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Effect of Particle Size on the Bioavailability of Digoxin

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Summary. The bioavailability of digoxin in three tablets prepared from materials with different particle sizes was measured in healthy volunteers in a cross-over study using an alcoholic solution of digoxin as a reference standard. Its bioavailability in tablets with particle sizes of 7 μ or 13 μ was 78-97% of that of digoxin in solution. The tablet with largest particle size (102 μ) showed markedly lower bioavailability than the reference solution, namely 39%. It is obvious that particle size is an important determinant of the dissolution rate and bioavailability of digoxin.

Key words. Particle size, bioavailability, digoxin.

Major differences have recently been demonstrated in the bioavailability of different digoxin products (1,2,3,4,5,6). Since the dose-response curve of digoxin is rather steep and the range between the therapeutic and toxic levels narrow, differencies in bioavailability constitute a potential risk factor in the treatment of patients.

The mechanisms underlying the differences in the bioavailability of various digoxin products are poorly understood. Factors concerned with the dosage-form of the tablet preparations undoubtedly play an important role. The rate at which digoxin preparations dissolve in gastrointestinal fluids is also critical for the absorption of digoxin. This is supported by numerous reports demonstrating the close correlation between dissolution rate of digoxin tablets in vitro and parameters of bioavailability in vivo (6,7,8,9). The dissolution rate of the tablet form of a drug is known to be influenced by several factors, such as particle size, physical state of the drug and composition and amount of inert agents employed in tablet manufacture (10). As far as digoxin is concerned, surprisingly few studies have been reported on the effects of these factors on bioavailability (11, 12,13). The present paper demonstrates the importance of the particle size of the basic material on the bioavailability of digoxin from tablets.

Material and Methods

Drugs

Three varieties of digoxin tablets, A, B and C, were made from commercially available materials. They fulfilled the requirements of USP (23a) as well as of the BP (23b). The material was either sieved (A and B) or micronized by fluid energy mill (C). The subsequent particle sizes were measured microscopically and by use of a HIAC counter, and the mean particle surface diameter was estimated by a method described elsewhere (14). The mean diameters of the particles in A, B and C are recorded in Table 1.

Direct compression with starch was used to make tablets containing 0.25 mg of each of the three forms of digoxin. The strengths of the tablets were tested with a Pfizer tester, and the disintegration times were measured according to USP XVIII (Table 1). All batches met the USP XVIII requirements for uniformity of content.

The dissolution rate of the tablets was measured

Table 1. Particle size, disintegration time and dissolution rate of three varieties of digoxin tablets

Tablet	Particle size, mean diameter μ (90% confidence limits)	Disintegration time, min	Dissolution rate, percentage in solution at 15 min	30 min	60 min
A	13 (5 - 30)	5.5	58	59	71
B	102 (80 - 150)	4.5	12	18	18
C	7 (3 - 10)	5.5	49	55	62

as described in USP XVIII. Six tablets were placed in 600 ml of water in the basket, and the speed of rotation was 120 ± 5 r.p.m. 10 ml of solution was withdrawn after 15, 30 and 60 min. The digoxin concentration in the solutions was measured fluorometrically, as described in USP XVIII; the results are shown in Table 1.

A solution of digoxin (product D), containing 0.25 mg digoxin, 0.4 g propylene glycol and 0.1 g ethanol per ml in aqueous solution served as the reference standard in the tests of bioavailability.

Subjects

Seven healthy males, 24 to 38 years of age, weighing 65 - 78 kg, participated in the study. Before the study a history, physical examination and laboratory tests (complete blood count, urinalysis, serum creatinine and electrocardiogram) were obtained. The results of the examinations were within normal limits for all the volunteers.

Administration of the Drugs

The absorption studies were done in the steady state situation. A cross-over design was used. Each subject received daily one 0.25 mg tablet of digoxin product A, B or C, or the same oral dose of digoxin solution D. The drug was administered with 100 ml of water at 8.30 a.m. after an overnight fast. The drugs were taken in random order for two weeks. During the absorption study, the fast was continued for the first five hours after administration of the drug.

Sampling

Venous blood samples were collected on the 13th and 14th day at 8.30 a.m., just before administration of the drug. In addition, blood samples were taken on the 14th day 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10 and 24 hours after dosing. The mean steady state 24 hour level was calculated from the three 24 hour values so obtained. Urine was collected on the 13th and 14th days for 24 hour periods. Serum was separated by centrifugation and the serum and aliquots of each urine collection kept frozen until analyzed.

Analysis of Digoxin

Serum levels of digoxin were estimated by the radioimmunoassay method described by Smith and co-workers (15). To determine the digoxin concentration in urine, the urine was diluted with appropriate amounts of digoxin-free human serum and analysed as above.

Analysis of Results

The area under the serum digoxin concentration-time curve (AUC) during the 24 hrs following the single daily dose of digoxin after two weeks of administration of the drug was calculated by the trapezoidal rule. The AUC's, the steady state 24 hour digoxin serum levels and peak serum levels,

Table 2. Statistical analysis of areas under the digoxin serum concentration-time curves (AUC) after administration of three preparations of digoxin with different particle sizes

Preparation[a]	AUC[c] (ng.hr.ml^{-1}), mean ± SE)	% of D
A	16.52 ± 1.51	96
B	6.49 ± 0.41	37
C	13.50 ± 1.58	78
D (alcoholic solution)	17.30 ± 1.41	100

Analysis of variance:

Source of variance	df	ss	ms	F
Between individuals	6	158.5	26.4	3.59
Between products	13	509.4	169.8	23.05
Error	18	132.6	7.4	
Total	27	800.5		

SE of varietal mean=1.025, $F_{0.05}(6;18)$= 2.66, $F_{0.05}(3;18)$=3.16

Multiple range analysis:

p		(2)	(3)	(4)
Shortest significant ranges		3.04	3.20	3.29
Product	B	C	A	D
AUC[b]	6.49	13.50	16.52	17.30

D-B=10.81>3.29; significant

D-C= 3.80>3.20; significant

D-A= 0.78<3.04; not significant

A-B=10.04>3.20; significant

A-C= 3.02<3.04; not significant

C-B= 7.01>3.04; significant

[a]Mean particle size: A = 13 μ, B = 102 μ, C = 7 μ, D = solution

[b]Any of two or more means not underscored by the same line are significantly different ($p<0.05$)

[c]AUC during 24 hrs after administration of the single daily dose of digoxin (0.25 mg) following two weeks of treatment with the drug

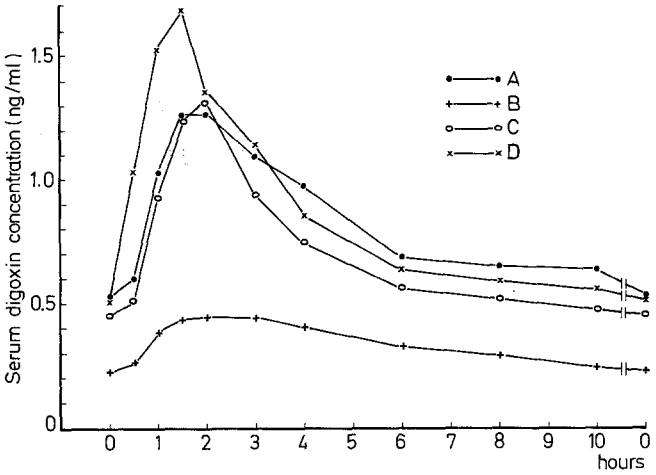

Fig. 1. Steady state serum digoxin concentration after administration of 0.25 mg of four varieties of digoxin products; mean values from 7 subjects.

Table 3. Peak serum concentration of digoxin, the time to reach the peak concentration, and the steady state serum concentration after administration of three preparations of digoxin with different particle sizes

Preparation[a]	Peak Concentration (ng/ml)	Peak Time[b] (hr)	Steady State Plasma Concentration[b] (24 hr after the dose, ng/ml)
A	1.66 ± 0.12[c]	1.64 ± 0.28	0.52 ± 0.04
B	0.51 ± 0.04	3.21 ± 0.55[d]	0.22 ± 0.01[d]
C	1.43 ± 0.24	1.79 ± 0.10	0.45 ± 0.16
C	2.19 ± 0.22	1.14 ± 0.24	0.51 ± 0.05

[a] mean particle size: A = 13 μ, B = 102 μ, C = 7 μ, D = solution

[b] values are mean ± SE (n = 7)

[c] all the means significantly different, except A and D ($p<0.05$)

[d] significantly different from A, C and D ($p<0.05$)

the time taken to reach the peak level and the urine data were analyzed by two-way analysis of variance. Whenever significant differences ($p<0.05$) between products were found, Duncan's multiple range test was applied to find the source of the differences (16). Simple linear regressions were calculated between different parameters of bioavailability to reveal statistically significant correlations.

Results

Serum Data

The steady state serum digoxin concentrations after administration of 0.25 mg of the four varieties of digoxin products are shown in Fig. 1. Table 2 shows the corresponding areas under the serum concentration-time curves (AUC). The largest area was obtained with D, the solution of digoxin. The AUC following ingestion of tablet A was almost the same (96% of D), whereas AUC of tablet C was significantly smaller (78% of D) than the AUC of D. By far the smallest area was seen after tablet B (37% of D).

The peak serum concentration of digoxin was highest (mean 2.2 ng/ml) and the time to reach it (1.1 hr) shortest after administration of the solution D (Table 3). Tablet A did not significantly differ from the solution in these parameters (1.7 ng/ml and 1.6 hr, respectively). Tablet C gave a significantly lower peak concentration (1.4 ng/ml) than D or A. The peak concentration following administration of tablet B was only about one quarter (0.5 ng/ml) of the peak after D, and it appeared much later (at 3.2 hr) than the peaks of the other products.

The steady state serum concentration, measured 24 hours after the last dose, was virtually the same after D and A; it was slightly lower after C, and significantly lower after administration of tablet B (Table 3).

Urine Data

The excretion of digoxin in urine during 24 hours at steady state is presented in Table 4 as the mean of two 24 hour excretions. Again, the mean amount of digoxin excreted during administration of solution D was largest (139 μg). Almost as much digoxin (134 μg; 97% of D) was excreted during administration of tablet A. Excretion on treatment with tablet C was slightly (121 μg; 88% of D) but significantly lower, and that during B significantly (95 μg; 42% of D) so than the excretion of digoxin after administration of solution D.

Fig. 2 shows the correlation between the excretion of digoxin in urine and the corresponding area under the serum concentration-time curve. The value of $r = 0.802$, was highly significant ($p<0.001$). A statistically significant correlation was also found between the amount of digoxin excreted in urine and the peak digoxin serum level ($r = 0.730$, $p < 0.001$), as well as the steady state 24 h digoxin serum levels ($r = 0.610$, $p<0.001$). A significant negative correlation was observed between the amount of digoxin excreted in urine

Table 4. Excretion of digoxin in urine during 24 hours after administration of four preparations of digoxin with different particle sizes

Preparation[a]	Digoxin in Urine (µg/24 hr, mean ± SE)	% of D
A	134.4 ± 8.9	97
B	58.6 ± 6.2	42
C	121.1 ± 8.6	88
D	139.1 ± 10.7	100

Analysis of variance:

Source of variance	df	ss	ms	F
Between individuals	6	11016	1836	19.82
Between products	3	29196	9734	83.86
Error	18	2088	116	
Total	27	42302		

SE of varietal mean=4.071, $F_{0.05}(6;18)$= 2.66, $F_{0.05}(3;18)$= 3.16

Multiple range analysis:

p		(2)	(3)	(4)
Shortest significant ranges		12.1	12.7	13.1
Product	B	C	A	D
Mean amount excreted in urine[b] (µg)	58.6	121.1	134.4	139.1

D-B=80.6 > 13.1; significant

D-C=18.0 > 12.7; significant

D-A= 4.7 < 12.1; not significant

A-B=75.8 > 12.7; significant

A-C=13.3 > 12.1; significant

C-B=62.6 > 12.1; significant

[a]Mean particle size: A = 13 µ, B = 102 µ, C = 7 µ, D = solution

[b]Any two or more means not underscored by the same line are significantly different ($p<0.05$).

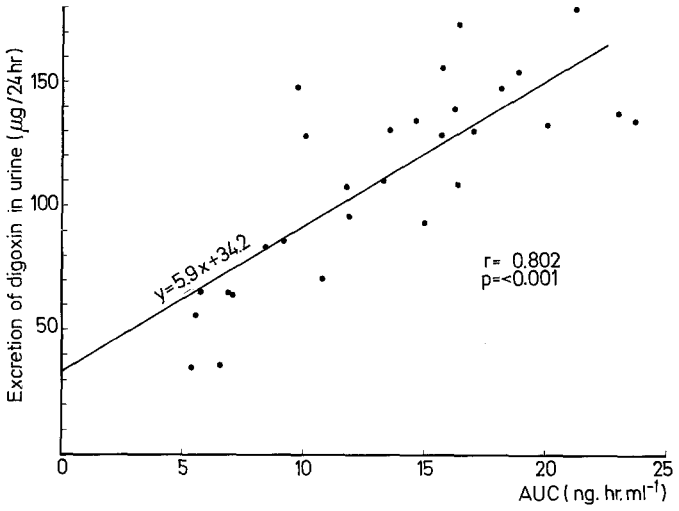

Fig. 2. Correlation between the area under serum digoxin concentration-time curve (AUC) and the excretion of digoxin in urine. AUC was calculated during 24 hr after administration of the single daily dose of digoxin.

and the peak serum level time ($r = -0.643$, $p<0.001$), and between the peak serum level and the time of the peak level ($r = -0.626$, $p<0.001$).

Discussion

Bioavailability studies can be performed as single dose studies or after multiple doses in the steady-state situation. For drugs like digoxin, which is usually given daily on a long-term basis and has a rather long elimination half-life, the latter method offers several advantages over single dose studies. To measure the total amount of drug absorbed after a single dose, blood levels should be followed for about 4-5 half-lives, or the cumulative excretion in urine measured for the same period. The short periods of time for which blood levels have been monitored in many bioavailability studies may reflect only the rate of absorption and not the total quantity of drug absorbed, as stressed by Sorby and Tozer (17). Also, the marked day-to-day variation in absorption of digoxin (18) may make erroneous results more likely after single doses than in steady state studies. On the other hand, the latter method is cumbersome for widespread bioavailability tests, and the excretion of digoxin in urine over one to six days after administration of a single oral dose has been reported to be a reliable parameter in this connection (19).

The two week period of drug administration used in the present study was long enough for the steady state condition to have been reached, in view of the 1.6 day plasma half-life of digoxin.

Multiple parameters of bioavailability were measured. The peak plasma levels and the time to reach the peak indicate the rate of absorption. The steady state serum levels, the AUC during the dosing interval, as well as the excretion of unchanged drug in urine reflect the total amount of digoxin absorbed. The combined use of all these methods has given a reliable picture of the bioavailability of the product (20).

An alcoholic solution of digoxin is essentially completely absorbed from the gastrointestinal tract (21,22). Therefore, this form of digoxin can be used as an oral reference standard in bioavailability tests. In the present study, 56% of the daily dose was recovered in urine as unchanged digoxin following administration of digoxin in solution. This agreed closely with the results of Huffman and Azarnoff (21).

The present results suggest that the particle size of the basic compound is an important determinant of the bioavailability of digoxin. The poor availability of digoxin from tablet B, made of the coarsest material, was strikingly apparent by any of the measures of bioavailability employed. Both the rate of absorption and the amount of digoxin absorbed from this product were significantly smaller than after administration of other products. To our knowledge, basic material (B) of this particle size has been used for manufacture of digoxin tablets.

Micronization of the raw material to yield very small particles improved the bioavailability, as shown by the results obtained with tablets A and C. In fact, after administration of tablet A, the serum concentrations and excretion of digoxin in urine proved to be almost the same as after administration of solution D; this result was in agreement with that of Steiness *et al.*, who found little if any difference between the bioavailability of "high potency" tablets with a fast dissolution rate and digoxin in alcoholic solution (22).

The amount of digoxin available from tablet C in the present study was slightly smaller than the amount available from tablet A, although the particle size of the raw material of C was the smallest of the three forms employed. It should be noted that the method used for preparation of product C differed from that of A and B. It is possible that loss of the edges of particles during micronization to yield material C has reduced the total surface of the particles, which is known to influence the dissolution rate of many drugs. This assumption was in agreement with the slightly slower dissolution rate of tablet C than tablet A.

There is a limit for particle size beyond which it is impossible to improve bioavailability. The present results indicate that this limit may be somewhere between 13 and 102 μ.

The particle size of raw material to be used in drug formulation has been shown to be an important determinant of the dissolution rate and bioavailability of several drugs. When the particle size is made smaller, the surface area of the drug which comes into contact with solvent is increased, and this leads to a faster dissolution rate; examples of such drugs are griseofulvin, sulphadiazine and chloramphenicol (10). In the light of the present study it is apparent that this is true, for digoxin, too. Using different methods to test bioavailability, Shaw and coworkers (12) have presented similar data that are confirmed by the present results, and which were consistent with our previous findings (11).

In order to obtain digoxin products with the best possible bioavailability, control of the particle size of the digoxin seems to be one critical factor.

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