Clinical Biochemistry 70 (2019) 14–17

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Experience with therapeutic drug monitoring of three antifungal agents using an LC-MS/MS method in routine clinical practice

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ARTICLE INFO

Keywords:
LC-MS/MS
Simple protein precipitation
Voriconazole
Itraconazole
Posaconazole
Therapeutic drug monitoring

ABSTRACT

Therapeutic drug monitoring (TDM) of voriconazole, itraconazole, and posaconazole is a useful tool for treatment of fungal infections. We validated a simple and reliable LC-MS/MS method using simple protein precipitation for simultaneous determination of voriconazole, itraconazole, and posaconazole. The linearity, accuracy, precision, carryover, and matrix effects were validated. Total sample preparation time was < 30 min per batch, and analytical run time was 3.8 min per sample. We also presented clinical experience of TDM at 1183 serum concentrations over one year using this validated assay method. About 77%, 85%, and 96% of measured voriconazole, itraconazole, and posaconazole concentrations were within the therapeutic range, respectively. The number of respective measurements per patient was 1–63, 1–8, and 1–4 for voriconazole, itraconazole, and posaconazole. All three antifungal agents showed large intra-individual variability (2 to 181% CV) and drug-drug interaction with proton pump inhibitors or rifampin. In conclusion, we developed and validated a simple and fast method that was successfully applied in a routine clinical setting.

1. Introduction

Voriconazole, itraconazole, and posaconazole are commonly administered antifungal agents that play crucial roles in both prophylaxis and treatment of fungal infection [1]. Because these agents show significant intra- and inter-individual variability within a narrow therapeutic index, therapeutic drug monitoring (TDM) can be useful for adjusting dosage to optimize treatment [1,2]. Some validating reports using liquid chromatography–tandem mass spectrometry (LC-MS/MS) to measure concentrations of voriconazole, itraconazole, fluconazole, and posaconazole simultaneously have been published [3–6]. These methods use liquid-liquid extraction (LLE) or solid-phase extraction (SPE) to prepare the samples. However, routine TDM is difficult to apply in a laboratory setting due to labor and time requirements, and presentation of large-scale TDM data from simultaneous detection of antifungal agents in clinical settings is rare [7–10].

Here, we describe clinical TDM experience with 1183 serum concentrations of voriconazole, itraconazole, 4-hydroxy itraconazole (4-OH-itraconazole), and posaconazole from 355 patients using our developed and validated assay method. This assay method is more effective and faster than conventional methods by using simple protein precipitation and was applied successfully in routine clinical TDM.

2. Materials and methods

A commercial In Vitro Diagnostic-Conformite European (IVD-CE) certified antifungal TDM kit including voriconazole, itraconazole, posaconazole, voriconazole-internal standard (IS), itraconazole-IS, 4-OH-itraconazole-IS, and posaconazole-IS, along with calibrators (No. REF92051) and quality control (QC) materials (No. REF0254) was purchased from Chromsystems Instruments & Chemicals GmbH (München, Germany). LC-MS/MS-grade acetonitrile, methanol, and distilled water (DW) were purchased from Burdick & Jackson (Muskegon, MI, USA). Here, 10 µL samples of patient sera, calibrators, or QC materials were treated with 150 µL of methanol/acetonitrile (50:50 (*v*/v)) containing the IS. The mixtures were mixed for 30 s to achieve homogeneity and centrifuged at 15000 rpm for 10 min. Finally, 3 µL of supernatant of the protein-precipitated sample was injected into the LC-MS/MS.

Analyses were performed on an Agilent 6460 tandem mass

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https://doi.org/10.1016/j.clinbiochem.2019.06.003
Received 9 January 2019; Received in revised form 28 May 2019; Accepted 3 June 2019
Available online 05 June 2019

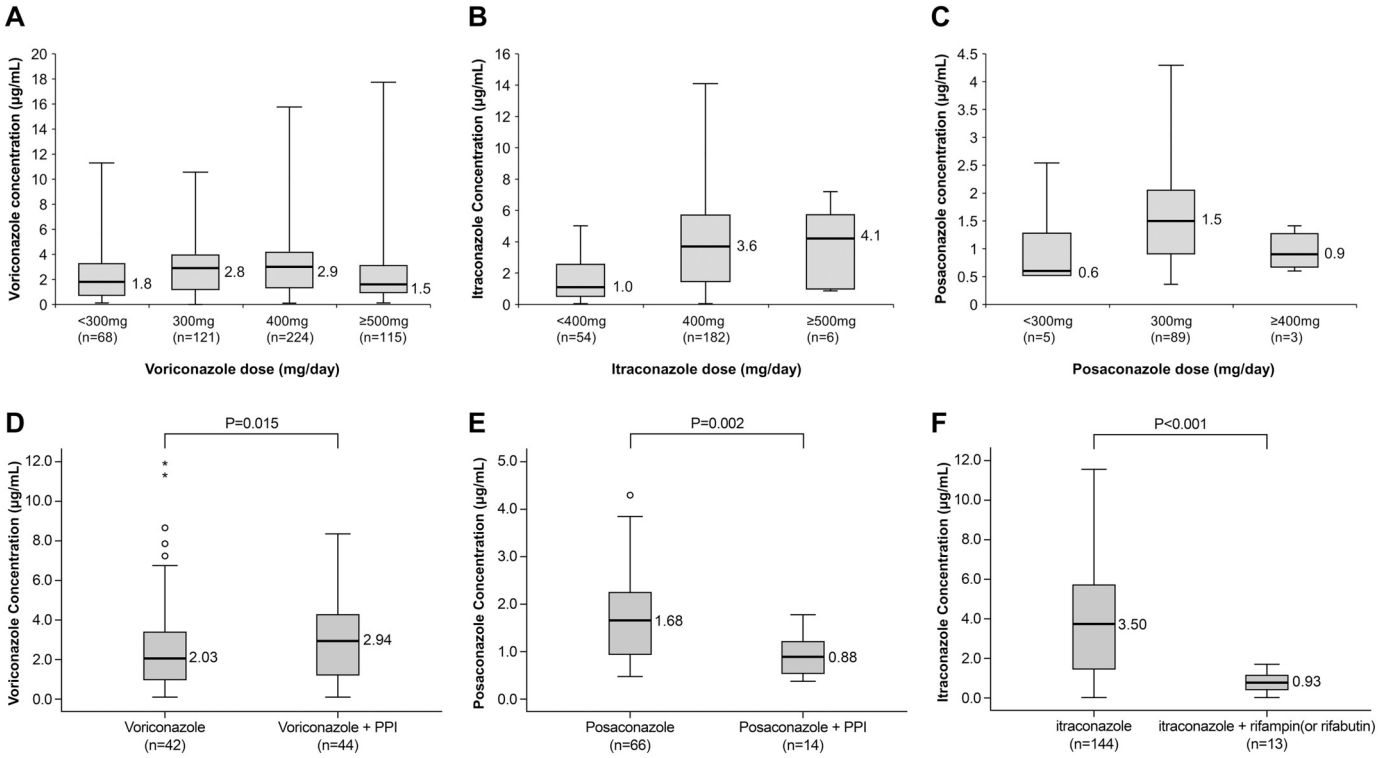

Fig. 1. Plasma concentration distributions of (A) voriconazole, (B) itraconazole, and (C) posaconazole by daily dose, and drug interactions of a proton pump inhibitor (PPI) with (D) voriconazole, (E) posaconazole, and (F) rifampin (or rifabutin) with itraconazole.

spectrometer equipped with an Agilent 1260 high-performance liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) and an electrospray ionization source operating in positive-ion detection mode. Chromatographic separation was performed on a Kinetex C18 column (3 μm × 2.1 mm × 50 mm; Phenomenex, Torrance, CA, USA). Mobile phases consisted of water containing 2 mM ammonium acetate and 0.1% formic acid (mobile A) and acetonitrile containing 0.1% formic acid (mobile B). The flow rate was 0.5 mL/min, and column temperature was 45 °C. Quantitative analysis was performed in multiple reaction-monitoring modes (m/z 350.3 > 281.1, m/z 705.2 > 392.2, m/z 721.3 > 408.2, m/z 701.4 > 683.3, m/z 353.3 > 284.1, m/z 714.2 > 401.2, m/z 729.2 > 416.2, and m/z 706.4 > 687.3 for voriconazole, itraconazole, 4-OH-itraconazole, posaconazole, voriconazole-IS, itraconazole-IS, 4-OH-itraconazole-IS, and posaconazole-IS, respectively). The selectivity, linearity, lower limit of quantitation (LLOQ), accuracy, precision, matrix effect, and extraction recovery were assessed according to Clinical and Laboratory Standards Institute guidelines [11]. Selectivity was validated with serum samples from multiple patients (n = 10) to observe the presence of interfering peaks. Samples for linearity were prepared by spiking a pooled serum to produce nine levels of analytes (Supplementary Fig. 1). LLOQ was determined as the lowest level with a signal to noise ratio > 10:1 and CV < 20%. The carryover effect was evaluated by consecutive analyses of blank matrix samples after running a high-concentration sample at the upper limit of quantification. Accuracy and precision were evaluated with three levels of QC materials, which were traceable to verified materials and reference tests, and two levels of spiked samples. The intraday (N = 6) and interday precision (N = 5) were reported as %CV. The accuracy was assessed by % bias calculated from measured versus expected concentrations (Supplementary Table S1). We participated in proficiency testing provided by the Korean Association of External Quality Assessments Service (KEQAS) and Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology (KKGT). The matrix effect and extraction recovery were evaluated by a post-extraction spike method using 4 samples for each analyte and its respective IS. The matrix effect was evaluated by comparing the peak areas of spiked neat solution and spiked serum samples. Extraction recovery was evaluated by comparing the peak areas of pre- and post-spiked samples.

From July 2016 to June 2017, a total of 1183 serum concentrations of the three antifungal agents (835 voriconazole, 246 itraconazole, and 102 posaconazole) were measured from 355 patients at Samsung Medical Center (Seoul, Republic of Korea). We retrospectively reviewed patient medical records for loading dose, loading time, and concomitant medication history. The study protocol was approved by the Institutional Review Board of Samsung Medical Center (SMC IRB No. 2018–01-049).

The therapeutic range of antifungal agents was defined as trough concentrations of 0.5–6.0 μg/mL for voriconazole and ≥ 0.5 μg/mL for itraconazole and posaconazole. In the case of itraconazole, the sum of itraconazole and 4-OH-itraconazole was considered the total itraconazole concentration [12]. The toxic level of voriconazole was defined as > 6.0 μg/mL, while toxic levels for itraconazole and posaconazole were not defined. Steady-state concentration was defined as no change in dosage measured over at least seven days of continuous administration [13–15]. Intra-individual variability was calculated after more than three measurements.

Statistical analyses were conducted using IBM SPSS statistics 25. This material is based upon work supported by the Ministry of Trade, Industry, & Energy (MOTIE, Korea) under the Industrial Technology Innovation Program (No.10080648).

3. Results and discussion

The chromatograms showed no interfering peak for voriconazole, itraconazole, 4-OH-itraconazole, or posaconazole (Supplementary Fig. 2) and no significant carryover effect. The linear assay range was 0.1–50 μg/mL for voriconazole and 0.05–10 μg/mL for itraconazole, 4-OH-itraconazole, and posaconazole (Supplementary Fig.1 and Supplementary Table S2). The intra- and inter-day precision values of

Table 1
Therapeutic drug monitoring of three antifungal agents in 355 patients.

	Voriconazole	Itraconazole	Posaconazole
No. of patients	114	157	84
Age (y), mean ± SD	52.2 ± 21.8	57.0 ± 18.7	54.2 ± 14.8
Sex, n, male:female	76:38	102:55	62:22
BMI (kg/m^2), mean ± SD	20.7 ± 4.3	19.1 ± 3.4	23.7 ± 3.0
Underlying disease, n (%)			
Pulmonary disease[a]	14 (12%)	128 (82%)	0
Leukemia/lymphoma	52 (46%)	7 (4%)	84 (100%)
Solid tumor[b]	7 (6%)	10 (6%)	0
Transplantation	15 (13%)	4 (3%)	0
Dose (mg/day), median (range)	400 (100–840)	400 (24–840)	300 (40–600)
Oral formulation, n (%)	86 (69.9%)	157 (100%)	80 (95.2%)
Dose per body weight (mg/kg), median (range)	7.6 (2.4–26.0)	7.4 (2.7–12.5)	4.8 (3.2–11.7)
No. of total drug measurements	835	246	102
No. of measurements per patient, median (range)	4 (1–63)	1 (1–8)	1 (1–4)
Drug concentration (μg/mL), median (range)	2.63 (0.05–10.0)	2.78 (0.05–9.8)	1.41 (0.36–4.3)
Distribution of drug concentration, n (%)			
Subtherapeutic	99 (12%)	35 (15%)	4 (4%)
Therapeutic	646 (77%)	211 (85%)	98 (96%)
Toxic	90 (11%)	NA	NA
Measurements at steady-state, n (%)	390 (47%)	226 (92%)	88 (86%)

Abbreviations: BMI, body mass index; NA, not applicable.
[a] Pneumonia, bronchitis, chronic obstructive pulmonary disease, nontuberculous mycobacteria infection.
[b] Lung cancer, liver cancer, stomach cancer, sarcoma.

all assays were < 6.0% CV. The accuracy ranged from 82.0% to 107.0% (Supplementary Table S1). The matrix effects ranged from 96.4% to 105.0%, and extraction recovery ranged from 84.8% to 91.0% (Supplementary Table S2). The results of proficiency tests provided by KEQAS and KKGT were all acceptable. Sample preparation time was < 30 min for a batch, and total analytic time for a batch was about 4 h. We interpreted the assay result and provided a TDM report in text format. The turn-around time of TDM reporting was within 24 h from the beginning of sample collection to verification of the final report.

The baseline characteristics of 355 patients are shown in Table 1. Along with variable underlying disease, voriconazole (36/114, 31.6%) was used in ICU patients much more than itraconazole (9/123, 7.3%) or posaconazole (3/89, 3.4%). This may cause more frequent and substantial dose changes and explains why the number of voriconazole samples drawn at steady state was lower than the number of the other agents.

Distribution of trough concentration by daily oral dose in described in Fig. 1. Large intra-individual variability was observed in group of patients whose drug concentrations were measured at least three times. In the voriconazole group (n = 71), the median CV was 61% (range, 5 to 181%); in the itraconazole group (n = 20), the median CV was 39% (range, 2 to 141%); and for the posaconazole group (n = 3), the median CV was 56% (range, 19 to 61%). Among the voriconazole group, 31 out of 71 patients (43.7%) changed dose several times (median 2, range 1–5), while itraconazole (n = 20) and posaconazole (n = 3) groups had no change in regimen. Of the above 31 voriconazole patients, the intra-individual variability of 11 patients who were measured at least three times at a single dose was 46.7% (median) of CV (range, 11.4 to 99.6%). A large intra-individual variability may be due to well-known properties, such as non-linear pharmacokinetics, variable oral availability, and drug interaction. For example, of 123 patients who were using voriconazole, 86 (69.9%) had an oral formulation and 44 (51.2%) had proton pump inhibitor (PPI). Although the effect of a PPI on voriconazole is controversial [16,17], observed voriconazole concentrations were higher when a PPI was used concurrently in the present study (median of 2.94 vs. 2.03 μg/mL; p = .015) (Fig. 1D). For posaconazole, 80 of 84 patients (95.2%) were prescribed an oral formulation and 14 (17.5%) received concomitant PPI. PPI is known to reduce oral bioavailability of posaconazole by increasing gastric pH, and the present study showed similar results that the posaconazole concentration of the PPI group was lower than that of patients not given a PPI (median, 0.88 vs. 1.68 μg/mL; p = .002) (Fig. 1E) [18]. The formulation of itraconazole was oral tablet (145 of 157 patients, 90.5%) or oral suspension (15 of 157 patients, 9.5%) among 157 patients receiving itraconazole treatment. For patients (13/157, 8%) treated with rifabutin (N = 12) or rifampin (N = 1), itraconazole concentration was significantly lower than that of patients who did not due to induction of cytochrome P450 by rifampin or rifabutin (median, 0.93 vs. 3.50 μg/mL; p < .001) (Fig. 1F) [1,19].

In sequential measurements performed at least two times, 272 out of 346 measurements (78.6%) in the voriconazole group were within the therapeutic range at the first measurement, and 310 measurements (310/346, 89.7%) achieved the therapeutic range at the final measurement after dose change following TDM. With itraconazole, 87 out of 101 measurements (86.1%) were within the therapeutic range at the first measurement, and 101 measurements (101/101, 100%) achieved the therapeutic range at the final measurement after dose change. In the posaconazole group, 1 of the 25 initial measurements was below the therapeutic range, and all measurements achieved the therapeutic range at the final measurement.

Although there are a few TDM reports of simultaneous determination of antifungal agents using LC-MS/MS [8,9], TDM with LC-MS/MS has several limitations due to labor-intensive and time-consuming sample preparation protocols. In this study, we demonstrate the successful application of a simple and reliable validated LC-MS/MS method using simple protein precipitation and produced substantial clinical data to support the usefulness and necessity of TDM of antifungal agents.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.06.003.

Disclosure of potential conflicts of interest

The authors have no conflict of interest to declare.

Acknowledgments

This material is based upon work supported by the Ministry of Trade, Industry & Energy(MOTIE, Korea) under Industrial Technology Innovation Program. No.10080648,'Antibiotics monitoring point-of-care test'

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