

## Original Article

# Evaluating a physiologically based pharmacokinetic model for predicting the pharmacokinetics of midazolam in Chinese after oral administration

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**Aim:** To evaluate the SimCYP simulator ethnicity-specific population model for predicting the pharmacokinetics of midazolam, a typical CYP3A4/5 substrate, in Chinese after oral administration.

**Methods:** The physiologically based pharmacokinetic (PBPK) model for midazolam was developed using a SimCYP population-based simulator incorporating Chinese population demographic, physiological and enzyme data. A clinical trial was conducted in 40 Chinese subjects (the half was females) receiving a single oral dose of 15 mg midazolam. The subjects were separated into 4 groups based on age (20–50, 51–65, 66–75, and above 76 years), and the pharmacokinetics profiles of each age- and gender-group were determined, and the results were used to verify the PBPK model.

**Results:** Following oral administration, the simulated profiles of midazolam plasma concentrations over time in virtual Chinese were in good agreement with the observed profiles, as were AUC and  $C_{max}$ . Moreover, for subjects of varying ages (20–80 years), the ratios of predicted to observed clearances were between 0.86 and 1.12.

**Conclusion:** The SimCYP PBPK model accurately predicted the pharmacokinetics of midazolam in Chinese from youth to old age. This study may provide novel insight into the prediction of CYP3A4/5-mediated pharmacokinetics in the Chinese population relative to Caucasians and other ethnic groups, which can support the rational design of bridging clinical trials.

**Keywords:** midazolam; pharmacokinetics; PBPK model; CYP3A4/5; Chinese

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## Introduction

As of 2014, China is the second largest pharmaceutical market, behind the United States<sup>[1]</sup>. Many large pharmaceutical companies have increased their presence in China, resulting in a dramatic increase in the number of clinical trials in the last decade<sup>[2, 3]</sup>. It is not surprising that an area of strategic focus for industry is faster product registration and entry into this emerging market; however, delays occur due to the critical concern that potential ethnic differences may result in substantial variability in the dose-response relationship, which ultimately affects safety and efficacy<sup>[4, 5]</sup>.

Physiologically based pharmacokinetic modeling (PBPK) is a key component of model-based drug development and is increasingly embraced by industry<sup>[6, 7]</sup> and regulatory authorities<sup>[8–10]</sup>. It is well-recognized that PBPK modeling can assess ethnicity sensitivity in virtual populations, thereby informing the need for and design of real studies. Currently, PBPK

models have been widely applied to the prediction of pharmacokinetics and drug-drug interactions in healthy subjects<sup>[11–15]</sup> and in specific populations (eg, cancer patients) in China<sup>[16–18]</sup>; however, limited demographic and physiological knowledge (eg, liver weight, cardiac output, and enzyme abundance, etc) impede the development of the PBPK approach in the Chinese population. Presently, commercially available PBPK software (eg, SimCYP and Gastroplus) affords the opportunity to predict drug exposure and disposition in different ethnic groups. These population-based PBPK approaches have been widely used to address critical concerns such as pharmacokinetics in pediatric populations, organ impairment, and metabolic drug-drug interactions<sup>[19, 20]</sup>; however, as these models use parameters gathered from primarily Western populations (ie, Caucasians) their capacities for predicting pharmacokinetics (PK) in Chinese are rather limited.

Midazolam is a short-acting hypnotic-sedative drug with anxiolytic and amnesic properties. Since the 1980s, it has become the most frequently used benzodiazepine for the induction of anesthesia and conscious sedation, as well as for long-term sedation in patients receiving mechanical ventila-

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tion. Midazolam is primarily metabolized by CYP3A4/5, and greater than 95% of the dose is metabolized by CYP3A4/5<sup>[21-23]</sup>. Consequently, the FDA has recommended midazolam for use as a typical substrate to characterize the CYP3A metabolic pathway<sup>[24]</sup>. Recently, Barter *et al* developed an ethnicity population database for the prediction of differences in cytochrome P450 (CYP)-mediated pharmacokinetics between Chinese and Caucasians using alprazolam and midazolam as probe drugs for the CYP3A metabolic pathway<sup>[11]</sup>. This study was the first systematic attempt at investigating inter-ethnic differences in the Chinese population using mechanistic PBPK modeling. Unfortunately, the predicted oral clearance of midazolam in Chinese was 2-fold higher than previously reported, while the prediction for alprazolam was in good agreement with observed data. With the limitations to the gut physiology and enzymology of the Chinese population model<sup>[11, 12]</sup>, the scarcity of quality of clinical data and appreciable inter-study variability may account for the overprediction<sup>[11]</sup>. Moreover, the PK data used for model verification were mostly acquired from young Chinese male subjects (19-31 years) with homogeneous demographics<sup>[25-27]</sup> and special populations such as the elderly were not involved.

It is well-recognized that CYP3A is an important metabolic pathway that accounts for the metabolism of approximately 50%-60% of therapeutic drugs<sup>[28]</sup>; therefore, it is necessary to evaluate the feasibility of the CYP3A PBPK models for the Chinese population. This study aimed to evaluate the SimCYP Chinese population model for midazolam (a CYP3A4/5 substrate) using a top-down approach and to investigate whether the pharmacokinetics of drugs that are predominantly metabolized by CYP3A4/5 were well captured.

## Materials and methods

### Clinical study of midazolam in Chinese after oral administration

To acquire reliable pharmacokinetic data for the model evaluation and investigate midazolam pharmacokinetics in Chinese, an open-label, parallel group single-dose study was performed. The study was performed in accordance with Good Clinical Practice regulations and the ethical principles stated in the Declaration of Helsinki and was approved by the Ethical Committee of the Peking Union Medical College Hospital (Beijing, China) prior to subject enrollment. After giving written informed consent, 40 Chinese subjects were enrolled based on the following inclusion and exclusion criteria: men and women of Chinese ethnicity (three generations living in mainland China), 20 to 85 years of age, and a body mass index of 19-32 kg/m<sup>2</sup>. For subjects ≤65 years, there was no evidence of clinically significant abnormalities in cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological and psychiatric function as determined by medical history, physical examination and laboratory screens; no history of drug or substance abuse; and no allergic history to benzodiazepines. For elderly subjects (>65 years), minor stable diseases frequently encountered in persons (*eg*, mild hypertension or arthritis) were permitted. Female subjects were required to be surgically incapable of pregnancy, or practicing an acceptable

double-barrier birth control method such as a condom or diaphragm with spermicide. For all the volunteers, we excluded those that used medications with a pronounced effect on the central nervous system within the last 3 months, or had impaired hearing or epilepsy. Intake of concomitant drugs was not permitted.

Forty Chinese subjects were enrolled and divided into 4 groups (20-50, 51-65, 66-75, and >76 years). Each age group included 10 subjects (half men and half women). On the study day, the subjects were given one tablet of midazolam (15 mg/tablet, Nhwa Pharmaceutical Co, Xuzhou, China) with 250 mL of water following overnight fasting. Standard meals were provided at 4 and 10 h post-dose. Serial blood samples (5 mL at each time point) were collected at the following time points: 0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h post-dose. A validated HPLC-MS/MS method was utilized to determine the plasma concentrations of midazolam. The assay was linear between 0.5 and 250 ng/mL, and the lower limit of quantification (LLOQ) was 0.5 ng/mL. Three quality control samples (1.5, 100 and 200 ng/mL) were also analyzed to ensure bioassay quality.

Pharmacokinetic parameters were calculated using non-compartmental analysis (NCA) with WinNonlin (Version 6.3; Pharsight, NC, USA).  $C_{max}$  was the maximum plasma concentration in a profile, and  $T_{max}$  was the time to  $C_{max}$ . The elimination half-life ( $t_{1/2}$ ) was estimated by linear regression of log-transformed concentration-time data. The area under the plasma concentration-time curve from time zero to time  $t$  ( $AUC_{0-t}$ ), where  $t$  is the time of last measurable sample, was calculated according to the linear trapezoidal rule. The AUC from time zero to infinity ( $AUC_{0-\infty}$ ) was estimated as  $AUC_{0-t} + C_t/\lambda_z$ , where  $C_t$  was the plasma concentration of the last measurable sample. The apparent total clearance ( $CL/F$ ) was calculated as  $Dose/AUC_{0-\infty}$  and the apparent total volume of distribution ( $V/F$ ) as calculated as  $CL/\lambda_z$ . Statistical analysis was performed using SPSS software (version 19, SPSS Inc, Chicago, IL, USA). Descriptive statistics were expressed as the mean±standard deviation (SD).  $C_{max}$  and AUC data were transformed by natural logarithm prior to statistical analysis. Differences among the age groups were identified by analysis of variance (ANOVA) and were considered to be significant when  $P < 0.05$ .

### PBPK model development

The PBPK model was developed using the SimCYP population-based simulator<sup>®</sup> (version 13.0, SimCYP Ltd, Sheffield, UK).

### Virtual population

There are two Chinese population databases in the SimCYP population-based simulator. One database is constructed based on demographic data of 8118 Han Chinese obtained from the China Health and Nutrition Survey 2006<sup>[29]</sup>, and the other is a volunteer database (594 healthy Chinese) provided by Pfizer Ltd (Sandwich, UK). In the current study, the first database was selected for simulation, with ages ranging between 20 and 80 years and with a proportion of females

of 0.5. The age distributions were fitted independently in males and females using a Weibull 2 parameter model<sup>[30]</sup>. The mean bodyweights for males and females were 62 and 60 kg, respectively, and the mean heights were 1.73 and 1.58 m, respectively. The mean BSA of the general Chinese population were 1.73 and 1.55 m<sup>2</sup> for males and females, respectively. Moreover, the mean abundances of CYP3A4 and 3A5 in the liver were 120 and 82 pmol/mg protein, respectively. As no data were available regarding intestinal CYP abundances in Chinese, the Chinese CYP3A4/5 intestinal abundances (58 and 21.5 pmol/mg) in SimCYP were scaled from Caucasians<sup>[11, 31]</sup>.

#### Midazolam PBPK model

The SimCYP compound file for midazolam was used for modeling and the input parameters are summarized in Table 1. The SimCYP segmental advanced dissolution absorption metabolism (ADAM) model<sup>[32]</sup> was used for absorption. As the midazolam tablets used in this study were formulated for immediate release (IR), IR tablet dissolution profiles were acquired by *in vitro* dissolution assays and incorporated into the ADAM model. Given the high lipophilicity of midazolam, a full PBPK model was utilized to improve recovery of plasma concentration-time profiles. The volume of distribution was predicted using the Rodgers *et al* method<sup>[33]</sup>, and the default SimCYP enzyme kinetics of midazolam were used to model elimination. Given the lack of hepatic and intestinal CYP3A4/A5 abundance data in Chinese populations, the following assumptions were made, as previously mentioned in Barter *et al*<sup>[11]</sup>: (1) the abundances of hepatic CYP3A5 in Chinese and Japanese were assumed identical; (2) the abundance of Chinese intestinal CYP3A4/5 was scaled from that of Caucasians based upon the relationship between hepatic CYP abundances in Caucasians and Chinese<sup>[31]</sup>.

#### Simulation of midazolam pharmacokinetics in Chinese after oral administration

Simulations were performed for subjects in 4 age groups

(20–50, 51–65, 66–75 and >76 years) using the SimCYP Chinese general population profile. Each simulation was performed for 10 trials with 100 subjects (total  $n=1000$ , with the proportion of females being 0.5). The trial design was identical to the real clinical study: a single dose of 15 mg of midazolam was administered to fasted subjects with 250 mL of water at 8:00 AM, and the study was 12 h in duration.

## Results

### Demographics

Forty Chinese subjects completed the clinical study and demographics are shown in Table 2. The distributions of mean demographics (age, height, weight and body mass index) of these subjects were comparable to the virtual Chinese population simulated by SimCYP.

### Pharmacokinetics of midazolam in Chinese at 20–80 years

To obtain reliable PK data for evaluation of the model, a clinical study was designed and performed in which forty Chinese subjects (age 23–80 years) were treated with 15 mg of midazolam. The mean pharmacokinetic parameters and mean concentration-time profiles of oral midazolam are shown in Table 3 and Figure 1. Midazolam was absorbed rapidly, with median  $t_{max}$  of 0.7 h, and an elimination  $t_{1/2}$  of 3–5 h following oral administration. Mean oral clearance ( $CL/F$ ) was 65–75 L/h and the mean volume of distribution ( $V/F$ ) was 350–420 L. Statistical analysis indicated that there were no significant differences in system plasma exposure ( $AUC$  and  $C_{max}$ ),  $CL/F$  and  $V/F$  for midazolam among the 4 age groups ( $P>0.05$ ). Moreover, there were no significant differences between female and male subjects with regards to these parameters ( $P>0.05$ ). These results suggest that the pharmacokinetics of midazolam following oral administration were not affected by either subject age or gender.

### Simulation of midazolam plasma concentration-time profiles

The simulated and observed plasma concentration-time pro-

**Table 1.** Summary of input parameters for midazolam PBPK model.

Parameter	Midazolam	Method/reference
<b>Absorption</b>		
Absorption model	ADAM	
Caco-2 permeability ( $\times 10^{-6}$ cm/s)	6.59	Calculated using SimCYP
fuGut	1	Assumed
Formulation	Immediate release tablet	
<b>Dissolution</b>		
Distribution	Dissolution profile (>95% was released within 0.5 h)	From an <i>in vitro</i> dissolution test
Distribution model	Full PBPK model	
$V_{ss}$ (L/kg)	10.048	Calculated using SimCYP
<b>Enzymes kinetics</b>		
	1-OH/CYP 3A4: $V_{max}$ 5.23, $K_m$ 2.18*	Ref 11
	1-OH/CYP 3A5: $V_{max}$ 19.7, $K_m$ 4.16	Ref 11
	4-OH/CYP 3A4: $V_{max}$ 5.2, $K_m$ 31.8	Ref 11
	4-OH/CYP 3A4: $V_{max}$ 4.03, $K_m$ 34.8	Ref 11

\* $V_{max}$ , pmol·min<sup>-1</sup>·pmol<sup>-1</sup> CYP enzyme;  $K_m$ , μmol/L.

**Table 2.** Subject demographics (mean±SD).

	Cohort	Age (year)	Weight (kg)	Height (m)	BMI (m/kg <sup>2</sup> )
Observed subjects	20–50 years (n=10)	32 (23–43) *	61.5±8.6	1.64±0.07	22.3±2.1
	51–65 years (n=10)	57 (55–62)	73.9±13.4	1.66±0.07	26.9±4.7
	66–75 years (n=10)	72 (67–74)	67.4±9.4	1.57±0.08	27.3±2.5
	>76 years (n=10)	77 (76–80)	70.8±9.8	1.63±0.09	26.7±2.7
Virtual subjects	20–50 years (n=1000)	40 (20–50) *	59.0±10.5	1.63±6.83	22.0±3.4
	51–65 years (n=1000)	57 (51–65)	57.0±10.3	1.61±6.7	23.2±6.6
	66–75 years (n=1000)	69 (66–75)	56.0±10.4	1.59±6.5	22.1±3.6
	>76 years (n=1000)	77 (76–80)	54.0±9.6	1.57±6.73	22.6±3.7

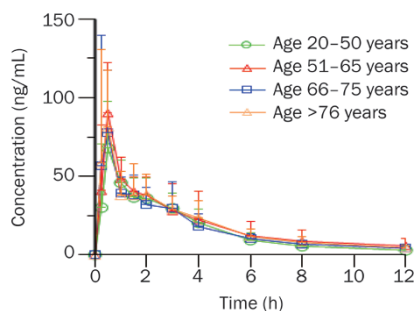
\*, range; BMI, body mass index.

**Table 3.** Pharmacokinetic parameters of the Chinese subjects after oral administration of 15 mg midazolam (mean±SD).

PK parameter	Group 1		Group 2		Group 3		Group 4	
	(Age 20–50 years)		(Age 51–65 years)		(Age 66–75 years)		(Age >76 years)	
	Male (n=5)	Female (n=5)	Male (n=5)	Female (n=5)	Male (n=5)	Female (n=5)	Male (n=5)	Female (n=5)
$C_{max}$ (ng/mL) <sup>a</sup>	68.6±17.5	88.4±25.3	100.3±29.5	95.7±25.1	80.5±18.5	115.4±52.6	100.7±69.3	92.9±51.0
$T_{max}$ (median, range)	1 (0.5–1.5)	0.5 (0.25–0.5)	0.5 (0.5–0.5)	0.5 (0.25–3)	0.5 (0.5–1.5)	0.5 (0.25–0.5)	0.5 (0.25–0.5)	0.5 (0.25–4)
$T_{1/2}$ (h)	2.9±0.9	3.6±1.0	3.7±0.6	6.1±0.9	4.7±1.3	4.8±1.9	3.8±0.9	4.4±1.3
$AUC_{0-t}$ (h·ng/mL)	223.8±52.5	199.3±55.6	274.3±123.4	278.0±167.0	195.7±74.8	262.2±42.9	233.8±77.8	274.2±100.3
CL/F (L/h)	70.0±16.0	80.7±24.8	63.1±24.7	66.7±28.9	84.8±28.4	64.3±20.4	70.1±22.9	61.9±25.4
V/F (L)	306.4±149.6	398.7±86.6	326.2±107.7	568.9±194.3	559.7±238.6	387.2±102.5	382.6±172.5	387.6±172.6

<sup>a</sup> $P>0.05$ .

files of midazolam in Chinese after oral administration are shown in Figure 2, and Table 4 summarizes the observed and predicted PK parameters. These data indicate that use of the SimCYP ADAM and full PBPK model resulted in the recovery of reasonable plasma concentration-time profiles in virtual Chinese. For the 20–50 years subjects, the predicted  $C_{max}$  and  $AUC_{0-t}$  were 63.4 ng/mL and 184.1 h·ng/mL, respectively. These values were in good agreement with the observed data of 68.7 ng/mL and 192.7 h·ng/mL. Moreover, for the middle- and old age subjects (51–65 years and 66–80 years), the predictions of  $C_{max}$  and AUC were also comparable to the observed values (Table 4).

**Figure 1.** Mean plasma concentration-time profiles of midazolam in Chinese subjects after oral administration (n=40).

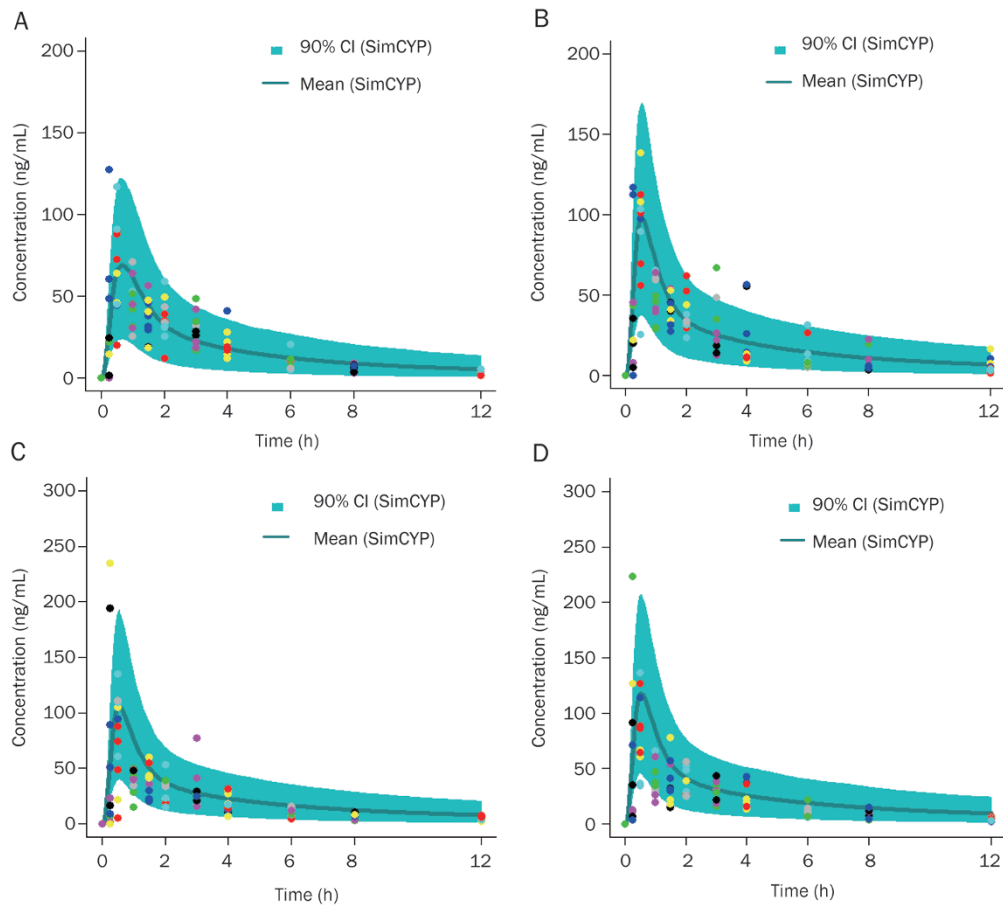
### Midazolam clearance prediction

After simulation in 1000 virtual Chinese for each age group (20–50, 51–65, 66–75 and >76 years), the predicted oral clearances were 81.5, 66.5, 60.7 and 53.8 L/h, respectively, and the observed values were 73.0, 59.8, 66.5 and 62.4 L/h, respectively. The ratios of predicted to observed clearances were between 0.8 and 1.25 (Table 4). These simulations demonstrated that the present midazolam PBPK model could recover midazolam clearance very well in Chinese.

Furthermore, we attempted to apply the PBPK model to investigate the effects of age and gender on midazolam pharmacokinetics in Chinese. After simulation in virtual Chinese subjects (n=1000, with a proportion of females of 0.5), we plotted the regression of AUC,  $C_{max}$  and CL versus age (Figure 3). These data indicated that predicted AUC,  $C_{max}$  and CL in virtual Chinese subjects were not closely related to age, consistent with our observations from the clinical study. Moreover, one-way ANOVA analysis demonstrated that there were no significant differences between female and male virtual subjects with regards to the predicted AUC,  $C_{max}$  and CL ( $P>0.05$ ).

### Discussion

CYP3A is one of the most important metabolic pathways and accounts for the metabolism of approximately 50%–60% of the drugs on the market<sup>[28]</sup>. Recently, Barter *et al* reported an



**Figure 2.** Simulated mean plasma concentration–time (line) and observed plasma concentration–time (points) profiles of midazolam in Chinese after oral administration. (A) 20–50 years; (B) 51–65 years; (C) 66–75 years; (D) >76 years. The blue line represents the 90th percentile of the virtual population.

ethnic-specific PBPK model for predicting CYP3A-mediated pharmacokinetics in Chinese and Caucasian populations<sup>[11]</sup>. Unfortunately, the predicted oral clearance of midazolam in Chinese was 2-fold higher than that in previous publications,

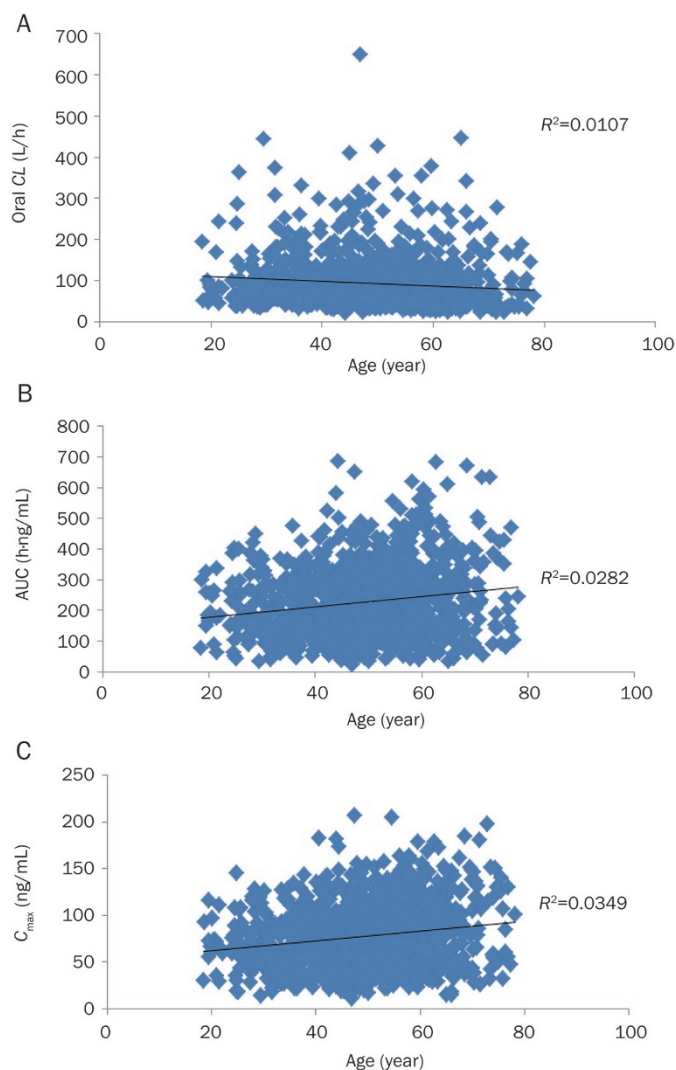
and the scarcity of quality of clinical data and appreciable interstudy variability in the outcome was assumed to potentially account for the overprediction.

In this study, we developed a midazolam PBPK model for

**Table 4.** Geometric mean of observed and predicted PK parameters (95%CI in brackets) in Chinese.

	PK Parameter	Observed	Predicted	Ratio*
Age 20–50 years	$C_{max}$ (ng/mL)	68.7 (51.6–91.3)	63.4 (61.5–65.4)	0.94
	$AUC_{0-t}$ (h·ng/mL)	192.7 (159.7–232.2)	184.1 (177.3–191.1)	0.96
	$CL_{po\#}$ (L/h)	73.0 (60.6–88.1)	81.5 (78.5–84.6)	1.12
Age 51–65 years	$C_{max}$ (ng/mL)	94.5 (76.7–116.3)	90.3 (87.7–92.9)	0.96
	$AUC_{0-t}$ (h·ng/mL)	218.8(164.6–290.8)	225.6(217.8–233.8)	1.03
	$CL_{po\#}$ (L/h)	59.8 (43.5–82.3)	66.5 (64.2–68.9)	1.11
Age 66–75 years	$C_{max}$ (ng/mL)	93.2 (64.6–134.2)	97.1 (94.3–99.6)	1.04
	$AUC_{0-t}$ (h·ng/mL)	199.6 (163.1–244.5)	247.3 (238.8–256.2)	1.23
	$CL_{po\#}$ (L/h)	66.5 (53.6–83.6)	60.7(58.6–62.8)	0.91
Age >76 years	$C_{max}$ (ng/mL)	83.1 (54.6–126.3)	108.8(105.7–111.2)	1.19
	$AUC_{0-t}$ (h·ng/mL)	215.3 (167.8–276.2)	278.7 (268.9–288.9)	1.29
	$CL_{po\#}$ (L/h)	62.4 (48.5–80.3)	53.8(53.9–58.8)	0.86

# $CL_{po}$  for oral administration; \*ratios of predicted to observed.



**Figure 3.** Relationships of the predicted oral CL, AUC and  $C_{max}$  to age ( $n=1000$ ).

CYP3A4/5 using the SimCYP Chinese population database, and we validated our model using a top-down study in which we performed a clinical study of forty Chinese subjects (23–80 years).

Furthermore, the PBPK model was used to investigate the effects of age and gender on midazolam pharmacokinetics in Chinese after oral administration.

#### Midazolam pharmacokinetics in Chinese after oral administration

The pharmacokinetics of orally administered midazolam were studied in 40 Chinese subjects from a wide age range (23–80 years). This is the first study to characterize midazolam PK properties in Chinese during middle and old age (>50 years). In this study, we demonstrated that midazolam was absorbed rapidly, with an overall time of peak concentration of 0.7 h after oral administration, consistent with the highly lipophilic properties of midazolam leading to rapid absorption. Statisti-

cal analysis indicated that there were no significant differences in system plasma exposure (AUC and  $C_{max}$ ),  $CL/F$  or  $V/F$  among the 4 age groups (20–50, 51–65, 66–75 and >76 years;  $P>0.05$ ). The same results were obtained with regards to gender ( $P>0.05$ ). These data suggest that midazolam pharmacokinetics in Chinese after oral administration are unaffected by either age or gender, and this conclusion is consistent with studies of Caucasian subjects<sup>[34–37]</sup>.

As an oral hypnotic, midazolam has been in the market for many years in China; however, there have been few pharmacokinetic studies<sup>[25–27]</sup>, and there is significant inter-study variability (Table 5). Surprisingly, double-peaks were observed in the midazolam plasma concentration-time profiles of some Chinese subjects given domestic midazolam tablets (NHwa Pharm Co China)<sup>[26]</sup>; however, this was not observed in other studies in which the drug was formulated as a solution or as a tablet from other suppliers<sup>[25, 27]</sup>. These studies suggested that effects of formulation should be included in PBPK modeling for oral midazolam.

#### Midazolam PBPK modeling

The Chinese database within the SimCYP population-based simulator was used to predict midazolam pharmacokinetics. Because the tablet used in the clinical study was formulated for immediate release, the SimCYP simulator first order model that treats the gut as a single compartment was used as the starting absorption model. Assuming that the  $F_a$  value (fraction of the drug available from dosage) was 1, the first-order absorption rate constant ( $k_a$ ) was input as 0.7 L/h and the lag time was 0.25 h. These values were estimated from the observed plasma concentration data; however, the simulation indicated that the first-order absorption model could not capture the midazolam plasma concentration-time profile. In contrast, the ADAM absorption model, which considered the impact of formulation, provided better predictions, especially when the midazolam dissolution profile was included. Given the high lipophilicity of midazolam and large volume of distribution, a full PBPK model was applied to improve the recovery of the plasma concentration-time profile. In contrast with the previous model for oral midazolam (2-fold over-prediction)<sup>[11]</sup>, this study contained two major modifications: (1) the single adjusting compartment (SAC) used previously was replaced by the full PBPK model with consideration of the high lipophilicity and large volume of distribution of midazolam, and (2) given the impact of formulation, *in vitro* midazolam dissolution profiles were acquired and incorporated into the ADAM model. Despite other potential factors (eg, intestinal transit time, stomach emptying time and variations in organ size and blood flow) that may influence drug absorption and distribution but were not considered in the PBPK model, the simulated plasma concentration-time profiles derived using the SimCYP Chinese population database were in good agreement with the observed data, as were the AUC and  $C_{max}$ . Furthermore, the ratio of the geometric mean of predicted clearance to observed clearance was between 0.8 and 1.25. These results demonstrated that despite lack of Chinese

**Table 5.** Summary of the midazolam PK in Chinese after oral administration.

PK parameter	Current study	Study 1	Study 2	Study 3
	Age 20–45 years (n=10) Dose 15 mg	Age 23±2.3 years (n=10) Dose 15 mg	Age 20–28 years (n=22) Dose 7.5 mg	Age 22–32 years (n=42) Dose 7.5 mg
$C_{max}$ (ng/mL)	73.5±27.3	103.1±26.4	53.4±22.1 (POR*28, CC) 57.6±14.6 (POR*28, CT) 60.7±19.3 (POR*28, TT)	30–40
$T_{max}$ (median)	0.5 (0.25–1.5)	1.5±0.7	0.5±0.2 (POR*28, CC) 0.4±0.1 (POR*28, CT) 0.4±0.1 (POR*28, TT)	0.5–2
$T_{1/2}$ (h)	3.3±0.9	3.0±0.8	2.3±0.8 (POR*28, CC) 2.0±0.4 (POR*28, CT) 2.1±0.4 (POR*28, TT)	NA
$AUC_{0-t}$ (h·ng/mL)	198.7±51.1	369.8±103.6	95.7±34.4 (POR*28, CC) 88.6±20.5 (POR*28, CT) 94.6±14.0 (POR*28, TT)	154.0±17.5 (CYP3A5*3) 132.2±12.8 (CYP3A5*1*3)
$AUC_{0-∞}$ (h·ng/mL)	211.6±52.6	397.3±124.1	104.8±39.6 (POR*28, CC) 93.7±23.4 (POR*28, CT) 100.1±14.6 (POR*28, TT)	NA
CL/F (L/h)	75.3±20.5	41.2±13.1	80.1±27.1 (POR*28, CC) 84.3±19.7 (POR*28, CT) 76.2±10.1 (POR*28, TT)	NA
Reference		[26]	[25]	[27]

NA: not available.

hepatic and gut CYP3A4/5 information, the PBPK model generated using the SimCYP Chinese population database could effectively recover the pharmacokinetics of midazolam, a typical probe of CYP3A4/5. Therefore, it is suggested that the current SimCYP Chinese population model incorporating limited CYP3A4/5 enzyme data from Chinese<sup>[11, 38, 39]</sup> might offer an opportunity for predicting CYP3A-mediated pharmacokinetics in Chinese.

#### Investigation of the effects on midazolam pharmacokinetics using the PBPK model

Previous studies in Caucasians demonstrated that the pharmacokinetics of midazolam were unaffected by age<sup>[34–37]</sup>; however, this topic has never been examined in Chinese. In this study, the verified PBPK model was used to investigate the effects of age on midazolam pharmacokinetics in Chinese from a physiological perspective.

Midazolam is a moderate extraction ratio drug and its clearance depends on hepatic blood flow and intrinsic clearance<sup>[40, 41]</sup>. Based on the simulation, liver blood flow (calculated as a 19% of the cardiac output<sup>[11]</sup>) appeared to be related to age (Figure 4A). In contrast, intrinsic CYP3A midazolam clearance was not expected to differ among elderly and young subjects as CYP3A activity did not change with age<sup>[42, 43]</sup>, and the PBPK model simulation supported this assumption (Figure 4B). Assuming that both  $F_a$  and  $F_g$  (fraction of drug entering enterocytes and escaping the first-pass gut wall metabolism) were unaffected by age, only protein binding was expected to affect midazolam bioavailability. As midazolam moderately binds to proteins<sup>[35]</sup>, we expected no difference in midazolam

exposure between young and elderly subjects. These hypotheses were supported by our simulations using virtual Chinese subjects; therefore, the current PBPK model can identify the effects of age on midazolam pharmacokinetics from a physiological perspective.

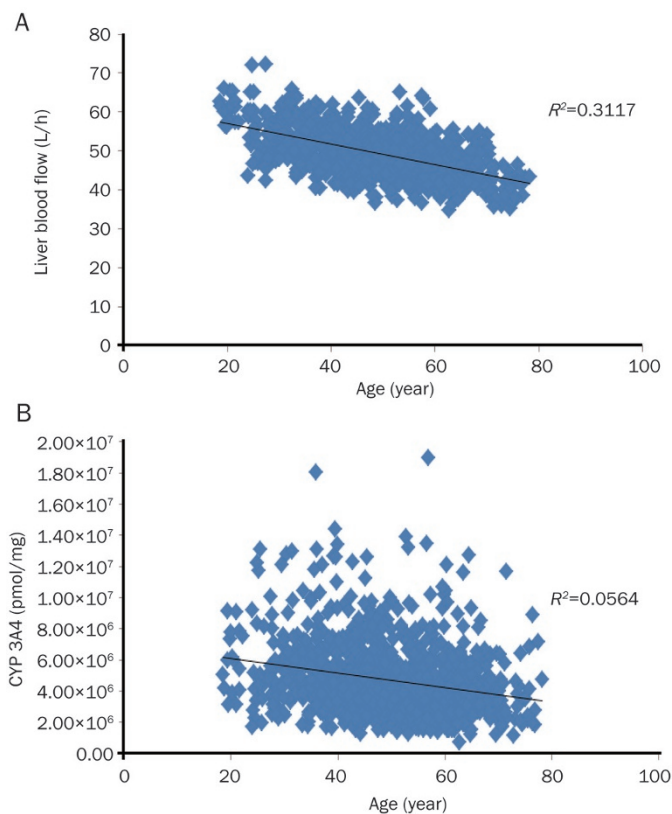
In summary, the SimCYP Chinese population model for midazolam was verified by comparing its predictions with our observations. Although there are limitations and assumptions regarding the abundances of hepatic and gut CYP3A4/5 in Chinese, it is believed that the Chinese population model of the SimCYP simulator can adequately predict CYP3A4/5-mediated pharmacokinetics in Chinese. Consequently, this capacity may lead to the early identification of ethnic sensitivity for the Chinese population, as well as the need for and design of real studies for drugs that are predominantly metabolized by CYP3A4/5. Notably, because limited data for the abundances of CYP3A4/5 in Chinese were used, this PBPK model is still evolving model and should be updated with additional physiological and genetic data. Moreover, use of this model for additional drugs may further demonstrate its strengths and weaknesses.

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#### Author contribution

Pei HU, Ji JIANG and Jun SHI designed the research; Xia



**Figure 4.** Relationship between simulated liver blood flow and CYP3A4 abundance ( $n=1000$ ).

CHEN and Hong-yun WANG performed the research; Hong-yun WANG analyzed the data and wrote the paper; Jun SHI revised the paper.

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