Evaluation of acarbose bioequivalence in healthy Chinese populations using novel pharmacodynamics endpoints

Linling Que¹, Zhenzhong Qian¹, Xuemei Xiang¹, Ying Ding¹, Kai Huang¹, Yichuan Bai¹, Huanan Zhao¹, and Qing He¹

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Abstract

Background and Objective Acarbose is a widely used α-glucosidase inhibitor to control postprandial hyperglycemia in type 2 diabetes mellitus patients. Recently, quite a few pilot studies on acarbose bioequivalence (BE) in Asian populations have defined new pharmacodynamic (PD) parameters as reliable endpoints. However, pivotal studies utilizing these new PD parameters were rarely reported. The study aims to explore acarbose BE using the new PD parameters and compare their applicability and sensitivity. Methods The study was conducted with an open, randomized, two-period crossover design using the test (T) or reference (R) drug at the dose of 2*50 mg. 64 subjects were recruited, with a one-week washout period. Serum glucose and insulin concentrations were determined after sucrose administration (baseline) and sucrose/acarbose co-administration. Results Using the parameters of rectifying method, which conducts pre-dose value deduction, the geometric mean ratios of Cmax,r and AUC0-2h,r were 102.91% and 105.29%, respectively. The 90% CIs of Cmax,r and AUC0-2h,r were all within acceptance limits (80.00-125.00%). The new parameters exhibited superior applicability and sensitivity in the evaluation of acarbose BE in healthy subjects. The incidence of AEs after the T drug or R drug was comparable, and healthy subjects were well tolerated. Conclusions The results from our study manifested that the PD parameters of the rectifying method demonstrate superior applicability and sensitivity in the evaluation of acarbose BE in healthy subjects. The T and R drug were bioequivalent using the novel PD parameters as primary endpoints, and both drugs demonstrated good safety and tolerability.

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**Methods**

The study was conducted with an open, randomized, two-period crossover design using the test (T) or reference (R) drug at the dose of 2*50 mg. 64 subjects were recruited, with a one-week washout period. Serum glucose and insulin concentrations were determined after sucrose administration (baseline) and sucrose/acarbose co-administration.

**Results**

Using the parameters of rectifying method, which conducts pre-dose value deduction, the geometric mean ratios of $C_{\text{max,r}}$ and $\text{AUC}_{0-2h,r}$ were 102.91% and 105.29%, respectively. The 90% CIs of $C_{\text{max,r}}$ and $\text{AUC}_{0-2h,r}$ were all within acceptance limits (80.00-125.00%). The new parameters exhibited superior applicability and sensitivity in the evaluation of acarbose BE in healthy subjects. The incidence of AEs after the T drug or R drug was comparable, and healthy subjects were well tolerated.

**Conclusions**

The results from our study manifested that the PD parameters of the rectifying method demonstrate superior applicability and sensitivity in the evaluation of acarbose BE in healthy subjects. The T and R drug were bioequivalent using the novel PD parameters as primary endpoints, and both drugs demonstrated good safety and tolerability.

**Abbreviations:**

The measured value method: $C_{\text{max}}$ (Maximum serum glucose concentration after co-administration of sucrose and acarbose) and $\text{AUC}_{(0-2h)}$ (The area under the curve of the serum glucose concentration-time profile 0-2h after co-administration of sucrose and acarbose), $\text{AUC}_{(0-4h)}$ (The area under the curve of the serum glucose concentration-time profile 0-4h after co-administration of sucrose and acarbose);

The difference method: $\Delta C_{\text{max}}$ (Maximum reduction in serum glucose concentration), $\text{AUEC}_{(0-2h)}$ (Reduction in the $\text{AUC}_{(0-2h)}$ of glucose between baseline and acarbose formulation), $\text{AUEC}_{(0-4h)}$ (Reduction in the $\text{AUC}_{(0-4h)}$ of glucose between baseline and acarbose formulation);

The ratio method: Radio $C_{\text{max}}$ (Ratio of maximum serum glucose concentration between sucrose-acarbose co-administration and baseline), Radio $\text{AUC}_{(0-2h)}$ (Ratio of $\text{AUC}_{(0-2h)}$ of glucose between sucrose-acarbose co-administration and baseline), Radio $\text{AUC}_{(0-4h)}$ (Ratio of $\text{AUC}_{(0-4h)}$ of glucose between sucrose-acarbose co-administration and baseline);

The rectifying method: $C_{\text{max},r}$, $\text{AUC}_{(0-2h),r}$, $\text{AUC}_{(0-4h),r}$ (Maximum serum glucose concentration with deduction of glucose concentration at 0h), $\text{AUC}_{(0-2h),r}$ after co-administration of sucrose and acarbose with deduction of baseline $\text{AUC}_{(0h),d}$, $\text{AUC}_{(0-4h),r}$ after co-administration of sucrose and acarbose with deduction of baseline $\text{AUC}_{(0h),d}$;

Insulin parameters: $\text{ISL}_{\text{max}}$ (Maximum serum insulin concentration after co-administration of sucrose and acarbose), $\text{ISL}_{\text{AUC}_{0-4h}}$ (The area under the curve of the serum insulin concentration-time profile 0-4h after co-administration of sucrose and acarbose)

**Keywords**

acarbose, bioequivalence, hypoglycemic effect, individual difference

**Introduction**

Type 2 diabetes mellitus is a growing concern worldwide, with more and more patients diagnosed at younger ages than in previous years. Acarbose is widely prescribed to treat type 2 diabetes mellitus as an adjunct to diet only or diet and exercise. It acts within the gastrointestinal tract as an intestinal $\alpha$-glucosidase
inhibitor to block digestion and absorption of starch, disaccharides and dextrin, leading to a blunting of the postprandial rise in blood glucose [1-2].

As a locally acting drug, less than 2% of acarbose gets absorbed. Due to this low systemic bioavailability, evaluation of its absorption rate and degree cannot be based on PK endpoints. Food and Drug Administration (FDA) of the United States of America (US) has issued draft guidance using PD parameters related to blood glucose level ΔC_{max} and AUEC_{0-4h} to evaluate acarbose BE since 2009[3]. However, the rationality of acarbose PD BE has not got fully explored. Since pharmaceutical manufacturers were allowed biowaiver of acarbose PD BE in abbreviated new drug application (ANDA) application based on certain requirements by both FDA and European Medicines and Healthcare Products Regulatory Agency (MHRA)[3-4].

National Medical Products Administration (NMPA) of China has required initiation of quality and efficacy consistency evaluation of generic drugs on Chinese pharmaceutical manufacturers since 2016, and re-evaluation of acarbose PD BE following the FDA guidance was one of them. In turn, it has re-aroused the attention on in vivo acarbose BE evaluation. Recently, quite a few articles discussing the rationality of the protocol design and primary PD parameters were reported[5-8]. The authors proposed new parameters with pre-dose value deduction instead of FDA parameters considering both applicability and sensitivity.

In 2022, FDA revised its guidance on acarbose BE. The main change was using the baseline-corrected maximum reduction in plasma glucose concentration C_{max} and baseline-corrected area under the plasma glucose reduction versus time curve through 2 hours as PD endpoints [9]. We have also published our current thoughts on the eligible protocol design of acarbose PD BE based on some subtly designed pilot studies. The change of using baseline corrected parameters and limiting the AUEC calculation under 2h after dosing is in accordance with the results of our previous pilot studies. The conclusions drawn from these pilot studies have not been validated in the main study. At the meantime, individual differences in response to acarbose treatment were observed. Strikingly, it has been reported around 13% of study population exhibited marginal hypoglycemic effect of acarbose [10], which brought a rising variance to the study. However, most of the data revealed were preliminary results from pilot studies. Reports on large populations from main studies were rarely found. Basing on the data from the main study, the current paper was to analyse the scientific rationality of the newly proposed parameters and the cause of individual differences in hypoglycemic effect in response to acarbose.

Methods

The studies were conducted at Wuxi People’s Hospital Affiliated to Nanjing Medical University, Jiangsu Province, China, and were performed in accordance with the principles of the World Medical Association (WMA) Declaration of Helsinki. The study protocols were approved by the Wuxi People’s Hospital Ethics Committee (Ethics approval No: 2018LLPJ-I-09 and 2018LLPJ-I-09-01). Written informed consent was obtained from each volunteer before screening process.

Acarbose Formulations

The reference formulation was Glucobay® 50 mg tablets produced by Bayer, Germany, batch number BXHLAJ1; the test formulation was acarbose 50 mg tablets produced by Beijing Bokangjian Gene Technology Co., Ltd., batch number 20171206.

Subjects

Eligible subjects were selected from healthy Chinese volunteers (including males and females). Inclusion and exclusion criteria were described as previous studies [7].

Study Protocol

The study design of the three studies was consistent. The studies were conducted with an open, randomized, two-period crossover design using the test (T) or reference (R) drug at the dose of 2*50 mg. The studies aimed to compare the BE between the R and T drug. 64 subjects were recruited with a one-week washout period.
For the sucrose challenge, subjects received 75 g sucrose dissolved in 150 mL of water (sucrose solution) followed by 100 mL of water. For sucrose/acarbose co-administration, subjects received the R or T drug at the dose of 2*50 mg with the sucrose solution followed by 100 mL of water in a randomized sequence.

Pharmacodynamic Analysis of Blood Samples

On the day of sucrose challenge and the day of sucrose/acarbose co-treatment, venous blood samples (4 mL) were collected from an intravenous indwelling catheter before dosing and at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 150, 180, 210, 240 min after dosing. After at least 30-minute clotting, serum was separated by centrifugation at 1300 g for 10 minutes at 20°C and stored at -20°C within 4 h after sample collecting and transferred to -80°C within 24 h.

Serum glucose concentrations were determined by the hexokinase method using Beckman Coulter AU680 with a lower limit of quantitation (LLOQ) of 0.56 mmol/L (calibration range, 0.56 to 44.40 mmol/L) as previously described [7]. The precision on three concentrations has been validated. The within-run precisions of glucose concentration at 3.32, 6.53, and 20.21 mmol/L were 1.18%, 1.52%, and 1.82%, respectively.

On the day prior to dosing and on the dosing day, plasma insulin concentrations were also tested before dosing and at 10, 20, 30, 40, 60, 90, 120, 240 min after dosing at clinic laboratory in Wuxi People’s Hospital.

Safety Assessments

Safety was assessed by subject interviews and adverse event (AE) monitoring according to National Cancer Institute Common Terminology Criteria Adverse Events (NCI CTCAE, Version 4.03). The clinical significance of abnormal laboratory test values was determined by physicians. If clinical abnormalities were present, further follow-ups were required until the laboratory test or vital sign of the abnormal items returned to a normal or stable state.

Data Analysis

Pharmacodynamic parameters were calculated using the noncompartmental model (NCA module), WinNonlin7.0 (Pharsight Corporation, Mountain View, CA, USA). Analysis of Variance (ANOVA) was performed on primary PD parameters, including the rectifying method ($C_{\text{max}, r}$, $AUC_{0-2h, r}$); secondary PD parameters, including the rectifying method ($AUC_{0-4h, r}$), the measured value method ($C_{\text{max}, r}$, $AUC_{0-2h}$, $AUC_{0-4h}$) and the measured value method of insulin level ($ISL C_{\text{max}, r}$ and $ISL AUC_{0-4h}$). In the ANOVA model, sequence, period, and treatment were fixed factors, and the subject within a sequence was assigned as a random factor. The geometric mean ratios (GMRs) and the corresponding 90% confidence intervals (CIs) of the T formulation to the R formulation were calculated for the PD parameters. The GMRs and the corresponding 90% CIs of the diarrhea group to the non-diarrhea group were also calculated to verify the correlation of diarrhea with PD parameters. When the 90% confidence intervals (CIs) for the T/R ratio fall between 80.00-125.00%, it will be considered equivalent. Intra-individual variations were also calculated. The statistical calculations were performed using the SAS software (Version 9.4).

Results

Characteristic of study participants

A total of 64 healthy volunteers were enrolled in this pivotal study. The baseline demographics of the participants were detailed in Table 1. All the participants were eligible for the inclusion and exclusion criteria. Except one subject who withdrew before taking pills, all subjects completed the study.

Pharmacodynamics Analysis

We compared the glucose lowering effect between the T drug and R drug. The serum glucose concentration-time profiles (Fig.1) showed that the sucrose load taken the day before the T drug or the R drug was almost coincident. The $C_{\text{max}}$ of serum glucose occurred between 30’50min after taking sucrose, and returned to baseline level around 2 h. The glucose curve manifested a down-regulation lower than the glucose level at 0 h during 2’4 h after sucrose taken. Addition of acarbose shortened the $T_{\text{max}}$ of serum glucose to 20’30min.
and decreased the $C_{\text{max}}$ of serum glucose by around 12.5%. Unlike the curve of sucrose load, the glucose level after acarbose fluctuated steadily around baseline between 2’4 h after dosing. The curve after the T drug was similar to that after the R drug. The insulin level was under negative feedback regulation of blood glucose. The serum insulin concentration-time profiles (Fig.2) manifested a similar changing process to serum glucose. The administration of acarbose also shortened the $T_{\text{max}}$ and decreased the $C_{\text{max}}$ of serum insulin. The curve was almost coincident between the T and R drug. The PD parameters were summarized in table 2. Both primary endpoints corrected by baseline were close between the T and R drug.

BE evaluation of the two groups is shown in Table 3. The geometric mean ratios of primary PD parameters $C_{\text{max},r}$ and $\text{AUC}_{0-2h,r}$ were 102.27% and 96.47%, respectively, and the geometric mean ratios of secondary PD parameters $\text{AUC}_{0-4h,r}$, $C_{\text{max}}$, $\text{AUC}_{0-2h}$, $\text{AUC}_{0-4h}$, ISLC$_{\text{max}}$, ISL_{AUC}$_{0-4h}$ were 97.55%, 100.52%, 99.21%, 99.62%, 101.16%, 96.17, respectively. The 90% CIs were all within acceptance limits (80.00-125.00%). The intra-individual variation of the baseline-corrected method was around 26%, which was consistent with the result of 25% in our previous pilot study. The intra-individual variations of the measured value method were less than 8%, and in the previous pilot study it was less than 10%. The results indicated bio-equivalence between the T and R drug utilizing the novel PD parameters.

Safety evaluation

Gastrointestinal diseases were the most common AEs occurred in this study. The incidences of other AEs were all below 5%. The results were coincident with results from pilot studies we reported before. The incidence of AEs after the T drug was slightly higher than the R drug (85.7% vs. 79.4%). All AEs were mild (Grade I) or moderate (Grade II). The outcomes of all AEs were back to normal. No subjects withdrew from the study due to AEs, and no serious AEs occurred.

Therefore, we concluded that the incidence of AEs after the T drug or R drug was comparable, and healthy subjects were well tolerated. The data were summarized in Table 4.

Discussion

The present study compared the bioequivalence between a test acarbose produced by Beijing Bo Kang Gene Technology Co., Ltd. and the reference drug. The results demonstrated that using the baseline-corrected pharmacodynamics endpoints $C_{\text{max},r}$ and $\text{AUC}_{0-2h,r}$ as primary endpoints, the T and R drug were bioequivalent. The 90% confidence intervals of the geometric mean ratios of all the secondary endpoints, including baseline-corrected $\text{AUC}_{0-4h,r}$, the measured value method ($C_{\text{max}}$, $\text{AUC}_{0-2h}$, $\text{AUC}_{0-4h}$) and the measured value method of insulin level (ISLC$_{\text{max}}$ and ISL_{AUC}$_{0-4h}$) between the T and R drug were within the range of 80.00%-125.00%. The data in this pivotal study were consistent with that in the pilot study we previously reported [7]. The results in our pivotal study revealed that the new baseline-corrected PD endpoints were practical for discriminating the differences between preparations. The PD parameters of the measured value method were almost the same between the two preparations with a particularly low intra-individual variation (less than 8%). The results proved again the measured value method was not sensitive enough to differentiate between preparations.

The inhibition of α-glucosidases delays carbohydrate digestion and absorption, and increases the amount of fermentable carbohydrate reaching the colon. This results in gastrointestinal symptoms, which seems to be another side of acarbose’s hypoglycemic effect. Acarbose’s effect on postprandial hyperglycemia was reported to be correlated with T2DM patients’ abdominal symptoms [11]. The more severe abdominal symptoms a subject had, the better effect on postprandial blood glucose acarbose manifested. We compared the primary PD endpoints among groups of different degrees of abdominal symptoms. However, no significant differences were found between groups using the t-test method ($p>0.05$) (data not shown). The previous study used T2DM patients and calculated M value [12] each day using the 7 measurements of blood glucose level. They designed a more sophisticated scale to monitor the abdominal symptoms. In our study, we used healthy populations and different PD parameters. The abdominal symptoms were just recorded as the frequency of diarrhea. The differences in study populations and variables may partially explain the contradictory results. In addition, we compared the primary PD endpoints in groups with and without diarrhea. The GMRs ratios

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between the diarrhea group and non-diarrhea group for C\textsubscript{max} and AUC
\textsubscript{0-2} were 88.61% and 89.29%, and the 90% CI of the GMRs for C\textsubscript{max} and AUC
\textsubscript{0-2} between the diarrhea group and the non-diarrhea group were 76.78−102.27 and 74.65−106.80. The primary PD endpoints were not bioequivalent between the two groups. The results indicated that the degree of diarrhea was somehow related to acarbose’s blood glucose lowering effects. However, the results need to be validated in large populations of T2DM patients using a more sophisticated evaluation of abdominal symptoms.

In our previous pilot studies, we observed that acarbose exerted minimal glucose lowering effect in around 16% of the subjects. In this pivotal study with 64 subjects, we’ve again observed some subjects insensitive to acarbose. Firstly, we calculated the baseline fluctuation of blood glucose C\textsubscript{max} and AUC
\textsubscript{0-2} on the sucrose loading day between 2 periods. The absolute value change for C\textsubscript{max} and AUC
\textsubscript{0-2} on the sucrose loading day between 2 periods were 0.66±0.57mmol*L\textsuperscript{-1} and 55.76+-43.32 min*mmol*L\textsuperscript{-1}, respectively and it was used as a cut-off to select the insensitive population. If the changes in blood glucose C\textsubscript{max} and AUC
\textsubscript{0-2} after acarbose treatment were less than the cut-off value, the subject was counted as insensitive. The results were summarized in table 5. Using a single cut-off value, around 20% of the subjects were defined as insensitive. Using combined cut-off values, around 10% of the subjects could still be defined as insensitive. We analyzed the correlation between the primary PD endpoints and various physiological indexes using multivariate analysis of variance. The results showed no significant correlation (data not shown). However, marked inter-individual variability in response to acarbose existed in healthy volunteers, which was also reported in T2DM patients\textsuperscript{[13-14]}. The underlying mechanism may be attributed to the different individual intestinal microenvironment. As acarbose was hardly absorbed through the microvillar membrane, it was retained in the intestinal lumen and transported to colon until excretion. The inter-reaction between acarbose and intestinal flora was thus predicted. On the one hand, long term use of acarbose in pre-diabetes, newly T2DM, and prevalent T2DM patients could modulate the composition of the fecal bacterial community\textsuperscript{[15-17]}, which generally produced a significant increase in lactobacillus and bifidobacterium, and a significant decrease in bacteroides \textsuperscript{[18]}. On the other hand, acarbose, as a pseudo-tetrasaccharide can be split by digestive enzymes and undergo biotransformation reactions in the gut. Some maltogenic amylase or glycosyltransferase catalyzing acarbose hydrolyzation and transglycosylation extracellularly have been reported\textsuperscript{[19-21]}. A novel \textalpha-glucosidase (RoaG1) from human gut microbiome has been found to interact with acarbose\textsuperscript{[22]}, this indicated a potential interaction between human gut flora and acarbose. In addition, a phosphotransferase (AcbK) can modify acarbose by phosphorylation at its 7-position and renders it much less active in \textalpha-glucosidase inhibition\textsuperscript{[23]}. A preliminary analysis of faecal samples from 16 T2DM patients treated with acarbose showed that the AcbK expression in flora macrogene was negatively related with the blood glucose lowering effect of acarbose \textsuperscript{[24]}. In summary, the interaction between acarbose and the intestinal flora may account for the significant inter-individual variances of its therapeutic effect. Metagenomic analysis of the gut microbiome, paired with detailed blood glucose lowering effect in a large number of patients or healthy volunteers treated with acarbose will be necessary to assess whether the carriage of some acarbose metabolizing genes predicts therapeutic efficacy.

Conclusions

In summary, the results of this pivotal study manifested that using the baseline-corrected pharmacodynamics endpoints C\textsubscript{max} and AUC
\textsubscript{0-2} as primary endpoints, the T and R drug were bioequivalent, and both drugs demonstrated good safety and tolerability. Marked inter-individual variability in response to acarbose was observed in our study and further assessment of the relating metabolizing genes expression in human gut flora with the clinical response after acarbose treatment may provide better insights into this question.

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Declarations of competing interest

None.
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