The Measurement of Plasma Digoxin Concentration: A Comparison of Two Methods

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Summary. No significant differences were found between plasma digoxin concentrations in samples assayed by both radioimmunoassay and "Rb uptake. The mean plasma digoxin concentration in 27 patients on a stable maintenance dose of digoxin was 1.1 ± 0.6 ng/ml, while that in a group of 12 patients with clinical evidence of full or overdigitalization was 2.2 ± 0.6 ng/ml, which was significantly greater (p < 0.05).

Key words: Plasma digoxin concentration, radioimmunoassay, "Rb uptake method.

Despite many years experience in the use of digoxin, problems in the management of digitalized patients remain common. The incidence of digoxin intoxication in patients treated in a Belfast Hospital was found to be 20% (Hurwitz and Wade, 1969) and 23 to 29% in a Boston Hospital (Beller et al., 1971). Despite claims that measurement of plasma digoxin concentration is of little clinical value (Fogelman et al., 1971), most investigators believe that such measurement can be helpful (Chamberlain et al., 1971) and "that its intelligent application will lead to better patient care" (Doherty, 1972).

Two sensitive methods for the measurement of plasma digoxin concentration have been described. One method is dependent on the inhibition by cardiac glycosides of "Rb flux across the human red cell membrane in vitro (Lowenstein and Corrill, 1966; Grahame-Smith and Everest, 1969). The other method utilizes radioimmunoassay (Smith, Butler and Haber, 1969; Evered and Chapman, 1971).

A direct comparison of results obtained by these two methods has not been reported. Furthermore, estimates of plasma digoxin concentration obtained by some investigators using the Rb uptake method (Grahame-Smith and Everest, 1969) have been greater than those reported following radioimmunoassay (Smith and Haber, 1970). It is not certain whether this difference is attributable to technical factors associated with the assays or whether other causes are responsible.

The present paper describes the measurement of digoxin concentration in the same plasma samples by both the Rb uptake and radioimmunoassay methods. Results are also presented to support the value of measurements of plasma digoxin concentration in the identification of patients who may be overdigitalized.

Materials and Methods

Radioimmunoassay

The Lanoxitest Digoxin Radioimmunoassay Kit (Wellcome Reagents Limited) was used throughout this study. Details of this assay are as follows:

Production of antibody

Random-bred rabbits were immunised with a digoxin-albumin conjugate (Butler and Chen, 1967) containing 1% digoxin in Freund's complete adjuvant. Intramuscular, multi-site injections were given at four-weekly intervals, and bleedings were taken from ear veins 10—14 days after booster injections.

Assay procedure

The following reagents were added to duplicate glass test tubes (80 × 7.5 mm) and incubated for not less than 15 min at room temperature: 0.1 ml plasma obtained by routine venepuncture, or 0.1 ml standard digoxin solution (prepared as described under Rubidium assay); antidigoxin serum at a dilution which bound approximately 40% of labelled digoxin in the absence of unlabelled digoxin; 0.5 ng tritiated digoxin (digoxin 12T, specific activity 7Ci/mM (prepared at the Wellcome Research Laboratories, Beckenham, Kent, and the Radiochemical Centre, Amersham); and sufficient phosphate buffer, pH 7.4 containing 0.5 g% bovine serum albumin, to bring the incubation volume to 0.8 ml. Normal serum or plasma (0.1 ml) was added to all control tubes to compensate for the presence of test plasma in the unknowns. Following incubation, during which time competition for specific binding sites on the antibody between the radio-active and unlabelled digoxin took place, free and bound forms of digoxin were separated by the addition of 0.2 ml of albumin-
coated charcoal suspension (Norit A Charcoal, Sigma Chemicals).

After 5—10 min the tubes were centrifuged at 2,000 g for 15 min. The supernatant containing the antibody-bound digoxin was decanted into a counting vial, 10 ml of Toluene-Triton X-100 scintillator (2 : 1) added and the radioactivity measured in a Beckmann Liquid Scintillation Counter.

The assay was designed to minimise differences in quenching between samples, so that quench correction was generally not required in routine assays. Where necessary, it was carried out by means of automatic external standardisation.

The concentration of digoxin in plasma unknowns was estimated by reference to a standard curve set up on each assay occasion. The specificity of the antiserum was determined by in vitro cross-inhibition reactions with other cardiac glycosides and steroid hormones. (Crystalline digoxin, digoxigenin, digitoxin and lanatoside C obtained from Burroughs Wellcome (U.K.) and the Wellcome Research Laboratories; cortisol, corticosterone, oestrone and progesterone from Koch Light Laboratories; aldosterone from Ciba Chemicals).

86Rubidium uptake method

The method of Grahame-Smith and Everest (1969) was followed with some modifications. 86RbCl obtained from the Radiochemical Centre, Amersham, was diluted with non-radioactive RbCl to a concentration of 100 μCi in 0.5 ml; to this was added 2.0 ml of a 22 mM solution of RbCl to provide a working solution of 18.8 mM.

Freshly prepared red cells from the same subject were used in each experiment. Heparinised venous blood (ammonium heparin, 12.5 I.u/ml whole blood) was centrifuged at 2,000 g for 10 min at 4°C. The red cells were washed twice with ice-cold Krebs Ringer solution, the final pellet of cells being used immediately for the incubation procedure.

Digoxin standard solutions were prepared by dissolving 100 mg digoxin in 40 ml hot ethanol and diluting to 100 ml with saline. A stock solution of 100 ng/ml was prepared by serial dilution and stored at 4°C. Working strength standards at concentrations between 8 and 0.5 ng/ml were stored frozen for up to 4 weeks with no deterioration. For incubation, the dried plasma extract (prepared as described by Grahame-Smith and Everest) was dissolved in 0.3 ml Krebs Ringer solution and 0.3 ml packed red cells added. The suspension was incubated for one hour at 37°C with continuous gentle shaking. 30μl

Comparison of the two assay methods

Samples of plasma from patients receiving digoxin were split and digoxin concentration measured by the two methods using the same standard digoxin solutions. Digoxin concentration was also measured in samples of normal human plasma to which known amounts of digoxin had been added.

Clinical methods

Assays were performed on plasma samples obtained from 25 healthy subjects and from 25 non-digitalized hospital patients. To investigate the specificity of the radioimmunoassay in patients with high concentrations of circulating steroids, measurements were also made in plasma samples from 50 women in the third trimester of pregnancy, 12 women taking oral contraceptives, 5 patients receiving oral prednisone (15—60 mg daily) and 12 patients with hypercholesterolaemia (serum cholesterol 301—560 mg/100 ml).

Digitalized patients

Blood was collected into lithium heparin bottles (for radioimmunoassay alone) or ammonium-heparin bottles (for assay by both methods) from 39 patients who had been on a maintenance dose of digoxin for longer than one week. Eighty-two percent of plasma samples were collected three hours after the last dose of digoxin had been given. In the group of 27 non-toxic patients, 24 samples were collected at three hours, 2 at six hours and 1 at seven

1 Rohm and Haas, Trade Mark.