Population Characteristics of Biological Systems Influenced by Multicomponent Random and Uniform Variation

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Received February 7, 1991; accepted September 6, 1991

The total variability associated with the pharmacokinetic disposition of seven therapeutic agents was decomposed into its source components. After differentiating and isolating random components from fixed uniform components of variance, the proportional contribution of random intersubject and intrasubject variance was evaluated. Statistical analysis utilized a mixed-effects probabilistic model which incorporated both random effects and fixed day-to-day effects. In some cases, time-within-day diurnal effects were also incorporated. While significant intersubject effects were found for all seven drugs studied, three of them were characterized by predominant intrasubject variance. Since intrasubject variance represents a measure of the stability of drug disposition, characterization of its relative magnitude is fundamentally important in assessing the therapeutic consequences of a given treatment at any given time. Significant diurnal effects were found which were strikingly invariant.

KEY WORDS: mixed-effects analysis of variance; random effects; fixed effects; intrasubject variance; intersubject variance; diurnal variance.

INTRODUCTION

The variability in a biological system composed of a population of individuals and an appropriately chosen measurable characteristic of those individuals can usually be ascribed to two random factors. The distribution of random fluctuations in the state of any specified individual results in an intrasubject variance. The distribution of the average states of individuals results in an intersubject variance. The variability associated with any randomly chosen population subset of the biological system is thus jointly dependent on these two source components. In pharmacokinetic systems, both sources of variability are well established (1–5).

Historically, intersubject variance has tacitly been assumed to be the predominate source component of variance. Virtually no evidence, however, has been reported to support this position.

In clinical therapeutics, it is common practice to correct drug therapy for intersubject differences by individually titrating patient dosing in order to achieve an acceptable pharmacologic response. Although one would expect improved therapy from treatment individualization, the actual magnitude of the therapeutic consequences depends on the relative proportion of intersubject variance to total population variance.

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Recently, a random effects analysis of the components of variance of the disposition of CGS 16617, an antihypertensive drug, showed that while differences between individuals were statistically distinguishable, the predominant source of variability was fluctuations within individuals (6). A predominant intrasubject source component of variance has important therapeutic implications with regard to treatment stability and therapeutic agent selection. In order to investigate further the scope of this finding, five drugs, fadrozole hydrochloride, metoprolol tartrate, cimetidine, carbamazepine, and prinomide tromethamine, were evaluated with regard to their population source components of variance and pharmacokinetic disposition.

MATERIALS AND METHODS

This was a retrospective investigation utilizing replicate data resulting from pharmacokinetic studies designed to evaluate the disposition of fadrozole hydrochloride, metoprolol tartrate, cimetidine, carbamazepine, and prinomide tromethamine when multiply dosed to steady state. Subjects employed in all studies were healthy volunteers meeting the usual entrance criteria for phase I clinical trials (Table I). Concomitant medications, including over-the-counter medications, were excluded during the course of these studies. Fadrozole hydrochloride was administered as a 2-mg formulated immediate release investigational tablet every 12 hr for 5 days. Metoprolol tartrate was administered as a 100-mg immediate release commercial tablet every 6 hr for 7 days. Cimetidine was administered as a 300-mg immediate release commercial tablet every 6 hr for 6 days. Carbamazepine was administered as a 200-mg immediate-release commercial tablet every 12 hr for 24 days. Prinomide tromethamine was administered as two 500-mg (1000 mg) formulated immediate-release investigational tablets every 12 hr for 28 days.

The pharmacokinetic variable retrieved for statistical analysis from these studies was the minimum drug plasma concentration at steady state (C_{min}). In each study, a blood sample was collected at the end of a dosage interval and just prior to the subject receiving the next dose. Plasma drug concentrations were determined from replicated end-of-interval samples. The number of C_{min} collections were as follows: four replicates for fadrozole, six replicates for metoprolol, two replicates for cimetidine, three replicates for carbamazepine, and three replicates for prinomide. All C_{min} replicates were obtained at the same time of day except in the cases of fadrozole and metoprolol, where balanced time-of-day and day-to-day replicates were obtained.

The concentrations of drugs in plasma were determined by well-established chromatographic methods. These assays were validated with regard to accuracy, precision, linearity, sensitivity, and specificity. The accuracy and precision of the methods, evaluated from quality control samples analyzed over the analytical time course of each study, are reported in Table II. Reported values are those pertinent to the observed C_{min} values from each study.

Statistical Methodology

The components of variance were evaluated using a pre-

Table I. Subject Demographics by Study

Drug	Sex	Number	Age (years)[a]	Weight (kg)[a]
Fadrozole hydrochloride	Female	18	55.5 (30–72)	66 (52–83)
Metoprolol tartrate	Male	18	29.5 (22–24)	71 (63–84)
Carbamazepine	Male	9	42 (32–48)	75 (64–92)
Cimetidine	Male	12	26 (20–32)	78 (60–84)
Prinomide tromethamine	Male	12	46 (31–54)	87 (63–97)

[a] Median (range).

viously defined statistical model (6). This analysis of variance (ANOVA) model included both random and fixed effects. Subject effects were regarded as a random factor and phase effects were regarded as a fixed factor. In some cases when balanced data were available, the ANOVA model was modified to include short-term phase effects, long-term phase effects, and the interaction between these two phases. Short-term phase effects were defined as the uniform effects on drug disposition resulting from within-day diurnal variation and were categorized as those resulting from either nighttime or daytime dosing. Long-term phase effects were defined as the uniform effects on daily drug disposition resulting from day-to-day variation. Both phase effects were again regarded as fixed factors. All subject × phase interactions were assumed to be zero, and phase × phase interactions were assumed to be zero in the nominal model only. Population estimates of intersubject and intrasubject variability were determined, and the significance of the sample fixed phase effects was evaluated.

The modified version of the assessed model was

$$Y_{ijk} = \mu + \tau_j + \pi_i + \delta_k + (\pi\delta)_{ik} + e_{ijk}$$

where μ is the general population mean and is constant, Y_{ijk} is the C_{min} value at steady state for the jth subject on the ith day and during the kth diurnal phase, τ_j is the random effect of the jth subject, π_i is the fixed effect of the ith day, δ_k is the fixed effect during the kth diurnal phase, $(\pi\delta)_{ik}$ is the interaction effect on the ith day and during the kth diurnal phase, and e_{ijk} is the residual effect corresponding to Y_{ijk}.

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 effects are assumed to be mutually independent and normally distributed with an intrasubject variance of σ^2 about a zero mean. The fixed short-term and long-term phase effects and the interaction effect are defined to sum to zero.

The components of variance can be estimated from standard multifactor ANOVA as follows:

$$E(\text{MSE}) = \sigma^2$$
$$E(\text{MSA}) = \sigma^2 + bc[\Sigma\pi_i^2/(a-1)]$$
$$E(\text{MSB}) = \sigma^2 + ac[\Sigma\delta_k^2/(b-1)], \quad \text{where} \quad b = 2$$
$$E(\text{MSC}) = \sigma^2 + ab\hat{\sigma}_\tau^2$$
$$E(\text{MSAB}) = \sigma^2 + c[\Sigma\Sigma(\pi\delta)_{ik}^2/(a-1)(b-1)]$$

where A is the day-to-day phase effect with a levels, B is the diurnal phase effect with two levels (i.e., nighttime and daytime), and C is the subject effect with c levels.

The population point estimate of the intersubject variability is computed as

$$\hat{\sigma}_\tau^2 = (\text{MSC-MSE})/ab$$

The contribution of diurnal variability to the total sample variability is computed as

$$s_\delta^2 = (\text{MSB-MSE})/ac$$

Standard F values were calculated from the ratios MSA/MSE, MSB/MSE, MSC/MSE, and MSAB/MSE, and inferences were made with regard to the probable contribution of intersubject, diurnal, day-to-day, and phase × phase interaction sources of variance.

Heteroscedasticity in univariate phase variances was evaluated by the Bartlett test.

The probable contribution of any effect was considered significant when $P < 0.05$.

RESULTS

In each of the five drugs tested, significant intersubject effects were detected, thereby demonstrating the unique dependence of pharmacokinetic disposition on specified subjects for those drugs studied (Table III). No significant day-to-day phase effects or phase × phase interactions were detected. The equivalence of daily phases suggests that the dosage regimens employed and times of sample collection resulted in C_{min} values that were representative of the steady

Table II. Analytical Accuracy and Precision

Drug	Theoretical concentration	N	Mean assayed concentration	Variance of assayed concentration
Fadrozole hydrochloride	2.5 ng/ml	23	2.43 ng/ml	0.014 ng/ml
	5.0	23	4.89	0.066
Metoprolol tartrate	125 ng/ml	25	122.8 ng/ml	38.44 ng/ml
	375	25	367.1	98.01
Carbamazepine	5.0 μg/ml	20	5.08 μg/ml	0.017 μg/ml
Cimetidine	300 ng/ml	15	281.0 ng/ml	1849 ng/ml
	500	14	489.3	1161
Prinomide tromethamine	50 μg/ml	35	50.9 μg/ml	8.01 μg/ml
	75	11	76.7	5.71

Table III. Analysis of the Components of Variance, C_{min} at Steady State

	Drug administered				
	Fadrozole hydrochloride	Metoprolol tartrate	Carbamazepine	Cimetidine	Prinomide tromethamine
Dose	2 mg CT[a]	100 mg CT	200 mg CT	300 mg CT	1000 mg CT
Dosage interval	12 hr	6 hr	12 hr	6 hr	12 hr
C_{min} units	ng/ml	ng/ml	μg/ml	ng/ml	μg/ml
F statistic (P value)					
For intersubject effect	38.1 (<0.001)	78.7 (<0.001)	15.0 (<0.001)	2.72 (0.056)	2.40 (0.039)
For day-to-day phase effect	0.926 (0.341)	1.13 (0.327)	1.29 (0.302)	3.65 (0.083)	0.308 (0.738)
For diurinal phase effect	25.1 (<0.001)	12.5 (<0.001)			
For phase × phase interaction	1.55 (0.219)	2.17 (0.121)			
Mean (±SE)					
Nighttime	3.7 (0.30)	287 (32.1)	5.0 (0.26)	448 (21.2)	57.2 (3.58)
Daytime	3.2 (0.30)	261 (32.1)			
Random population variance					
Intersubject (σ_τ^2)	1.61	18,320	0.572	3,427	89.5
Intrasubject (σ^2)	0.173	1,415	0.122	3,977	192
$\sigma_\tau^2/(\sigma_\tau^2 + \sigma^2)$, %	90.3	92.8	82.4	46.3	31.8
Fixed sample variance					
Diurinal phase (s_δ^2)	0.116	301			
s_δ^2/s^2, %	6.5	1.6			

[a] Commercial tablet.

state. In the two cases where diurnal effects were determinable, highly significant time-of-day effects were detected. The magnitude of the nighttime diurnal effect was 14 and 9% relative to the daytime values for fadrozole and metoprolol, respectively. The proportional contribution of the diurnal variance to the total observed sample variance was less than 7% and considered negligible. Experimental design effects with regard to within-day time of plasma sampling therefore did not contribute any substantial additional variance to that estimated from random effects alone.

The expected relative reproducibility of a randomly selected C_{min} value from a population or the average C_{min} value of a sample randomly selected from a population varied between cases. The largest coefficient of variation (CV) was 51.3% and the largest relative standard error (RSE) was 11.7%. Both of these values corresponded to metoprolol. Carbamazepine exhibited the smallest CV value, 16.7%; and cimetidine the smallest RSE value, 4.7%.

Random source components of variance were isolated from the specific fixed effects attributable to the diurnal and day-to-day variations. The intersubject and intrasubject variances when calculated from a sample of C_{min} values randomly selected from a larger population are unbiased point estimates of the corresponding population values. The estimated proportion of the intersubject source component of variance from the respective populations studied ranged from 31.8% for prinomide to 92.8% for metoprolol.

Of the two random effects evaluated, only the intersubject effect is uniquely causally related to its variance. The intrasubject effect, being the residual effect, represents the sum of all unidentified sources of random variation. Included in this effect is the variation in analytical determination of plasma concentrations. From Tables II and III, the intrasubject variance was partitioned into intrasubject variance resulting from "pure" intrasubject error and intrasubject variance due to assay variation (Table IV). The proportion of intrasubject variance resulting from assay variance ranged from a low of 3.8% for prinomide to a high of 33.7% for cimetidine. The estimated proportion of intersubject variance based on "pure" intrasubject variance ranged from 32.6% for prinomide to 93.2% for metoprolol. While in some instances, assay variance contributed substantially to intra-

Table IV. Proportion of Intrasubject Variance Due to Analytical Variance

	Intrasubject variance	Assay variance at C_{min}[a]	Proportion due to assay (%)	Pure intrasubject variance	$\sigma_\tau^2/(\sigma_\tau^2 + \sigma^2)$ adjusted (%)
Fadrozole hydrochloride	0.173	0.035	20.2	0.138	92.1
Metoprolol tartrate	1415	73.9	5.2	1341	93.2
Carbamazepine	0.122	0.017	13.9	0.105	84.5
Cimetidine	3977	1340	33.7	2637	56.5
Prinomide tromethamine	192	7.37	3.8	185	32.6

[a] Linear interpolation of proximal values.

subject variance, i.e., fadrozole and cimetidine, its effect on the estimation of the relative contribution of the intersubject and intrasubject variances was negligible.

In addition to the equivalence of long-term phase means, the appropriateness of the steady-state ANOVA employed in this study was further evaluated by testing the equivalence of all univariate phase variances for each diurnal phase on each day. In no case was significant heteroscedastic phase variance detected (Table V).

DISCUSSION

Ideally, steady state represents a state in which any measurement thereof is independent of time. In real biological systems, steady state can be known only in a time-averaged sense or average steady state. At any specified time, time-dependent accumulation or depletion in concentration can occur due to the negative or positive divergence of the drug input rate to the drug elimination rate. In pharmacokinetic studies, when the measured dependent variable is steady state trough plasma concentrations, the contribution of measurement (assay) error is directly accessible and can be easily evaluated with regard to its variance. Assay errors from serial steady-state concentration measurements can be assumed to be mutually independent and, therefore, independent of time. Other sources of residual error, however, may not be independent of time and therefore must be tested by probabilistic models in order to substantiate the attainment of steady state as well as differentiate any factors that may uniformly perturb the steady state. Within individuals, the variance of any remaining fluctuations, provided that they are random, is defined as the intrasubject variance. Causative factors contributing to the intrasubject variance may be local heterogeneities in the biological system presumed to result from the variation in pharmacokinetic "constants" usually descriptive of such systems. Intrasubject variance as such is fundamentally descriptive of the pharmacokinetic system and equally valuable as a descriptive parameter as the "constants" are themselves since it is indicative of the stability of the system under investigation.

Fluctuations about the average steady-state concentration which are not random and occur at regular fixed times are the result of predisposing fixed factors and are similar to treatment effects. Causal–effect relations can generally be established for these phase effects and usually are attributable to environmental factors such as the administration of food or metabolic activity level. These fluctuations contribute to the steady-state variance but are not fundamental in that they result from specific predisposing factors dictated by the experimental design which may be controlled. Fixed effects are important to identify, however, so that they may be accounted for in the statistical model in order to isolate random effects.

Table V. Bartlett Test for Heteroscedastic Phase Variance

	N	B statistic (P value)
Fadrozole hydrochloride	4	1.74 (0.63)
Metoprolol tartrate	6	3.92 (0.56)
Carbamazepine	3	3.16 (0.21)
Cimetidine	2	0.082 (0.78)
Prinomide tromethamine	3	1.33 (0.51)

From the distribution of the average steady state of individuals, a population average steady state may be defined which includes both intersubject and intrasubject variances. The population average and intersubject variance are fundamental characteristics of the population from which the experimental sample was obtained. The intersubject variance may be correlated with specific population subtypes. These correlations provide an explanation of the observed variability from which predictions may be made with regard to drug development, regulation, and therapy. Unexplained variability in populations, while less attractive, is nevertheless equally important. Once quantifiable, drugs exhibiting large intersubject variances can be identified and evaluated in terms of specific causal–effect relations.

Analysis of variance of both random and fixed effects contributing to pharmacokinetic characteristics provides a useful and powerful means for determining the source of variability in biological systems. It is important to recognize, however, the constraints which standard ANOVA imposes upon steady-state analysis. Standard ANOVA assumes the mutual independence of residual errors and a common error variance. Important precautions which should be employed to support the validity of any steady-state analysis are as follows: (i) statistical models should always incorporate a fixed phase effect in order to evaluate the equivalence of phase means, and (ii) heteroscedasticity in univariate estimates of phase variances should be tested by an appropriate method such as the Bartlett or Hartley test. Equivalent phase means and variances support the premise of independent normally distributed residual errors.

In order to broaden further the findings reported here with regard to the predominant source component of variance, the literature was searched for suitable replicate pharmacokinetic data which could be evaluated by random effects analysis. Two studies were found whereby phenacetin and trazodone hydrochloride were single-dosed on multiple occasions (2,7). The reported areas under the plasma concentration time curves (AUC) were subsequently analyzed for their components of variance according to the nominal model defined above. The results of this analysis are summarized in Table VI. In both cases, a predominant intrasubject proportion of variance was found. Indeed, due to the large contribution of intrasubject variance, a 57% increase in power was reportedly achieved by incorporating internal control on intrasubject variance using d_4-trazodone in a bioavailability comparison of two formulations. In addition to these studies, one study reported the components of variance of terodiline in support of a double-isotope bioequivalence trial (8). These investigators found 89.9% of the random variation attributable to intersubject variance. Even in this case, an improvement in power was reportedly achievable.

Surveying those drugs with known population characteristics, prinomide, phenacetin, trazodone, and CGS 16617 (6) were found to exhibit a predominant intrasubject source of variability. Carbamazepine, metoprolol, cimetidine, fadrozole, and terodiline were found to exhibit a predominant intersubject source of variability. An attempt to asso-

Table VI. Analysis of the Components of Variance, Single-Dose AUC[a]

	Drug administered	
	d_4-Trazodone hydrochloride	Phenacetin
Dose	50 mg	900 mg
Dosage form	Oral solution	Oral suspension
AUC units	ng · hr/ml	μg · min/ml
F statistic (P value)		
For intersubject effect	3.87 (0.033)	4.43 (0.004)
For phase effect	0.877 (0.446)	0.467 (0.759)
Mean (±SE)	5,542 (532.2)	288 (67.1)
Random population variance		
Intersubject (σ_τ^2)	1,010,301	19,932
Intrasubject (σ^2)	1,317,158	35,588
$\sigma_\tau^2/(\sigma_\tau^2 + \sigma^2)$, %	43.4	35.9
Ref. No.	7	2

[a] Reported data from literature sources.

ciate a predominant source of variability with the modality of drug elimination was unsuccessful. Fadrozole, carbamazepine, prinomide, phenacetin, trazodone, and metoprolol all are metabolically eliminated to an extent of greater than 90%, yet their population characteristics differ. Similarly, CGS 16617 and cimetidine are really eliminated to an extent of greater than 70% and exhibit different predominant sources of variability. Caution must be exercised in drawing conclusions about this apparent lack of association since the contribution of absorption rate or bioavailable fraction variance to C_{min} or AUC variance is presently unknown. It may be concluded, however, that the population characteristics of any particular drug are unique to its pharmacokinetic disposition. Further investigation will be required to elucidate causal–effect relationships between drug disposition and specific sources of random variability.

Carbamazepine represents a particularly interesting case because its clearance variability has been reported to be accounted for by induction of its metabolism through the epoxide-diol pathway (9,10). In view of our findings that intersubject variance represents 85% of its variability after 24 days of dosing, it may be concluded that the metabolic capacity for induction is subject specific and of a limited capacity which remains stable once fully induced.

Diurnal rhythms have been shown to affect drug disposition in a number of studies (11–13). The diurnal effects observed in this study are in agreement with these reports. Characteristically, the diurnal effect is strikingly invariant. Although the contribution of diurnal variation to the total population variance is minimal, its magnitude relative to the intrasubject variance is substantial. The diurnal variance was 67 and 21% of the intrasubject variances for fadrozole and metoprolol, respectively. This illustrates the need to isolate diurnal effects when analyzing for intrasubject effects. The average increase of 9–14% in C_{min} overnight suggests a reduction in clearance during this period since both fadrozole and metoprolol are well absorbed.

In conclusion, the characterization of population effects can effectively be accomplished using classical statistical methods employing a random effects probabilistic model. The primary criterion for such analysis is replication of measures within subjects and the mutual independence of these measures.

Intrasubject variance was demonstrated to be an important source of population variability for some drugs. This conclusion is not expected to be altered when populations are expanded to include disease state, age, etc., provided that such correlates are properly accounted for in the statistical models. Conversely, investigations of larger more diverse populations may lead to such correlations.

ACKNOWLEDGMENT

The author wishes to thank Dr. John V. Castellana, Department of Biostatistics, CIBA-GEIGY Corporation, for his careful review of the manuscript and helpful comments.

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