 (85–2186), 663 (85–1994), and 497 (43–1872), respectively. Thirteen patients (11%) developed proven (1/13), probable (2/13), and possible IFIs (10/13). A significant relationship existed between lower steady-state posaconazole concentrations and a higher breakthrough IFI incidence by binary logistic regression ($P = 0.0108$). Posaconazole value of ≥338 ng/ml on day 3 predicted the achievement of ≥500 ng/ml at day 7 (sensitivity: 78.5%, specificity: 66.7%, AUC: 0.747). Food intake ($P = 0.0014$) and proton pump inhibitor ($P = 0.0063$) were significantly associated with higher and lower posaconazole concentrations, respectively.
Conclusions: TDM of posaconazole oral suspension formulation is recommended based on the exposure–response relationship of the present study.

1. Introduction

Invasive fungal infections (IFIs) are major causes of morbidity and mortality in hematologic malignancy patients who are undergoing remission induction chemotherapy [1]. Therefore, primary antifungal prophylaxis has been used widely as a strategy to prevent IFI development [2,3].

Posaconazole is a potent, extended-spectrum triazole with in vitro activity against a broad spectrum of fungi and has clinical activity against various fungal pathogens, including *Aspergillus*, *Candida*, *Zygomycetes*, and *Fusarium* species [4]. It is currently used as a curative IFI treatment [5,6], and, it is the most recently approved triazole for the prophylaxis of neutropenic patients after induction chemotherapy for acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) [7] or of patients with graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) [4)].

A tablet formulation of posaconazole was recently approved by the United States Food and Drug Administration (FDA) and by the European Medicines Agency [8]. However this study focused on oral suspension formulation of posaconazole, which is widely used and remains available worldwide [9]. The oral suspension formulation is a 40 mg/ml oral suspension that contains polysorbate 80 as an emulsifying agent [10]. Similar to other triazoles (itraconazole, voriconazole) [11], the posaconazole oral suspension formulation is associated with significant inter-patient variability, with a reported inter-subject variability of 35–50% in healthy volunteers, and variability of 71–85% in patients [5].

Additionally, a number of factors have been demonstrated to influence posaconazole absorption of oral suspension formulation, including food intake (with fat specifically) [6], gastric pH [12], mucosa integrity [13], and administration frequency [14]. The significant inter-patient variability and the various factors affecting the posaconazole bioavailability underscore the need for therapeutic drug monitoring (TDM) for

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this formulation of posaconazole. In this study we performed a prospective single center study of prophylactic posaconazole oral suspension formulation in neutropenic patients with AML/MDS, to evaluate the association between posaconazole concentrations and the occurrence of IFIs and to analyze factors that have an impact on posaconazole plasma concentrations.

2. Materials and methods

2.1. Patients

This prospective study was conducted at the Catholic Blood and Marrow Transplantation Center at Seoul St. Mary's Hospital in Korea. Patients aged ≥18 y, who were admitted from March 2013 through September 2013, were eligible if they had, or were anticipated to have neutropenia with an absolute neutrophil count of ≤500 cells/mm^4, lasting for ≥7 days, which resulted from remission induction chemotherapy for newly diagnosed subjects, or re-induction chemotherapy after a first relapse of AML or MDS. Patients were excluded from the study if they had any of the following: an IFI history within recent 1 y, clinically significant hepatic or renal dysfunction, abnormal QTc interval, a history of hypersensitivity or idiosyncratic reactions to azoles, or a requirement for drugs that are and known to interact with azoles and that may lead to life-threatening side effects [15]. The decision to use posaconazole for prophylaxis in a patient was made based on a consensus discussion between the hematologists and infectious diseases specialists. The daily dosage of posaconazole for prophylaxis was 200 mg in an oral suspension three times/day as recommended, and patients were instructed to take each dose with meals. However, patients at risk of IFI are usually critically ill and may have difficulty eating solid foods in the course of treatment. Therefore a small fraction of patients were temporarily on NPO during posaconazole prophylaxis at the discretion of his/her treating physicians. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of Seoul St. Mary's Hospital.

2.2. Pharmacokinetic assessment

Plasma samples for the enrolled patients were collected before the first of the three daily doses of posaconazole on days 2, 3, 7, 14, and 21 post-initiation of posaconazole prophylaxis to represent trough levels. Posaconazole has a long half-life of approximately 35 h, and steady-state is achieved by 7 days of administration [6,16,17]. Additionally, there is minimum fluctuation around the mean values once steady-state is reached [18]. Therefore, the plasma concentration on day 7 was regarded as the average steady-state posaconazole plasma concentration (C_{avg}) for each patient. A sub-therapeutic concentration was defined as a C_{avg} value <500 ng/ml target concentration at steady-state based on the literature [11,13,18].

2.3. LC-MS/MS conditions

Plasma posaconazole concentrations were measured using a newly developed and validated LC-MS/MS method. At the time of development, posaconazole-d4 [19], an isotope of posaconazole, was not readily available, and we used ketoconazole as an internal standard [20] for the present study. Posaconazole powder was provided by MSD and internal standard ketoconazole was purchased from Sigma-Aldrich. A 3-point set of lyophilized calibrators from Chromsystems was used together with three levels of lyophilized quality control materials from the same vendor.

After protein precipitation (20 μl of plasma plus 80 μl of zinc sulfate plus 200 μl of acetonitrile, which contained the internal standard, ketoconazole at 100 ng/ml), 120 μl of the supernatant was injected into the LC-MS/MS system, which consisted of an Alliance 2795 HT HPLC (Waters) module that was coupled to a Quattro Premier XE tandem mass spectrometry system (Waters). Separation was achieved using an XBridge C_{18} 50.0 × 2.1 mm (3.5 μm particle size) column.

For HPLC, mobile phase A, which contained 0.1% (*v*/*v*) formic acid in de-ionized water, was introduced and was coupled with 0.1% (*v*/*v*) formic acid that was dissolved in acetonitrile for mobile phase B. A flow of 90% mobile phase A was introduced from the initial sample injection, and was switched to 0% mobile phase A at time 3.00, then to 90% mobile phase A at time 4.60. The column flow rate was 0.3 ml/min and the column temperature was maintained at 40 °C. A strong wash consisting of 60%, 30%, and 10% of acetonitrile, propan-2-ol, and acetone, respectively, was used between the sample injections.

The electrospray ionization source was operated in positive mode with a capillary voltage of 3 kV and cone voltage of 50 V. Compounds were detected by via multiple reaction monitoring mode employing the ion transitions of *m*/*z* 701.05 → 683.15 (collision energy: 35 eV) for posaconazole and *m*/*z* 531.00 → 489.00 (collision energy: 30 eV) for the internal standard, ketoconazole. The linearity of the standard curve ranged from 100 to 5000 ng/ml. The within-run coefficient of variation was 9.11%, 5.11%, and 4.30% for target concentrations of 550 ng/ml, 1140 ng/ml, and 4600 ng/ml, respectively. The between-run coefficient of variation was 12.02%, 14.20%, and 14.02% for target concentrations of 550 ng/ml, 1140 ng/ml, and 4600 ng/ml, respectively. Representative LC–MS/MS chromatograms for posaconazole and the internal standard as well as data regarding the LC-MS/MS assay validation are available as online Supplementary data.

2.4. IFI assessment

IFIs were adjudicated by an independent, infectious diseases specialist panel who were blinded to the posaconazole plasma concentrations. IFI assessments were made during each admission episode for remission induction or re-induction chemotherapy including the full period of neutropenia. Additionally, the IFIs were categorized as proven, probable, or possible, according to the 2008 European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria (EORTC/MSG) [21]. The *Aspergillus* galactomannan test (Platelia; Bio-Rad Labs) was used as one of the microbiological criteria for a diagnosis of invasive aspergillosis using the FDA-approved 0.5 cut-off value.

2.5. Data collection and variables of interest

The following variables were analyzed: gender, age, height, body weight, body surface area (BSA), underlying disease, IFI diagnosis, baseline (on or before day 7) aspartate aminotransferase (AST), alanine aminotransferase (ALT), total-bilirubin, and gamma-glutamyl transferase (GGT) levels. For each posaconazole concentration measurement, histamine$_2$ (H_2)-receptor antagonist use, proton pump inhibitor (PPI) use, concomitant use of inhibitors (e.g., verapamil, cyclosporine, quinidine, clarithromycin, erythromycin, etc.) or inducers (e.g., rifampin, rifabutin, certain anticonvulsants) of posaconazole clearance pathways [15], as well as vomiting, diarrhea, and food intake were documented.

2.6. Statistical analyses

Continuous data are presented as medians (ranges) for non-normally distributed data and categorical data are presented as numbers (%). Normality was assessed using the D'Agostino and Pearson normality test. Univariate analyses between the individual variables and C_{avg} were performed using the Mann–Whitney U-test for continuous variables and the Chi-square test for categorical variables. Then multiple regression analysis was performed between the statistically significant variables on univariate analysis ($P < 0.05$) and C_{avg}.

The association between longitudinal posaconazole levels was analyzed using the repeated measures analysis of variance with a Bonferroni