Journal of Pharmacokinetics and Biopharmaceutics, Vol. 7, No. 3, 1979

Bioavailability of Imipramine Tablets Relative to a Stable Isotope-Labeled Internal Standard: Increasing the Power of Bioavailability Tests

Henry d'A. Heck,[1,2,6] Sidney E. Buttrill, Jr.,[1] Norman W. Flynn,[1,3] Robert L. Dyer,[1] Michael Anbar,[1,4] Thomas Cairns,[5] Shrikant Dighe,[5] and Bernard E. Cabana[5]

Received June 6, 1978—Final January 9, 1979

A new methodology for comparative bioavailability testing is described in which each drug formulation is compared with a stable isotope-labeled variant of the drug that is consumed orally in solution at the same time the tested formulation is ingested. The methodology is used to determine the comparative bioavailabilities of two commercially available brands of imipramine hydrochloride. The power of the new methodology to detect differences between drug formulations, when, in fact, such differences exist, is shown to be superior to that of conventional bioavailability tests.

KEY WORDS: bioavailability; confidence intervals; hypothesis testing; imipramine; internal standard; mass spectrometry; power; relative bioavailability.

INTRODUCTION

The probability that a comparative bioavailability test will detect differences between formulations of a drug, when, in fact, such differences exist, is known as the "power" of the test (1). The statistical definition of

This study was supported by Contract 223-75-3006, DHEW/Public Health Service, Food and Drug Administration, Rockville, Maryland.

[1]Mass Spectrometry Research Center, Stanford Research Institute, Menlo Park, California 94025.

[2]Present address: Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, North Carolina 27709.

[3]Present Address: Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143.

[4]Present address: Department of Biophysical Sciences, State University of New York at Buffalo, Amherst, New York 14226.

[5]DHEW/Public Health Service, Food and Drug Administration, Rockville, Maryland 20852.

[6]Address correspondence to H. d'A. H.

0090-466X/79/0600-0233$03.00/0

power is $1-\beta$, where β is the probability of a type II ("failure to reject") error (2). In the special case of analysis of variance, power can be related to a variable, ϕ, defined as (1,2)

$$\phi^2 = \sum_{i=1}^{k} (\bar{X}_i - \bar{\bar{X}})^2 / k \Big/ s^2/N \qquad (1)$$

where $\bar{X}_i$ is the mean value of a selected bioavailability parameter for treatment i, $\bar{\bar{X}}$ is the overall mean value of the same parameter for all treatments [hence $(\bar{X}_i - \bar{\bar{X}})^2$ is a measure of the dispersion of the mean values], k is the number of treatments, s^2 is the residual mean square from the analysis of variance for crossover design (hence s^2 is a measure of the intrinsic error of the test due to analytical and intrasubject variability), and N is the number of subjects. Equation 1, together with appropriate tables (3), can be used to calculate the number of subjects required to achieve a given value of power, if the other parameters in the equation are known or can be assumed.

This article describes a new methodology for *in vivo* bioavailability testing that is notable primarily for the considerable increase in power that it achieves in comparison with conventional bioavailability tests. The method, which is hereafter denoted by the term "relative bioavailability," is based on a comparison between the tested formulation and a stable isotope-labeled variant of it that is consumed orally in solution at the same time the tested formulation is ingested. Since the labeled drug is already dissolved, it can be regarded as being "optimally bioavailable" and thus can logically be used to determine the relative efficiency of absorption of each drug formulation. All of the conventional parameters of bioavailability, such as plasma concentrations at various times, time of concentration maximum, peak concentration, area under the plasma concentration–time profile, and half-life, can be determined relative to the corresponding parameter for the internal standard. It will be seen that the coefficient of variation of this procedure is considerably smaller than that of a conventional bioavailability measurement.

The measurement of relative bioavailability requires that one or more stable isotope-labeled variants of the drug be synthesized and that the analytical laboratory have access to a mass spectrometer. The methodology that was developed in our laboratory for the study of the relative bioavailabilities of two commercially available brands of imipramine (IP) has been described (4). The tablets, designated A (Ciba-Geigy Lot No. 1100681) and B (Biocraft Laboratories, Elmwood Park, New Jersey), were reported to contain 25 mg of imipramine hydrochloride. The tablets were purchased from local pharmacies.

MATERIALS AND METHODS

Analysis of Tablets

The amounts of imipramine hydrochloride in tablets A and B were determined by a combination of high-performance liquid chromatography (HPLC) and stable isotope dilution-field ionization mass spectrometry (4). Six tablets of each kind were weighed and dissolved separately in 500 ml volumetric flasks, using 60% (v/v) H_2O/CH_3OH as solvent. Aliquots of approximately 10 ml were withdrawn from each flask and were centrifuged to remove undissolved particulate matter, of which there was a considerable quantity. A 5-μl sample from each supernatant solution (representing 250 ng of IP hydrochloride if the active ingredient dissolved completely and the tablets were correctly labeled) was then transferred to a centrifuge tube that contained 0.6 ml of H_2O and 363 ng of a multilabeled isotopic diluent, IP-d_6 hydrochloride (4). The solutions were then extracted, using 1.2 ml of 1.5 M Na_2CO_3 and 1.2 ml of hexane. After centrifugation, the hexane layer was withdrawn and was stored in a silanized centrifuge tube. Purification of the extract by HPLC and mass spectrometric isotope ratio analysis was performed as described previously (4).

Subjects

Eight healthy adult males, ranging in age from 18 to 57 years and in weight from 62 to 80 kg, participated as volunteers in the study after their informed consent was obtained. The bioavailability tests began at 8 A.M. Food or liquids other than water had not been consumed for the previous 12 hr. Initial blood samples were drawn into heparinized tubes, then the subjects were given a tablet of either brand A or brand B. The source of the IP was not disclosed to the subjects.

To provide an internal standard against which to compare the bioavailability of each tablet, the subjects drank 120 ml of water that contained 25 mg of IP-d_2 hydrochloride [deuterated in the ethylene bridge (4)]. Blood samples were drawn at selected times for up to 48 hr after ingestion. The samples were centrifuged as soon as possible after drawing, and the plasma was frozen. Normal feeding was resumed 4 hr after the tablets and solution had been ingested.

One week after the study was begun, the same subjects reassembled and were given a new tablet of imipramine together with a new sample of the internal standard. Those subjects who received brand A in the first trial received brand B in the second and *vice versa*. The same procedure was followed in the second trial as in the first with respect to blood sampling.

Analysis of Plasma Samples

The procedure that was used for analysis of plasma samples has been published (4). Briefly, plasma samples were thawed on the day of analysis and were spiked with a known amount (363 ng) of multilabeled IP-d_6 hydrochloride, which was used as an analytical standard. Sodium carbonate solution was added, then the plasma samples were extracted with 95% (v/v) hexane/isoamyl alcohol. Purification of the extracts was achieved by back extractions into HCl solution and, after addition of Na_2CO_3, into hexane, followed by HPLC. The concentrations of IP and IP-d_2 hydrochlorides in the plasma relative to IP-d_6 hydrochloride were simultaneously determined by mass spectrometric isotope ratio analysis.

Data Analysis

Analysis of variance for crossover design was performed by computer using the PROPHET time-sharing computer system (see Acknowledgments). Areas under curves were calculated by the trapezoidal rule. Half-lives were estimated from a least-squares straight line drawn through the logarithms of the last three data points (obtained at 24, 34, and 48 hr) of each subject.

Table I. Isotope Dilution Analysis of Imipramine Hydrochloride Contents of Pharmaceutical Tablets

Tablet	Total tablet mass (mg)	Mass of IP hydrochloride (mg)	Percent of label per tablet
	92.3	24.7	98.8
	100.5	24.7	98.8
	90.8	25.1	100.4
Brand A	90.4	24.1	96.4
	92.9	26.8	107.2
	89.6	26.5	106.0
(Ave.)[a]	92.8±4.0	25.3±1.1	101.3±4.4
	174.8	25.5	102.0
	173.9	23.6	94.4
	170.2	23.8	95.2
Brand B	190.8	27.6	110.4
	172.5	26.1	104.4
	170.5	26.8	107.2
(Ave.)[a]	175.5±7.7	25.6±1.6	102.3±6.4

[a] Errors are standard deviations.

Table II. Results of Conventional Bioavailability Comparisons of 25-mg Imipramine Hydrochloride Tablets

Parameter	Treatment average[a]		Result of ANOVA test for between-treatment averages
	Brand A	Brand B	
Imipramine hydrochloride plasma concentration (ng/ml)			
1 hr	0.73 (81.2)	1.08 (74.6)	NS ($0.25 > p > 0.1$)
1.5 hr	3.31 (62.5)	3.26 (53.2)	NS ($p > 0.5$)
2 hr	6.29 (52.4)	6.22 (45.4)	NS ($p > 0.5$)
3 hr	8.49 (33.4)	8.96 (32.7)	NS ($p > 0.5$)
4 hr	8.96 (27.9)	9.39 (36.7)	NS ($p > 0.5$)
5 hr	8.24 (23.0)	9.09 (32.4)	NS ($p > 0.25$)
7 hr	6.46 (35.3)	6.83 (29.4)	NS ($p > 0.5$)
10 hr	4.66 (27.2)	5.09 (36.9)	NS ($p > 0.25$)
24 hr	1.57 (47.3)	1.64 (42.9)	NS ($p > 0.5$)
34 hr	0.84 (46.0)	0.83 (39.9)	NS ($p > 0.5$)
48 hr	0.43 (67.1)	0.45 (46.4)	NS ($p > 0.5$)
Peak concentration (ng/ml)	9.45 (29.6)	9.79 (30.2)	NS ($p > 0.5$)
Time of peak concentration (hr)	3.62 (25.3)	4.00 (23.1)	NS ($p > 0.5$)
Area 0–48 hr [(ng/ml) × hr]	124. (31.6)	132. (31.2)	NS ($p > 0.5$)
Half-life (hr)	12.2 (21.5)	13.5 (22.0)	NS ($p > 0.25$)

[a] Average values are followed by coefficients of variation (percent standard deviation).

RESULTS

Tablet Analyses

The total weights and imipramine hydrochloride contents of six tablets of brand A and six of brand B are listed in Table I. It is seen that the average imipramine hydrochloride contents were essentially identical and corresponded well with the values reported on the labels.

Comparative Bioavailabilities of Tablets A and B

The parameters used to assess the comparative bioavailabilities of the two imipramine formulations are given in Table II. It is evident that no significant differences were found, according to these conventional bioavailability criteria.

A plot of the average plasma concentrations of imipramine hydrochloride produced by tablets A and B is shown in Fig. 1. Very similar average concentrations were produced by each drug preparation. Since, as shown in Table II, the differences at each time are not significant ($p > 0.1$), the superimposition principle (5) is fulfilled, and the two preparations can be regarded as bioequivalent.

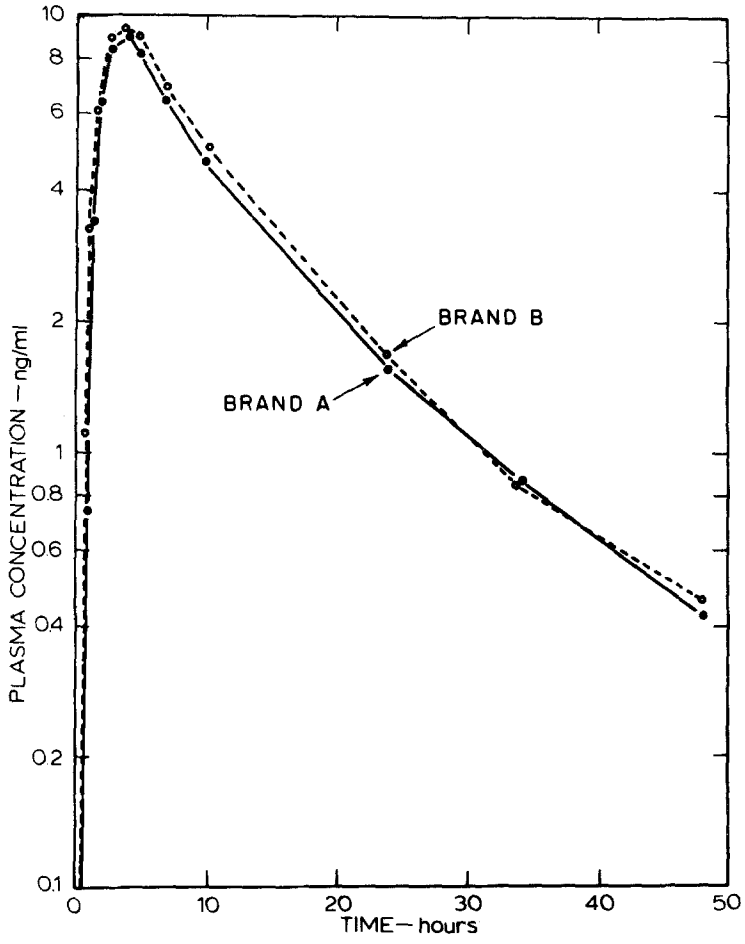

Fig. 1. Mean plasma concentration of imipramine hydrochloride following ingestion of a 25-mg tablet of brand A (solid line) or brand B (dashed line).

Relative Bioavailabilities of Tablets A and B

The various parameters used to assess bioavailability in Table II were also determined relative to the corresponding values for the internal standard, IP-d_2 hydrochloride (4). The results of these measurements are summarized in Table III. It is again obvious that the relative measurements also fail to detect any significant differences between brands A and B. Notable, however, is the decreased coefficient of variation (percent standard

Table III. Results of Relative Bioavailability Comparisons of 25-mg Imipramine Hydrochloride Tablets Using a 25-mg IP-d_2 Hydrochloride Internal Standard

Parameter	Treatment average[a]		Result of ANOVA test for between-treatment averages
	Brand A	Brand B	
Relative imipramine hydrochloride plasma concentration			
1 hr	0.331 (55.9)	0.399 (51.6)	NS ($p > 0.5$)
1.5 hr	0.615 (15.9)	0.612 (26.8)	NS ($p > 0.5$)
2 hr	0.790 (13.7)	0.760 (19.9)	NS ($p > 0.5$)
3 hr	0.896 (7.5)	0.919 (6.1)	NS ($0.25 > p > 0.1$)
4 hr	0.930 (12.5)	0.954 (4.5)	NS ($p > 0.25$)
5 hr	0.997 (9.4)	0.984 (6.4)	NS ($p > 0.5$)
7 hr	0.986 (7.8)	0.993 (4.7)	NS ($p > 0.5$)
10 hr	0.968 (10.0)	0.962 (8.2)	NS ($p > 0.5$)
24 hr	0.919 (14.7)	0.997 (7.5)	NS ($0.25 > p > 0.1$)
34 hr	1.010 (22.6)	1.087 (10.5)	NS ($p > 0.25$)
48 hr	1.245 (36.8)	1.200 (10.9)	NS ($p > 0.25$)
Relative peak concentration[b]	0.887 (9.8)	0.931 (4.5)	NS ($0.25 > p > 0.1$)
Relative time of peak concentration[c]	1.0 (0.0)	1.1 (17.9)	NS ($0.25 > p > 0.1$)
Relative area 0–48 hr	0.935 (5.5)	0.960 (4.4)	NS ($0.25 > p > 0.1$)
Relative half-life	1.18 (13.5)	1.13 (7.6)	NS ($p > 0.25$)

[a] Average values are followed by coefficients of variation (percent standard deviation).
[b] The relative peak concentration is defined as the ratio of the maximum concentration of IP to the concentration of IP-d_2 found at the same time.
[c] The relative time of the peak concentration is the ratio of the time of the peak concentration of IP to the time of the peak concentration of IP-d_2.

deviation) of each parameter in Table III in comparison with the corresponding parameter in Table II.

A plot of the average of the ratio of unlabeled to labeled imipramine hydrochloride produced by tablets A and B is shown in Fig. 2. The general form of these curves illustrates the marked kinetic differences that exist between the plasma concentration–time profiles of orally administered imipramine tablets and solution. It may be noted that IP-d_2 hydrochloride administered orally in solution appears much more rapidly in the plasma than does IP hydrochloride ingested as tablets. This result is, of course, to be expected, since tablet dissolution should not occur instantaneously. The period of tablet dissolution, which takes place simultaneously with stomach emptying and absorption of IP-d_2 hydrochloride, is shown as the initial steeply rising portion of the two curves. Thereafter, the concentration of unlabeled IP was essentially identical to that of the internal standard [although both concentrations were changing rapidly (Fig. 1)], which resulted in the IP/IP-d_2 ratios being very close to unity. The last data point, at $t = 48$ hr, exhibits a slightly increased value for the ratio, indicating a slightly

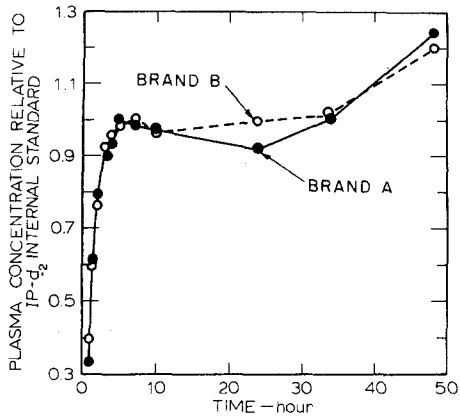

Fig. 2. Mean plasma concentration of imipramine hydrochloride relative to imipramine-d_2 hydrochloride following ingestion of a 25-mg tablet of brand A (solid line) or brand B (dashed line) and a 25-mg dose of imipramine-d_2 hydrochloride dissolved in water.

higher concentration of the unlabeled compound than of the labeled compound in plasma. This difference suggests a possible inverse isotope effect in the elimination of IP-d_2 hydrochloride, but further studies are needed to establish whether the observed difference is persistent and repeatable.

Relative Efficiency of Absorption of Tablets A and B

The area under the curve of each preparation, relative to that of the internal standard (Table III), can be regarded as a measure of the relative absorption efficiency, since, as stated previously, the internal standard should be optimally bioavailable. In both cases, the relative areas are slightly smaller than unity. Application of the *t*-test (6) indicates that the small difference is statistically significant ($0.005 > p > 0.0005$ for brand A and $0.025 > p > 0.01$ for brand B; one-tailed test, 7 df). Hence neither drug preparation appears to be as fully bioavailable as the solution, although the difference between tablet and solution is quite small.

DISCUSSION

Bioequivalence of Tablets A and B

The data of Tables I–III clearly demonstrate that the two imipramine formulations are bioequivalent within the limits of precision of these tests. It

Table IV. Selected Bioavailability Parameters, Corresponding Residual Mean Squares, and Estimated Power Values

Parameter	Brand A	Brand B	Residual mean square[a]	Power $(1-\beta)^b$
Area 0–48 hr [(ng/ml) × hr]	124	132	698	0.04
Peak concentration (ng/ml)	9.45	9.79	5.18	0.01
Relative area 0–48 hr	0.935	0.960	0.0014	0.19
Relative peak concentration	0.887	0.931	0.0026	0.31

[a] Based on analysis of variance for crossover design.

[b] Tables of power (actually graphs) given by Dixon and Massey (3) do not include values of ϕ smaller than 1.2 for $\alpha = 0.05$ and $\nu_1 = k - 1 = 1$ df for treatment variability. To estimate $1-\beta$ for small values of ϕ, the tables were redrawn using linear graph paper, and a smooth curve was extrapolated from the lowest reported value of $1-\beta = 0.30$ down to $1-\beta = 0$.

should not, of course, be inferred that all formulations of imipramine are bioequivalent.

Power of Conventional and Relative Bioavailability Tests

Although the two formulations of IP are bioequivalent according to both conventional and relative tests, it is important to consider whether one test may be intrinsically more powerful than the other. An increase in power would enable fewer subjects to be employed and therefore would reduce the risk, cost, and time of *in vivo* testing.

To estimate the power of each test, the areas under the plasma concentration–time curves and the peak plasma concentrations will be used as the measures of bioavailability. The absolute and relative values of these parameters are given in Tables II and III, respectively. For ease of reference, these values are repeated in Table IV, together with the corresponding residual mean squares from the analysis of variance for crossover design and the estimated power values calculated from equation 1.

It is evident from the last column of Table IV that the relative bioavailability measurements are considerably more powerful than the corresponding absolute measurements, consistent with their smaller coefficients of variation. It is equally obvious, however, that the power values achieved by both testing methods are too small to reliably demonstrate differences of the magnitudes shown in Table IV. Such small differences might, however, be regarded as clinically unimportant. Although there is no general rule pertaining to every drug formulation that specifies how large a difference must be before it becomes therapeutically significant, a possible criterion suggested by Wagner (5) in accordance with FDA

guidelines (7) is that a difference of 20% should be demonstrable when $\alpha = 0.05$ and $1-\beta = 0.8$.[7]

If the above recommendation is accepted, we can calculate the number of subjects that would be required to detect (with a probability of 0.8) a difference of 20% between brand A and a *hypothetical* impramine preparation having a (presumably) lesser bioavailability. We assume the same values for the residual mean square error as in the present case (Table IV).

The procedure of calculation is to assume values of N and then to calculate ϕ^2 (equation 1). Values of ϕ^2 are also obtained from tables (3), corresponding to $\nu_2 = (k-1)(N-2)$ degrees of freedom for the residual mean square error, $\alpha = 0.05$, and $1-\beta = 0.8$. N is varied until approximate agreement is obtained between the calculated and tabulated values of ϕ^2.

An example of this calculation is given below for the measurement of absolute areas, assuming a 20% difference in means between brand A and a hypothetical imipramine formulation. Using equation 1, we find that

$$\phi^2 = \frac{[(124-111.6)^2+(99.2-111.6)^2]/2}{698/N}$$

$$= 0.22N \qquad (2)$$

Values of ϕ^2 corresponding to different values of N, the corresponding values of ν_2, and the tabulated values of ϕ^2 are assembled in the upper part of Table V. It is seen that the solution lies between $N = 19$ and $N = 20$. Since a two-way crossover study requires an even number of subjects, we conclude that about 20 subjects would be needed.

The same procedure can be used to estimate the numbers of subjects that would be needed to detect a difference of 20% (with a probability of 0.8) in the peak concentrations, relative areas, and relative peak concentrations. The results of these calculations are also shown in Table V. The estimated numbers of subjects are 26 for the measurement of peak concentrations, but are less than 3 for relative areas and approximately 4 for relative peak concentrations. (Values of $N < 4$ are, of course, unrealistic, since N must be at least 4 to ensure that ν_2 is nonzero and that N is even.) We conclude that no more than 4 subjects would be needed by the relative tests but that 20–26 subjects would be required by the conventional absolute methods of testing. It is clear that the increase in power achieved by using relative bioavailability permits a substantial reduction to be made in the

[7]Elsewhere in his book (p. 304 of reference 1), Wagner remarks that the criterion that $1-\beta = 0.8$ may require an excessively large number of subjects (using conventional bioavailability techniques). He suggests that the required number of subjects be estimated using the confidence interval approach (8) as well as by means of power calculations to determine how different the estimates are. Both methods are used in this article (see Tables V and VII).

Table V. Estimation of Number of Subjects, N, Required to Achieve $1-\beta=0.8$, Assuming 20% Difference in Selected Bioavailability Parameters for Brand A and a Hypothetical Imipramine Tablet[a]

Parameter	N	ϕ^2	ν_2[b]	From tables (3) ϕ	From tables (3) ϕ^2
Area 0–48 hr	18	3.97	16	2.11	4.45
	19	4.19	17	2.10	4.41
	20	4.41	18	2.09	4.36
	21	4.63	19	2.08	4.32
Peak concentration	24	4.13	22	2.078	4.32
	25	4.30	23	2.073	4.30
	26	4.47	24	2.069	4.28
	27	4.64	25	2.066	4.27
Relative area	3	18.7	1	~3.3[c]	~11[c]
Relative peak concentration	3	9.09	1	~3.3[c]	~11[c]
	4	12.12	2	~3.0[c]	~9[c]

[a] For $\alpha=0.05$ and residual mean squares given in Table IV.
[b] Degrees of freedom of residual mean square, equal to $(k-1)(N-2)$, where k is the number of treatments.
[c] Dixon and Massey (3) do not tabulate values of ϕ at $1-\beta=0.8$ for $\nu_2<6$. The ϕ values given here were estimated by graphic extrapolation of the Dixon and Massey tables (3) to lower values of ν_2 and should be regarded as approximate only.

number of subjects required to perform these bioavailability comparisons at the desired level of significance.

Application of Relative Bioavailability to the Method of Confidence Intervals

An alternative procedure for comparing the bioavailabilities of two drug formulations is the use of confidence intervals (8). In this procedure, two drug formulations are concluded to be bioequivalent if it can be stated with 95% confidence that their bioavailabilities are within a certain small percentage of each other. The magnitude of this percentage will depend upon the particular drug, but Westlake (8) suggested as an example that two drug formulations might be regarded as being essentially bioequivalent if their bioavailabilities differ by no more than 15% (95% confidence limit).

If we accept Westlake's criterion, we can test the proposition that tablets A and B are bioequivalent according to the method of confidence intervals. In this method, two different values of Student's t are determined, k_1 and k_2, such that the integral under the t-distribution curve from k_2 to k_1 is 0.95. The values of k_1 and k_2 must also satisfy the equation (8),

$$k_1+k_2=(\bar{X}_i-\bar{X}_j)(2N)^{1/2}/s \tag{3}$$

where $\bar{X}_i$ and $\bar{X}_j$ are mean values of a particular bioavailability parameter for two treatments i and j, N is the number of subjects in a two-way crossover study, and s is the square root of the residual mean square calculated from the analysis of variance for crossover design (9). Individual values of k_1 and k_2 are obtained by assuming a value for one of them, calculating the other using equation 3, and determining the area under the t-distribution profile from k_2 to k_1 for $N-2$ df using tables of the probability distribution function for Student's t (10). The assumed values are adjusted until this area is equal to 0.95.

Properly chosen values of k_1 and k_2 can then be used to calculate a parameter, Δ, defined as (8)

$$\begin{aligned}\Delta &= k_1 s/(N/2)^{1/2} - (\bar{X}_i - \bar{X}_j) \\ &= -[k_2 s/(N/2)^{1/2} - (\bar{X}_i - \bar{X}_j)]\end{aligned} \tag{4}$$

where Δ is the 95% confidence limit for the possible deviation that could exist between the true treatment means. The ratio, $\Delta/\bar{X}_i$, is the fractional deviation of the mean of sample j from the mean of sample i that might exist with 95% confidence. According to Westlake's suggestion (8), this ratio should not exceed 0.15 if the formulations are to be considered bioequivalent.

This procedure was applied to the bioavailability results that were obtained in this investigation, using the parameters given in Table IV as four measures of bioavailability. The results of these calculations are summarized in Table VI.

According to Table VI, if we had chosen a maximum acceptable deviation of 15% for practical bioequivalence, we would have been unable by the conventional methods of testing to conclude that the two tablets are bioequivalent, since the mean areas and mean peak concentrations could differ by as much as 28.6% and 30.2%, respectively, and still fall within the

Table VI. Comparison of Selected Bioavailability Parameters of Tablets A and B Using the Method of Confidence Intervals

Parameter	From tables (10)		Δ	95% confidence limit for percent deviation of tablet B from tablet A
	k_1	k_2		
Area 0–48 hr	3.29	−2.08	35.5[a]	28.6
Peak concentration	2.81	−2.21	2.85[b]	30.2
Relative area	4.64	−1.97	0.0618	6.6
Relative peak concentration	5.41	−1.96	0.0940	10.6

[a] Units: (ng/ml) × hr.
[b] Units: ng/ml.

Table VII. Estimation of Number of Subjects, N, Required to Conclude with 95% Confidence That Selected Bioavailability Parameters of Tablet B Are Within 15% of Those of Tablet A

Parameter	Δ	N	k_1	k_2	Confidence limit (%)
Area 0–48 hr	18.6[a]	34	4.15	−1.65	94.6
		36	4.27	−1.70	95.1
Peak concentration	1.418[b]	26	2.78	−1.71	94.4
		28	2.89	−1.77	95.2
Relative area	0.140	4	6.25	−4.36	96.3
Relative peak concentration	0.133	4	4.91	−2.47	91.4
		6	6.01	−3.02	97.9

[a] Units: (ng/ml) × hr.
[b] Units: ng/ml.

95% confidence range. However, by using relative bioavailability, we can conclude with 95% confidence that the mean relative areas and mean relative peak concentrations differ by no more than 6.6% and 10.6%, respectively. Since these deviations fall within the acceptable range of 15%, we infer with 95% confidence that the two formulations are bioequivalent for all practical purposes.

A further application of the method of confidence intervals is to calculate the minimum numbers of subjects required to conclude with 95% confidence that the deviations between sample means are no larger than 15%. We assume the same values of $\bar{X}_i$, $\bar{X}_j$, and s that are given in Table IV, and using equation 4 we calculate values of k_1 and k_2 for different values of N, given that $\Delta/\bar{X}_i = 0.15$. The desired value of N in each case is that which yields an integral under the t-distribution profile from k_2 to k_1 equal to 0.95 or slightly greater. The final results are assembled in Table VII.

For the conventional bioavailability measurements, we find that 36 and 28 subjects, respectively, would be required to conclude with at least 95% confidence that the areas under the plasma concentration–time curves and the peak plasma concentrations of imipramine produced by brands A and B differ by no more than 15%. By using relative bioavailability, however, the same conclusion would be reached with only 4 and 6 subjects, respectively. Thus the possibility of demonstrating the practical bioequivalence of two formulations by means of confidence intervals represents a further useful application of relative bioavailability.

SUMMARY AND CONCLUSIONS

This article describes the first example of the use of an internal standard for bioavailability comparisons. It is important to note that although the

internal standard has presumably similar pharmacological effects to the tested drug, it is pharmacokinetically distinct from the tested formulations primarily as a result of the differing dosage forms (solution vs. tablets), and possibly as a result of (presumably minor) isotope effects in absorption, distribution, and elimination. By administering the internal standard at the same dose and by the same route as the tested formulations, however, possible kinetic differences between IP and IP-d_2 that could be caused by nonlinear effects were presumably avoided. In a comparative bioavailability test, kinetic differences between the internal standard and the tested formulations would, of course, exist in each case and therefore should not affect comparisons *between the formulations*, except by reducing the level of precision.

The kinetic differences that exist between IP and IP-d_2 did not prevent the present study from achieving an increased level of precision. Dissolution of the tablets was sufficiently rapid that the plasma concentrations of IP and IP-d_2 were similar over most of the profile. For comparisons among more slowly dissolving or "controlled release" drug formulations, however, predissolution of the internal standard might be undesirable if it resulted in a plasma profile that differed too greatly from those of the tested formulations.

The calculations presented in this discussion have shown that the measurement of relative bioavailability is a superior method for detecting differences between drug formulations, when, in fact, such differences exist. Conventional bioavailability measurements using 8–12 subjects may be inadequate to detect medically significant differences with a high probability, either by hypothesis testing or by the method of confidence intervals, particularly in cases where the residual mean square error is large. In contrast, by using only 4–6 subjects, relative bioavailability tests can readily detect differences of 20% with a probability of 0.8, or, alternatively, can demonstrate with 95% confidence the practical bioequivalence of two formulations. It is expected that the use of relative bioavailability techniques will become particularly important for resolving difficult problems of bioavailability and their associated legal ramifications.

ACKNOWLEDGMENTS

We thank Dr. Leo Hollister and Hamp Gillespie of the Palo Alto Veterans Administration Hospital for obtaining the blood samples. Computer analysis of variance was carried out using the PROPHET System, a national computer resource sponsored by the NIH. Information about PROPHET can be obtained from the Director, Chemical/Biological Information-Handling Program, Division of Research Resources, National Institutes of Health, Bethesda, Maryland 20014.

Table IA. *In Vitro* Dissolution Tests of Tablet A (10 Tablets) and Tablet B (6 Tablets)

Time (min)	Percent dissolved[a]		Significance level for two-tailed test[b]
	Tablet A	Tablet B	
5	0.39± 0.33	3.21± 0.72	Sig ($0.001 > p$)
10	0.81± 0.75	8.98± 3.19	Sig ($0.001 > p$)
20	14.75±12.97	25.19±12.39	NS ($0.2 > p > 0.1$)
40	56.50±24.22	55.58±18.90	NS ($p > 0.5$)
60	80.48±14.87	82.68±10.21	NS ($p > 0.5$)
75	98.35± 4.67	96.03± 1.96	NS ($0.3 > p > 0.2$)
90	100.3 ± 1.88	97.59± 3.33	NS ($0.1 > p > 0.05$)

[a] Average value is followed by the standard deviation.
[b] From Student's t, 14 df.

APPENDIX: *IN VITRO* DISSOLUTION TESTS OF TABLETS A AND B

S. Dighe and B. E. Cabana

A comparison between the tablet dissolution rates of the two imipramine formulations was made in the laboratories of the Food and Drug Administration. The dissolution rates were determined using a Hanson paddle run at 50 rpm for 60 min and at 150 rpm for an additional 30 min in 500 ml of 0.1 M HCl. A total of 10 tablets of brand A and 6 of brand B were used for the determination. The results are summarized in Table IA.

It is seen that under the test conditions brand B dissolves significantly more rapidly than brand A during the initial 10 min of the test. Thereafter, for $20 \leq t \leq 90$ min, the differences between brands A and B are not significant.

The initial more rapid dissolution of brand B than brand A was not observed *in vivo*. Imipramine was not detected in plasma from either formulation at concentrations significantly greater than zero until 1 hr had elapsed following ingestion. Since the plasma concentrations were not significantly different at 1 hr, it can be inferred that equivalent amounts of dissolved drug were produced by each tablet by the time detectable absorption had occurred.

REFERENCES

1. J. G. Wagner. *Fundamentals of Clinical Pharmacokinetics*, Drug Intelligence Publications, Hamilton Press, Hamilton, Ill., 1975, pp. 301–305.
2. W. J. Dixon and F. J. Massey, Jr. *Introduction to Statistical Analysis*, McGraw-Hill, New York, 1957, pp. 244–259.
3. W. J. Dixon and F. J. Massey, Jr. *Introduction to Statistical Analysis*, McGraw-Hill, New York, 1957, pp. 426–433.

4. H. d'A. Heck, N. W. Flynn, S. E. Buttrill, Jr., R. L. Dyer, and M. Anbar. Determination of imipramine in plasma by high pressure liquid chromatography and field ionization mass spectrometry: Increased sensitivity in comparison with gas chromatography mass spectrometry. *Biomed. Mass Spectrom.* **5**:250–257 (1978).
5. J. G. Wagner. *Fundamentals of Clinical Pharmacokinetics*, Drug Intelligence Publications, Hamilton Press, Hamilton, Ill., 1975, p. 346.
6. R. L. Wine. *Statistics for Scientists and Engineers*, Prentice-Hall, Englewood Cliffs, N.J., 1964, p. 250.
7. J. P. Skelly. Bioavailability Policies and Guidelines. 13th Annual International Industrial Pharmacy Conference, Austin, Tex., February 26–28, 1974. Cited in J. G. Wagner. *Fundamentals of Clinical Pharmacokinetics*, Drug Intelligence Publications, Hamilton Press, Hamilton, Ill., 1975, p. 354.
8. W. J. Westlake. Use of confidence intervals in analysis of comparative bioavailability trials. *J. Pharm. Sci.* **61**:1340–1341 (1972).
9. J. G. Wagner. *Fundamentals of Clinical Pharmacokinetics*, Drug Intelligence Publications, Hamilton Press, Hamilton, Ill., 1975, pp. 297–300.
10. S. L. Meyer. *Data Analysis for Scientists and Engineers*, Wiley, New York, 1975, pp. 484–495.