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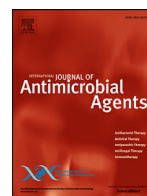
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Posaconazole trough concentrations are not influenced by inflammation: A prospective study

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ABSTRACT

During inflammation, several cytochrome P450 enzymes are downregulated. Recently it was shown that voriconazole metabolism is reduced during inflammation. Posaconazole, another triazole with broad-spectrum antifungal activity, is metabolised only to a limited extent by cytochrome P450 enzymes and to a wider extent by phase 2 enzyme systems. The aim of this study was to investigate posaconazole concentrations during inflammation. Patients aged ≥ 18 years receiving posaconazole prophylaxis or treatment for fungal infections were enrolled in a prospective observational study. Samples for posaconazole and C-reactive protein (CRP) concentrations were collected routinely for each patient. Longitudinal data analysis was performed to analyse the correlation between posaconazole serum trough concentrations and CRP values, corrected for potential factors that could influence the posaconazole concentration. Between August 2015 and June 2017, 64 patients were recruited to this study. Data for 55 patients (511 posaconazole samples) were included in the final analysis. The overall median posaconazole concentration was 1.8 mg/L [interquartile range (IQR) 1–2.9 mg/L, range 0.1–7.94 mg/L] and the overall median CRP concentration was 23.5 mg/L (IQR 5–75 mg/L, range 0–457 mg/L). Longitudinal data analysis showed that only the posaconazole daily dose (in mg/kg body weight) had a significant influence on posaconazole concentration after correction for other factors ($P < 0.0001$). Posaconazole concentrations were not influenced by CRP concentrations ($P = 0.77$). Posaconazole concentrations are not influenced by inflammation, reflected by CRP concentration. Therefore, more frequent therapeutic drug monitoring of posaconazole during inflammation or after an infection subsides is not necessary.

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1. Introduction

Posaconazole, a broad-spectrum triazole antifungal, is widely used for prophylaxis of invasive aspergillosis [1]. Whilst posaconazole is the first-line choice for prophylaxis of invasive aspergillosis, voriconazole is recommended as first-line treatment. Posaconazole is used as a second choice for treatment of invasive aspergillosis [1].

Until recently, it was difficult to maintain therapeutic plasma concentrations of posaconazole administered as oral suspension owing to inadequate absorption as well as drug–drug interactions (e.g. concomitant use of proton pump inhibitors) and insufficient food intake [2]. These issues led to substantial intra- and inter-individual patient variability in the pharmacokinetics of posaconazole, and adequate trough concentrations were not easily achieved [3]. After these studies, there was a general consensus to routinely perform posaconazole therapeutic drug monitoring (TDM). [4–9]. The introduction of the new modified-release tablet and an intravenous (i.v.) formulation have further improved drug absorption, and thereby exposure, compared with the previously used suspension [10,11].

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As the use of posaconazole is becoming more widespread, other factors influencing the pharmacokinetics of posaconazole should be investigated, e.g. the presence of inflammation. Recently it has been shown that inflammation, expressed by C-reactive protein (CRP) concentration, affects the serum concentrations of voriconazole, which can be explained by downregulation of cytochrome P450 isoenzymes [12–14]. Posaconazole, however, is metabolised only to a limited extent by cytochrome P450 enzymes [15,16] and is mostly metabolised through phase 2 enzyme systems using uridine diphosphate-glucuronosyltransferase (UGT) enzyme pathways [17]. UGT enzymes are inhibited during inflammation, thus there is also a chance of posaconazole concentrations being affected [18]. However, as cytochrome P450 isoenzymes are not involved to a large extent, it is likely that posaconazole clearance is not influenced by inflammation expressed by elevated CRP concentrations. None the less, there are no published data to support the hypothesis that posaconazole exposure is not influenced by inflammation expressed by CRP concentrations.

Therefore, the objective of this study was to determine whether posaconazole drug exposure is influenced during inflammation.

2. Materials and methods

2.1. Study design

A prospective, observational study was performed at the University Medical Center Groningen (UMCG), Groningen, the Netherlands. Patients aged ≥ 18 years who received posaconazole for treatment or (primary and secondary) prophylaxis of fungal infections were eligible to enter this study. Patients were excluded if they concomitantly used a strong cytochrome P450 inhibitor or inducer.

Both for prophylaxis and invasive aspergillosis the loading dose on Day 1 was 300 mg twice daily and the daily dose was 300 mg once daily [1]. Samples used for routine TDM of posaconazole as well as posaconazole concentrations that were measured from discarded blood (taken for other clinical reasons) were included in the analysis. Discarded blood was collected from all hospitalised patients during their hospital stay (4–5 days). Only confirmed trough concentrations were included in the analysis. Steady-state was considered to have been achieved by Day 6, and all levels of samples that were taken before Day 6 were excluded from the final data analysis [19]. During the study period, patients received oral (modified-release tablets) or i.v. posaconazole. Posaconazole dosing was done according to Infectious Diseases Society of America (IDSA) guidelines [1].

2.2. Ethics

Treatment with posaconazole and the use of TDM for posaconazole were part of routine care and were neither initiated nor altered for study purposes. If the posaconazole concentration was low, the attending hospital pharmacist provided the attending physician with a dosage advice. Posaconazole concentrations from discarded blood samples were measured afterwards. This study was evaluated by the Medical Ethics Committee of UMCG. Written Informed consent was obtained from each patient. The study was registered at ClinicalTrials.gov under ID no. NCT02492802.

2.3. Posaconazole concentrations

Posaconazole plasma concentrations were measured using a validated liquid chromatography–tandem mass spectrometry assay with a lower limit of quantification of 0.1 mg/L [20]. This assay was externally confirmed by an international proficiency testing programme [21].

2.4. Data collection

Data were documented in a case report form and included patient age, sex, weight, height, underlying disease, posaconazole dose (mg/kg/day), time and route of administration, and potentially interacting cytochrome P450 co-medications.

To assess the level of inflammation, CRP concentrations were collected from each patient's medical record. Besides CRP, routine laboratory parameters, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ -GT), total bilirubin and albumin were collected from each patient's medical record.

2.5. Statistical analysis

Continuous variables were summarised as the median and interquartile range (IQR), and nominal variables as the frequency and percentage. Longitudinal data on the posaconazole concentration was analysed using a linear mixed model.

As the data were not normally distributed, a log transformation was performed on posaconazole concentrations. To determine the differences in concentrations between patients, a random additive effect was used. In addition, a first-order autoregressive correlation was used to correct for differences in intervals between observations. The Wald type III test was used to assess the influence of inflammation on posaconazole concentration. The test was corrected for age, sex, posaconazole dose, route of administration, ALP, ALT, AST, γ -GT and total bilirubin. A second test was performed and additionally corrected for albumin.

Statistical analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC). A *P*-value of <0.05 was considered statistically significant.

3. Results

Between August 2015 and June 2017, a total of 64 patients were enrolled in this study, of which 9 were eliminated from the final analysis (2 because no CRP was measured for these patients and 7 because the measured posaconazole samples were not collected at steady-state). Thus, 55 patients with a median age of 62 years (IQR 56–69 years) were included for final analysis of the data. Patient characteristics are presented in Table 1.

In total, 511 posaconazole trough samples with an overall median concentration of 1.8 mg/L (IQR 1–2.9 mg/L, range 0.1–7.94 mg/L) were obtained, and a median of seven samples (IQR 3–12, range 1–28) were collected per patient. Of the 511 posaconazole samples, 217 were measured as part of routine TDM and a dosage change was recommended by the clinical pharmacist for 53 (24.4%) of these. The other 294 samples were discarded blood samples from routine laboratory analyses. The number of CRP concentrations obtained was 386, with an overall median CRP concentration of 23.5 mg/L (IQR 5–75 mg/L, range 0–457 mg/L).

Two patients received interacting co-medication. One patient received omeprazole while also receiving posaconazole suspension, and another patient had received rifampicin <2 weeks before the start of posaconazole treatment. As the interacting medications could have affected drug concentrations, the samples taken during treatment and 1 week after treatment with interacting medications were eliminated from the final analysis.

Fig. 1 shows a scatterplot with posaconazole trough concentrations (mg/L) divided by daily dose (mg/kg body weight) and CRP values (mg/L) to visually describe the correlation between these variables. It can be seen that the CRP concentration does not appear to affect the dose concentration ratio.

A longitudinal data analysis was performed to confirm these findings. The posaconazole concentrations were not normally

Table 1
Characteristics of patients included in the study ($n=55$).

Characteristic	No. (%) of patients or median (IQR)
Male sex	33 (60)
Age (years)	62 (56–69)
Weight (kg)	80 (71–86)
Height (cm)	176 (168–185)
BMI (kg/m ²)	25.7 (23.4–26.8)
Underlying conditions	
AML	35 (64)
MDS	8 (15)
Other ^a	12 (22)
Stem cell transplant	
Allogeneic	22 (40)
Autologous	2 (4)
No transplant	31 (56)
Posaconazole treatment	
Therapeutic issue	
Prophylaxis	40 (73)
Treatment	15 (27)
Route of administration	
Oral	50 (91)
Intravenous and oral	5 (9)
Daily dose (mg/kg body weight)	3.75 (3.4–4.9)

IQR, interquartile range; BMI, body mass index; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome.

^a Other includes X-linked gammaglobulinaemia, T-cell prolymphocytic leukaemia, follicular lymphoma, chronic myelomonocytic leukaemia, Burkitt's lymphoma, blastic plasmacytoid dendritic cell neoplasm, enteropathy-associated T-cell lymphoma type 2, systemic mastocytosis, primary cutaneous T-cell lymphoma, aplastic anaemia, primary myelofibrosis and acute promyelocytic leukaemia.

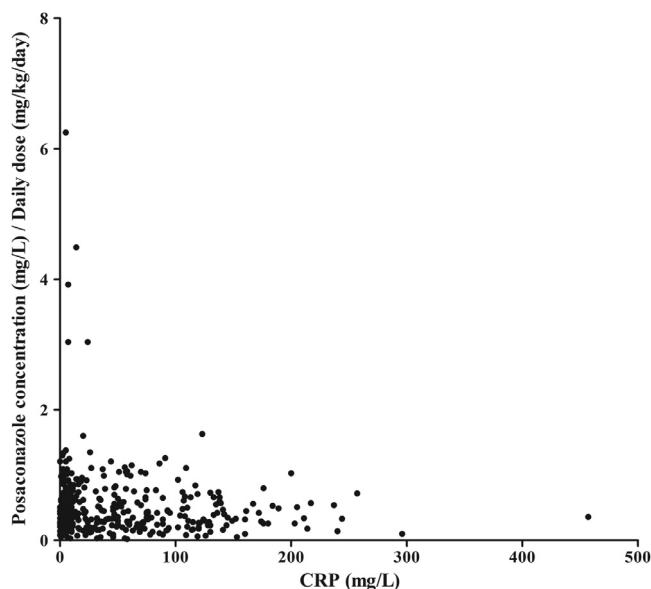


Fig. 1. Scatterplot showing posaconazole concentration divided by daily dose (mg/kg body weight) and the corresponding C-reactive protein (CRP) values.

distributed, thus a log transformation was performed. This analysis showed that the posaconazole daily dose (mg/kg) had a significant influence on posaconazole concentration ($P < 0.0001$) and that posaconazole concentrations were not significantly influenced by CRP concentrations ($P=0.77$) after correction for other potential confounding factors (i.e. sex, age, underlying disease, posaconazole dose, ALP, AST, ALT, γ -GT, total bilirubin and CRP, and excluding albumin).

To confirm the results, a post-hoc analysis was performed to determine whether a clinically relevant change of CRP would re-

sult in a change of posaconazole concentration. Based on the estimated standard error and the degrees of freedom for CRP association in the analysis, the t -distribution was used to calculate the anticipated power if the association would be truly larger than the clinical association. We aimed to see whether a change of 10 units of CRP would result in an absolute change of 0.3 mg/L posaconazole concentration. The association of CRP with posaconazole concentration was determined through multiple regression (log-transformed posaconazole concentrations were regressed on CRP concentrations). For this reason, the previously mentioned clinically relevant association was translated to a percentage change of 14.78% ($=100\% \times 0.3/2.03$) with respect to the average posaconazole concentration. This means a percentage change of posaconazole concentration of ca. 1.5% for every unit change in CRP. The standard error per unit change CRP was estimated at 0.3825% with 312 degrees of freedom. This results in an estimated post-hoc power of 97.4%.

The second longitudinal analysis was corrected for all previously mentioned variables, including albumin ($n=184$). This analysis included less samples as albumin was not routinely measured for all patients. There was still a significant interaction between the daily dose (mg/kg body weight) and posaconazole concentration ($P=0.04$). In addition, the albumin level had a significant influence on posaconazole concentration ($P=0.0012$). At lower albumin concentrations, lower posaconazole trough concentrations were observed and CRP still did not have an effect ($P=0.21$).

4. Discussion

This study investigated the effect of inflammation expressed by CRP values on posaconazole concentrations. CRP was chosen as a marker of inflammation as it is routinely measured in clinical practice and it has been previously shown that high CRP concentrations are associated with high voriconazole concentrations [14]. The results of this study showed that posaconazole exposure was not influenced by CRP concentrations ($P > 0.05$) and only posaconazole daily dose (mg/kg body weight) had a significant effect on drug concentrations. An additional power analysis confirmed that these results have a post-hoc power of 97.4%. The CRP range is comparable with the range reported in the voriconazole study by Veringa et al., where it was shown that in the case of a CRP increase to 200 mg/L, voriconazole concentration is expected to increase approximately to 4 mg/L [14]. In the current study with posaconazole, a clinically significant effect of CRP on posaconazole concentrations was not observed.

On the other hand, we are making assumptions over only a part of posaconazole metabolism as it is eliminated mostly through faecal excretion and to a lesser extent through urinary excretion. Most of the excreted drug is shown to be unchanged in the faeces [22]. Moreover, as mentioned before, the majority of remaining posaconazole is metabolised through phase 2 enzyme systems using UGT enzyme pathways (mostly UGT1A4) [17]. It is also known that enzyme UGT1A4, UGT2B7 and UGT2B4 mRNA levels are influenced by inflammation expressed through interleukin-1 β (IL-1 β) mRNA, tumour necrosis factor- β and IL-6 [18,23]. As CRP is produced by IL-6 stimulation, we can assume that the metabolism of UGT is taken into consideration [24].

The median CRP concentration was 23.5 mg/L and the majority of included patients received posaconazole for prophylaxis. We saw that according to the severity of inflammation, expressed by CRP concentration, there is no indication that inflammation influences posaconazole metabolism. A possible limitation to this study is that the majority of patients had a low CRP value and thus a low grade of inflammation.

In the secondary longitudinal analysis it was observed that with decreasing albumin concentrations, lower posaconazole

concentrations were observed. This effect could be caused by the high protein binding of posaconazole (>98%, mainly to albumin) [19]. The free fraction of highly protein-bound drugs can be significantly affected by small changes in protein binding [25,26]. Ideally, the free concentration of a drug is measured, since total blood concentrations are affected by hypoalbuminaemia [26]. However, in clinical practice it is an analytical challenge to measure free drug concentrations for posaconazole as the high protein binding results in very low free concentrations of the drug. These low concentrations are far below the lower limits of quantification of the currently available assays [20,27,28]. As the current study was designed to analyse the effect of inflammation on total posaconazole concentrations and a limited number of albumin concentrations were included in this analysis, a separate study should be performed collecting detailed information on free drug concentrations and plasma proteins to further explore this observation.

The discussion whether to perform routine posaconazole TDM for modified-release tablets is ongoing. A study in lung transplant recipients who received modified-release tablets showed that posaconazole plasma concentrations are variable [29]. The current study contributes to this debate by eliminating the possible effect of inflammation, expressed by CRP concentration, as a potential source of pharmacokinetic variability of posaconazole.

In conclusion, the results of this study show that posaconazole levels are not influenced by inflammation expressed by CRP concentrations. Therefore, more frequent TDM of posaconazole during inflammation or after an infection subsides appears unnecessary.

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Competing interests

JWCA reports fees from Pfizer, MSD and Gilead for financial support of ‘Noordelijk Schimmel Symposium’ and grants from Pfizer, Astellas and MSD. All other authors declare no competing interests.

Ethical approval

This study was evaluated by the Institutional Review Board of University Medical Center Groningen (Groningen, the Netherlands) [METc 2015.151]. Written Informed consent was obtained from each patient. The study was registered at ClinicalTrials.gov under ID no. NCT02492802.

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