Evaluation of Clinical Drug Interaction Potential of Clofazimine Using Static and Dynamic Modeling Approaches[§]

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ABSTRACT

The 2016 World Health Organization treatment recommendations for drug-resistant tuberculosis (DR-TB) positioned clofazimine as a core second-line drug. Being identified as a cytochrome P450 (P450) inhibitor *in vitro*, a P450-mediated drug interaction may be likely when clofazimine is coadministered with substrates of these enzymes. The P450-mediated drug interaction potential of clofazimine was evaluated using both static [estimation of the R_1 and area under the plasma concentration-time curve ratio (AUCR) values] and dynamic [physiologically based pharmacokinetics (PBPK)] modeling approaches. For static and dynamic predictions, midazolam, repaglinide, and desipramine were used as probe substrates for CYP3A4/5, CYP2C8, and CYP2D6, respectively. The AUCR static model estimations for clofazimine with the substrates midazolam,

repaglinide, and desipramine were 5.59, 1.34, and 1.69, respectively. The fold increases in the area under the curve (AUC) predicted for midazolam, repaglinide, and desipramine with clofazimine (based on PBPK modeling) were 2.69, 1.60, and 1.47, respectively. Clofazimine was predicted to be a moderate-to-strong CYP3A4/5 inhibitor and weak CYP2C8 and CYP2D6 inhibitor based on the calculated AUCR by static and PBPK modeling. Additionally, for selected antiretroviral, antitubercular, antihypertensive, antidiabetic, antileprotics, and antihyperlipidemic CYP3A4/5 substrate drugs, approximately 2- to 6-fold increases in the AUC were predicted with static modeling when coadministered with 100 mg of clofazimine. Therefore, the possibility of an increase in the AUC of CYP3A4/5 substrates when coadministered with clofazimine cannot be ignored.

Introduction

Tuberculosis (TB) is an airborne infectious disease caused by organisms of the *Mycobacterium tuberculosis* complex. Over the past two decades, the incidence of TB has declined in most regions of the world; however, the emergence of resistance to anti-TB drugs is a threat to the gains in TB control. Drug-resistant TB (DR-TB) cases are generally of three types: 1) rifampicin-resistant TB, caused by bacteria that do not respond to rifampicin; 2) multidrug-resistant TB (MDR-TB), caused by bacteria that do not respond to, at least, isoniazid and rifampicin; and 3) extensively drug-resistant TB, a form of MDR-TB that is also resistant to fluoroquinolones and second-line injectable drugs [see the World Health Organization (WHO) MDR-TB factsheet; <http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/en/>].

Clofazimine is an antimycobacterial agent originally developed in the 1950s for TB and currently approved for the treatment of lepromatous leprosy and its complication, erythema nodosum leprosum (Fajardo et al., 1999; Hwang et al., 2014). Clofazimine has been used off-label as

a second-line TB drug in a multidrug regimen for DR-TB (see the companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis; <https://www.ncbi.nlm.nih.gov/books/NBK247431>). Publication of various drug regimens used by the Damien Foundation in Bangladesh (Van Deun et al., 2010), which included clofazimine as part of the treatment protocol, has drawn the attention of researchers, and authors have continued to study clofazimine as part of a multidrug regimen in the treatment of MDR-TB (Dooley et al., 2013). Among the five different regimens used in Bangladesh, the regimen containing clofazimine for MDR-TB had a low failure rate and a treatment default rate of 7.9% without any relapses up to 2 years in cured patients (Van Deun et al., 2010). In the follow-up study, 84.4% of patients had bacteriologically favorable treatment outcomes after 2 years (Aung et al., 2014). A similar outcome has been reported from countries in Africa (Piubello et al., 2014; Kuaban et al., 2015). The 2016 WHO DR-TB treatment guidelines positioned clofazimine as a core second-line drug (group C) (<http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/en/>).

In 2015, an estimated 10.4 million new (incident) TB cases were reported worldwide, and among these, 1.2 million (11%) cases had been living with human immunodeficiency virus (HIV). In addition to the 1.4 million TB deaths in 2015, 0.4 million deaths were reported among people living with HIV (see the WHO global tuberculosis report; www.who.int/tb/publications/global_report/en/). TB is one of the most

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ABBREVIATIONS: AUC, area under the curve; AUCR, area under the curve ratio; DDI, drug-drug interaction; DR-TB, drug-resistant tuberculosis; f_a , fraction absorbed; F_g , fraction of dose that escapes first-pass elimination in the intestine; f_m , fraction metabolized by an enzyme; f_{up} , fraction unbound in plasma; HIV, human immunodeficiency virus; $[I_{gut}]$, gut concentration; k_a , first-order absorption rate constant *in vivo*; $K_{i,u}$, unbound inhibition constant; MDR-TB, multidrug-resistant tuberculosis; P450, cytochrome P450; PBPK, physiologically based pharmacokinetics; PK, pharmacokinetics; PopPK, population pharmacokinetics, TB, tuberculosis; WHO, World Health Organization.

common opportunistic infections and a leading cause of death in HIV patients (www.who.int/tb/publications/global_report/en/). The augmented reports of MDR-TB and synergistic interactions with the HIV epidemic are posing difficult challenges for effective management and control of TB (Zumla et al., 2013).

Clofazimine is always prescribed as part of a multidrug regimen for the treatment of DR-TB. Given that TB contributes to comorbidity in patients with HIV, concomitant administration of anti-HIV drugs with clofazimine is most likely. In vitro cytochrome P450 (P450) inhibition results (Supplemental Table 1) have suggested that clofazimine has reversible inhibitory effects on CYP3A4/5, CYP2C8, and CYP2D6 at concentrations of up to 10 μ M. Therefore, it becomes imperative to evaluate the drug interaction potential of clofazimine with drugs that are substrates for CYP3A4/5, CYP2C9, and CYP2D6. This study evaluated the CYP3A4/5, CYP2C8, and CYP2D6 inhibition-mediated drug interaction potential of clofazimine (as a perpetrator) using static and dynamic [i.e., physiologically based pharmacokinetics (PBPK)] models.

Materials and Methods

The drug interaction potential of clofazimine (P450 mediated) was evaluated using static and mechanistic dynamic model predictions according to the Food and Drug Administration Guidance for Industry for drug interaction studies (<https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf>).

Static Model Predictions

P450 inhibition parameters were collected from data obtained using pooled human liver microsomes, which indicated that clofazimine reversibly inhibits CYP3A4/5, CYP2C8, and CYP2D6 with unbound dissociation constant values of 0.000786, 0.00372, and 0.00246 μ M, respectively (see Supplemental Table 1). The human plasma protein binding of clofazimine was reported as 99.9%, ranging from 99.9% to 99.96% (see the TB Alliance report on clofazimine in clinical trials for tuberculosis; <http://www.resisttb.org/wp-content/uploads/2013/08/Global-Alliance-Clinical-Work-with-Clofazimine.pdf>). Therefore, a correction for plasma protein binding of 99.9% [fraction unbound in plasma (f_u) of 0.001] was used for all calculations in this analysis. Although there is one report of potential weak (≤ 2 -fold) CYP3A4 activity induction in vitro (Horita and Doi, 2014), there are no published reports that have implicated clofazimine as a clinical inducer of CYP3A4 (see the University of Washington Drug Interaction Database; <https://didb.druginteractioninfo.org>). Therefore, it was assumed in the models that any potential CYP3A4 induction would be negligible, and for the worst-case scenario CYP3A4 inhibition would predominate. A population pharmacokinetics (PopPK) model was developed for clofazimine using plasma concentration-time data obtained from healthy volunteers and leprosy patients (internal data). Simulations were performed using the PopPK parameter estimates obtained from the final model. A steady-state plasma C_{max} value of 890 ng/ml (1.89 μ M) (after repeat dose administration of 100 mg once daily) was obtained from the model, which was used for the R_1 and area under the curve (AUC) plasma concentration-time ratio (AUCR) calculations.

Determination of the R_1 Value. The R_1 value was calculated using the following formula:

$$R_1 = 1 + [I]/K_{i,u} \quad (1)$$

where [I] is the maximum anticipated C_{max} of clofazimine in patients [1.89 μ M (total C_{max}) or 0.00189 μ M (unbound C_{max})] and $K_{i,u}$ is the unbound inhibition constant determined in vitro. The $K_{i,u}$ values used in the models for P450 inhibition were 0.00372 μ M (CYP2C8), 0.00246 μ M (CYP2D6), and 0.000786 μ M (CYP3A4/5), vide supra.

Since clofazimine is a CYP3A4/5 inhibitor that will be administered orally, the R_1 value was also determined using the gut concentration [I_{gut}], to take into account intestinal CYP3A inhibition

$$R_1 = 1 + [I_{gut}]/K_{i,u} \quad (2)$$

where [I_{gut}] = dose/250 ml (or 845 μ M for 100 mg dose). Initially, the R_1 value for P450 (CYP3A4/5, CYP2C8, and CYP2D6) inhibition by clofazimine was

calculated; if the R_1 values were >1.1 (systemic) or >11 (gut; only for CYP3A4/5), then the AUCR and $AUC_{inhibitor}/AUC_{control}$ were estimated using the mechanistic static (net effect) model.

Determination of the AUCR Value by Using the Mechanistic Static (Net Effect) Model. The net effect model was used to calculate the AUCR (Fahmi et al., 2008, 2009; Einolf et al., 2014). The AUCR value was calculated using the following formula:

$$AUCR = \left[\frac{1}{(A_g \times B_g \times C_g) \times (1 - F_g) + F_g} \right] \times \left[\frac{1}{(A_h \times B_h \times C_h) \times f_m + (1 - f_{mP450})} \right] \quad (3)$$

where F_g is the fraction of dose that escapes first-pass metabolism in the intestine; f_{mP450} is the fraction of total systemic clearance of the substrate that is metabolized by an individual P450 enzyme; and subscripts “h” and “g” denote liver and gut, respectively.

Since clofazimine exhibits reversible inhibition but not time-dependent inactivation or clinically relevant induction

$$A_g = 1/(1 + [I_{ent}]/K_i) \quad (4)$$

$$A_h = 1/(1 + [I_h]/K_i) \quad (5)$$

and B_g , C_g , B_h , and C_h were set as 1.

In eq. 4, [I_{ent}] is the enterocyte concentration of clofazimine defined as follows:

$$[I_{ent}] = f_a \times k_a \times \text{dose}/Q_{ent} \quad (6)$$

where f_a is the fraction of clofazimine absorbed after oral administration (0.7) (Vischer, 1969); k_a is the first-order absorption rate constant in vivo (0.088 hour^{-1} , internal data); and Q_{ent} is the enterocytic blood flow (248 ml/min) (Obach et al., 2006).

In eq. 5, [I_h] is the hepatic concentration of clofazimine defined as follows:

$$[I_h] = f_u \times [C_{max, total} + (k_a \times f_a \times \text{dose}/Q_h)] \quad (7)$$

where Q_h is the hepatic blood flow [1470 ml/min for a 70-kg individual (21 ml/min per kilogram)] and f_u is the fraction unbound in plasma (0.001) (<http://www.resisttb.org/wp-content/uploads/2013/08/Global-Alliance-Clinical-Work-with-Clofazimine.pdf>). The dose was defined as 100 mg and the C_{max} total value (at steady state) was 1.89 μ M. The input data [fraction metabolized by an enzyme (f_m) values] for midazolam, repaglinide, and desipramine were 0.9 (Einolf et al., 2014), 0.71 (Kajosaari et al., 2005; Varma et al., 2013), and 0.9 (Obach et al., 2006), respectively. The input data (f_m values) for other drugs were calculated from the published literature (Supplemental Table 2).

The f_{mP450} value was calculated as follows:

$$f_m = [1 - (1/AUC_i)] \quad (8)$$

where, AUC_i represents the fold increase in the exposure of a substrate after coadministration with a strong inhibitor (Rowland and Matin, 1973). This equation assumes complete inhibition of the enzyme. The F_g values were not available for a majority of these substrates; a value of 0.51 (F_g value of midazolam, which undergoes predominant gut metabolism) was used (Gertz et al., 2010).

Selection of Drugs for AUCR Determination

To predict the magnitude of CYP3A4/5-, CYP2C8-, and CYP2D6-mediated inhibition by clofazimine, the AUCR values were estimated using midazolam, repaglinide, and desipramine as probe substrates, respectively. Various classes of possible coadministered drugs such as antituberculars, antiretrovirals, antidiabetics, antihypertensives, antileptotics, and antihyperlipidemics (statins) were considered for further evaluation.

PBPK Model Predictions

Input Parameters. The platform used for the PBPK modeling was the Simcyp Simulator (version 15, release 1; Certara, Princeton, NJ). The healthy volunteer

TABLE 1
PBPK model (Simcyp Simulator) input parameters for clofazimine

Parameter	Value	Source
Physicochemical properties		
Molecular weight (g/mol)	473	
Log $P_{o:w}$	7.66	ChemIDplus database
pK_a	8.51	Quigley et al. (1990)
Compound type	Monoprotic base	
B/P	0.55	Internal data
f_{up}	0.001	TB Alliance ^a
Absorption		
Model	First-order absorption	
f_a	0.7 (CV 10%)	Vischer (1969)
k_a (h^{-1})	0.25 (CV 30%)	Optimized to predict PK
T_{lag} (h)	0.55 (CV 30%)	Optimized to predict PK
Q_{gut} (l/h)	12.7	Predicted in Simcyp
f_{ugut}	0.001	Assumption, same as f_{up}
$P_{eff,man}$ ($\times 10^{-4}$ cm/s)	4.38	Predicted in GastroPlus
Distribution		
Model	Minimal PBPK model	
Q (l/h)	42.6	Internal PopPK analysis
V_{sac} (l/kg)	32.7	Internal PopPK analysis
V_{ss} (l/kg)	47.5 (CV 20%)	Internal PopPK analysis
Elimination		
CL_{iv} (l/h)	4.18 (CV 30%)	Internal PopPK analysis
CL_R (l/h)	0	Levy (1974)
Interaction		
Reversible inhibition		
CYP3A4 $K_{i,u}$ (μM)	0.000786	Measured ^b
CYP2C8 $K_{i,u}$ (μM)	0.00372	Measured ^b
CYP2D6 $K_{i,u}$ (μM)	0.00246	Measured ^b

B/P, blood-to-plasma ratio; CL_{iv} , in vivo intravenous clearance; CL_R , renal clearance; f_{ugut} , fraction unbound in the enterocyte; log $P_{o:w}$, logarithmic partition coefficient octanol:water; $P_{eff,man}$, effective permeability in man; Q , inter-compartment clearance; T_{lag} , lag time; V_{sac} , single adjusted compartment volume; V_{ss} , volume of distribution at steady state.

^aGlobal Alliance for TB Drug Development (<http://www.resisttb.org/wp-content/uploads/2013/08/Global-Alliance-Clinical-Work-with-Clofazimine.pdf>).

^bSupplemental Table 1.

population library file provided by the software was used for all simulations. For simulations that included patients up to 70 years of age, the default maximum age for the healthy volunteer population file was increased from 65 to 70. The PBPK model input parameters are summarized in Table 1 and described in detail subsequently.

The molecular weight of clofazimine is 473 g/mol, and the log P octanol:water value used was 7.66 (ChemIDplus database; U.S. National Library of Medicine, Bethesda, MD, <https://chem.nlm.nih.gov/chemidplus/>). The compound type was entered as a monoprotic base with an acid dissociation constant pK_a value of 8.51 (Quigley et al., 1990). The blood-to-plasma ratio was entered as 0.5 (minimally distributed into blood; internal data), and the f_{up} was entered as 0.001 (<http://www.resisttb.org/wp-content/uploads/2013/08/Global-Alliance-Clinical-Work-with-Clofazimine.pdf>). A first-order absorption model was used and the fraction

of dose absorbed (f_a) was entered as 0.7 (Vischer, 1969). A parameter sensitivity analysis was performed on the impact of the clofazimine f_a value on the drug interaction with the sensitive CYP3A4 substrate, midazolam (Supplemental Fig. 1A). Varying the f_a value from 0.42 to 0.98 ($0.7\% \pm 40\%$ of the value used in the model) resulted in a predicted midazolam AUCR range of 2.2–3.7, which was within 38% of the predicted AUCR of 2.7 with a f_a value of 0.7. The results of this parameter sensitivity analysis suggested that the CYP3A drug-drug interaction (DDI) was not highly sensitive to the clofazimine f_a values evaluated and would remain categorized as a moderate CYP3A4 inhibitor. The absorption rate constant (k_a) used was 0.25 hour^{-1} and the lag time was entered as 0.55 hour. These values were optimized to predict the pharmacokinetics (PK) parameters (e.g., the C_{max} and time to reach maximum plasma concentration values). The effective permeability in humans was user defined as $4.38 \times 10^{-4} \text{ cm/s}$, predicted based

TABLE 2
Summary of clofazimine PK simulations

The elimination half-life of clofazimine is approximately 25 days, thus it is assumed that the exposure clofazimine reaches steady state in plasma by day 126 (approximately five half-lives).

Study (Model)	Clofazimine Dosing Regimen	Simulated Population	Observed Population	PK Measurement of Substrate
Model development				
Clofazimine PK (Internal data)	50 mg once daily for 43 days (fasting)	Age range: 35–64 years; proportion of females: 25% $n = 120$ (10 trials of 12 subjects)	Age range: 35–64 years; proportion of females: 25% $n = 12$	Day 1: T_{max} , C_{max} , and $AUC_{0-24 \text{ h}}$
Clofazimine PK (Internal data)	100 mg once daily for 43 days (fasting)	Age range: 45–70 years; proportion of females: 50% $n = 120$ (10 trials of 12 subjects)	Age range: 45–76 years; proportion of females: 50% $n = 12$	Day 1: T_{max} , C_{max} , and $AUC_{0-24 \text{ h}}$
Model application				
Clofazimine PK	100 mg once daily for 126 days (fasting)	Age range: 18–70 years; proportion of females: 50% $n = 100$ (10 trials of 10 subjects)	NA	Day 126: C_{max} and $AUC_{0-24 \text{ h}}$

$AUC_{0-24 \text{ h}}$, area under plasma-drug concentration curve between 0 and 24 hours; NA, not applicable; T_{max} , time to reach maximum concentration.

TABLE 3
DDI predictions of clofazimine with P450 substrates

Model Application (P450 Substrate)	Study	Dosing Regimen	PK Measurement of Substrate
CYP3A4/5	Midazolam + clofazimine	Midazolam 5 mg on day 126 + clofazimine 100 mg once daily on days 1–126	Day 126: AUC _{inf} , C _{max}
CYP3A4	Saquinavir + clofazimine	Saquinavir 1200 mg on day 126 + clofazimine 100 mg once daily on days 1–126	Day 126: AUC _{inf} , C _{max}
CYP3A4	Efavirenz + clofazimine	Efavirenz 600 mg on day 126 + clofazimine 100 mg once daily on days 1–126	Day 126: AUC _{inf} , C _{max}
CYP2C8	Repaglinide + clofazimine	Repaglinide 0.25 mg on day 126 + clofazimine 100 mg once daily on days 1–126	
CYP2D6	Desipramine + clofazimine	Desipramine 50 mg on day 126 + clofazimine 100 mg once daily on days 1–126	

AUC_{inf}, area under the plasma concentration-time curve from 0 to infinity.

on the clofazimine chemical structure by using the absorption, distribution, metabolism, excretion, and toxicity predictor in GastroPlus (Simulations Plus, Inc., Lancaster, CA). The Q_{gut} value was predicted as 12.7 l/h by the Simcyp Simulator. The fraction unbound in the enterocyte value was set at 0.001 (assumed to be the same as f_{up}). The coefficient of variation (30%) for the input parameters was the default value in the Simcyp Simulator. In addition, sensitivity analyses of the clofazimine fraction unbound in the enterocyte as well as the k_a value were performed to determine the impact of these values on the drug interaction with midazolam. The results of the sensitivity analysis can be found in Supplemental Fig. 1, B and C. Variation of the fraction unbound in the enterocyte (range of 0.001–1) resulted in minimal impact in the midazolam AUCR (range of 3.0–3.9) and categorization of the DDI. The predicted DDI of clofazimine with midazolam was not sensitive to k_a values ranging from 0.088 to 0.25 hour⁻¹ (i.e., values used in either the static or PBPK models). The minimal PBPK model in the Simcyp Simulator was used with a single adjusting compartment. The input parameters for distribution were optimized to best fit the PK data obtained from the clinical trial (PopPK analysis, internal data); the volume of distribution at steady state, intercompartment clearance (Q), and single adjusted compartment volume were estimated to be 47.5 l/kg, 42.6 l/h, and 32.7 l/kg, respectively. The in vivo intravenous clearance was user defined as 4.18 l/h, also optimized to best fit of the PK data from the clinical trial. Renal clearance of clofazimine was deemed to be negligible since less than 1% of the administered drug was reported to be eliminated in the urine (Levy 1974). The inhibition parameters entered in the Simcyp Simulator were already corrected for microsomal protein binding (Supplemental Table 3).

Model Performance and Application

PK Trial Simulations. The PK parameter inputs for the simulations were estimated using the clinical PK data of clofazimine. The simulated data were qualified using the observed PK data from the same study (Table 2).

DDI Predictions of Clofazimine as a Perpetrator of P450 Substrates. For DDI simulations using PBPK modeling, midazolam and selected antivirals such as saquinavir or efavirenz were considered as CYP3A4/5 substrates. Repaglinide and desipramine were used as CYP2C8 and CYP2D6 substrates, respectively. The Supplemental Material contains details of the input parameters for the substrates used in the PBPK model (Supplemental Tables 4–8). A total of 10 trials including 10 subjects were simulated; the age range of the simulated subjects was 18–70 years, with 50% of the population being women (Table 3).

Results

Static Model Predictions

R_1 Values for CYP3A4/5 Inhibition. The R_1 values for P450 inhibition by clofazimine are listed in Table 4. Since the calculated R_1 values of clofazimine were >1.1 for both total and unbound maximum anticipated C_{max} (inhibitor concentration) and >11 for $[I_{\text{gut}}]$, the AUCR was determined using the mechanistic static (net effect) model and by incorporating estimated f_m and F_g values of the interacting substrates.

AUCR Values for Clofazimine with Sensitive Substrates

The AUCR values were determined to estimate the extent of risk with respect to P450 inhibition in vivo when clofazimine was coadministered with P450 substrates. The AUCR values of midazolam, repaglinide, and desipramine with clofazimine were estimated as 5.59, 1.34, and 1.69, respectively. Based on these calculated net effect values, clofazimine was predicted to be a strong CYP3A4/5 inhibitor and a weak CYP2C8 and CYP2D6 inhibitor in vivo.

Predicted AUCR for Inhibition of CYP3A4/5-Mediated Clearance. For the selected substrates evaluated, approximately 2- to 6-fold increase in the AUC was predicted when coadministered once daily with 100 mg clofazimine (Table 5).

PBPK Model Predictions

Simulations of Clofazimine PK on Days 1 and 126. The predicted the AUC, C_{max} , and time to reach maximum plasma concentration values on day 1 using the PBPK model were within 2-fold of the observed values. The observed and simulated clinical PK parameters on day 1 for clofazimine following single oral doses (50 and 100 mg) are summarized in Table 6. The simulated clinical PK parameters for clofazimine at day 126 following multiple oral doses of 100 mg once daily can be found in Table 7. The predicted C_{max} value (797 ng/ml) on day 126 after multiple doses was comparable to the C_{max} value (890 ng/ml) predicted from the PopPK model, which was used for the static model.

DDI Simulations of CYP3A (Midazolam, Saquinavir, and Efavirenz), CYP2C8 (Repaglinide), and CYP2D6 (Desipramine) Substrates with Clofazimine. The predicted AUC and C_{max} ratios for midazolam with clofazimine were predicted as 2.69 and 1.68, respectively. The predicted AUC and C_{max} ratios of saquinavir when

TABLE 4
Input parameters and R_1 values for P450 inhibition by clofazimine

Input Parameter/ R_1 Value	P450 Enzyme		
	CYP3A4/5	CYP2C8	CYP2D6
Input parameter			
$K_{i,u}$ (μM)	0.000786	0.00372	0.00246
$[I]$ (μM); total C_{max}	1.89 ^a	1.89 ^a	1.89 ^a
$[I]$ (μM); unbound C_{max}	0.00189	0.00189	0.00189
$[I_{\text{gut}}]$ (μM); total	845	— ^b	—
R_1 Value			
Systemic: $[I] = C_{\text{max,total}}$	2406	509	769
Systemic: $[I] = C_{\text{max,u}}$	3.40	2	2
Gut: $[I] = [I_{\text{gut}}]$	1,075,065	—	—

$[I]$, inhibitor concentration (maximum anticipated C_{max}).

^aPredicted concentration after repeat administration of 100 mg/day clofazimine.

^b—, not applicable.

TABLE 5

Predicted fold change in the AUC of selected substrates of CYP3A4/5

For the weak CYP3A4/5 inhibitor the AUCRs are >1.25 and <2 . For the moderate CYP3A4/5 inhibitor the AUCRs are >2 and <5 . For the strong CYP3A4/5 inhibitor the AUCR is ≥ 5 .

Class/Substrate	Predicted AUCR
Antiretrovirals	
Atazanavir	2.10
Amprenavir	2.39
Darunavir	2.50
Delaviridine	2.57
Dolutegravir	3.00
Efavirenz	2.48
Elvitegravir	2.62
Etravirine	2.57
Indinavir	4.48
Lopinavir	2.52
Nelfinavir	2.41
Raltegravir	2.81
Ritonavir	2.57
Rilpivirine	2.57
Saquinavir	2.96
Simeprevir	5.16
Tipranavir	5.83
Antituberculars	
Bedaquiline	2.25
Clarithromycin	2.93
Delamanid	5.59
Antihypertensives	
Losartan	2.35
Verapamil	2.50
Diltiazem	2.62
Nitrendipine	3.37
Amlodipine	3.79
Guanfacine	3.79
Nifedipine	3.85
Eplerenone	4.72
Felodipine	4.88
Lercanidipine	5.96
Antidiabetics	
Alogliptin	2.23
Pioglitazone	2.39
Repaglinide	2.48
Teneligliptin	2.57
Saxagliptin	3.46
Antihyperlipidemics	
Pravastatin	2.90
Atorvastatin	4.88
Simvastatin	5.83
Lovastatin	6.23
Antileprotics	
Dapsone	1.97

coadministered with clofazimine were predicted to be 2.89- and 2.42-fold, respectively. No change in the AUC or $C_{\max}$ ratios was predicted by the PBPK model for efavirenz in the presence of clofazimine. In addition, the predicted AUCRs for repaglinide (CYP2C8 substrate) and desipramine (CYP2D6 substrate) with clofazimine were 1.60 and 1.47, respectively (Table 8).

Discussion

Clofazimine is currently being considered as a core second-line drug for treatment of DR-TB (see the WHO treatment guidelines; <http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/en/>). As per the WHO TB treatment guidelines, clofazimine is administered as part of a multidrug regimen; therefore, evaluating the drug interaction potential of clofazimine will be beneficial to arrive at appropriate dosing regimens for the coadministered drugs. In the absence of clinical drug interaction studies, data derived from in vitro experiments and modeling were used to predict the drug interaction potential of clofazimine.

TABLE 6

Observed and simulated clinical PK parameters for clofazimine in patients following single oral doses (50 and 100 mg)

Parameter	Clofazimine (Mean $\pm$ S.D.)	
	Oral Dose (50 mg)	Oral Dose (100 mg)
$C_{\max}$ (ng/ml)		
Observed ^a	71.8 $\pm$ 34.1	131 $\pm$ 59.9
Predicted	83.3 $\pm$ 219	178 $\pm$ 465
PE (%) ^b	+16%	+36%
AUC _{0-24 h} (ng·h/ml)		
Observed ^a	1084 $\pm$ 409	1856 $\pm$ 821
Predicted	976 $\pm$ 1633	2090 $\pm$ 3691
PE (%) ^b	-10%	+13%
$T_{\max}$ (h) ^c		
Observed ^a	8	6
Predicted	9.5	9.5
PE (%) ^b	+19%	+58%

AUC_{0-24 h}, area under plasma-drug concentration curve between 0 and 24 hours; PE, prediction error (%); $T_{\max}$, time to reach maximum concentration.

^aClinical PK parameters were not available on day 43; hence, the day 1 PK parameters were used for comparison; picomoles per grams was converted to nanograms per milliliter as follows: pmol/g = (pmol/g) $\times$ molecular weight (g/mol) = pmol/mol = ng/g = ng/ml, where plasma density assumes a value of ~ 1 g/ml.

^bPrediction error = [(predicted value - observed value)/observed value] $\times$ 100.

^cMedian.

In the static model, the clofazimine R_1 values for CYP3A4/5, CYP2C8, and CYP2D6 were found to be >1.1 and >11 for systemic inhibitor concentration and $[I_{\text{gut}}]$, respectively. Therefore, the possibility of a P450 inhibition-mediated interaction for clofazimine cannot be ruled out (<https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf>). The DDI potential was further evaluated by the mechanistic static (net effect) model, where the AUCR was determined using the probe substrates (midazolam, repaglinide, and desipramine for CYP3A4/5, CYP2C8, and CYP2D6, respectively). P450 inhibitors are typically classified as strong, moderate, or weak based on the magnitude of changes in the plasma AUC of the probe substrates: ≥ 5 -fold, strong inhibitor; between 2- and 5-fold, moderate inhibitor; and between 1.25- and 2-fold, weak inhibitor (<https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf>). The estimated AUCR values of clofazimine are 5.59, 1.34, and 1.69 for midazolam (CYP3A4/5), repaglinide (CYP2C8), and desipramine (CYP2D6), respectively. Similar results were obtained for clofazimine when the DDI was simulated using a PBPK model for repaglinide and desipramine. Thus, clofazimine can be classified as a weak inhibitor of CYP2C8 and CYP2D6.

The fold increase in exposure predicted using the PBPK model (AUCR of 2.69) was approximately 50% lower than that calculated using the mechanistic static (net effect) model (AUCR of 5.59) for midazolam. No change in the AUC or $C_{\max}$ value was predicted by the PBPK model for efavirenz in the presence of clofazimine; however, the static model predicted a 2.48-fold increase in the AUC. Considering the fold increase in the AUC predicted using both models, clofazimine can be classified as a moderate-to-strong inhibitor of CYP3A4/5.

The findings from the drug interaction predictions were further qualified against the available clinical evidence in Balakrishnan and Seshadri (1981), where clofazimine was coadministered with dapsone (a CYP3A4 substrate). The study concluded that there was no significant effect on the exposure of dapsone when administered with clofazimine (Balakrishnan and Seshadri, 1981). The AUCR value for dapsone with clofazimine was estimated to be 1.97 and predicted weak inhibition by clofazimine on dapsone PK. This is in agreement with the clinical data where no interaction was observed. The minimal difference between the findings from the predicted and observed value can be attributed to the fact that dapsone is also metabolized by CYP2C9 (Gill et al., 1995).

TABLE 7

Simulated clinical PK parameters for clofazimine on day 126 following multiple oral doses of 100 mg once daily
An example of the summary model input, output, and PK statistical output for this scenario can be found in the Supplemental Tables 9–11.

Parameter	Value
Mean $C_{\max}$ (ng/ml)	797 (S.D.: 392)
Mean AUC_{0-24h} (ng·h/ml)	16,627 (S.D.: 5367)
Median $C_{\max}$ (ng/ml)	703 (minimum: 255; maximum: 2966)
Median AUC_{0-24h} (ng·h/ml)	15,919 (minimum: 5868; maximum: 36,902)
Geometric mean $C_{\max}$ (ng/ml)	728 (CV%: 49)
Geometric mean AUC_{0-24h} (ng·h/ml)	15,797 (CV%: 32)

AUC_{0-24h} , area under plasma-drug concentration curve between 0 and 24 hours.

In view of the most frequent comorbidities associated with TB and the multidrug regimen used for DR-TB treatment, various classes of antituberculars, antiretrovirals, antidiabetics, antihypertensives, antileptotics, and antihyperlipidemics (statins) that undergo metabolism by CYP3A4/5 were considered for AUCR evaluation. Clofazimine is always administered as part of a multidrug regimen, either as a shorter or conventional regimen (WHO guidelines; <http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/en/>). The shorter regimen contains kanamycin (an injectable agent), moxifloxacin, prothionamide, clofazimine, isoniazid, pyrazinamide, and ethambutol, given together in an initial phase of 4 months, and followed by 5 months of treatment with four of the medicines (moxifloxacin, clofazimine, pyrazinamide, and ethambutol). For the conventional regimen, the intensive phase includes pyrazinamide and four second-line drugs (fluoroquinolones: levofloxacin, moxifloxacin, and gatifloxacin; second-line injectables: amikacin, capreomycin, kanamycin, and streptomycin; and core second-line agents: ethionamide/ prothionamide, cycloserine/terizidone, linezolid, and clofazimine). If the minimum of effective TB medicines cannot be composed, either a group D2 drug (bedaquiline or delamanid), and possibly group D3 agents (*p*-aminosalicylic acid, imipenem-cilastatin, meropenem, amoxicillin-clavulanate, or thioacetazone) could be added. With the exception of bedaquiline and delamanid, no P450-mediated interaction was predicted for other antitubercular drugs (fluoroquinolones, second-line injectables, core second-line agents, and D1 and D3 agents) that are currently recommended by WHO for treatment of DR-TB (both shorter and conventional regimens).

For most of the tested antiretroviral drugs known to be metabolized by CYP3A4/5, moderate interactions were assessed, with an estimation of between 2- and 5-fold increase in the AUC of the antiretroviral drug

[except simeprevir (5.13-fold) and tipranavir (5.83-fold)] when administered concomitantly with clofazimine 100 mg/daily. Moderate inhibitions were predicted for some of the dipeptidyl peptidase-4 inhibitors tested. Moderate-to-strong inhibitions were predicted for antihypertensives and antihyperlipidemic drugs (pravastatin, atorvastatin, simvastatin, and lovastatin) when administered concomitantly with clofazimine 100 mg/d.

In conclusion, clofazimine was predicted to be a weak CYP2C8 and CYP2D6 inhibitor. Thus, the possibility of a clinically significant interaction when coadministered with CYP2C8 and CYP2D6 substrate is minimal. Clofazimine is predicted to be a moderate-to-strong CYP3A4/5 inhibitor based on the net effect and PBPK modeling predictions. In the absence of a clinical DDI study, the possibility of an increase in the AUC of CYP3A4/5 substrates when coadministered with clofazimine cannot be ignored. Thus, caution is recommended when clofazimine is prescribed along with a CYP3A4/5 substrate.

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Authorship Contributions

Participated in research design: Sangana, Gu, Chun, Einolf.
Conducted experiments: Sangana, Gu, Chun.
Performed data analysis: Sangana, Gu, Einolf.
Wrote or contributed to the writing of the manuscript: Sangana, Gu, Chun, Einolf.

TABLE 8

Predicted clinical PK parameters in plasma for midazolam, saquinavir, efavirenz, repaglinide, and desipramine
Single dose given on day 126 in the presence and absence of clofazimine (100 mg once daily, days 1–126).

Substrate (dose) Simulated $n = 100$	Mean (S.D.)		Geometric Mean (CV%)		Geometric Mean Ratio (90% CI)	
	AUC_{inf}	$C_{\max}$	AUC_{inf}	$C_{\max}$	AUC	$C_{\max}$
	ng/ml·h	ng/ml	ng/ml·h	ng/ml		
Midazolam (5 mg)	72.1 (55.8)	25.1 (16.9)	56.0 (77)	20.4 (68)		
Midazolam (5 mg) + clofazimine	214 (174)	42.3 (27.0)	151 (81)	34.2 (64)	2.69 (2.58, 2.80)	1.68 (1.62, 1.74)
Saquinavir (1200 mg)	2197 (1519)	388 (309)	1810 (69)	304 (80)		
Saquinavir (1200 mg) + clofazimine	6164 (3730)	886 (567)	5224 (60)	736 (64)	2.89 (2.87, 2.90)	2.42 (2.30, 2.53)
Efavirenz (600 mg)	118,424 (78,432)	2376 (1653)	97,306 (66)	1950 (70)		
Efavirenz (600 mg) + clofazimine	124,951 (86,932)	2389 (1671)	101,211 (64)	1958 (70)	1.04 (1.04, 1.04)	1.00 (1.00, 1.00)
Repaglinide (0.25 mg)	8.27 (4.16)	3.83 (1.36)	7.14 (50)	3.56 (36)		
Repaglinide (0.25 mg) + clofazimine	13.6 (8.75)	5.04 (1.78)	11.4 (59)	4.69 (35)	1.60 (1.58, 1.76)	1.32 (1.29, 1.34)
Desipramine (50 mg)	2836 (7808)	17.3 (11.4)	688 (275)	14.5 (66)		
Desipramine (50 mg) + clofazimine	3125 (7745)	20.4 (11.6)	1009 (248)	17.7 (57)	1.47 (1.44, 1.50)	1.27 (1.24, 1.30)

AUC_{inf} , area under plasma-drug concentration curve from 0 to infinity; CI, confidence interval.

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