

Radioimmunoassay

Measurement of Serum Cardiac Glycoside Levels: Using Pharmacologic Principles to Solve Crossreactivity Problems

Thomas J. Persoon

The University of Iowa, Iowa City, Iowa

Laboratories performing analyses for serum cardiac glycosides are sometimes faced with the problem of distinguishing between digoxin and digitoxin in a specimen. The antibodies to the cardiac glycosides supplied with radioimmunoassay kits for these drugs have some measurable degree of crossreactivity. Therapeutic levels of digitoxin are approximately ten times greater than those of digoxin, and the half-lives of these drugs in serum differ by a factor of four. These facts have been combined into a series of rules which allow the technologist to distinguish between digoxin and digitoxin in a sample and provide a level of the drug that has been corrected for crossreactivity.

In 1972 Edmonds et al. (1) published data on the crossreactivity of digitoxin in the digoxin radioimmunoassay (Tables 1 and 2). They showed the slopes of digoxin-digitoxin cross reactivity plots to be linear. Kuno-Sakai et al. (2) published similar data, relating actual digoxin values in the presence of digitoxin to observed digoxin levels by a linear equation. In a later report (3) these authors noted that the value of the slope varied with the lot of antisera used, thus making their equation useful only as an approximation. However, these data can be combined with a knowledge of the pharmacokinetics of the cardiac glycosides to solve the problems created by crossreactivity or the presence of both drugs.

Materials and Methods

In our laboratory, digoxin concentrations were measured by radioimmunoassay using kits purchased from Schwarz/Mann, Inc. (Orangeburg, NY) and Kallestad Laboratories (Chaska, MN). Digitoxin concentrations were measured using the digitoxin radioimmunoassay kit purchased from Schwarz/Mann. The standards from that kit, containing digitoxin in human serum, were used in the crossreactivity studies. All kits were used according to the manufacturer's instructions.

Results

Data collected in our laboratory are similar to those published by Kuno-Sakai. Tables 3 and 4 show the measured concentrations of digoxin and digitoxin from patient sera known to contain only one drug. Figure 1 is a plot of measured digoxin levels versus actual digitoxin concentration of serum digitoxin standards. The slope and intercept of the line were determined by the linear least-squares technique.

Discussion

Figure 2 shows the chemical structures of four cardiac glycosides: digoxin, digitoxin, cedilanid, and

TABLE 1. Crossreactivity of Digitoxin in Digoxin RIA

Digitoxin added (ng/ml)	Measured level of digoxin (ng/ml)
5	0.20
10	0.34
20	0.75
30	0.90
40	1.08
50	1.29

From Edmonds et al., N Engl J Med 286: 1266, 1972.

TABLE 2. Crossreactivity of Digoxin in Digitoxin RIA

Digoxin added (ng/ml)	Measured level of digitoxin (ng/ml)
5	2.88
10	5.10
20	12.7
30	26.1
40	45.4
50	>50.0

From Edmonds et al., N Engl J Med 286: 1266, 1972.

For reprints contact: Thomas J. Persoon, Dept. of Pathology, 254 Medical Research Center, University of Iowa, Iowa City, IA 52242.

TABLE 3. Digoxin Crossreactivity in Actual Patient Specimens

Measured digoxin level (ng/ml)	Measured digitoxin level (patients on digoxin) (ng/ml)
3.9	<4.0
2.5	<4.0
0.7	<4.0
1.7	<4.0
0.9	<4.0
0.9	<4.0
1.1	<4.0

TABLE 4. Digitoxin Crossreactivity in Actual Patient Specimens

Measured digoxin level (ng/ml)	Measured digitoxin level (patients on digitoxin) (ng/ml)
0.6	5
1.6	19
1.7	24
1.8	36
1.8	47
2.0	38
2.4	33
3.2	54

deslanoside. Digitoxin differs from digoxin in the absence of a hydroxyl group in the 12 position on the steroid moiety. Digoxin, cedilanid, and deslanoside have identical steroid moieties, but the latter two have four sugars instead of three. Therapeutic digoxin levels are in the range of 1–2 ng/ml, with levels of 2.1 ng/ml or greater being potentially toxic and 10.0 ng/ml usually fatal (4). Cedilanid and deslanoside are less commonly used and therapeutic serum levels are not well established. The data in Tables 2 and 3 demonstrate that levels of digoxin less than 10 ng/ml are essentially below the limits of detection in the digitoxin radioimmunoassay, so for all practical purposes digoxin does not crossreact in the digitoxin assay. Therefore, if one gets a significant digitoxin level upon assaying a sample, it is certain that the patient has received digitoxin. If the patient also received digoxin, it is not obvious from the digitoxin result. The case where the patient has both drugs circulating is more complicated and will be reviewed later.

The knowledge of digitalis pharmacology may be used to establish a set of rules for solving the problem when the nature of the digitalis preparation prescribed is unknown. Generally, three preparations are available: digitalis leaf, pure digoxin, and pure digitoxin. Digitalis leaf preparations are generally made from *digitalis purpurea*, which has digitoxin as its major cardioactive moiety. If a patient is taking digitalis leaf it is best to contact the physician and ask for the exact

prescription the patient is taking. The *Physician's Desk Reference* (PDR) (5) can then be consulted for the composition of that prescription. Digitalis preparations made from *digitalis lanata* have digoxin as their more active component. However, digoxin is more commonly available as the pure compound in tablet, elixir, or iv injection.

Digoxin and digitoxin have vastly different pharmacokinetics. Digoxin is the faster acting of the two drugs, with onset of action 5 to 30 min after iv administration. Peak activity is reached in 90 min to 5 h, and action begins regressing after 8 to 10 h. The average half-life in a person with normal renal function is 34–38 h (6, 7), but individual variations exist. Digitoxin activity, on the other hand, begins ½ to 2 h after iv

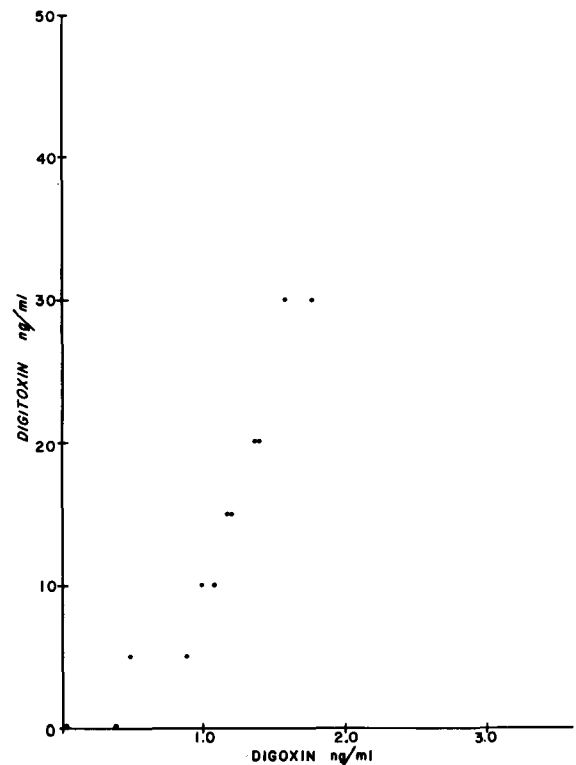


FIG. 1. Crossreactivity plot for digitoxin in radioimmunoassay for digoxin. Least-squares determination of slope gives crossreactivity factor of 0.05 (slope = 0.053). Nonzero intercept is due to experimental error.

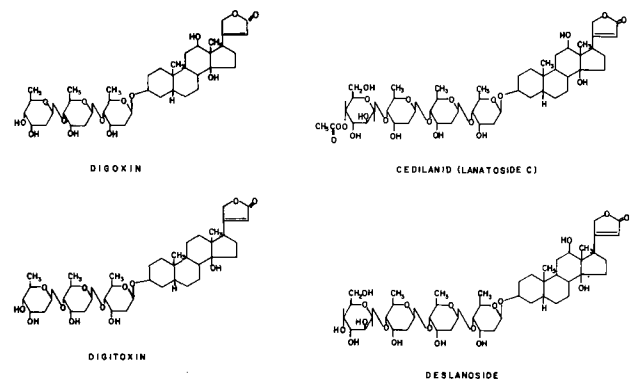


FIG. 2. Common cardiac glycosides.

dose, is maximal at 4–12 h, and begins to subside only after 48–72 h. The half-life of digitoxin in the normal individual is around 120 h (8).

Of the digoxin present in serum, 25–30% is bound to proteins, primarily albumin (9). Digitoxin, on the other hand, is 95% bound to albumin. This vast difference in protein binding is a major reason for the difference in half-lives. Digoxin is eliminated via glomerular filtration, and thus its elimination parallels creatinine elimination (10). Impaired renal function results in a given digoxin dose remaining in the circulation longer, and dose schedules must be corrected accordingly. Digitoxin is primarily removed by the liver (11), at a much slower rate.

Based on this information, the following rules can be used to determine which assay to perform in those cases where the exact digitalis preparation taken by the patient is unknown.

Rule 1: If the patient has taken an unknown digitalis leaf preparation, the sample should be assayed for digitoxin. *Rationale:* Most digitalis leaf preparations are *digitalis purpurea*, which contains digitoxin as the cardioactive component.

Rule 2: If the digitalis preparation is unknown, and renal function is normal or nearly normal, and it has been more than 108 h since the last dose, measure digitoxin first. If the digitoxin level is less than 4.0 ng/ml, confirm the absence of cardiac glycosides with a digoxin level assay. *Rationale:* After 108 h (three half-lives) only 12.5% of digoxin remains in circulation, whereas less than one half-life has elapsed for digitoxin. If toxic symptoms are observed and renal function is normal, digitoxin is more likely to be present.

When no information at all is available regarding the medication that the patient has taken, there is no choice but to do both assays. Rule 3 allows the analyst to decide which glycoside is present.

Rule 3: If both digoxin and digitoxin assays are performed on a sample from a living patient, the following criteria may be used to determine which drug is present.

1. If the digoxin value is greater than 0.4 ng/ml and the digitoxin value is *greater* than 4 ng/ml, report the digitoxin value. Check for the absence of digoxin by using rule 4.
2. If the digoxin value is greater than 0.4 ng/ml and the digitoxin value is *less* than 4 ng/ml, report the digoxin value.
3. If both values are below detection limits, report this for both tests.

Rationale: The data in Tables 1–4 provide the rationale for this rule.

Occasionally it becomes necessary for a physician to administer digoxin to a patient who has recently been taking digitoxin. The usual reason for this is that the digitalizing dose must be increased quickly. In

such cases both digoxin and digitoxin will be present, and rule 4 applies.

Rule 4: If both digoxin and digitoxin are known to be present in a sample, assay for both. The digitoxin value may be reported directly, since the levels of digoxin required to elevate the digitoxin value significantly (more than 5 ng/ml) are not possible in a living patient (Table 2). Correct the digoxin value for digitoxin interference as follows (3):

$$\text{actual digoxin} = \text{measured digoxin} - [\text{factor} \times \text{measured digitoxin}],$$

where the factor is crossreactivity. The factor should be determined for each lot of antiserum and is most easily obtained by running samples of known digitoxin concentration through the digoxin assay. The slope of the line through the concentration data (measured digoxin versus known digitoxin) is the crossreactivity factor.

Occasionally physicians request serum levels of cardiac glycosides for which radioimmunoassays are not available. These glycosides include deslanoside and cedilanid (Lanatoside C). The structures of these drugs are shown in Fig. 2. Notice that the steroid moiety in both is identical to that of digoxin, while the glycoside moieties differ. Since the antibody to digoxin used in the radioimmunoassay is usually directed toward the steroid moiety, these other glycosides might be expected to have a high degree of crossreactivity. The literature (12) confirms that crossreactivity is nearly 100%. Thus the radioimmunoassay for digoxin can be modified to assess serum levels of these other drugs by substitution of appropriate standard solutions.

Acknowledgment

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