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Equivalence Lack in Digoxin Plasma Levels

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In an eight-subject two-way crossover study, 0.5-mg oral doses of digoxin were administered as two 0.25-mg tablets made by two different manufacturers. Treatment "B" yielded average peak plasma levels and areas under the curves that were 59% and 55%, respectively, of those attained following treatment "A". Two of the eight subjects also received the same dose in 5% dextrose both orally and by constant rate intravenous infusion during a one-hour period. Average peaks were 4.19, 3.76, 1.46, and 0.70 ng/ml following the intravenous infusion, solution orally, treatment A (tablet) orally, and treatment B (tablet) orally, respectively. Relative areas under the curves were 100%, 80.2%, 56.7%, and 30.7% for the treatments in the same order.

Previous investigations by others[1-6] and this report clearly delineate four distinct types of bioavailability problems with use of digoxin therapeutically. These are as follows: (1) There is a "route of administration effect." The area under the plasma concentration, time curve (hereafter called *area*) following oral administration of digoxin in aqueous solution averaged 80% of the area generated when the same dose was given intravenously. This difference may be partly due to the "first pass effect"[7] and partly due to incomplete absorption. (2) The label dose of digoxin in the original manufacturer's United States product (treatment A) is not fully available as evidenced by the difference in plasma levels observed following oral administration of that tablet and the drug dissolved in 5% dextrose. A recent editorial[6] stated the bioavailability of digoxin from treatment A type tablets made in Great Britain was doubled by a change in the manufacturing process. (3) Reports of Manninen et al,[1] Lindenbaum et al,[2] Shaw et al,[5] and this report indicate that the composition and method of manufacture of some other commercial digoxin tablets are such that less digoxin reaches the circulation following their administration than following oral administration of treatment A. In this report it is shown that the poor results achieved in man with some of these brands of digoxin tablets are well correlated with results of a simple in vitro rate of dissolution test. (4) Some manufacturers of digoxin tablets have a mixing problem during preparation of their tablets, resulting in excessive tablet-to-tablet variation in potency. The existence of this problem with a given lot of tablets is readily discerned by multiple tablet assays in vitro and does not require human testing. A knowledge of mixing techniques, multiple assays of the powder blend during the mixing process, and good manufacturing practices can readily solve this fourth problem; it really should not exist at all.

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Materials and Methods

Crossover Study.—Eight normal adult white volunteers were fully informed of the nature of the study and signed consent forms. Subjects received no known enzyme-inducing agents for a period of 30 days preceding initiation of the study. They received no other medication nor alcoholic beverages for a period of seven days preceding initiation of the study nor during it. A medical history was taken and a physical examination was performed on each subject before the study. Values for the following tests were in the normal range: electrocardiogram, hematocrit, white blood cell count, differential cell

Table 1.—Eight-Subject Two-Way Crossover Study Comparing Two Digoxin Tablets Both Passing All USP Specifications

Hours	Average Plasma Level (ng/100 ml) Treatment A	Treatment B	P*	Ratio†
0	0	0	...	...
0.25	0.26	0.07	.025>P>.01	26.9
0.50	0.94	0.38	<.001	40.4
0.75	1.24	0.67	<.001	54.0
1.0	1.37	0.81	<.001	59.1
1.5	1.21	0.72	<.001	59.5
3.0	0.71	0.32	<.001	45.1
5.0	0.41	0.22	<.001	53.7
12.0	0.27	0.12	<.001	44.4
24.0	0.24	0.12	<.001	50.0
48.0	0.16	0.10	<.001	62.5
72.0	0.10	0.07	.005>P>.001	70.0
96.0	0.08	0.04	.005>P>.001	50.0
	Average Peak Level (ng/ml)			
...	1.47	0.87	<.001	59.2
	Average Area $\left(\frac{ng}{ml} \times hr\right)$			
0 to 5	3.98	2.12	.005>P>.001	53.3
0 to 96	19.4	10.6	<.001	54.6

*P values for significance of difference between treatment averages from analysis of variance for crossover design.
†(Average for treatment B/average for treatment A) × 100.

Table 2.—Digoxin Plasma Concentrations in Two Subjects Administered Three Different Oral Treatments

Hours	Plasma Concentration of Digoxin (ng/ml) 0.5 mg Digoxin in 5% Dextrose, Orally 1	2	0.5 mg Digoxin as Treatment A, Orally 1	2	0.5 mg Digoxin as Treatment B, Orally 1	2
0	0	0	0	0	0	0
	0*	0	0	0	0	0
0.25	1.29	1.49	0.03	0.11	0	0
	1.48	1.40	0.02	0.11	...	0.02
0.5	3.96	4.45	0.39	1.13	0.18	0.33
	3.46	3.17	0.43	1.20	0.20	0.40
0.75	3.21	3.07	0.65	1.70	0.34	0.72
	3.57	3.26	0.64	1.67	0.37	0.77
1.0	2.02	2.74	0.74	2.32	0.49	0.83
	1.85	3.09	...	2.02	0.45	0.87
1.5	1.53	0.86	0.79	1.66	0.52	0.67
	1.62	0.85	0.71	1.53	0.56	0.76
3.0	0.78	0.61	0.60	0.71	0.25	0.22
	0.55	0.40	0.55	0.69	0.26	0.25
5.0	0.52	0.37	0.38	0.32	0.24	0.22
	0.63	0.45	0.37	0.36	0.25	0.25
12.0	0.22	0.32	0.28	0.31	0.10	0.10
	0.20	0.35	0.27	0.34	0.09	0.15
24.0	0.18	0.15	0.21	0.18	0.11	0.09
	0.28	0.21	0.20	0.17	0.09	0.09
48.0	0.31	0.25	0.13	0.14	0.06	0.09
	0.29	0.21	0.15	0.14	...	0.07
72.0	0.22	0.13	0.09	0.11	0.05	0.15
	0.07	0.10	0.09	0.08	0.03	0.10
96.0	0.18	0.18	0.08	0.02	0.01	0.06
	0.14	0.17	0.04	0.05	...	0.04

*Duplicate assays were run on each plasma sample.

count, urinalysis, alkaline phosphatase, total bilirubin, serum glutamic oxaloacetic transaminase, serum creatinine, and chest x-ray film. They were divided into two groups of four subjects and the averages and ranges of their vital statistics were as follows: group 1: 27 (24 to 32) years of age; 78 (70.5 to 86) kg; 1.96 (1.86 to 2.04) sq m body surface area; group 2: 24 (22-25) years; 67 (55 to 74) kg; and 1.80 (1.65 to 1.89) sq m body surface.

Subjects fasted overnight and for four hours after administration of digoxin. On the mornings when medication was to be administered, each subject drank 240 ml of water within the first hour after arising, and 240 ml of water after the two tablets of digoxin were swallowed intact. No food or other beverages were taken until four hours after the dose was administered. From four hours after digoxin was given food and beverages were taken ad libitum.

Treatment A consisted of two 0.25-mg tablets of digoxin (Lanoxin) (lot 999A) manufactured by Burroughs Wellcome & Co., Tuckahoe, NY. Sixty individual tablets, assayed in the laboratories of the Food and Drug Administration had an average potency of 102.3% (range 90.5% to 109.5%) of label. The tablets passed all specifications of the United States Pharmacopeia (USP). Treatment B was two 0.25-mg digoxin tablets (lot 1510) manufactured by Fougera & Co., Inc., Hicksville, NY. Sixty individual tablets, assayed in the laboratories of the Food and Drug Administration, had an average potency of 102.7% (range, 88.5% to 117.1%) of label. The tablets passed all USP specifications.

On the first day of phase 1, subjects 1 through 4 received treatment A and subjects 5 through 8 received treatment B. On the first day of phase 2, subjects 1 through 4 received treatment B and subjects 5 through 8 received treatment A. The two doses were separated by a 14-day period.

Ten milliliters of whole blood were taken from a forearm vein just before the dose was administered at "zero time" and at 0.25, 0.5, 0.75, 1, 1.5, 3, 5, 12, 24, 48, 72, and 96 hours after the dose. The blood was drawn into specimen tubes containing citrate. Each blood sample was centrifuged within 30 minutes after collection, and the plasma was quick-frozen. All plasma samples were assayed by the radioimmunoassay method reported by Stoll et al.[8] This method is sensitive to about 0.08 ng/ml. The method utilizes antiserum available in a commercial kit. Stoll et al[8] showed that metabolites of digoxin also react with the antiserum. However, since it has been reported[9] that about 90% of the absorbed dose of digoxin is excreted unchanged in the urine and only about

10% is metabolized, and since the metabolites, with the exception of digoxigenin, are cardioactive, we believe that the assay is completely satisfactory for the bioavailability studies reported.

Oral Solution Study.—Subjects 1 and 2 of the eight-subject panel of the crossover study were used. Their vital statistics were as follows: 25 and 24 years of age; weighing 70 and 82 kg; and 1.91 and 2.02 sq m body surface area, respectively. The subjects were fully informed of the nature of the study and signed consent forms. One-half mg of USP reference standard digoxin was dissolved in 250 ml of sterile 5% dextrose. An aliquot of the same solution was used for both subjects, and the solution assayed 104.5% of label. Conditions in this study were the same as in the crossover study with the exception that the subjects ingested the 250 ml of digoxin solution at zero time instead of the two tablets followed by 240 ml of water. Blood samples were withdrawn at the same times as in the crossover study and the plasma samples were collected, stored, and assayed as described above. A 12-lead ECG was taken and interpreted prior to and at several times following administration of the digoxin. Subjects were asked about side effects or intolerance and cardiac rhythm was monitored by auscultation.

Intravenous Infusion Study.—The same two subjects who were used in the oral solution study were used in this study. The subjects were fully informed of the nature of the study and signed consent forms. The contents of three ampules of digoxin injection (each containing 2 ml and 0.5 mg digoxin) were diluted aseptically with sterile 5% dextrose to make 720 ml of sterile solution. The solution assayed 115.7% of label potency. Each subject received 240 ml equivalent to 0.5 mg of digoxin during a one-hour period at a rate of 4 ml/min. The subjects fasted overnight, then at 6:30 AM ate a breakfast of orange juice, one boiled egg, two strips of bacon, two pieces of toast, and one cup of coffee. At 8 AM the infusion pump was started and the 240-ml solution, containing 0.5 mg of digoxin, was infused at a rate of 4 ml/min into a forearm vein. Blood samples were withdrawn from the contralateral arm just before the pump was started, and at 15, 30, and 45 minutes, and just when the pump was cut off at one hour, then at 0.25, 0.5, 0.75, 1, 1.5, 3, 5, 12, 24, 48, 72, and 96 hours measured from the end of the infusion. No food or beverage other than water was taken from 8 AM to 4 PM. During this period water was taken ad lib and the subjects were encouraged to drink at least four glasses of water. After 4 PM, food and beverages (excluding alcoholic beverages) were taken ad lib. The subjects were standing during the infusion and ambulatory from the time the infusion ceased until 4 PM. This precaution was taken to assure that in the infusion study the circulatory hemodynamics of the subjects were as similar as possible to those during the oral studies.

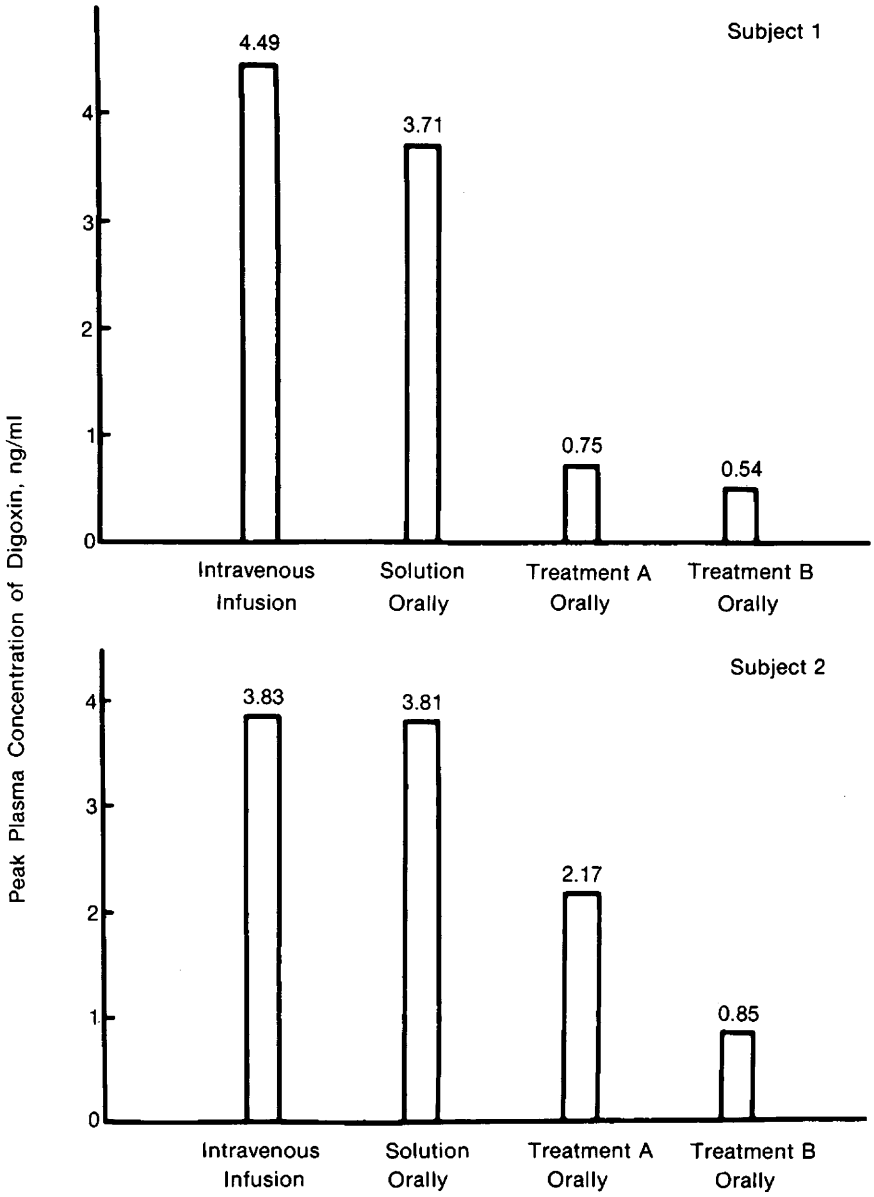

Fig 1.—Comparison of peak plasma digoxin concentrations in two subjects given four different treatments.

Statistical Analysis.—Plasma concentrations at each sampling time, peak plasma concentrations, and areas were analyzed by analysis of variance for crossover design using the data collected in the crossover study. The sources of variation in this type of analysis are groups, subjects within the group, time periods, treatments, and residual. In Table 1, only the significance level of the treatment mean square is reported, since reporting of the complete analyses would require extensive tabulation. The complete data for the two sub-

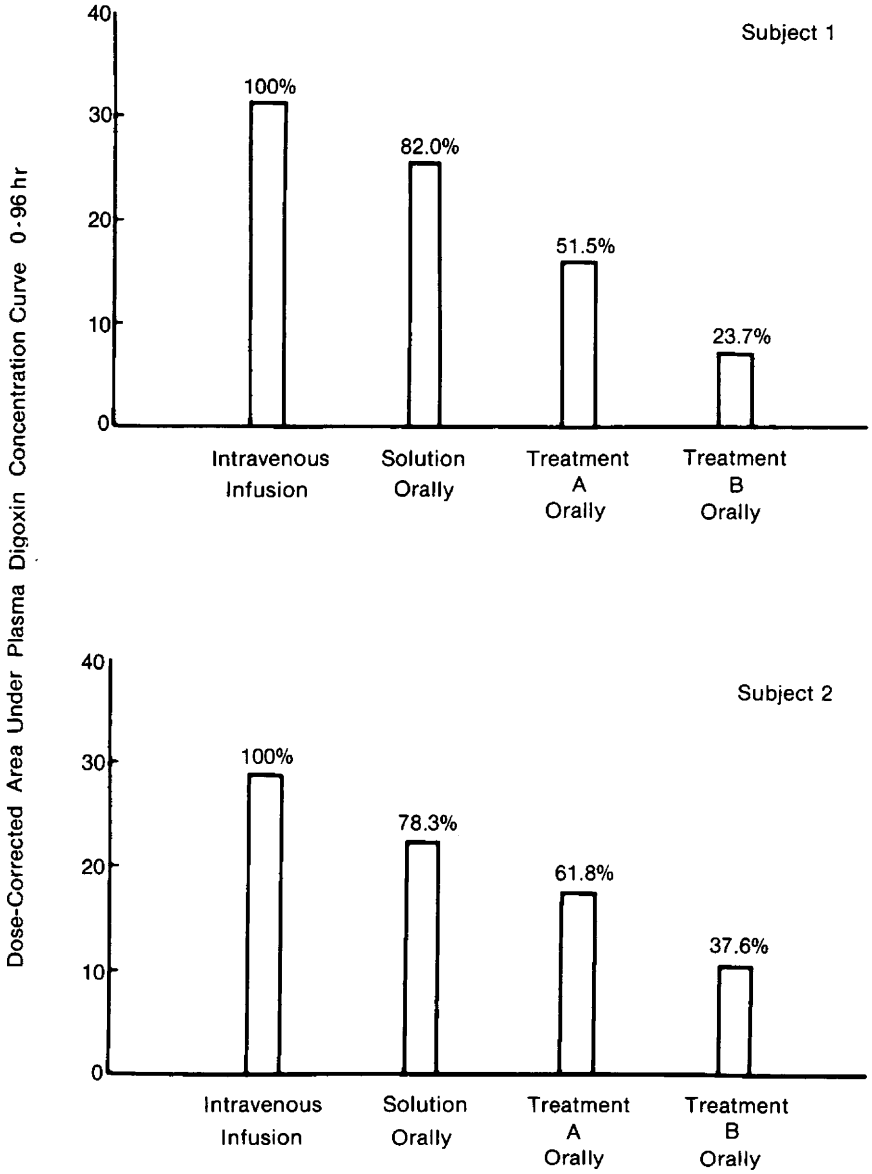

Fig 2.—Comparison of areas under the digoxin plasma concentration, time curves in two subjects given four different treatments. Percentages are normalized values assuming infusion is 100% in each case.

jects administered four different treatments are reported in Tables 2 and 3, since, to our knowledge, such intensive sampling has not been reported previously in digoxin radioimmunoassay studies.

Dissolution Rate Tests.—Tablets of lots A_1, B_1, and B_2, studied in man by Lindenbaum et al[2] were obtained by the Food and Drug Administration and tested for dissolution rates. Tablets of lot C, studied by Lindenbaum et al,[2] were not available. Tablets used as treatments A and B in our studies were also tested in vitro by the same dissolution rate method. Tablets were studied individually and four tablets of each lot were tested. Tests were performed in a three-necked, round-bottom, 1,000-ml flask containing 500 ml of water at 37±1 C. The water was stirred at 50±3 rpm with the stirrer blade described by Wagner,[10] using a standard servodyne power drive system. Ten-milliliter samples of the solution were taken at frequent time intervals with immediate replacement by 10 ml of fresh preheated deionized water. The digoxin was then determined by the fluorometric method of Wells et al.[11] The percent of the labeled amount of digoxin in solution was plotted vs time. A smooth curve was drawn through the points, then the percent in solution at 15, 80, and 120 minutes was estimated for each tablet. For the tablets used in the crossover study, the times required to dissolve 20%, 30%, and 40% of the labeled amount of digoxin were also estimated, and, for each set, an analysis of variance was performed. Sources of variation were brands of tablets, different tablets of a given brand, and residual or unexplained variation.

Results

Crossover Study.—Table 1 lists the average digoxin plasma concentrations at each of the 12 sampling times, the average peak digoxin plasma concentrations, and the average areas measured over both of the intervals 0 to 5 hours and 0 to 96 hours. The significance level of the treatment mean square from the analysis of variance for crossover design is given in the fourth column of Table 1. In the fifth column the average for treatment B is expressed as a percentage of the average for treatment A.

Although both of these tablets passed all USP specifications, and had essentially the same average potency and tablet-to-tablet variation in potency as measured by in vitro assays of 60 individual tablets of each lot in the laboratories of the Food and Drug Administration, their in vivo performance in man was markedly different. Based on the total area over the four-day sampling period, the bioavailability of treatment B was only 55% of that of treatment A. Surprisingly, the corresponding value was 53% based on measurement of only the five-hour area. This similarity in values supports the comparison and data of Lindenbaum et al[2] who only measured plasma concentrations of digoxin over a five-hour period.

Comparison of Four Treatments in Two Subjects.—Table 2 lists plasma concentrations of digoxin measured in two subjects following three different oral treatments containing the same 0.5-mg label dose of digoxin. Table 3 lists plasma concentrations of digoxin measured in the same two subjects following administration of a 0.5-mg label dose in 5% dextrose by constant rate intravenous infusion over a one-hour period. The average areas (0 to 96 hours) for these two

subjects, namely 17.4 and 9.37 ng/ml times the hours following treatments A and B, respectively, were very similar to the average areas, namely 19.4 and 10.6 ng/ml times the hours, respectively, for all eight subjects used on the crossover study. Hence, results achieved with these two subjects with the two tablets are representative of those obtained with the panel of eight subjects.

Figure 1 compares peak plasma concentrations of digoxin in the two subjects following the four treatments; values plotted are the averages of duplicate assays. Figure 2 compares dose-corrected areas following the four treatments in the same two subjects. To correct for the actual dose, as determined by assay for each preparation, the area was divided by the values 1.157, 1.045, 1.023, and 1.027 for the intravenous infusion, oral solution, treatment A, and treatment "B", respectively, these values being based on the potencies determined by assay. Above the bars in Fig 2 are the ratios of areas, expressed as a percentage, assuming the area for the intravenous infusion was 100% in each case. Pretreatment and posttreatment ECGs were normal and remained unchanged throughout the oral solution study.

In Vitro Rate of Dissolution Tests.—Table 4 summarizes results of in vitro rate of dissolution tests performed on four tablets of each of three lots studied by Lindenbaum et al[2] and the two lots studied by the authors in man. The percent of the labeled amount of digoxin in solution at 15, 80, 120 minutes, shown in the Table, was estimated by drawing smooth curves through more extensive data when the dissolution media were sampled at very frequent time intervals.

Correlation of In Vitro and In Vivo Results.—Table 5 shows the correlation between some of the in vitro results reported in Table 4 and the in vivo results reported by Lindenbaum et al[2] and in this paper. The areas under the plasma concentration curves over the 0- to 5-hour period are compared in Table 5 since Lindenbaum et al[2] only measured plasma levels over the five-hour period.

Table 3.—Digoxin Plasma Concentrations in Two Subjects Administered 0.5 mg Digoxin by Constant Rate Intravenous Infusion

Time After Start of Infusion, hr	Plasma Concentration of Digoxin (ng/ml)			
	Subject 1		Subject 2	
	Assay 1	Assay 2	Assay 1	Assay 2
0	0	0	0	0
0.25	2.26	2.26	3.36	3.30
0.50	4.39	4.09	3.47	3.48
0.75	4.50	4.47	3.78	3.76
1.0*	3.47	3.31	3.83	3.93
1.25	3.09	2.88	2.81	2.58
1.5	2.12	2.19	1.97	2.05
1.75	1.99	1.78	1.69	1.71
2.0	1.20	1.14	1.09	1.07
3.0	0.63	0.52	0.49	0.51
4.0	0.58	0.66	0.53	0.65
5.0	0.69	0.62	0.44	0.46
6.0	0.65	0.66	0.56	0.53
8.0	0.70	0.69	0.57	0.55
25.0	0.42	0.38	0.43	0.38
49.0	0.27	0.26	0.20	0.21
73.0	0.17	0.16	0.17	0.20
97.0	0.17	0.12	0.19	0.16

*Time infusion ceased.

Table 4.—Summary of In Vitro Rate of Dissolution Tests Performed on Four Tablets of Each Lot

Tablet	% of Labeled Amount in Solution (min)					
	15		80		120	
	Average	SD*	Average	SD	Average	SD
Treatment A†	40.8	2.6	73.6	1.2	80.3	2.0
Lot A_1‡	30.1	4.7	62.6	5.0	71.3	4.2
Lot B_1‡	45.7	6.8	73.0	5.4	79.6	7.1
Treatment B†	6.2	2.6	36.1	2.6	43.7	2.0
Lot B_2‡	1.3	0.2	0.9	1.7	1.2	1.7

*SD, standard deviation.
†Wagner et al.
‡Lindenbaum et al.[2]

Table 5.—Correlation of In Vivo Results Obtained in Human Studies With In Vitro Rate of Dissolution for Five Lots of Digoxin Tablets

Tablet	In Vitro	In Vivo	
	Average Amount in Solution in 120 min as % of Label	Average Peak Plasma Digoxin Level (ng/ml)	Average Area 0-5 hr (ng/ml × hr)
Lot A_1*	71.3	2.10	4.97
Treatment A†	80.3	1.47	3.98
Lot B_1*	79.6	1.40	3.52
Treatment B†	43.7	0.87	2.11
Lot B_2*	1.2	0.30	0.69

*Lindenbaum et al.[2]
†Wagner et al.

Comment

In a recent article, apparently intended to promote repeal of anti-substitution laws, E. G. Feldmann[12] of the American Pharmaceutical Association wrote:

Moreover, many purported instances of inequivalence, either therapeutic or biological, are given wide publicity, and subsequently it is quietly reported that the unsatisfactory product has been taken off the market quite some time earlier, or that the products did not pass compendial requirements. A recent example of such a chain of events was the page one story in the *New York Times* a few months ago about digoxin tablets.

The authors believe that Dr. Feldmann's summary was not objective and that his judgment of the Lindenbaum[2] data was premature for the following reasons. This report shows

a large difference in bioavailabilities of two commercial digoxin tablets sold in the United States. Both of these lots of tablets were assayed in the laboratories of the Food and Drug Administration and passed all USP specifications. Dr. Feldmann failed to point out the differences in in vivo results reported by Lindenbaum et al[2] with his lots A_1 and B_2, both of which passed all USP specifications. In this report, we give results of in vitro rate of dissolution tests performed with the Lindenbaum lots A_1, B_1, and B_2. Tablets of lot B_2, which gave the lowest digoxin plasma concentrations in the Lindenbaum study released an average only 1.2% of their digoxin content when stirred at 50 rpm in 500 ml of water at 37 C for two hours. When the stirring rate was increased to 200 rpm for an additional hour, the tablets had released a total of only 3.6% of their digoxin content. Hence, the problem with the marketed lot B_2 was not only tablet-to-tablet variation in potency,[3] but also extremely poor dissolution characteristics. Such a dissolution test, which apparently is capable of distinguishing good or bad lots of digoxin tablets, is not an official specification at present.

Both good and bad digoxin tablets meet the present USP limit of 30 minutes in the USP tablet disintegration test. Treatment A, used in the present studies, had a USP disintegration time of 18 seconds, while treatment B had a USP disintegration time of 5 minutes, 20 seconds. Although the average disintegration times were different, both types of tablets readily passed the test. In the dissolution test, the brand averages were 3.9 and 36.3 minutes for $t_{20\%}$, 6.6 and 61.8 minutes for $t_{30\%}$, and 10.3 and 104.5 minutes for $t_{40\%}$, for treatments A and B, respectively. These differences in brand averages were highly significant ($P<.005$) in the analyses of variance.

The area measured over a sufficiently long time period is generally accepted as a measure of bioavailability.[13] For a drug with as long apparent half-life as digoxin, one does not usually expect that the area measured over a short time interval such as five hours after the dose was administered to reflect relative bioavailability. However, in the case of digoxin, the area measured over the interval 0 to 5 hours correlates extremely well with the area measured over 0 to 96 hours as shown by the data in Table 1. This supports the results of Lindenbaum et al[2] who measured plasma levels of digoxin by radioimmunoassay only over the 0- to 5-hour period.

The dose-corrected average area (0 to 96 hours) following oral administration of digoxin in 5% dextrose solution was 80% of the area generated when the same label dose was administered by constant rate intravenous infusion. This suggests that average absolute absorption was 80% of the dose when digoxin was administered orally in aqueous solution. This value agrees very well with the values of 85% reported by Doherty et al[14] and 80% reported by Doherty[15] for absolute absorption of digoxin in man, based on administration of tritiated digoxin orally and intravenously and measurement of radioactivity in gastrointestinal contents and stool.

When digoxin was administered as treatment A, 51.5% and 61.8% (average 56.7%) of the dose (by assay) was absorbed, but when digoxin was administered as treatment B, only 23.7% and 37.6% (average 30.7%) of the dose (by assay) was absorbed (Fig 2).

The marked differences in peak plasma levels of digoxin observed at the end of the one-hour infusion period and at 1 to 1.5 hours after oral administration of the drug in solution compared with the peaks attained following commercial tablets (Fig 1) are noteworthy from a clinical point of view. Such differences may be important when digitalization is performed with different preparations and by different routes of administration.

Although not plotted in this report, an inspection of the data in Table 3 shows that following the intravenous infusion, the plasma levels of digoxin plateau from about two to seven hours after the infusion ceased. Plots of radioactivity vs time following rapid intravenous injection of tritiated digoxin show the same characteristic as evidenced by the trends of the points in Figures 6C and 6D of the paper of Doherty.[15] This plateau may be due to enterohepatic cycling of digoxin as reported by Doherty et al.[14] Such a plateau suggests that from a pharmacokinetic viewpoint the description of a digoxin plasma concentration curve following a rapid intravenous injection by a two-term exponential equation, as reported by Doherty et al,[15] or by a three-term exponential equation, as reported by Chiou,[16] is questionable. When radioactivity is measured following tritiated digoxin and when the radioimmunoassay procedure is used following cold digoxin, some metabolites of digoxin are measured as well as unchanged drug.[8] These considerations make extensive pharmacokinetic analysis tenuous.

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Nonproprietary Name and Trademarks of Drug

Digoxin—*Lanoxin, Davoxin.*

References

1. Manninen V, Melin J, Härtel G: Serum-digoxin concentrations during treatment with different preparations. *Lancet* 2:934-935, 1971.
2. Lindenbaum J, et al: Variation in biologic availability of digoxin from four preparations. *N Engl J Med* 285:1344-1347, 1971.
3. Vitti TG, Banes D, Byers TE: Bioavailability of digoxin. *N Engl J Med* 285:1433-1434, 1971.
4. Hibble AG, Isaac P, Grahame-Smith DG: Bioavailability of digoxin. *Lancet* 2:90-91, 1972.
5. Shaw TRD, Howard MR, Hamer J: Variation in the biological availability of digoxin. *Lancet* 2:303-307, 1972.
6. The bioavailability of digoxin, editorial. *Lancet* 2:311-312, 1972.
7. Harris PA, Riegelman S: Influence of the route of administration on the area under the plasma concentration time curve. *J Pharm Sci* 58:71-75, 1969.
8. Stoll RG: Specificity of the digoxin radioimmunoassay procedure. *Res Com Chem Path Pharmacol* 4:503-510, 1972.
9. Marcus FI, Kapadia GJ, Kapadia GG: The metabolism of digoxin in normal subjects. *J Pharmacol Exp Ther* 145:203-209, 1964.
10. Wagner JG: *Biopharmaceutics and Relevant Pharmacokinetics.* Hamilton, Ill, Drug Intelligence Publications, 1972, p 112.
11. Wells D, Katzung B, Myers FH: Spectrofluorometric analysis of cardiotonic steroids. *J Pharm Pharmacol* 13:389-395, 1961.
12. Feldmann EG: Drug product selection-freedom with responsibility. *J Am Pharm Assoc* 12:368-374, 1972.
13. *Guidelines for Biopharmaceutical Studies in Man,* APhA Academy of Pharmaceutical Sciences. Washington, D.C., American Pharmaceutical Association, 1972, p 10.
14. Doherty JE, et al: Tritiated digoxin: XIV. Enterohepatic circulation, absorption, and excretion studies in human volunteers. *Circulation* 42:867-873, 1970.
15. Doherty JE: The clinical pharmacology of digitalis glycosides: A review. *Am J Med Sci* 255:382-414, 1968.
16. Chiou WL: A simple equation to estimate the fraction of drug remaining in the body after an intravenous injection. *J Pharm Pharmacol* 34:342-343, 1972.