COMMENTARY

High Variability in Isavuconazole Unbound Fraction in Clinical Practice: A Call to Reconsider Pharmacokinetic/Pharmacodynamic Targets and Breakpoints

Anouk M. E. Jansen[1,2] · Rob ter Heine[1] · Paul E. Verweij[2,3] · Roger J. M. Brüggemann[1,2]

Abstract

Isavuconazole exposure–response relationships have been studied with a focus on total rather than unbound exposure, assuming a constant unbound fraction of 1%. We observed a median (range) unbound fraction of 1.59% (0.42–5.30%) in patients. This highly variable protein binding asks for re-evaluation of current pharmacokinetic and pharmacodynamic targets for isavuconazole.

Key Points

Isavuconazole protein binding in both critically ill and non-critically ill patients is highly variable.

Findings from pharmacokinetic and pharmacodynamic research for isavuconazole thus far, assuming a constant unbound fraction, should be reconsidered.

1 Introduction

Isavuconazole is a triazole antifungal agent recommended for the treatment of mould infections, such as invasive aspergillosis and invasive mucormycosis [1–3]. To increase survival in patients with invasive fungal disease, early antifungal treatment initiation at the right dose and subsequent adequate drug exposure are crucial [4]. To achieve this goal, it is vital to understand the exposure–response relationship and determine pharmacokinetic/pharmacodynamic (PK/PD) targets associated with both beneficial outcome as well as toxicity. Although the PK/PD driver for the effect of isavuconazole, described by the area under the concentration–time curve (AUC) related to the minimal inhibitory concentration (MIC) is known, a clear exposure target in humans has not been established to date [5].

Only the unbound fraction (f_u) of a drug exerts a pharmacological effect. This dictates that for highly protein bound drugs, studies on target concentrations should be aimed at resolving relationships with unbound drug exposure, i.e. free AUC (fAUC), as small changes in protein binding have a significant impact on this pharmacologically active drug exposure. Isavuconazole is highly protein bound, with a reported plasma protein binding of 99.2–99.4% in healthy volunteers [6]. Studies investigating isavuconazole exposure–response relationships thus far are employed under the assumption of a fixed f_u of 1% [6]. To date, the protein binding of isavuconazole in patients is unknown. Consequently, it can be challenged whether the assumption of a fixed f_u correctly reflects the clinical situation. With this report, we aim to add knowledge on isavuconazole plasma protein binding in patients and discuss the far-reaching implications.

✉ Anouk M. E. Jansen
anouk.me.jansen@radboudumc.nl

1 Department of Pharmacy, Radboud university Medical Center, Radboud Institute for Medical Innovations, Geert Grooteplein-Zuid 10, Postbox 9101, 6500 HB Nijmegen, The Netherlands

2 Radboud University Medical Center-Canisius Wilhelmina Ziekenhuis Center of Expertise for Mycology, Nijmegen, The Netherlands

3 Department of Medical Microbiology, Radboud University Medical Center, Radboud Institute for Medical Innovations, Nijmegen, The Netherlands

2 Methods

We determined total and unbound isavuconazole concentrations in 205 plasma samples from clinical patients receiving isavuconazole for the prevention or treatment of an invasive fungal infection in three hospitals. A part of the data originates from a previously published prospective study [7]. Samples were locally centrifuged and processed to plasma samples and analysed centrally at the Laboratory of the Department of Pharmacy at the Radboud University Medical Center, Nijmegen, the Netherlands. Total and unbound isavuconazole concentrations in plasma were quantified using a fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. The accuracy range was 95.20–100.22% and 97.80–101.50% for total and unbound isavuconazole concentrations, respectively. Within-day and between-day precision for total and unbound isavuconazole concentrations were all below 3% and 12%, respectively. The lower limit of quantification was 0.001 and 0.05 mg/L for unbound and total concentrations, respectively. To determine the unbound concentration, ultrafiltration at 37 °C at 1650g for 20 min using an Amicon® 30K Ultra Centrifugal filter was performed. The measured unbound isavuconazole concentration was divided by the measured total isavuconazole concentration to calculate the isavuconazole f_u. The correlation between f_u and unbound concentration was calculated with the Spearman's rank correlation coefficient (Spearman's rho) using SPSS Statistics (IBM, version 27.0.1.0). Patient demographics were collected from the electronic patient record.

3 Results

Patient characteristics are described in Table 1. Total and unbound isavuconazole concentrations ranged from 0.70–10.42 mg/L and 0.010–0.178 mg/L, respectively. The median (interquartile range) observed f_u based on measured total and unbound isavuconazole concentrations was 1.59% (1.29–2.18%). The minimum and maximum observed f_u were 0.42% and 5.30%, respectively, corresponding to a 12.6-fold range. Isavuconazole f_u versus unbound concentrations are presented in Fig. 1. Most (86.8%) observed f_u were higher than the assumed constant f_u of 1%. Furthermore, it can be observed that f_u increases non-linearly with unbound concentration. The statistically significant correlation between f_u and unbound concentration (Spearman's

Table 1 Patient characteristics

Characteristic	Median (range)
Total number of patients, N	26
Age (years)	63 (5–73)
< 18 years of age, n (%)	6 (23)
Sex, female, n (%)	17 (65)
Weight (kg)	73.7 (25.3–130.0)
Height (cm)	171 (121–184)
Body mass index (kg/m²)	24.4 (13.5–47.8)
Serum albumin (g/L)	23.0 (14.0–33.4)
Primary underlying condition, n (%)	
Haematological malignancy	10 (38.5)
Severe viral pneumonia	9 (34.6)
Solid organ transplant	5 (19.2)
Chronic obstructive pulmonary disease	1 (3.8)
Other	1 (3.8)
Indication for isavuconazole therapy, n (%)	
Invasive pulmonary aspergillosis (IPA)	10 (38.5)
Viral infection-associated pulmonary aspergillosis (VAPA)	9 (34.6)
Allergic broncho-pulmonary aspergillosis (ABPA)	1 (3.8)
Aspergillus tracheitis	1 (3.8)
Combined aspergillosis and fusariosis	1 (3.8)
Mucormycosis	2 (7.7)
Secondary prophylaxis	2 (7.7)
Isavuconazole daily maintenance dose at time of sample collection (mg)	200 (100–500)
Duration of isavuconazole therapy at time of sample collection (days)	6 (3–35)

Fig. 1 Unbound isavuconazole fraction versus unbound isavuconazole concentration observed in patients. Spearman's rho = 0.445, $P < 0.001$. The dashed horizontal line represents the assumed constant unbound fraction of 1%
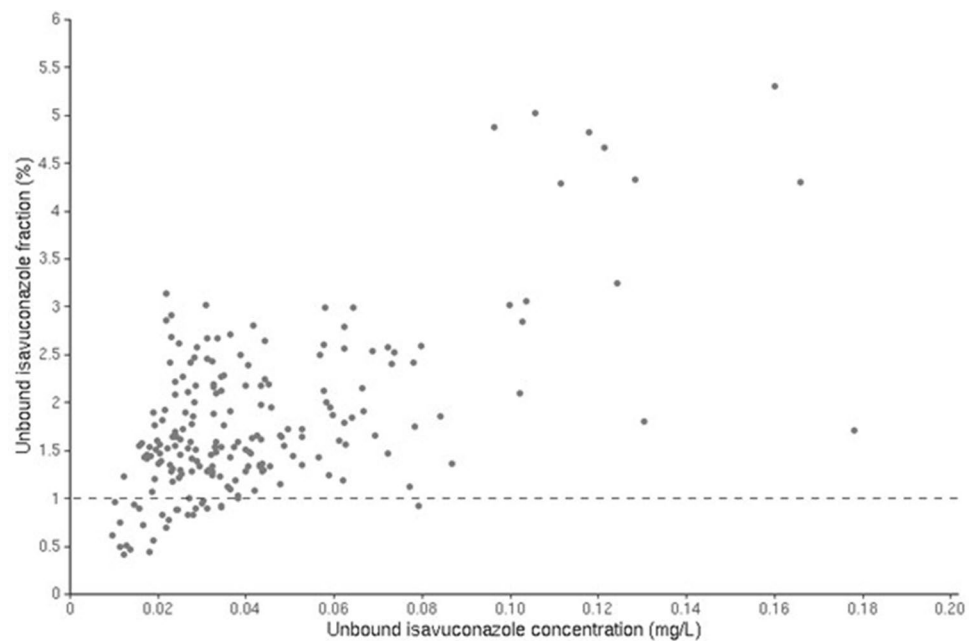
rho = 0.445; $P < 0.001$) confirmed this positive, non-linear trend.

4 Discussion

The present report shows that isavuconazole protein binding in patients is highly variable and that observed f_u strongly deviate from the assumed constant f_u of 1%. This further emphasizes the need for a focus on free drug, as already highlighted in the European Medicines Agency (EMA) public assessment report on isavuconazole [6]. Our observations have far-reaching implications, as to date, researchers have used total rather than unbound drug exposure in animal and human studies, as well as in isavuconazole clinical breakpoint determination.

From early developmental stages onwards, isavuconazole pharmacodynamics (PD) have been examined using total concentrations [5, 8]. In one in vivo experiment, Lepak et al. calculated the fAUC/MIC ratio under the assumption of a constant f_u of 1% [5]. The higher median f_u we observed in patients would call for a recalculation. More importantly, the extreme variability in observed f_u does not allow a calculation of an fAUC/MIC ratio from a total AUC/MIC ratio at all. In essence, PD experiments will require measurement of unbound isavuconazole concentrations, rather than calculations based on assumptions.

In establishing isavuconazole PK/PD breakpoints for *Aspergillus fumigatus*, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) antifungal susceptibility testing committee even so made use of total AUC/MIC instead of fAUC/MIC ratios in their probability of target attainment (PTA) analyses [9]. The subsequently derived PK/PD breakpoints dictating whether a pathogen with a certain MIC could be treated with isavuconazole require reassessment as well since these are established on the total AUC/MIC ratios derived from the aforementioned in vivo experiments [9]. Under these potentially inadequate assumptions, patients infected with pathogens with currently assumed attenuated MICs might possibly benefit from isavuconazole treatment after all. As most of the observed f_u were higher than the presumed 1%, it is likely that the PTA percentages are higher when re-evaluated. Consequently, patients infected with *A. fumigatus* with an attenuated MIC of 2 mg/L, categorized as 'area of technical uncertainty' (ATU), or even 4 mg/L can still be managed with isavuconazole. This contradicts current PTA analyses where these pathogens are considered untreatable from a PK/PD perspective. Once these aforementioned fAUC/MIC ratios are established, analyses leading to these PK/PD breakpoints should subsequently be re-evaluated.

In PK/PD analyses based on data from the SECURE trial, a relationship between total isavuconazole exposure and clinical response could not be identified [10]. Consequently, no human PK/PD targets for efficacy and toxicity are defined, although in practice, a total AUC between 60 and 233 mg*L/h at steady state is thought to be associated with successful and safe therapy [10, 11]. Yet again, unbound exposure was not evaluated. We contend that the human exposure–response relationship should be reconsidered as well. The inability to find such a relationship in humans may be the consequence of the high variability in f_u. Patients with treatment failure and those with treatment success showed no statistically significant difference in

total isavuconazole exposure [10]. Potentially, there is a difference in unbound, and thus, pharmacologically active, exposure between these groups. For example, if patients with treatment failure had an overall low f_u and patients with treatment success a high f_u, a difference in exposure may actually predict treatment outcome. The same applies to the relationship between exposure and toxicity. To assess this, exposure–response analyses should be iterated using measured unbound exposure, rather than assumed unbound exposure.

For this, and to be able to use these unbound PK/PD targets for isavuconazole dosage optimization in the future, a validated method to measure unbound concentrations is required. Alternatively, pharmacometric models could aid in estimating the unbound concentration from measured total concentrations. A minimal precondition for this approach is the availability of a model adequately describing isavuconazole protein binding. Currently, such a model predicting isavuconazole unbound concentrations without significant bias and imprecision is not available, dictating the actual measurement of unbound concentrations. A first step towards model informed prediction of unbound isavuconazole concentrations has been made [7]. Finally, our observations affect the interpretation of results from (population) PK studies and other studies that were performed under the assumption of and using the results from the aforementioned analyses. In line with EMA's conclusion, it is unclear why the focus has not been on unbound isavuconazole exposure from the start of the development and clinical evaluation of this drug [6]. For all highly protein bound drugs, we argue for emphasis on free drug from early developmental stages onwards.

Variations in observed f_u may partly be explained by the saturability of the protein binding and the dependency of alterations in serum levels of the main binding protein albumin seen in clinical practice. This underlines the incorrectness of the assumption of a fixed f_u. Extreme variations in albumin concentrations and occurrence of hypoalbuminemia are specifically observed in patients admitted to the intensive care unit, where isavuconazole has been increasingly used recently due to a growing number of viral infection-associated cases of invasive fungal infection. This even more underlines the importance of understanding the PK/PD relationships of this drug.

In conclusion, we provide evidence that isavuconazole f_u in patients is highly variable, which contradicts the current common assumption of a fixed f_u of 1%. Our results show that total exposure is not a good surrogate for unbound exposure, necessitating a reassessment of isavuconazole PK/PD studies that have been performed so far. To be able to use isavuconazole to its full potential, it seems that there is a need to go back to the drawing board.

Declarations

References

1. European Medicines Agency. Cresemba (isavuconazole). Summary of Product Characteristics; 2015. https://www.ema.europa.eu/en/documents/product-information/cresemba-epar-product-information_en.pdf. Accessed Jan 5, 2023.

2. Ullmann AJ, Aguado JM, Arikan-Akdagli S, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect. 2018;24(Suppl 1):e1–38.

3. Maertens JA, Raad II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by *Aspergillus* and other filamentous fungi (SECURE):

a phase 3, randomised-controlled, non-inferiority trial. Lancet. 2016;387(10020):760–9.

4. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother. 2005;49(9):3640–5.

5. Lepak AJ, Marchillo K, Vanhecker J, Andes DR. Isavuconazole (BAL4815) pharmacodynamic target determination in an in vivo murine model of invasive pulmonary aspergillosis against wild-type and cyp51 mutant isolates of *Aspergillus fumigatus*. Antimicrob Agents Chemother. 2013;57(12):6284–9.

6. European Medicines Agency. Cresemba (isavuconazole). Assessment Report; 2015. https://www.ema.europa.eu/en/documents/assessment-report/cresemba-epar-public-assessment-report_en.pdf. Accessed Jan 5, 2023.

7. Jansen AME, Mertens B, Spriet I, Verweij PE, Schouten J, Wauters J, Debaveye Y, ter Heine R, Brüggemann RJM. Population pharmacokinetics of total and unbound isavuconazole in critically Ill patients: implications for adaptive dosing strategies. Clin Pharmacokinet. https://doi.org/10.1007/s40262-023-01305-8

8. Seyedmousavi S, Bruggemann RJ, Meis JF, Melchers WJ, Verweij PE, Mouton JW. Pharmacodynamics of isavuconazole in an Aspergillus fumigatus mouse infection model. Antimicrob Agents Chemother. 2015;59(5):2855–66.

9. European Committee on Antimicrobial Susceptibility Testing. Isavuconazole and *Aspergillus* spp.: rationale for the clinical breakpoints, version 2.0; 2020. http://www.eucast.org.

10. Desai AV, Kovanda LL, Hope WW, et al. Exposure–response relationships for isavuconazole in patients with invasive aspergillosis and other filamentous fungi. Antimicrob Agents Chemother. 2017;61(12): e01034-17.

11. Keirns J, Desai A, Kowalski D, et al. QT interval shortening with isavuconazole: in vitro and in vivo effects on cardiac repolarization. Clin Pharmacol Ther. 2017;101(6):782–90.