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Report

Intra- and Intersubject Variability: Mixed-Effects Statistical Analysis of Repeated Doses of an Angiotensin Converting Enzyme Inhibitor, CGS 16617

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The intrasubject and intersubject variabilities for CGS 16617, an angiotensin converting enzyme inhibitor, were evaluated in an open-label, repeat single-dose bioavailability trial. Eight healthy male volunteers each received a 20-mg oral dose of CGS 16617 as an aqueous solution on four separate occasions. Components of variance were evaluated for a mixed-effects statistical model in which subjects were regarded as a random factor. While intersubject variability was statistically significant ($P < 0.05$) for all pharmacokinetic variables measured, AUC, C_{max}, $t_{1/2}$, and t_{max}, its contribution to the total observed variability was relatively small for AUC, $t_{1/2}$, and t_{max}. The proportion of variation due to intrasubject variability was 70, 19, 61, and 72% for AUC, C_{max}, $t_{1/2}$, and t_{max}, respectively. Ramifications of the large intrasubject source component of variability as related to bioavailability trials and biological variation are discussed.

KEY WORDS: random-effects statistical model; bioavailability trials; biological variation; intersubject variability; intrasubject variability; CGS 16617.

INTRODUCTION

Several investigators (1–4) have studied the effects of intrasubject and intersubject variability on pharmacokinetic disposition. Such studies have usually employed only a single replicate dosing for any given treatment. While these studies generally report both intersubject and intrasubject variability separately, these variabilities are actually confounded by each other (5). By considering a random-effects model and evaluating the components of variance, intrasubject and intersubject variability can be isolated, and inferences to a population made. The objective of the present study was to determine the intrasubject and intersubject variability of CGS 16617, 3-[(5-amino-1-carboxy1*S*-pentyl)-amino]-2,3,4,5-tetrahydro-2-oxo-3*S*-1*H*-benzazepine-1-acetic acid, an angiotensin converting enzyme inhibitor, utilizing a mixed-effects model in which subjects were regarded as a random factor.

CLINICAL AND ANALYTICAL METHODOLOGY

This was an open-label, repeat single-dose study. CGS 16617 (20 mg) was administered orally as an aqueous solution (150 ml) to each subject on four occasions, separated by washout periods of 2 weeks. Subjects were confined to the Clinical Pharmacology Unit throughout the duration of the study (54 days) in order to standardize the sample as much as possible. Eight healthy male volunteers participated in the study, with an average age of 28 years (range, 24–34 years). Their mean weight was 76.4 kg (range, 65.0–89.6 kg). The subjects took no medication, including nonprescription preparations, for 2 weeks prior to and during the study. On each dosing occasion the volunteers fasted for at least 10 hr from the previous evening and remained fasted until 4 hr postdose.

Serial samples of venous blood (7 ml) were collected from 0 to 240 hr in heparinized (143 U) blood collection tubes. Following centrifugation, plasma was harvested and stored frozen until analysis. Plasma samples were analyzed for CGS 16617 by a radioenzymatic inhibition assay (6).

PHARMACOKINETIC ANALYSIS

Plasma concentration–time profiles for each subject at each drug administration were characterized in terms of the peak drug concentration in plasma (C_{max}), the time to peak concentration (t_{max}), the area under the plasma concentration–time curve extrapolated to infinity (AUC), and the half-life of the terminal decline in plasma concentrations ($t_{1/2}$). AUC was calculated using the following equation:

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$$\text{AUC} = \text{AUC}_{0\text{-}T} + C_T/\lambda(z)$$

where $\text{AUC}_{0\text{-}T}$ is the area under the curve between 0 and the last time point at which the plasma concentration was quantifiable (T), C_T is the predicted plasma concentration at time T, and $\lambda(z)$ is the terminal rate constant. $\text{AUC}_{0\text{-}T}$ was estimated using the trapezoidal approximation method. C_T and $\lambda(z)$ were determined by least-squares regression analysis of the terminal phase of the log-linear plasma concentration–time profile. The terminal half-life was calculated using the following equation:

$$t_{1/2} = 0.693/\lambda(z)$$

STATISTICAL METHODOLOGY

Pharmacokinetic variables were evaluated by analysis of variance (ANOVA) utilizing a mixed-effects model (7,8). The model was comprised of two factors. Subject effects were regarded as a random factor and phase effects were regarded as a fixed factor. Subject-by-phase interactions were assumed to be zero. With the model defined as such, estimates of intersubject and intrasubject variability were obtained. A typical model with the above characteristics can be written as follows.

$$Y_{ij} = \mu + (\mu_j - \mu) + \pi_i + \epsilon_{ij} = \mu + \tau_j + \pi_i + \epsilon_{ij}$$

where μ is the general population mean and is constant, Y_{ij} is the value of the pharmacokinetic variable Y for the jth subject during the ith phase of the trial, μ_j is the mean of Y for the jth subject, τ_j is the random effect of the jth subject, π_i is the fixed effect of the ith phase of the trial, and ϵ_{ij} is the residual effect corresponding to Y_{ij}.

The subject effects are assumed to be mutually independent and normally distributed with an intersubject variance of σ_τ^2 about a zero mean. The residual effect are assumed to be mutually independent and normally distributed with an intrasubject variance of σ_ϵ^2 about a zero mean. The fixed phase effects are defined to sum to zero.

Accordingly, the total variance of Y is the sum of the intersubject and intrasubject variances and phase sum of squares, such that

$$\sigma^2(\text{Y}) = \sigma_\tau^2 + \sigma_\epsilon^2 + b[\Sigma\pi_i^2/(r-1)]$$

The σ_τ^2 and σ_ϵ^2 can be determined from standard two-factor ANOVA as follows:

$$E(\text{error mean square; MSE}) = \sigma_\epsilon^2$$
$$E(\text{phase mean square; MSP}) = \sigma_\epsilon^2 + b[\Sigma\pi_i^2/(r-1)]$$
$$E(\text{subject mean square; MSS}) = \sigma_\epsilon^2 + r\sigma_\tau^2$$

where r is the number of phases and b is the number of subjects, each for a balanced block design.

The point estimate of the intersubject variability is thus calculated as

$$\hat{\sigma}_\tau^2 = \frac{\text{MSS} - \text{MSE}}{r}$$

Standard F values may be calculated for inferences concerning subject and phase effects. If $\sigma_\tau^2 = 0$, then the test statistic

$$F^* = \frac{\text{MSS}}{\text{MSE}}$$

has an F distribution with $b-1$ and $(b-1)(r-1)$ degrees of freedom. If there are no phase effects, then the test statistic

$$F^* = \frac{\text{MSP}}{\text{MSE}}$$

has an F distribution with $r-1$ and $(b-1)(r-1)$ degrees of freedom.

The exact $1-\alpha$ confidence intervals for σ_ϵ^2 and $\sigma_\tau^2/(\sigma_\tau^2 + \sigma_\epsilon^2)$ are, respectively, as follows:

$$P\left\{\frac{(r-1)(b-1)\cdot\text{MSE}}{\chi^2(1-\alpha/2;(r-1)(b-1))} \leq \sigma_\epsilon^2 \leq \frac{(r-1)(b-1)\cdot\text{MSE}}{\chi^2(\alpha/2;(r-1)(b-1))}\right\} = 1-\alpha$$

and

$$P\left\{\frac{LL}{1+LL} \leq \frac{\sigma_\tau^2}{\sigma_\tau^2+\sigma_\epsilon^2} \leq \frac{UL}{1+UL}\right\} = 1-\alpha$$

where

$$LL = \frac{1}{r}\left[\frac{\text{MSS}}{\text{MSE}}\cdot\frac{1}{F[1-\alpha/2;\, b-1,\,(r-1)(b-1)]} - 1\right]$$
$$UL = \frac{1}{r}\left[\frac{\text{MSS}}{\text{MSE}}\cdot\frac{1}{F[\alpha/2;\, b-1,\,(r-1)(b-1)]} - 1\right]$$

and $\chi^2(\alpha;\nu)$ represents the critical point for a chi-square distribution with ν degrees of freedom; $F(\alpha;\nu_1,\nu_2)$ represents the critical point for an F distribution with ν_1 and ν_2 degrees of freedom.

The confidence interval for σ_τ^2 is complex. A second-order approximate confidence interval as determined by Scheffé (8) is given by

$$P\{\text{MSE}\cdot g_L \leq \sigma_\tau^2 \leq \text{MSE}\cdot g_U\} = 1-\alpha$$

where the functions g are

$$g_U = F(\alpha/2;\infty,b-1)\cdot\frac{\text{MSS}}{\text{MSE}} - 1 + \frac{\text{MSE}}{F[\alpha/2;(r-1)(b-1),\,b-1]\cdot\text{MSS}}\left[1 - \frac{F(\alpha/2;\,\infty,\,b-1)}{F[\alpha/2;\,(r-1)(b-1),\,b-1]}\right]$$

$$g_L = \frac{\text{MSS}}{\text{MSE}\cdot F(\alpha/2;\,b-1),\infty)} - 1 - \frac{\text{MSE}\cdot F[\alpha/2;\,b-1,\,(r-1)(b-1)}{\text{MSS}}\left\{\frac{F[\alpha/2;\,b-1,\,(r-1)(b-1)]}{F(\alpha/2;\,b-1,\infty)} - 1\right\}$$

where $F(1-\alpha/2;\nu_2,\nu_1) = 1/[F(\alpha/2;\nu_1,\nu_2)]$.

For the general mean μ, the overall variable mean $\bar{Y}$ is an unbiased estimator with variance

$$\sigma^2(\bar{Y}) = \frac{\sigma_\tau^2}{b} + \frac{\sigma_\epsilon^2}{br}$$

An unbiased estimation of $\sigma^2(\bar{Y})$ is

$$s^2(\bar{Y}) = \frac{\text{MSS}}{br}$$

The $1 - \alpha$ confidence interval about the general mean is thus

$$P\{\bar{Y} - t(1 - \alpha/2; b - 1) \cdot s(\bar{Y}) \leq \mu \leq \bar{Y} + t(1 - \alpha/2; b - 1) \cdot s(\bar{Y})\} = 1 - \alpha$$

RESULTS

The overall mean plasma concentration–time curve is illustrated in Fig. 1. Four primary variables, AUC, $t_{1/2}$, C_{max}, and t_{max}, were chosen as descriptors of the pharmacokinetic disposition of CGS 16617. Of these variables, AUC and $t_{1/2}$ are indicators of elimination rate, intercompartmental distribution, and absolute extent of absorption. The variables C_{max} and t_{max} are indicators of the relative rate of drug absorption to the rate of drug elimination.

The univariate mean and standard error of each variable for each subject are presented in Table I. The multivariate mean and standard error of each variable are presented in Table II. Notably, the composite standard error (Table I) is not equivalent to the standard error when factor levels are taken into account (Table II).

Globally, an estimate of the population relative variability is defined by the coefficient of variation (CV), where

$$\text{CV} = \sqrt{\hat{\sigma}_\epsilon^2 + \hat{\sigma}_\tau^2}/\hat{\mu}$$

The CV values for AUC, C_{max}, $t_{1/2}$, and t_{max} were 22.8, 55.9, 29.6, and 36.1%, respectively. Comparatively, those parameters with an input rate dependence (C_{max}, t_{max}) showed a larger degree of relative total variability than those parameters independent of, or generally stable to, the input rate

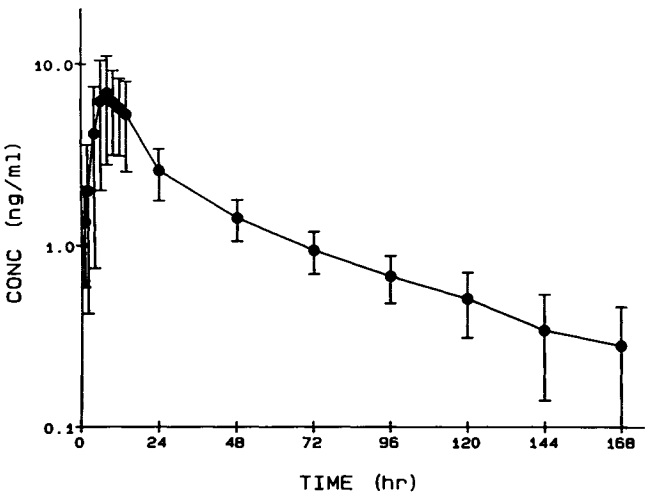

Fig. 1. Composite mean (±SD) plasma concentration–time profile for all subjects and all phases following oral administration of CGS 16617 as a 20-mg, single-dose oral solution.

Table I. Summary of Statistics by Subject

Subject No.	Pharmacokinetic variable: AUC (ng · hr/ml)	C_{max} (ng/ml)	$t_{1/2}$ (hr)	t_{max} (hr)
1				
Mean	329.75	14.88	47.37	7.50
SD[a]	82.68	2.56	6.74	4.43
2				
Mean	255.50	4.68	69.00	12.00
SD	17.02	0.93	12.50	2.83
3				
Mean	281.50	5.52	58.45	9.50
SD	16.66	1.89	5.15	3.00
4				
Mean	284.50	7.22	75.72	9.00
SD	78.92	2.79	23.62	3.46
5				
Mean	288.50	10.65	48.72	9.00
SD	29.69	2.46	16.11	2.00
6				
Mean	204.25	6.57	37.80	7.50
SD	47.71	2.22	11.08	1.00
7				
Mean	244.50	5.64	53.32	12.00
SD	41.16	2.07	9.62	2.31
8				
Mean	213.75	3.94	63.02	13.00
SD	28.14	0.82	13.72	2.00
All				
Mean	262.78	7.39	56.68	9.94
SE[b]	10.36	0.69	2.95	0.56

[a] Standard deviation ($N = 4$).
[b] Standard error ($N = 32$).

(AUC, $t_{1/2}$). The parameter exhibiting the lowest degree of total variability of those measured was AUC.

The total variability was partitioned into each of its source components (Table II). No significant ($P \geq 0.05$) phase effects were detected with respect to all four variables tested. The intersubject variability was statistically significant ($P < 0.05$) with respect to all four variables tested. However, its relative contribution to the total demonstrated variability was small for three of these variables, AUC, $t_{1/2}$, and t_{max}. Only C_{max} exhibited appreciable intersubject variability.

DISCUSSION

CGS 16617 is a free dicarboxylic acid angiotensin converting enzyme inhibitor (ACEI). It is characterized by a poor oral bioavailability, which is typical of nonesterified ACEIs (9). Its activity is assumed to be related to its relatively high degree of potency. Serious adverse reactions have been rare with nonsulfhydryl ACEI therapy (10) and apparently unrelated to dose. Similar behavior is expected with the newer ACEIs being developed. Thus a primary concern regarding ACEI therapy for free dicarboxylic acid drugs is the consistency with which drug delivery to the general circulation can be produced, both between individuals and within an individual. To evaluate this consistency we designed a trial with four repetitions of a single dose.

While in general the total variability was not unusual,

Table II. Summary of Analysis of Variance When Correcting for Factor Effects Utilizing a Mixed-Effects Model

	Pharmacokinetic variable			
	AUC (ng · hr/ml)	C_{max} (ng/ml)	$t_{1/2}$ (hr)	t_{max} (hr)
F statistic (*P* value)				
For intersubject variability	2.75 (0.034)	10.82 (<0.001)	3.56 (0.011)	2.53 (0.047)
For phase effect	0.60 (0.623)	0.05 (0.983)	1.40 (0.270)	1.78 (0.181)
Mean				
Point estimate (±SE)	263 (±15)	7.39 (±1.29)	56.7 (±4.4)	10 (±1)
95% confidence interval	(228, 298)	(4.34, 10.44)	(46.3, 67.0)	(8, 12)
Intrasubject SD				
Point estimate	50	2.22	13.1	3
95% confidence interval	(39, 72)	(1.71, 3.17)	(10.1, 18.8)	(2, 4)
Intersubject SD				
Point estimate	33	3.48	10.5	2
95% confidence interval	(0, 81)	(2.12, 7.34)	(3.6, 24.3)	(0, 4)
Proportion of variation due to intersubject variability				
Point estimate	0.30	0.71	0.39	0.28
95% confidence interval	(0.00, 0.74)	(0.40, 0.92)	(0.05, 0.79)	(0.00, 0.72)

the contributing sources of variation were very interesting. A significant subject effect was detected, indicating a positive contribution of intersubject variability. As a consequence each subject may be assumed to possess his own characteristic set of disposition parameters. Surprisingly, however, the contribution of intersubject variability to total variability was only 30 to 40% for AUC, $t_{1/2}$, and t_{max}. Thus for these three variables the population mean point estimate is influenced primarily by the random error associated with each individual. Only in the case of C_{max} were differences between subjects important. These results are of general interest since biological variation between individuals is usually regarded as the primary determinant of pharmacokinetic variability. This clearly was not the case in this study and may not be the case in general. The evaluation of a pharmacokinetic parameter based on a single determination, albeit specified for an individual, may thus be severely compromised, particularly when the coefficient of variation is large. The impact of such error would be reflected, for example, in the calculation of the absolute bioavailable fraction when the intravenous dose AUC and oral dose AUC are measured on two separate occasions.

Another important implication is in the design of bioavailability trials. Currently, the subject sample size usually employed is between 12 and 24 individuals. With intrasubject variability being the primary determinant of the observed total variability from any trial, an equivalent power could be obtained with a smaller sample size if the intrasubject variability could be reduced. This would be the case when stable isotopes are employed as internal standards and coadministered as a solution with the test formulations. By having this internal standard, intrasubject variability could be severely reduced. The magnitude of the sample size reduction can be evaluated for an equivalent noncentrality parameter,[6] ψ.

[6] An equivalent noncentrality parameter does not precisely imply an equivalent power since the power of an *F* test for differences among phase means also depends on the degrees of freedom which must change with *b*. However, for a first degree of approximation in estimating sample size reductions, both may be regarded as approximately equivalent.

$$\frac{\sigma(Y)}{\sqrt{b}} = \frac{1}{\psi}\sqrt{\frac{\Sigma\alpha_i^2}{a}} = \text{constant}$$

where a is the number of treatments, b is the number of subjects per treatment group, and α_i are the treatment deviations. Thus, $b' = b(\sigma'/\sigma)^2$ where b' is the reduced sample size and σ' is the reduced total variability. Conversely, when larger sample sizes are utilized and stable isotopes employed, sufficient evidence may be generated to support the relaxation of regulatory criteria for proof of bioequivalence. When intrasubject biological variation is the major source of intrasubject variability, as may be with organic nitrates, and this source is severely reduced or eliminated, a maximal degree of power could be ascertained for any specificied sample size. Practical limits on sample size may be prescribed in the absence of important clinical ramifications.

In conclusion, the major source of random variability for CGS 16617 was intrasubject variability. Based upon the estimated total population variability, the larger source of variability related to the disposition of CGS 16617 is apparently the absorption rate.

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