Digoxin Bioavailability: Evaluation of a Generic Tablet and Proposed FDA Guidelines

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Abstract □ The relative bioavailability of a generic digoxin tablet formulation was investigated according to proposed FDA guidelines. After administration of digoxin in three different oral formulations on separate occasions to 12 subjects, multiple serum samples were obtained over the first 5 hr and assayed for digoxin by radioimmunoassay. The average 0–5-hr area under the serum digoxin concentration–time curve (*AUC*) for the generic tablet was 99.6% of the combined mean of the average *AUC* values for the reference tablet and a solution. In addition, the average peak heights, peak times, and *AUC* values for the two tablet formulations were not significantly different. The currently proposed FDA method of data analysis utilizes a combination of the means of three experimental measurements and provides no measure of the variability of the relative bioavailability estimate. Three other methods of data analysis that do provide variance estimates were evaluated: (*a*) the FDA method on an individual subject basis followed by averaging, (*b*) a logarithmic transformation of the *AUC* values, and (*c*) comparison of the *AUC* values of the generic and reference tablets using a paired *t*-test. A consideration of the experimental design required by the FDA and the statistics involved indicated that the last method may be the most appropriate for examination of the bioavailability of digoxin tablets relative to a market standard. It is also suggested that the proposed regulations be amended to make the standard a solution rather than a specific manufacturer's product.

Keyphrases □ Digoxin—bioavailability, various formulations compared, proposed guidelines evaluated, humans □ Bioavailability—digoxin, various formulations compared, proposed guidelines evaluated, humans □ Cardiotonic agents—digoxin, bioavailability of various formulations compared, proposed guidelines evaluated, humans

The lack of equivalent bioavailability among various digoxin tablets has been the subject of numerous publications (1–12). When tested against the current market standard[1], several digoxin tablets have been shown to be absorbed to a lesser extent. This type of evidence, along with clinical reports of a lack of pharmacological response to several generic brands of digoxin tablets, led the Food and Drug Administration (FDA) to propose bioavailability standards for digoxin tablets (13). These standards indicate that the extent of drug absorption is to be measured by the area under the 0–5-hr serum digoxin concentration–time curve (*AUC*) and that the "bioavailability of the test product shall be demonstrated if a mean absorption of at least 75 percent of the combined mean of the two reference standards is observed"[2]. The reference standards are a tablet supplied by the FDA[1] and an aqueous digoxin solution, and all treatments are at a dose of 0.5 mg. The suggested protocol requires the use of 12 normal subjects in a three-way crossover study and 11 serum samples during the 5-hr period.

The obvious objective of such a regulation is to assure that all marketed digoxin tablets are not appreciably different from a bioavailability standpoint and that, regardless of the brand dispensed to the patient, there are only inconsequential differences in the amount of drug absorbed. Therefore, the method used to evaluate bioavailability should permit measurement of the precision of the bioavailability estimate so that the data may be interpreted correctly. This paper reports the results of a bioavailability study of a generic digoxin tablet[3] conducted according to the protocol outlined by the FDA[4]. The method employed is then examined with respect to permitting a valid estimate of precision and the detection of unsuitable tablet formulations.

EXPERIMENTAL

The three treatments discussed in this paper were part of a larger study in which digoxin was administered in two doses and four dosage forms to 12 healthy male volunteers in a seven-way crossover study. Each subject was given a physical examination with appropriate laboratory tests prior to entering the study. Blood chemistry and hematological values were within normal limits. Subjects were informed of the nature and hazards of the study and gave written consent.

Subjects fasted for 12 hr before and 4 hr after drug administration. The tablet treatments (two 0.25-mg tablets) were administered with 250 ml of water, and the solution (0.5 mg/200 ml) was given with a 50-ml rinse. A minimum of 2 weeks separated consecutive treatments to allow for the complete elimination of the previous dose. The two commercial digoxin tablet preparations were tested and found to meet compendial standards.

A 10-ml blood sample was drawn before each treatment to provide the blank and the diluent for standards used in the assay of samples from that treatment. Blood samples (5 ml) were withdrawn from a forearm vein at 10, 20, 30, 45, 60, and 90 min and at 2, 3, 4, and 5 hr after drug administration. Samples taken during the initial 3–4 hr were withdrawn through a 19-gauge infusion set kept open by a normal saline drip; remaining samples were withdrawn by venipuncture.

All samples were centrifuged, and the serum was kept frozen until assay. Serum samples were assayed using a ^{125}I-radioimmunoassay[5], modified slightly from the kit instructions to increase accuracy and precision (14).

Serum digoxin concentrations at each experimental time for each treatment in each subject were determined and plotted against time. The area under each curve from 0 to 5 hr was calculated using the trapezoidal method.

RESULTS AND DISCUSSION

The averaged serum digoxin concentration–time data from the 12 subjects for the three treatments are plotted in Fig. 1. The average peak heights and peak times are summarized in Table I. Table II contains the areas under the curve from 0 to 5 hr for each treatment in each subject and the relative bioavailability of the generic tablet calculated according to the FDA procedure but on an individual subject basis.

The generic digoxin tablet met the proposed FDA bioavailability requirements, yielding a mean absorption (ratio of the mean area for the test tablet to the combined mean area for the standards) of 99.6%. The bioequivalence of the two tablet formulations is further substantiated by Fig. 1; the serum digoxin concentration–time curves for the two tablet

[1] Lanoxin (Lot 022-1, Burroughs Wellcome and Co.) was used in this study.

[2] The complete text of this section of the proposed bioavailability standards is given in the *Appendix*.

[3] Lot 7161B3, Philips Roxane Laboratories, Columbus, Ohio (a new formulation not previously tested for bioavailability).

[4] The complete protocol used in this study was approved by the FDA prior to the start of the study.

[5] Catalog No. 0750-06, Schwarz/Mann.

Table I—Summary of Peak Heights and Peak Times for the Three Treatments

Treatment	Peak Height[a], ng/ml	Peak Time[a], min
Generic tablet	2.62 (0.87)	65 (29)
Reference tablet	1.89 (1.16)	94 (77)
Aqueous solution	3.39 (0.85)	41 (9)

[a] Mean with standard deviation in parentheses.

preparations are similar. In addition, the peak heights and peak times (Table I) and areas under the curve (0–5 hr) for the two tablet treatments were not significantly different (paired t test, $p > 0.05$). Therefore, it may be concluded that, under the conditions of this study and based upon both the criteria proposed by the FDA and the lack of significant differences between peak heights, peak times, and areas under the curve, the generic digoxin tablet is bioequivalent to the market standard.

As stated previously, the purpose of the proposed bioavailability standards is to detect batches of tablets that are not as well absorbed as current market standards. This conclusion should be based on establishing the existence or lack of statistically significant differences. Therefore, in addition to providing an estimate of relative bioavailability, any test must also provide a measure of the variability of that estimate, which the FDA method of combining means obviously does not. Using the FDA method but calculating relative bioavailability for each subject and *then* averaging yields an estimate of 106% with 95% confidence limits of 79.1–133%. The standard error (12.2%) is large relative to the mean (percent coefficient of variation of 11.5%) and indicates that the sensitivity of this technique for detecting unsuitable formulations is low.

If this variability is typical of other studies, tablet formulations with a "true" relative bioavailability of considerably less than the minimum 75% could still, purely by random chance, appear to meet the requirements. If one assumes that the standard error of the mean is equal to 11.5% of the mean and that there are 11 degrees of freedom, the data in Table III can be calculated and indicate that a formulation must have a true relative bioavailability of 64% or less before the probability of obtaining an experimental value ≥75% decreases below even 10%; a more acceptable level of 5% requires a true relative bioavailability ≤62%.

This apparent lack of sensitivity may be due to the method of data analysis since three different experimental measurements, each with its own error distribution, are included in the relative bioavailability ratio. A logical and statistical basis for using three measurements is not readily apparent to the authors, regardless of whether the means are used to estimate the ratio (as recommended by the FDA) or whether the ratio is calculated for each subject and these ratios are then averaged. The FDA states in the *Federal Register* (13) that the method used should "be capable of detecting the difference between the reference tablet and the reference oral solution." This requirement is fine as a test of the experimental design, methodology, and precision of measurement, but it is not justification for the use of the average of two reference treatments as a basis for a relative bioavailability calculation. Therefore, the authors propose alternative methods of data analysis which are more correct statistically, thus allowing for a greater probability of the regulations performing their intended task.

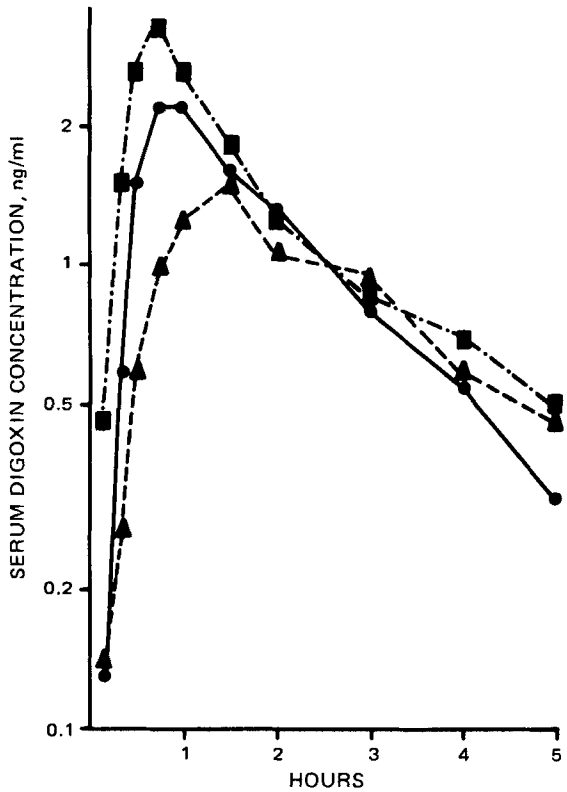

Figure 1—*Semilogarithmic plot of the averaged serum digoxin concentration–time data for the three treatments. Key:* ●, *generic digoxin tablet;* ▲, *current market standard tablet; and* ■, *aqueous solution.*

If all three of the treatments suggested by the FDA must be used in the estimation of one relative bioavailability number, then a method suggested by Finney (15) for estimating relative potency in biological assays (which can include bioavailability studies) utilizing a logarithmic transformation may be applied. Let the average area under the curve (0–5 hr) for the test tablet and the average mean reference (Table II, columns 2 and 5) be the two experimental means whose ratio is to be calculated. Each individual measurement is converted to its common logarithm, and the logarithms are averaged. The average logarithms are then subtracted to give the ratio, and a pooled estimate of the standard deviation of the ratio is calculated. This estimate is then used to calculate fiducial limits (similar to confidence limits) for the potency ratio.

This method gives a ratio of 101% with 95% fiducial limits of 84–122%. Although the result is similar, *i.e.*, the mean absorption of the test tablet is >75% of the mean absorption of the standards, there is now some measure of precision and, in this case, some certainty that the probability of the relative bioavailability actually being <75% is extremely low. There

Table II—Areas under the Serum Digoxin Concentration–Time Curves (in Nanogram Hours per Milliliter) from 0 to 5 hr[a] and the Relative Bioavailability[b] of the Test Tablet

Subject	Test Tablet	Reference Tablet	Reference Solution	Mean Reference[c]	Relative Bioavailability
M.G.	4.23	3.15	6.21	4.68	90.4
R.R.	4.85	6.81	7.54	7.18	67.5
P.H.	5.59	3.22	6.08	4.65	120.
C.N.	4.52	4.03	7.55	5.79	78.1
H.P.	4.21	4.43	6.05	5.24	80.3
D.S.	7.36	0.81	5.60	3.21	229.
R.S.	6.07	5.20	7.67	6.44	94.3
J.M.	5.55	4.08	7.07	5.58	99.5
S.R.	5.82	3.39	6.99	5.19	112.
H.B.	3.90	2.91	5.11	4.01	97.3
W.W.	6.40	9.20	6.32	7.76	82.5
R.M.	5.39	3.37	5.31	4.34	124.
Mean	5.32	4.22[d]	6.46	5.34	106.
SD	1.03	2.12	0.89	1.31	42.3

[a] Areas were calculated using the trapezoidal rule. [b] Relative bioavailability is defined according to the proposed FDA regulations as the ratio of the area under the curve (0–5 hr) for the test tablet to the mean of the areas under the curve for the reference tablet and solution. [c] Mean of the reference tablet and reference solution. [d] Significantly different from reference solution (paired t test, $p < 0.05$).

Table III—Probability of Obtaining a Mean Relative Bioavailability Estimate[a] of 75% or Greater

True Relative Bioavailability	*SEM*[b]	Calculated[c] *t* Value	Probability[d], %
74	8.51	0.12	45.4
73	8.40	0.24	40.8
72	8.28	0.36	36.2
71	8.16	0.49	31.7
70	8.05	0.62	27.4
69	7.93	0.76	23.3
68	7.82	0.90	19.5
67	7.70	1.04	16.1
66	7.59	1.19	13.0
65	7.47	1.34	10.4
64	7.36	1.49	8.16
63	7.24	1.66	6.29
62	7.13	1.82	4.78
61	7.01	2.00	3.57
60	6.90	2.17	2.62

[a] Defined as in Table II. [b] Equal to 11.5% of the mean or true relative bioavailability. [c] Calculated according to: $t = (75 - \bar{X})/s$, where $\bar{X}$ = true relative bioavailability and s = standard error of the mean. [d] Probability of obtaining t greater than the calculated value (equal to the area under the t distribution curve from the calculated t to infinity) due to random error or chance.

are, however, two drawbacks to this method. First, it still utilizes three experimental measurements in the relative bioavailability calculation. Second, even though the studies are done in a crossover manner, the influence of subject-to-subject variability is not minimized because of the utilization of only the means and standard deviation estimates. Thus, the advantage of a crossover study, *i.e.*, being able to look at differences independent of intersubject variation, is ignored. But if one *must* utilize the FDA method as currently written, then this type of data analysis may be preferred.

The authors believe the intent of the FDA at the present time is to guarantee that all digoxin tablets are as good as the market standard. Consequently, the most direct method would be to do a two-way crossover study with the test and reference tablets or to do the FDA three-way crossover study, comparing the reference tablet and solution *only* as a means of testing the methodology and then calculating relative bioavailability using only the test and reference tablets. Since the same subjects are to be used in all treatments, a comparison of the data using the paired t test may be done, thus minimizing the influence of subject-to-subject variability, and a determination of whether the products are significantly different can be made. When using this method (data in Table II, columns 2 and 3), the average difference was 1.11 units with 95% confidence limits of from −0.386 to 2.61, not significantly different from zero ($p > 0.05$).

The last method probably provides as good an estimate as any of the bioavailability of a test tablet relative to the current market standard under the conditions of the FDA protocol. However, it does require that the regulations utilize a specific manufacturer's product as a standard. It may be better to utilize as a standard the oral solution and to require that *all* manufacturers, including the manufacturer of the market standard, demonstrate that the absorption of their tablets is greater than some minimum percent of the absorption of the solution. This requirement would allow for future changes in the regulations to increase the bioavailability of digoxin from tablets to more closely approximate that of the solution.

The FDA has taken a major step forward by suggesting bioavailability requirements for digoxin tablets. However, these standards need to be reevaluated and some changes made before final regulations are enacted.

APPENDIX

Regulation 130.51, part (d), from Ref. 13 reads as follows:

The protocol for the in vivo bioavailability tests required in paragraphs (a) and (c) of this section shall employ a three-way crossover design using the digoxin test product; a reference digoxin tablet supplied, on request, by the Food and Drug Administration; and bulk digoxin USP in an oral solution. Appropriate venous blood and urinary samples are to be collected and analyzed. This method shall be capable of detecting the difference between the reference tablet and the reference oral solution. Bioavailability of the test product shall be demonstrated if a mean absorption of at least 75 percent of the combined mean of the two reference standards is observed. Assistance in developing a protocol for a particular dosage formulation may be obtained by contacting the Food and Drug Administration, Bureau of Drugs (HFD-220), 5600 Fishers Lane, Rockville, MD 20852.

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