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Research Article

Guidance for Rifampin and Midazolam Dosing Protocols To Study Intestinal and Hepatic Cytochrome P450 (CYP) 3A4 Induction and De-induction

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Abstract. Cytochrome P450 3A4 (CYP3A4) catalyses the metabolism of > 30% of clinically used small molecule drugs. Induction of CYP3A4 is often associated with clinically important metabolic drug–drug interactions (DDIs). To collate published data regarding induction of CYP3A4 expression by rifampin and identify an optimal protocol to study DDIs using physiologically based pharmacokinetic (PBPK) modelling. The University of Washington Drug Interaction Database was searched for published data regarding induction of CYP3A4 by rifampin. A verified PBPK model was used to define the optimal dose, duration, timing and route of administration of rifampin and midazolam to assess induction of intestinal and hepatic CYP3A4 by rifampin. Sensitivity analysis was performed to evaluate the impact of participant characteristics including sex, race and age. The maximal induction of intestinal CYP3A4 (9.5-fold) was almost double that of hepatic CYP3A4 (5.5-fold). Maximal induction of intestinal and hepatic CYP3A4 was achieved in > 90% of participants within 5 and 10 days, respectively. Intestinal CYP3A4 expression returned to baseline in > 90% of participants within 7 days of rifampin cessation, whereas induction of hepatic CYP3A4 persisted for greater than 7 days in > 50% of participants. There was a significant difference in magnitude, but not time course, of CYP3A4 induction between males and females. Age and race did not significantly affect either the magnitude or time course of CYP3A4 induction. Maximal induction of intestinal CYP3A4 is achieved faster than hepatic CYP3A4. To assess maximal hepatic CYP3A4 induction, oral rifampin (600 mg daily) should be dosed for > 10 days.

KEY WORDS: CYP3A4; induction; physiologically based pharmacokinetic modelling; rifampin; study protocol.

INTRODUCTION

Cytochrome P450 (CYP) 3A4 is the drug metabolizing enzyme of greatest clinical importance as it plays a major role in the clearance of more than 30% of clinically used small molecule drugs (1,2). Consistent with this important role, CYP3A4 is abundantly expressed in the liver and throughout the intestine (duodenum, jejunum, ileum) with the exception of the colon (3,4). Total hepatic CYP3A4 protein abundance (approximately 225 nmol/organ) is considerably greater than the summed total intestinal CYP3A4 protein abundance (approximately 13 nmol/organ) (4). Despite the lower absolute protein abundance, CYP3A4 in the intestine remains physiologically and clinically important for drugs with a small f_g, for example midazolam (*i.e.* $f_g = 0.26$), where only a small fraction of the oral dose reaches the portal blood circulation intact (5).

Metabolic drug–drug interactions (DDIs) that induce CYP3A4 activity are an important source of variability in the exposure of drugs that are metabolized by this enzyme, as they can result in a loss of therapeutic efficacy (6–8). The characteristics of an induction DDI that determine clinical importance include the magnitude of induction, and the induction and de-induction time courses. Despite marked advances in the pre-clinical prediction of induction interactions, such as the development of inducible *in vitro* systems (*i.e.* HepaRG cell line) (9–11), induction DDIs remain the most challenging to characterize in pre-clinical *in vitro* studies because of the complex nature of these reactions, which often involve multiple pathways, and the difficult to translate time course (12). Notably, no *in vitro* model readily facilitates evaluation of the comparative impact of hepatic and intestinal induction of CYP3A4 expression (13).

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Accordingly, for any new molecular entity (NME) where CYP3A4 catalysed metabolism is identified as the predominant clearance pathway, evaluation of the impact of CYP3A4 induction on drug exposure is required by regulatory agencies including the US Food and Drug Administration (FDA) (14) and European Medicines Agency (EMA) (15). By way of example, when considering the clinical assessment of CYP induction, FDA guidance for clinical drug interaction studies (14) recommends that the induction perpetrator should be dosed for a sufficient duration to ensure maximal induction of the pathway of interest, noting that this may take two or more weeks of daily dosing. Specifically, when considering induction of CYP3A4 by rifampin, whilst there is no prescriptive duration required for rifampin, the general rule of thumb applied to NME studies is to dose rifampin for at least 10 but preferably 14 days at a daily dose of 600 mg (16–19). This practice is consistent with sparse direct (20) and anecdotal indirect evidence that maximal induction of hepatic CYP3A4 activity is achieved after 9 to 11 days of rifampin dosing (University of Washington Drug Interaction Database (UWDIDB)).

Assessment of CYP3A4 probe exposure pre- and post-rifampin dosing has also become a routine approach to characterize aspects of CYP3A4 phenotype in academic scientific literature. In this setting, there is less guidance regarding study design, and protocols are largely based on accepted convention. When considering the design of studies involving induction of CYP3A4 by rifampin, 7 days oral dosing at a daily dose of 300 to 600 mg is routinely accepted (21–23), although several studies report 'induction endpoints' for CYP3A4 derived from shorter courses of rifampin dosing (*i.e.* 4 to 5 days) (24–26).

This study collated existing data for induction of CYP3A4 by rifampin and used physiologically based pharmacokinetic (PBPK) modelling to characterize the magnitude, time course and variability in 'observable' and 'true' hepatic and intestinal CYP3A4 induction. Observable induction of CYP3A4 expression was evaluated as the change in the area under the midazolam plasma concentration–time curve (AUC), true induction of CYP3A4 expression was evaluated as the change in hepatic and intestinal CYP3A4 protein expression. The outcomes of PBPK modelling were used to define the optimal dose, duration, timing and route of administration of rifampin and midazolam to assess induction of intestinal and hepatic CYP3A4 by rifampin. The impact of participant characteristics, including sex, race and age on CYP3A4 induction was also assessed. In addition, to contextualize and explain indirect clinical findings reported in the UWDIDB, simulation data presented provide important additional insights that complement prior PBPK studies reporting the importance of dynamic simulation when considering induction DDIs involving CYP3A4 (27), the influence of time between substrate and inducer administration on induction kinetics (28) and the potential role of transporter–enzyme interplay for interaction victims that undergo significant transporter mediated uptake and/or efflux (29).

METHODS

Literature Search

A literature search was performed using the UWDIDB to identify all DDI studies involving rifampin as an interaction perpetrator (Supplemental Table 1). Identified studies were evaluated to determine the frequency of rifampin dosing duration, dose and route of administration.

Structural Model

Simulations were performed using the Simcyp population-based simulator® (version 17.0; Certara, Sheffield, UK) (30). The Simcyp® software uses a 'bottom–up' mechanistic modelling approach to extrapolate *in vitro* data on drug absorption, distribution, metabolism and excretion (ADME) with pharmacodynamic outcomes to simulate differences in *in vivo* drug exposure within virtual populations (30). The differential equations used by the simulator describing enzyme kinetics and the impact of co-variates have been described previously (31).

Population Profiles and Demographic Variables

Unless specified otherwise, simulations were performed using the in-built healthy volunteer (Sim-Healthy Volunteers) population profile with virtual study cohorts comprising of an equal distribution of males and females aged 20 to 35 years old. Simulations were performed as ten trials, each with ten subjects (total $n = 100$). For simulations performed to assess

Table I. Demographics of the Virtual Cohort for Both Male and Female Participants ($n = 100$)

Parameter	Females			Males		
	Geometric mean	95% confidence interval		Geometric mean	95% Confidence interval	
		Lower	Upper		Lower	Upper
Age (years)	27.1	21.5	33.2	25.8	20.7	32.0
Weight (kg)	66.7	48.8	93.1	77.3	60.9	100.2
Height (cm)	164	154	175	176	167	184
Kidney weight (g)	287	187	432	330	246	455
Liver weight (g)	1522	1216	1984	1613	1335	2021
BMI (kg/m^2)	24.8	18.4	34.0	25.0	19.4	32.2
Albumin (g/L)	43.6	39.0	48.9	47.2	39.5	57.7
GFR ($mL/min/1.73\ m^2$)	146	107	188	130	98.1	159

BMI body mass index, *GFR* glomerular filtration rate

the impact of sex, all-male and all-female Sim-Healthy Volunteer populations were used (Table I). For simulations performed to assess the impact of race, in-built Caucasian (Sim-NEurCaucasian), Chinese (Sim-Chinese) and Japanese (Sim-Japanese) population profiles were used along with the previously reported South African (V15R1_SouthAfrican_Population_CPTR_20170308) population profile (32). Table II defines the comparative CYP3A4 and CYP3A5 abundance in Caucasian, Chinese, Japanese and South African populations.

Compound Profiles

Simulations performed to assess CYP3A4 activity used the in-built substrate profile for midazolam (Sim-Midazolam) (Table III) (30). Midazolam area under the plasma concentration time–curve (AUC) and maximal plasma concentration (C_{max}) were simulated using a 'minimal PBPK model' comprising a liver compartment and a merged compartment representing all other organs (33–35). Unless specified otherwise, 5 mg of midazolam was dosed orally in a fasted state. Induction of hepatic and intestinal CYP3A4 was modelled using the in-built inhibitor profile for rifampin (compound file 'rifampicin' based on BAN generic name) including specification of multiple dose induction parameters (Sim-rifampicin-MD) (Table II) (36). Unless specified otherwise, 600 mg of rifampin was dosed orally every 24 h. When assessing the impact of rifampin dose on the magnitude and time course of induction, the two most commonly studied doses of 300 mg and 600 mg were evaluated.

Verification of Time Course of Hepatic and Intestinal CYP3A4 Induction and De-induction

The simulated time course of CYP3A4 induction by rifampin and de-induction upon cessation of rifampin was verified by comparing the virtual time course of hepatic and intestinal CYP3A4 expression with observed data from clinical studies (20,37,38). Simulated hepatic and intestinal CYP3A4 expression was monitored over 1056 h (20 observations/hour). Midazolam (5 mg oral) was dosed daily at 9:00 am from day 1 to day 44 (44 doses), rifampin (600 mg oral) was dosed daily at 9:00 am from day 3 to day 20 (17 doses). The magnitude and time course of induction of hepatic and intestinal CYP3A4 activity was monitored as the per cent baseline activity for the duration of the study. Immediately preceding each daily dose of rifampin, the extent of hepatic and intestinal CYP3A4 induction was assessed.

Optimal Study Design To Evaluate CYP3A4 Induction and De-induction (44 Days to 28 Days)

An optimized protocol for assessment of hepatic and intestinal CYP3A4 induction and de-induction was defined with respect to rifampin dose, dosing duration and midazolam dosing schedule to assess baseline, half-maximal and maximal induction, and half-maximal and maximal de-induction for hepatic and intestinal CYP3A4. The pre-specified criteria that were applied to define maximal induction and de-induction time courses were >90% induction in at least 90% of participants for induction, and at least 90% of participants returning to within 25% of baseline activity for de-induction. To avoid confounding of results due to drug accumulation, the minimal wash-out period between midazolam doses was also considered.

Impact of Dose on the Magnitude and Time Course of Hepatic and Intestinal CYP3A4 Induction

In DDI studies, FDA guidance recommends using the highest proposed clinical dose of the perpetrator drug to maximize the likelihood of detecting a potential clinical

Table II. Population Mean CYP3A Expression in Different Racial Groups

Organ	Enzyme	Population	CYP expression (nmol P450/organ)			Significance
			Geometric mean	95% confidence interval		
				Lower	Upper	
Liver	CYP3A4	Caucasian	8269	7210	9328	0.000
		Chinese	6537	5583	7490	
		Japanese	6477	5155	7799	
		South African	9997	8547	11,446	
	CYP3A5	Caucasian	1423	504	2342	0.000
		Chinese	1722	812	2631	
		Japanese	967	372	1563	
		South African	5807	4711	6904	
Small intestine	CYP3A4	Caucasian	62	52	72	0.005
		Chinese	54	45	64	
		Japanese	44	37	52	
		South African	69	57	81	
	CYP3A5	Caucasian	6	2	11	0.000
		Chinese	7	3	10	
		Japanese	5	2	7	
		South African	15	12	19	

Table III. Compound Parameters for Midazolam and Rifampin Substrate Profiles

Parameter	Sim-midazolam	SV-rifampin-MD
Physiochemical properties		
Molecular weight	325.8	823
log $P_{o:w}$	3.53	4.01
Species	Ampholyte	Ampholyte
Protein binding		
B/P	0.603	0.9
f_{up}	0.032	0.116
Absorption (first-order absorption model)		
f_a	1	0.925
k_a (1/h)	3	0.939
Distribution (minimal PBPK model)		
V_{ss} (L/kg)	0.88	0.42
CYP metabolism (K_m (μM))		
CYP3A4 (1-OH)	2.16	
CYP3A5 (1-OH)	4.16	
CYP3A4 (1-OH)	31.8	
CYP3A5 (4-OH)	34.8	
CYP metabolism V_{max} (pmol/min/pmol P450)		
CYP3A4 (1-OH)	5.23	
CYP3A5 (1-OH)	19.7	
CYP3A4 (1-OH)	5.2	
CYP3A5 (4-OH)	4.03	
UGT metabolism K_m (μM)		
UGT1A4	40.3	
UGT metabolism V_{max} (pmol/min/mg protein)		
UGT1A4	445	
Additional organ clearance (L/h)		
Renal clearance	0.085	1.26
Competitive inhibition (K_i; μM)		
CYP3A4		15
Induction (IND_{MAX}; maximum fold induction)		
CYP3A4		16
CYP3A5		16
Induction ($INDC_{50}$; μM)		
CYP3A4		0.32
CYP3A5		0.32

interaction. To thus assess the effect of dose on the magnitude and time course of hepatic and intestinal CYP3A4 induction, daily doses of 300 mg and 600 mg of rifampin were used. To study the dose relationship of CYP3A4 induction, rifampin was administered daily for a 14-day period (336 h). Following each dose, (percent) induction of intestinal CYP34 activity was compared to induction of hepatic CYP3A4 activity.

Impact of the Route of Drug Administration on the Magnitude and Time Course of Hepatic and Intestinal CYP3A4 Induction

When multiple routes of drug administration are developed for clinical use, FDA guidance recommends that DDI studies consider the expected mechanisms of potential clinical interactions following all routes of drug administration. To assess whether the route of drug administration can influence the magnitude and time course of hepatic and intestinal CYP3A4 induction, midazolam was administered intravenously (IV) and orally (PO). Respective per cent induction of intestinal and hepatic CYP3A4 activity was then compared.

Statistical Comparisons

Simulation data are presented as the geometric mean and 95% confidence interval (CI). One-way ANOVAs were performed to assess the statistical significance of the impact of race and sex on the magnitude and time course of rifampin-induced induction of hepatic and intestinal CYP3A4. Time to reach 90% maximal induction in 90% of the cohort was the primary end-point. The magnitude of maximal induction, percentage of individuals that achieved maximal induction and the time to return to baseline were also evaluated as secondary outcomes. When considering the statistical significance of differences between sexes and races in terms of the duration of rifampin dosing required, duration of follow-up required and magnitude of induction, probability (p) value less than 0.05 is considered statistically significant. For continuous variables, the coefficient of determination (r^2) was also evaluated and is reported.

RESULTS

Published DDI Study Designs that Used Rifampin as the Interaction Perpetrator

Analysis of the UWDIDB identified 530 DDI studies of rifampin as the interaction perpetrator, 276 involved a predominant CYP3A4 substrate as the interaction victim. Of the studies involving predominant CYP3A4 studies, 276 were evaluable for rifampin and probe route of administration, 275 were evaluable for rifampin dose and 263 were evaluable for rifampin dosing duration. There was consistency regarding the rifampin route of administration and dose, rifampin was administered orally in all studies and the dose was 600 mg in 233 (85%) studies (Fig. 1a). In contrast, the rifampin dosing duration ranged from 4 to 64 days; rifampin was dosed for 7 days or less in 104 (39.8%) studies and 233 (85%) studies had a dosing duration of 14 days or less (Fig. 1b).

Published Effect of Rifampin on Verified CYP3A4 *In Vivo* Probe Exposure

Forty-nine studies identified in the UWDIDB have reported the effect of rifampin on exposure to a CYP3A4 probe substrate: 2 buspirone, 44 midazolam, 2 simvastatin and 1 triazolam. The CYP3A4 probe was administered orally in 34 studies and IV (midazolam only) in 15 studies. No correlation between rifampin dose duration (5 to 28 days) and magnitude of induction was observed in studies involving an orally administered probe (probe for intestinal and hepatic CYP3A4; Fig. 2a). However, a significant ($p = 0.011$; Pearson correlation coefficient (r) = 0.634) correlation was observed between rifampin dose duration (5 to 14 days) and magnitude of induction for probes administered intravenously (probe for hepatic CYP3A4 only; Fig. 2b).

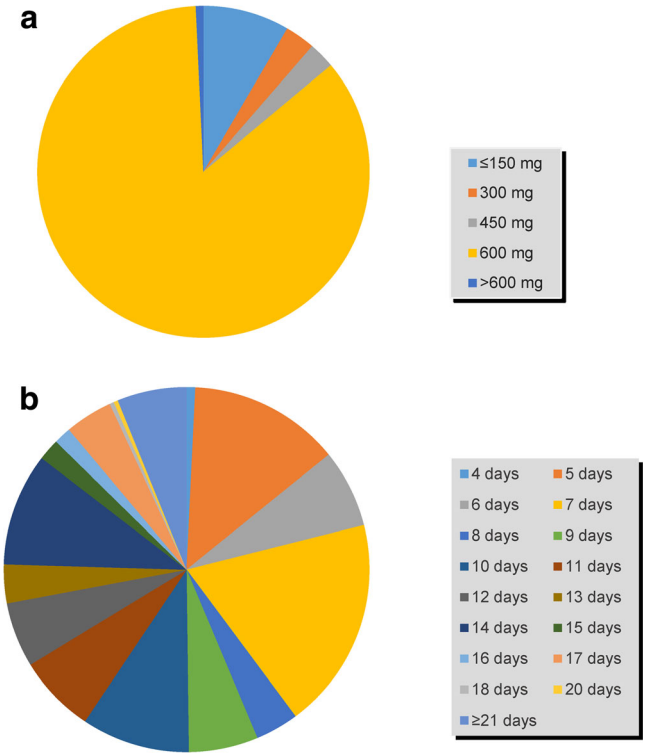

Fig. 1. Pie charts describing dosing conditions for rifampin in reported DDI studies involving a predominant CYP3A4 substrate; Panel **a**: rifampin dose (mg), Panel **b**: rifampin dose duration (days)

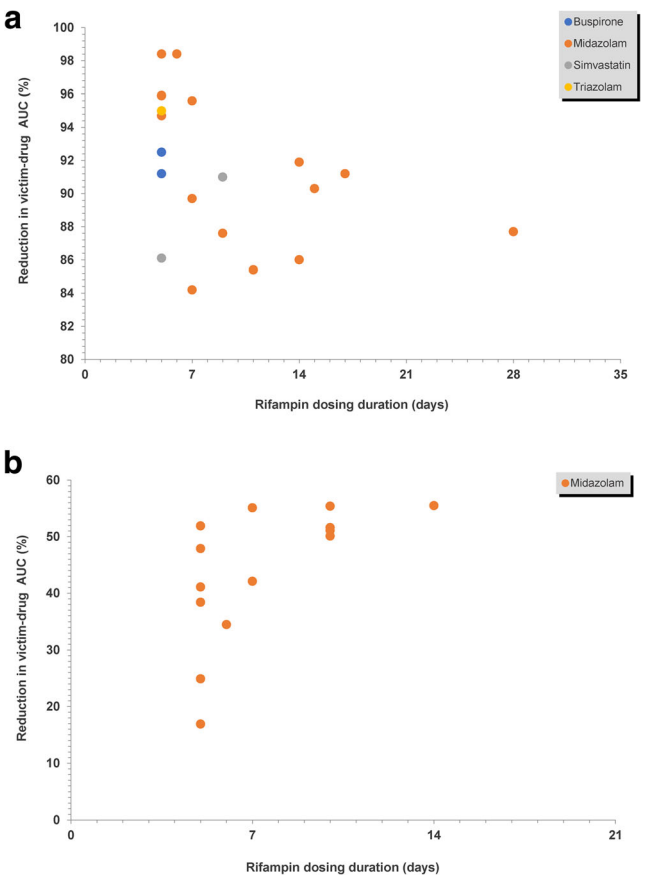

Fig. 2. Correlation between rifampin dose duration and reduction in victim AUC for CYP3A4 probe substrates. Panel **a**: orally administered probes. Panel **b**: IV-administered probe

Rifampin Dosing Duration To Assess Induction of Hepatic and Intestinal CYP3A4

The profile of CYP3A4 induction was simulated with respect to the observable change in CYP3A4 expression based on the decrease in probe (midazolam) AUC and C_{max} and the true change in CYP3A4 expression based on increase in hepatic and intestinal protein concentration (Fig. 3). Consistent with reported studies involving orally administered CYP3A4 probes (hepatic and intestinal), the mean magnitude of true intestinal CYP3A4 induction was equivalent at days 7 and 14. In contrast, observed and true hepatic CYP3A4 induction was 12% and 16% greater, respectively, following administration of rifampin for 14 days compared to 7 days (Table IV). This finding is consistent with the observed correlation between rifampin dose duration and extent of induction for IV-administered dose. Paired-sample T test of individual participant data further demonstrated that the greater magnitude of observed and 'true' hepatic CYP3A4 induction at day 14 was statistically significant ($p < 0.001$) for all parameters (AUC, C_{max} and protein expression).

When informing the protocol for assessment of hepatic and intestinal CYP3A4 induction by rifampin, the minimal duration of rifampin dosing was determined based on the prespecified criteria of the number of doses required to achieve 90% maximal 'true' induction of hepatic and intestinal CYP3A4 expression in at least 90% of participants. Whilst 90% maximal induction of intestinal CYP3A4 was achieved in all participants within 5 days, at least 9 days of rifampin dosing was required to achieve 90% maximal induction of hepatic CYP3A4 in at least 90% of participants (Fig. 4; Supplemental Table 2).

Follow-Up Duration To Assess De-induction of Hepatic and Intestinal CYP3A4

The duration of follow-up to assess de-induction of CYP3A4 expression following cessation of rifampin dosing was determined based on returning to within 25% of 'true' baseline CYP3A4 expression in at least 90% of the participants. Whilst 90% of participants returned to within 25% of baseline intestinal CYP3A4 expression within 6 days, CYP3A4 induction persisted in 17% of participants, where greater than 125% baseline hepatic CYP3A4 expression was exhibited at 14 days post rifampin cessation (Supplemental Table 3). The threshold of 90% of participants returned to within 25% of baseline hepatic CYP3A4 expression was achieved on day 17 post cessation of rifampin.

Optimal Midazolam Dosing Protocol

An optimal midazolam dosing schedule was defined with respect to the assessment of baseline, half-maximal induction, maximal induction, half-maximal de-induction and return to baseline in both the intestine and liver. Based on the data presented, midazolam dosing at baseline (day 0), day 1 (intestinal), day 2 (hepatic) and day 14, and rifampin dosing

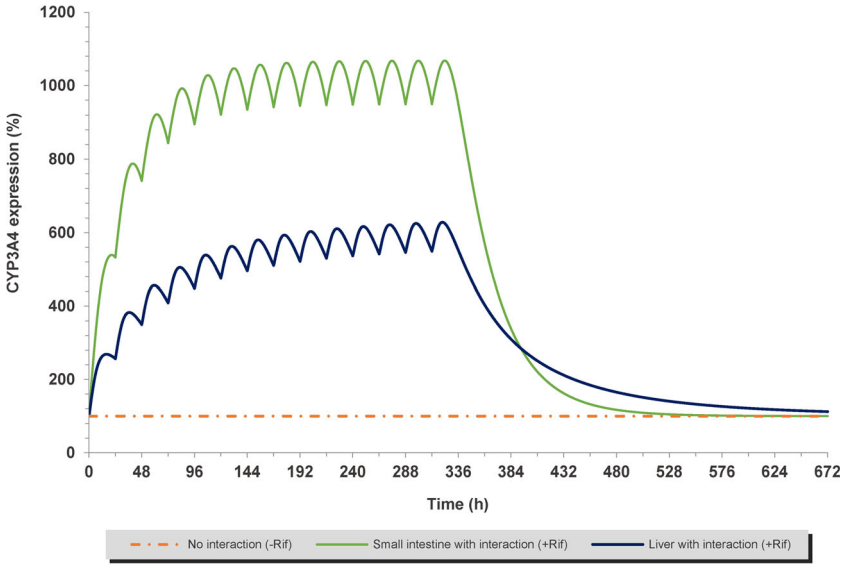

Fig. 3. Simulated geometric mean time course of intestinal and hepatic CYP3A4 induction. Green line: intestinal CYP3A4 expression with interaction. Dark blue line: hepatic CYP3A4 expression with interaction. Orange line: control CYP3A4 expression (no interaction)

on days 1 to 14, will facilitate assessment of baseline, half-maximal, and maximal, induction of intestinal and hepatic CYP3A4; midazolam dosed on day 15 (1 day post rifampin cessation) to assess half-maximal intestinal de-induction, day 16 (2 days post rifampin cessation) to assess half-maximal hepatic de-induction and day 28 (14 days post rifampin cessation) to assess full return to baseline. Following rifampin cessation, all participants within the virtual cohort intestinal CYP3A4 expression returned to baseline within 8 days.

Impact of Virtual Participant Demographics on Induction and De-induction Profile

Sex. Following dosing of rifampin, the magnitude of CYP3A4 induction was greater in the intestine as compared to the liver in both male and female participants. Consistent with published data (39,40), the magnitude of observable maximum induction in females (5.0-fold) was significantly ($p = 0.008$) lower than males (6.1-fold). Minor apparent differences in the induction and de-induction time courses were also observed; female participants appeared to achieve 90% maximal intestinal and hepatic CYP3A4 induction and returned to within 25% of baseline following cessation of rifampin faster (Supplemental Tables 4 and 5). However, differences in induction and de-induction time courses were not statistically significant and did not impact the appropriate protocol for assessment of induction with respect to the optimal days for midazolam dosing. Analysis of hepatic rifampin concentration over a 24-h period (hepatic AUC) demonstrated that the mean (range) exposure in females and males was comparable ($p = 0.162$) at 60.9 (27.4 to 136.6) and 67.0 (18.4 to 127.2), respectively.

Race. The magnitude of CYP3A4 induction differed between Caucasian, Chinese, Japanese and South African populations. Following the final dose of rifampin (day 15), the mean change in midazolam AUC was lowest in Japanese participants and highest in South African participants (Fig. 5). However, statistical analysis demonstrated that differences in the magnitude of induction between races was not statistically significant ($p = 0.221$). Similarly, the time course of CYP3A4 induction and de-induction did not vary significantly between races. It is plausible that difference in CYP3A4 and CYP3A5 enzyme abundance (Table II) contribute to the apparent observed differences in enzyme activity. It is further worth noting that the percentage of virtual participants expressing

Table IV. Mean Statistics for Midazolam With and Without Rifampin Interaction for 7 and 14 Days

	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (ng/mL)	AUC (ng/mL h)	C_{max} ratio[a]	AUC ratio[a]
No interaction	0.50	4.12	24.3	72.5		
Rifampin 7 days	0.50	3.10	4.57	8.69	0.19	0.12
Rifampin 14 days	0.50	2.92	4.18	7.83	0.17	0.11

AUC area under the plasma concentration–time curve, C_{max} maximal plasma concentration, $T_{1/2}$ half-life, T_{max} time to reach the maximum concentration

[a] Ratio of post-interaction/pre-interaction parameter

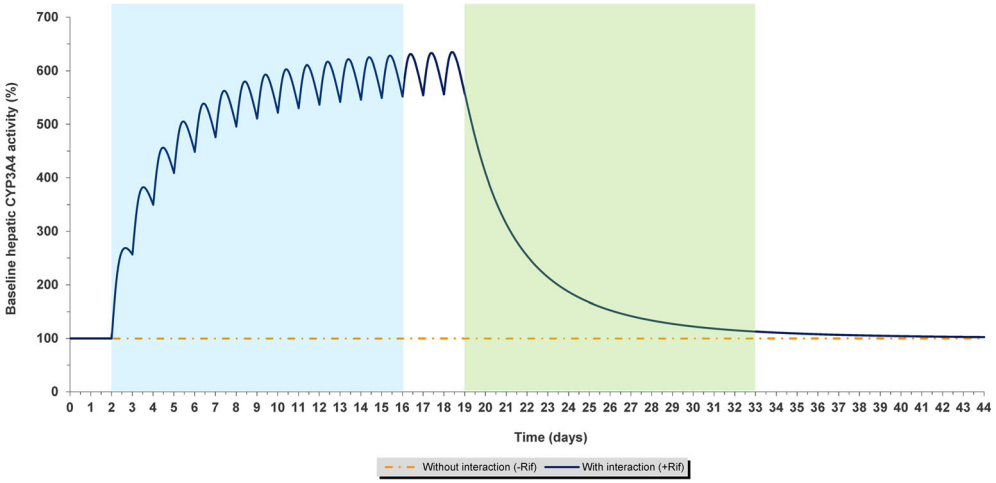

Fig. 4. Proposed study duration to assess CYP3A4 induction and de-induction using rifampin as the interaction perpetrator based on the induction time course for hepatic CYP3A4 (time limiting factor). Blue box: induction phase. Green box: de-induction phase

active CYP3A5 (*1) were significantly different ($p = 0.000$) between Caucasian (20%), Chinese (34%), Japanese (28%) and South African (86%) populations.

Age. Variability in the magnitude of intestinal and hepatic CYP3A4 induction was observed within the 20- to 35-year-old age groups; however, this variability was not associated with age and not statically significant ($p \geq 0.497$). By way of example, the r^2 for the association of age with maximal induction (at day 14) equalled 0.002.

Impact of Dosing Conditions

Impact of Rifampin Dose on Induction Profile. Over the 14-day (336 h) dosage period, greater CYP3A4 induction was demonstrated in the intestine as compared to the liver upon administration of both the 600 mg and 300 mg dose of rifampin. The larger dose of rifampin (600 mg) resulted in 29% greater induction of intestinal and 16% greater induction of hepatic CYP3A4, compared to the lower dose (300 mg) of rifampin.

Impact of Route of Midazolam Administration on Induction Profile. Greater magnitude of induction was achieved using oral dosing. Following IV administration, the midazolam AUC ratio (on day 14) was higher compared to oral administration, at 0.114 and 0.552 respectively. In addition, greater variability in induction on day 7 compared to day 14 was maintained with both oral and IV dosing. The concordance of the simulation data with observed data for midazolam AUC is graphically represented in Fig. 6. Individual participant data from a healthy volunteer trial (38) was overlayed against a simulation matched for sex, age and midazolam dose. The mean simulated and observed AUC values were 12.1 and 13.9 ng/mL h, respectively, whilst mean simulated and observed C_{max} values were 5.1 and 6.5 ng/mL, respectively.

DISCUSSION

Before an investigational new drug is tested in phase II and phase III clinical trials, it is important to determine the potential risk of DDIs in order to assist in design of the study and highlight any possible safety issues or loss of efficacy. The

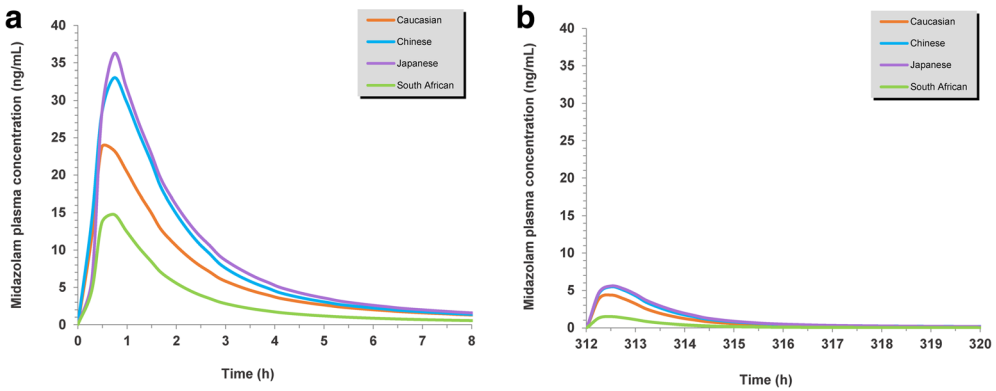

Fig. 5. **a**, **b** Midazolam steady state plasma concentration time profile in Caucasian, Chinese, Japanese and South African populations in the presence of rifampin (600 mg daily)

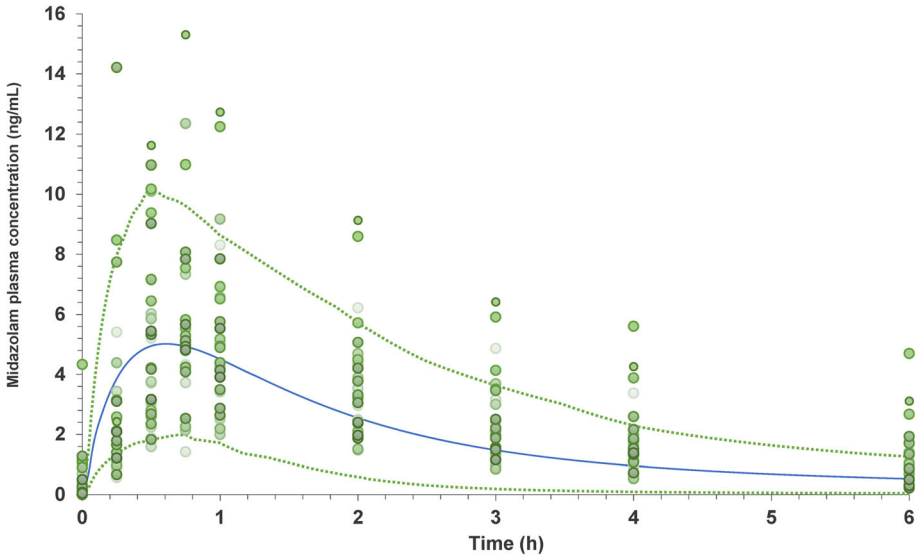

Fig. 6. Observed participant data for 30 healthy male volunteers showing variability in midazolam plasma concentration time profiles (green dots) with simulated mean (blue line) and 95% confidence intervals (green lines) over 6 h

AUC and C_{max} for a new chemical entity or new molecular entity (NCE/MNE) are measured prior and post administration of the perpetrator drug, such as rifampin (600 mg PO daily). In 1997, the USA FDA first published guidance to document industry conduct of drug interaction studies (41). For industry DDI studies, it reports the sponsor is required to administer the inducer as multiple doses to ensure maximal induction, noting this will equate to 2 or more weeks of daily drug administration (42). However, less guidance is provided to academic DDI studies. As such, years of convention has resulted in a frequent use of shorter dosing course of rifampin at 7 days or less (Fig. 1b). Therefore, the current study aimed to define the duration of rifampin dosing required to achieve steady state maximal induction, as well as the duration of follow-up required to return to within 25% of baseline activity, over a 44-day study duration. Simulation data showed that between day 6 and 10, changes in exposure of orally administered midazolam are associated with intestinal CYP3A4 induction. During this period, intestinal CYP3A4 expression is fully induced and hepatic CYP3A4 expression is only partially induced. As such, changes in CYP3A4 clearance, based on midazolam AUC ratio (*e.g.* day 7/day 1), are likely to be an underestimate.

Comparison of simulated and observed data confirmed that the Simcyp midazolam profile accurately represented this drug as a sensitive substrate of CYP3A4, based on the profound (> 5-fold) (7) decrease in exposure when co-administered with the strong CYP3A4 inducer rifampin. Simulation results indicated both intestinal and hepatic CYP3A4 activity was clearly induced by rifampin administration. This finding was expected, as induction of CYP3A4 is mediated by the activation of the nuclear pregnane X receptor (PXR), which is highly expressed in both the intestine and liver (20). Variation in the magnitude of CYP3A4 induction existed between the organs, such that CYP3A4 activity was greater in the intestine. This is believed to be due to more pronounced zonal expression of CYP3A4 in the intestine than the liver, accounting for ~ 80% and ~ 10% of total P450 enzymes, respectively (21,22). These data were consistent with previously reported clinical studies comparing direct deconvolution of intestinal and hepatic CYP3A4 activity (25,43). Differences also existed in the time course of induction in the intestine and liver. Furthermore, simulation results from the 44-day study demonstrated that the liver was the limiting organ in terms of rate of CYP3A4 induction and de-induction. Consistent with the indirect reported differences between induction time course for oral and IV administration reported in the UWDIDB (Fig. 2), maximal simulated induction of intestinal CYP3A4 was achieved faster than that of hepatic CYP3A4 (Fig. 3), such that 90% maximal induction of intestinal CYP3A4 was achieved in 100% of the cohort within 5 days, whereas at least 9 days of rifampin dosing was required to achieve 90% maximal induction in the liver. Furthermore, following cessation of rifampin, 90% of the cohort returned to within 25% baseline intestinal CYP3A4 activity within 5 days. However, residual induction of the liver persisted in 17% of the cohort for 14 days, where baseline hepatic CYP3A4 activity was greater than 125%. Rifampin thus appears to cause more sustained induction in the liver than the intestine. Therefore, the optimized 28-day protocol was defined by the time required to measure induction and de-induction in the liver.

The basis for this discrepancy between intestine and liver responsiveness to rifampin-induced induction and de-induction is believed to be related to the natural degradation time of the enzyme and the elimination of the inducing agent (43). Time-dependent changes in liver and intestinal CYP3A4 are key drivers of altered drug exposure. In the intestine, drug exposure is transient as gastrointestinal motility and absorption rapidly reduce drug concentration, allowing the rate of enzyme recovery to be primarily determined by the endogenous degradation rate constant of the enzyme (*kdeg*) and the half-life of the inducer. The time course of intestinal CYP3A4 induction is thus primarily determined by the duration of exposure to rifampin, not the concentration of rifampin. A faster turnover of enterocytes compared to hepatocytes (23) may have also contributed to the faster time course of induction and de-induction observed in the intestine.

It should be discouraged to administer rifampin for 7 days or less when evaluating the potential clinical significance of CYP3A4 induction during DDI studies. Simulation results presented supported the indirect evidence reported in the UWDIDB that assessment of midazolam exposure after 7 days of rifampin dosing resulted in a statistically significant under-prediction of the maximal potential induction of hepatic CYP3A4. Results displayed in Supplemental Table 3 indicate induction of hepatic CYP3A4 persisted for greater than 7 days in more than 50% of healthy individuals. Therefore, contrary to academic convention, to measure baseline and maximal induction of CYP3A4, midazolam exposure should be monitored prior to and after at least a 10-day course of rifampin (600 mg PO daily).

To define the appropriate study population to assess induction and de-induction of intestinal and hepatic CYP3A4, the effect of participant characteristics on the magnitude and time course of induction was investigated. Population characteristics examined included sex, race and age. Each characteristic was assessed by determining the significance of differences in induction and de-induction time course, maximum absolute induction and proportion of cohort achieving maximal induction.

Simulations revealed induction of CYP3A4 activity varied between males and females, specifically a significant difference was recorded in the magnitude ($p = 0.008$), but not time course of CYP3A4 induction ($p > 0.1$). During co-administration of rifampin and midazolam, females demonstrated a higher systematic concentration of midazolam than males (Supplemental Tables 4 and 5). As such, induction of intestinal and liver CYP3A4 by rifampin was more pronounced in males than females. This may be due in part to the female-predominant expression of CYP3A4, which may have led to higher clearance of rifampin, thus greater CYP3A4 activity (40). These differences in CYP3A4 induction that were observed between males and females were not explained by the differences in hepatic rifampin exposure ($p = 0.162$).

Assessment of inter-racial variability found variation in CYP3A4 activity between races. Specifically, differences in the magnitude of induction were recorded between races; greatest induction was achieved in the South African population (Fig. 5 and Table II). However, from the perspective of conducting routine drug metabolism studies, the magnitude of these differences is inconsequential, especially when one considers the wide inter-individual variation in CYP3A4 activities within any group examined. Critically, the time course of induction and de-induction did not vary (Supplemental Tables 2 and 3).

Simulations indicate that for healthy volunteers between 20 and 35 years old, age is not associated with altered intestinal and hepatic CYP3A4 activity, as determined by the clearance of midazolam. Amongst the participants of the healthy virtual cohort, there was no significant difference in the magnitude and time course of CYP3A4 induction and de-induction between those aged 20 to 35 years old. As such, it is appropriate to include participants of any age within this range without the risk of confounded results.

The mechanism-based induction of CYP3A4 by rifampin was shown to be clearly dose-dependent. The 600 mg dose of rifampin produced a higher fold-increase in CYP3A4 activity compared to the 300 mg dose of rifampin. As such, to maximize the likelihood of identifying potential DDIs administration of 600 mg rifampin would be preferable to lower doses. Furthermore, induction of CYP3A4 activity was greater and occurred more rapidly in the intestine than the liver for both the 600 mg and 300 mg dose of rifampin, which further supports the liver as the limiting organ in terms of induction, irrespective of dose.

The extent of intestinal and hepatic CYP3A induction was influenced by the route of administration. Simulation results presented CYP3A4 activity was lower when rifampin was dosed intravenously as compared to orally. This suggests if rifampin is administrated by IV, it may overestimate the variability and underestimate the magnitude of CYP3A4 induction. Therefore, for DDI studies where rifampin is the perpetrator drug, an oral route of administration recommended.

In the design of a clinical study assessing CYP3A4 induction that involves healthy female participants, an important consideration is the reduced effectiveness of oral contraceptives, which are predominantly cleared by CYP3A4. Simulation results demonstrated that in female participants, sufficient induction of CYP3A4 activity to reduce oral contraceptive efficacy is achieved almost immediately following the commencement of rifampin dosing (600 mg PO daily) and persists in >90% of participants for at least 14 days following cessation of rifampin. This may consequently result in faster excretion of the steroid and lead to unexpected pregnancy. As such, females involved in healthy volunteer trials should be aware of this at the time of obtaining informed consent. In addition, the current study uses mechanistic dynamic modelling to highlight the importance of considering perpetrator dosing duration as a potential confounder when assessing induction of CYP3A4 expression. However, it is important to acknowledge that whilst physiologically based, the findings of the current analysis are based on simulation, no model is perfect (36). As such, whilst the core recommendations are justified, a real-world clinical trial should be undertaken to confirm the timings outlined in this manuscript. A further limitation of the current study was the availability of clinical data to directly compare the time course of simulated enzyme induction. Indeed, one of the studies used in the verification (20) was based on an endogenous biomarker for CYP3A4, the validity of which has been questioned (44).

CONCLUSION

Results documented in clinical drug interaction studies that measure induction of CYP3A4 following an insufficient dose and course of perpetrator drug should be considered with caution. Results of DDI stimulations highlight DDI studies that have administered rifampin for 7 days or less have likely under-predicted the absolute magnitude of induction and over-predicted the variability of induction by measuring partial enzyme induction in a proportion of individuals. Instead, findings of this manuscript suggest to accurately assess complete intestinal and hepatic CYP3A4 induction, dosing of rifampin should continue for at least 11–14 days. Following cessation of rifampin, CYP3A4 activity should then be monitored for a further 14 days as induction of

hepatic CYP3A4 persists for at least 2 weeks in a significant proportion of individuals. In addition, to maximize the likelihood of identifying potential DDIs, the dose of the perpetrator drug, rifampin should be orally administered at the highest proposed clinical dose (600 mg). Regarding inter-individual variability amongst participants, based on the predicted impact of sex, age and race on CYP3A4 induction and de-induction on the healthy virtual population, it is appropriate to include both female and male participants, aged 20 to 35 years old of different race in a study cohort without confounded results.

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