Measurement of Plasma Digoxin Concentration by Radioimmunoassay

D. C. EVERED,* M.B., B.SC., M.R.C.P. ; C. CHAPMAN, † B.SC., PH.D. ; C. J. HAYTER, ‡ M.B., B.SC., M.R.C.P.

British Medical Journal, 1970, **3**, 427-428

Summary: A rapid, sensitive, and precise method for measuring the plasma digoxin concentration has been developed with the radioimmunoassay technique. Seventy patients receiving digoxin were shown to have plasma digoxin concentrations between 0.4 and 5 ng./ml. Preliminary studies show that though there is a positive correlation between total daily dose and the plasma digoxin concentration, the relationship is not close, and a relatively wide range of plasma digoxin concentrations appear to be consistent with effective digitalization.

Introduction

Although cardiac glycosides have been used extensively for nearly two centuries the lack of specific chemical techniques which are sensitive enough to measure the low concentrations of glycosides present in the blood has hampered studies of these compounds. Lowenstein (1965) and Lowenstein and Corrill (1966) developed an assay for cardiac glycosides based on the inhibition of ^{86}Rb uptake by red cells, and this method has recently been further refined (Grahame-Smith and Everest, 1969). A double isotope dilution derivative assay for digitoxin (Lukas and Peterson, 1966) and a radioimmunoassay for digitoxin (Oliver, Parker, Brasfield, and Parker, 1968) have recently been described. Butler and Chen (1967) successfully raised digoxin-specific antibodies and Smith, Butler, and Haber (1969) developed an immunoassay for digoxin. An immunoassay for digoxin has been developed independently in this department and preliminary studies with this technique are reported here.

Materials and Methods

Stable digoxin, pure, U.S.P., was obtained from Koch-Light Laboratories. Tritiated digoxin was obtained initially from Burroughs Wellcome, U.S.A., with an activity of 0.098 Ci/mM. A later supply was obtained from NEN Chemicals with an activity of 9 Ci/mM. Polylysine HBr (minimum MW 50,000) was obtained from Koch-Light Laboratories.

Preparation of Antigen.—Digoxin was conjugated to polylysine HBr by a modification of the periodate-oxidation technique described by Butler and Chen (1967), which yielded up to 11 digoxin residues/mole polylysine. Free digoxin was removed by prolonged dialysis against distilled water.

Immunization Procedures.—The digoxin-polylysine conjugate was suspended in complete Freund's adjuvant to give a final concentration of 5-7 mg./ml. Six New Zealand white rabbits were immunized, each animal receiving 1 ml. of the conjugate subcutaneously weekly for four weeks and thereafter fortnightly. Three rabbits were found to have an adequate antibody response after 10 weeks.

Identification of Antibody.—Sera from the successfully immunized rabbits were shown to bind ^3H -digoxin. Full details of the preparation will be published separately.

Assay Procedure.—A series of standard solutions of non-labelled digoxin (0.8 ng./ml.) were prepared to normal human plasma. One-millilitre volumes of each standard solution were prepared in triplicate and 1-ml. volumes of each unknown

plasma in duplicate. To each sample 5 ng. of tritiated digoxin in 0.1 ml. of phosphate buffer and 0.1 ml. of a suitable dilution of antiserum were added. Sufficient antiserum to bind 80-90% of the ^3H -digoxin was added. Each sample was incubated at 30°C. for 15 minutes. Dextran-coated charcoal 0.5 ml. (Herbert, Lau, Gottlieb, and Bleicher, 1965) was added to each sample, which was then centrifuged after one minute. One millilitre of the supernatant of each sample was dissolved directly in 10 ml. of a xylene-hyamine-octanol mixture (Evered, 1969) and the bound fraction was assayed in a Packard Tri-Carb Liquid Scintillation Counter. A standard curve was constructed for the solutions of known concentration and the unknowns were read.

Patients

Seventy patients who were receiving digoxin were selected at random. Sixty-seven were on regular maintenance doses of digoxin and none had gross evidence of digoxin toxicity; three were being "digitalized" and had received a total of 1-1.25 mg. of digoxin over the preceding 18 to 24 hours.

Results

Precision.—A series of standards was run with each batch of unknowns. The standard curve was shown to vary very little from batch to batch. Triplicate determinations were initially carried out on all standard samples, and the standard deviation was found to be ± 0.128 ng./ml.

Specificity.—Twelve plasma samples were taken from patients not receiving digoxin; all gave results of less than 0.2 ng./ml.

Rapidity.—It is possible to assay a single sample with one or two standards to check the standard curve and provide a result in less than 40 minutes; therefore the method is applicable in situations where an early result is required.

Clinical Studies.—Plasma digoxin concentrations ranging from 0.4 to 5 ng./ml. were found in 70 patients, with a mean of $1.63 \text{ S.D.} \pm 1.13$ ng./ml. The three subjects from whom samples were taken during digitalization had plasma digoxin concentrations of 0.6, 0.8, and 1.5 ng./ml. The remaining 67 subjects were all on maintenance doses of digoxin, and though none of them had clinical evidence of digoxin toxicity it is noteworthy that all three subjects with plasma digoxin concentrations greater than 4 ng./ml. had renal failure; if this group is excluded then the highest recorded level was 3.7 ng./ml. The results obtained in patients on digoxin are shown in the Chart, and the mean and the standard deviation at each dose level are shown in Table I. There is a positive correlation ($r = +0.306$, S.E. = 0.123) between total daily dose and plasma digoxin concentration which is just significant. The blood samples in this group were not time-related to administration of dose. There was no significant difference between males and females. Samples taken immediately before and hourly after the morning (oral) dose of digoxin in four subjects on maintenance therapy showed an increase in plasma digoxin concentration of 0.5-1 ng./ml. between two and four hours after administration of the drug. The concentration was shown to fall to the initial value between three and four hours after the peak (Table II). Much larger changes in concentration have been noted in subjects during digitalization.

*Registrar.

†Biochemist.

‡Consultant Physician.

Department of Nuclear Medicine, the General Infirmary at Leeds.

TABLE I.—Plasma Digoxin Concentrations. Mean Values and Standard Deviations at Each Dose Level

Total Daily Dose (mg.)						No. of Patients	Plasma Digoxin Concentration (ng./ml.)	
							Mean	S.D.
0.25 alternate days	..	..	..	..	..	3	0.90	—
0.25	..	..	..	..	..	17	1.14	0.91
0.5	..	..	..	..	..	37	1.86	1.23
0.75	..	..	..	..	..	10	2.03	1.02

TABLE II.—Plasma Digoxin Concentrations. Rise and Fall Following Administration of Oral Dose

Case No.	Initial Concentration (ng./ml.)	Rise (ng./ml.)	Baseline—Peak (Hours)	Peak—Baseline (Hours)
1	0.5	1.0 (200%)	2	3
2	1.0	0.5 (50%)	2	4
3	1.5	0.7 (46%)	4	4
4	3.2	0.9 (28%)	2	3

Discussion

The plasma digoxin concentrations reported here are similar to those reported (Marcus, Burkhalter, Cuccia, Pavlovich, and Kapadia, 1966) after administration of tritiated digoxin to normal subjects, in whom a mean plasma digoxin concentration of 1.4 ng./ml. was found. The lower value in normal people is readily explicable in terms of diminished renal function in many patients treated with digoxin. Reports of plasma digoxin concentrations assayed by the inhibition of ⁸⁶Rb uptake of red cells (Lowenstein and Corroll, 1966; Binnion, Morgan, Stevenson, and Fletcher, 1969; Grahame-Smith and

Everest, 1969) and by radioimmunoassay (Smith *et al.*, 1969) are similar to those reported here. The observation that the plasma concentration rises by 0.5–1 ng./ml. and falls again to the baseline value in five to eight hours after an oral dose of digoxin in subjects on maintenance therapy emphasizes that single measurements of plasma digoxin concentration, particularly if they are not time-related to the administration of the dose, are of limited value.

The relatively wide range of plasma digoxin concentrations observed cannot be attributed to experimental error. It might be argued that this range may result from variable plasma protein-binding of digoxin. This is unlikely, since the standard solutions are prepared in normal human plasma and the standard curve is identical with a theoretical curve calculated on the basis of a “one for one” molecular displacement of tritiated digoxin by stable digoxin. The plasma protein-binding of digoxin varies very little (23–33%) in the therapeutic range, and the binding constant for the plasma protein-digoxin reaction is greater than that of the antibody-digoxin reaction by a factor of more than 10⁶ litres/mole (Evered and Chapman, 1970).

The range of concentrations observed appears to be compatible with effective therapy based on clinical criteria, and this is in keeping with the wide range of myocardial: serum digoxin concentration ratios which have been reported (Doherty, Perkins, and Flanagan, 1967; Binnion *et al.*, 1969). Surawicz and Mortelmans (1969) in a review of factors affecting individual tolerance to digitalis, collected reports of altered tolerance or myocardial binding of the glycosides in relation to age, sex, body weight, serum cholesterol concentration, sympathetic activity, serum potassium, sodium, calcium, and magnesium concentrations, disease of the myocardium, anaesthesia, adrenalectomy, thyroid disease, and obstructive jaundice. Clearly the relation between plasma digoxin concentration and recognized digitalis toxicity (which is based on clinical and electrocardiographic criteria) is unlikely to be a simple one. Further studies are necessary before the final value of the measurement of plasma digoxin concentration in the control of digoxin therapy can be assessed.

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