Radioimmunoassay and Enzyme Immunoassay Compared for Determination of Digoxin

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Patients' sera were analyzed for digoxin by using two different radioimmunoassays and an enzyme immunoassay. Quantitative results obtained by enzyme immunoassay (I) were compared to results obtained on aliquots of the same sample by the radioimmunoassays (II and III). The correlation coefficients were: I vs. II 0.90, n = 108; I vs. III 0.94, n = 102; and II vs. III 0.95, n = 158. Day-to-day precision (10 days) on a low control (1.3 μg/liter) and a high control (3.0 μg/liter), expressed as coefficients of variation, were: I, 13% and 7.8%; II, 4.0% and 4.7%; and III, 8.9% and 4.2%. Ten digoxin-supplemented samples (0–8 μg/liter) were analyzed by the three methods. Correlation coefficients were: supplemented sample vs. I, 0.99; supplemented sample vs. II, 0.97; supplemented sample vs. III, 0.98.

Analytical methods for determination of digoxin in pharmaceuticals by colorimetry (I), fluorometry (II), gas-liquid chromatography (3, 4), and polarography (5) have been reported. However, methods with sufficient specificity and sensitivity for routine clinical assays of digoxin in small serum samples were not available until the radioimmunoassay for digoxin was introduced by Smith et al. in 1969 (6). Smith and Haber (7) demonstrated correlation between clinical presentation and serum digoxin concentration. In 1975, Chang et al. (8) reported an enzyme immunoassay for digoxin with derivatives of digoxin covalently bound to glucose-6-phosphate dehydrogenase from Leuconostoc mesenteroides. The objective of this study was to compare the quantitative results obtained for digoxin by this enzyme immunoassay (8) and two commercially available radioimmunoassays.

Materials and Methods

Materials

We assayed serum from 158 patients receiving digoxin therapy. Samples were assayed by the two radioimmunoassays and the enzyme immunoassay where sufficient sample remained.

Serum for precision and recovery studies was prepared by adding weighed amounts of digoxin to digoxin-free human serum.

Control sera were obtained from Ortho Diagnostics, Raritan, N.J. 08869 and Beckman Instruments, Inc., Fullerton, Calif. 92634.

Methods

The reagents for the enzyme immunoassay (EMIT®) of digoxin (I) were obtained from Syva Corp., Palo Alto, Calif. 94304, and used according to manufacturer's suggestions. Absorbance was read at 340 nm on a Beckman Model 24/25 double-beam spectrophotometer with a heated sipper cell and a Beckman Model 701 printer/calculator, Beckman Instruments, Inc., Irvine, Calif. 92713.

The first radioimmunoassay (II) used an 125I-labeled digoxin tracer, rabbit anti-digoxin antibody, and as a second precipitating antibody, goat anti-rabbit gamma globulin. A water-soluble polymer was used as a precipitation accelerator. This kit was obtained from Beckman Instruments, Inc., Fullerton, Calif. 92634.

The second radioimmunoassay (III) used an 125I-labeled digoxin tracer, sheep anti-digoxin antibody and dextran-coated charcoal as an absorbant to separate the bound and unbound digoxin. This kit was obtained from Schwarz/Mann, Orangeburg, N.Y. 10962.

Statistical Analysis

Concentrations of digoxin found by the radioimmunoassays (II and III) were compared to each other and to the concentrations found by enzyme immunoassay (I). Errors were estimated from the least-squares parameters (slope of the least-squares line, y intercept, standard error of estimates, Sxy), as recommended by Westgard and Hunt (9).

| Table 1. Statistical Comparison of Radioimmunoassay and Enzyme Immunoassay for Digoxin |
|----------------------------------|----|----|----|----|----|
| Patients' sera                   | m  | b  | Sxy| r  | n  |
| a. RIA (II) vs. EMIT (I)         | 1.00| 0.17| 0.53| 0.90| 108|
| b. RIA (III) vs. EMIT (I)        | 1.09| 0.06| 0.42| 0.94| 102|
| c. RIA (II) vs. RIA (III)        | 0.96| 0.12| 0.38| 0.95| 158|
| Digoxin-supplemented sera        |     |    |    |    |    |
| d. vs. EMIT (I)                  | 1.06| −0.21| 0.20| 0.99| 10 |
| e. vs. RIA (II)                  | 0.84| 0.21| 0.43| 0.97| 10 |
| f. vs. RIA (III)                 | 0.89| 0.05| 0.37| 0.98| 10 |

m = slope of least-squares regression line
b = intercept of least-squares regression line
Sxy = standard error of the estimate
r = correlation coefficient
n = no. samples

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Results

Patients' Sera

Radioimmunoassay (II) vs. enzyme immunoassay (I) (Table 1, line a): the slope of the line is 1.06, which indicates a proportional error less than 1.0%. Constant error is estimated at 0.17 µg/l from the intercept. Random error is estimated at 0.55 µg/l from the standard error of the estimate, S_{xy} (Figure 1).

Radioimmunoassay (III) vs. enzyme immunoassay (I) (Table 1, line b): the slope of the line is 1.09, which indicates a proportional error of 9%. Constant error is estimated at 0.06 µg/l from the intercept. Random error is estimated at 0.42 µg/l from the standard error of the estimate, S_{xy} (see Figure 2).

Radioimmunoassay (II) vs. radioimmunoassay (III) (Table 1, line c): the slope of the line is 0.96, which indicates a proportional error of 4%. Constant error is estimated at 0.12 µg/l and random error at 0.38 µg/l.

Digoxin-Supplemented Samples

Ten supplemental serum samples containing weighed amounts of pure digoxin to make up serum concentrations of 0.7, 1.3, 6.4, 4.5, 3.0, 2.5, 3.5, 2.0, 1.6, and 7.5 µg/l were assayed by methods I, II, and III. The values obtained by the assay were compared to the amount of digoxin added, by least-squares regression.

Digoxin-supplemented vs. enzyme immunoassay (I) (Table 1, line d): the slope of the line is 1.06, which indicates a proportional error of 6%. Constant error is estimated at −0.21 µg/l and random error at 0.20 µg/l.

Digoxin-supplemented vs. radioimmunoassay (II) (Table 1, line e): the slope of the line is 0.84, which indicates a proportional error of 16%. Constant error is estimated at 0.21 µg/l and random error at 0.43 µg/l.

Digoxin-supplemented vs. radioimmunoassay (III) (Table 1, line f): the slope of the line is 0.89, which indicates a proportional error of 11%. Constant error is estimated at −0.05 µg/l and random error at 0.37 µg/l.

Controls

High (3.0 µg/l) digoxin controls and low (1.3 µg/l) digoxin controls were assayed in duplicate on 10 successive days. Digoxin concentrations obtained were used to calculate standard deviation and coefficients of variation for methods I, II, and III. Table 2, lines b and d, coefficients of variation are (I) 13.0% and 7.8%; (II) 4.0% and 4.7%; (III) 8.9% and 4.2%.

<table>
<thead>
<tr>
<th>Control value</th>
<th>Av</th>
<th>SD</th>
<th>CV, %</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 0.9 ± 0.3 µg/liter</td>
<td>0.72</td>
<td>0.07</td>
<td>9.7</td>
<td>16</td>
</tr>
<tr>
<td>RIA (II)</td>
<td>0.79</td>
<td>0.09</td>
<td>11.4</td>
<td>16</td>
</tr>
<tr>
<td>RIA (III)</td>
<td>1.38</td>
<td>0.18</td>
<td>13.0</td>
<td>10</td>
</tr>
<tr>
<td>RIA (II)</td>
<td>1.23</td>
<td>0.05</td>
<td>4.0</td>
<td>10</td>
</tr>
<tr>
<td>RIA (III)</td>
<td>1.23</td>
<td>0.10</td>
<td>8.9</td>
<td>10</td>
</tr>
<tr>
<td>b. 1.3 ± µg/liter</td>
<td>2.19</td>
<td>0.19</td>
<td>8.6</td>
<td>16</td>
</tr>
<tr>
<td>RIA (II)</td>
<td>3.19</td>
<td>0.25</td>
<td>7.8</td>
<td>10</td>
</tr>
<tr>
<td>RIA (II)</td>
<td>2.95</td>
<td>0.13</td>
<td>4.7</td>
<td>10</td>
</tr>
<tr>
<td>RIA (III)</td>
<td>2.89</td>
<td>0.12</td>
<td>4.2</td>
<td>10</td>
</tr>
<tr>
<td>e. 4.8 ± 1.0 µg/liter</td>
<td>4.14</td>
<td>0.23</td>
<td>5.6</td>
<td>16</td>
</tr>
<tr>
<td>RIA (II)</td>
<td>4.22</td>
<td>0.25</td>
<td>5.9</td>
<td>16</td>
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</tbody>
</table>

Ortho RIA control serum I (0.6 to 1.2 µg/l, average 0.9 µg/l digoxin), lot 3N402, and Ortho RIA control serum II (3.8 to 5.8 µg/l, average 4.8 µg/l digoxin), lot 3N502, were assayed in duplicate on 16 days by radioimmunoassays (II) and (III). Table 2, lines b and e, coefficients of variation were (II) 9.7% and 5.6%, and (III) 11.4% and 5.9%.

A control serum provided by the manufacturer of radioimmunoassay (II) (1.8 to 2.6 µg/l digoxin) was assayed in duplicate on 16 days by radioimmunoassay (II). The coefficient of variation is 8.6%.

Discussion

The digoxin enzyme immunoassay (I) required 75 minutes for assay of approximately 40 tubes. Pretreatment of the sample at room temperature for 20 minutes and incubation at 30 °C for 30 minutes were required. The time and temperature of incubation are critical. The many manual pipetting
steps and the requirement for precise test parameters may have contributed to the higher coefficients of variation (less precision) achieved with the digoxin enzyme immunoassay. We expect that with the use of automatic pipettor-dilutors, precision would improve.

Radioimmunoassay III showed intermediate precision between radioimmunoassay II and enzyme immunoassay I. Radioimmunoassay II had slightly better precision in the day-to-day comparison.

Assay II is an equilibrium assay requiring an incubation of 30 minutes at 37 °C, however, the time and temperature did not seem to be critical. Time was varied from 20 minutes to 2 hours, and temperature varied ±5 degrees without harm. The radioimmunoassay II required 1.5 hours to assay approximately 40 tubes. The second radioimmunoassay III averaged 1.5 hours to assay approximately 40 tubes. Counting times were not included.

The enzyme immunoassay gave slightly higher digoxin concentration than did the radioimmunoassays with the control sera and patient sera. For instance, with the 1.3 μg/l control, the enzyme immunoassay result averaged 1.38 μg/l and the radioimmunoassays both averaged 1.23 μg/l. However, the difference was not statistically significant. Therefore, substitution of enzyme immunoassay for radioimmunoassay in the determination of therapeutic concentrations of digoxin would not require any change in the accepted therapeutic or toxic ranges.

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References

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Stabilization of Blood Glucose by Cooling with Ice: An Effective Procedure for Preservation of Samples from Adults and Newborns

Yuan Ly Lin, Carl H. Smith, and David N. Dietzler

Glycolysis causes a considerable decrease in blood glucose when whole blood is kept at room temperature without preservative. The most commonly used preservative, NaF, makes analysis of other serum constituents such as sodium and calcium and urea difficult or impossible, an especially serious limitation when sample size must be restricted. In samples at room temperature without preservative, plasma glucose decreased 36 mg/liter per hour in blood from adults and 60 mg/liter per hour in blood from newborns. Cooling on ice slowed these rates to 3.9 and 11, respectively. Plasma potassium increased 0.3 mmol/liter per hour in cooled specimens from both adults and newborns. Sodium, calcium, chloride and urea values were unaffected. We conclude that cooling effectively stabilizes plasma glucose for 4 h in samples from both adults and newborns and that potassium may be measured with negligible change for as long as 1 h and other constituents for the entire period.

Blood glucose decreases considerably and rapidly when whole blood is kept at room temperature (1–4). Some precaution must be taken to ensure against a significant change in the blood glucose concentration during the time between obtaining the sample and performing the analysis. Two procedures are commonly used to minimize the error introduced by the metabolism of glucose by blood cells: rapid separation of the cells from plasma or serum, and addition of an inhibitor of glycolysis to the blood sample. Rapid separation is frequently inconvenient and its success depends on a knowledgeable individual making sure that the sample is rapidly delivered to the laboratory. NaF has been the inhibitor most widely used for the preservation of blood samples (5). Addition of NaF renders the specimen definitely usable for assay of calcium or sodium and may interfere with measurement of urea nitrogen by a urease procedure (6, 7). In general, a separate blood sample is required exclusively for the analysis of glucose. This limitation is especially serious when sample size must be restricted. A less-common procedure is dilution of the sample at the bedside, either with water or isotonic NaF (8, 9). This procedure is effective, but it makes accurate measurement of glucose dependent on the precision of the dilution by the blood drawer at the bedside.

Because these three procedures possess disadvantages, we investigated cooling as an alternative means of preservation. There are conflicting statements as to how effectively cooling...