Intra- and Inter-Subject Variabilities of CGP 33101 after Replicate Single Oral Doses of Two 200-mg Tablets and 400-mg Suspension

Wing K. Cheung,[1,2] Farid Kianifard,[3,4] Audrey Wong,[3] Josette Mathieu,[1,6] Terry Cook,[5] Vivian John,[1] Elliot Redalieu,[1] and Keith Chan[1]

Received June 5, 1995; accepted August 15, 1995

Purpose. The purpose of this study was to use a replicate designed trial to assess the overall, intra- and inter-subject variabilities in pharmacokinetic parameters of CGP 33101 after oral administration of tablets relative to that of powder suspended in water, and to determine the relative proportion of the intra-subject variance to the overall variability.

Methods. Sixteen healthy subjects were randomly assigned to four groups to receive tablets and suspension twice in four different treatment sequences. The plasma concentration-time profile of CGP 33101 was characterized in terms of C_{max}, T_{max}, and AUC. Bioavailability of tablets relative to suspension and intra- and inter-subject variability were assessed by statistical analysis.

Results and Conclusions. The overall variabilities in absorption kinetics of CGP 33101 in healthy subjects were small with CV's of the population mean values for AUC and C_{max} less than 26% for both tablets and suspension. Contribution of intra-subject variability to the overall variability was also small (~20%). Both the overall and intra-subject variabilities of AUC and C_{max} after suspension were larger than after the tablets. However, the differences in variability between tablets and suspension were not statistically significant ($p > 0.05$). The tablet formulation was bioequivalent to suspension in terms of rate and extent of absorption based on 90% conventional confidence intervals (for AUC and C_{max}) and Wilcoxon rank-sum test (for T_{max}).

KEY WORDS: CGP 33101, intra-subject variability; inter-subject variability; pharmacokinetics; healthy subjects; bioavailability.

INTRODUCTION

Most clinical trials, especially for bioavailability studies (1), are conducted with crossover design to avoid any possible inter-subject differences in absorption and disposition characteristics of the drug. The crossover design, which uses each subject to serve as his/her own control, assumes that an individual does not have any day-to-day variations in his/her physiological absorption and disposition characteristics. In other words, absence of intra-subject variability in these characteristics is a key element for the success of a crossover designed trial. However, an individual may vary from time to time in his/her physiological condition such that this assumption may not be valid (2). For example, studies have demonstrated that drugs such as theophylline (3), digoxin (4), furosemide (5), prinomide, phenacetin, trazodone, and CGS 16617 (6) exhibited a predominant intra-subject source of variability.

The contribution of intra-subject variability can be reduced by a proper design of the clinical trial. For example, intra-subject variability can be minimized by controlling the times of dosing on each dosing date (diurnal effects), concomitant medications and diet consumed by the subjects during the trial, food consumption during drug administration, posture of the subjects, and location and environment of the trial (2).

There are, however, some intrinsic sources of intra-subject variability which cannot be eliminated by any means. For example, compounds with low solubility in gastric fluid and enteric-coated formulations are prone to have large day-to-day variations in drug absorption characteristics; unstable physiological conditions due to changes in disease severity will lead to large day-to-day variation in drug responses (2); and inter-day assay variability is another intrinsic source of intra-subject variability (2,7).

To compensate for the contribution of intra-subject variability as a confounding factor in the analysis of bioequivalence data, a larger number of subjects would be required in a classical crossover study. On the other hand, by quantifying the intra-subject variability, the number of subjects required in bioavailability studies could be reduced (5,8). Grahnén et al. (5) showed that a crossover study in replicate (subjects received each tested formulation twice or more), randomized complete blocks, which could separate the intra-subject variability from the total variability, required only 16 subjects to detect a true 20% difference between two dosage forms with a power of 80% (significance level = 0.05), compared to between 20 to 37 subjects that would have been required in a non-repeated classical crossover study (9).

During drug development, it is important to characterize the contribution of intra-subject variance to the overall variability, especially for those that have a narrow therapeutic plasma concentration range. One can maintain the drug concentrations within the therapeutic range by individualizing the dosing regimen if the variability is due predominantly to inter-subject variance (10,11,12). However, if the variability is due largely to intra-subject variance, individualization will not help to improve the drug therapy. It is not commendable to develop a drug product if its intra-subject variability is so large that the span of inter-day fluctuation of plasma drug concentrations after a given dose of the drug is wider than its therapeutic dose range.

CGP 33101 [1-(2,6-difluorbenzyl)-1H-1,2,3-triazole-4-carboxide], a potential antiepileptic drug structurally unrelated to any commercially available antiepileptic drugs, is being studied in clinical trials as a potential antiepileptic drug. The drug has a limited solubility in 0.1 N HCl and

[1] Bioanalytics and Pharmacokinetics, Ciba Pharmaceuticals Division, Summit, New Jersey, 10502.
[2] Current address: Drug Metabolism Department, PRI, Route 202, P.O. Box 300, Raritan, New Jersey 08869.
[3] Medical Information Management and Statistics, Ciba Pharmaceuticals Division, Summit, New Jersey, 07901.
[4] Current address: Biostatistics Department, Hoechst-Roussel Pharmaceuticals, P.O. Box 2500, Somerville, New Jersey 08876-1258.
[5] CNS Clinical Research, Ciba Pharmaceuticals Division, Summit, New Jersey, 07901.
[6] To whom correspondence should be addressed.

0724-8741/95/1200-1878$07.50/0

simulated intestinal fluid (63 and 59 mg/L, respectively; data on file, Ciba US) and it was anticipated that there would be a large variability in oral absorption of the drug. The objective of this study was to use a replicate designed trial to assess the overall, intra- and inter-subject variabilities in pharmacokinetic parameters of CGP 33101 after oral administration of two 200-mg tablets of CGP 33101 relative to that of 400 mg powder suspended in 100 ml water (presumably free of any formulation factors) as a reference standard, and to determine the relative proportion of the intra-subject variance to the overall variability.

MATERIALS AND METHODS

Clinical Procedure

This was a single-center, single-dose, open-label, four-sequence, four-period, randomized crossover trial. Sixteen healthy male subjects who satisfied all admission criteria were enrolled. Their ages ranged from 21 to 50 years with a mean ± S.D. value of 33 ± 11 years. Their weights ranged from 60.5 to 88.2 kg with a mean ± S.D. value of 75.6 ± 7.9 kg. Each subject received the following two treatments: Treatment A—a single oral dose of two 200-mg tablets; Treatment B—a single oral dose of 400 mg powder suspended in 100 ml water (suspension). There was a 1-week time interval between dosings. The 16 subjects were randomized into 4 groups to receive the 2 treatments in 4 different dosing sequences: AABB, BBAA, ABBA, and BAAB. This four-sequence, four-period design for comparing two treatments allows the assessment of intra-subject variabilities and possesses some optimal statistical properties (13).

Subjects were admitted to the Clinical Research Facility the evening before each dosing day, and remained as inpatients in the facility until 48 hours after dosing. Subjects received the suspension or tablet formulation immediately after a standardized breakfast at about 8 a.m. The two 200-mg tablets were administered with 200 ml water. The 100 ml of suspension was administered with another 100 to 250 ml of water rinses of the bottle to ensure complete dosing. Blood samples (7 ml each) were collected at 0 (pre-dose), 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, and 48 hr after each drug administration for determination of plasma levels of CGP 33101.

Analytical Procedure

Plasma levels of CGP 33101 were measured using an established high performance liquid chromatography method (14). The validated concentration range of the assay was 50 to 4000 ng/ml. Accuracy (expressed as recovery) and precision (expressed as CV), as determined from the quality control samples, ranged from 98% to 123% and 11% to 23%, respectively.

Pharmacokinetic Analysis

The plasma concentration-time profile of CGP 33101 was characterized in terms of peak concentration (C_{max}), time to peak concentration (T_{max}), and area under the curve (AUC). AUC from time zero to time 48 hr [AUC(0–48)] was calculated using the linear trapezoid rule. AUC from time zero to time infinity [AUC(0–∞)] was calculated by adding AUC(0–48) to C(48)/K, where C(48) is the plasma concentration at time 48 hr and K is the terminal rate constant. K was estimated by linear regression using a minimum of three quantifiable data points from the terminal log-linear region of the plasma concentration-time profiles. Half-life ($T_{1/2}$) was calculated by dividing ln 2 by K. $T_{1/2}$ was not reported if coefficient of determination of the linear regression was less than 0.95. AUC(0–48) instead of AUC(0–∞) was used in the statistical analyses because there were six non-reportable half-life values among the two treatments, and the difference in mean values of AUC(0–48) and AUC(0–∞) was less than 5% of the AUC(0–∞) mean value.

Statistical Analysis

The SAS statistical package was used for the statistical analysis.

Bioavailability of Tablets Relative to Suspension. The relative bioavailability of tablets compared to suspension was assessed by comparing AUC(0–48), C_{max}, and T_{max} values. The original values of AUC(0–48), C_{max} and T_{max} were analyzed using an analysis of variance (ANOVA) for a four-sequence, four-period crossover design with sequence, subject within sequence, period, treatment, and carryover as factors. The primary equivalence criterion for AUC(0–48) and C_{max} was that the 90% conventional confidence intervals for the difference between least-squares means of the two formulations (tablet minus suspension), expressed as a percentage of the suspension (reference) least-squares mean, should be contained in the interval (−20%, 20%). The power to detect a 20% difference from the reference least-squares mean at the 0.05 significance level was also calculated.

T_{max} was also analyzed using the Wilcoxon rank-sum test. The analysis was performed on the average of the two T_{max} values of the same formulation computed for each subject. Under the assumption of no carryover effects, these averages could be modeled as two "blocks" of standard 2×2 crossover designs depending on the periods that were pooled to obtain the averages. The Wilcoxon rank sum statistics computed within each "block" were combined into a single test statistic for testing the equality of treatment effects (15). Equivalence was to be claimed if no statistically significant difference between the two formulations was detected.

Intra-Subject Variability. The intra-subject variabilities of AUC(0–48) and C_{max} values for each formulation were

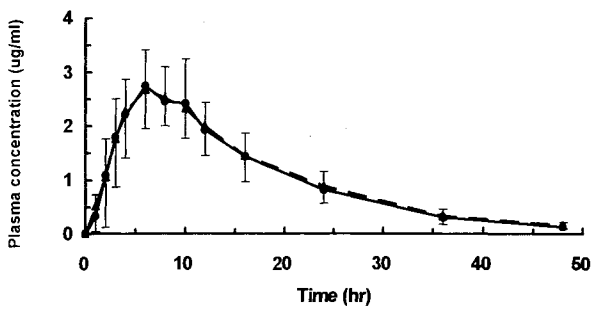

Fig. 1. Mean (SD) plasma concentrations of CGP 33101 as a function of time in healthy subjects after two single oral doses of 2 × 200-mg tablets given on two occasions.

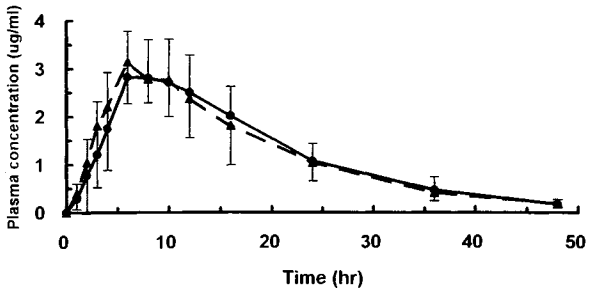

Fig. 2. Mean (SD) plasma concentrations of CGP 33101 as a function of time in healthy subjects after two single oral doses of 400 mg suspension given on two occasions.

estimated using a two-way ANOVA with period and subject as factors. The mean-square errors (MSE) were considered as the estimates of intra-subject variances. The coefficient of variation (CV) of intra-subject variability was estimated by dividing the square root of MSE by the sample mean value. Ratios of mean-square errors of the two formulations (tablet to suspension) were calculated and used to test for equality in intra-subject variability between formulations utilizing the F-distribution.

Inter-Subject Variability. Let $Y_{ij/k}$ be the j observation for Subject i after Formulation k, where j = 1 and 2, i = 1 to 16, and k = tablet (A) or suspension (B). For Subject i at observation 1 after Formulation A,

$$Y_{i1/A} = S_{i/A} + e_{i1/A}$$

where $S_{i/A}$ is the subject effect for the population and $e_{i1/A}$ is the intra-subject effect for Subject i at observation 1. Similarly at Observation 2 after Formulation A,

$$Y_{i2/A} = S_{i/A} + e_{i2/A}$$

where $e_{i2/A}$ is the intra-subject effect for Subject i at Observation 2. Let A_i^* be the average value for Subject i for the tablet formulation, i.e.,

$$A_i^* = \tfrac{1}{2}(Y_{i1/1} + Y_{i2/A})$$
$$A_i^* = \tfrac{1}{2}(S_{i/A} + e_{i1/A} + S_{i/A} + e_{i2/A})$$
$$A_i^* = S_{i/A} + \tfrac{1}{2}(e_{i1/A} + e_{i2/A})$$

Similarly,

$$B_i^* = S_{i/B} + \tfrac{1}{2}(e_{i1/B} + e_{i2/B})$$

The variance of A_i^* (overall variance) may be expressed as follows: Variance of A_i^* = Inter-Subject Variance + ½(Intra-Subject Variance) The Pitman-Morgan test was employed to test for equality of the variances of A_i^* and B_i^* in C_{max} and AUC, and was adjusted for period, sequence, and carryover effects (16,17,18). The variance of A_i^* (or B_i^*) is the pooled (over sequences) variance. The above relationship was then used to compute inter-subject variance. The CV of inter-subject variability for tablets and suspension was estimated by dividing the square root of the inter-subject variance by the sample mean.

RESULTS

Mean (SD) plasma concentration-time profiles of CGP 33101 after the tablets and suspension (given on two different occasions) are shown in Figure 1 and Figure 2, respectively. The mean Pharmacokinetic parameters of CGP 33101 after the tablets and suspension are shown in Table 1. The mean ± S.D. values for AUC(0–48) after the tablets and suspension were 49.4 ± 10.1 and 57.0 ± 13.5 μg·hr/ml. The corresponding mean ± S.D. values were 3.03 ± 0.64 and 3.32 ± 0.75 μg/ml for C_{max}, and 8.82 ± 1.69 and 9.12 ± 1.37 hr for $T_{1/2}$. The median (range) values for T_{max} were 6.5(4–8) and 6.5(5–12) hr for the tablets and suspension, respectively.

A summary of bioequivalence test statistics between tablets and suspension is listed in Table II. The 90% conventional confidence intervals of AUC and C_{max} (−19.4% to −7.40% for AUC and −15.0% to −2.94% for C_{max}) did not contain zero but they were within the ± 20% of the mean for the suspension, therefore meeting the criteria for bioequivalence. No statistically significant difference in T_{max} was detected ($p>0.05$) between formulation base on the Wilcoxon rank-sum test.

A summary of variability statistics is listed in Table III. There were small variabilities in the pharmacokinetic parameters for CGP 33101 observed in this study. Coefficient of variation (CV) of the overall mean AUC(0–48) were 20.4% and 23.8% for the tablets and suspension, respectively (Table I). The corresponding CV values for C_{max} were 21.0% and 22.5%. CV values of intra-subject variability of AUC(0–48) were 13.9% and 15.2% for the tablets and suspension, respectively. The corresponding CV values for C_{max} were 12.7% and 14.4%. CV of inter-subject variance of AUC(0–48) were 19.7% and 22.6% for the tablets and suspension, respectively). The corresponding CV values for C_{max} were 18.3% and 22.6%.

Table I. Mean and Median Pharmacokinetic Parameters for CGP 33101

		C_{max} (μg/ml)	T_{max} (hr)	AUC(0–48) (μg · hr/ml)	$T_{1/2}$ (hr)
Tablets	Mean	3.03	6.56	49.4	8.82
	S.D.	0.64	1.26	10.1	1.69
	CV (%)	21.0	19.2	20.4	19.2
	Median (Range)	—	6.5(4–8)	—	—
	N	16	16	16	16
Suspension	Mean	3.32	7.22	57.0	9.12
	S.D.	0.75	2.27	13.5	1.37
	CV (%)	22.5	31.5	23.8	15.0
	Median (Range)	—	6.5(5–12)	—	—
	N	16	16	16	16

Table II. Summary of Statistical Analyses for Bioequivalence Between Tablets and Suspension

	C_{max}	T_{max}	AUC(0–48)
Analysis of variance	P = 0.016	N.S.	P = 0.001
Power of test	>0.99	0.77	>0.99
90% Conventional[a] confidence interval	−15.%, −2.94%	−21.3%, 3.14%	−19.4%, −7.40%
Wilcoxon rank-sum test[b]	—	P = 0.97	—
Statistical significance based on primary test	N.S.[c]	N.S.	N.S.

[a] Primary test for AUC(0–48) and C_{max}.
[b] Primary test for T_{max}.
[c] N.S. Not statistically significant at 0.05 level.

Ratios of intra-subject variability estimates (tablet/suspension) were 0.63 and 0.64 for AUC(0–48) and C_{max}, respectively. The test based on the F-distribution showed that the differences in variabilities between the two formulations were not statistically significant ($p > 0.05$) for both AUC(0–48) and C_{max}.

Ratios of overall variability estimates (tablet/suspension) were 0.58 and 0.56 for AUC(0–48) and C_{max}, respectively. The Pitman-Morgan test showed that the differences in overall variabilities between the two formulations were not statistically significant ($p > 0.05$) for both AUC(0–48) and C_{max}.

For AUC(0–48), intra-subject variability accounted for 20% and 18% of the overall variability for tablets and suspension, respectively; and for C_{max}, the corresponding contributions of the intra-subject variability to the overall variability were 19% and 17%.

DISCUSSION

This study documented that CGP 33101, despite its low solubility in aqueous solution, had very small variabilities in its absorption kinetics as indicated by the small CV values for C_{max} (rate of absorption) and AUC (extent of absorption). In fact, CV values of the overall mean values were comparable to the CV values of the assay.

Table III. Summary of Variability Statistics

	AUC(0–48)	C_{max}
Overall variance (CV, %)		
Tablets	118 (22.0)	0.380 (20.4)
Suspension	203 (25.0)	0.680 (24.8)
Ratio of variance (tablet/suspension)	0.58	0.56
Pitman-Morgan test p-value	0.168	0.207
Intra-subject variance (CV, %)		
Tablets	47.0 (13.9)	0.147 (12.7)
Suspension	74.8 (15.2)	0.229 (14.4)
Ratio of variance (tablet/suspension)	0.63	0.64
F-test p-value	0.414	0.430
Contribution to overall variance		
Tablets	20%	19%
Suspension	18%	17%
Inter-subject variance (CV, %)		
Tablets	94.6 (19.7)	0.307 (18.3)
Suspension	166 (22.6)	0.565 (22.6)

Contributions of intra-subject variability to the overall variability were small (~20%). This was probably because the study was conducted with a design which eliminated most of the preventable sources of intra-subject variability. For example, the study was conducted with healthy subjects (constant physiological factors and no concomitant medications) in a single research facility (constant location and environment). The drug was always administered in the morning (same diurnal effects) immediately after a standardized breakfast (same diet and food effects). The observed intra-subject variability, therefore, was due predominately to intrinsic factors such as physiochemical properties of CGP 33101 and assay variability. The small overall variability indicated that the physicochemical properties of CGP 33101 (low solubility in gastric fluid) did not lead to high variabilities in the absorption characteristics of the drug.

Although the overall variability observed in this study was small, under realistic clinical situations, intra-subject variability and, consequently, the overall variability would be larger for the following reasons. The physiological conditions of patients might not be stable; concomitant medications taken by patients could interact with the drug; and food, which has been demonstrated to increase the AUC of CGP 33101 by 40% (data on file, Ciba-Geigy Limited, Basle, Switzerland), might not have been controlled.

Suspension was used in the study because we intended to compare the variabilities of the tablet formulation with a reference standard that is presumably free of any formulation factors. It was quite unexpected to find that the suspension actually had larger variabilities in C_{max} and AUC(0–48) values than the tablets. Although results of ANOVA (for intra-subject variability) and Pitman-Morgan test (for overall variability) indicated that the differences in variabilities between formulations were not statistically significant ($p > 0.05$) for both Cmax and AUC(0–48). In addition, the 90% conventional confidence intervals (for AUC and C_{max}) and the Wilcoxon rank-sum test (for T_{max}) also showed that the two formulations were bioequivalent. CGP 33101 has limited solubility in 0.1 N HCl and simulated intestinal fluid, the presence of solubilizing agents in the tablet could have been a factor leading to smaller variability in absorption.

CONCLUSIONS

The variabilities of CGP 33101 in healthy subjects were small with overall CV for AUC(0–48) and C_{max} less than 26% for both tablets and suspension. Contribution of intra-subject variability to the overall variability was also small (~20%). Between the two formulations, variabilities of AUC(0–48) and C_{max} after suspension were larger than after the tablets. However, the differences in variability were not statistically significant ($p > 0.05$). The tablet formulation was bioequivalent to suspension in terms of rate and extent of absorption based on 90% conventional confidence intervals (for AUC and C_{max}) and the Wilcoxon rank-sum test (for T_{max}).

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