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PHARMACOKINETICS AND DISPOSITION

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Population pharmacokinetics of fluconazole given for secondary prevention of oropharyngeal candidiasis in HIV-positive patients

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Abstract *Objectives*: To determine fluconazole population pharmacokinetics and explore the relationships between fluconazole average concentration and treatment effectiveness or microbiological resistance induction during a study aimed at evaluating the efficacy, tolerability and resistance induction after secondary prevention with fluconazole (150 mg weekly) versus placebo in human immunodeficiency virus-positive (HIV+) patients with oropharyngeal candidiasis.
Methods: Population pharmacokinetic parameters of fluconazole determined from 458 serum drug concentration measurements obtained over 37 months in 132 HIV+ patients not receiving highly active antiretroviral therapy. Mean estimates and variabilities were generated using non-linear regression analysis. Logistic and linear regression analyses were used to explore the relationships between the estimated average concentration of fluconazole and candidiasis relapse or fungal resistance towards fluconazole.
Results: Fluconazole kinetics were best described by a one-compartment model with first-order oral absorp tion from the gastrointestinal tract. The pharmacokinetics were influenced only by body weight. No effect was observed for gender, age, height or lymphocyte CD4 counts. The mean apparent population clearance was 0.79 l/h, the volume of distribution 57 l and the absorption constant (k_a) 0.93 h^{-1}. Inter-occasion variability in clearance (45%) was large relative to inter-subject variability (21%). Taking into account the average fluconazole concentration or the time above the minimal inhibitory concentrations did not clinically improve the prediction of the occurrence of oropharyngeal relapse or microbiological resistance.
Conclusion: The relationship between fluconazole concentrations and preventive effectiveness was poor. Together with the rather large inter-occasion variability in fluconazole clearance, this suggests no role of therapeutic drug monitoring in optimising fluconazole treatment for secondary prevention.

Keywords Fluconazole · PK · Population · Candidiasis · HIV · Patients

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Introduction

Oropharyngeal candidiasis is observed in many human immunodeficiency virus-positive (HIV+) patients and still represents the most frequent opportunistic infection in HIV-infected individuals. The efficacy and safety of fluconazole in the treatment of oral thrush associated with HIV infection has been demonstrated [1, 2], but the frequency of relapses is high and increases with the patient immunodeficiency. The management of oropharyngeal candidiasis in HIV+ patients using a secondary prevention with fluconazole (50–150 mg once a week) has thus been proposed, and the efficacy and safety of this approach are now established [3, 4, 5]. This strategy presents advantages with respect to cost, compliance and drug interactions [6]. However, increased resistance to fluconazole has been reported during prophylactic therapy [7].

A prospective, double-blind, randomised, placebo-matched study was conducted in a cohort of HIV+ patients over a period of 37 months to assess the effect of secondary prevention by weekly fluconazole on the development of clinical and microbiological resistance to fluconazole (Pagani et al., unpublished observations). Blood samples were collected during this study primarily

to assess patient's adherence to treatment. The aims of this analysis were to characterise the population pharmacokinetics of fluconazole in this group of patients. As a secondary exploratory endpoint, we evaluated the relationships between the average blood concentrations or the time over the minimal inhibitory concentrations (MICs; according to a posteriori Bayesian estimation) and the probability of oropharyngeal candidiasis relapse or of clinical or microbiological resistance to fluconazole.

Materials and methods

Patients

Data from 132 patients with 458 serum concentrations were available for the population pharmacokinetic analysis. The patients were randomised to receive orally either fluconazole (150 mg weekly; $n = 66$) or placebo ($n = 66$). In case of candidiasis relapse, a treatment of fluconazole (200 mg per day) was undertaken for 7 days. Serum samples were drawn at least every 3 months on follow-up visits and during candidiasis relapses to check for compliance. For convenience, blood samples were taken at each visit. No predefined sampling strategy was applied, but the exact time of the last administered dose was recorded by the physician at each visit to get accurate dosing information. In addition to accurate dosing information and time of sampling, the following data were collected for each patient: gender, body weight (BW), height, age, number of CD4 lymphocytes and serum creatinine concentration. There were 93 males and 39 females. Their body weights ranged from 41 kg to 97 kg (mean 61 kg), their height from 146 cm to 192 cm (mean 172 cm) and their age from 25 years to 63 years (mean 37 years). The serum creatinine concentration ranged from 53 μmol/l to 181 μmol/l (mean 83 μmol/l); the mean creatinine clearance estimated using the equation of Cockcroft and Gault [8] was 85 ml/min (five patients < 60 ml/min). The CD4 lymphocytes ranged from 0/mm^3 to 605/mm^3 (mean 101/mm^3). The number of fluconazole serum concentration measurements per patient was 2.7 (range 1–8).

Analytical methods

Serum fluconazole concentrations were measured using reverse-phase high-performance liquid chromatography after solid-phase extraction, adapted from the method published by Inagaki [9]. The chromatographic equipment consisted of a Hewlett-Packard 1090 instrument (Series II; Hewlett-Packard, Germany) equipped with a spectrophotometric ultraviolet–visible (UV–VIS) diode-array detector (DAD) set at 200 nm. The separations were carried out on a Macherey-Nagel ChromCart 125/4 Nucleosil 100 C18AB (Düren, Germany) using an isocratic elution of acetonitrile + 0.1% acetic acid/bidistilled water (17/83) for 10 min at a flow rate of 1 ml/min, followed by rinsing and equilibration steps. The calibrations, using the internal standard method (UK-54373) are linear ($r^2 > 0.999$) over the 0.1- to 25-mg/l concentration range. The detection limit was 0.1 mg/l. Quality control samples at 0.75-, 7- and 20-mg/l concentrations had, overall, an inter-day relative standard deviation within 1.4–7.5%. The mean inter-assay deviations from their nominal concentrations were comprised within the range –3.3% to +0.4%. Calculated precision and accuracy of the analytical method were therefore in accordance with the ±15% recommendations of the Conference Report on Bioanalytical Method Validation [10].

Population pharmacokinetic analysis

The analysis was performed using the computer program NONMEM, version V, developed by Beal and Sheiner [11]. It uses mixed-effects (fixed and random) non-linear regression modelling to estimate the mean and the variance of the pharmacokinetic parameters in the study population and factors that may influence them. A stepwise procedure was used to find the model that best fitted the data. First, we compared one- and two-compartment models with first- or zero-order absorption. The influence of each recorded patient characteristics on the kinetic parameters was tested sequentially. A proportional error distribution was assumed for the inter-individual variability of the pharmacokinetic parameters of the form shown below:

$$CL_j = TVCL \cdot \left(1 + \eta_j^{CL}\right)$$

$$Vd_j = TVVd \cdot \left(1 + \eta_j^{Vd}\right)$$

$$Ka_j = TVKa \cdot \left(1 + \eta_j^{Ka}\right)$$

in which CL_j, Vd_j and Ka_j are the true values of the pharmacokinetic parameters for the jth individual; TVCL, TVVd and TVKa are the typical values of the pharmacokinetic parameters in the population and η is a random effect with mean zero and variance ω^2. No covariance was assumed between the parameters.

To allow for an evaluation of pharmacokinetic–pharmacodynamic relationships, an additional inter-occasion variability $\left(\eta_{2ij}^{P} \cdot Occ_1 + \eta_{3ij}^{P} \cdot Occ_2 + ... + \eta_{nij}^{P} \cdot Occ_n\right)$ was introduced on clearance, although only one concentration sample per occasion was available, thus making inter-occasion and intra-individual variabilities confounded. The equation of the inter-individual and inter-occasion variability of the jth subject was expressed as follows [12]:

$$CL_j = TVCL \cdot \left(1 + \eta_{1ij}^{CL} + \eta_{2ij}^{CL} \cdot Occ_1 + \eta_{3ij}^{CL} \cdot Occ_2 + ... + \eta_{nij}^{CL} \cdot Occ_n\right)$$

The parameter Occ_n takes the value of 1 on the nth occasion and 0 otherwise, and the occasion-related variations, η_{2ij} to η_{nij}, are constrained to have the same variance. An additive and proportional error distribution was assumed for description of the intra-individual (residual) variability: $Y_j = F_j + F_j \cdot \varepsilon_{1j} + \varepsilon_{2j}$ where F_j is the model prediction concentration for the jth individual, ϵ_1 and ϵ_2 are the residual intra-individual error terms with mean zero and variance σ^2. All drug concentration data below the detection limit were set to half the detection limit. To limit their influence, the variance of the additive intra-individual error component was forced to equal or exceed the squared half of the detection limit. the difference in the minimum value of the objective function (ΔOF) was used to compare two models.

For model selection, we compared the plots of predicted response versus observations and used the Akaike criterion [13]. In addition, the following goodness-of-fit parameters were considered when choosing between models: residual plots, standard error and correlation matrix of the parameter estimates, size of the inter-individual variance of the pharmacokinetic parameters, and size of the residual error. A simulation based on the pharmacokinetic results including 1000 individuals was performed using NONMEM to calculate the 95% predicted interval depicted in Fig. 1. The concentrations at the 2.5th and 97.5th percentile at each time point were retrieved to construct the prediction interval.

Analysis of effect data

Oropharyngeal candidiasis relapse was determined clinically and confirmed by means of bacteriological examinations (direct examination, primary strain isolation, identification of colonies). The susceptibility of *Candida Albicans* towards fluconazole was determined at each visit with a disk diffusion agar test using 50 μg fluconazole [7]. The inhibition diameter (mm) was measured, and microbiological resistance was defined as an inhibition diameter on an agar plate smaller than 25 mm.

The individual average concentrations of fluconazole were estimated using the post-hoc estimates of clearance. Both the area

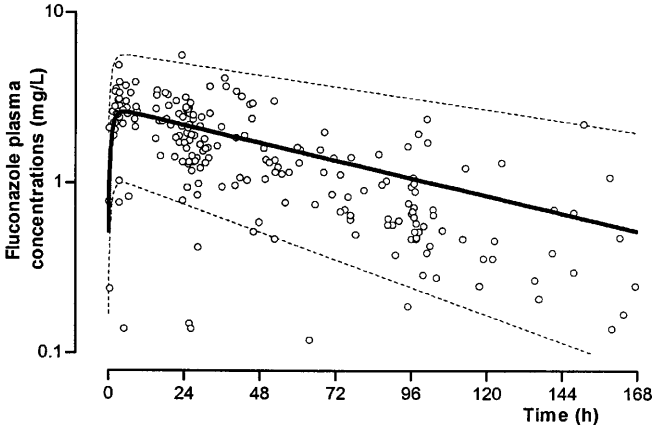

Fig. 1 Fluconazole plasma concentrations in 66 human immunodeficiency virus-positive patients receiving 150 mg fluconazole per week (*open circles*), with the average population prediction (*solid line*) and 95% prediction interval (*dashes lines*)

under the concentration–time curve (AUC) and the time over the MIC (defined as 0.39 mg/l for *C. albicans*) were used for the statistical analysis. The AUC was defined as D_{ose}/CL and the time above MIC was simulated using the post-hoc individual pharmacokinetic estimates in the software program Excel (version 7, Windows 98). The relationship between fluconazole AUC or time above MIC and candidiasis relapses was assessed using logistic regression. To take into account a potential difference in the time until relapse between both groups, a weighting variable was also introduced in the analysis. The independent variables were treatment (fluconazole or placebo) and fluconazole AUC or time above MIC, the dependent variable the candidiasis relapse and the weighting variable the logarithm of the time until relapse. The resistance to treatment was assessed using linear regression, with the inhibition diameter (mm) defined as the dependent variable and treatment (nested within subject) and fluconazole AUC or time above MIC as the independent variables. All the standard statistical tests were performed using the Statistix software (version 4.1).

Results

Population pharmacokinetic analysis

A one-compartment model with first-order absorption from the gastrointestinal tract was found to describe the data adequately (two- vs one-compartment model: $\Delta OF = 0.0$, $\Delta n_{par} = 2$). The absorption kinetics were difficult to estimate with precision since only trough concentration samples were generally taken. An inter-subject variability was assigned on apparent clearance (CL) ($\Delta OF = -17.0$, $\Delta n_{par} = 1$) and on apparent volume of distribution (V_d) ($\Delta OF = -7.9$, $\Delta n_{par} = 1$). Without any covariates, the population estimate of CL was 0.65 l/h. The inter-subject variability in this parameter, expressed as percentage coefficient of variation (CV%), was 34%; when allowing for an inter-subject and inter-occasion variability, the CV values were 30% and 45%, respectively ($\Delta OF = -186.0$). The mean population V_d (CV%) was 70.7 l (30%). The absorption was rapid, with a half-life of 42 min. The additive and proportional intra-subject variability were 0.78 mg/l (SD) and 34% (CV%), respectively.

Covariates assessment

The relationship between various covariates and the individual estimates of fluconazole CL and V_d were examined. Body weight significantly influenced both the CL and the V_d of fluconazole ($\Delta OF > -14.4$, $\Delta n_{par} = 1$), reducing the CL variability from 34% to 31% and explaining the overall variability on the V_d. The regression model for fluconazole CL and V_d accounting for BW was $CL = 0.79 + 1.1 \times BWE$ and $V_d = 87.4 + 44.2 \times BWE$, where BWE expresses the relative deviation of the individual BW from the mean BW in the population ($BWE = -1 + BW/mean\ BW$). No effect of age ($\Delta OF = -0.3$, $\Delta n_{par} = 1$) or gender ($\Delta OF = 0.0$, $\Delta n_{par} = 1$) was observed. The number of lymphocytes CD4 and the height influenced the CL of fluconazole ($\Delta OF > -3.3$, $\Delta n_{par} = 1$) but did not remain statistically significant in the multivariate analysis ($\Delta OF = -1$, $\Delta n_{par} = 1$), being too highly correlated with body weight. Similarly, no effect of creatinine CL on fluconazole CL was observed ($\Delta OF = -0.3$, $\Delta n_{par} = 1$). The remaining inter-subject and inter-occasion variabilities in CL were, respectively, 21% and 45%. The values of the population parameters for the final regression model are given in Table 1. Plasma concentrations of fluconazole with population prediction and 95% prediction interval are presented in Fig. 1.

Fluconazole effects

The population pharmacokinetic analysis was used to assess whether taking into account either the average concentration of fluconazole or the time above MIC could improve the prediction of the response to fluconazole prophylactic treatment. As assessed by Pagani et al. (unpublished observations), significantly more patients experienced a relapse in the placebo than in the fluconazole group (90% vs 61%, $P = 0.001$). The average fluconazole concentration failed to improve the prediction of the response ($P = 0.7$), even while using the time interval to relapse as a weighting variable ($P = 0.5$). Similarly, the time above MIC failed to predict the response ($P = 0.8$). A tendency to a significant difference could be detected between both groups for the occurrence of microbiological resistance in Candida isolates ($P = 0.03$). However, when considering the millimetres of inhibition measured in the disk diffusion agar test, a difference of 3.2 mm diameter was noticed between the placebo (mean 46.5 mm) and the fluconazole (43.3 mm, $P < 0.0001$) groups. This trend towards some decrease in susceptibility to fluconazole is however small, far from the cut-off value of 25 mm defined for microbiological resistance to treatment and, therefore, probably not clinically significant. Here, again, taking into account the average fluconazole concentration or the time above

Table 1 Population pharmacokinetic parameters of oral fluconazole. Mean population estimates. All parameters are apparent values. *CL* apparent clearance, V_d apparent volume of distribution, k_a absorption constant

Parameter	Population mean		Inter-individual variability[a]		Inter-occasion variability[a]	
	Estimate	SEM[b]	Estimate	SEM[b]	Estimate	SEM[b]
CL (l/h)	0.79	4%	21%	49%	45%	53%
CL_{bw}[d] (l/h)	1.14	17%				
Vd (l)	57.4	6%				
Vd_{bw}[d] (l/h)	44.2	29%				
Ka (h^{-1})	0.93	19%				
σ_{add} (mg/l)[e]	0.19	17%				
σ_{prop} (CV%)[e]	31%	45%[c]				

[a]Estimates of variability expressed as coefficient of variation
[b]Standard error of the estimates, expressed as coefficient of variation
[c]Standard error of the variance components, taken as $\sqrt{s.e_{estimate}}/estimate$, expressed as a percentage
[d]Proportionality term relating CL and Vd to a relative increase or decrease in body weight (kg) from the average value (61 kg) in the population
[e]Residual intra-individual variability of the serum concentration, expressed as standard deviation (add, SD mg/l) and coefficient of variation (prop, CV%)

MIC did not improve statistically the prediction of this effect.

Discussion and conclusions

This study determined the population pharmacokinetics of fluconazole and demonstrated that only BW but not gender nor any of the other studied covariates influences fluconazole pharmacokinetics. Intra-individual as opposed to inter-subject variability in CL was rather large, and individual fluconazole concentrations did not correlate with therapeutic outcome, indicating that therapeutic drug monitoring would probably not improve use effectiveness of fluconazole.

The mean population pharmacokinetic parameter estimates were in the same range as those already reported [14, 15, 16, 17]. Although a previous study reported no effect of BW on fluconazole pharmacokinetics [16], in this study BW significantly influenced fluconazole CL and V_d. In contrast to previous studies [16, 18], this study included female and male patients. Since neither the covariate plots suggested any gender differences nor the inclusion of additional parameters for gender differences in CL and/or V_d improved the fit, a clinically relevant effect of gender on fluconazole pharmacokinetics can be excluded.

A reduction in fluconazole dose was previously suggested for people with HIV infection who are seriously ill and/or who have compromised renal function [16, 18]. However, in this study, after inclusion of BW, neither lymphocyte CD4 count nor creatinine CL exhibited a significant effect on fluconazole CL. The initially detected statistical significance of CD4 counts in our analysis probably occurred due to an inverse correlation of lymphocyte CD4 counts – as a marker surrogate of disease progression – and BW. A possible explanation why this study failed to detect a relevant effect of creatinine CL on fluconazole CL, despite the fact that fluconacole is mainly excreted unchanged renally, might be that this study included only a few patients with moderately impaired and nobody with severely impaired renal function. Therefore, a dose reduction in patients with considerably impaired renal function still seems justified, although we would not recommend the same for patients with low CD4 counts.

In contrast to a previous population pharmacokinetic study on fluconazole, which mainly included only one sampling occasion per patient, this study included blood sampling on several occasions for most patients. This sampling schedule allowed us to distinguish between inter-subject and inter-occasion variability. After inclusion of the covariate BW, the remaining inter-subject variability was rather small compared with the inter-occasion variability. No relationship between the patients average fluconazole concentration and the occurrence of oropharyngeal relapse or clinical or microbiological resistance could be detected. The large inter-occasion variability of pharmacokinetics might explain why drug concentrations at one occasion failed to explain overall fluconazole response. However, also, the sparse sample collection may have compromised the power of the study to detect association of limited strength. In conclusion, this study demonstrated that fluconazole pharmacokinetics are gender independent and do not depend on CD4 counts. The rather large inter-occasion variability in fluconazole CL and the absent correlation between drug exposure and treatment response suggests that therapeutic drug monitoring does not represent a sensible approach for improving fluconazole's therapeutic effectiveness.

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