Comparative Bioavailability and Tolerability of a Single 2-mg Dose of 2 Repaglinide Tablet Formulations in Fasting, Healthy Chinese Male Volunteers: An Open-Label, Randomized-Sequence, 2-Period Crossover Study

Xue-jia Zhai, MD¹, Kai Hu, MD², Fen Chen, MS¹, Yong-ning Lu, MD¹,∗

¹ Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People’s Republic of China
² China Ship Development & Design Center, Wuhan, People’s Republic of China

Abstract

Background: Repaglinide, an oral insulin secretagogue, was the first meglitinide analogue to be approved for use in patients with type 2 diabetes mellitus.

Objective: In our study, the bioavailability and tolerability of the proposed generic formulation with the established reference formulation of repaglinide 2 mg were compared in a fasting, healthy Chinese male population.

Methods: This 2-week, open-label, randomized-sequence, single-dose, 2-period crossover study was conducted in 22 healthy native Han Chinese male volunteers. Eligible subjects were randomly assigned in a 1:1 ratio to receive a single 2-mg dose of the test or reference formulation, followed by a 7-day washout period and administration of the alternate formulation. After an overnight fast, subjects received a single oral dose of repaglinide (2 mg). Blood samples were drawn at predetermined time points (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 hours). All plasma concentrations of repaglinide were measured by LC-MS/MS. The observed Cmax, T max, t 1/2, and AUC were assessed. The formulations were to be considered bioequivalent if the ln-transformed ratios of Cmax and AUC were within the predetermined bioequivalence range of 80% to 125% established by the State Food and Drug Administration of the People’s Republic of China. Tolerability was assessed throughout the study via subject interview, vital signs, and blood sampling.

Results: The mean (SD) age of the subjects was 24.2 (2.3) years; their mean (SD) weight was 62.6 (5.8) kg, their mean (SD) height was 172 (5.7) cm, and their mean (SD) body mass index was 21.0 (1.1). The mean (SD) Cmax for repaglinide with the test and reference formulations were 20.0 (5.1) and 18.7 (8.7) ng/mL. The AUC0–t for the test formulation was 46.3 (15.1) and AUC0–1 was 47.9 (16.5) ng·h/mL. With the reference formulation, the corresponding values were 46.4 (26.1) and 49.0 (31.3) ng·h/mL. The mean (SD) Tmax values with the test and reference formulations were 1.2 (0.7) hours and 1.5 (0.8) hours and the mean (SD) values t 1/2 values were 1.0 (0.3), and 0.9 (0.3) hours, respectively. The ln-transformed ratios of Cmax, AUC0–t, and AUC0–1 were 113.6:1, 105.6:1, and 104.7:1. The corresponding 90% CIs were 99.8 to 129.2, 93.4 to 119.5, and 91.8 to 119.5, respectively.

Conclusions: This single-dose study found that the test and reference formulations of repaglinide met the regulatory criteria for bioequivalence in these fasting, healthy Chinese male volunteers. Both formulations appeared to be well tolerated.

Introduction

Repaglinide is a prandial glucose regulator for the treatment of type 2 diabetes mellitus. As a short-acting insulin secretagogue, it reduces blood glucose concentrations by enhancing glucose-stimulated insulin release in pancreatic β-cells.1,2 Available clinical data indicate that repaglinide, given 2 to 4 times daily before each main meal, is an effective agent in the management of type 2 (noninsulin-dependent) diabetes mellitus. During short-
long-term administration repaglinide has been well tolerated and few patients have withdrawn from treatment because of adverse effects.\(^3,4\)

The pharmacokinetic profile of repaglinide has been reported in humans.\(^5\)–\(^7\) \(C_{\text{max}}\) is reached 30 to 60 minutes after administration. After reaching \(C_{\text{max}}\), plasma levels decrease rapidly and the drug is eliminated within 4 to 6 hours. Its absolute bioavailability is 63%, and its absorption is not affected by food. In humans, it is highly bound (< 98%) to plasma protein albumin. It is rapidly absorbed after oral administration with a bioavailability ~60%. During elimination, repaglinide is first taken up from the blood to hepatocytes by human organic anion–transporting polypeptides 1B1 (OATP1B1) expressed on the basolateral membrane of hepatocytes and then transformed into inactive metabolites via cytochrome P–450 (CYP) 2C8 and CYP3A4 in the liver, which are genetically polymorphic enzymes.\(^8\)–\(^11\)

Despite repaglinide having been in widespread clinical use for many years, there is little information on its pharmacokinetics in the Chinese population.\(^12\) A search of the literature for reports on the pharmacokinetics of repaglinide identified a small number of published pharmacokinetic studies in white patients.\(^3\)–\(^7\) Few reports were identified concerning the pharmacokinetic properties of repaglinide in a Chinese population.\(^12\)

Before allowing the marketing of generic repaglinide, the State Food and Drug Administration (SFDA) of China requires pharmacokinetic studies of the bioequivalence of generic and branded formulations. Therefore, the purpose of our single-dose study was to compare the bioavailability and tolerability of the proposed generic formulation with the established reference formulation of repaglinide in a fasting, healthy Chinese male population.

**Study Population and Methods**

**Trial design**

This single-center, randomized, open-label, 2-phase study was designed and conducted at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People’s Republic of China. The study was conducted according to the principles of the Declaration of Helsinki and its amendments for biomedical research involving human subjects and the principles of the Good Clinical Practice Guidelines. The clinical trial was approved by the SFDA (approval No. 2012LO1684), and the clinical protocol and the informed consent form were approved by the local ethics committee at Tongji Medical College, Huazhong University of Science and Technology (approval No. [2012]162). All eligible participants were informed of the aim and risks of the study by the clinical investigators and provided written informed consent before participation.

Subjects were hospitalized at 10 PM the night before the beginning of the study. They were randomly assigned, in a 1:1 ratio using a computer-generated table of random numbers, to receive a single 2-mg dose of the test formulation or the reference formulation of repaglinide under fasting condition during Period 1 and the alternate formulation during Period 2. The 2 periods of administration were separated by a 7-day washout period. The test formulation of repaglinide (1 mg; lot No. 120801) was provided by Sichuan Deren Pharmaceutical Co, Ltd (Sichuan, China). The reference formulation of repaglinide (1 mg; lot No. AM70752) was provided by Beijing Novo Nordisk Pharma Co Ltd (Beijing, China).

During each period, before administration of the drug, an indwelling venous catheter (Becton Dickinson Medical Devices Co, Ltd, Suzhou, People’s Republic of China) was placed in a suitable forearm vein, and a 5-mL blood sample was drawn into a vacuum tube with heparin sodium (Tianjin Biochemical Pharmaceutical Factory Co, Ltd, Tianjin, People’s Republic of China). Then, according to the US Food and Drug Administration guidelines on repaglinide, the patients were administered, under the supervision of study investigators, 2 repaglinide tablets orally with 250 mL 20% v/v glucose solution. Sixty milliliters of 20% v/v glucose solution were administered every 15 minutes until 4 hours after administration. Intake of food was not permitted after drug administration; a standardized lunch (200 g cooked rice, 200 g vegetables, 50 g pork, and 50 mL tomato soup) was provided at 4 hours after administration.

Additional blood samples were drawn at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 hours after administration. Plasma was obtained by centrifugation at 1000 g for 10 minutes at 5°C (LDZ5-2 Auto-balance Table Centrifuge, Beijing Medical Centrifuges Ltd, Beijing, People’s Republic of China) and stored at −80°C until analyzed using an LC-MS/MS method.

Intense physical activity, smoking, and consumption of beverages containing xanthine derivatives or alcohol were not allowed during the course of the study. Subjects were under continuous medical supervision at the study site throughout the 2-week study period.

**Inclusion and exclusion criteria**

Healthy, nonsmoking, male Han Chinese volunteers aged 18 to 40 years with body mass index 19 to 24 were enrolled in the study. Before study entry, subjects were interviewed (regarding their occupation, smoking and drinking habits, and medical history) and underwent a routine physical examination, including vital sign monitoring (ie, blood pressure, heart rate, respiratory rate, and temperature), ECG, chest radiograph, and laboratory analysis (ie, hematology, blood biochemistry, hepatic and renal function, and urinalysis) to ensure that they were healthy enough to participate in the study.

Subjects were excluded if they had a history or evidence of a renal, gastrointestinal, hepatic, or hemotologic abnormality; any acute or chronic disease; or an allergy to any chemicals. Subjects who had used drugs of any kind within the 2 weeks before the start of or during the study were excluded, as were those who consumed a moderate amount of alcohol daily (ie, > 1 L beer or its equivalent [ie, 50 g/day alcohol]).

**Determination of plasma concentrations**

The analysis of the concentrations of repaglinide in plasma was conducted at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People’s Republic of China, after the completion of both periods. The concentration of repaglinide in plasma was measured using a validated LC-MS/MS method. The LC-MS/MS system was a Shimadzu LC-30AD pump (Shimadzu, Kyoto, Japan) and a SIL-30AC autosampler (Shimadzu, Kyoto, Japan) coupled to an API QTRAP 5500 triple quadrupole mass spectrometer with an electrospray ionization source (AB Sciex, Concord, Ontario, Canada). The tandem mass spectrometer was operated under multiple reaction monitoring (MRM) using an electrospray atmospheric pressure ionization source in positive ion mode. The optimized condition consisted of a collision-activated dissociation gas of the medium, a curtain gas of 25 psi, a nebulizer gas of 50 psi, a TurbolonSpray gas of 50 psi, an ionspray voltage of +5500 V, a source temperature of 550°C, and an entrance potential of +10 V for repaglinide and the internal standard (nateglinide). Quantification was operated in MRM of the transitions m/z 453.1 → m/z 230.2 for repaglinide, and m/z 318.0 → m/z 166.1 for nateglinide. MRM data were acquired and the chromatograms were integrated with Analyst 1.6.1 software (AB Sciex, Concord, Ontario, Canada).
An aliquot of 20 μL internal standard solution (IS, nateglinide 2100 ng/mL in mobile phase) and 20 μL 50% v/v methanol-water were added to a 200 μL plasma sample in a screw-cap glass tube. After vortex mixing for 20 seconds, 600 μL acetonitrile was added to the mixture to precipitate protein and the sample was vortex mixed for 60 seconds and centrifuged at 13,000 rpm at 4°C for 10 minutes; 150 μL of the upper layer was transferred to an injection bottle that was loaded into an autosampler cabinet and 5 μL aliquot was injected into the LC-MS/MS system.

Chromatographic separation was performed on a Ultimate C18 column (150 mm x 2.1 mm, 5 μm; Welch Materials, Potomac, Md). The mobile phase consisted of acetonitrile: 0.1% aqueous acetic acid (70:30 v/v) at an isocratic flow rate of 0.3 mL/minute, and the injection volume was 5 μL and the run time was 4.5 minutes. The temperatures of the analytical column and autosampler were set at 40°C and 4°C, respectively. Under these conditions, the retention time for repaglinide and nateglinide were 3.72 minutes and 2.75 minutes, respectively.

Tolerability assessment

Tolerability was assessed using monitoring of vital signs (ie, blood pressure, body temperature, heart rate, and respiratory rate), physical examination, ECG, and routine blood and urine tests, along with blood biochemical tests (ie, hepatic and renal function), at the start as well as at the end of the study. Blood tests, along with blood biochemical tests (ie, hepatic and renal function), were measured at 3 concentration levels was acceptable (varied from 85.42%–114.71%) and the relative SD values were all <3.50%. Precision was likewise acceptable (between 1.66% and 8.23%). Repaglinide and the internal standard nateglinide were stable in plasma at room temperature for at least 24 hours, as well as for 30 days, at –80°C after 3 freeze–thaw cycles. The analytic method for repaglinide quantitation in plasma samples was validated and applied to the bioequivalence study according to international guidelines.

Repaglinide pharmacokinetics

Table I summarizes the mean [SD] pharmacokinetic parameters of repaglinide after single-dose administration of 2 2-mg tablet formulations in 22 healthy Chinese male volunteers. The mean [SD] Cmax for repaglinide with the test formulation was 20.0 [5.1] ng/mL and Tmax was 1.2 [0.7] hours. With the reference formulation, the corresponding values were 18.7 [8.7] ng/mL and 1.5 [0.8] hours, respectively. With ANOVA, no period or sequence effects were observed for any pharmacokinetic property. The mean plasma concentration-time profiles of the 2 formulations after administration of a single oral dose of repaglinide are shown in the Figure. Table II shows the 90% Cs of the ratios (test/reference) for the ln-transformed values of pharmacokinetic parameters, as well as the probability of exceeding the limits of acceptance for bioavailability and the power of the test in the 22 healthy Chinese male volunteers. The results were shown to meet the predetermined criteria for bioequivalence.

Method validation and stability testing

The method was shown to be suitable for the determination of repaglinide in human plasma over the range of 0.306 to 30.6 ng/mL (r = 0.999). Using weighted least-squares regression, the lower limit of quantitation was 0.306 ng/mL plasma. Accuracy measured at 3 concentration levels was acceptable (varied from 85.42%–114.71%) and the relative SD values were all <3.50%. Precision was likewise acceptable (between 1.66% and 8.23%). Repaglinide and the internal standard nateglinide were stable in plasma at room temperature for at least 24 hours, as well as for 30 days, at –80°C after 3 freeze–thaw cycles. The analytic method for repaglinide quantitation in plasma samples was validated and applied to the bioequivalence study according to international guidelines.

### Table I

| Parameter          | Reference (n = 22) | Test (n = 22) |
|--------------------|-------------------|--------------|
|                    | Mean (SD)         | Mean (SD)    |
| Cmax, ng/mL        | 18.7 (8.7)        | 20.0 (5.1)   |
| Tmax, h            | 1.5 (0.8)         | 1.2 (0.7)    |
| t1/2, h            | 0.9 (0.3)         | 1.0 (0.3)    |
| AUC0–t, ng·h·mL    | 46.4 (26.1)       | 463 (15.1)   |
| AUC0–∞, ng·h·mL    | 49.0 (31.3)       | 479 (16.5)   |
| AUC0–t/AUC0–∞      | 0.963 (0.04)      | 0.971 (0.02) |

### Table II

| Parameter          | Reference (n = 22) | Test (n = 22) |
|--------------------|-------------------|--------------|
|                    | Mean (90%)        | Mean (90%)   |
| Cmax, ng/mL        | 18.7 (8.7)        | 20.0 (5.1)   |
| Tmax, h            | 1.5 (0.8)         | 1.2 (0.7)    |
| t1/2, h            | 0.9 (0.3)         | 1.0 (0.3)    |
| AUC0–t, ng·h·mL    | 46.4 (26.1)       | 463 (15.1)   |
| AUC0–∞, ng·h·mL    | 49.0 (31.3)       | 479 (16.5)   |
| AUC0–t/AUC0–∞      | 0.963 (0.04)      | 0.971 (0.02) |

### Results

#### Subjects

A total of 22 subjects (mean [SD] age, 24.2 [2.3] years [range, 20–29 years]; weight, 62.6 [5.8] kg [range, 51–74 kg]; height, 172 [5.7] cm [range, 161–185 cm]) were enrolled in the study. All subjects completed both periods, with no protocol violations. Vital signs, physical examinations, ECGs, and clinical laboratory assessments remained within normal limits for all subjects, with no clinically meaningful differences between groups.
Repaglinide appeared to be well tolerated by all volunteers. No adverse events were reported by volunteers or found on analysis of vital signs or laboratory test results.

**Discussion**

Our study assessed the bioequivalence of single dose of 2 tablet formulations of repaglinide. There were no significant differences between formulations in pharmacokinetic properties in this small, selected, fasting, healthy Chinese male volunteer population. Although the branded repaglinide tablet formulation was already marketed in China, published information regarding the pharmacokinetics of repaglinide and the bioequivalence of these formulations in Chinese populations had never been reported. No significant differences were observed between the 2 oral formulations in terms of pharmacokinetic parameters.

The peak plasma concentrations occurred 1.2 to 1.5 hours after single-dose administration of 2 2-mg tablet formulations of repaglinide. Both formulations were apparently readily absorbed from the gastrointestinal tract, and repaglinide was measurable at the first sampling time (at 0.25 hour). The mean plasma profiles and bioavailability of the volunteers were similar between the 2 formulations of repaglinide in this single-dose pharmacokinetic analysis.

In our study, no period or sequence effects for any pharmacokinetic property were found using ANOVA in the healthy Chinese male volunteers. The absence of a sequence effect in both parameters suggests that there was no carryover effect for these 22 subjects. These results indicate that a washout period of 7 days was adequate for total elimination of the drug between the 2 administration periods.

There are several studies on the clinical pharmacokinetic properties of repaglinide published in the literature. However, most of them have been performed with Western volunteers. Pharmacokinetic studies of repaglinide conducted with Asian races have seldom been reported. In a study of pharmacokinetics of a single-dose administration of 2-mg tablet formulations of repaglinide in healthy American volunteers (N = 24), the pharmacokinetics of repaglinide were similar in healthy young adult and elderly subjects. In the study, the AUC for repaglinide was 69.0 ng·h/mL and Cmax was 47.9 ng/mL. The Tmax value was 0.8 hour, and the t1/2 value was 1 hour. In another pharmacokinetics study of a single-dose administration of 2-mg tablet formulations of repaglinide carried out in Germans, the AUC for repaglinide was 36.03 ng·hour/mL and Cmax was 30.96 ng/mL. The Tmax value was 0.8 hours. Thus, the repaglinide values of Cmax were higher in whites than in our Chinese subjects and the values of Tmax were much lower. However, drug was not administered with water containing glucose and followed by administration of oral glucose solution at specified intervals in the studies performed with Western volunteers. Therefore, additional glucose solution in our study may have resulted in the reduction of Cmax. However, the prolonged Tmax and changed AUC may be attributed to the difference between Asians and whites.

Repaglinide is first taken up from the blood to hepatocytes by OATP1B1 and then metabolized in the liver mainly by CYP3A4 and CYP2C8, which are genetically polymorphic enzymes. In 1 study in China,12 the results showed that the polymorphism of SLC01B1 (the encoding gene of the human OATP1B1) has significant influence on the pharmacokinetics of repaglinide. The SLC01B1 variants were found to be relatively common in Chinese patients. These frequencies were found to be similar to those observed in healthy Chinese and Japanese individuals, but significantly different from whites and blacks.13 The CYP2C8 enzyme, which is another genetic factor effect on the pharmacokinetics of repaglinide, has been mainly found in Africans (frequency, 0.04–0.18) and whites (frequency, 0.10–0.23), but such frequencies have not been observed in Asians.19 Some studies indicate that there are ethnic differences in the expression levels of adult liver CYP3A mRNAs between Japanese and whites, and that the mechanism regulating
the hepatic CYP3A expression may be different between these ethnic groups. In our study, the intersubject variation in the pharmacokinetics of repaglinide was relatively large. The high intersubject variation maybe explained by the natural interindividual variation in activity of OATP1B1, CYP3A4, and CYP2C8.

No subject withdrew from our study, and no adverse events were found on analysis of vital signs or laboratory test results. However, the study was limited by its short duration, its inclusion of only healthy male volunteers under fasted conditions, and by its single-dose, open-label design. The mean age of our healthy subjects was 24.2 years (range, 20–29 years) and, therefore, the study results cannot be extrapolated to an older population. The results also cannot be used to predict performance in patients in clinical practice.

Conclusions

Our single-dose study found that the test and reference formulations of repaglinide met the regulatory criteria for bioequivalence in these fasting, healthy Chinese male volunteers. Both formulations appeared to be well tolerated.

Acknowledgments

This research was sponsored by Sichuan Deren Pharmaceutical Co, Ltd, Sichuan, People’s Republic of China. The authors thank Sichuan Deren Pharmaceutical Co, Ltd for providing the repaglinide tablets used in this study. Dr. Zhai contributed to the study design, data interpretation, and writing. Dr. Hu provided data collection and analysis. Ms. Chen conducted the literature search and assisted with the study design. Dr. Lu contributed to the study design and provided consultation (including manuscript review before submission).

Conflicts of interest

This research was sponsored by Sichuan Deren Pharmaceutical Co, Ltd, Sichuan, People’s Republic of China. The sponsor ensured that the study was conducted according to clinical protocol but had no role in the design, conduct, analysis, or publication of the results. There were no benefits from commercial sources for the work reported in this article. The authors have indicated that they have no conflicts of interest regarding the content of this article.

References

1. Scott LJ. Repaglinide: a review of its use in type 2 diabetes mellitus. Drugs. 2012;72:249–272.
2. Raskin P. Oral combination therapy: repaglinide plus metformin for treatment of type 2 diabetes. Diabetes Obes Metab. 2008;10:1167–1177.
3. Johansen OE, Birkeland KI. Defining the role of repaglinide in the management of type 2 diabetes mellitus: a review. Am J Cardiovasc Drugs. 2007;7:319–335.
4. Germino FW. Noninsulin treatment of type 2 diabetes mellitus in geriatric patients: a review. Clin Ther. 2011;33:1868–1882.
5. Thomsen MS, Chassard D, Evéne E, et al. Pharmacokinetics of repaglinide in healthy Caucasian and Japanese subjects. J Clin Pharmacol. 2003;43:23–28.
6. Schumacher S, Abbas i, Weise D, et al. Single- and multiple-dose pharmacokinetics of repaglinide in patients with type 2 diabetes and renal impairment. Eur J Clin Pharmacol. 2001;57:147–152.
7. Hatop V, Huang WC, Strange P. Repaglinide pharmacokinetics in healthy young adult and elderly subjects. Clin Ther. 1999;21:702–710.
8. Bidstrup TB, Damkier P, Olsen AK, et al. The impact of CYP2C8 polymorphism and grapefruit juice on the pharmacokinetics of repaglinide. Br J Clin Pharmacol. 2006;61:49–57.
9. Hatop V, Hansen KT, Thomsen MS. Influence of drugs interacting with CYP3A4 on the pharmacokinetics, pharmacodynamics, and safety of the prandial glucose regulator repaglinide. J Clin Pharmacol. 2003;43:649–660.
10. Kalliokoski A, Backman JT, Kurkinen KJ, et al. Effects of gemfibrozil and atorvastatin on the pharmacokinetics of repaglinide in relation to SLC01B1 polymorphism. Clin Pharmacol Ther. 2008;84:488–496.
11. Kalliokoski A, Backman JT, NeuvenEN P, Niemi M. Effects of the SLC01B1*1B haplotype on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. Pharmacogenet Genomics. 2008;18:937–942.
12. Xiang Q, Cui YM, Zhao X, et al. The influence of MDR1 G2677T/a genetic polymorphisms on the pharmacokinetics of repaglinide in healthy Chinese volunteers. Pharmacology. 2012;89:105–110.
13. Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products-General Considerations. Silver Spring, Md: US Food and Drug Administration, Center for Drug Evaluation and Research; 2003.
14. Chen ZY, Zheng QS, Sun RY. Functions of the DAS software for pharmacological and the lipid-lowering effects of atorvastatin and simvastatin in Chinese individuals. Eur J Clin Pharmacol. 1998;36:636–641.
15. Fu Q, Li YP, Gao Y, et al. Lack of association between SLC01B1 polymorphism and the lipid-lowering effects of atorvastatin and simvastatin in Chinese individuals. Eur J Clin Pharmacol. 2013;69:1269–1274.
16. Totah RA, Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. Clin Pharmacol Ther. 2005;77:341–352.
17. Yamaori S, Yamazaki H, Iwano S, et al. Ethnic differences between Japanese and Caucasians in the expression levels of mRNAs for CYP3A4, CYP3A5 and CYP3A7, lack of co-regulation of the expression of CYP3A in Japanese livers. Xenobiota. 2005;35:69–83.