Spironolactone-Associated Digoxin Radioimmunoassay Interference

Bernie Silber,1,2 Lewis B. Shelnor,1,3,4 James L. Powers,1 Michael E. Winter,1,5 and Wolfgang Sadée1,2

Apparent digoxin was measured in the serum of 21 patients receiving spironolactone and in 21 controls, by use of a sequential-saturation 3H-radioimmunoassay (RIA) and an equilibrium 125I-RIA. No patient had been given digoxin for at least four weeks. "Digoxin" values in the former group were significantly (p < 0.05) higher than in the control group, and often were in or near the "therapeutic" range by the equilibrium 125I-RIA, but not by the sequential-saturation 3H-RIA. Canrenone (a major active metabolite of spironolactone) in the serum of the former group was measured by a newly developed liquid-chromatographic technique and correlated (r = 0.73) with "digoxin" concentrations by the 125I-RIA. However, external addition of canrenone to control serum in comparable concentrations did not cause appreciable "digoxin" values by the 125I-RIA. These findings suggest that other metabolites of spironolactone are responsible for the assay interference, the degree of which appears to depend on antibody specificity. Therefore, assay specificity should be established in clinical laboratories by using digoxin-free serum from patients ingesting spironolactone, and not by using spironolactone- or canrenone-fortified digoxin-free serum.

Accurate radioimmunoassay of digoxin in serum may depend upon antibody specificity, incubation conditions, and the radiolabel used. Furthermore, erroneously high or low digoxin values may result from assay interference caused by steroids (1) or radioisotopes (2).

Spironolactone (Aldactone, Searle), a potassium-sparing diuretic with steroid-like structure, is commonly prescribed for patients receiving digoxin. Interference by spironolactone in the radioimmunoassay for digoxin has been examined by several workers (1, 3–7), with conflicting results. Most of these studies involved either small numbers of patients, low daily doses of spironolactone, or in vitro methods (externally adding spironolactone to control serum), and so the extent of spironolactone-associated interference and its mechanism(s) remain unresolved questions.

Spironolactone is extensively metabolized, with canrenone as a major active metabolite (8–10); it has been suggested that this metabolite is responsible for the interference (5). However, canrenone comprises only a small fraction of the total radioactivity in plasma after doses of [3H]spiroloclactones in humans (8). Therefore, adding spironolactone or canrenone to control serum in order to test for assay interference may be invalid, because unknown metabolites could account for part of the interference observed in vivo.

We used two rather different digoxin radioimmunoassays in our study, one of which was significantly interfered with by spironolactone administration. Our results demonstrate that assay interference is indeed caused by spironolactone metabolites other than canrenone.

Materials and Methods

Experimental Design

Twenty-one hospitalized male patients who had received at least 75 mg of spironolactone daily for a minimum of five days were selected as the experimental group; 21 male patients not receiving the drug served as controls. No patient had received any radioisotope during the previous two weeks, and none had been given a cardiac glycoside or steroid drug within the previous four weeks. Controls had not received spironolactone for at least four weeks before this study.

Venous blood was obtained for the determination of albumin, total bilirubin, urea nitrogen, and creatinine in the serum, by standard clinical laboratory techniques. In general, patients who had received spironolactone had higher concentrations of bilirubin and lower concentrations of albumin than did controls (Table 1). Blood was sampled from both groups in the early morning, at least 8 h subsequent to the last previous dose of spironolactone (in the experimental group). Plasma samples were analyzed, in duplicate, for digoxin by the Clinical Pharmacokinetics Laboratory (CPL) of the University of California, San Francisco, CA, by a sequential-saturation radioimmunoassay involving tritium (3H-RIA), and by the Riverside Hospital Clinical Laboratory (RHCL), North Hollywood, CA, using a commercially available equilibrium radioimmunoassay involving radiiodine (125I-RIA). Plasma samples were also analyzed, in duplicate, for canrenone by the first laboratory, using "high-performance" liquid chromatography.

Procedures

Sequential-saturation 3H-RIA (CPL). The method used is a modification of that of Smith et al. (11). Fifty microliters of serum is added to 150 μL of phosphate-buffered saline (pH 7.4; per liter, 0.1 mol of Na2HPO4, 8 g of NaCl, and 1 g of NaNO3). Three hundred microliters of diluted digoxin antibody (Antibodies Inc., Davis, CA 95616; lot no. P10610) is added to this solution, which is mixed, and then incubated for 45 min at 25 °C. Fifty microliters of a 10 μg/L solution of [3H]digoxin (New England Nuclear, Boston, MA 02118; lot no. 772-067) is added to the incubation mixture, mixed, and then incubated again for 15 min. Free and bound digoxin are
Table 1. Comparison of Patient Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental group</th>
<th>Control group</th>
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</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>55 ± 12*</td>
<td>53 ± 13</td>
</tr>
<tr>
<td>Total bilirubin, mg/L</td>
<td>31 ± 22</td>
<td>14 ± 15</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>31 ± 9</td>
<td>35 ± 15</td>
</tr>
<tr>
<td>Serum urea nitrogen, mg/L</td>
<td>210 ± 140</td>
<td>180 ± 140</td>
</tr>
<tr>
<td>Serum creatinine, mg/L</td>
<td>12 ± 3</td>
<td>13 ± 10</td>
</tr>
</tbody>
</table>

* Mean ± SEM, throughout n = 21 for both groups.

Separated by adding 0.5 mL of dextran-coated charcoal (per liter, 20 g of activated charcoal and 200 mg of dextran, both from Sigma Chemical Co., St. Louis MO 63178). The suspension is incubated for 5 min, and then centrifuged at 1800 X g for 10 min. Ten milliliters of Oxifluor scintillation fluid (New England Nuclear) is added to the supernate, which is then counted for 2 min in a Mark III scintillation counter (Searle Analytical Inc., Des Plaines, IL 60018). Percent digoxin bound is converted to digoxin concentration by reference to a standard curve, prepared daily. The CV is 5 and 10% at digoxin concentrations of 3.0 and 1.0 µg/mL, respectively.

Equilibrium 125I-RIA (RHCL). The DIGI-TAB® digoxin kit containing [125I]digoxin (lot no. DB-19), and digoxin antibody (lot no. DB-19) were used according to manufacturer's directions (12). The CV is 10% at a digoxin concentration of 1.0 µg/L.

Canrenone assay. Five hundred microliters of serum is added to 4 mL of diethyl ether. The solution is mixed and then centrifuged at 100 X g for 5 min. The aqueous layer is frozen in a solid CO2/acetone bath, and the supernate is then transferred and dried under nitrogen. To the residue is added 200 µL of the chromatographic eluent [an equilibrium mixture of acetonitrile (Buridick and Jackson Laboratories, Muskogon, MI 49442) and 5 mmol/L phosphate buffer (pH 4.0)]. After this mixture has stood for 30 min, 50 µL is injected into the high-performance liquid chromatograph equipped with U6K Injector, Model 6000A pump, and a C18 micro-Bondapak column (3.9 mm i.d. × 30 cm) (Waters Associates, Milford, MA 01757). Canrenone is measured at its ultraviolet maximum of 293 nm, with a variable-wavelength detector (Schoeffel Instrument Corp., Westwood, NJ 07675). At a flow rate of 2 mL/min, the retention time is 4.5 min. The sensitivity of the assay is 20 µg/L at an attenuation of 0.01 A full-scale. The analytical recovery is 76% and the CV is 7% at a concentration of 100 µg/L.

Results

Spironolactone administration interfered with the equilibrium 125I-RIA of digoxin. Serum from most of the patients receiving spironolactone had significantly (p < 0.05) higher "digoxin" values by this assay than did controls (Mann-Whitney U test). By the sequential-saturation 3H-RIA, few measurable "digoxin" values were observed in either group and there was no significant difference between results for the two groups.

Both RIA techniques for digoxin were tested for interference by spironolactone, canrenone, and canrenoate (also a metabolite of spironolactone) by assaying control serum with added parent drug or metabolites at concentrations ranging from 10 to 10,000 µg/L. Spironolactone and canrenoate did not interfere with either RIA, but canrenone in concentrations of 10,000 µg/L and 1000 µg/L resulted in apparent digoxin values of 1.1 µg/L and 0.5 µg/L by the equilibrium 125I assay, and 0.5 µg/L and <0.3 µg/L (unmeasurable) by the sequential-saturation 3H assay. When the 3H assay was performed as an equilibrium rather than as a sequential-saturation technique, the "digoxin" values were higher, but the differences were not significant.

We measured values for canrenone in serum as a result of spironolactone administration in the experimental group, using a newly developed assay that is specific for canrenone in the presence of spironolactone. We found a correlation (r = 0.73) between canrenone and "digoxin" concentrations by the equilibrium 125I-RIA (Table 2 and Figure 1), but interference in the 125I-RIA observed in the experimental group was substantially larger than would have been predicted from the canrenoate concentrations alone.

To determine whether metabolites other than canrenoate were contributing to the observed interference, we extracted pooled serum from the experimental group. Canrenoate would be extracted into the ether fraction under these conditions. We found that the ether extractable fraction of serum accounted for less than 20% (0.3 µg/L) of the interference, whereas the aqueous fraction accounted for >80% (0.7 µg/L).

There was no significant correlation between either serum albumin, urea nitrogen, or creatinine and "digoxin" in either group, by either assay. Bilirubin correlated (r = 0.70) with "digoxin" in the experimental group by the 125I-RIA. However, neither digoxin assay was affected by externally added bilirubin in concentrations up to 180 mg/L, although the 3H assay required color-quench correction when bilirubin concentrations exceeded 40 mg/L. Therefore, the correlation between bilirubin and "digoxin" 125I-RIA interference must be indirect.

Discussion

Although Ravel (3) and Müller et al. (7) reported that spironolactone administration should not appreciably affect the digoxin RIA because antibodies of high specificity are currently available, Lichey et al. (6) recently found that both oral spironolactone and intravenous canrenoate-K therapy significantly affected results of digoxin RIA.

Table 2. Sequential-Saturation 3H- and the Equilibrium 125I-Digoxin Radioimmunoassays Compared

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sequential-saturation 3H</th>
<th>Equilibrium 125I</th>
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<tbody>
<tr>
<td></td>
<td>Subjects</td>
<td>Controls</td>
</tr>
<tr>
<td>Daily dose of spironolactone, mg</td>
<td>187 ± 99</td>
<td>—</td>
</tr>
<tr>
<td>Canrenone, µg/L</td>
<td>307 ± 307</td>
<td>—</td>
</tr>
<tr>
<td>&quot;Digoxin,&quot; µg/L</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Patients with &quot;digoxin&quot; values &gt;0a</td>
<td>7 (33%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Correl. between canrenone and &quot;digoxin&quot; values</td>
<td>-0.02</td>
<td>—</td>
</tr>
<tr>
<td>Correl. between total bilirubin and &quot;digoxin&quot; values</td>
<td>0.28</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

* Concentrations of canrenone and "digoxin" <20 µg/L and 0.3 µg/L, respectively, are reported as zero. a Mean ± SEM. b p < 0.05. c p < 0.001.
The present study indicates that spironolactone administration can significantly interfere with at least one digoxin RIA. Our results suggest that antibody specificity is the major variable influencing the degree of assay interference, because assay specificity was not consistently associated with either the type of tracer or the incubation method utilized (3, 5, 6).

Our in vitro screening of spironolactone, canrenoate, and canrenone showed that only canrenone interfered with the digoxin RIAs tested in this study and only at concentrations greater than about 1000 μg/L. When the in vitro results are examined in conjunction with the "digoxin" and canrenone concentrations measured in the experimental group (Table 2 and Figure 1), only a small fraction of the observed 125I-RIA interference can be accounted for by canrenone alone. Huffman (5) also reported a correlation between canrenone concentrations and digoxin assay interference, and concluded that canrenone was the cause of this interference. However, he did not determine the antibody affinity of externally added canrenone, which is necessary to support this conclusion. Sadée et al. (13) reported that an aldosterone RIA was interfered with by a spironolactone metabolite other than canrenone or canrenoate, and for that assay Finn et al. (14) identified the metabolite as 20-hydroxycanrenone.

Canrenone and 20-hydroxycanrenone both are extracted from serum by ether. Because we found that the ether-extractable fraction of pooled serum (from the experimental group) accounted for only a small fraction (<20%) of the total 125I-RIA interference, other non-ether-extractable and unknown spironolactone metabolites appear to account for most of the spironolactone-associated interference with the 125I-RIA for digoxin.

Since our results confirm that digoxin antibodies of varying specificity are now available, clinicians should consider the potential of spironolactone administration to interfere with a digoxin RIA, unless assay specificity has been tested with digoxin-free serum from patients ingesting spironolactone, and not by using digoxin-free serum with externally added spironolactone or canrenone, or both.

This study was supported in part by USPHS grant no. GM16496, from the NIGMS, NIH, Bethesda, MD. We thank Drs. William Alberts and Joseph Auerbach, and Lee Corallo for performing the 125I digoxin radioimmunoassays.

References