Br J Clin Pharmacol 1996; **41**: 291–298

Pharmacokinetics of fluconazole in people with HIV infection: a population analysis

A.J. McLACHLAN & S.E. TETT*
Clinical Pharmacology & Toxicology, St Vincent's Hospital Darlinghurst, Australia.

1 The population pharmacokinetics of fluconazole have been investigated in 113 male subjects with HIV infection and AIDS. Plasma concentration–time data (between 1 and 17 observations per dose) were collected from individuals as part of a pharmacokinetic investigation (13 subjects) or during routine fluconazole therapy (100 subjects) for the treatment or prophylaxis of fungal infection.

2 A one-compartment pharmacokinetic model was used to describe the disposition of fluconazole after oral and intravenous infusion doses. Population pharmacokinetic parameters were generated using the NONMEM and P-PHARM computer programs.

3 The population estimates (calculated using NONMEM) of fluconazole clearance and volume of distribution were 0.78 l h^{-1} and 47.6 l, respectively. The intersubject variability for these parameters was 41% and 8%, respectively. The model-dependent estimate of the extent of absorption was 0.99 with an intersubject variability of 6%. Mean population estimates generated by NONMEM and P-PHARM were in close agreement.

4 Examination of the relationship between patient covariates and pharmacokinetic parameters indicated that intersubject variability in fluconazole clearance could in part be explained by the severity of disease (as indicated by CD4 + T-lymphocyte count) and renal function (indicated by estimated creatinine clearance). Other pharmacokinetic parameters were unaffected by these covariates.

5 Fluconazole clearance (estimated using NONMEM) in subjects with a CD4 + T-lymphocyte count less than and greater than 200 cells mm^3 was 0.73 l h^{-1} (95% CI ; 0.64–0.82 l h^{-1}) and 0.99 l h^{-1} (95% CI ; 0.86–1.12 l h^{-1}), respectively. The regression model for fluconazole clearance that accounted for changes in renal function and disease severity was CL (l h^{-1}) = 0.25 (33%) + 0.0057 (32%) × CLcr (in ml min^{-1}) + 0.00068 (10%) × CD4 cell count (in cells mm^{-3}) where intersubject variability (expressed as %CV) is shown in brackets.

6 Based on pharmacokinetic considerations a reduction in the dose of fluconazole would appear to be warranted in people with HIV infection who are seriously ill or who have compromised renal function. However, the emergence of resistance to fluconazole must also be considered when thinking of dosage adjustments.

Keywords fluconazole AIDS population pharmacokinetics antifungal

* Present address: Department of Pharmacy, University of Queensland, Brisbane QLD 4072, Australia.
Correspondence: Dr Andrew J. McLachlan, Clinical Pharmacology and Toxicology, St Vincent's Hospital, Victoria Street, Darlinghurst, NSW 2010 Australia.

Introduction

Fluconazole is used in the treatment and prevention of opportunistic fungal infections, such as candidiasis and cryptococcal meningitis, in people with human immunodeficiency virus (HIV) infection [1–3]. Optimal fluconazole dosage regimen design may involve the targeting of specific plasma concentrations to ensure anti-fungal efficacy, whilst avoiding excessive concentrations which may lead to toxicity. However, to date, fluconazole dose recommendations for people with HIV infection and AIDS are based on pharmacokinetic data obtained from healthy subjects [5, 6] with only a few studies being conducted in HIV infected subjects [4, 7, 8]. The results of a pharmacokinetic study with intensive blood sampling suggested that people with low (less than 200 cells mm^{-3}) CD4 + T-lymphocyte counts ($n = 4$) had a lower clearance of fluconazole than either non-infected healthy subjects ($n = 10$) or those infected by HIV with higher (greater than 200 cells mm^{-3}) CD4 + T-lymphocyte counts ($n = 9$) [4]. Despite prolonged efforts, it was not possible to recruit further subjects with low CD4 + T-lymphocyte counts into this study. High quality information is needed about the pharmacokinetics of fluconazole, particularly about changes in pharmacokinetic parameters with disease states, in order to accurately individualize therapy and provide safe and effective dosing strategies. This information is best obtained using data collected from individuals receiving the drug as part of their normal therapy. Using a population approach it is possible to characterise a pharmacokinetic model capable of describing and predicting pharmacokinetic behaviour in the target population and to examine factors affecting disposition within that population [9, 10].

The aims of the present analysis were to determine the population pharmacokinetics of fluconazole in people with HIV infection and to examine factors affecting fluconazole disposition in this population.

Methods

Study population

Concentration–time data from 113 male subjects with HIV infection was obtained from two separate investigations. Covariate information was available for 109 people. The mean (and range) age of the subjects was 38 (23–60) years and weight was 63 (42–88) kg. The mean CD4 + T-lymphocyte count of the study group, a measure of disease severity, was 69 cells mm^{-3} and this ranged from 0 to 1050. The mean creatinine clearance for the subjects, estimated from serum creatinine concentration, age and weight, using the equation of Cockcroft & Gault [11], was 68 ml min^{-1} and this ranged from 36 to 138 ml min^{-1}. There was no relationship between disease severity (as measured by CD4 + T-lymphocyte count) and estimated creatinine clearance ($r^2 = 0.04$). The majority of patients were receiving other drugs, none of which were known to affect the pharmacokinetics of fluconazole. These included anti-retroviral nucleosides (zidovudine, didanosine and zalcitabine) and co-trimoxazole. The majority of people had or previously had an AIDS defining illness including Kaposi's sarcoma (21), pneumocystis carinii pneumonia (35), cytomegalovirus retinitis (25) and herpes simplex virus infection (12).

Dose administration and plasma sampling

The data set consisted of observations from individuals from two separate studies. In the first study (Study 1) 13 male subjects received between one and three oral doses (Diflucan™ capsules) and one intravenous infusion (administered at a rate of 50 mg per 15 min Diflucan™) at doses of 50 mg, 100 mg or 400 mg fluconazole as part of a pharmacokinetic investigation [4]. In Study 1 doses were separated by at least 2 weeks and between 12 and 17 plasma samples were taken after each dose administration at or near the following times; 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 32, 48, 72, 96, 120 and 168 h. Study 2 was a cross-sectional study conducted in male subjects receiving between 50 to 800 mg as an oral dose for the treatment or prophylaxis for fungal infections (including oral candidiasis and cryptococcal meningitis). The frequency of dosing ranged from twice daily to once weekly with the majority of subjects receiving fluconazole as a single daily dose. All subjects had been on their present dose regimen for at least 7 days and were considered to be at steady-state. One plasma sample was obtained for each subject at an accurately recorded time after the previous dose (mean 20.9 h; range 0.42–51.6 h). Informed and written consent was obtained from each subject. Study 1 and Study 2 had the approval of the Research Ethics Committee of St Vincent's Hospital (Sydney, Australia).

Fluconazole assay

Concentrations of fluconazole were determined in plasma samples using a modified version of the gas liquid chromatographic method of Debruyne *et al.* [12] as previously published by Tett *et al.* [4]. The limit of quantitation of the assay was 0.1 mg l^{-1}. Within run coefficients of variation were less than 6% at plasma drug concentrations between 0.5 and 10 mg l^{-1} [4]. This was confirmed in the present study; the mean coefficient of variation of duplicate analyses was 6%. The conversion factor to obtain fluconazole concentration in SI units is 1 mg l^{-1} is equivalent to 3.26 μM.

Data analysis

Concentration-time data were analysed using non-linear mixed effects modelling implemented in P-PHARM Ver 1.3 [13] and NONMEM IV [14]. Computations were performed on an IBM PC. The population approach examines fixed (e.g. pharmacokinetic model parameters such as clearance and volume) and random (e.g.

intersubject variance of pharmacokinetic parameters and residual variability) effects [9].

Pharmacokinetic model

Preliminary analysis of the concentration-time data from Study 1 [4] indicated that a linear one-compartment pharmacokinetic model with first-order elimination and with first-order input after an oral dose and zero-order input after an intravenous infusion best described the disposition of fluconazole. A single dose model was use for data from Study 1 and a multiple-dose steady-state model was used to fit data from Study 2. The pharmacokinetic model was parameterized using clearance (CL), volume of distribution (V), a first order absorption rate constant (ka) and the fraction of the dose absorbed (F).

Pharmacostatistical model

Random effects are considered to consist of inter-individual variability (termed η with a standard deviation ω) in each pharmacokinetic parameter (that is ω_{CL}, ω_V, ω_{ka} and ω_F) with the remaining variability being termed the residual or unexplained variability (termed ε with a standard deviation σ) within subjects [9]. Preliminary analysis of the pattern of residuals and the reduction in the standard error of the parameter estimates indicated that the interindividual variability was best described by a normal distribution, rather than a log-normal distribution, when using P-PHARM [13] and a multiplicative model (as described in Equation 1), rather than an additive error model, when using NONMEM [14].

$$p_j = p_{pop}\,(1+\eta_{pj}) \qquad (1)$$

where p_j is a parameter (CL, V, ka or F) of the jth individual, p_{pop} is the population mean value of the parameter and η_{pj} is the interindividual error. Residual variability, which encompasses measurement error, model misspecification and intraindividual variability, was described by the following model for the NONMEM analyses [14],

$$C_{ij}(t) = f(p_j, t_{ij}) + \varepsilon_{ij} \qquad (2)$$

where $C_{ij}(t)$ and $f(p_j, t_{ij})$ are the observed and predicted fluconazole concentrations in the jth individual at time t_{ij} and ε_{ij} is the residual error. A comparison of the size of the residual error between competing error models (0.07 mg l^{-1} *vs* 0.39 mg l^{-1}, respectively) indicated that a heteroscedastic error model (proportional to the squared value of the prediction), rather than a homoscedastic error model, best described the residual variability when using P-PHARM [13].

Data analysis strategy

Firstly, mean population pharmacokinetic estimates were obtained using data from 13 subjects (Study 1) for whom numerous observations (670) were available. A second analysis was then conducted which included the sparse data obtained from 100 subjects (Study 2) so that the combined data set consisted of 113 subjects and 770 observations. Both P-PHARM and NONMEM were used to define the population parameters and estimates of random effects. The factors (covariates) affecting fluconazole pharmacokinetic parameters were then examined in data for 109 subjects (where covariate information was available) using different strategies for P-PHARM and NONMEM.

P-PHARM generates posterior Bayesian parameter estimates (for CL and V) for each subject which are then compared with patient specific factors (e.g. CD4 + T-lymphocyte count, creatinine clearance and weight) using stepwise multiple linear regression. This approach selects a covariate or combinations of covariates and examines the relationship with pharmacokinetic parameters using a partial F test to judge the statistical significance [13]. Alternatively, parameters and covariates can be individually selected to examine the relationship between each.

The influence of body weight, renal function (assessed using predicted creatinine clearance) and disease severity (assessed using the CD4 + T-lymphocyte count) were tested on clearance and volume of distribution. The relationship between covariates and the absorption rate constant and the fraction of the dose absorbed were not investigated. A number of regression models were examined to test the relationship between pharmacokinetic parameters and covariates (see Table 4). The difference in the NONMEM objective function between competing models was compared with a chi-squared statistic [14].

Results

Population pharmacokinetic analysis

The mean population pharmacokinetic parameters for fluconazole obtained from data for 13 subjects are shown in Table 1. The population estimates of clearance obtained using P-PHARM and NONMEM were 0.80 l h^{-1} and 0.88 l h^{-1}, respectively. The intersubject variability in this parameter, expressed as percent coefficient of variation (%CV), was approximately 20% (for both P-PHARM and NONMEM) in the data from Study 1. The estimated population mean volume of distribution (%CV) was 44.8 l (8%) and 47.4 l (8%), from P-PHARM and NONMEM, respectively. Population model dependent estimates of absorption indicated that the rate of fluconazole absorption was rapid (absorption half-life estimated to be 8 or 14 min) and variable (P-PHARM %CV for ka was 41%) but essentially complete (F; 0.93 and 0.98, using P-PHARM and NONMEM, respectively) with little variability in the extent of absorption across the 13 subjects (%CV was 5% and 6%). The residual error was estimated to be 0.07 mg l^{-1} using a heteroscedastic model

Table 1 Mean population pharmacokinetic estimates for fluconazole obtained using data from Study 1 containing 13 subjects (12–17 observations per sample and up to four doses; 670 observations)

	P-PHARM			*NONMEM*		
	Mean	*95% CI**	*%CV†*	*Mean*	*95% CI*	*%CV*
CL ($l\ h^{-1}$)	0.80	0.71–0.89	21	0.88	0.73–1.03	20
V (l)	44.8	42.8–46.8	8	47.4	43.6–51.2	8
ka (h^{-1})	3.05	2.35–3.75	41	5.03	4.19–5.87	246
F	0.93	0.90–0.96	5	0.98	0.91–1.05	6
Residual error ($mg\ l^{-1}$)			0.07			0.52

*Upper and lower 95% confidence interval of parameter estimate. †Intersubject variability expressed as percent coefficient of variation of population.

(implemented in P-PHARM) and 0.52 $mg\ l^{-1}$ using an additive error model (implemented in NONMEM).

The results of the second population analysis using the combined rich and sparse data (770 observations) from 113 individuals are shown in Table 2. The mean population estimates of clearance were 0.73 $l\ h^{-1}$ (32%) and 0.78 $l\ h^{-1}$ (41%), obtained using P-PHARM and NONMEM, respectively, which tended to be lower than that observed in the analysis of data from Study 1 (Table 1). The mean population estimates (and %CV) of V, ka and F did not change notably from the analysis of the data from 13 subjects. The estimate of residual error similarly was unchanged.

Covariate analyses: examining the factors affecting fluconazole pharmacokinetics

The relationship between covariates and the estimates of fluconazole clearance and volume of distribution were examined. The reason a patient was taking fluconazole (treatment or prevention of fungal infection) did not significantly explain the variability in either clearance or volume of distribution. Similarly, the co-administration of zidovudine, didanosine and co-trimoxazole did not affect these parameters. Furthermore, there was no association between concurrent pathophysiology and fluconazole clearance and volume.

Multiple linear regression using P-PHARM on data obtained from 13 subjects (Study 1) indicated that whether a subject had a CD4 + T-lymphocyte count greater or less than 200 cells mm^{-3} was capable of describing 59% of the variability in fluconazole clearance, while weight and age significantly influenced volume of distribution, with 34% and 29% of the variability in this parameter being explained by these covariates, respectively. Estimated creatinine clearance was not selected as a covariate that significantly affected clearance probably because the 13 subjects in Study 1 had a narrow range of estimated creatinine clearance (80–138 ml min^{-1}). When this analysis was repeated using data from 109 subjects (for whom covariate data were available from Studies 1 and 2) P-PHARM did not select any covariate as significantly influencing clearance or volume of distribution. In this revised analysis, P-PHARM indicated that the combined influence of CD4 + T-lymphocyte count, patient weight and the estimated creatinine clearance accounted for 11% of the variability in clearance and only 4% of the variability in the volume of distribution. Based on the hypothesis raised by Tett *et al.* [4] the combined data were re-analysed with the clearance split for subjects with a CD4 + T-lymphocyte count less than or greater than 200 cells mm^{-3}. This cut-off was selected based on clinical information and corresponds to the degree of immunosuppression where people are more prone to opportunistic infections. In this P-PHARM analysis the values of V, ka and F were fixed to the population mean (Table 2). Table 3 shows the population mean and 95% CI of the mean estimates of fluconazole clearance in subjects with a CD4 + T-lymphocyte count above ($n = 12$) and below ($n = 97$) 200 cells mm^{-3}. This analysis indicated that people with a lower CD4 + cell count had a lower clearance of fluconazole (0.73 $l\ h^{-1}$ *vs* 0.88 $l\ h^{-1}$, respectively). To investigate further the relationship

Table 2 Mean population pharmacokinetic estimates for fluconazole obtained using the combined data set (observations from Study 1 and Study 2) containing 113 subjects (1–17 observations after single and multiple dosing; 770 observations)

	P-PHARM			*NONMEM*		
	Mean	*95% CI**	*%CV†*	*Mean*	*95% CI**	*%CV†*
CL ($l\ h^{-1}$)	0.73	0.69–0.77	32	0.78	0.69–0.86	41
V (l)	44.7	44.0–45.4	8	47.6	45.4–49.8	8
ka (h^{-1})	3.07	2.83–3.31	41	5.02	4.19–5.85	234
F	0.96	0.95–0.97	5	0.99	0.91–1.06	6
Residual error ($mg\ l^{-1}$)			0.07			0.52

*Upper and lower 95% confidence interval of parameter estimate. †Intersubject variability expressed as percent coefficient of variation of population.

Table 3 Fluconazole clearance ($l\ h^{-1}$) in 109 HIV-infected people with a CD4 + T-lymphocyte count less than and greater than 200 cells mm^{-3}. Data for other pharmacokinetic parameters are shown in the Results section.

	P-PHARM			*NONMEM*		
	Mean	*95% CI**	*%CV†*	*Mean*	*95% CI**	*%CV†*
CD4 < 200	0.73	0.68–0.77	33	0.73	0.64–0.82	40
CD4 > 200	0.88	0.77–1.00	22	0.99	0.86–1.12	19

*Upper and lower 95% confidence interval of parameter estimate. †Intersubject variability expressed as percent coefficient of variation of population.

between fluconazole clearance and renal function a linear regression analysis of the posterior Bayesian estimates of clearance (generated using P-PHARM) and predicted creatinine clearance was conducted. The equation for fluconazole clearance derived from P-PHARM generated Bayesian estimates was CL ($l\ h^{-1}$) = $0.39 + 0.0025 \times CL_{cr}$ (in ml min^{-1}).

Table 4 presents the results of the comparison between regression models incorporating patient covariates and fluconazole clearance and volume of distribution using NONMEM. Patient weight did not influence either clearance or volume of distribution. The relationship between clearance and CD4 + cell count was examined using both a linear regression model and a categorical model which split clearance into two sub-populations with CD4 + cell counts above and below 200 cells mm^{-3} (as done previously for the P-PHARM analysis). Both models resulted in a significant reduction in the NONMEM objective function (Table 4) indicating that disease severity influences fluconazole clearance. The equation for estimating fluconazole clearance using CD4 + cell count derived from NONMEM using a regression model was CL ($l\ h^{-1}$) = 0.70 (11%) + 0.001 (38%) × CD4 cell count (in cells mm^{-3}), where the intersubject variability (as %CV) is shown in brackets. When fluconazole clearance was split between people who had a CD4 + T-lymphocyte count less than ($n = 97$) or greater than ($n = 12$) 200 cells mm^{-3} the mean population and 95% confidence interval estimates generated by NONMEM for each group are presented in Table 3. People with a lower CD4 + T-lymphocyte count had a mean fluconazole clearance of 0.73 $l\ h^{-1}$ while people with a higher CD4 + T-lymphocyte count had a clearance of 0.99 $l\ h^{-1}$. The mean (and intersubject variability) of other pharmacokinetic parameters remained unchanged in these two groups (*V*; 47.4 l (8%), *k*a; 4.99 h^{-1} (234%) and *F*; 0.98 (7%)). Residual error was unchanged at 0.52 mg l^{-1}. Using a similar approach to examine the volume of distribution indicated that disease severity did not influence this parameter. The relationship between fluconazole clearance and renal function was assessed using two regression models. The most appropriate model to describe the relationship between estimated creatinine clearance and fluconazole clearance was CL ($l\ h^{-1}$) = 0.20 (9%) + 0.0068 (34%) × CL_{cr} (ml min^{-1}) indicated by a significant

Table 4 Models employed to examine the influence of covariates on fluconazole clearance and volume of distribution using NONMEM.

Question	*Model*	*LLD**	*Answer†*
Does weight influence CL?	$CL = \theta_1 \times wt$	8	no
Does disease severity influence CL?‡	$CL = \theta_1 + \theta_2 \times CD4$	−21	yes
	$CL = \theta_1 + CD4^{\theta 2}$	1050	no
	CL_1 *vs* CL_2§	−18	yes
Does renal function influence CL?‡	$CL = \theta_1 \times CL_{cr}$	−28	yes
	$CL = \theta_2 + \theta_1 \times CL_{cr}$	−34	yes
Are there combined influences of renal function and disease severity on CL?			
	$CL_1 = \theta_1 \times CL_{cr}$ *vs* $CL_2 = \theta_2 \times CL_{cr}$	−29	yes
	$CL_1 = \theta_1 + \theta_2 \times CL_{cr}$ *vs* $CL_2 = \theta_3 + \theta_4 \times CL_{cr}$	−36	no
	$CL = \theta_1 + \theta_2 \times CL_{cr} + \theta_3 \times CD4$	−41	yes
Does weight influence *V*?	$V = \theta_3 \times wt$	5	no
Does disease severity influence *V*?‡	V_1 *vs* V_2§	−1	no

*Difference in the NONMEM objective function compared to the simple model where $CL = \theta_1$ or $V = \theta_3$. †A difference of 8 or more was considered statistically significant. ‡Assessed by CD4 count (in units of cells mm^{-3}). §Subscripts 1 and 2 represent subpopulations above and below a CD4 count of 200 cells mm^{-3}, respectively. ¶Assessed using predicted creatinine clearance (CL_{cr}; ml min^{-1}).

decrease in the value of the NONMEM objective function (Table 4). Values of other pharmacokinetic parameters were unchanged.

The combined influence of estimated creatinine clearance and CD4 + T-lymphocyte count on fluconazole clearance were examined. As before this was tested using both a regression and categorical model approach (Table 4). The regression model for fluconazole that resulted in the largest reduction in the NONMEM objective function (-41) of all the models tested (Table 4) accounted for both changes in renal function and disease severity, such that CL ($l\,h^{-1}$) = 0.25 (33%) + 0.0057 (32%) × CL_{cr} (in ml min^{-1}) + 0.00068 (10%) × CD4 cell count (in cells mm^{-3}), where intersubject variability (expressed as %CV) is shown in brackets after each parameter estimate. Mean population estimates (and intersubject variability expressed as %CV) of other pharmacokinetic parameters were 47.5 l (8%), 4.98 h^{-1} (234%) and 0.98 (7%) for volume of distribution, absorption rate constant and bioavailability. Residual error was determined as 0.51 mg l^{-1}.

Discussion

A linear one-compartment model best described the concentration–time data for fluconazole in the present investigation [4]. DeMuria *et al.* [7] employed a two-compartment pharmacokinetic model to describe the disposition of fluconazole after oral and intravenous administration of fluconazole using an iterative two-stage population approach. However, the population estimate of intercompartmental or distributional clearance (CL_d) from the study by DeMuria *et al.* [7] was high (85.4 l h^{-1} and 20.7 l h^{-1} after intravenous and oral doses, respectively) indicating very rapid transfer of fluconazole between the central and peripheral compartments, suggesting that a one-compartment model provides an adequate representation of the data. Furthermore, the sampling protocol employed in Study 1 was extensive (which like the DeMuria *et al.* study drew the first sample at 15 min) and therefore capable of adequately characterizing the disposition of fluconazole after a single dose.

In the present study two methods have been used to obtained population parameter estimates. P-PHARM and NONMEM software are both designed to examine the central tendency of population data but they approach the analysis of these data differently. P-PHARM uses an EM-like algorithm [13] whereas NONMEM uses a first order Taylor series expansion to obtain a linear random effects model [14]. Despite different analysis procedures both software packages provided similar population estimates. NONMEM had difficulty estimating the intersubject variability for the absorption rate constant (*k*a), probably because of the very few fluconazole concentrations taken prior to the peak concentration. However, the population mean estimates of *k*a obtained from P-PHARM and NONMEM were in good agreement.

Humphery *et al.* [6] examined the kinetics of fluconazole in healthy subjects and found estimates of clearance ranging from 0.82 to 1.20 l h^{-1} and a volume of distribution of 48 l. Other than the study by Tett *et al.* [4], data from which were used in this population analysis, three reports have been published on the pharmacokinetics of fluconazole in people with HIV infection. The most comprehensive study to date was conducted by DeMuria *et al.* [7] in 10 people with AIDS (all having a CD4 + T-lymphocyte count below 200 cells mm^{-3}) and found a mean (%CV) clearance of 1.06 l h^{-1} (31%) and volume of distribution of 53.8 l (14%). In comparison to the findings of the present study, the estimate of clearance determined by DeMuria *et al.* is at the upper end of the confidence interval for fluconazole clearance estimated in the group of patients with a higher CD4 cell count (Table 3), despite there being no difference in the predicted creatinine clearance between the two study populations. One possible reason for this difference is that DeMuria *et al.* [7] collected plasma samples up to 72 h (only 24 h in four individuals) and used an assay with a sensitivity limit of 200 ng ml^{-1}. These limitations have the potential to provide an underestimate of area-under-the-concentration-time curve and therefore overestimates of clearance, as has been demonstrated by Tett & Cutler [15] for chloroquine. Furthermore, the optimal sampling time (estimated using the method detailed by Bourne [22]) to provide the best estimate of fluconazole clearance after a single dose was at or near 48 h after a single dose. For four individuals in the DeMuria *et al.* study, sampling did not continue to this time. Yeates *et al.* [8] studied fluconazole disposition in 10 healthy and 10 HIV infected subjects (CD4 + T-lymphocyte count ranged from 5 to 99 cells mm^{-3}), collecting plasma samples up to 120 h after an intravenous infusion, and found, using non-compartmental pharmacokinetic methods, that clearance was lower in the HIV infected individuals (1.02 l h^{-1} *vs* 1.38 l h^{-1}) while volume of distribution was unchanged (57 l). Chin *et al.* [16] estimated the clearance of fluconazole in one subject with AIDS being treated for cryptococcal meningitis. Fluconazole clearance (0.57 l h^{-1}) was in good agreement with the results of the present analysis, when the estimated creatinine clearance (62 ml min^{-1}) was taken into consideration.

In the present study, the population estimate of clearance was lower in the combined analysis (113 subjects) of data from Studies 1 and 2 (Table 2) compared with data from Study 1 alone (13 subjects; Table 1) while other parameters (*V*, *F* and *k*a) remained unchanged. The cross-sectional data that comprised Study 2 consisted mainly of samples collected at the latter end of the dosing interval (mean sample collection time; 20.9 h). The optimal sampling time [22] for volume of distribution was between 1 and 3 h and for clearance between 12 and 18 h during a 24 h dosing interval at steady-state. Hence in the present analysis the value of clearance changes when Study 2 data was introduced into the analysis but the estimate of volume of distribution remained unchanged. The reason for the observed decrease in the estimate of clearance between Table 1 and 2 was due to the increased proportion of

people with a CD4+cell count below 200 cells mm^{-3} and lower predicted creatinine clearance in Study 2. The volume of distribution of fluconazole (Table 2; approximately 0.76 l kg^{-1}) indicates that this drug effectively distributes into the aqueous space (0.6 l kg^{-1}; [17]) throughout the body. This finding is supported by human tissue concentration–time data presented by Fischman *et al.* [18] and is in keeping with the very low plasma protein binding and the polar nature of this compound [19]. Concentration–time data for fluconazole after an oral and intravenous dose was simultaneously modelled allowing a population estimate of the fraction of the dose absorbed, as proposed by Kaniwa *et al.* [20]. Fluconazole absorption was rapid and essentially complete, which is in good agreement with the work of DeMuria *et al.* [7] in people with AIDS and Humphery *et al.* [6] in healthy subjects.

The fact that residual error did not change when the single observations from Study 2 were included in the analysis (Table 1 *vs* Table 2), nor when regression models were used to account for variability in clearance using covariates, is most likely because the residual error, representing intraindividual error, could not be further assessed with these additional data as only a single observation per individual was available. Unadkat & Agosti [21] have highlighted the heterogeneity of the HIV infected subject population and discussed the implications of this with respect to pharmacokinetic studies.

Covariate analysis suggests that both disease severity (as judged by CD4+T-lymphocyte count) and estimated creatinine clearance explain some of the variability in fluconazole clearance. However, there was no correlation ($r^2 = 0.04$) between CD4+T-lymphocyte count and estimated creatinine clearance in the study group and a combined model for fluconazole that accounted for both these covariates was the most appropriate. The effect of a decline in renal function (as measure by predicted creatinine clearance) on fluconazole clearance is not surprising as this drug is eliminated mainly by renal clearance [5, 6, 19]. NONMEM provided an intercept of the relationship between fluconazole clearance and estimated creatinine clearance (mean $\pm$ s.e. mean; 0.20 ± 0.12) that is in good agreement with the non-renal clearance found in traditional pharmacokinetic studies [22].

The reason for the relationship between CD4+T-lymphocyte count and fluconazole clearance is not clear. One hypothesis is that HIV infection may affect the processes involved in renal drug handling. Fluconazole is a weak base (pKa 2) that is only weakly bound in plasma suggesting that potential disease-related changes in urine pH and protein binding are unlikely to account for the observed influence of disease severity. HIV infection can affect the physiology of many organ systems, including the kidney and the liver, the major organs of drug elimination [23, 25]. The disposition of xenobiotics can thus be altered in people with HIV infection relative to healthy volunteers. It has been reported that clearance of clindamycin is lowered in people with HIV infection [26], bioavailability of zidovudine [27] and folic acid [28] is significantly reduced in people with more severe immunosuppression due to HIV infection and the activity of the drug metabolizing enzymes is altered in this group [29]. Fluconazole undergoes net reabsorption in the kidney, perhaps HIV infection alters a secretion process or the nature of the proximal tubule, thereby influencing reabsorption of this drug in the kidney. An alternative hypothesis is that standard nomograms used for the prediction of creatinine clearance [11], based on age, weight and serum creatinine concentration, which have been devised in otherwise healthy populations are not applicable to patients with severe HIV infection. Perhaps then the correlation between CD4+T-lymphocyte count and fluconazole clearance is an artefact of an undetected decline in the renal drug elimination process with HIV disease progression.

In conclusion, this population analysis has found that clearance of fluconazole in HIV infected people is influenced by the severity of disease (as indicated by CD4+T-lymphocyte count) and renal function (indicated by predicted creatinine clearance). People with advanced HIV infection and compromised renal function would appear to need lower doses of fluconazole, than otherwise healthy people, to achieve the same concentration required for antifungal efficacy, without the undue risk of toxicity. However, the emergence of resistance to fluconazole must also be considered when contemplating dosage adjustments.

Sarah Moore and Jonathan Benjamin are thanked for their expert technical assistance in sample collection and analysis. The authors thank Dr Leon Aarons for helpful comments. Support in part by a research grant from Pfizer (Australia) Pty Ltd is acknowledged. Dr McLachlan is a Commonwealth AIDS Research Grant Postdoctoral Fellow and Dr Tett was supported by a National Health and Medical Research Council (Australia) R D Wright Award.

References

1 Sugar AM. Treatment of fungal infections in patients infected with the human immunodeficiency virus. *Pharmacother* 1990; **10**: 154s–158s.

2 Nelson MR Fisher M, Cartledge J, Rogers T, Gazzard BG. The role of azoles in the treatment and prophylaxis of cryptococcal disease in HIV infection. *AIDS* 1994; **8**: 651–654.

3 Milatovic D, Voss A. Efficacy of fluconazole in the treatment of systemic fungal infections. *Eur J Clin Microbiol Infect Dis* 1992; **11**: 395–402.

4 Tett S, Moore S, Ray J. Pharmacokinetics and bioavailability of fluconazole in two groups of males with HIV infection compared with a group of males without HIV infection. *Antimicrob Agents Chemother* 1995; **39**: 1835–1841.

5 Brammer KW, Farrow PR, Faulkner JK. Pharmacokinetics and tissue penetration of fluconazole in humans. *Rev Infect Dis* 1990; **12** (Suppl): s318–s326.

6 Humphery MJ, Jevons S, Tarbit MH. Pharmacokinetic evaluation of UK-49,858, a metabolically stable triazole antifungal drug, in animals and man. *Antimicrob Agents Chemother* 1985; **28**: 648–653

7 DeMuria D, Forrest A, Rich J, Scavone JM, Cohen LG,

Kazanjian PH. Pharmacokinetics and bioavailability of fluconazole in patients with AIDS. *Antimicrob Agents Chemother* 1993; **37**: 2187–2192.
8 Yeates RA, Ruhnke M, Pfaff G, Hartmann A, Trautmann M, Sarnow E. The pharmacokinetics of fluconazole after a single intravenous dose in AIDS patients. *Br J Clin Pharmacol* 1994; **38**: 77–79.
9 Whiting B, Kelman AW, Grevel J. Population pharmacokinetics: Theory and clinical application. *Clin Pharmacokinet* 1985; **11**: 387–401.
10 Aarons L. Population pharmacokinetics: theory and practice. *Br J Clin Pharmacol* 1991; 32: 669–670.
11 Cockcroft DW, Gault MH. 1976. Prediction of creatinine clearance from serum creatinine. *Nephron* **16**: 31–41.
12 Debruyne D, Ryckelynck J-P, Bigot M-C, Moulin M. Determination of fluconazole in biological fluids by capillary column gas chromatography with a nitrogen detector. *J Pharm Sci* 1988; **77**: 534–535.
13 Gomeni R, Pineau G, Mentre F. Population kinetics and conditional assessment of the optimal dosage regimen using the P-PHARM software package. *Anticancer Res* 1994; **14**: 2321–2326.
14 Beal SL, Sheiner LB. *NONMEM users guide*. San Francisco, University of California.
15 Tett SE, Cutler DJ. Apparent dose-dependence of chloroquine pharmacokinetics due to limited assay sensitivity and short sampling times. *Eur J Clin Pharmacol* 1987; **31**: 729–731.
16 Chin T, Fong IW, Vandenbroucke A. Pharmacokinetics of fluconazole in serum and cerebrospinal fluid in a patient with AIDS and cryptococcal meningitis. *Pharmacother* 1990; **10**: 305–307.
17 Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993; **10**: 1093–1095.
18 Fischman AJ, Alpert NM, Livni E, *et al*. Pharmacokinetics of 18F-labelled fluconazole in healthy human subjects by positron emission tomography. *Antimicrob Agents Chemother* **37**: 1270–1277, 1993.
19 Debruyne D, Ryckelynck J-P. Clinical pharmacokinetics of fluconazole. *Clin Pharmacokinet* 1993; **24**: 10–27.
20 Kaniwa N, Aoyagi N, Ogata H, Ishi M. Application of the NONMEM method to evaluation of the bioavailability of drug products. *J Pharm Sci* 1990; **79**: 1116–1120.
21 Unadkat JD, Agosti JM. Problems in pharmacokinetic investigations in patients with HIV infection. *Clin Pharmacokinet* 1990; **19**: 172–176.
22 Toon S, Ross CE, Gokal R, Rowland M. An assessment of the effects of impaired renal function and haemodialysis on the pharmacokinetics of fluconazole. *Br J Clin Pharmacol* 1990; **29**: 221–226.
23 Forsmark CE. AIDS and the gastrointestinal tract. *Postgrad Med* 1993; **93**: 143–148.
24 Bourne DWA. *Mathematical Modeling of Pharmacokinetic Data*, Pennsylvania: Technomic Publishing, 1995: 123–126.
25 Bourgoignie JJ. Renal complications of human immunodeficiency virus type 1. *Kidney Int* 1990; **37**: 1571–1584.
26 Gatti G, Flaherty J, Bubp J, White J, Borin M, Gambertoglio J. Comparative study of bioavailabilities and pharmacokinetics of clindamycin in healthy volunteers and patients with AIDS. *Antimicrob Agents Chemother* 1993; **37**: 1137–1143.
27 Macnab KA, Gill MJ, Sutherland LR, De Boer Visser N, Church D. Erratic zidovudine bioavailability in HIV seropositive patients. *J Antimicrob Chemother* 1993; **31**: 421–428.
28 Revell P, O'Doherty MJ, Tang A, Savidge GF. Folic acid absorption in patients infected with human immunodeficiency virus. *J Int Med* 1991; **230**: 227–231.
29 Lee BL, Wong D, Benowitz NL, Sullam PM. Altered patterns of drug metabolism in patients with acquired immunodeficiency syndrome. *Clin Pharmacol Ther* 1993; **53**: 529–535.

(*Received 23 June 1995, accepted 9 November 1995*)