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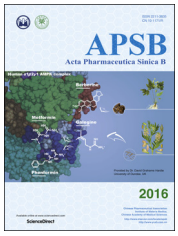

ORIGINAL ARTICLE

Implementation of a reference-scaled average bioequivalence approach for highly variable generic drug products of agomelatine in Chinese subjects

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7-Desmethyl-agomelatine;
Chinese subjects;
High variability;
Generic drug

Abstract The aim of this study was to apply the reference-scaled average bioequivalence (RSABE) approach to evaluate the bioequivalence of 2 formulations of agomelatine, and to investigate the pharmacokinetic properties of agomelatine in Chinese healthy male subjects. This was performed in a single-dose, randomized-sequence, open-label, four-way crossover study with a one-day washout period between doses. Healthy Chinese males were randomly assigned to receive 25 mg of either the test or reference formulation. The formulations were considered bioequivalent if 90% confidence intervals (CIs) for the log-transformed ratios and ratio of geometric means (GMR) of AUC and C_{max} of agomelatine were within the predetermined bioequivalence range based on RSABE method. Results showed that both of the 90% CIs for the log-transformed ratios of AUC and C_{max} of 7-desmethyl-agomelatine and 3-hydroxy-agomelatine were within the predetermined bioequivalence range. The 90% CIs for natural log-transformed ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of agomelatine (104.42–139.86, 101.33–123.83 and 97.90–117.94) were within the RSABE acceptance limits, and 3-hydroxy-agomelatine (105.55–123.03, 101.95–109.10 and 101.72–108.70) and 7-desmethyl-agomelatine (104.50–125.23, 102.36–111.50 and 101.62–110.64) were within the FDA bioequivalence definition intervals (0.80–1.25 for AUC and

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0.75–1.33 for C_{max}). The RSABE approach was successful in evaluating the bioequivalence of these two formulations.

1. Introduction

Agomelatine is a novel antidepressant for use in the European Union[1]. It is thought to act through a combination of antagonist activity at serotonin 5-HT_{2C} receptors and agonist activity at melatonergic MT_1/MT_2 receptors[2]. As such, its pharmacology is unique among licensed antidepressant drugs. In patients with major depression, agomelatine is as effective as paroxetine, setraline, venlafaxine and fluoxetine, with a lower relapse rate (23.9%) than placebo (50.0%)[3,4]. Agomelatine improves sleep quality and reduced waking after sleep onset in depressed patients[5,6]. At a therapeutic dose (25 mg once daily)[7], agomelatine preserves vigilance and memory in healthy volunteers[8]. Due to the risk of common liver enzyme elevation and rare serious liver complications, routine laboratory monitoring of liver function is recommended periodically throughout treatment[9].

The existing data on agomelatine metabolism, bioavailability and pharmacokinetics in Caucasians indicate that the absorption of agomelatine is rapid, with the median T_{max} 0.75–1.5 h and almost complete with at least 80% intestinal absorption[10,11]. However, the absolute oral bioavailability of this drug is low (approximately 3%–4%) and highly variable (estimated to 104%). These properties are attributed to the extensive first pass metabolism of agomelatine[7].

A systemically active generic drug is considered to be bioequivalent to the reference-listed drug if the rate and extent of absorption of the two products do not show a significant difference[12]. The US Food and Drug Administration (FDA) uses peak drug concentrations (C_{max}) in plasma or other appropriate biological fluid as an index of drug rate of absorption and the area under the drug plasma concentration *versus* time curve (AUC) as an index of a drug's extent of absorption[13]. Due to the highly variable features (highly variable drugs are defined as those for which within-subject variability [CV(%)] in bioequivalence measures is 30% or greater), a standard number of subjects (*e.g.*, 18–24) may not be able to demonstrate the bioequivalence of generic products or their corresponding reference product using a two-way crossover design. Although agomelatine pilot data are published for Caucasians, they may not be applicable to the bioequivalence in other populations due to ethnic differences. Pei et al.[14] investigated the intra-subject CV of agomelatine in healthy Chinese volunteers. Results showed notable intra-subject variability in AUC_{0-t} (CV = 43.52%) and C_{max} (CV = 78.34%). Wang et al.[15] evaluated the inter- and intra-individual variability in AUC and C_{max} of agomelatine tablets in Chinese healthy male subjects and found inter-individual CVs of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ to be 102.20%, 131.74% and 130.59%, respectively. The intra-individual CVs of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 84.34%, 49.61% and 50.83%, respectively. In preliminary experiments with a four-way crossover method, the within-subjects variability of AUC and C_{max} of agomelatine were 53% and 70%, respectively. Comparable values for 3-hydroxy-agomelagtine were 21.2% and 37.8%, and for 7-desmethyl-agomelatine were 42.6% and 61.4%. These results showed that although the within-subject CV of agomelatine could be reduced with a four-way crossover method, it was still difficult to evaluate the bioequivalence. Song et al.[16] found no differences in agomelatine pharmacokinetics between the rs2069514 GG homozygotes ($n = 35$) and the rs2069514 AG allele ($n = 35$) in all subjects, suggesting that the rs762551, rs2470890 and rs2472304 genetic polymorphisms might be associated with the marked inter-individual variability of agomelatine.

The topic of bioequivalence evaluation of highly variable drugs is one that has been intensely debated in many recent articles, conferences and meetings[17]. The FDA observed that studies of highly variable drugs generally used more subjects than studies of lower variability[18]. For the highly variable drug agomelatine, excessively large sample sizes would be required by a standard bioequivalence study, but the FDA discourages unnecessary human testing. These observations raise questions about the appropriate sample sizes for bioequivalence studies of drug products for which high variability does not appear to impact safety and efficacy. An additional concern is that the large sample sizes needed for bioequivalence studies of highly variable drugs may deter the development of new generic products[19,20]. A final concern is that a highly variable reference product may not be shown to be bioequivalent to itself in a crossover study using a relatively modest number of subjects (*e.g.*, 18–40)[21]. The commonly-accepted method for statistical analysis of bioequivalence data is the average bioequivalence (ABE) approach. Bioequivalence is established when the difference between the logarithmic means occur between preset regulatory limits, as shown below:

$$\left(\mu_T - \mu_R\right)^2 \le {\theta_A}^2 \quad (1)$$

where μ_T is the population average response of the log-transformed measure for the test (T) formulation, μ_R is the population average response of the log-transformed measure for the reference (R) formulation, and θ_A is equal to ln 1.25. So the limits are:

$$\ln 0.8 \le \left(\mu_T - \mu_R\right) \le \ln 1.25 \quad (2)$$

On one hand, only the average means of main pharmacokinetic parameters (*e.g.*, AUC and C_{max}) are taken into consideration in ABE method, and the individual variations of pharmacokinetic parameters are not considered. Thus, the two formulations showed ABE does not guarantee individuals' bioequivalence (IBE). On another hand, the bioequivalence criteria for the ABE method are identical for both low variability and high variability drugs.

For a time, the FDA worked toward implementing an individual bioequivalence (IBE) approach for studies submitted to New Drug Applications (NDAs) and Abbreviated New Drug Application (ANDAs, for generic drugs). It was argued that requiring drug products to meet an IBE rather than an ABE standard would improve formulation switchability[22,23]. The proposed criteria for acceptable IBE included the comparison of test and reference means, comparison of within-subject variances, assessment subject-by-formulation interactions, and ability to scale the bioequivalence limits if within-subject variability of the reference

product exceeded predetermined values. Under IBE, the inequality used to determine if two products are bioequivalent is as follows:

$$\frac{(\mu_T-\mu_R)^2+\sigma_D{}^2+(\sigma_{WT}{}^2-\sigma_{WR}{}^2)}{\sigma_{WR}{}^2}\le\theta_I \quad (3)$$

where $\sigma_D{}^2$ is the population subject-by-formulation interaction variance components, $\sigma_{WT}{}^2$ is the population within-subject variance of the test formulation, $\sigma_{WR}{}^2$ is the population within-subject variance of the reference formulation, and θ_I is the bioequivalence limit for IBE. From 1999 to 2001, at the FDA's request, the pharmaceutical industry applied the IBE study design and analysis to NDAs and ANDAs for modified-release drug products[24]. The IBE was used to evaluate the bioequivalence of modified-release drug products because it was thought that, due to the relative complexity of modified-release formulations, the likelihood was greatest of detecting subject-by-formulation interactions with these types of drug products. However, analysis of these data failed to detect the presence of clinically significant subject-by-formulation interactions[25].

To lower the sample size required for bioequivalence studies of highly variable drugs, the FDA and European Medicines Agency (EMA) have recommended the RSABE approach, whereby the bioequivalence acceptance limits are scaled to the variability of a reference product[26,27].

The RSABE for both AUC and C_{max} is evaluated as below:

$$\frac{(\mu_T-\mu_R)^2}{\sigma_{WR}{}^2}\le\theta_S \quad (4)$$

where μ_T is the population average response of the log-transformed measure for the test (T) formulation, μ_R is the population average response of the log-transformed measure for the reference (R) formulation, and $\sigma_{WR}{}^2$ is the population within-subject variance of the reference formulation, $\theta_S=\frac{(\ln 1.25)^2}{\sigma_{W0}{}^2}$ is the bioequivalence limits, and $\sigma_{W0}{}^2$ is a predetermined constant set by the regulatory agency.

Under this model, the implied limits (which represent FDA's desired consumer risk model) on $\mu_T-\mu_R$ are:

$$-\left(\ln 1.25\frac{\sigma_{WR}}{\sigma_{W0}}\right)\le\mu_T-\mu_R\le\ln 1.25\frac{\sigma_{WR}}{\sigma_{W0}} \quad (5)$$

If $\sigma_{WR}{}^2=\sigma_{W0}^2$, the implied limits are equal to the standard unscaled bioequivalence limits of $\pm\ln 1.25$ (0.80–1.25). If $\sigma_{WR}{}^2>\sigma_{W0}{}^2$, the implied limits are wider than the standard limits. If $\sigma_{WR}{}^2<\sigma_{W0}{}^2$, the implied limits are narrower than the standard limits.

The Agency has determined that it is acceptable for the implied limits to be wider than the standard limits only when σ_{WR} is large (as for highly variable drugs). The mixed scaling model is as shown below.

T and *R* are considered bioequivalent if:

$$\frac{(\mu_T-\mu_R)^2}{\sigma_{W0}{}^2}\le\frac{(\ln 1.25)^2}{\sigma_{W0}{}^2}\quad\text{if}\quad\sigma_{WR}\le\sigma_{W0} \quad (6)$$

and if:

$$\frac{(\mu_T-\mu_R)^2}{\sigma_{WR}{}^2}\le\frac{(\ln 1.25)^2}{\sigma_{W0}{}^2}\quad\text{if}\quad\sigma_{WR}\ge\sigma_{W0} \quad (7)$$

The FDA sets the value of σ_{W0} at 0.25[26,28].

The FDA implemented RSABE for highly variable generic drug wide therapeutic index to ease the regulatory burden. In the RSABE approach recommended by the FDA, the reference product is administered twice in order to determine its within-subject variability. As such, the bioequivalence study can use either a partial replicate (three-way crossover: RTR, RRT or TRR) or full replicate (four-way crossover: RTRT or TRTR) design, but should enroll a minimum of 24 subjects[28,29]. The implied bioequivalence limits scale to reference within-subject variability once $\sigma_{WR}=\sigma_{W0}=0.25$ or greater. However, to preserve an acceptable (<5%) type I error rate, applicants cannot apply reference scaling to calculate bioequivalence limits until the reference product within-subject SD in the bioequivalence study is at least 0.294.

Therefore, the aim of this study was to investigate the pharmacokinetic properties of agomaletine and the bioequivalence of a test agomelatine tablet (Chongqing FuAn Pharmaceutical Group Qingyutang Pharmaceutical Co., Ltd., Chongqing, China; lot No. 130301) and a reference agomelatine tablet (Servier, French; lot No. 893158) to obtain regulatory approval for the test formulation. In this study, we used the RSABE method to evaluate the bioequivalence of two formulations with parent agomelatine for the first time in healthy Chinese male subjects.

2. Materials and methods

2.1. Study design and procedures

A single-dose, randomized-sequence, open-label, four-way crossover study was conducted at the phase I Clinical Research Unit of the Third Xiangya Hospital of Central South University (Changsha, China) from November 2012 to April 2014. The study (Chinese National Registry Code: 2013L00911) was performed in accordance with the 2008 version of the World Medical Association Declaration of Helsinki[30], the International Conference on Harmonization Guideline for Good Clinical Practice[31], and the local regulatory guidelines of the State Food and Drug Administration (SFDA) of People's Republic of China[32]. The study protocol, protocol amendment, and informed-consent form were approved by the independent ethics and research committee at the Third Xiangya Hospital of Central South University prior to the initiation of the study. Before undergoing any study procedure, all participants provided written consent after they had been informed of the study's purpose, nature, procedures, and risks by clinical investigators.

A computer-generated random number table of SPSS 17.0 was applied to assign subjects in a ratio of 1:1 to receive a single 25-mg dose of (administered with 250 mL of tap water at room temperature) either the test or the reference formulation of agomelatine. Volunteers were admitted into the phase I clinical research unit at 9:00 p.m. the day before study and fasted 10 h before each drug administration. Neither caffeine-containing nor alcoholic beverages were allowed until 24 h after dosing. Smoking was forbidden during the same interval after the dose administration. As the $t_{1/2}$ of agomelatine is approximately 1–2 h, a one day washout period was used following administration of the initial formulation, after which the alternate formulation was administered. The design scheme of the study is summarized in Table 1.

2.2. Subjects

Formulations were considered bioequivalent if the 90% CIs for the log-transformed ratios and ratio of geometric means (GMR) of AUC and C_{max} of agomelatine were within the predetermined bioequivalence range based on RSABE method. Both the 90% CIs

Table 1 Study design for the bioequivalence evaluation of test and reference agomelatine tablet formulations.

Study period	Group	Agomelatine formulation administered
First	1	One tablet (25-mg dose) of agomelatine reference formulation
	2	One tablet (25-mg dose) of agomelatine test formulation
One day wash-out period		
Second	1	One tablet (25-mg dose) of agomelatine test formulation
	2	One tablet (25-mg dose) of agomelatine reference formulation
One day wash-out period		
Third	1	One tablet (25-mg dose) of agomelatine reference formulation
	2	One tablet (25-mg dose) of agomelatine test formulation
One day wash-out period		
Forth	1	One tablet (25-mg dose) of agomelatine test formulation
	2	One tablet (25-mg dose) of agomelatine reference formulation

for the log-transformed ratios of AUC and C_{max} of 7-desmethyl-agomelatine and 3-hydroxy-agomelatine were within the bioequivalence range of ABE method. The sample size was calculated by the within-subject variability (37.8%) of 3-hydroxy-agomelatine from pre-experiment as follows[33]:

$$n = \left[\left(t_\alpha + t_\beta\right)\sigma_d/\delta\right]^2 \quad (8)$$

where t_α is t value of the α inspection standards, t_β is the type II error rate. δ is the requirements of discrimination, and σ_d is the within-subject variability. As $t_\alpha = 1.6449$, $t_\beta = 1.2816$, $\delta = 0.2$, $\sigma_d = 0.378$, the sample size used was $n = 31$.

Based on the above, a minimum of 32 subjects were required. Taking into account the test management and lost cases, 44 subjects were enrolled in the four-way crossover study.

Forty-four healthy Chinese male volunteers aged 18–40 years with body mass indices (BMI) between 19 and 25 kg/m^2 were assessed for inclusion in the study. As females can be influenced by additional variables such as menstruation and pregnancy, the guidelines of the Chinese State Food and Drug Administration (SFDA) generally recommend selecting healthy males for bioequivalence studies[32]. Subjects were judged to be eligible for the study when no clinically significant abnormal findings existed on a complete medical examination. The exam included medical history, physical examination, 12-lead electrocardiogram, hematology, blood biochemistry and urinalysis.

2.3. Blood sampling

Blood samples (5 mL) were collected from a suitable forearm vein into anticoagulant tube by an indwelling catheter at the following time point: 0 (before administration), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0 and 8.0 h after drug administration. After washout and administration of the alternate formulation, blood samples were drawn and analyzed in the same way.

2.4. Tolerability assessments

Subjects were carefully monitored by vital signs (sitting blood pressure, heart rate, breathing rate, and oral body temperature), clinical laboratory tests (hematology, blood biochemistry, and urinalysis), 12-lead ECGs, and physical examinations at baseline and at the end of each study period. National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0 was used to describe and grade all toxicities and adverse events. The relationship of adverse events to study drug was documented by the investigator as unrelated or unlikely, possibly, probably, or definitely related.

2.5. Pharmacokinetic evaluations

An LC–MS/MS validated method for the simultaneous determinations of agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine concentrations in human plasma. The analytes were quantified by use of phenacetin as the internal standard. The plasma sample clean-up procedure was performed by liquid-liquid extraction. Aliquots (5 μL) were injected onto the analytical column (Phenomenex ODS3, 150 mm × 4.6 mm, 5 μm, USA). The mobile phase consisted of methanol and formic acid aqueous solution (20%) within 5 mmol/L ammonium formate (70:30, *v/v*) was delivered with a flow rate of 0.8 mL/min, with a run time of approximately 7 min. Positively charged ions, created at atmospheric pressure, were transferred to an Agilent 6460 triple-quadrupole LC–MS (Aligent, USA). The transitions for agomelatine were selected from *m/z* 244.1 → 185.1, 3-hydroxy-agomelatine from *m/z* 260.1 → 201.1, 7-desmethyl-agomelatine from *m/z* 230.1 → 171.1, and the internal standard from *m/z* 180.1 → 110.1.

2.6. Pharmacokinetic analysis

A non-compartmental analysis was used to determine the pharmacokinetic parameters using WinNonlin 6.1. C_{max} and T_{max} were obtained directly from the plasma concentration-time curves. The AUC_{0-t} was calculated according to the trapezoidal rule[34]. $AUC_{0-\infty}$ was calculated as follows:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/k_e \quad (9)$$

where C_t was the last measured concentration at time t, and k_e was the terminal elimination rate constant estimated by log-linear regression analysis of data visually assessed to be a terminal log-linear phase. At least 3 points were used for estimation of k_e. The apparent terminal elimination $t_{1/2}$ was calculated as follows:

$$t_{1/2} = 0.693/k_e \quad (10)$$

Intra-individual variability for the considered pharmacokinetic parameters was assessed by CV(%).

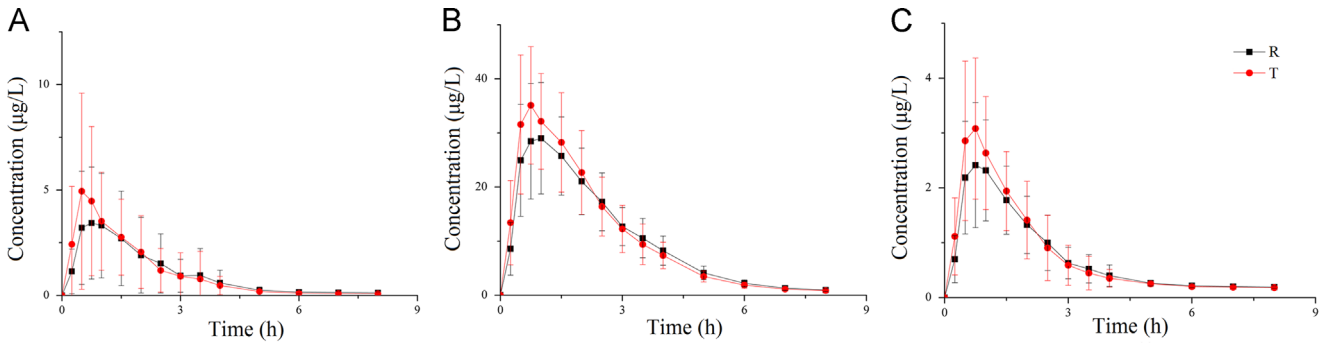

Figure 1 Plasma concentration-time curves of (A) agomelatine, (B) 3-hydroxy-agomelatine and (C) 7-desmehyl-agomelatine following a single 25-mg oral dose of a test (Chongqing FuAn Pharmaceutical Group Qingyutang Pharmaceutical Co., Ltd., Chongqing, China) or reference (Servier, French) formulation of agomelatine 25-mg tablet in healthy fasted Chinese adult males. Data are expressed as Mean (SD), $n=44$.

2.7. *Statistical analysis*

To test the bioequivalence of the formulations, ANOVA was performed on log-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. The nonparametric signed rank test was used to complete the T_{max} for the 2 formulations. $P \leq 0.05$ was considered statistically significant. The ratios of the log-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of parent agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine for both formulations were calculated, and 90% CIs were obtained. The probability of exceeding the limits of acceptance was obtained by two 1-side t tests. The 2 formulations were considered bioequivalent if the 90% CIs of the parent agomelatine of two formulations ratios of AUC and C_{max} were within the limits according to RSABE method shows below:

$$\text{Bioequivalence limits, upper, lower} = e^{\pm 0.223\sigma_{WR}/\sigma_{W0}} \quad (11)$$

For 3-hydroxy-agomelatine and 7-desmethyl-agomelatine, the test/reference ratios of AUC were within the predetermined bioequivalence range of 0.80 to 1.25 and C_{max} ratios were within 0.75–1.33, according to the guidelines of the SFDA of the China[32].

The bioequivalence assessment of the parent drug agomelatine was an essential goal of the present study. Evaluation of the bioequivalence of the two metabolites was considered as possibly supportive evidence for the bioequivalence of the parent drug.

3. Results

A total of 44 male subjects were enrolled in the study. Index, mean (range): age, 22.8 (2.5) years (range, 19–28 years); height, 170 (10) cm (range, 157–181 cm); weight, 60.5 (6.3) kg (range, 51–74 kg); BMI, 20.7 (1.6) kg/m^2 (range, 19.0–24.0 kg/m^2). Each subject received the test formulation and the reference formulation twice, respectively. All volunteers completed the study.

3.1. *Tolerability*

There were no protocol violations or serious adverse events observed in the study. Twenty subjects experienced a total of 37 mild adverse events in this four-way crossover study. The most frequently recorded were somnolence (17), dizziness (6), insomnia (6), epigastric pain (1). Somnolence, dizziness and insomnia were considered to be definitely related to the study treatment, and epigastric pain was considered to be probably related to the study medication. There were no withdrawals from the study due to adverse events.

3.2. *Method validation*

The calibration curves for agomelatine, 7-desmethyl-agomelatine and 3-hydroxy-agomelatine were linear over the concentration ranges of 0.0457–100 μg/L, 0.1372–300 μg/L and 0.4572–1000 μg/L in human plasma, respectively. The mean regression equation of the calibration curve for agomelatine was $Y=0.1188X-0.0005$ ($r^2=0.9962$), for 7-desmethyl-agomelatine was $Y=0.0734X-0.0003$ ($r^2=0.9975$), and for 3-hydroxy-agomelatine is $Y=0.0543X-0.0007$ ($r^2=0.9978$) with lower limits of quantitation being 0.0457, 0.1372 and 0.4572 μg/L, respectively. Precision values were all $<15\%$, and accuracy was between 85% and 115%. Technically, the assay for the determination of agomelatine and its metabolites from human plasma was highly reproducible, sensitive, and accurate method.

3.3. *Pharmacokinetic properties*

Following single 25-mg oral doses of the test and reference formulations, the mean plasma concentration–time curve of agomelatine, 3-hydroxy-agomelatine and 7-desmesthyl-agomelatine are shown in Fig. 1A–C, respectively. Mean pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, and $t_{1/2}$) and CV(%) are summarized in Table 2.

For the parent agomelatine, no period or sequence effects were detected for any pharmacokinetics properties on ANOVA. A significant subject effect was observed for AUC_{0-t}, $AUC_{0-\infty}$, C_{max}. There were no significant differences between the two formulations in regard to AUC_{0-t}, $AUC_{0-\infty}$, C_{max} or $t_{1/2}$ by two 1-side t tests, with the exception of T_{max} (1.44 [0.75] h for the test formulation and 1.22 [0.86] h for the reference formulation ($P<0.05$ by Mann–Whitney U test). For the metabolite 3-hydroxy-agomelatine and 7-desmethyl-agomelatine, no period, formulation, or sequence effects were observed for any pharmacokinetic properties by ANOVA, and there were no significant differences between the two formulations in AUC_{0-t}, $AUC_{0-\infty}$, C_{max} by two 1-side t test or in T_{max} by Mann–Whitney U test.

3.4. *Bioequivalence evaluation*

The 90% CIs of the ratios (T/R) for the log-transformed AUC_{0-t}, $AUC_{0-\infty}$, C_{max} are listed in Table 3. There were no significant differences between the test and reference formulations. The 90%

Table 2 Pharmacokinetic parameters and CV (%) of agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine after a single 25-mg oral dose of a test or a reference formulation of agomelatine 25-mg tablet in healthy fasted Chinese adult males.

Parameter	Agomelatine		3-Hydroxy-agomelatine		7-Desmethyl-agomelatine	
	Test	Reference	Test	Reference	Test	Reference
AUC_{0-t} (μg · h/L)	8.59 (10.17)	7.99 (10.15)	88.30 (31.81)	84.44 (32.49)	5.75 (2.64)	5.34 (2.38)
CV (%)	54.1	60.1	14.4	23.7	24.2	26.1
$AUC_{0-\infty}$ (μg · h/L)	8.72 (10.16)	8.31 (10.23)	89.78 (32.27)	86.11 (33.06)	6.16 (2.76)	5.76 (2.51)
CV (%)	52.6	54.7	14.4	23.4	23.1	25.1
C_{max} (μg · h/L)	7.55 (10.11)	5.74 (6.91)	50.09 (25.45)	43.30 (22.45)	4.43 (3.04)	3.77 (2.44)
CV (%)	84.4	80.0	43.9	42.0	53.5	46.7
T_{max} (h)	1.14 (0.75)	1.22 (0.86)	1.13 (0.72)	1.25 (0.81)	1.07 (0.72)	1.23 (0.80)
$t_{1/2}$ (h)	1.24 (1.40)	1.58 (1.32)	1.24 (0.24)	1.29 (0.26)	1.55 (1.97)	1.47 (0.92)

Data are expressed as Mean (SD), unless otherwise specified; $n=44$.

Table 3 Comparison of 90% CIs of natural log(ln)-transformed parameters of agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine for a test or reference formulation of agomelatine 25-mg tablet after a single 25-mg oral dose in healthy fasted Chinese adult males ($n=44$).

Parameter	Ratio	90% CI	Power
Agomelatine			
$\ln C_{max}$	1.21	1.04–1.40	0.808
$\ln AUC_{0-t}$	1.12	1.01–1.24	0.978
$\ln AUC_{0-\infty}$	1.07	0.98–1.18	0.989
3-Hydropxy-agomelatine			
$\ln C_{max}$	1.14	1.06–1.23	0.999
$\ln AUC_{0-t}$	1.05	1.02–1.10	1.000
$\ln AUC_{0-\infty}$	1.05	1.02–1.09	1.000
7-Desmethyl-agomelatine			
$\ln C_{max}$	1.14	1.05–1.25	0.991
$\ln AUC_{0-t}$	1.07	1.02–1.12	1.000
$\ln AUC_{0-\infty}$	1.06	1.02–1.11	1.000

CIs for natural log-transformed ratios of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ of agomelatine (104.42–139.86, 101.33–123.83, and 97.90–117.94, respectively) were within the RSABE acceptance limits (8.99%–204.13%, 59.48%–170.99%, 61.38%–162.91% for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, respectively). The metabolites 3-hydroxy-agomelatine and 7-desmethyl-agomelatine were within the predetermined regulatory 90% CI ranges for bioequivalence (80%–125% for AUC_{0-t} and $AUC_{0-\infty}$, 75–133% for C_{max} for the T/R ratio).

4. Discussion

According to US FDA guidelines[35], only the parent compound released from the formulation rather than the metabolite is generally recommended for bioequivalence studies. However, when a metabolite contributes meaningfully to the drug's pharmacologic effects or when a parent compound is difficult to analyze in plasma, metabolite quantification is also recommended. Although the pharmacokinetic parameters of agomelatine itself are the most essential criteria for bioequivalence evaluation, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine were assessed in the present study to provide supporting evidence. The median values of T_{max} for 3-hydroxy-agomelatine and 7-desmethyl-agomelatine confirmed the rapid disappearance of the parent compound which was comparable between the two formulations.

The FDA has recommended the RSABE approach to evaluate the bioequivalence of highly variable drugs (*e.g.*, agomelatine). Accordingly, the acceptance limits for such a study is to be scaled to the variability of the reference formulation. In the present study, we used the RSABE approach to assess the bioequivalence of two formulations of parent compounds for the first time in Chinese healthy male subjects. The standard criteria were used to evaluate the bioequivalence of the test formulation and the reference formulation, along with studies of the metabolites 3-hydroxy-agomelatine and 7-desmethyl-agomelatine.

The aim of this study was to apply the RSABE approach to evaluate the bioequivalence of 2 formulations of agomelatine, a drug with highly variable kinetics, and to investigate the pharmacokinetic properties of agomelatine in Chinese healthy male subjects. There are a few reports in the literature on the pharmacokinetics of agomelatine in Chinese population. Pei et al.[14] investigated the CV(%) of agomelatine in 16 Chinese healthy male volunteers and showed significant ethnic differences between Chinese and Caucasian subjects in C_{max} and AUC_{0-t} whereas no ethnic differences in T_{max} or $t_{1/2}$ were found. Less obvious first-pass effects in Chinese subjects may partially account for why both C_{max} and AUC of Chinese males were much higher than those of Caucasian males. In this study, the mean (SD) agomelatine and its metabolites AUC_{0-t}, T_{max}, and C_{max} for Chinese subjects (summarized in Table 3) are presented for the first time.

The 90% CIs of the test/reference ratios of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ for agomelatine and metabolites were all located within RASBE and the standard criteria range, respectively. The %CV of the main pharmacokinetic parameters of agomelatine and metabolites varied greatly. The large inter-subject variability in pharmacokinetic behavior observed in our study was consistent with the previous literature in other populations[7]. Agomelatine is rapidly absorbed from the gastrointestinal tract and immediately transported to the liver, where it is extensively metabolized by cytochrome P450 (CYP) isoenzymes CYP1A1, CYP1A2 and CYP2C9[36]. 7-Desmethyl-agomelatine and 3-hydroxy-agomelatine were identified as the two metabolites of agomelatine, which have less activity than the parent drug, and no significant differences of absorption and metabolism were found among agomelatine, 3-hydroxy-agomelatine and 7-desmethyl-agomelatine for the two formulations in 44 subjects.

The present study had several limitations that should be considered. The pharmacokinetic data of this study were obtained only from Chinese healthy males who were administered a single dose. Therefore, the pharmacokinetics might be different in other targeted populations or after other dosage regimens.

5. Conclusions

The RSABE approach was successfully applied to evaluate the bioequivalence of two formulations of the highly variable drug agomlatine in Chinese male volunteers. This study found that the test and reference formulations of aogmelatine 25-mg tablet met the regulatory definition.

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