Bioavailability and Dissolution Properties of Two Commercial Digoxin Tablets

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Abstract □ Six healthy male subjects were given 0.5 mg of digoxin on three separate occasions in the form of an elixir and two different commercially available brands of compressed tablets. Blood levels were measured by a radioimmunoassay technique at various time intervals up to 48 hr following drug administration. A previously unreported variable in the radioimmunoassay method was found. Varying amounts of digoxin-free serum added to aqueous samples such as urine or hydroalcoholic solutions led to significant changes in the apparent digoxin concentration measured in the sample. The elixir form gave much higher blood levels than either tablet during the first two sampling times, but the two tablets showed nearly identical blood levels and relative bioavailabilities. A previously reported dissolution rate test method showed wide differences between the two tablets and thus failed to correlate with the similar bioavailabilities observed *in vivo*.

Keyphrases □ Digoxin tablets and elixir—comparative bioavailability, variable in radioimmunoassay technique reported, tablet bioavailability compared to dissolution rate □ Bioavailability of digoxin tablets and elixir—compared using radioimmunoassay technique, variable in technique reported, tablet bioavailability compared to dissolution rate □ Radioimmunoassay—analysis, digoxin blood levels, variable in technique reported

Marked differences in the bioavailability of digoxin from commercial tablet dosage forms have been reported (1–6). Other reports (7, 8) indicated that digoxin is less than 100% absorbed from tablet dosage forms as compared to an oral solution or an intravenous injection. As a result of these reports, it has become increasingly apparent that the bioavailability of digoxin from a particular manufacturer's dosage form may be significantly reduced. Such incomplete or variable bioavailability of digoxin preparations represents a potential hazard to the patient because a change in the source of manufacture of digoxin or lot-to-lot variation from the same manufacturer may result in toxicity or underdigitalization.

Various methods have been used to estimate or predict the bioavailability of digoxin from commercially available products. With the advent of the radioimmunoassay technique for digoxin, a rapid, precise, and sensitive assay became available for repetitive assays of digoxin in biological fluids. However, as illustrated in this and other reports (9), certain limitations are inherent in the radioimmunoassay for digoxin. Similarly, other reported methods of predicting therapeutic effectiveness of the cardiac glycosides, including *in vitro* dissolution rate testing (5), are subject to limitations. Thus, the objectives of this article are to: *(a)* present a previously unreported variable in the radioimmunoassay technique for the determination of serum digoxin concentrations, *(b)* report the relative bioavailability of two commercially available digoxin tablets in comparison to an elixir, and *(c)* report the applicability of dissolution rate testing in predicting the relative bioavailability of digoxin from tablet dosage forms.

EXPERIMENTAL

Six healthy volunteer male subjects, ranging in age from 20 to 22 years and in weight from 54.6 to 74.2 kg, were selected. The subjects had no history of GI, liver, or kidney disease, and none admitted to taking any medication regularly. Each subject received a thorough physical examination. Values for the following clinical pathology laboratory tests were within normal ranges: hemoglobin, hematocrit, complete blood count, platelet count, differential count, serum creatinine, blood urea nitrogen, sodium, potassium, serum alkaline phosphatase, serum total bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, chest X-ray, and ECG. Urine was also collected and analyzed for creatinine to enable the determination of creatinine clearance. Digoxin elixir[1] USP was administered first to each subject to determine whether any malabsorption problems existed, a phenomenon previously reported for digoxin (10). Either digoxin tablets[2] USP (hereafter referred to as Treatment I) or another group of digoxin tablets[3] (Treatment II) were then administered to the same six subjects in a crossover manner.

All volunteer subjects were asked to abstain from alcoholic beverages for 48 hr preceding each experiment. They were also asked not to ingest any drug of any kind for at least 1 week preceding each experiment. All subjects were informed as to the nature of the experiments and signed a form prior to each experiment consenting to special studies that are ordinarily not a part of normal diagnosis and treatment of disease states.

Beginning about 8 am on the day of the experiment, two tablets, each containing 0.25 mg of digoxin, were administered orally with 100 ml of water, followed by an additional 100 ml of water 2 hr later. Representative samples from each lot of tablets and elixir were previously determined to meet USP XVIII specifications as to potency, disintegration time, and content uniformity.

Food was not permitted for 8 hr before and 4 hr after digoxin administration. Blood samples of 5–7 ml each were obtained by venipuncture or *via* an intravenous administration set at 0, 0.5, 1, 1.5, 2, 3, 5, 7, 12, 24, and 48 hr following drug administration. The samples were analyzed for digoxin utilizing commercially available radioimmunoassay kits[4] and solid crystal scintillation counting. The bound radioactivity separated from the free ^{125}I-digoxin derivative during the assay procedure was counted by an automatic control gamma scintillation spectrometer[5] for 5 min/sample.

Subjects were ambulatory during the experiment. All subjects were requested not to be in a horizontal position for at least 4 hr after the test dose of digoxin was administered, but they were permitted to sit in a semireclining position.

The dissolution method described by Wagner *et al.* (5) was employed to investigate the applicability of an *in vitro* dissolution rate method in predicting relative bioavailabilities of digoxin from tablet dosage forms. The dissolution medium in the current study was 900 ml of distilled water. One-milliliter samples were

[1] Lanoxin Elixir, Burroughs Wellcome & Co., Research Triangle Park, NC 27709
[2] Lanoxin Tablets, lot 059G, Burroughs Wellcome & Co., Research Triangle Park, NC 27709
[3] Lot 047105, Towne Paulsen and Co., Monrovia, CA 91016
[4] ^{125}I-Digoxin radioimmunoassay kit, Schwarz/Mann, Division of Becton, Dickinson and Co., Orangeburg, NY 10962
[5] Model 578, Packard Instrument Co., Downers Grove, IL 60515

Table I—Mean Digoxin Serum Levels (ng/ml ± Standard Error) and Areas under the Serum Level–Time Curves (ng/ml × hr) for Six Subjects following Oral Administration of 0.5 mg Digoxin in Three Different Forms along with the F Ratio and Level of Significance (p) Resulting from an Analysis of Variance of the Data

	Digoxin Elixir		Treatment I		Treatment II			
Hours	Mean	*SE*	Mean	*SE*	Mean	*SE*	F^a	p
0.0	0.00	0.00	0.00	0.00	0.00	0.00	—	—
0.5	2.76	0.51	0.83	0.30	0.19	0.12	14.92	<0.01
1.0	2.38	0.33	1.38	0.43	0.67	0.20	6.66	<0.01
1.5	1.59	0.16	1.12	0.31	1.56	0.37	0.82	n.s.[b]
2.0	1.09	0.14	0.81	0.14	1.27	0.21	1.61	n.s.
3.0	0.66	0.05	0.64	0.08	0.67	0.10	0.05	n.s.
5.0	0.33	0.06	0.47	0.06	0.37	0.09	0.96	n.s.
7.0	0.22	0.06	0.26	0.03	0.22	0.06	0.20	n.s.
12.0	0.15	0.06	0.22	0.04	0.17	0.07	0.39	n.s.
24.0	0.16	0.03	0.24	0.06	0.19	0.07	0.16	n.s.
48.0	0.04	0.03	0.09	0.02	0.12	0.04	1.91	n.s.
AUC_5	5.51	0.58	3.68	0.66	3.54	0.52	3.42	n.s.
AUC_{12}	6.97	0.56	5.59	0.77	5.11	0.82	1.71	n.s.
AUC_{24}	8.81	0.48	8.04	0.87	7.30	1.44	1.07	n.s.
AUC_{48}	11.17	0.33	11.32	1.08	11.10	2.58	0.01	n.s.

[a] Degrees of freedom (2, 15). [b] Not significant ($p > 0.05$).

obtained at 30, 60, and 120 min; they were filtered through a 0.22-μm membrane filter[6], diluted to 25 ml with 25% ethanol, and then assayed by the radioimmunoassay previously described for digoxin. The only variation in this procedure was that 50 μl of digoxin-free serum was added to each tube immediately after the addition of a 50-μl dissolution sample dilution.

RESULTS AND DISCUSSION

Radioimmunoassay—Linear standard curves were obtained by least-squares linear regression analysis of the reciprocal counts per minute of the bound ^{125}I-digoxin derivative *versus* digoxin concentration. Excellent correlation coefficients were obtained for each standard curve prepared during the assay of each group of samples. The mean correlation coefficient (±standard error) for 25 consecutive standard curves plotted in this way was 0.9988 ± 0.0001. These findings are consistent with reported results (12). Thus, it was concluded that the precision of the method of plotting corrected reciprocal counts per minute *versus* known digoxin concentrations is satisfactory for constructing a standard curve for the determination of unknown digoxin concentrations in samples of similar digoxin concentration.

The radioimmunoassay for digoxin was also investigated for its accuracy and reproducibility in the quantitative determination of digoxin in serum, urine, and hydroalcoholic samples to which a known quantity of digoxin had been added. Within experimental error, complete recovery was demonstrated for each. However, an interesting observation was made as to the necessity of maintaining a uniform quantity of serum in the samples used to prepare the standard curve and in the samples to be assayed. For example, two standard curves prepared simultaneously with different quantities of the same blank serum resulted in nonsuperimposable curves (Fig. 1). One standard curve prepared from 50 μl of blank serum/tube resulted in a Y-intercept of 0.00073999 and a slope of 0.00034044. The other standard curve prepared from 200 μl of blank serum resulted in a Y-intercept of 0.0016223 and a slope of 0.0012828. The correlation coefficients obtained by linear regression analysis were 0.99949 and 0.99977, respectively, indicating that both standard curves were linear. As illustrated in Fig. 1, a reciprocal count read as 1.0 ng/ml from the standard curve prepared from 200 μl of blank serum would be read as 6.4 ng/ml from the standard curve prepared from 50 μl of blank serum—an approximate sixfold difference in concentration resulting from the same reciprocal count. As can be seen from these plots, the error would become even greater at higher reciprocal counts. The total absence of blank serum from the standard curve and samples yielded erratic results and thus was not investigated further.

In light of this discussion, the results of the urinary excretion studies by Huffman and Azarnoff (7) might be subject to question. To determine the digoxin concentration in urine, they added 0.2 ml of blank plasma to each 0.1 ml urine sample except when the concentration exceeded the highest standard, in which case they simply diluted the sample with phosphate buffer (pH 7.4) containing bovine serum albumin (1 g/liter) to bring the concentration within the range of the standard curve. Unless the serum, plasma, or protein content was adjusted beyond that reported, different quantities of plasma and/or serum albumin were apparently included in the final dilution of some samples. As suggested by the current study, differences in the serum, plasma, or protein

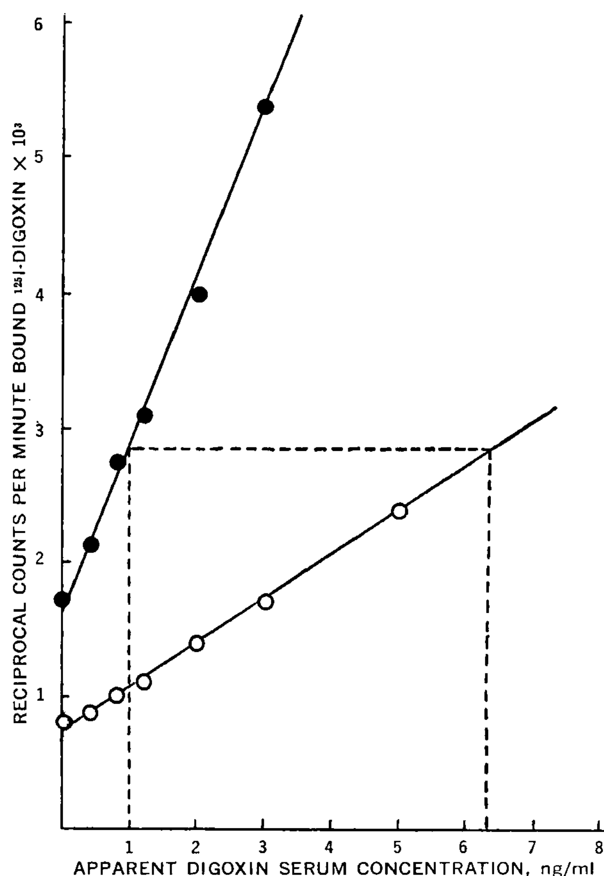

Figure 1—*Reciprocal plots of two standard curves prepared simultaneously utilizing 200 (●) and 50 (○) μl blank serum/tube, illustrating the markedly different apparent digoxin concentrations (indicated by the dashed lines) obtained from reciprocal counts read from the two plots.*

[6] Millipore Corp., Bedford, MA 01730

Table II—Mean Percent[a] Bioavailabilities (±Standard Error) of Commercial Digoxin Tablets Estimated from Area under the Serum Level–Time Curve during Selected Finite Time Periods following the Oral Administration of Two 0.25-mg Digoxin Tablets to Six Male Subjects

Hours	Bioavailability, %			
	Treatment I	Treatment II	t^b	p
0–5	70.98 ± 8.14	64.88 ± 8.92	0.616	n.s.[c]
0–12	83.28 ± 5.62	73.53 ± 13.13	0.931	n.s.
0–24	95.64 ± 7.17	85.17 ± 19.23	0.676	n.s.
0–48	106.38 ± 10.27	100.75 ± 24.09	0.238	n.s.

[a] Calculated as a percentage of the corresponding area under the serum level–time curve obtained from the elixir data. [b] Degrees of freedom = 5. [c] Not significant ($p > 0.05$).

content of either the samples used to prepare the standard curve or the unknown samples could result in markedly different apparent digoxin concentrations.

Relative Bioavailability Studies—Table I summarizes the mean serum levels at all times for the three treatments, along with the area under the serum level–time curves up to 5, 12, 24, and 48 hr as calculated by the trapezoidal rule. The serum levels at each sampling time, the individual serum levels at each sampling time, and the individual areas were analyzed by a one-way analysis of variance. Thus, the F-ratios and the significance levels of the treatment mean square are also shown in Table I. As can be seen, significant differences resulting from the treatments occurred only at the 0.5- and 1.0-hr periods, probably due to the much higher levels obtained following administration of the elixir. Thus, it would appear that there was very little difference in the rate and extent of digoxin absorption from the two brands of compressed tablet dosage forms investigated.

One criticism of the study by Lindenbaum *et al.* (1) was that serum concentrations of digoxin were determined for less than one-third of one half-life, a period generally thought to be too short to reflect accurately the total absorption and, thus, bioavailability. However, in a recent report (5), the area under the curve (AUC) for the 0–5-hr interval correlated "extremely well" with the area measured from 0 to 96 hr. Similar results were obtained in the current study, as can be seen from the various areas (Table I). However, relative bioavailabilities of the two tablet dosage forms appeared to be somewhat lower when calculated from data over shorter periods, as can be seen by comparing the relative bioavailabilities and taking the elixir data as 100% (Table II). The increase in the apparent relative bioavailabilities of both commercial digoxin tablets when estimated from data recorded over a longer period may be accounted for on the basis of a larger proportion of the total area under the curve occurring during the latter part of the time interval for the tablets in comparison to the elixir, which showed much earlier and higher peak levels. However, the relative bioavailabilities of the two tablets remained quite similar regardless of the time used to calculate the respective areas. When the individual values were statistically evaluated by a t test for paired observations, there were no significant differences between the two treatments at any time interval (Table II). Consequently, further treatment of the serum level data tends to provide additional evidence that there is indeed very little difference in the rate and extent of digoxin absorption from these two commercially available tablet dosage forms.

Blood levels of various drugs are frequently used by clinicians to determine the extent of an expected pharmacological or toxic response. However, such data have little meaning in the absence of supportive information. Concentrations obtained at the time of peak blood levels may lead to a different conclusion from concentrations obtained during the equilibrium plateau of the blood level curve. In the case of digoxin, as demonstrated in this study, individual peak serum levels may exceed 4 ng/ml[7] following a single oral dose of 0.5 mg of digoxin in the elixir dosage form (upper limit of 0.5-hr level). Such levels have been reported to be in the toxic range (11, 12).

Dissolution Studies—A commonly employed method of estimating bioavailability is testing the rate of dissolution of the drug from a compressed tablet. Recently, Wagner *et al.* (5) correlated rate of dissolution of digoxin from commercially available tablets to values for areas under the curve, and they concluded that their dissolution test was capable of distinguishing between "good" and "bad" lots of digoxin tablets. From the results of the current study, it appears that this dissolution method (5) may not be applicable to the comparative evaluation of the two brands of tablets employed in the current study. The mean percent (±standard error) of the labeled amount of digoxin in solution at 120 min for five Treatment I tablets (Table III) was 59.1 ± 3.9% whereas the mean percent in solution at 120 min for five Treatment II tablets was 19.4 ± 3.5%, a more than threefold difference. Although the Treatment I tablets were only about 59% dissolved after 2 hr as contrasted to a reported 80.3% dissolved in the study of Wagner *et al.* (5) for the same brand, this should not be surprising in light of a recent report by Lindenbaum *et al.* (6). These investigators showed a fairly wide range in dissolution rates for three different lots of tablets prepared by the manufacturer of the Treatment I tablets. The percent dissolved after 2 hr, estimated from Fig. 1 of the Lindenbaum *et al.* (6) report, ranges from slightly less than 60% to slightly more than 80% for the various lots tested. Although the methodology employed by these investigators was somewhat different than was utilized in the present study, the lower rate obtained compared to that reported by Wagner *et al.* (5) can be explained by an apparent, large batch-to-batch variation in the dissolution rate properties of digoxin tablets made by this manufacturer.

Table III—Mean Percent (±Standard Error) of Labeled Digoxin in Solution at Various Times following *In Vitro* Dissolution Testing on Five Tablets of Each Formulation

Minutes	Dissolved, %	
	Treatment I	Treatment II
30	34.9 ± 4.3	6.0 ± 1.2
60	46.9 ± 6.1	8.0 ± 0.8
120	59.1 ± 3.9	19.4 ± 3.5

However, as shown by the data in Tables I and II, no such difference was observed in the bioavailability of the tablets. This would suggest that the drug is readily available for absorption from both tablets and does, indeed, yield relatively high areas under the curve. The slower dissolution observed with Treatment II tablets did not have a significantly adverse effect on the bioavailability as shown in this study. It is possible that when certain differences in formulation or manufacturing specifications are involved between two brands, it may not be possible to correlate dissolution results and bioavailability for digoxin tablets. Thus, it is apparent that *in vivo* testing in the target species (humans) is necessary to prove bioavailability of a particular dosage form and that additional work is needed before a dissolution rate method can be used to predict digoxin bioavailability consistently.

[7] Although individual data have not been reported, Subject 2 showed a serum level of 4.70 ng/ml 30 min after drug administration.

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GLC Determination of Plasma Concentration of Phenylbutazone and Its Metabolite Oxyphenbutazone

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Abstract □ Sensitive specific methods are described for the determination of phenylbutazone and its metabolite oxyphenbutazone from the same plasma sample. The sample, to which an internal standard 5-(4-hydroxyphenyl)-5-phenylhydantoin (Standard II) is added, is first extracted with ether to remove interfering substances and then with *n*-heptane under acidic conditions to separate phenylbutazone, which is determined on a gas chromatograph by flash methylation (310°) with trimethylanilinium hydroxide using 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane as the external standard (Standard I). The aqueous acidic residue from which the phenylbutazone has been selectively removed is shaken again with ether to extract the oxyphenbutazone, which is analyzed with a different GLC system with flash methylation (310°) against Standard II. The methods are of sufficient sensitivity to determine plasma levels in humans after a 200-mg dose of phenylbutazone (phenylbutazone, 1 μg/ml; oxyphenbutazone, 0.5 μg/ml).

Keyphrases □ Phenylbutazone and its metabolite oxyphenbutazone—GLC analysis in plasma □ Oxyphenbutazone metabolite and phenylbutazone—GLC analysis in plasma □ GLC—analysis, phenylbutazone and oxyphenbutazone metabolite in plasma

Although several spectrophotometric methods (1-6) and a GLC method (7) have been reported for the estimation of phenylbutazone in biological fluids, fewer methods have been described for the estimation of oxyphenbutazone [1-phenyl-2-(*p*-hydroxyphenyl)-3,5-dioxo-4-*n*-butylpyrazolidine] and none for hydroxyphenylbutazone [1,2-diphenyl-3,5-dioxo-4-(3-hydroxybutyl)pyrazolidine], which are the two main metabolites of phenylbutazone.

The classical method of Burns *et al.* (1) is not of sufficient sensitivity to estimate phenylbutazone and oxyphenbutazone in biological fluids following single doses of phenylbutazone. Although the need for a sensitive specific method for estimating phenylbutazone in plasma has been met by the recently reported high-speed liquid chromatography (HSLC) and GLC procedures (8, 9), no suitable methods are available for estimating oxyphenbutazone and hydroxyphenylbutazone following single doses of phenylbutazone in humans.

For the current pharmacokinetic study in these laboratories (10), a GLC method that is specific and sensitive for measuring oxyphenbutazone in plasma following single doses of phenylbutazone has been developed. The liquid chromatographic method reported earlier (9) for phenylbutazone is adequate, but the present method measures the levels of oxyphenbutazone as well as phenylbutazone in the same plasma sample.

EXPERIMENTAL

Reagents—Ether[1] and *n*-heptane[2] were distilled in glass prior to use. Stock solutions of phenylbutazone[3] containing 100 μg/ml were prepared (9) and diluted with phosphate buffer (pH 7.4) to the concentrations required (2–64 μg/ml) before use. Stock solutions of oxyphenbutazone[3] containing 16 μg/ml in ether were freshly prepared daily and diluted to the required range (1–16 μg/ml). Stock solutions of 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane[4] (Standard I), containing 50 μg/ml in *n*-heptane, and 5-(4-hydroxyphenyl)-5-phenylhydantoin[5] (Standard II), containing 22.2 μg/ml in 0.01 *N* NaOH, were prepared. A solution of Standard I was prepared daily and used as such. A solution of Standard II was prepared every week and diluted to 1.11 μg/ml with 0.01 *N* NaOH daily before use. A 0.2 *M* phosphate buffer (pH 11.2 ± 0.2) and 1 *N* and 2 *N* HCl were employed. A 0.2 *M* solution of trimethylanilinium hydroxide in methanol was synthesized according to the method of Barret (11).

Glassware—All glassware was silanized before use by soaking for 1 hr in 1% hexamethyldisilazane in ether and rinsing with ether, methanol, and finally water (distilled in glass) before oven drying. Evaporation tubes with narrow bore bases (12) were custom made from Teflon-lined screw-capped test tubes[5].

Plasma Level Study—Phenylbutazone was administered to two healthy male volunteers, in one case in a solution buffered at pH 7.2 [Subject 1, 30 years, 90.8 kg (200 lb)] and in the second case in 2 × 100-mg tablets[6] [Subject 2, 30 years, 95.3 kg (210 lb)]. Samples of blood (10 ml) were withdrawn from the cubital vein by means of heparinized containers[7] at 14 appropriate time intervals after dosing. The blood samples were centrifuged and the plasma was transferred before storing at −10°.

General Procedure—*Extraction of Phenylbutazone*—To 1-ml plasma samples (spiked or from dosed volunteers) in Teflon-lined screw-capped centrifuge tubes (20 ml) are added 1 ml of Standard

[1] Diethyl ether (anhydrous), Mallinckrodt Chemical Works Ltd., Montreal, Quebec, Canada.
[2] Caledon Laboratories Ltd., Georgetown, Ontario, Canada.
[3] Ciba-Geigy, Canada.
[4] Aldrich Chemical Co., Milwaukee, WI 53233.
[5] Canadian Laboratory Supplies Ltd., Montreal, Quebec, Canada.
[6] Butazolidin, Geigy Pharmaceuticals, Montreal, Quebec, Canada.
[7] Vacutainers, Becton Dickinson & Co., Mississauga, Ontario, Canada.