Research Article

Acarbose Bioequivalence: Exploration of New Pharmacodynamic Parameters

Min Zhang,¹ Jin Yang,^{1,4} Lei Tao,² Lingjun Li,² Pengcheng Ma,^{2,4} and John Paul Fawcett³

Received 14 December 2011; accepted 21 February 2012; published online 15 March 2012

Abstract. To investigate bioequivalence (BE) testing of an acarbose formulation in healthy Chinese volunteers through the use of recommended and innovative pharmacodynamic (PD) parameters. Following the Food and Drug Administration (FDA) guidance, a randomized, cross-over study of acarbose test (*T*) and reference (*R*) (Glucobay®) formulations was performed with a 1-week wash-out period. Preliminary pilot studies showed that the appropriate dose of acarbose was 2×50 mg, and the required number of subjects was 40. Serum glucose concentrations after sucrose administration (baseline) and co-administration of sucrose/acarbose on the following day were both determined. Three newly defined PD measures of glucose fluctuation (glucose excursion (GE), GE' (glucose excursion without the effect of the homeostatic glucose control), and fAUC (degree of fluctuation of serum glucose based on AUC)), the plateau glucose concentration (C_{ss}), and time of maximum reduction in glucose concentration (T_{max}) were tested in the evaluation. The adequacy of the two parameters recommended by the FDA, $\Delta C_{SG,max}$ (maximum reduction in serum glucose concentration) and $AUEC_{(0-4h)}$ (reduction in the $AUC_{(0-4h)}$ of glucose between baseline and acarbose formulation) was also evaluated. The T_{max} values were comparable, and the 90% confidence intervals of the geometric test/reference ratios (T/R) for $\Delta C_{SG,max}$, C_{ss} , GE, and fAUC were all within 80–125%. The parameter GE' was slightly outside the limits, and the parameter $AUEC_{(0-4h)}$ could not be computed due to the presence of negative values. In acarbose BE evaluation, while the recommended parameter $\Delta C_{SG,max}$ is valuable, the combination of C_{ss} and one of the newly defined glucose fluctuation parameters, GE, GE', and fAUC is preferable than $AUEC_{(0-4h)}$. The acarbose test formulation can be initially considered to be bioequivalent to Glucobay®.

KEY WORDS: acarbose; bioequivalence (BE); degree of fluctuation of serum glucose based on AUC (fAUC); glucose excursion (GE); pharmacodynamic.

INTRODUCTION

Acarbose, a biosynthetic hypoglycemic drug used to treat type 2 diabetes, was the first α -glucosidase inhibitor approved by the US Food and Drug Administration (FDA). It reduces production of monosaccharides and their absorption from the gut by inhibiting α -glucosidase in the brush border of the small intestine. Acarbose leads to a significant lowering of postprandial blood glucose levels after dietary intake of carbohydrates. The initial oral dosage of 50 mg once daily may be increased to 200 mg three times daily if necessary.

Being a very hydrophilic polyol, acarbose has extremely low bioavailability due to poor absorption from the gastrointestinal tract, its intended primary target. This means that bioequivalence (BE) testing of generic acarbose formulations cannot be based on a pharmacokinetic endpoint. Of the other means of demonstrating BE based on pharmacodynamic

(PD), clinical, or *in vitro* endpoints (1), the hypoglycemic action of acarbose offers a convenient and accessible method. The question of how best to demonstrate BE based on this hypoglycemic action is the subject of this paper.

Bae *et al.* (2007) applied this PD-based BE method to acarbose tablets in a placebo-controlled cross-over (3×3) study in 23 healthy volunteers (2). The subjects received a single oral dose of placebo, reference drug, or test drug just before breakfast with strict dietary control. The serum glucose concentration *versus* time profile after placebo treatment was taken as baseline from which differences between placebo and formulations were analyzed by ANOVA. However, BE statistical results were not given in the paper, nor was a clear conclusion drawn as to whether the two formulations were bioequivalent. Nevertheless, this study facilitated the development of a new practical protocol for acarbose BE evaluation.

In 2009, the FDA released draft guidelines for acarbose BE evaluation based on PD parameters (3). Since acarbose reduces the serum glucose level produced by a dose of sucrose, it was recommended that BE should be based on a comparison of the areas under the glucose concentration *versus* time curves over 4 h ($AUC_{(0-4h)}$) following simultaneous acarbose/sucrose administration and following sucrose

¹ Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, 210009, China.

² Dermatological Hospital, Chinese Academy of Medical Sciences, Nanjing, 210042, China.

³ School of Pharmacy, University of Otago, Dunedin, New Zealand.

⁴ To whom correspondence should be addressed. (e-mail: yjcpu@yahoo.cn; mpc815@163.com)

administration alone (serum glucose baseline). The recommended evaluation parameters were (1) $\Delta C_{SG,max}$, the maximum reduction in serum glucose concentration and (2) $AUEC_{(0-4h)}$, the reduction in the $AUC_{(0-4h)}$ from baseline subsequent to co-administration of acarbose/sucrose. Subjects were to receive 75 g sucrose on the day prior to administration of the first acarbose formulation to determine the serum glucose baseline followed by the acarbose formulations together with 75 g sucrose on the assessment days. The recommended protocol was a randomized, balanced, two-way cross-over design with a 1-week washout period between treatments. It was also recommended that pilot studies be carried out at the smallest dose of acarbose to determine the appropriate dosage and the number of subjects for the pivotal study.

At the time the FDA formulated its draft guidelines, Koytchev *et al.* (2009) reported a pivotal BE trial of acarbose incorporating some features of the recommended protocol (4). They showed that sucrose (rather than starch) was the most appropriate carbohydrate load, and a dose of 100 mg acarbose was the most appropriate dose. However, they used the baseline-adjusted area under the breath hydrogen response as their PD endpoint and determined that 100 subjects would be needed to prove BE. Since no other BE study of acarbose has been reported, we decided there was a need to validate the method and BE parameters recommended by the FDA. This paper reports the results of a pivotal study performed subsequent to a cross-over pilot study in four subjects and a parallel pilot study in 22 subjects. In the pivotal study in 40 subjects, some newly defined statistical parameters for BE evaluation were investigated along with those recommended by the FDA.

MATERIALS AND METHODS

Acarbose Formulations

The reference formulation (R) was Glucobay® 50 mg tablets produced by Bayer, Germany, batch number 119084; the test formulation (T) was acarbose 50 mg tablets produced by Zhejiang Hisun Pharmaceutical Co., Ltd, batch number 10053101.

Study Protocol

Eligible subjects were selected from healthy Chinese male volunteers aged 19–28 years, body weight ≥ 50 kg, and BMI 19–24 kg/m². A full medical examination was performed on all subjects including a physical examination, biochemical tests (routine blood and urine chemistry), and electrocardiogram. An oral glucose tolerance test was also performed in order to ensure normal oral glucose tolerance. In all subjects, fasting blood glucose was ≤ 6.1 mmol/L and postprandial glucose concentration at 2 h (2hPBG) ≤ 7.8 mmol/L. Medical history was obtained, and those reporting allergic reactions to acarbose were excluded from the study.

Participants were required to avoid all drugs for 2 weeks before and throughout the study period and to abstain from drink alcoholic beverages or coffee from 1 week before until the whole study ended. Standard meals were supplied from the time subjects were hospitalized 1 day before the study

began. Diet and exercise were strictly controlled, and any excessive exertion or lying supine for long periods were not allowed. Before each treatment, the subjects observed an overnight fast for at least 10 h.

The whole study involved the pilot studies and the randomized, balanced, two-way cross-over pivotal study with a 1-week washout period. The pilot studies included a randomized, balanced, two-way cross-over study in four subjects and a parallel study in 22 subjects both at a dose of 50 mg. In the cross-over study, the four subjects received the acarbose formulations in the sequence T then R or R then T; in the parallel study in the 22 subjects, 11 subjects received the T formulation and the other 11 received the R formulation.

Subject to a successful parallel study at a dose of 50 mg, the intention was to proceed to a cross-over study in these subjects in order to determine the within-subject variability (coefficient of variation (CV)) from which to predict the sample size for the pivotal study. However, since the 50-mg dose was found to be inadequate in the parallel study, it was considered appropriate to use a dose of at least 2×50 mg for the pivotal study and perform it using a sample size determined by the CV of the first pilot study.

In the pivotal trial, a baseline sucrose challenge was performed on the day prior to each of the drug treatment. For this challenge, subjects received 75 g sucrose dissolved in 150 mL water followed by 100 mL water. Venous blood samples (3 mL) were collected from an intravenous indwelling catheter prior to the dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h after the dose. For the subsequent drug administrations, subjects were randomized to receive acarbose together with 75 g sucrose following the sequence T then R or R then T with a 1-week wash-out period. Drug was administered with 100 mL water, and blood was then sampled in the same way as after sucrose alone. Subjects were not allowed to drink water for 2 h and were given a standard lunch 4 h after sucrose and sucrose/acarbose administration. Blood samples were drawn into BD Vacutainers® (SST™ II Advance REF 367957) with no anticoagulant. After clotting, serum was separated by centrifugation at 4,000 rpm for 15 min and stored in BD Vacutainers® at 4°C until analysis within 48 h.

The studies, conducted at the Dermatological Hospital Affiliated to Chinese Academy of Medical Sciences, Nanjing, China, were performed in accordance with the principles of the WMA Declaration of Helsinki. The protocols were approved by the Chinese SFDA (State Food and Drug Administration) and the Hospital Ethics Committee. Before the study, written informed consent was obtained from all subjects after potential adverse reactions were clearly described.

PD Parameters

The statistical parameters for BE evaluation recommended by the FDA ($\Delta C_{SG,max}$ and $AUEC_{(0-4h)}$) are both baseline-adjusted. To demonstrate BE between the T and R formulations, the 90% confidence intervals (90% CIs) of the geometric test/reference (T/R) ratios for $AUEC_{(0-4h)}$ and $\Delta C_{SG,max}$ should fall within the range 80–125%.

In addition to the recommended parameters, we defined others in an attempt to more accurately assess therapeutic BE between the two acarbose products. These parameters are glucose excursion (GE), GE' (glucose excursion without the effect of the homeostatic glucose control), and fAUC (the degree of fluctuation in serum glucose concentration based on $AUC_{(0-4h)}$). GE is calculated as the difference between the peak (C_{max}) and trough (C_{min}) serum glucose concentrations in the 4-h study period:

The parameter GE' is calculated as follows:

$$GE' = C_{max} - C'_{min}$$

where C'_{min} is the minimum glucose concentration in the time interval $0-t_{max}$ where t_{max} is the time at which the C_{max} is reached.

fAUC is calculated as follows:

$$fAUC = AUC(C \geq C_{ss}) + AUC(C \leq C_{ss})$$

$$C_{ss} = AUC_{\tau} / \tau.$$

where $AUC(C \geq C_{ss})$ is the AUC for concentrations $\geq C_{ss}$, $AUC(C \leq C_{ss})$ is the AUC for concentrations $\leq C_{ss}$ and C_{ss} is the plateau concentration of glucose in each case. In our study, τ was 4 h. The definition is made visual in Fig. 1.

We also compared the parameter T_{max} , defined as the time of maximum glucose reduction. The 90% CIs of the geometric T/R ratios for $\Delta C_{SG,max}$, $AUEC_{(0-4h)}$, C_{ss} , GE, GE', and fAUC were calculated using the two one-sided t tests after In-transformation of the data. The presence of a sequence effect was determined from the mean square ratios of the sequence and the subject (sequence) using the subject (sequence) as the error term. The non-parametric Wilcoxon signed-rank test was used to test differences in the non-transformed T_{max} values for the two formulations.

RESULTS

All subjects completed the two pilot studies. In the cross-over study in four subjects, the CV of the In-transformed $\Delta C_{SG,max}$ values was approximately 34% with a 20% difference in $\Delta C_{SG,max}$ between the two formulations. On

Fig. 1. Glucose concentration (millimoles per liter) versus time (hours) where C_{ss} is the plateau concentration of glucose and fAUC is the sum of the shaded areas

this basis, the number of subjects needed to detect this difference in the pivotal study with 80% power at the 5% level of significance was 40. As for the $AUEC_{(0-4h)}$ parameter, one of the eight values obtained was negative.

In the parallel study in 22 subjects, the average glucose level showed only a small hypoglycemic effect of acarbose (Fig. 2), indicating the 50-mg dose was inadequate. The dose needed to elicit a measurable response relative to baseline in the pivotal study was therefore deemed to be at least 2×50 mg. The sample size needed in the pivotal study at this dose is somewhat smaller than the 40 predicted by the first pilot study because, in a cross-over study at this higher dose, the signal-to-noise is perforce higher than that observed at 50 mg dose and consequently, the corresponding CV will be reduced. While the predicted sample size of 40 is therefore likely to be more than adequate, it would nevertheless be preferable to predict the number of participants for the pivotal study based on a larger pilot study.

All of the 40 enrolled subjects successfully completed the pivotal study. The serum glucose concentration versus time curves (Fig. 3) show a definite hypoglycemic effect after administration of 2×50 mg acarbose tablets.

Values of the parameters recommended by the FDA for BE testing of acarbose formulations ($\Delta C_{SG,max}$ (maximum reduction in serum glucose concentration), and $AUEC_{(0-4h)}$) and the related parameter $AUC_{(0-4h)}$ are listed in Table I.

As a large percentage of subjects gave negative values of $AUEC_{(0-4h)}$ for both the R and T formulations (35% versus 45% respectively), the GeOM of $AUEC_{(0-4h)}$ could not be determined. Values for parameters in our expanded approach to BE testing ($\Delta C_{SG,max}$, C_{ss} , GE, GE', fAUC, and T_{max}) are summarized in Table II where the sequence differences (T_{max} excluded) and corresponding P values are also given.

After subject 5 received sucrose alone, the glucose concentration of the first serum sample was C_{max} , i.e., t_{max} was 0 h. This resulted in a zero value for the corresponding GE'. Similar results were obtained for subjects 6 and 17 after acarbose/sucrose administration. Reasons for the very early C_{max} values in these subjects could not be ascertained.

As shown in Table II, the 90% CI of GE' after sucrose administration was in the range 80–125%, but this was not the

Fig. 2. Average serum glucose concentration versus time curves for baseline and simultaneous acarbose/sucrose co-administration (either Glucobay® or the test formulation) in the parallel study in 22 subjects (acarbose dose, 50 mg)

Fig. 3. Serum glucose concentration *versus* time curves for **a** baseline (sucrose alone) and acarbose reference formulation (Glucobay®)+sucrose and **b** baseline and acarbose test formulation+sucrose. Data are mean±SD ($n=40$)

case after acarbose/sucrose administration. The 90% CIs of the remaining four parameters, $\Delta C_{SG,max}$, C_{ss} , GE, and fAUC, were all within the acceptance range for both administrations. The P value for the non-parametric Wilcoxon signed-rank test of T_{max} was 0.643, showing that the T_{max} values were not significantly different for the two formulations. The P values for the other parameters also indicated that the sequence difference was not significant between the two formulations. Although the P value of the baselines for GE' was significant at 0.03, the actual difference of 11.7% could still be accepted, and it may be due to the exclusion of the three extreme values.

Post-study calculations of the number of subjects required to achieve 80% statistical power ($\beta=20\%$) for the five parameters (T_{max} excluded) based on their respective within-subject variations are shown in Table III.

Safety was assessed based on adverse events (AEs). The observed gastrointestinal AEs were diarrhea, flatus, and moderate abdominal pain with diarrhea combined with watery stool being the most common. This osmotic diarrhea and flatus are related to the pharmacological action of acarbose. The incidence of AEs after T was a little higher than after R (35% *vs.* 22.5%), but none of the subjects suffered a serious AE, and all AEs disappeared within 2–3 days. Therefore, we conclude that both acarbose formulations were well tolerated. In fact, a number of abnormal initial biochemical indices in several individuals returned to normal in less than 16 days.

DISCUSSION

FDA Guidelines

As a disaccharide, sucrose cannot be systemically absorbed unless it is hydrolyzed to glucose and fructose by α -glucosidase. After a dose of acarbose, the decrease in absorption of glucose produced from sucrose reflects the activity of α -glucosidase and indirectly reflects the efficacy of the drug. Thus, in the FDA guidelines for demonstrating acarbose BE published in 2009, administration of sucrose was recommended to provide a better baseline measure of α -glucosidase activity. As found by Koytchev *et al.* (2009), administration of sucrose is more suitable than eating a meal since it produces a more reproducible change in serum glucose concentration (4).

The FDA also recommended that the lowest acarbose dose producing a measurable PD response relative to baseline should be validated. To do this, the first dose to be tested should be the smallest denomination of the two formulations available, in this case, 50 mg. The change in serum glucose observed in the pivotal study showed that 2×50 mg was sufficient to elicit a measurable glucose-lowering response which was not near the top of the dose-response curve. At the same time, the FDA recommended measuring the change in the area under the serum glucose concentration *versus* time profile between baseline (sucrose alone) and the T and R formulations (acarbose/sucrose) in

Table I. Values of the Recommended BE Parameters, $\Delta C_{SG,max}$, $AUC_{(0-4h)}$, and $AUEC_{(0-4h)}$, for Evaluation of Acarbose BE in 40 Subjects

Parameter	Treatment	Mean	SD	GeoM	Minimum	Median	Maximum
$\Delta C_{SG,max}$ (mmol/L)	T	2.27	0.84	2.09	0.53	2.27	4.07
	R	2.22	0.89	2.06	0.73	1.97	4.64
$AUC_{(0-4h)}$ (mmol h/L)	Baseline of T	20.0	1.91	19.9	16.1	19.9	24.0
	Baseline of R	20.0	1.60	20.0	16.9	20.2	22.8
	T	19.5	1.36	19.5	16.7	19.7	22.9
	R	19.4	1.40	19.4	16.9	19.2	22.9
$AUEC_{(0-4h)}$ (mmol h/L)	T	0.48	1.55	^a	-3.12	0.15	3.82
	R	0.64	1.33	^a	-1.45	0.51	5.01

BE bioequivalence, SD standard deviation, T acarbose test, R reference

^a Values could not be computed due to existence of negative values

Table II. Values of Parameters Used in Expanded BE Evaluation of Acarbose Formulations in 40 Subjects with Calculated 90% CIs for T/R Values and P Value for $T_{\max}$

Parameter	Treatment	Mean	SD	GeoM	Minimum	Median	Maximum	90% CI for T/R (or P value)	Sequence difference
$\Delta C_{SG,\max}$ (mmol/L)	T	2.27	0.84	2.09	0.53	2.27	4.07	89.9–113.9%	0.13
	R	2.22	0.89	2.06	0.73	1.97	4.64		
C_{ss} (mmol/L)	Baseline of T	5.00	0.48	4.98	4.03	4.98	6.00	97.7–101.8%	0.17
	Baseline of R	5.01	0.39	4.99	4.21	5.05	5.70		
	T	4.88	0.34	4.87	4.19	4.93	5.71	99.3–102.1%	0.57
	R	4.85	0.34	4.84	4.23	4.80	5.73		
GE (mmol/L)	Baseline of T	4.07	1.10	3.92	1.79	4.05	6.33	99.7–113.6%	0.13
	Baseline of R	3.84	1.07	3.69	1.49	3.76	6.57		
	T	1.46	0.65	1.33	0.56	1.32	3.31	89.3–117.9%	0.31
	R	1.45	0.78	1.30	0.64	1.26	4.19		
GE' (mmol/L) ^a	Baseline of T	3.08	0.91	2.93	1.12	3.17	4.84	102.7–123.3%	0.03
	Baseline of R	2.76	0.98	2.60	1.15	2.61	5.78		
	T	1.03	0.59	0.89	0.19	0.82	2.55	78.6–120.4%	0.95
	R	1.08	0.68	0.91	0.30	0.83	2.85		
fAUC (mmol h/L)	Baseline of T	3.87	1.56	3.55	1.18	3.66	7.90	95.4–115.2%	0.20
	Baseline of R	3.68	1.56	3.39	1.30	3.15	9.30		
	T	1.38	0.66	1.25	0.51	1.21	3.37	93.7–122.0%	0.61
	R	1.27	0.55	1.17	0.56	1.06	2.92		
$T_{\max}$ (h)	T	0.61	0.32	^b	0.00	0.50	2.00	$P=0.64$	^b
	R	0.62	0.23	^b	0.25	0.50	1.00		

BE bioequivalence, CI confidence interval, T/R test/reference ratio, GE glucose excursion, fAUC degree of fluctuation of serum glucose based on AUC

^a Data for subjects 5, 6, and 17 were excluded from analysis

^b Data were not computed

each subject. Compared with giving placebo as a baseline measurement in a three-way cross-over design (2), this method provides a more accurate assessment of acarbose efficacy.

Disadvantages of the AUEC_(0-4h) Parameter

In our study, the variability in AUEC_(0-4h) for both T and R formulations was very large resulting in non-normal distributions. Furthermore, the AUEC_(0-4h) values for more than 30% of subjects were negative meaning that ln-transformation was not possible in these subjects. This can be illustrated by reference to the serum glucose concentration *versus* time profiles for subject 10 in the pivotal study for whom a negative AUEC_(0-4h) value was obtained (Fig. 4).

Table III. Number of Subjects Required to Detect Differences in Five Parameters with 80% Statistical Power ($\beta=20\%$) Based on ln-Transformed Data

Parameter	Treatment	CV (%)	Sample size needed
$\Delta C_{SG,\max}$		32.2	28
	Baseline	5.44	12
C_{ss} (mmol/L)	Acarbose administration	3.62	12
	Baseline	17.5	18
GE (mmol/L)	Acarbose administration	38.2	42
	Baseline	23.6	64
GE' (mmol/L)	Acarbose administration	58.5	82
	Baseline	22.6	28
fAUC (mmol h/L)	Acarbose administration	36.2	70
	Baseline		

CV coefficient of variation, GE glucose excursion, fAUC degree of fluctuation of serum glucose based on AUC

Here, it can be seen that, after administration of sucrose (baseline), the serum glucose level increased rapidly and then decreased rapidly to a value of 4–5 mmol/L after 0.75 h whereas, after administration of Glucobay®/sucrose, the glucose level remained steady in the desired range of 5–6 mmol/L. As a result, the AUC_(0-4h) for glucose after sucrose alone was lower than after sucrose/acarbose and the AUEC_(0-4h) was negative. This can be ascribed to homeostatic control of the glucose concentration. Thus, the difference in the two curves, *i.e.*, baseline correction by subtraction, reflects not only the efficacy of acarbose but also the effect of homeostatic glucose regulation.

These results indicated that the baseline corrected parameter, AUEC_(0-4h), may be unsuitable as an evaluation parameter for acarbose BE. However, AUEC_(0-4h) may still be a useful parameter using data obtained through a hyper-insulinemic–euglycemic clamp to reflect glucose absorption by insulin infusion rate after sucrose administration and acarbose/sucrose co-administration. This is because such data excludes the effect of glucose homeostatic regulation and avoids negative AUEC_(0-4h) values.

The situation is different for $\Delta C_{SG,\max}$, the other parameter recommended to be baseline-corrected. Here, the corresponding $T_{\max}$ values occurred primarily during the first 1 h post-dose, a timeframe within which the blood glucose homeostatic regulation mechanisms were just beginning to be expressed.

The New Evaluation Parameters

In clinical practice, the glucose level in diabetic patients should be maintained in a normal, safe, and acceptable range.

Fig. 4. Serum glucose concentration *versus* time profiles after sucrose alone (baseline) and after Glucobay®+sucrose in a subject (subject 10 in the pivotal study) giving a negative value of $AUEC_{(0-4h)}$

The amplitude of fluctuations of blood glucose levels is therefore an important surrogate biomarker of glycemic control. In fact, it has been reported that glucose fluctuation contributes more to promoting lipid peroxidation and decreasing antioxidant capacity than chronic sustained hyperglycemia (as measured by the HbA1c) (5). On this basis, glucose fluctuation may be considered an independent risk factor for diabetic complications (6). Using data generated by a continuous glucose monitoring system, glucose fluctuation can be defined as either the mean amplitude of glucose excursion or the mean postprandial glucose excursion (5). Besides the newly defined parameter, GE, we also introduced GE' and $fAUC$ over the 4-h period to evaluate glucose

fluctuation. The former evaluates GE without interference from homeostatic glucose control whereas the latter describes GE based on a sum of areas which reflect deviations from C_{ss} . Thus, BE of acarbose should be based on comparisons of parameters that reflect both the glucose level (the C_{ss}) and its fluctuation (GE, GE' , $fAUC$).

Insomuch as they are based on only two glucose concentrations [C_{max} and C_{min} (or C_{min}')], GE and GE' are only rough estimates of glucose fluctuation. Thus, to a certain extent, comparability of the parameters C_{ss} , GE, and GE' , following administration of sucrose alone probably reflects stability of a subject's glucose self-regulation. Similarly, comparability of C_{ss} and GE after acarbose/sucrose treatment reflects stability of a subject's overall capacity for glucose control including homeostatic control and acarbose efficacy whereas comparability of C_{ss} and GE' may reflect stability of acarbose efficacy specifically. In fact, the GE' values for three subjects were zero excluding them from statistical analysis and reducing the reliability of GE' as a parameter for BE evaluation of acarbose. The parameter, $fAUC$, which is based on all glucose concentrations in the 0–4-h interval, is probably more reliable because it is based on deviations from C_{ss} and is positively correlated with glucose fluctuation. A larger $fAUC$ value reflects a larger glucose fluctuation and a relatively low efficacy of acarbose. At the same time, we did not make baseline corrections for the parameters C_{ss} , GE, GE' , and $fAUC$, since, like $AUEC_{(0-4h)}$, the corrected parameters do not specifically reflect acarbose efficacy due to the effect of homeostatic glucose control.

Plateau concentration of glucose

Fig. 5. Virtual data of glucose concentration to illustrate the relationship between BE and the two independent indices of BE, the C_{ss} of glucose and glucose fluctuation (*plus sign* stands for comparability and *minus sign* stands for dissimilarity of the two formulations)

Figure 5 serves to illustrate this theory that the glucose baselines before administration of the acarbose formulations A and B are comparable. In part B of Fig. 5 (glucose fluctuation, BE; C_{ss} : not BE), formulation B shows superior efficacy to formulation A due to a more acceptable C_{ss} , although they have a similar glucose fluctuation. Similarly, in part C (glucose fluctuation: not BE; C_{ss} : BE), formulation B shows superior efficacy to formulation A due to a smaller glucose fluctuation although they have a similar C_{ss} . Neither C_{ss} nor glucose fluctuation alone were sufficient to evaluate acarbose efficacy. Given that our BE evaluation of acarbose is based on these two independent indices, only when the 90% CIs of both are within the criteria range can BE of the two formulations be declared.

We did not apply the classical fluctuation index, DF, to describe glucose fluctuation as is done in BE evaluation of controlled release formulations. DF is calculated using the equations:

$$DF = (C_{\max} - C_{\min})/C_{ss}$$

$$C_{ss} = AUC_{\tau}/\tau$$

In part D of Fig. 5 (glucose fluctuation: not BE; C_{ss} : not BE), the more acceptable value of C_{ss} and the smaller glucose fluctuation for formulation B demonstrate its superior efficacy to formulation A despite the two formulations having similar DF. Similarly, in part B of Fig. 5 (glucose fluctuation: BE; C_{ss} : not BE), the two formulations have similar glucose fluctuation, but formulation B shows a better glycemic control due to a more acceptable C_{ss} , despite DF being higher for formulation B than for A. DF is a hybrid parameter involving both C_{ss} and glucose fluctuation and does not reflect the two indices separately.

Overall, we have evaluated a combination of six parameters, C_{ss} , GE, GE', fAUC, $\Delta C_{SG,\max}$, and $T_{\max}$, in which GE, GE', and fAUC are newly defined in an attempt to establish the best means of demonstrating BE of acarbose formulations. However, we have no evidence to support the appropriateness of the classical 90% CI of 80–125% for the GE, GE', and fAUC parameters since they all showed relatively high variability after administration of acarbose/sucrose (CVs of 38.19%, 58.53%, and 36.18% for GE, GE', and fAUC, respectively). Clinical trials comparing formulations in diabetic patients are needed in order to validate which parameter or combination of parameters is the best to demonstrate BE of acarbose formulations. However, in using more parameters than recommended by the FDA, the probability of committing a type 1 error (the risk of failing

to demonstrate BE when it actually exists) is probably increased.

CONCLUSIONS

The FDA recommended parameter, $\Delta C_{SG,\max}$, is useful in reflecting efficacy of acarbose since it is a measure of the maximum reduction in serum glucose concentration. However, since the other FDA recommended parameter, $AUEC_{(0-4h)}$, can be negative due to homeostatic glucose control, it is a less reliable measure of acarbose efficacy. The new PD parameters, GE, GE', and fAUC, reflect glucose fluctuation and, in this regard, are better reflections of acarbose efficacy. The 90% CIs of GE' (78.6–120.4%) were slightly outside the acceptance range of 80–125%, but the intervals for C_{ss} , $\Delta C_{SG,\max}$, GE, and fAUC were all completely within it. Thus, based on these four parameters, BE of the two acarbose formulations can be initially considered to have been demonstrated. However, more clinical trials may be needed to validate which one of the three new parameters is suitable to demonstrate acarbose BE and whether the acceptance range of 80–125% can be applied.

ACKNOWLEDGMENTS

The authors are indebted to Laurence Yu for providing suggestions in preparing this manuscript.

REFERENCES

1. Midha KK, McKay G. Bioequivalence; its history, practice, and future. *AAPS J.* 2009;11(4):664–70.
2. Bae JW, Jang CG, Lee SY. Methods for pharmacodynamic analysis and proposed protocols for bioequivalence study of acarbose. *Yakhak Hoeji.* 2007;51(6):440–6.
3. U.S. Department of Health and Human Services, Food and Drug Administration. Contains nonbinding recommendations: draft Guidance on Acarbose. http://www.fda.gov/downloads/Drugs/Guidance/Compliance_Regulatory_Information/Guidances/UCMI170242.pdf
4. Koytchev R, Richter W, ErKent U, Kirkov V, Dimitrova V, Nern A, Kunter U. Influence of acarbose on blood glucose and breath hydrogen after carbohydrate load with sucrose or starch. *Arzneimittelforschung.* 2001;59(11):557–63.
5. Wang Z, Li L, Zheng F, Jia C, Ruan Y, Li H. Correlation between the amplitude of glucose excursion and the oxidative/antioxidative system in subjects with different types of glucose regulation. *Biomed Environ Sci.* 2011;24(1):68–73.
6. Marcovecchio ML, Lucantoni M, Chiarelli F. Role of chronic and acute hyperglycemia in the development of diabetes complications. *Diabet Technol Therap.* 2011;13(3):389–94.