Moricizine Bioavailability via Simultaneous, Dual, Stable Isotope Administration: Bioequivalence Implications

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The relative bioavailability of a 200 mg film-coated tablet of [^{12}C]moricizine•HCl in comparison to a 200 mg [$^{13}C_6$]moricizine•HCl oral solution was determined after simultaneous administration to 8 young healthy male subjects. Concentrations of [^{12}C]moricizine•HCl and [$^{13}C_6$]moricizine•HCl were determined by thermospray liquid chromatography-mass spectrometry (LC-MS) using [$^{2}H_{11}$]moricizine•HCl as the internal standard. The mean absorption and disposition parameters of the tablet versus the solution were the following (% CV): maximum concentration, 0.83 (39%) versus 0.79 (39%) μg/mL; time of maximum concentration, 0.81 (40%) versus 0.65 (28%) hours; area under the concentration-time curve (AUC), 1.58 (39%) versus 1.49 (37%) μg•h/mL; apparent oral clearance, 150.7 (52%) versus 158.1 (50%) L/h; and $t_{1/2}$, 1.9 (42%) versus 1.9 (42%) hours. The AUC for the tablet averaged 106% of the solution, which likely reflects a greater first-pass effect with the oral solution. Partitioning sources of variation confirmed the low (< 6%) intrasubject coefficient of variation (cv_ε) afforded via the single-period, dual-isotope design. In contrast, a previous study using the conventional two-period crossover design determined the cv_ε about moricizine metrics to be in excess of 30%, resulting in classification of this drug as having highly variable absorption. The results of this study further illustrate the benefits of dual, stable isotopes to assess bioavailability and bioequivalence. This paradigm results in a reduction in experimental time and subject inconvenience and lower costs in comparison with the standard crossover study. Perhaps most important is the improved statistical power for the evaluation of bioavailability or bioequivalence in the absence of period and sequence effects that confound the assessment of intrasubject variation in the standard crossover design.

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Moricizine•HCl, [10-[3-(4-morpholinyl)-1-oxopropyl]- 10H-phenothiazin-2-yl]carbamic acid ethyl ester monohydrochloride, or Ethmozine (the proprietary name frequently found in the early literature) is a novel phenothiazine derivative that is an effective agent for treating ventricular arrhythmias.[1]

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Moricizine is both rapidly and completely absorbed after oral administration; however, due to extensive first-pass metabolism, the absolute oral bioavailability of moricizine is only 34% to 38%.[2,3] A pilot relative bioavailability (tablet to solution) study employing 24 subjects in a standard crossover design was conducted as an additional arm of a food interaction study[4] and resulted in an inadequately powered study to discern a statistically meaningful difference.[5] The mean results from the initial study are shown in Figure 1. These results confirmed the classification of moricizine as being a drug with highly variable absorption given that the intrasubject variability in log-transformed metrics exceeded 30%. It was estimated that a sample size of 80 would be required to pass average equivalence criteria with the desired statistical power. Such a study is both time and cost prohibitive and is ethically questionable in light of the number of healthy volunteer drug

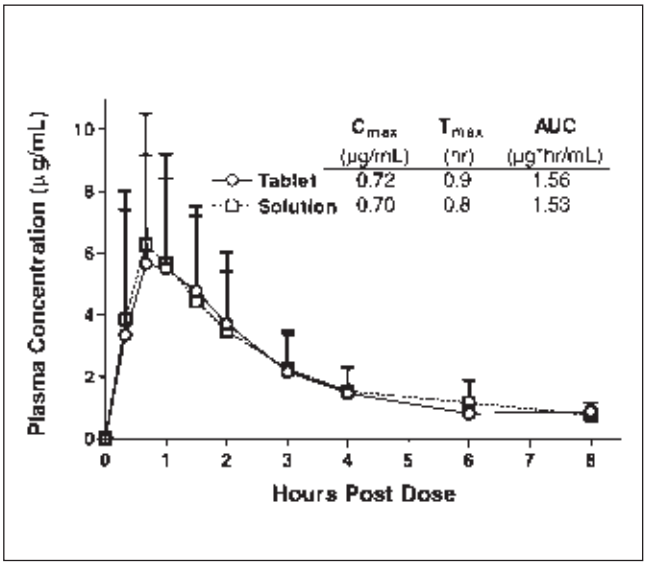

Figure 1. Mean (+ standard deviation) plasma concentration-time profile and pharmacokinetic summary from 24 healthy male volunteers administered a 250 mg tablet and a 250 mg oral solution of moricizine on separate occasions.

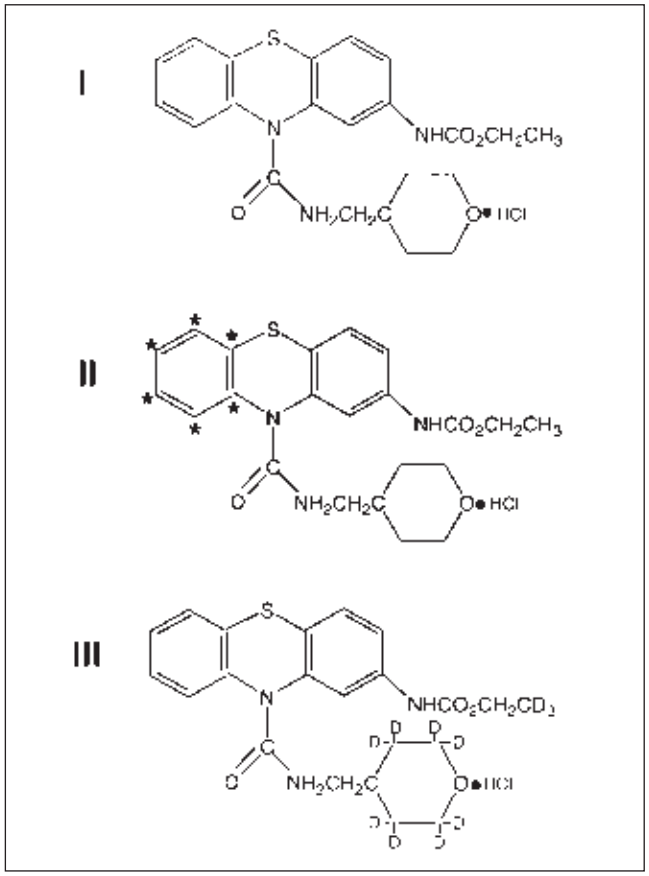

*Figure 2. Chemical structures for unlabeled [^{12}C]moricizine•HCl (I), [$^{13}C_6$]moricizine•HCl (II), and [$^{2}H_{11}$]moricizine (III). The positions of the ^{13}C- and ^{2}H-atoms are denoted * and D, respectively.*

exposures required. An alternative experimental design was chosen—simultaneous oral dosing of both tablet and oral solution dosage forms, which would eliminate the concern of within-subject differences in clearance that may occur with dosing on two different occasions. The experimental design requires that two different isotopic forms of the drug be incorporated separately into the dosage forms. This approach results in a substantial reduction in the total number of subjects needed to examine average equivalence and provides greater statistical power to evaluate true differences.[6,7] Moreover, given that period and subject with sequence effects are not incurred in such a design, the estimation of intrasubject variability can be more accurately assessed. As both formulations are assessed simultaneously, balanced groups are ensured. We report the results of such a study in 8 subjects using ^{12}C- and $^{13}C_6$-labeled moricizine.

METHODS

Drug Preparations

The [$^{13}C_6$]moricizine•HCl was synthesized under GMP conditions (DuPont—NEN Research Products, Boston, MA) and was determined to be 98.0% chemically and 99.2% isotopically pure. An internal standard for the analytical method was synthesized (DuPont Co., Wilmington, DE) by initially reacting [$^{2}H_3$]carbamic acid and (10H-phenothiazin-2-yl) ethyl ester (DuPont—NEN Research Products, Boston, MA) with 3-chloropropionyl chloride. The resulting product was further reacted with [$^{2}H_8$]morpholine (Merck Sharp & Dohme Isotopes, Montreal, Canada) to give [$^{2}H_{11}$]moricizine free base (95.9% chemically and 99.1% isotopically pure). All moricizine amounts and concentrations are expressed in terms of the hydrochloride salt. The structures of unlabeled, $^{13}C_6$-labeled, and $^{2}H_{11}$-labeled moricizine are shown in Figure 2.

Clinical Protocol

Subjects. Eight healthy nonsmoking male Caucasian volunteers participated in this study after providing written informed consent. The mean (± standard deviation) age, weight, and height of the study participants were 27 ± 7 years, 77 ± 12 kg, and 71 ± 2 inches, respectively. Healthy status was established on the

basis of medical history, physical examination (including a 12-lead electrocardiogram), and clinical laboratory findings. Exclusion criteria were known sensitivity to phenothiazines or heparin, use of any drug known to alter liver function within 1 month of the study, use of any prescription drug within 2 weeks of the study, use of any over-the-counter product or ethanol within 1 day of the study, and greater than 10% variation from ideal body weight for age and height.

Protocol. The study was based on a single-dose, open-label, two-treatment, single-period design. On the morning of the study, following an overnight fast, each subject ingested an oral 200 mg dose of a film-coated moricizine•HCl tablet (with 8 ounces of water) followed immediately with a 200 mg dose of [$^{13}C_6$]moricizine•HCl as a solution. The [$^{13}C_6$]moricizine•HCl was supplied as a powder in a sealed vial and was reconstituted with 11 mL water immediately prior to dosing. Once the powder was completely dissolved, a volume (10 mL) of solution corresponding to the exact dose required was removed via syringe. The solution dose was administered orally using the syringe followed by a water rinse of the syringe and subsequent ingestion of the rinse solution.

Subjects remained seated prior to and for 2 hours following dosing. After that time, the subjects were free to move about, but any strenuous activity was forbidden. They were not permitted any beverage 1 hour prior to dosing, and food was administered in the form of a standard meal, low in fat content, 4 hours after dosing. A standard meal was also served 10 hours following dosing. No other food was permitted, but subjects were allowed to ingest fluids freely postdose. Blood samples were obtained via an indwelling venous catheter placed into the forearm vein and collected into glass tubes containing lithium heparin. Prior to obtaining the blood sample, the heparin contained in the catheter was removed (1 mL) and discarded. A blood sample was taken, and heparin was then reinstilled to maintain patency. Blood samples were taken prior to and at the following times relative to dosing: 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours. Plasma was obtained within 1 hour of sample collection and stored frozen in polypropylene cryotubes (Vanguard International Co., Neptune, NJ) until analyzed.

Vital signs (heart rate, blood pressure, respiratory rate, and oral temperature) were obtained just prior to dosing, 4 hours after dosing, and after the last blood sample was obtained. At each blood sampling time, subjects were asked how they felt and were queried for any physical complaints. The study was conducted at the University of Arizona Health Sciences Center (Tuscon, AZ), and the institutional review was performed and approved by the University of Arizona Health Sciences Center Institutional Review Committee (Tucson, AZ).

Analytical

Plasma samples were assayed for unlabeled and $^{13}C_6$-labeled moricizine•HCl by a quantitative thermospray liquid chromatography-mass spectrometry assay.[8] After addition of the [$^2H_{11}$]moricizine as an internal standard, plasma samples were extracted under alkaline conditions into dichloromethane. Reconstituted extracts were chromatographed on an ODS column using a volatile mobile phase consisting of methanol/0.1 M ammonium acetate containing 0.2% triethylamine (65:35). The mass spectrometry system consisted of a Finnigan 4600 TSQ and a Vestec thermospray interface. Multiple ions at m/z 428, 434, and 439 were scanned using selected ion monitoring. The assay was linear over a plasma concentration range of 0.01 to 0.8 g/μL. Plasma samples that assayed greater than 0.8 g/μL were diluted with control plasma and reassayed. Intraday precision and accuracy ranged from 1.8% to 13.3% and 1.9% to 15.8%, respectively. Interday precision ranged from 1.9% to 12.0%.

Pharmacokinetics

The plasma concentration-time data for unlabeled and $^{13}C_6$-labeled moricizine were individually analyzed by noncompartmental methods. The natural log concentration-time data in the postabsorptive, postdistributive phases were analyzed by linear regression to obtain an estimate of the terminal disposition rate constant (λ_n) and from which the disposition half-life ($t_{1/2}$) was calculated ($LN2/\lambda_n$). The area under the plasma concentration-time curve (AUC) was estimated by the linear trapezoidal rule with extrapolation to time infinity by dividing the last measured concentration on the regression line (C_n) by the terminal disposition rate constant (λ_n). The apparent oral clearance (CL_o) was calculated by dividing the oral dose by the corresponding AUC. The maximum plasma concentration (C_{max}) and the time of its occurrence (t_{max}) were recorded as the actual observed values. Relative pharmacokinetic values for AUC, C_{max}, t_{max}, and λ_n were calculated by dividing the pharmacokinetic results of the [^{12}C]moricizine tablet by those of the [$^{13}C_6$]moricizine solution; relative AUC and C_{max} were then normalized by the ratio 1.0125 since the molecular weight of the isotope-labeled moricizine (solution) is 1.25% greater

than that of the moricizine tablet. The natural logarithms of the relative pharmacokinetic parameters AUC, C_{max}, and λ_n were then computed. Mean ratios (differences in logarithmic scale) were calculated, and these transformed parameters were used in all subsequent statistical analyses.

Statistics

All statistical analyses were performed using PC SAS version 6.12. The ratio of test to reference means (point estimates) and 90% confidence intervals about the log-transformed pharmacokinetic metrics AUC and C_{max} were estimated from ANOVA and least squares mean estimates using the relations below.[9]

$$\text{Ratio of Means} = 100*\exp[LSM_t - LSM_r], \quad (1)$$

where LSM_t and LSM_r are the least squares means for test (tablet) and reference (solution) formulations, respectively.

$$\text{Confidence Interval for Mean Ratios} = 100*\exp[LSM_t - LSM_r \pm t_{df,0.05} * SE_t], \quad (2)$$

where SE_t is the standard error of the least squares mean of the test formulation.

A student's *t*-test on the untransformed parameters was also performed assuming independent variances across formulation. Results from the ANOVA were also used to partition the variation in log-transformed metrics (AUC and C_{max}).[10] The intrasubject coefficient of variation, CV_ε, defined as $CV_\varepsilon = [\exp(\sigma_\varepsilon^2) - 1]^{1/2}$ was estimated by

$$cv_\varepsilon = [\exp(MS_\varepsilon) - 1]^{1/2}, \quad (3)$$

where MS_ε is the residual mean square error. Similarly, the intersubject coefficient of variation, CV_s, defined as $CV_s = [\exp(\sigma_s^2) - 1]^{1/2}$, was estimated by

$$cv_s = [\exp(MS_s - MS_\varepsilon)/2 - 1]^{1/2}, \quad (4)$$

where MS_s is the subject component of the model sum of squares. The power (i.e., probability of detecting a 20% difference relative to the reference formulation LSM_r at the 5% significance level using a *t*-test under the null hypothesis of zero-difference) was calculated for the log-transformed parameters AUC and C_{max} using the following equation:

$$\text{Power}(\%) = 100 \times \text{Prob}\left(\frac{LN(1.25)}{SE_{t-r}} - t_{df,0.025} > t_{df}\right). \quad (5)$$

Power curves based on Schuirmann's two one-sided test procedure[9] were calculated using Liu and Chow's approximate formula[11] (see equation (6)), based on a 2 × 2 crossover design under scenarios in which the difference in bioavailability (θ) of test and reference formulations were θ = 0, 5%, and 10%. The bioequivalence limit (∇) was set to ± 20% of the average reference bioavailability, which is commonly employed in designing most bioequivalence studies according to the current FDA guidelines.

$$n \geq [t_{\alpha,2n-2} + t_{\beta,2n-2}]^2 \times \left[\frac{cv_\varepsilon}{(\nabla - \theta)}\right]^2. \quad (6)$$

RESULTS

The 8 subjects originally enrolled completed the study. Several adverse reactions noted by the subjects appeared to be drug related. All such instances were of short duration and of mild to moderate severity (i.e., the subject was aware of the symptom, but it was easily tolerated). The symptoms noted (number of subjects) were the following: "lightheaded" or "dizzy" (5), "nausea" (2), "fatigue" or "drowsy" or "relaxed" (3), "numb throat" (1), "fingers tingle" (1), "cold hands" or "cold arm" (1), and "stomach cramp" (1). With the exception of three of these incidents, which were judged to be of moderate severity (i.e., discomfort associated with the symptom; "lightheaded," "fatigue," and "drowsy"), all reported adverse effects were of mild severity. With the exception of one incident of "fatigue" (4.5 hours) and one incident of "cold hands" (2 hours), all of the symptoms were reported within 1 hour of dosing. The majority of the symptoms lasted for no more than 1 hour after they were noted with the following exceptions (duration; number of subjects): "fatigue" (1.25 hours; 1), "nausea" (1.17 hours; 1), "cold hands" (3.0 hours; 1), and "cold left arm" (2.0 hours; 1). Within 4 hours of dosing, vital signs were normal and not statistically different from predose values.

Figure 3 shows the individual plasma concentration-time profiles for [^{12}C]moricizine and [$^{13}C_6$]moricizine in the 8 volunteers. These data confirm the relative contributions of intra- and intersubject variation. While the profiles are extremely consistent within subject, the disparity in subject 3 relative to subjects 7 and 8 illustrates the large intersubject variability. In the majority of the cases, the data were uniform in appearance with the exception of increasing concentrations between 6 and 12 hours in subjects 1 and 3 and amenable to noncompartmental analysis.

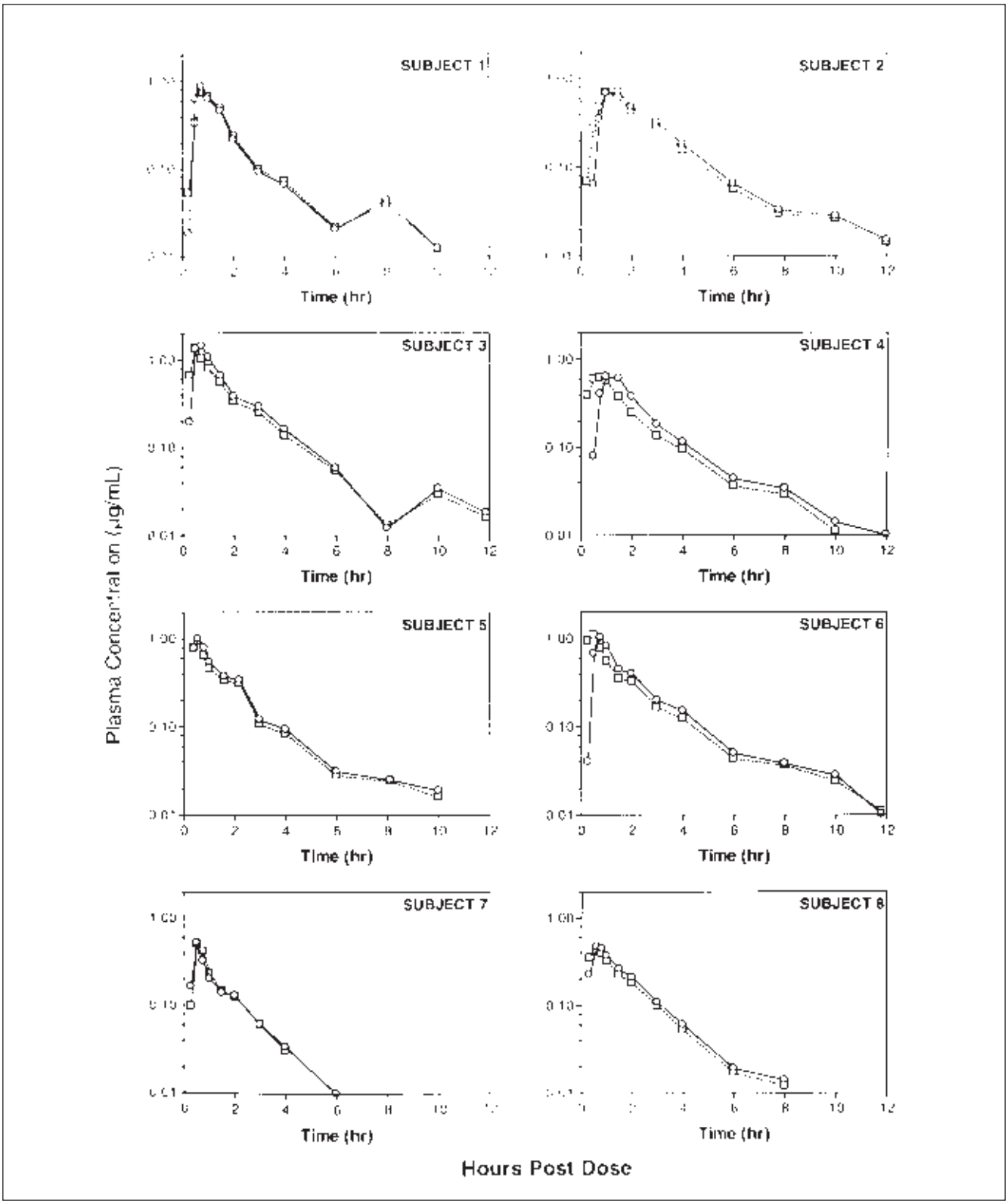

Figure 3. Individual plasma concentration versus time profiles of [^{12}C]moricizine (open circles) and [$^{13}C_6$]moricizine (open squares) following simultaneous administration of tablet (open circles) and solution (open squares) 200 mg dosage forms to healthy male volunteers.

Table I Absorption and Disposition Parameters of Moricizine following the Simultaneous Oral Administration of a 200 mg Film-Coated Tablet of [^{12}C]moricizine•HCl and a 200 mg [$^{13}C_6$]moricizine•HCl Oral Solution to Healthy Young Males

	Tablet [^{12}C]moricizine					Solution [$^{13}C_6$]moricizine				
Subject	C_{max} (μg/mL)	t_{max} (h)	AUC (μg•h/mL)	CL_o (L/h)	$t_{1/2}$ (h)	C_{max} (μg/mL)	t_{max} (h)	AUC (μg•h/mL)	CL_o (L/h)	$t_{1/2}$ (h)
1	0.87	0.75	1.37	146.1	2.3	0.74	0.75	1.33	150.3	2.6
2	0.71	1.50	2.01	99.7	3.1	0.70	1.00	1.91	104.6	3.3
3	1.42	0.75	2.54	78.9	2.1	1.33	0.50	2.26	88.4	2.2
4	0.63	1.00	1.52	131.3	2.3	0.62	0.77	1.43	139.9	2.0
5	1.00	0.58	1.71	117.2	2.5	0.92	0.58	1.53	130.6	2.5
6	1.03	0.77	1.95	102.7	2.2	1.11	0.50	1.92	104.1	2.4
7	0.52	0.50	0.63	319.5	1.2	0.50	0.50	0.62	320.5	1.1
8	0.47	0.62	0.95	210.0	1.2	0.42	0.62	0.88	226.8	1.2
Mean	0.83	0.81	1.58	150.7	1.9[a]	0.79	0.65	1.49	158.1	1.9[a]
SD	0.32	0.32	0.61	78.9	0.8	0.31	0.18	0.55	78.3	0.8

a. Harmonic mean and pseudo-standard deviation.

Absorption and disposition parameters for each subject for both tablet and solution treatments are displayed in Table I. The ratios of tablet to solution parameters are displayed in Table II. Ratios ([^{12}C]moricizine/[$^{13}C_6$]moricizine) for C_{max} and AUC were analyzed by comparing the observed ratio with a theoretical ratio of 1.0125; ratios for λ_n were compared to the theoretical ratio of 1.0. The increased exposure (6% increase in AUC) with the tablet is deemed clinically insignificant.

Table III contains the metrics for bioavailability equivalence assessment via the two one-sided test procedure for the current study relative to those obtained from a previous study in which a traditional crossover design was employed.[4] Intra- and intersubject variation about the log-transformed metrics and the power of the two one-sided procedure to detect a 20% difference are shown as well as indices of variation (total %CV) about each individual concentration-time data pair and across the concentration-time profile (mean

Table II Ratio of Moricizine Pharmacokinetic Metrics following Simultaneous Administration of a 200 mg Film-Coated Tablet of [^{12}C]moricizine•HCl and a 200 mg [$^{13}C_6$]moricizine•HCl Oral Solution to Healthy Young Males

	Ratio of Tablet to Solution				
Subject	C_{max}	t_{max}	AUC	CL_o	$t_{1/2}$
1	1.18	1.00	1.03	0.97	1.14
2	1.01	1.50	1.05	0.95	1.07
3	1.07	1.50	1.12	0.89	1.02
4	1.03	1.30	1.07	0.94	0.89
5	1.08	1.00	1.11	0.90	0.99
6	0.93	1.54	1.01	0.99	1.09
7	1.04	1.00	1.00	1.00	0.92
8	1.10	1.00	1.08	0.93	1.01
Mean	1.05	1.23	1.06	0.95	1.02
SD	0.07	0.26	0.04	0.04	0.09
p-value	0.08	—[a]	0.02	—[a]	0.64

a. Treatment effect not evaluated.

Table III Comparison of Bioequivalence Metrics and Indices of Variability for Moricizine Tablet and Solution Formulations Studied in Conventional Two-Way Crossover and Dual-Isotope Study Designs

	Crossover Study Two Period (n = 24)		Dual-Isotope Study Single Period (n = 8)	
Bioequivalence Metrics	**LN(AUC)[a]**	**LN(C_{max})**	**LN(AUC)**	**LN(C_{max})**
Ratio of means (%)	110.8	105.3	107.5	106.7
90% confidence interval	(98.7-124.6)	(92.6-119.9)	(105.4-109.6)	(103.0-110.4)
Intrasubject variation; CV_ε%	34.2	38.2	2.8	5.1
Intersubject variation; CV_s%	53.0	46.2	46.3	39.3
Power, $(1 - \beta)$%	63.2	50.7	> 99	> 99
Indices of Variation	**Tablet**	**Solution**	**Tablet**	**Solution**
CV% range across concentration-time profile	[30-139]	[24-92]	[30-146]	[18-83]
Mean CV% across concentration-time profile	61	58	56	44

a. Truncated AUC (AUC to last observed concentration-time pair instead of AUC to infinity) due to inadequately defined terminal phase (assay lower limit of quantification was 60 ng/mL for pilot crossover study).

%CV). As expected, a significant reduction in the true intrasubject variation is observed in the log-transformed metrics using the stable isotope design with a cv_ε of 2.8% and 5.1% for LN(AUC) and LN(C_{max}), respectively, compared with 34.2% and 38.2% observed in the crossover study. The ratio of means confirms the increased bioavailability with the tablet and is consistent in both studies. The 90% confidence intervals about the log-transformed metrics confirm the equivalence of tablet and solution formulations and are the consequence of the low intrasubject variation relative to the standard crossover study that narrowly passes average equivalence criteria as well. The modest reduction in the range and mean total variation about the observed concentration-time data confirms that the intersubject variation is the largest component of the total variability.

The results of the pilot crossover study imply that greater sample size would be necessary to reduce the risk of failing a pivotal bioavailability or bioequivalence trial if a standard crossover design is employed, particularly if one assumes that the true difference in average bioavailability is greater than 0. Figure 4 depicts power curves based on the two one-sided test procedure for the typical intrasubject variability observed in the dual, stable isotope (5%) and crossover (35%) studies under conditions in which the average difference in bioavailability is 0, 5%, and 10%. Regardless of the difference in bioavailability, the stable isotope design resulted in an excess of 90% power with 8 subjects, and only 4 subjects were actually required for 80% power. The standard crossover design, burdened by the large intrasubject variation, cannot achieve 80% power with fewer than 40 subjects and only under the condition in which one assumes there to be no difference in average bioavailability.

DISCUSSION

The results presented here demonstrate the utility of the dual, stable isotope approach to accurately assess the relative bioavailability of the highly variable drug moricizine and confirm the bioequivalence of a moricizine oral solution to the marketed commercial tablet. The intrasubject variabilities assessed in a pilot crossover study suggested that the moricizine drug substance (and necessary pharmacokinetic processes) is indeed the major source of this variation and not the tablet drug product. The reduction in intrasubject variability of log-transformed metrics (7- to 10-fold) is dramatic and permits conclusions to be drawn with far fewer subjects (N = 8 vs. 24 in the crossover study) and increased power. It should be noted, however, that the estimates of intrasubject variation from the two-way crossover design encompass Subject × Formulation effects that are confounded in this design and are lumped in the residual error term along with measurement error and the true intrasubject variation. The modest reduction in the total variation about the observed concentration data (within group from crossover to stable isotope studies) indicates that the intersubject variance is the largest partition of the total variance and suggests that the intrasubject variance component of the log-transformed metrics in the

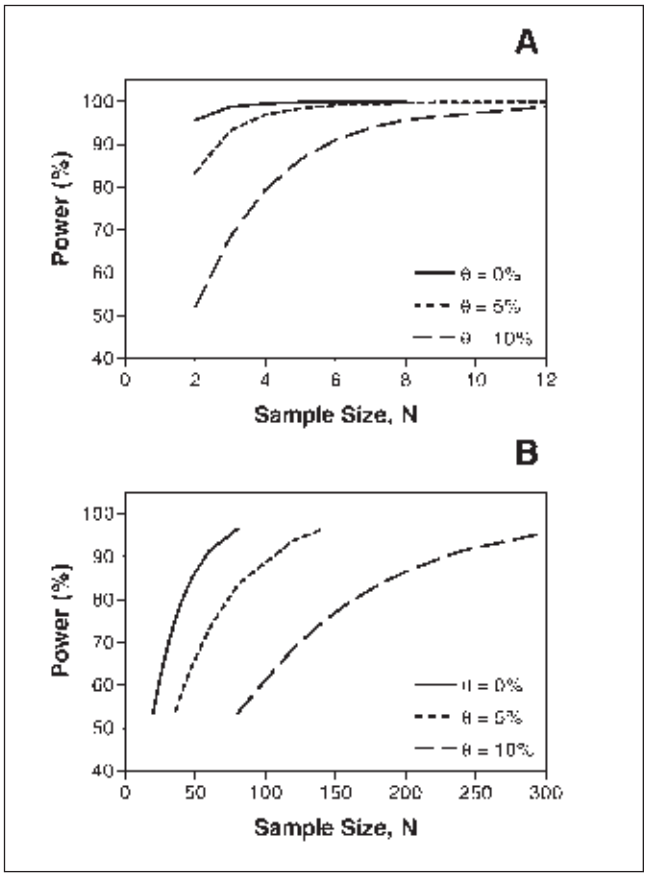

Figure 4. Power curves for the two one-sided tests procedure with a coefficient of variation of 5% representative of the stable isotope study (A) and 35% representative of the crossover study (B) under conditions in which the true difference in bioavailability (θ) is 0, 5%, and 10%. Note differences in the scales on the x-axes.

crossover study may indeed be inflated by Subject × Formulation effects. This is possibly due to the gastrointestinal presystemic component of the first-pass effect, given that moricizine may be a CYP 3A4 substrate.[12] Despite the moricizine tablet's 75% in 30 minutes dissolution specification,[5] the 5% to 10% increase in the ratio of mean bioequivalence metrics may be site specifically mediated given that 3A4 isozymes are most dense along the proximal gastrointestinal tract.[13]

The use of stable isotopes to evaluate relative bioavailability in situations where saturable processes may affect the rate and extent of absorption of one labeled formulation because of the presentation of the other should be used with caution as the principle of superposition will likely not apply, and the interpretation of results may be biased. Such instances have been inadvertently described in a treatise on similar techniques applied for drug interaction assessment by Veng-Pederson et al.[14]

Use of stable isotopes to examine the relative bioavailability and bioequivalence of highly variable drugs has been demonstrated previously.[15-19] The design advocated by Heck et al.[6] requires the isotropic drug to be administered in solution simultaneously with test and reference solid dosage forms. In this design, isotope bioequivalence metrics are used as covariates in the ANOVA. Statistical issues surrounding the appropriateness of this design have been discussed by Phillips,[20] and this design remains an alternative to large, time-consuming, and costly bioequivalence studies in which unrealistic numbers of subject exposures are required. The moricizine oral solution was examined here as a nonintravenous reference; in this setting, a single-period design was required. Relative to a repeat 80-subject study with adequate statistical power, the stable isotope study necessitated 72 fewer subject exposures and 720 fewer blood collections, incurred significantly less clinical costs due to the reduced number of subjects, and was completed in less time due to the single-period design (no washout or crossover treatment) and reduced bioanalytical time/effort. Some of the cost savings may be offset by the need to synthesize and purify GMP grade, stable label drug substance, and internal standard. Browne[21] has provided a compelling cost analysis that favors the dual-isotope paradigm.

While there is no regulatory power requirement in the formal assessment of bioavailability or bioequivalence, the strategy of using the minimum sample size producing 80% power is often used to plan these studies. Hence, the increase in power observed with the stable isotope study design with substantially fewer subjects and exposures is not trivial in light of the comparability of point estimates with the standard crossover study, particularly if one assumes that the true difference in bioavailability (θ) is greater than 0. In the advent of new FDA criteria designed to improve the science of bioequivalence and reward manufacturers for the marketing of drugs with less variable absorption, the use of stable isotope methodology has considerable merit. Further consideration should be given for the incorporation of these methods into new bioequivalence guidance proposals.

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