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Report

Between-Lot and Within-Lot Comparisons of Bioavailability of Macrocrystalline Nitrofurantoin Capsules

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Comparative bioavailability studies should be designed and the resulting data evaluated based on estimates of both intersubject and intrasubject variances in the kinetic parameters for the particular drug products(s) being studied. This report presents the results of two comparative bioavailability studies. In the first study, three production lots of macrocrystalline nitrofurantoin capsules (Macrodantin) were compared in 21 subjects, and in the second study, capsules from one production lot were administered to 21 different subjects on three occasions. Both model-independent kinetic parameters for urinary excretion and a one-compartment model with zero-order absorption were used to evaluate both the rate and the extent of bioavailability. Overall the results showed a very low variance between and within production lots and a relatively large intersubject variance in the rate and extent of absorption.

KEY WORDS: nitrofurantoin macrocrystals; bioavailability; urinary excretion; drug absorption.

INTRODUCTION

The increasing number of drug products no longer protected by U.S. patents and the legislation, enacted in 1984, affecting the approval of generic products (1) have stimulated renewed interest in the design and evaluation of comparative bioavailability studies. Recent presentations of FDA procedures at public meetings (2,3) indicate that randomized crossover studies, with analysis of variance and calculation of power, are the first line of evaluation. Other evaluation methods such as the 75/75 test (4,5) and confidence intervals may be employed when the first evaluation fails to show equivalence with sufficient power. Metzler and Huang (6) reviewed the various statistical methods employed to evaluate bioequivalence data. These methods attempt to address the fact that the rate and extent of absorption of a drug entity are functions of both the physical and chemical properties of the drug product and the physiological characteristics of the study subjects. Among a group of study subjects there are intersubject differences, and each subject may present for each dose period in a different condition, creating intrasubject differences, even if the differences are not obvious to the investigator and the subject is within the protocol. For example, despite well-controlled conditions, subtle differences in the acidity, motility, and other conditions of the gastrointestinal tract may significantly influence drug absorption. Drug products can have some variance in properties between production lots from the same manufacturer and even within the same production lot. In the commonly employed randomized crossover study design, each subject receives each drug product only once, and the intrasubject variance is confounded with the drug product or treatment variance, since the statistical model is usually partitioned into periods, subjects (i.e., intersubject), treatments (i.e., drug products), and an error term (residual sum of squares). The underlying assumption is that intrasubject variance is relatively small, and the variance for the interaction of the subject with each dosage form is attributed to the dosage form itself. The actual intrasubject variance will be specific to the drug product, since it is essentially an interaction of subject and product as noted above; it should thus be assessed for each drug product at some point to validate study design.

Macrocrystalline nitrofurantoin capsules (Macrodantin, Norwich Eaton Pharmaceuticals, Inc.) have been designed to provide a sustained release and absorption of nitrofurantoin as recently summarized (7). This report presents the urinary excretion data from two studies of macrocrystalline nitrofurantoin, which were designed to assess comparative bioavailability between production lots of the same manufacturer and also the combined intrasubject and within-lot variance for one production lot.

MATERIALS AND METHODS

In the first study, 50-mg capsules from three production lots (designated A, and B, and C)[4] were administered in single 100-mg (2-capsule) doses to 21 normal healthy male subjects using a randomized, three-way crossover design.

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[4] A, L.N. 54309; B, L.N. 54045; C, L.N. 54262.

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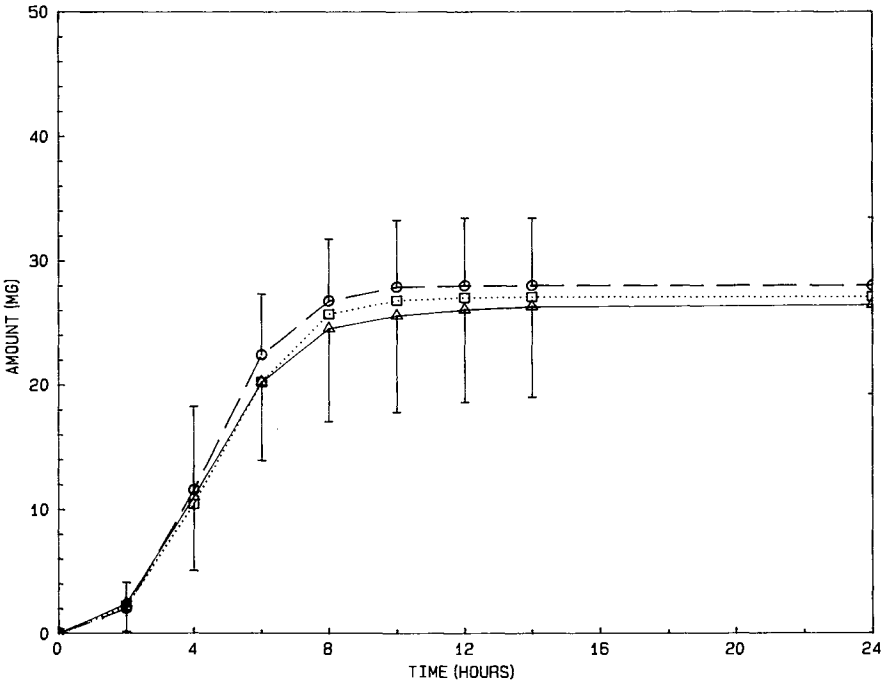

Fig. 1. Mean (±1 SE) cumulative nitrofurantoin excretion following 100-mg doses of three lots of macrodantin. (△) Lot A; (○) lot B; (□) lot C.

The second study was identical to the first in all aspects except the capsules from only one production lot, arbitrarily selected from the first three lots, were administered on all three occasions, and a different group of 21 subjects was employed. Both studies employed the same protocol, HPLC analysis, and pharmacokinetic and statistical evaluations as described previously (7).

RESULTS

Plots of the mean accumulation (N = 21) of nitrofurantoin in the urine for all three lots (A, B, C) are quite similar, as shown in Fig. 1. Data and summary statistics for each of the three model-independent kinetic parameters of the three lots are shown in Table I. The mean recovery over 24 hr for each lot is within ± 1% of the grand mean of 26.9%, and analysis of variance shows no difference between lots with the power to detect a 20% difference equal to 0.92 (taking lot C as the arbitrary reference). There are significant intersubject differences ($P < 0.013$) for dose recovery (DR), with DR% ranging from 4.1 to 39.0%. One would conclude that the 21-subject study does establish that the three lots provide equivalent amounts of nitrofurantoin.

Table I. Model-Independent Kinetic Parameters for Excretion of Nitrofurantoin After Administration of Capsules from Three Production Lots of Macrodantin[a]

	R_{max} (mg/hr)			T_{max} (hr)			DR% (% actual dose)		
	Lot A	Lot B	Lot C	Lot A	Lot B	Lot C	Lot A	Lot B	Lot C
Mean	5.88	6.72	6.02	4.7	4.3	4.6	26.27	27.86	26.54
±SD	2.12	1.37	1.59	2.1	1.0	1.4	7.26	5.43	6.25
Min.	0.49	3.95	2.63	3.0	3.0	3.0	4.05	19.37	13.09
Median	5.92	6.66	6.32	5.0	5.0	5.0	26.67	27.77	27.33
Max.	10.44	9.99	8.78	11.0	5.0	7.0	38.93	38.99	35.87
PR > F[b]	—	—	0.213	—	—	0.549	—	—	0.539
Power (=20%)[c]	—	—	64%	—	—	59%	—	—	92%
Power (=25%)[d]	—	—	82%	—	—	79%	—	—	—
N^e	—	—	30	—	—	34	—	—	—

[a] Two 50-mg capsules as a single 100-mg dose.
[b] Probability of difference by lot due to chance for the ANOVA model: Treatments (Lot), Periods, Subjects.
[c] Power to detect a 20% difference from lot C (14).
[d] Power to detect a 25% difference from lot C (14).
[e] Estimated number of subjects to attain a power of 80%, i.e., to detect a 20% difference from lot C (14).

The two rate-indicating parameters, R_{max} and T_{max}, have grand means of 6.21 mg/hr and 4.54 hr, respectively, and there are no significant differences between lots ($P > 0.05$); however, the statistical power in each case is less than 80%. About 34 subjects would be required to achieve an 80% probability of detecting a 20% difference from lot C. Alternatively, the power requirement could be met if the detectable difference were raised to 25%, as indicated in Table I. Neither R_{max} nor T_{max} would pass the 75/75 test in this group of 21 subjects. In this study both intrasubject and between-lot variances are attributed to the dosage form, as discussed in the Introduction.

Figure 2 presents the mean cumulative nitrofurantoin in the urine by period in the second study, in which 21 subjects received doses of capsules from lot C on each of three separate occasions. All three plots are quite similar, and analysis of variance shows no significant difference between periods in any of the three model-independent parameters, R_{max}, T_{max}, and DR%. The means for each period, as shown in Table II, are very close to the grand means of 6.16 mg/hr, 4.8 hr, and 30.6% of the acutal dose for R_{max}, T_{max}, and DR%, respectively. This compares well with the means of 6.02 mg/hr, 4.6 hr, and 26.54%, respectively, when the same lot had been administered to a different group of 21 subjects in the first study. Analysis of variance between subjects and within subjects (between periods) resulted in no significant intrasubject effects, with significant intersubject differences for both T_{max} and DR%; R_{max} was not significantly different ($P = 0.09$). The power to detect a 20% difference from the grand means was 54% for R_{max}, 63% for T_{max}, and 86% for DR%.

The mean range of a parameter over all subjects (i.e., the range for each subject averaged over all subjects) is an indicator of the intrasubject variation (between periods). Likewise, the mean range of a parameter over all three test periods (i.e., the range for each test period averaged over all three periods) is an indicator of intersubject effects. In this study the intrasubject variation was noticeably smaller than the intersubject variation. The mean range over subjects was 3.12 mg/hr, 2.24 hr, and 9.96% for R_{max}, T_{max}, and DR%, respectively. The mean range over periods was 7.85 mg/hr, 7.33 hr, and 29.98% for R_{max}, T_{max}, and DR%, respectively.

Of the 63 excretion-rate curves from the first study with lots A, B, and C, only 14 could not be described by a one-compartment model with first-order elimination and first- or zero-order absorption. These 14 had too few data points or a correlation coefficient less than 0.90. Comparison of residuals and correlation coefficients revealed the zero-order absorption model to be more appropriate for 46 of the curves and the first-order absorption model for 1 curve, with 2 curves described equally well by both models. Table III presents the summary statistics for the pharmacokinetic parameters as defined (7) for zero-order absorption. Overall, the mean elimination-rate constant was 0.686 per hr, which corresponds to an elimination half-life of 1.0 hr and shows no significant differences between lots. The grand means for the lag time (T_L) and absorption time (T_A) were 0.748 and 4.51 hr, respectively, with the only significant difference being the higher T_L for lot B, which is 0.39 hr (23.4 min) greater than lot A. As shown in Table III, only the T_A had a power to detect a 25% difference from lot C greater than 80%.

Also shown in Table III are the statistics for the parameter estimates of the same zero-order absorption model as applied to the data of the second study. In this study there were 63 excretion-rate curves for lot C in the 21 subjects. Based on the criteria stated above, 42 were most appropriately described by the zero-order absorption model, 2 by the

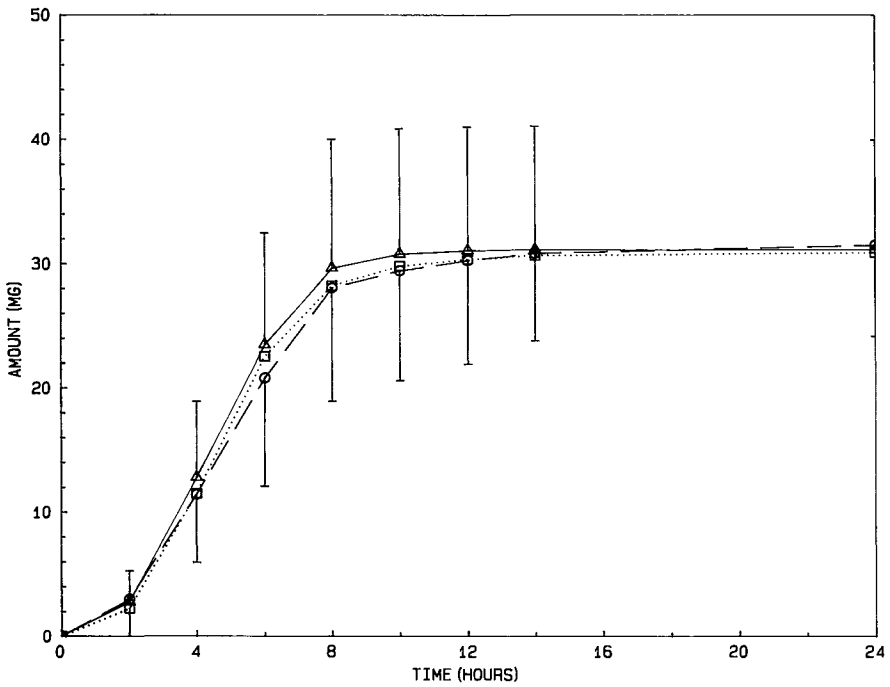

Fig. 2. Mean (±1 SE) cumulative nitrofurantoin excretion for one lot of macrodantin administered on three occasions. (△) Lot A; (○) lot B; (□) lot C.

Table II. Model-Independent Kinetic Parameters for Excretion of Nitrofurantoin After Administration of Capsules from the Same Lot of Macrodantin to 21 Subjects on Three Occasions[a]

	R_{max} (mg/hr), Period			T_{max} (hr), Period			DR% (% actual dose), Period		
	1	2	3	1	2	3	1	2	3
Mean	6.71	5.55	6.21	4.5	5.0	5.0	30.53	30.89	30.30
±SD	2.35	1.49	2.09	1.4	2.5	1.8	9.73	8.65	6.64
Min.	2.88	3.29	2.97	3.0	1.0	3.0	11.08	17.43	14.89
Median	6.20	5.18	5.90	5.0	5.0	5.0	30.75	31.40	31.58
Max.	11.85	8.67	12.16	7.0	13.0	9.0	47.79	44.42	41.14
PR > F[b]									
Intrasubject	—	—	0.972	—	—	0.704	—	—	0.809
Period	—	—	0.142	—	—	0.392	—	—	0.952
Intersubject	—	—	0.094	—	—	0.001	—	—	<0.001
Power (=20%)[c]	—	—	54%	—	—	63%	—	—	86%

[a] Two 50-mg capsules from lot C as a single 100-mg dose.
[b] Probability of difference due to chance for the ANOVA model: Treatments (Lot), Periods, Subjects.
[c] Power based on probability of detecting a 20% difference from the values in the position of lot C in the randomization schedule.

first-order model, and 2 by either model, and 17 did not meet the criteria for curve fitting. The parameters for lot C agree closely with the values estimated in the 19 subjects showing zero-order absorption in the first study.

DISCUSSION

In the randomized crossover design commonly employed in comparative bioavailability studies, each subject receives each treatment only once, with the variance being attributed to treatments [i.e., dosage form(s) tested], subjects (i.e., intersubject effects), and treatment periods, plus residual. In reality the kinetic parameters are functions of the physiological characteristics of the individual subject at the time of the treatment and of the physical and chemical properties of the specific dose unit(s) the subject receives, both of which fall under treatment in the statistical model. In the first study, in which capsules from three production lots from the same manufacturer were the treatments, the three lots were found to give nearly identical urinary recoveries of nitrofurantoin of about 27% of the dose, with significant intersubject differences. Recoveries ranged from 4.1 to 39%. Analysis of variance revealed no significant differences in R_{max} (power = 64%), T_{max} (power = 59%), or DR% (power = 92%) between lots. Only DR% had at least a power of 80% to detect a 20% difference, whereas about 34 subjects of similar variance would have been required to achieve 80% power for R_{max} and T_{max}.

Because controlled release is important for both reducing the incidence of adverse effects and assuring an adequate duration of antimicrobial urinary concentrations of macrocrystalline nitrofurantoin, testing the absorption-rate parameters is essential. As a possible alternative to using more than 30 subjects, one may consider the absorption time

Table III. Pharmacokinetic-Model Parameters for Zero-Order Absorption of Macrodantin

	K_e (hr^{-1})				T_L (hr)				T_A (hr)			
	Lot A	Lot B	Lot C	Lot C[a]	Lot A	Lot B	Lot C	Lot C[a]	Lot A	Lot B	Lot C	Lot C[a]
N	14	16	19	46	14	16	19	46	14	16	19	46
Mean	0.68	0.71	0.67	0.61	0.59	0.98	0.67	0.80	4.59	4.24	4.68	4.40
±SD	0.25	0.21	0.27	0.22	0.34	0.54	0.27	0.50	1.14	1.22	1.35	1.25
Min.	0.33	0.38	0.34	0.19	0.00	0.51	0.00	0.00	3.05	1.93	2.00	2.09
Median	0.62	0.76	0.61	0.50	0.70	0.93	0.75	0.78	4.27	4.17	4.45	4.07
Max.	1.23	1.01	1.32	1.00	0.96	2.92	0.96	2.77	6.42	6.00	6.78	8.01
PR > F[b]	—	—	0.809	—	—	—	0.018	—	—	—	0.513	—
Power (=20%)[c]	—	—	44%	—	—	—	18%	—	—	—	70%	
Power (=25%)[d]	—	—	62%	—	—	—	28%	—	—	—	87%	
N[e]			49				128				27	

[a] Capsules from Lot C were administered to 21 subjects, three times each, in the second study.
[b] Probability of difference in means due to chance for the ANOVA model: Treatments (Lot), Period, Subjects.
[c] Power to detect a 20% difference from lot C (14).
[d] Power to detect a 25% difference from lot C (14).
[e] Estimated number of subjects to attain a power of 80%, i.e., to detect a 20% difference from lot C (14).

(T_A), estimated by fitting the excretion-rate curves to a one-compartment model with zero-order absorption. Although only about 78% of the curves could be described by this model, the power of this parameter to detect a 20% difference in rate was about 70%; for a 25% difference, it was 87%.

Administration of capsules from the same production lot to 21 subjects on three occasions allowed an estimate of the intrasubject variance (i.e., within-subject and within-lot effects combined) to be calculated. Although different groups of subjects were employed in the two studies, all of the parameter estimates for capsules from lot C agreed within ±10% across the studies. The intrasubject variance was quite small compared with the intersubject variance on the same production lot and shows the lot of capsules tested to be highly consistent in absorption characteristics when administered three times to the same subject. Also, the power of the second study was quite similar to that of the first for both model-independent and kinetic-model parameters.

Clearly, the data from these studies revealed that the behavior of the capsules of macrocrystalline nitrofurantoin (Macrodantin) was highly reproducible, both within a production lot and between production lots. Also, the data suggest that more than 30 subjects would be required to test bioequivalence for both rate and extent, if model-independent kinetic parameters for excretion in urine are the basis of comparison. A reduction in the number of subjects may be possible if the data could be described by a zero-order absorption model with first-order elimination. An important note here is that these results were obtained with capsules produced by the innovator of the macrocrystalline nitrofurantoin product, Macrodantin, and the variances observed may or may not be replicated with products from alternative sources. Finally, one must keep in mind that the extent of absorption alone is not sufficient, but that the rate of absorption of macrocrystalline nitrofurantoin is also an important concern.

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