Instability of Digoxin in Acid Medium Using a Nonisotopic Method

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Received March 12, 1977, from *the Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.* Accepted for publication January 30, 1978.

Abstract □ A selective nonisotopic assay was used to investigate the digoxin hydrolysis rates at 37 ± 0.1° over the pH 1.1–2.2 range. The colorimetric method adopted is based on the use of a xanthydrol reagent after extraction with chloroform. The spectrofluorometric method specified in the dissolution test for digoxin tablets was nonspecific because of digoxigenin interference. Digoxin hydrolysis followed specific acid hydrolysis, and K values of the apparent first-order reaction varied from 0.0357 to 0.0027 min^{-1} over the pH range used. The effect of the dissolution medium on digoxin stability during the dissolution tests of the tablets also was studied. Water (the BP medium) and 0.6% HCl (the USP medium) were compared using the fluorometric method and the xanthydrol method. In the USP medium (pH 1.3), no hydrolysis was revealed by the fluorometric estimation whereas the xanthydrol method showed about 74% hydrolysis. In water, the two methods revealed no hydrolysis. The extent of hydrolysis after 1 hr in the USP medium was studied using three brands of digoxin tablets of differing dissolution characteristics. The fast dissolving brand showed relatively more hydrolysis than the slow dissolving tablets.

Keyphrases □ Digoxin—hydrolysis at gastric pH, colorimetric analysis □ Hydrolysis—digoxin at gastric pH, colorimetric analysis □ Colorimetry—analysis, digoxin, using xanthydrol reagent, aqueous acidic solutions □ Stability—digoxin at gastric pH, colorimetric analysis □ Cardiotonic agents—digoxin, hydrolysis at gastric pH, colorimetric analysis

The acid hydrolysis of digitalis glycosides was the subject of many studies. As early as 1897, it was observed that a decrease in the efficacy of the glycosides occurred after prior incubation with acidic gastric juices (1, 2). Later studies confirmed the *in vitro* hydrolysis of the glycosides in acid media, as determined by TLC separation followed by colorimetric estimation or by an isotopic technique (3–5).

Kuhlmann *et al.* (6), using ^3H-digoxin, found that the glycoside was rapidly hydrolyzed in the pH 1–2 range; at higher pH values, digoxin was stable. The present work examined digoxin hydrolysis rates at 37 ± 0.1° within the pH 1.1–2.2 range by a specific nonisotopic colorimetric method. The effect of the BP and USP dissolution media (specified for dissolution of digoxin tablets) on digoxin stability also was studied using three brands of digoxin tablets of differing dissolution properties.

EXPERIMENTAL

Materials—Digoxin powder BP[1], digoxigenin[2], and digitoxose[3] were used as received. Buffer solutions[4] (pH 1.1–2.2 ± 0.05, as measured by a digital pH meter[5]) and an acid pepsin solution (BPC, 1968) were used. For the xanthydrol reagent, 0.1 ml of a 10% solution of xanthydrol in methanol[6] was mixed with 1.0 ml of hydrochloric acid and diluted with acetic acid to 100 ml.

Three brands of digoxin tablets were used in dissolution studies. Brand A was a fast dissolving formulation prepared by coprecipitation of digoxin with polyethylene glycol 6000 in a ratio of 1:50 from an ethanol–chloroform mixture (1:2 v/v). Brands B[7] and C[8] were commercial lots.

Table I—Results of Replicate Assays of Synthetic Mixtures of Digoxin, Digitoxose, and Digoxigenin by the Fluorometric Method (1) and Modified Xanthydrol Method (2)

Composition, mg %			Hydrolysis, %	Digoxin Found, mg %[a]	
Digoxin	Digitoxose	Digoxigenin		1	2
1.00	0.000	0.000	0	1.001	1.020
0.90	0.057	0.050	10	1.007	0.889
0,75	0.142	0.125	25	1.013	0.760
0.50	0.285	0.250	50	0.997	0.493
0.00	0.570	0.500	100	1.005	0.000

[a] Average of four replicates (±1.8%).

Analyses of Synthetic Mixtures of Digoxin, Digoxigenin, and Digitoxose—To check the suitability of the method for stability studies, synthetic mixtures containing digoxin, digoxigenin, and digitoxose, in proportions corresponding to varying percentages of hydrolysis, were analyzed. Two methods were used:

Spectrofluorometric Determinations (7)—The procedure adopted was essentially as described in the USP for the determination of digoxin during the dissolution test of the tablets (8). Fluorescence was measured with a spectrofluorometer[9].

Modified Xanthydrol Method (9)—The sample to be assayed (10–50 μg) was diluted to 15 ml with water and extracted with chloroform (3 × 10 ml). Each chloroform extract was washed with the same 5-ml aliquot of water, and the combined chloroform extract was evaporated. Xanthydrol reagent (5 ml) was added, and the tube was placed in a boiling water bath for 3 min and cooled in an ice bath for 5 min. After cooling, the color intensity was measured after 20 min at 530 nm[10] against a blank. A standard was assayed simultaneously.

Stability Studies—These studies were carried out at 37 ± 0.1° using digoxin solutions of initial concentrations of 0.5–2.0 mg %. The media used were buffer solutions (pH 1.1–2.2), 0.1 N HCl (pH 1.1), 0.6% HCl (pH 1.3, the USP dissolution medium for digoxin tablets), and an acid pepsin solution (pH 1.3) of the BPC 1968. At specified time intervals, aliquots were assayed by the modified xanthydrol method. To stop the acid hydrolysis during the procedure, the sample withdrawn was added to water containing a few drops of ammonia TS before extraction with chloroform as already described.

Dissolution Test—The dissolution test of three brands of digoxin tablets was performed using the rotating-basket dissolution apparatus[11] of USP XIX (8). Following either the USP or BP (addendum 1975) methods, the percentages of digoxin remaining in solution after 1 hr were determined. In addition to the fluorometric method specified in both monographs, the digoxin content also was determined by the modified xanthydrol method.

To determine the dissolution rate in both the USP and BP media (0.6% HCl and water, respectively), six tablets were used; the volume of the dissolution medium was 600 ml. Aliquots were withdrawn at 15, 30, 45, and 60 min, filtered through a membrane filter[12] (0.2-μm mean pore diameter), and assayed by both the fluorometric and modified xanthydrol methods. Fresh aliquots of the dissolution medium were added at each sampling time to maintain a constant volume.

[1] Burroughs Wellcome, Kent, England.
[2] Control No. 90106, Boehringer Mannheim GMBH, West Germany.
[3] L. Light & Co., Colnbrook, England.
[4] Clark-Lub (potassium chloride–hydrochloric acid) buffer solutions.
[5] Model pH N75, Tacussel Electronique, France.
[6] E. Merck, Darmstadt, West Germany.
[7] Lanoxin tablets, batch No. 2042 X, Burroughs Wellcome, Kent, England.
[8] Digoxin tablets, batch No. 58, The Alexandria Co. for Pharmaceuticals and Chemical Industries, Alexandria, Egypt.
[9] Perkin-Elmer model 204 fluorescence spectrophotometer.
[10] Spekol spectrocolorimeter, Carl-Zeiss, Germany.
[11] Erweka Apparatebau GMBH, D-6056 Heusenstamm, West Germany.
[12] Sartorius membrane filter GMBH, 34 Gottingen, West Germany.

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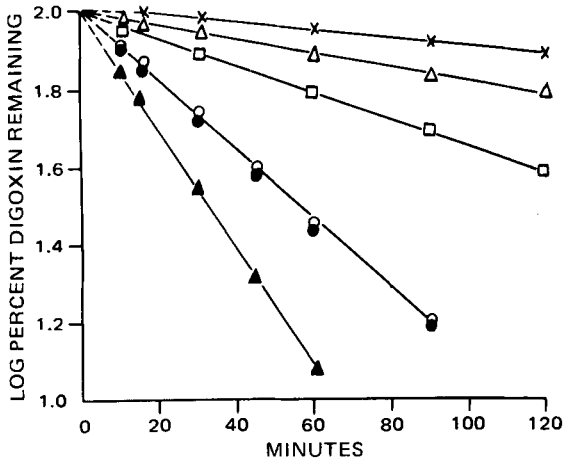

Figure 1—*First-order plot of digoxin stability at 37° in the following media: ▲, 0.1 N HCl, pH 1.1; ○, 0.6% HCl, pH 1.3; ●, acid pepsin solution, pH 1.3; □, buffer, pH 1.7; △, buffer, pH 2.0; and ×, buffer, pH 2.2.*

All three brands tested complied with the USP requirement for content uniformity.

RESULTS AND DISCUSSION

Method Selectivity—Of the nonisotopic assays for digoxin, the fluorometric method is the most sensitive. However, as with some colorimetric methods for digoxin (10), it lacks specificity. Wells *et al.* (7) reported that the sugar digitoxose did not influence the spectrofluorometric analysis of digoxin and digitoxin. They compared the fluorescence of digoxigenin and digitoxigenin with that of the parent glycosides and found identical spectra and equivalent fluorescence intensity when calculated on a molar basis.

The results obtained in the present work (Table I) confirm the findings of Wells *et al.* (7). The fluorometric method did not differentiate between the intact glycoside and its hydrolytic product digoxigenin in the synthetic mixtures tested. Irrespective of the extent of hydrolysis in the mixtures, the "apparent" digoxin contents recovered were almost the same. Even in the synthetic mixture containing no digoxin (corresponding to 100% hydrolysis), the percentage of apparent digoxin found was the same as in the unhydrolyzed mixture (Table I).

The modified xanthydrol method proved to be more selective; the percentages of digoxin recovered were in good agreement with the digoxin contents in the mixtures. Since the xanthydrol reagent reacts with the digitoxose moiety, any "free" digitoxose from hydrolysis should be separated from the intact digoxin to avoid interference. This separation was accomplished in the extraction step with chloroform, and any free digitoxose was retained in the aqueous phase. The modified xanthydrol method, although less sensitive than the fluorometric and isotopic methods, requires no TLC separation. It is simpler and less time consuming.

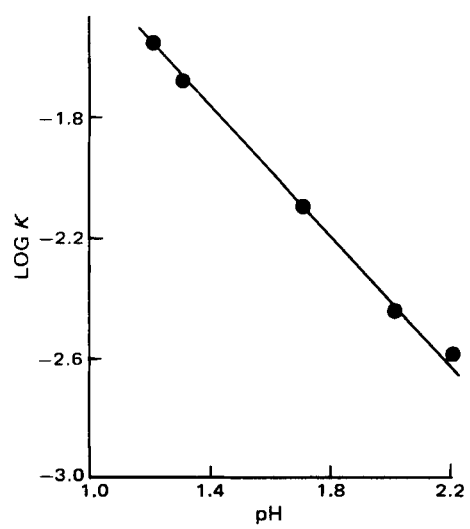

Figure 2—*Log K-pH plot for digoxin at 37°.*

Table II—Values of the Rate Constant (K) and Half-Life ($t_{1/2}$) of Digoxin Hydrolysis in Various Media at 37 ± 0.1°

Medium	K, min^{-1}	$t_{1/2}$, min
0.1 *N* HCl (pH 1.1)	0.0357	19.4
0.6% HCl (pH 1.3)[a]	0.0216	32.1
Acid pepsin solution (pH 1.3)[b]	0.0205	33.8
Buffer (pH 1.3)	0.0209	33.2
Buffer (pH 1.7)	0.0080	86.6
Buffer (pH 2.0)	0.0035	198.0
Buffer (pH 2.2)	0.0027	256.7

[a] The USP dissolution medium for digoxin tablets. [b] BPC (1968).

Table III—Results of Dissolution Testing of Three Brands of Digoxin Tablets in the BP Medium (Water) and USP Medium (0.6% HCl) Using the Modified Xanthydrol Method[a]

Brand	$t_{90\%}$ in Water, min	Digoxin Recovered after 1 hr, % In Water	In 0.6% HCl
A	13.8	101.4	24.9
B	30.0	96.8	26.2
C	45.5	92.6	38.5

[a] Average of three runs.

Stability Testing—Figure 1 shows the apparent first-order plots for digoxin hydrolysis within the pH 1.1–2.2 range. The calculated rate constant, K, and the half-lives, $t_{1/2}$, are shown in Table II. The results of stability testing did not significantly differ with the change in the initial digoxin concentration over the 0.5–2.0 mg % range. Within the pH range studied, digoxin was significantly hydrolyzed; the hydrolysis rate was pH dependent. The calculated half-lives ranged from 19.4 min to about 4.3 hr.

The presence of pepsin in the acid pepsin solution (pH 1.3) had an insignificant effect on the hydrolysis rate. At pH 1.3, the rate constant was 0.0216 min^{-1} in the buffer compared to 0.0205 min^{-1} in the acid pepsin solution (Table II). A plot of log K *versus* pH gave a straight line (Fig. 2) with a slope −1.01 (least-squares method), suggesting a specific acid catalysis.

The present results are in fair agreement with the findings of Kuhlmann *et al.* (6) who studied ^{3}H-digoxin hydrolysis at 37° by TLC followed by an isotopic technique. Their reported half-life in 0.1 *N* HCl was about 13 min, compared with 19.4 min in the present study. They also reported 18% hydrolysis after 30 min at pH 2.0, compared with 14.6% found in the present work.

Dissolution Studies—The results are shown in Fig. 3 and Table III. With the fluorometric method, almost identical dissolution plots were obtained for the same brand whether the medium was water or 0.6% HCl (Fig. 3*a*). Since significant hydrolysis was found in 0.6% HCl (Fig. 1) by the modified xanthydrol method, it follows that the fluorometric method failed to reveal the hydrolysis during dissolution testing.

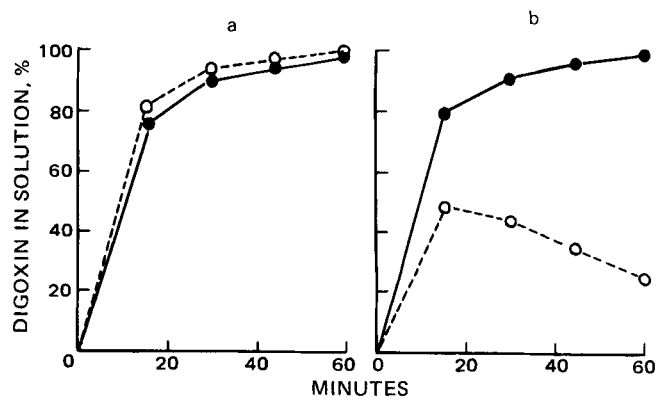

Figure 3—*Results of dissolution rate experiments of digoxin tablets, Brand B, using the spectrofluorometric method (a) and the modified xanthydrol method (b). Dissolution media used were water (●) and 0.6% HCl (○).*

Because of the nonselectivity of the fluorometric method, previously reported dissolution profiles in water and acidic media (11, 12) were almost identical. In the present work, however, this was not the case when the modified xanthydrol method was used. As shown in Fig. 3*b*, the percentage of digoxin in solution was significantly reduced in the USP medium. After 1 hr, only about 26% of the labeled digoxin remained. This result is in agreement with the value obtained from the stability studies at pH 1.3 where 27.5% hydrolysis occurred after 1 hr (Fig. 1). In water (the BP medium), no apparent hydrolysis took place.

Table III compared the amounts of digoxin recovered after 1 hr of dissolution of the tablets following the BP and USP monographs. The brands of digoxin tablets used had differing release profiles since their dissolution plots in water and the calculated $t_{90\%}$ (Table III) varied significantly. The extent of hydrolysis in the USP medium was dependent on the brand used; the fast dissolving Brand A showed more hydrolysis than Brand C (Table III).

Although the type of adjuvants used in the tablet formulations might have an effect, it is believed that the digoxin release rate from the tablets is the rate-determining factor in controlling the *in vitro* hydrolysis of the released digoxin. From the foregoing, it is concluded that digoxin undergoes rapid hydrolysis in acid solutions. As was shown by TLC separation (6), the main hydrolysis product is digoxigenin. Since the latter possesses only about one-tenth the cardioactivity of the parent glycoside (13), it follows that some loss of therapeutic efficacy may result if digoxin is exposed for a sufficient time to the acidic pH of the gastric juice. Normally, gastric pH may reach 1–2 (14, 15); but in hyperacidic patients as in Zollinger–Ellison syndrome (16), gastric pH may fall below 1. Under such conditions, some hydrolysis may occur.

Clark and Kalman (17) found 32 and 40% digoxin breakdown products in the urine of two patients but only 0–1% in four others. This result may be attributed to variations in the gastric pH since a decrease in the pH from 2.2 to 1.1 produced about a 13-fold increase in the value of K (Table II). Some reports indicated significant differences in serum digoxin levels between volunteers (18, 19). Since the gastric pH values were not given, the observed differences could be partly due to variations in the gastric pH of the volunteers.

REFERENCES

(1) P. Deucher, *Dtsch. Arch. Klin. Med.*, **58**, 47 (1897).

(2) C. L. Lhota, *Arch. Int. Pharmacodyn. Ther.*, **23**, 307 (1913).

(3) F. Svec, *Arch. Exp. Pathol. Pharmakol.*, **185**, 57 (1937).

(4) K. Kasahara and A. Ruiz-Torres, *Klin. Wochenschr.*, **47**, 1109 (1969).

(5) M. H. Gault, J. D. Charles, D. L. Sugden, and D. C. Kepkay, *J. Pharm. Pharmacol.*, **29**, 27 (1977).

(6) J. Kuhlmann, U. Abshagen, and N. Rietbrock, *Arch. Pharmacol.*, **276**, 149 (1973).

(7) D. Wells, B. Katzung, and F. H. Meyers, *J. Pharm. Pharmacol.*, **13**, 389 (1961).

(8) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 149.

(9) K. Jensen, *Arch. Pharm. Chem. Sci. Ed.*, **1**, 55 (1973).

(10) S. El-Masry and S. A. H. Khalil, *J. Pharm. Pharmacol.*, **27**, 35 P (1975).

(11) A. C. Caws, D. J. Jenkins, and J. A. McCrerie, *Postgrad. Med. J., Suppl. 6*, **50**, 37 (1974).

(12) D. A. Cowan and A. H. Beckett, *ibid.*, **50**, 52 (1974).

(13) G. Kroneberg, *Arch. Exp. Pathol. Pharmakol.*, **237**, 222 (1959).

(14) M. H. Sleisenger and J. S. Fordtran, "Gastrointestinal Disease—Pathophysiology–Diagnosis–Management," Saunders, Philadelphia, Pa., 1973, p. 720.

(15) G. J. Milton-Thompson, D. J. A. Williams, and J. J. Misiwicz, *Lancet*, **1**, 693 (1974).

(16) E. H. Ellison and S. D. Wilson, *Ann. Surg.*, **160**, 512 (1964).

(17) D. R. Clark and S. M. Kalman, *Drug Metab. Dispos.*, **2**, 148 (1974).

(18) D. H. Huffman, C. V. Manion, and D. L. Azarnoff, *J. Pharm. Sci.*, **64**, 433 (1975).

(19) J. Lindenbaum, *Clin. Pharmacol. Ther.*, **17**, 296 (1975).

ACKNOWLEDGMENTS

Presented in part at the 113th British Pharmaceutical Conference, St. Andrews, Scotland, September 1976.

The authors thank Dr. Ibrahim Abdullah, Chairman of the Alexandria Co. for Pharmaceuticals and Chemical Industries, for facilities and Professor Said A. Khalil for use of the spectrofluorometer.

Plasma Propranolol Levels in Beagle Dogs after Administration of Propranolol Hemisuccinate Ester

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Received August 16, 1977, from the *Biopharmacy Section, Pharmacy Research and Development Division, Ayerst Laboratories, Inc., Rouses Point, NY 12979.* Accepted for publication January 26, 1978.

Abstract □ The hemisuccinate ester of propranolol was administered to beagle dogs to test its applicability as a potential prodrug of propranolol. Following oral administration of propranolol hemisuccinate, plasma propranolol levels were eight times higher than after an equivalent dose of propranolol hydrochloride. The hemisuccinate was absorbed rapidly, with peak plasma levels observed at 0.5–1 hr. Following intravenous dosing, the disappearance half-life of the prodrug from the plasma was 0.5 hr while the propranolol half-life was 1.7 hr. This study demonstrated the potential usefulness of the prodrug approach when a highly metabolized drug such as propranolol is protected from first-pass elimination.

Keyphrases □ Propranolol—plasma levels after administration of hemisuccinate in dogs □ Plasma levels—propranolol after administration of hemisuccinate in dogs □ Prodrugs, potential—propranolol hemisuccinate, plasma levels in dogs □ Cardiac depressants—propranolol, plasma levels after administration of hemisuccinate in dogs

Propranolol (I) is a commonly used β-adrenergic receptor blocking drug. When compared to intravenous administration, the bioavailability of an oral propranolol dose is low and varies widely from patient to patient. This variation has been attributed to extensive first-pass elimination of the drug (1).

Several metabolites of propranolol have been identified (2–8). The major metabolites are propranolol *O*-glucuronide, *p*-hydroxypropranolol and its glucuronide conjugate, and naphthoxylactic acid. One major site of propranolol metabolism is the GI tract since *p*-hydroxypropranolol is detected after oral, but not after intravenous, adminis-

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