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ORIGINAL PAPER

Pharmacokinetics-pharmacodynamics of sertraline as an antifungal in HIV-infected Ugandans with cryptococcal meningitis

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Abstract

The ASTRO-CM dose-finding pilot study investigated the role of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis in HIV-infected Ugandan patients. The present study is a post hoc pharmacokinetic-pharmacodynamic analysis of the ASTRO-CM pilot study to provide insight into sertraline exposure–response–outcome relationships. We performed a population pharmacokinetic analysis using sertraline plasma concentration data and correlated various predicted PK-PD indices with the percentage change in $\log_{10}$ CFU/mL from baseline. Sertraline clearance was 1.95-fold higher in patients receiving antiretroviral (ART), resulting in 49% lower drug exposure. To quantify the clinical benefit of sertraline, we estimated rates of fungal clearance from cerebrospinal fluid (CSF) of ASTRO-CM patients using Poisson model and compared the clearance rates to a historical control study (COAT) in which patients received standard *Cryptococcus* therapy of amphotericin B (0.7–1.0 mg/kg per day) and fluconazole (800 mg/day) without sertraline. Adjunctive sertraline significantly increased CSF fungal clearance rate compared to COAT trial and sertraline effect was dose-independent with no covariate found to affect fungal clearance including ART. Study findings suggest sertraline response could be mediated by different mechanisms than directly inhibiting the initiation of protein translation as previously suggested; this is supported by the prediction of unbound sertraline concentrations is unlikely to reach MIC concentrations in the brain. Study findings also recommend against the use of higher doses of sertraline, especially those greater than the maximum FDA-approved daily dose (200 mg/day), since they unlikely provide any additional benefits and come with greater costs and risk of adverse events.

Keywords Sertraline · Pharmacokinetics · Pharmacodynamics · Fungal clearance · Survival · HIV-associated cryptococcal meningitis

Introduction

Cryptococcal meningitis (CM) is an opportunistic fungal infection of the central nervous system caused by the pathogenic encapsulated yeast *Cryptococcus neoformans*. *Cryptococcus* is one of the most common acquired immunodeficiency syndrome (AIDS)-defining opportunistic infections accounting for 15% of AIDS-related deaths worldwide [1]. The mortality rate in low and middle income countries is up to 70% [1, 2]. Cerebral spinal fluid (CSF) sterilization by 2 weeks is achieved only in 50–70% of patients receiving standard amphotericin B deoxycholate therapy [3–6]. Current drug therapies are expensive, toxic and not readily accessible, especially in resource-poor

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settings. This highlights the critical unmet need for new safe, effective, affordable and readily accessible antifungal therapies for cryptococcosis.

Sertraline, a selective serotonin reuptake inhibitor commonly prescribed for the treatment of depression and other mental disorders, was shown to have an in vitro and in vivo fungicidal activity against *C. neoformans* [7–9]. The sertraline effect was dose-dependent and believed to be mediated by the inhibition of protein synthesis of the fungus [7, 9, 10]. Other proposed sertraline mechanisms of antifungal activity include: non-specific lipophilicity-dependent cytotoxicity; membrane phospholipids disruption of acidic intracellular organelles; and elevation of plasma serotonin (5-HT) that is biological active against *Candida* and *Aspergillus* species [11–14]. The effect of sertraline in combination with fluconazole, a major component of standard induction, consolidation, and maintenance therapy for CM, was also synergistic against *Cryptococcus* [15–17].

Previous findings support the potential use of sertraline in treating HIV-related CM given its favorable physiochemical, pharmaceutical, and therapeutic properties. Sertraline has high lipophilic characteristics that enable sertraline to cross the blood brain barrier easily and concentrate in the brain at concentrations 10–57 times higher than in plasma [18]. Sertraline can also help with depression, a common co-morbidity in HIV-infected patients [19, 20]. More importantly, sertraline has a good safety profile: sertraline overdose are relatively safe and easily managed; sertraline drug–drug interactions are minimal providing an important advantage in this therapeutically complex setting; sertraline is available as a less-costly generic formulation; sertraline is orally bioavailable; and sertraline has long half-life that allows once-daily dosing [21].

Based on the above compelling evidence and therapeutic properties of sertraline, our group hypothesized that the addition of sertraline to amphotericin B and fluconazole would result in faster rates of fungal clearance from CSF and better survival. To test this hypothesis, we conducted and authored the first-in human dose-escalating clinical trial to investigate the safety and efficacy of adjunctive sertraline for the treatment of HIV-associated CM (ASTRO-CM, NCT01802385) [3]. The present study is a post hoc analysis of the ASTRO-CM pilot study using a pharmacokinetic-pharmacodynamic (PK-PD) modeling approach to provide insight into sertraline exposure–response–outcome relationships. An older study, COAT (Cryptococcal Optimal ART Timing, NCT01075152), was used as a control comparison to quantify sertraline added effect on CSF fungal clearance rate [4].

Methods

Patients cohort & sampling collection

This study included a subset of HIV-infected Ugandans with CM from the ASTRO-CM pilot study, who had sertraline plasma concentrations measured [3]. Venous blood samples were collected on day 1, 3, 7, 10 and 14 mostly within 8 h after dose administration and analyzed using a high-performance liquid chromatography (HPLC) method. Full details of study design and methods are outlined in our previously published manuscript [3].

Pharmacokinetic analysis

All sertraline plasma concentrations were fitted simultaneously using nonlinear mixed-effect regression to one- and two-compartment PK models with linear and nonlinear clearance. Between subject variability (BSV) was described by an exponential model and residual unexplained variability (RUV) was evaluated by proportional and combined error model. The effects of age, sex, weight, concomitant antiretroviral therapy (ART), parent-to-metabolite ratio (sertraline-to-desmethylsertraline ratio, SDR), serum creatinine (SCr) and liver function enzymes (AST and ALT) were visually screened against Empirical Bayes Estimates (EBEs) of the base model. Potential covariates were then tested as linear and power models for continuous covariates and as a fractional change for categorical ones. The statistical significance of covariates inclusion was tested using the likelihood ratio test (χ^2, $\alpha = 0.05$, df = 1) which corresponds to at least a 3.84-point drop in objective function value (OFV). The criteria to select the PK model were OFV, plausibility of parameter estimates, diagnostic plots and the prospective applicability of the model. The performance of the selected model was assessed by prediction-corrected visual predictive check (pcVPC) [22] and parameter precision was evaluated by the sampling importance resampling (SIR) method [23].

There were missing data in weight, SDR and time of dose administration and blood draws. A single imputation was performed to replace missing weights with the median value of known weights based on sex (54 kg for male and 46 kg for female). Within a patient, SDR, SCr, AST, and ALT were imputed with the last observation carried forward then the last observation carried backward. Missing dosing times were guided by the frequency of usual dosing times of patients. Missing blood draw times were imputed by visit times when available or by the most frequent blood draw time for other patients on the same day. When there was no information to make reasonable imputation, records were excluded. Furthermore, the impact of the imputation

on PK parameter estimates was assessed by sensitivity analysis to ensure the acceptability of the imputation.

Predictive PK-PD index exploration

This was a visual exploratory correlation between various predicted PK-PD indices with the percent change in $\log_{10}$ CFU/mL from baseline and was limited to patients who had fungal count quantifications in CSF and sertraline plasma concentration measurements. Quantitative CSF cultures were obtained by therapeutic lumber punctures using manometers at diagnosis, and on day 3, 7, 10, 14 and as needed for signs of elevated intracranial pressure. A previously described method was used for determining quantitative CSF cultures measured as CFU/mL [24]. The EBEs from the selected PK model were used to simulate the sertraline brain concentration–time profiles using literature brain-to-plasma concentration ratios and to calculate standardized PK-PD indices (C_{max}/*MIC, AUC*$_{24}$/*MIC and* $\%T_{>MIC}$) [18].

C_{max} is the highest (peak) concentration reached, MIC is the minimum inhibitory concentration of sertraline against *Cryptococcus*, AUC_{24} is the area under concentration–time profile over 24 h, and $\%T_{>MIC}$ is the cumulative percentage of 24-h period that the concentration is above MIC. *cAUC/MIC*, the ratio of cumulative area under concentration–time profile to MIC, is not a commonly used PK-PD index but it was calculated to evaluate whether the drug effect was better associated with cumulative drug exposure. The sertraline MIC was measured by broth microdilution in RPMI1640 media per protocol [25]. When MICs of clinical isolates were undetermined, susceptibility was imputed with the median MIC value (4 μg/mL).

PK-PD indices are generally based on the unbound (free) plasma or tissue concentrations, and indicated by the prefix f. However, because unbound plasma concentrations were not measured in our patients, the prefix f was not used and total, rather than unbound, brain sertraline concentrations were predicted using the literature median value (16.5-fold) of total brain-to-plasma concentration ratios [18]. Assuming measured fungal CFU/mL was unlikely affected by the most recent dose, PK-PD indices were calculated after the dose before the most recent dose for C_{max}/*MIC,* AUC_{24}/*MIC,* $\%T_{>MIC}$ and up to the dose before the most recent dose for *cAUC/MIC*. Drug effect was calculated as the percent change in $\log_{10}$ CFU/mL from the baseline culture and plotted against the predicted PK-PD indices to identify any general trend.

Fungal count analysis

Fungal CSF counts from the ASTRO-CM pilot study and COAT trial were modeled together to determine the added benefit of sertraline on brain CSF fungal clearance rate when given with standard induction therapy for 2 weeks [3]. COAT is an earlier study that included similar patients from the same hospital, who were treated with the same induction regimen of amphotericin and fluconazole without sertraline [4]. All CSF fungal counts were $\log_{10}$-transformed, rounded to nearest integer and fitted to a Poisson model (Eq. 1) [26].

$$P(Y_{ij} = n) = \frac{\lambda_{ij}^{n}}{n!} e^{-\lambda_{ij}} \tag{1}$$

The probability of observing Y_{ij} equal to n = 0, 1, 2, … is determined by lambda (λ_{ij}), the mean fungal count for individual i occurring at fixed time-interval j, and the factorial function (!) of n. λ_{ij} was further influenced by the preceding counts ($f(\lambda_{i(j-1)})$) and time ($f(t_j)$) (Eq. 2).

$$\lambda_{ij} = \mathrm{BASE_i} \cdot \ f\left(\lambda_{i(j-1)}\right) \cdot f\left(t_j\right) \tag{2}$$

A three-state transition Markov model (MM) for an increase, decrease and no change in $\log_{10}$ CFU/mL from the previous count was used to account for the correlation of fungal counts within a patient. Time effect was best modeled by a mono-exponential decline function with a separate random effect for each study (Eq. 3).

$$\lambda_{ij} = \mathrm{BASE_i} \cdot \mathrm{MM} \cdot e^{-[\mathrm{TE} \times \mathrm{K_i} + (1-\mathrm{SER})\cdot\eta_1 + (\mathrm{SER}\cdot\eta_2)\cdot t_j]} \tag{3}$$

MM is a multiplier factor for each Markov model state. SER is an indicator variable; SER equals 0 for COAT trial and 1 for ASTRO-CM study. TE is an exponential constant for the daily decrease in fungal counts (day^{-1}) and K is a TE multiplier that was fixed to 1 for the COAT trial and estimated for each dose arm (100, 200, 300, 400 mg/day) of the ASTRO-CM study. This model parameterization was purposely chosen to allow for easy comparison among different sertraline arms and historical control study.

Given the in vitro and in vivo animal evidence of sertraline dose-dependent effect against *Cryptococccous*, an exploratory sigmoidal E_{max} model was also used to understand what the dose–response relationship would be and to compare the results to the Poisson model (Eq. 4) [7–9].

$$\lambda_{ij} = BASE_i \cdot MM \cdot e^{-[(\mathrm{TE}+\eta 1)\cdot t]} \cdot \left[1 - \frac{\left(\frac{DOSE}{MIC}\right)^{\gamma}}{D_{50}^{\gamma} + \left(\frac{DOSE}{MIC}\right)^{\gamma}}\right] \tag{4}$$

D_{50} is sertraline dose to MIC ratio when sertraline effect is half maximal and γ is a shape parameter for the dose–response curve. The final count model was assessed by VPC and parameters uncertainty was estimated by SIR.

Survival analysis

This analysis was limited to patients included in the fungal count analysis. Two-week survival rate of patients from both (COAT & ASTRO-CM) studies was modeled as time to death using a time-varying exponential hazard function (Eq. 5).

$$h(t) = \lambda_0 \cdot e^{\beta_o \times \ln(t)} \quad (5)$$

λ_o is the baseline hazard and β_o is the shape parameter of the hazard function. Based on whether covariates were time-invariant or time-varying, they were modeled, respectively, as follows [27]:

$$h(t) = \lambda_0 \cdot e^{(\beta_0 \times \ln(t) + \beta_{c1} \times covariate_1)} \quad (6)$$

$$h(t) = \lambda_0 \cdot e^{\beta_0 \times \ln(t)} \cdot [1 + \beta_{c1} \times covariate_1(t)] \quad (7)$$

The selected model was validated internally by VPC and distributions of parameter uncertainty were calculated by SIR.

Software

Nonlinear mixed-effect, count and survival modeling analyses were performed in NONMEM 7.3 (ICON Development Solutions, Ellicott City, MD) using various ADVANs and estimation methods. The PK analysis was performed with ADVAN2 TRANS2 and FOCEI method; count analysis was done using PREDPP with the FO Laplacian method; and survival rates were estimated using ADAVN13 with the FO likelihood method. Data manipulation, imputation and plotting were done in R (version 3.5.0). Perl-speaks-NONMEM (PsN) was utilized to perform VPC and SIR analyses [28]. The Pirana interface was used to maintain and compare NONMEM and PsN runs [29].

Results

Pharmacokinetic model

The final analysis included 335 sertraline plasma concentrations from 137 patients whose characteristics are summarized in Table 1. Weights of 45 unique patients (32 males and 13 females), 94 dosing times and 19 blood draw times were imputed as described above. Nine observations below the lower limit of quantification (1 ng/mL) were excluded. PK parameter estimates were similar before and after including imputed records in the analysis; therefore, imputation was considered acceptable and all imputed records were included which improved the precision of parameter estimates.

A 1-compartment PK model with first-order absorption and elimination adequately described the sertraline data. A combined proportional and additive error model better fit the RUV. Oral clearance (*CL/F*) and volume of distribution (*V/F*) were allometrically scaled to a standard 70-kg person. The selected PK model included ART as a covariate on *CL/F*, reducing the OFV by 24 points (p value < 0.0001).

$$\frac{CL}{F} = \theta_1 \times \left(\frac{Weight}{70}\right)^{0.75} \times (1 + ART \times \theta_3) \times e^{\eta_1} \quad (8)$$

$$\frac{V}{F} = \theta_2 \times \left(\frac{Weight}{70}\right) \times e^{\eta_2} \quad (9)$$

Sertraline clearance was 1.95-fold higher in patients on ART and the effect was indifferent between patients receiving efavirenz- and nevirapine-based ART. The diagnostic and pcVPC plots showed the model reasonably captured the observed data (Figs. 1, 2). Table 2 summarizes the parameter estimates of the selected PK model and their SIR-based uncertainties.

Predictive PK-PD Index

In vitro susceptibilities were determined for 151 *Cryptococcus* clinical isolates (81 from ASTRO-CM and 70 from COAT) obtained from baseline CSF cultures from participants with a first episode of cryptococcosis. The MIC distributions were comparable for both studies. The median (range) of sertraline MIC were 4 (1–8) and 4 (1–12) µg/mL for ASTRO-CM and COAT *Cryptococcus* isolates, respectively. The undetermined MIC levels for the remaining 138 clinical isolates (34 from ASTRO-CM and 104 from COAT) were assumed 4 µg/mL. Figure 3 shows the correlation between the percent change in $\log_{10}$ CFU/mL from baseline and the predicted PK-PD indices of ASTRO-CM patients. None of the PK-PD indices appears to correlate well with the daily reduction in fungal count.

Rate of fungal clearance

A total of 947 fungal count observations (491 from ASTRO-CM and 456 from COAT) from 289 patients (115 from ASTRO-CM and 174 from COAT) were included in the final analysis. The distribution of fungal counts and individual mean–variance relationships were similar in the two studies, but ASTRO-CM had a slightly stronger correlation of counts than COAT (Fig. 4). ASTRO-CM treatment arms were comparable but fewer patients were initially assigned the 100 mg dose compared to the other arms since the 100 mg dose was less likely to produce therapeutic concentrations.

Table 1 Characteristics of patients included in PK analysis

	100 mg	200 mg	300 mg	400 mg	All arms
Subjects, n	14	49	36	38	137
Male, n (%)	8 (57)	33 (67)	20 (56)	26 (68)	87 (64)
Age, mean ± SD	38 ± 7	36 ± 8	38 ± 8	34 ± 8	36 ± 8
Weight, mean ± SD	53 ± 9	52 ± 11	51 ± 9	52 ± 10	52 ± 10
ART, n (%)	8 (57)	23 (47)	12 (33)	14 (37)	57 (42)

*Study arms were not statistically different from each other for characteristics

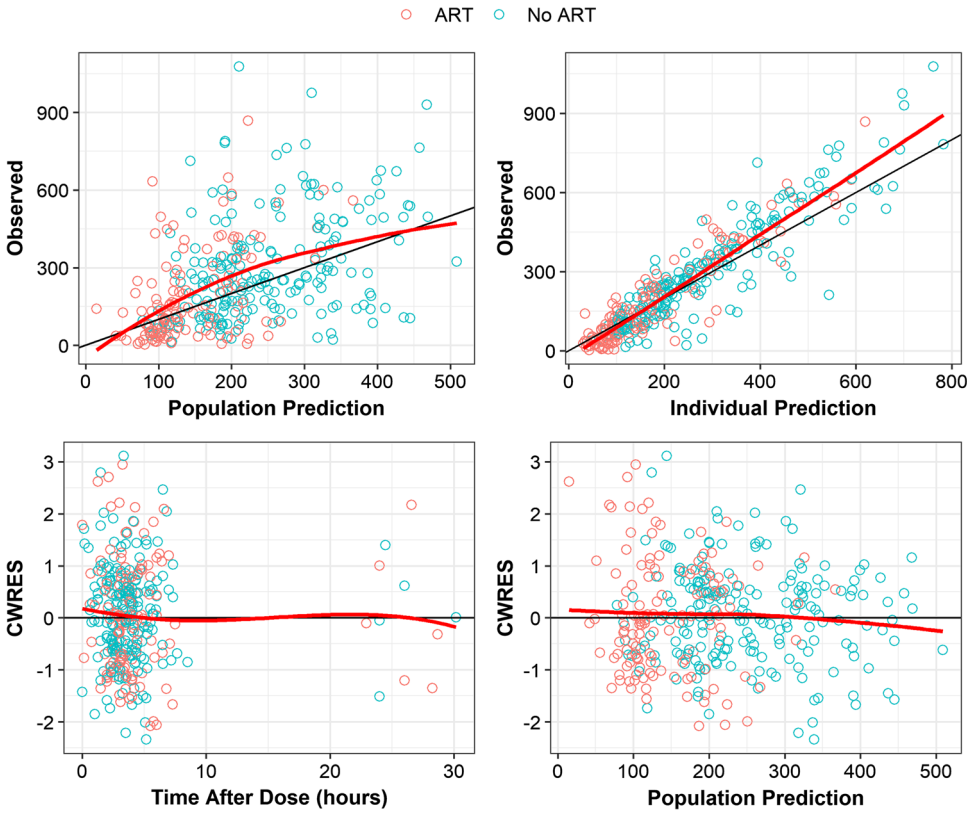


Fig. 1 Diagnostic plots for the selected PK model

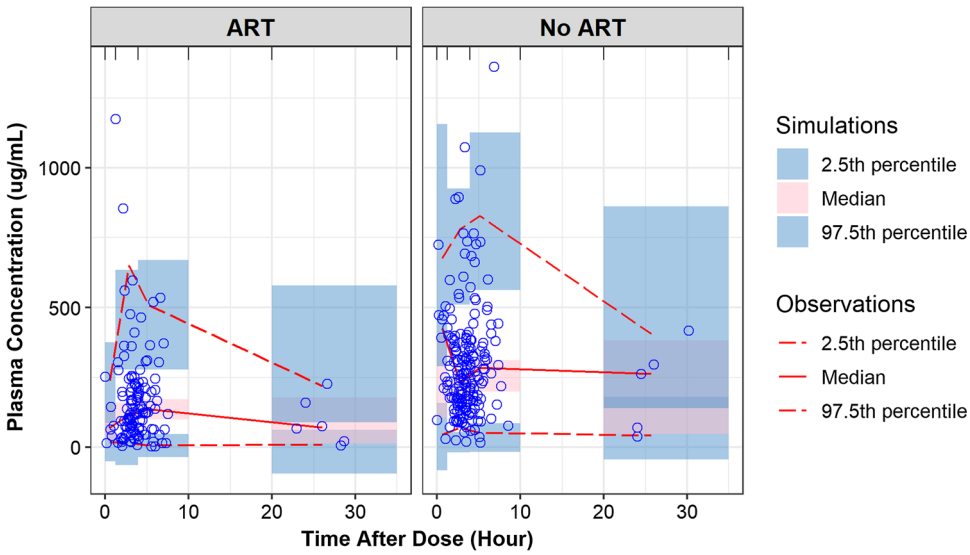


Fig. 2 Prediction-corrected and observed sertraline concentrations vs time after dose by ART status

Table 2 Parameter estimates for the selected PK model

Parameter	Definition	Estimate (RSE)	SIR median (95% CI)
*CL/F**	Oral clearance (L/h)	52.7 (8)	52.4 (42.7–62.2)
*V/F**	Volume of distribution (L)	1780 (18)	1883 (1261–2361)
ART_CL	Fraction change in CL for ART patients	0.948 (26)	1.02 (0.48–1.65)
K_a	Absorption rate constant (h^{-1})	0.101 (31)	0.110 (0.05–0.19)
BSV_CL	Between subjective variability in CL/F (%CV)	70.3 (10)	70.5 (58.9–91.4)
BSV_V	Between subjective variability in V/F (% CV)	60.4 (21)	63.2 (35.3–94.0)
RUV_1	Additive residual variability (SD)	44.3 (53)	44.6 (30.5–60.5)
RUV_2	Proportional residual variability (% CV)	10.2 (29)	10.3 (6.3–15.9)

*Parameters are allometrically scaled to a 70-kg person, RSE is relative standard of error, SIR is sampling importance resampling

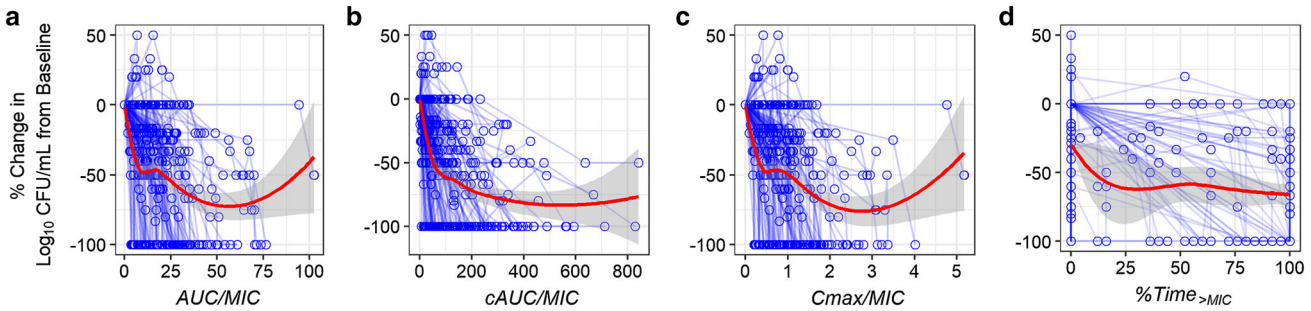


Fig. 3 Association between the percent change in $\log_{10}$ CFU/mL from baseline and PK-PD indices (**a**–**d**). Red line is lowess smoothing (Color figure online)

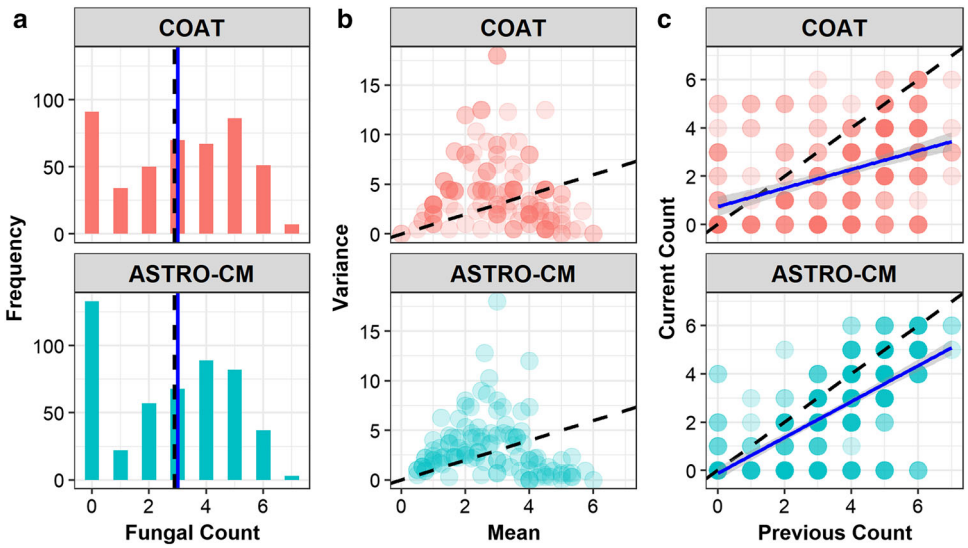


Fig. 4 Characteristics of log-transformed fungal counts by study. **a** Distribution of counts; **b** variance (of individual counts) versus mean (of individual counts); **c** correlation of counts. Dash black line represents the mean in **a** and identity line in **b**, **c**. Solid blue line is the median in **a** and is a linear regression line in **c** whose slope indicates the strength correlation of counts (Color figure online)

No covariate was found to affect fungal counts. The selected model (Eq. 3) found the addition of sertraline to standard induction antifungal therapy significantly increased CSF fungal clearance by 34 to 48%. The sertraline effect was similar irrespective of the daily dose patients received (Table 3). The adequacy of the selected model was supported by the VPC in which model predictions reasonably captured the observed data (Figs. 5, 6). The exploratory sigmoidal E_{max} model with fewer parameters (Eq. 4) produced similar results obtained by the Poisson model. Despite being inferior with unreliable estimate of D50, the sigmoidal E_{max} model had comparable VPC plots (results not shown). Modeling results were also similar when un-rounded $\log_{10}$-transformed were fitted to Poisson and sigmoidal E_{max} model, indicating minimum effect of rounding (results not shown).

Table 3 Parameter estimates for the selected count model

Parameter	Definition	Estimate (RSE)	SIR median (95% CI)
λ	Population mean fungal count (log10 CFU/mL)	4.43 (2)	4.42 (4.20–4.67)
MM 1	Fractional increase in mean fungal count from the previous one	1.55 (4)	1.55 (1.36–1.75)
MM -1	Fractional decrease in mean fungal count from the previous one	1.14 (4)	1.14 (1.02–1.27)
TE	Rate of daily decrease in fungal count (day^{-1})	0.156 (8)	0.154 (0.131–0.176)
K-100	Estimated multiplier for 100 mg arm of ASTRO-CM	1.41 (28)	1.42 (0.68–2.19)
K-200	Estimated multiplier for 200 mg arm of ASTRO-CM	1.42 (15)	1.45 (1.06–1.90)
K-300	Estimated multiplier for 300 mg arm of ASTRO-CM	1.34 (18)	1.35 (0.96–1.82)
K-400	Estimated multiplier for 400 mg arm of ASTRO-CM	1.48 (18)	1.51 (1.07–1.98)
BSV_1 in TE	Between subjective variability in TE for COAT (SD)	0.0744 (14)	0.0749 (0.0562–0.0953)
BSV_2 in TE	Between subjective variability in TE for ASTRO-CM (SD)	0.135 (13)	0.142 (0.111–0.175)

RSE is relative standard of error and SIR is sampling importance resampling

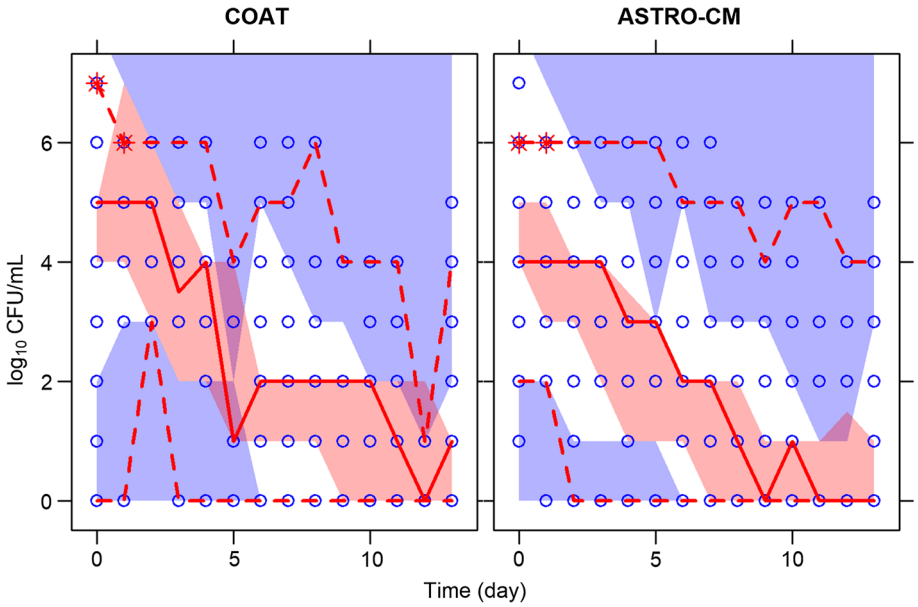


Fig. 5 Predicted and observed fungal counts over time by study. Blue open circles are the observed counts. Solid and dash red lines represent the median, 5th and 95th percentiles of the observed data. The shaded red and purple areas are simulated 95% CI for the median, 5th and 95th percentiles (Color figure online)

Survival rate

The selected survival model found hazard of death was significantly influenced by sex and sertraline daily dose as follows:

$$h(t) = \lambda_0 \cdot e^{\left(\beta_0 \times \ln(t) + \beta_{sex} \times SEX + \beta_{DOSE} \times DOSE_{100/400}\right)} \quad (10)$$

SEX equals 0 for female and 1 for male. $DOSE_{100/400}$ is 1 for those patients receiving 100 or 400 mg and 0 otherwise. Inclusion of sex reduced OFV by 13.9 points (p-value < 0.0005) and subsequent inclusion of $DOSE_{100/400}$ resulted in additional drop of 6.24 points in OFV (p-value < 0.02). The exponentiation of β's coefficients revealed that the hazard ratio of male to female is 0.25 and patients receiving sertraline 100 or 400 mg a day to those receiving no sertraline, 200 or 300 mg daily is 2.92 (Table 4). The internal validation by VPC confirmed the model adequate (Fig. 7).

Discussion

We found the ART co-administration increased sertraline oral clearance by 1.95-fold, resulting in 49% lower drug exposures. This has a clinical implication for sertraline use as an antidepressant in HIV-infected populations. Yet, ART did not affect fungal clearance from CSF or survival of patients. Of the PK-PD indices explored, none appeared to correlate well with the percent change in $\log_{10}$ CFU/mL from baseline. The addition of sertraline significantly increased the rate of CSF fungal clearance with a similar effect across dose arms. We also found that female patients

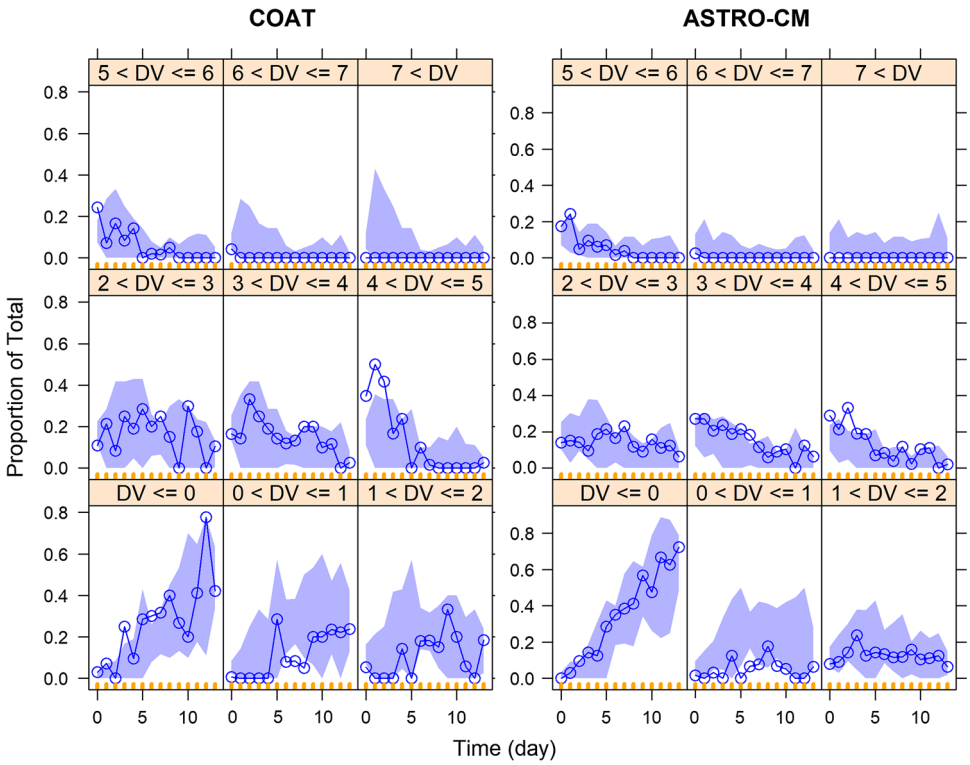


Fig. 6 Visual predictive checks of proportion of counts versus time by study. The blue line and purple shaded area are the proportion of observed and simulated 95% CI at each count level, respectively (Color figure online)

Table 4 Parameter estimates for the selected survival model

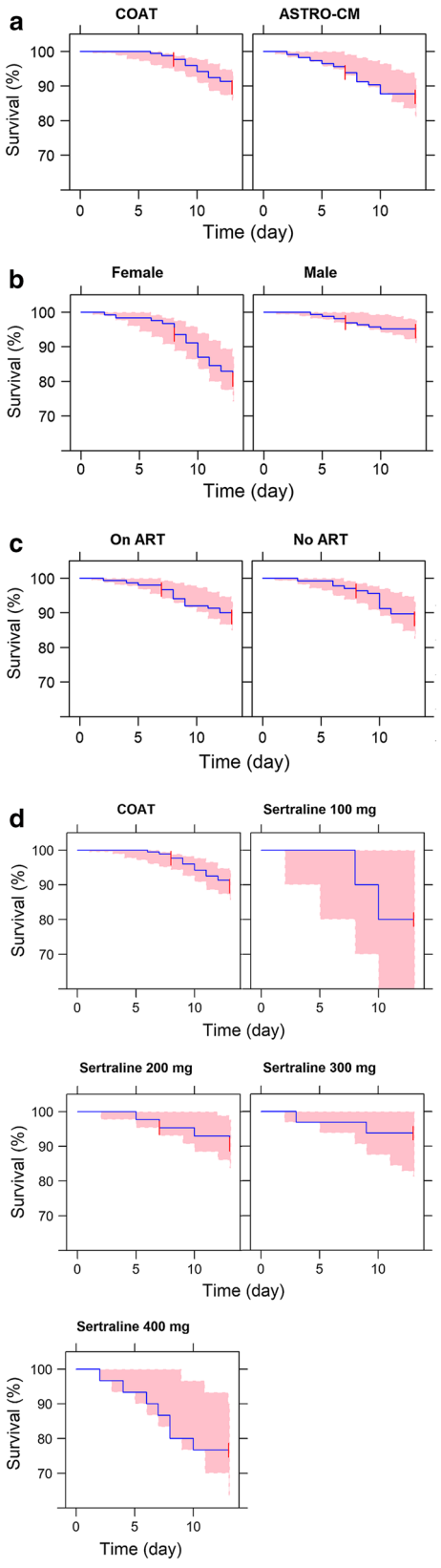
Parameter	Definition	Estimate (RSE)	SIR median (95% CI)
λ_o	Baseline hazard	0.0367 (17)	0.0365 (0.0241–0.0473)
β_o	Shape parameter of the hazard function	2.32 (16)	2.30 (1.61–3.25)
SEX_{male}	Effect of being male on the baseline hazard	− 1.38 (29)	− 1.38 (− 2.20 to − 0.695)
$DOSE_{100/400}$	Effect of receiving 100 or 400 mg of sertraline daily on the baseline hazard	1.07 (38)	1.03 (0.25–1.82)

RSE is relative standard of error and SIR is sampling importance resampling

and those receiving 100 or 400 mg of sertraline daily had lower 2-week survival rates.

Estimated sertraline clearance was similar to what was previously published, but the volume of distribution was 2–3-fold lower even after adjusting for body weight [30, 31]. The difference in volume of distribution could possibly be explained by the disease state and/or race given that our patients are HIV-infected sub-Saharan Africans. Metabolism is the main route of sertraline elimination and its clearance was expected to increase with ART co-administration because our patients received non-nucleotide reverse transcriptase inhibitors (NNRTI) (e.g. efavirenz, nevirapine) that are known inducers of cytochrome P450 metabolizing enzymes responsible for sertraline metabolism [32–34].

It is challenging to separate out a predictive PD index of sertraline effect in the current study due to variability and confounders. This may explain why we did not find a PD index that correlates well with the daily reduction in fungal count. Even if we did find an index, it would be unreliable PD metric of sertraline because our patients were on the CM standard therapy and the observed reduction in $\log_{10}$ CFU/mL cannot be solely attributed to sertraline. A reliable PD target of drug effect is usually determined from in vitro, ex vivo, or in vivo animal studies that can control for host and fungal factors. Findings from these studies can then be used to propose a rational dosage regimen of sertraline for confirmatory testing in humans. Our correlation analysis was exploratory and strictly visual that neither accounts for correlation of counts within a patient nor the effect of other covariates. Therefore, we did not expect to find a correlation, Rather, it was an exploratory exercised aimed to identify any general trend and more importantly



◀**Fig. 7** Kaplan–Meier plots for time to death by **a** study, **b** sex (female = 0 and male = 1), **c** ART (no ART = 0 and ART = 1) and **d** sertraline daily dose (DOSE = 0 for COAT). This is comparison of observed data (blue line) to the 95% prediction interval of the simulated data (pink area) with the time-to-event (TTE) model (Color figure online)

to select relevant sertraline exposure metric for formal testing in subsequent analyses.

Although ART decreased sertraline exposure, ART did not influence the rate of CSF fungal clearance. The sertraline effect was also similar across doses. Together, this suggests the observed clinical effect of sertraline is dose- and concentration-independent which contradicts previous evidence from in vitro and animal studies [7–9]. Possible explanations are unbalanced arms, large variability or a narrow range of tested sertraline doses. The range of studied sertraline doses were only 4-fold and seem to fall at the lower range of the sertraline dose–response curve (Fig. 8) assuming the sertraline dose–response curve is true. At least 10-fold range between the lowest and highest studied doses is recommended by the European Medicines Agency to sufficiently estimate dose–response relationships [35]. The dose to MIC ratio at which sertraline effect is half maximal (D_{50}) was estimated 500 mg. With MIC is 4 μg/mL, the sertraline daily dose required to achieve a 50% reduction in fungal counts would be 2000 mg. This is 10-fold greater than the maximum daily dose approved by the Food & Drug Administration (FDA) [36].

Sertraline effect in human could possibly be mediated by different mechanisms than directly inhibiting protein synthesis of fungi because predicted unbound brain sertraline concentration is unlikely to reach MIC

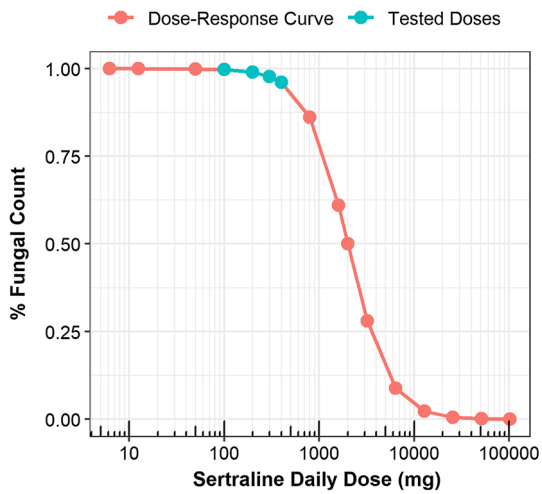


Fig. 8 Dose–response curve of sertraline. This is the exploratory sigmoidal E_{max} function describing the sertraline effect in Eq. 4 assuming sertraline has a dose-dependent effect with MIC of 4 μg/mL

concentrations [9, 11]. Sertraline is highly-protein bound in plasma and only unbound concentration is able to cross tissue membranes (e.g. blood–brain barrier and fungal cell wall), bind to therapeutic targets, and elicit pharmacological action [37]. The highest steady-state total sertraline plasma concentration observed in our patients was 1.078 μg/mL. With 98% protein binding and median of 16.5-fold higher concentration in brain tissue than in blood, the unbound brain sertraline concentration is predicted to be 0.356 μg/mL. This is lower than the lowest determined MIC level (1 μg/mL) for our clinical isolates. Despite consensus that unbound concentration is the driver of drug action in vivo, there is unfortunately still a persistent inclination to report total tissue concentrations, arguing total concentrations are better related to drug efficacy. In fact, total tissue concentrations are shown to be poor surrogates for drug efficacy because total concentrations are determined after tissue homogenization that are unlikely representative of concentrations at the site of action [38].

Enhancing fluconazole effect through beneficial pharmacokinetic interaction is another plausible mechanism for the observed clinical effect of sertraline. Fluconazole is a substrate for the efflux transport P-glycoprotein that is widely expressed in gut, blood–brain barrier, renal tubules and other human tissues [39–41]. Sertraline, on the other hand, is a substrate and inhibitor of P-glycoprotein [42, 43]. In theory, the inhibition of P-glycoprotein (P-gp) by sertraline would increase gut absorption, central nervous system (CNS) penetration producing higher brain concentrations and decrease renal clearance of fluconazole. Ultimately, sertraline would increase fluconazole exposure particularly in the brain which could possibly explain our results as pointed out by Veringa and his colleagues [44]. This was confirmed in preclinical species and recently in humans despite the skepticism about the clinical relevance of P-gp-mediated drug–drug interaction at blood brain barrier [45, 46].

Increasing 5-HT in plasma could also be the driver of the sertraline observed effect. 5-HT has direct and indirect anti-microbial effects against invading pathogens [47, 48]. 5-HT possesses antimicrobial properties by itself and it is a master regulator of innate and adaptive immune response through its widely expressed receptors on immune cells and via receptor independent signaling so-called serotonylation [49]. Depletion of 5-HT in blood and platelets is associated with impaired immune responses as in HIV-infected individuals [50]. On the other hand, elevated circulating level of 5-HT is documented in multiple autoimmune inflammatory diseases, such as rheumatoid arthritis, asthma, Crohn's disease and ulcerative colitis due to either over-production of 5-HT or/and reduced function of 5-HT transporter (SERT) [47]. Just like in the brain, SERT in the periphery functions to tightly regulate 5-HT signaling via the reuptake 5-HT back into the cell, terminate and prevent off-target 5-HT effect, protect 5-HT from degradation by monoaminoxidases and store 5-HT for later release upon subsequent stimulation [51, 52]. With that, it is plausible that SERT inhibition by sertraline could be the underlying mechanism for the improved fungal clearance in the ASTRO-CM patients.

Survival was not a primary outcome and the study was not powered to find survival difference between sertraline dose arms. However, there was statistical evidence that female patients (p-value < 0.0005) and those receiving sertraline 100 or 400 mg daily (p-value < 0.02) had lower survival rates. Either this finding represents an immunomodulatory effect of sertraline, which we are currently investigating, or this is biased findings. Analyzed patients represented a nonrandom sample since the analysis only included patients who have plasma measurements and fungal counts (115 out of 137). Results could also be biased by differential co-morbidities or unadjusted confounders. The first 60 patients were assigned non-randomly and it could possibly be that patients receiving sertraline 100 or 400 mg daily were prognostically different than the rest. Though, results should be taken into considerations for further studies to balance patients by sex and test at least two different sertraline doses (low and high) to further investigate these findings.

The current study has several limitations. The ASTRO-CM study was not designed for the pharmacometric analyses presented here. Considerable data were missing and needed to be imputed to complete the analyses. Pharmacokinetic data were also sparse and not optimally sampled. Sertraline concentrations were measured mostly in the absorption phase on different days. Moreover, our patients were diagnosed with multiple medical conditions and on poly-drug therapy. This created a challenging situation to account for all probable drug–drug interactions but did reflect reality. Lastly, sertraline arms were not balanced with fewer patients in the 100 mg arm. With these limitations, our findings should be interpreted cautiously. Nonetheless, these post hoc pharmacometric analyses illustrate the utility of leveraging data from previous studies to gain insight into exposure–response relationships.

Conclusions

Sertraline appeared to increase the fungal clearance rate from CSF when added to the standard combination therapy of amphotericin B and fluconazole compared to a prior study, but with no survival benefit. The sertraline effect is dose-independent, indicating higher doses, especially those greater than the maximum FDA-approved daily dose

(200 mg/day), may not provide any additional benefits and come with greater costs and risk of adverse events. Observed clinical effect of sertraline unlikely to be mediated by the inhibition of fungal protein synthesis since unbound sertraline concentrations do not reach MIC concentrations. Further studies are in need to confirm or refine current findings.

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