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Effect of Spironolactone, Potassium Canrenoate, and Their Common Metabolite Canrenone on Dimension Vista Digoxin Assay

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Spironolactone, a potassium sparing diuretic metabolized to canrenone, is often used with digoxin to treat various conditions including congestive heart failure. Potassium canrenoate is a similar drug that is also metabolized to canrenone. Due to reported interference of spironolactone, potassium canrenoate, and their common metabolite canrenone with digoxin immunoassays, we investigated potential interference of these compounds with Dimension Vista Digoxin immunoassay using Flex reagent cartridge. Aliquots of a drug-free serum pool were supplemented with various amounts of spironolactone, potassium canrenoate, or canrenone and apparent digoxin values were measured using Dimension Vista digoxin assay, we observed none-detected value except when aliquots were supplemented with higher amounts of spironolactone or canrenone. Similarly, when aliquots of a serum digoxin pool (prepared by pooling specimens from patients receiving digoxin) where further supplemented with various amounts of spironolactone, potassium canrenoate, or canrenone, we observed moderately falsely elevated digoxin values only in specimens containing higher amounts of spironolactone or canrenone. We conclude that spironolactone and canrenone but not potassium canrenoate may cause modest interference with Dimension Vista digoxin assay but such interferences may not be clinically significant except with very high amounts of canrenone. J. Clin. Lab. Anal. 24:413–417, 2010.

Key words: spironolactone; canrenone; potassium canrenoate; dimension vista digoxin

Digoxin is a cardiac glycoside used most frequently to increase the adequacy of circulation in patients with congestive heart failure and to slow ventricular rate in the presence of atrial fibrillation and flutter. Therapeutic drug monitoring of digoxin is essential and immunoassays are most commonly used for routine monitoring of digoxin. Due to the narrow therapeutic range for digoxin, interference from endogenous and exogenous factors is troublesome in accurately measuring serum digoxin concentration (1).

Spironolactone, a competitive aldosterone antagonist, has been used clinically in the therapy of hypertension and congestive heart failure. Spironolactone is rapidly and extensively metabolized to canrenone, which is an active metabolite. Although not used in the U.S. due to potential carcinogenic property, potassium canrenoate is used in Europe and in other countries throughout the world (2). Potassium canrenoate, like spironolactone, is also metabolized to canrenone (3,4). Potassium canrenoate, spironolactone, and canrenone all have structural similarity with digoxin (Fig. 1).

Because spironolactone and digoxin may be used concurrently in the management of a patient, interference of spironolactone and canrenone in therapeutic monitoring of digoxin is problematic. Morris et al. reported positive interference of spironolactone in digoxin measurement using the fluorescence polarization immunoassay (FPIA) marketed by the Abbott Diagnostics (Abbott Park, IL) for application on the

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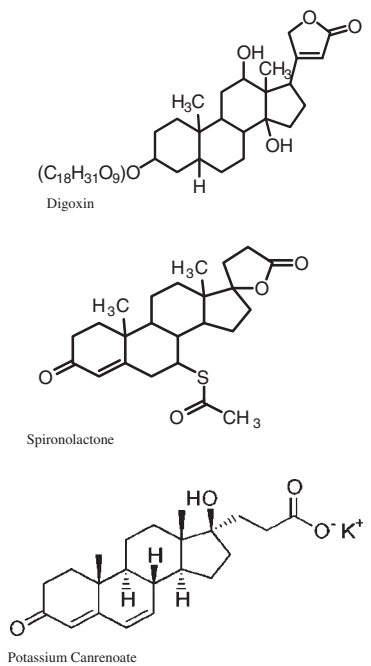

Fig. 1. Chemical structure of digoxin, spironolactone, and potassium canrenoate.

TDx/FLX analyzer (5). Later other authors verified the interference of spironolactone and canrenone with FPIA and other commonly used immunoassays for digoxin (6–8). However, recently Abbott Diagnostics discontinued the both TDx/FLX FPIA and IMx MEIA digoxin assays.

Okazaki et al. also reported falsely elevated digoxin level in patients receiving digoxin and potassium canrenoate. The authors reported two cases in which cross-reactivity of the assay system caused clinical problems and recommended use of the OPUS digoxin assay, which showed minimal cross-reactivity (9). Steimer et al. described negative interference of canrenone in digoxin measurements. Canrenone and spironolactone caused falsely low digoxin values due to negative interference with serum digoxin measurements when using a microparticle enzyme immunoassay (MEIA), Digoxin II. Misleading sub-therapeutic concentrations of digoxin as measured on several occasions led to erroneous digoxin-guided dosing that lead to serious digoxin toxicity in patients. In addition, negative interference of spironolactone, potassium canrenoate, and canrenone was also observed in the Dimension digoxin assay marketed by Dade Behring (Now Siemens Diagnostics, Deerfield, IL) (10,11). Recently, we acquired Dimension Vista platform in our clinical laboratory but potential interference of spironolactone and related compounds on this relatively new platform has not been reported before. Here we report our findings.

MATERIALS AND METHODS

Spironolactone and potassium canrenoate were purchased from the Sigma Chemical Company (St. Louis, MO). We prepared canrenone by acid-catalyzed lactonization of potassium canrenoate according to Tal except that we used *p*-toluenesulfonic acid as a catalyst (12). Canrenone was purified by crystallization using ethyl acetate as described by Tal. Digoxin immunoassay (Dimension Vista Digoxin Assay using Flex reagent cartridge) was obtained from Siemens Diagnostics and all assays were run on the Dimension Vista 1500 analyzer following manufacturer's recommended protocol. The analytical measurement range (AMR) of the digoxin assay is 0.1–5.0 ng/ml. Because the limit of quantitation is 1.0 ng/ml, any digoxin value less than 0.1 ng/ml was considered as "none detected" digoxin value. For this study we used serum pools that were prepared from left-over discarded specimens only after de-identifying specimens according to policy of our institutional review board. These specimens are routinely submitted to our laboratory for analysis and stored for one week after reporting all results to ordering physicians. These specimens are then discarded.

We used one drug-free serum pool for this study. No digoxin was detected in the drug-free serum pool. In order to ensure that the serum pool was indeed drug and DLIF (digoxin-like immunoreactive factors) free, the serum pool was further treated with activated charcoal (50 mg/ml of serum) for 20 min. Activated charcoal was purchased from Aldrich Chemical Company (Milwaukee, WI). After treatment with activated charcoal, the pool was centrifuged at a high speed in order to separate activated charcoal from the serum. The resulting supernatant was used for further experiments. Treatment of serum with activated charcoal is known to remove DLIF (Dasgupta et al., unpublished data). We also prepared a digoxin pool from surplus serum specimens that were submitted to our clinical laboratory for therapeutic drug monitoring of digoxin and that would have been discarded after testing. These specimens are stored in the laboratory for a week after performing and reporting results to the ordering clinicians. We used only leftover specimens that would have otherwise been discarded.

Stock solutions of spironolactone, potassium canrenoate, and canrenone (1 mg/ml) were prepared in methanol by dissolving 100 mg of each compound into 100 ml of methanol. Then, working solutions for all these compounds (0.01 mg/ml) were made by diluting stock solutions with methanol. In the first set of experiments, aliquots of drug- and DLIF-free serum pool were supplemented with various amounts of spironolactone, potassium canrenoate, and canrenone representing expected in vivo concentrations from

typical prescribed doses or in cases of moderate-to-severe overdose. For supplementing purposes, microliter quantities of working stock solutions of the appropriate compounds were added to dry test tubes. Residual methanol was evaporated under nitrogen at room temperature to obtain dry residue. The dry residue was reconstituted with drug-free serum pool. This extra step was taken to ensure that no methanol was present in the specimens analyzed for digoxin to avoid any matrix effect due to supplementation of these specimens with spironolactone, potassium canrenoate, or canrenone. After reconstitution with drug- and DLIF-free serum, digoxin concentrations were measured using Dimension Vista Digoxin assay. Each measurement was performed in triplicate and results were expressed as the mean and one standard deviation.

In another set of experiments, aliquots of a digoxin pool were further supplemented with various concentrations of spironolactone, potassium canrenoate, and canrenone and digoxin values were measured again with Dimension Vista digoxin assay for comparison with the digoxin concentration of the original pool. Again, we added microliter quantities of working solution of spironolactone, potassium canrenoate, and canrenone in dry test tubes and evaporated methanol under nitrogen prior to reconstitution of drug residue with digoxin pool in order to avoid matrix effects.

Statistical analyses were performed using independent *t*-test two tailed and we considered an increase statistically significant only at 95% confidence interval or higher ($P < 0.05$).

TABLE 1. Study of Cross Reactivity of Spironolactone, Potassium Canrenoate and Canrenone with Dimension Vista Digoxin Assay

Specimen	Digoxin concentrations (ng/ml), mean (SD), $n = 3$ Dimension Vista Digoxin Assay
Drug/DLIS free serum pool	None detected
+Spironolactone	
100 ng/ml	None detected
250 ng/ml	None detected
500 ng/ml	0.1 (0.00)
1,000 ng/ml	0.2 (0.06)
+Potassium Canrenoate	
100 ng/ml	None detected
250 ng/ml	None detected
500 ng/ml	None detected
1,000 ng/ml	None detected
2,000 ng/ml	None detected
+Canrenone	
100 ng/ml	None detected
250 ng/ml	0.2 (0.06)
500 ng/ml	0.3 (0.00)
1,000 ng/ml	0.4 (0.06)
2,000 ng/ml	0.4 (0.06)

RESULTS

When aliquots of drug-free serum pools were supplemented with various amounts of spironolactone, potassium canrenoate, or their common metabolite canrenone, we observed measurable apparent digoxin levels only in aliquots supplemented with high amounts of spironolactone or canrenone. Highest apparent digoxin concentration of 0.4 ng/ml was observed when an aliquot of a drug-free serum pool was supplemented with canrenone to achieve a final canrenone concentration of 2,000 ng/ml. No apparent digoxin level was observed even when another aliquot of a drug-free serum pool was supplemented even with 2,000 ng/ml of potassium canrenoate (Table 1).

Rigorous characterization of immunoassay interference due to a cross reactant should be performed in the presence of the primary analyte (13). Therefore, we added these cross reactants (spironolactone, potassium canrenoate, or canrenone) to various aliquots of a digoxin pool prepared from patients receiving digoxin and then measured apparent digoxin concentrations for comparison with the original digoxin concentration of the pool. We observed only modest increases in serum digoxin values in specimens supplemented with high amounts of spironolactone or canrenone. For example, when an aliquot of a digoxin pool was further supplemented with canrenone to achieve a final canrenone concentration of 2,000 ng/ml, the observed digoxin value was increased from 1.3 ng/ml (digoxin concentration of the original pool) to 1.6 ng/ml, a statistically significant increase. Again, no interference was observed when another aliquot of the digoxin pool was supplemented with potassium canrenoate to achieve a final concentration of 2,000 ng/ml (Table 2).

DISCUSSION

Oral administration of 100 mg spironolactone, the recommended dosage typically leads to a peak serum spironolactone concentration of 83 ng/ml and a peak canrenone concentration of about 202 ng/ml (14). After intravenous administration of potassium canrenoate, the peak plasma canrenone concentration usually reaches 2,066 ng/ml. However, the peak canrenone concentration can be as low as 1,117 ng/ml (15). Sadde et al. studied pharmacokinetics of spironolactone, canrenone, and potassium canrenoate in humans. The authors either used an oral dose of 400 mg of spironolactone or an intravenous dose of 380 mg

TABLE 2. Effect of Supplementing Aliquots of Digoxin Pool with Various Amounts of Spironolactone, Potassium Canrenoate and Canrenone on Digoxin Measurements by Dimension Vista Digoxin Assay

Specimen	Digoxin concentrations (ng/ml), mean (SD), $n = 3$ Dimension Vista Digoxin Assay
Drug/DLIS free serum pool	1.3 (0.06)
+Spironolactone	
100 ng/ml	1.3 (0.06)
250 ng/ml	1.3 (0.00)
500 ng/ml	1.3 (0.06)
1,000 ng/ml	1.4 (0.06)
+Potassium Canrenoate	
100 ng/ml	1.3 (0.00)
250 ng/ml	1.3 (0.06)
500 ng/ml	1.3 (0.06)
1,000 ng/ml	1.3 (0.00)
2,000 ng/ml	1.3 (0.06)
+Canrenone	
100 ng/ml	1.3 (0.00)
250 ng/ml	1.4 (0.06)
500 ng/ml	1.4 (0.06)
1,000 ng/ml	1.5 (0.06)*
2,000 ng/ml	1.6 (0.00)*

*Significantly higher than the digoxin value in the original digoxin pool by the independent *t*-test, two tailed.

of potassium canrenoate. Although initial plasma concentration of potassium canrenoate was high, the value rapidly dropped to 1,000 ng/ml after achieving steady state. The mean plasma concentration of canrenone was 1,400 ng/ml (16). These published reports and in vitro study of Okazaki et al. were the basis of selection of concentrations of spironolactone, potassium canrenoate, and canrenone for this study.

Interference of spironolactone, potassium canrenoate, and their common metabolite canrenone may be positive or negative in serum digoxin measurement using immunoassays. Spironolactone and its metabolite canrenone can falsely elevate serum digoxin levels if measured by FPIA or Elecsys (positive interference) or falsely lower digoxin levels if measured by MEIA, Imx, and Dimension (negative interference). The magnitude of interference is more significant with potassium canrenoate where concentration of its metabolite canrenone can be significantly higher. In one report authors observed a 42% decline in expected value of serum digoxin in the presence of 3,125 ng/ml of canrenoate using MEIA, 78% decline in using Dimension, and 51% decrease using IMx. EMIT 2000 and Vitros digoxin assay is free from such interference (11). Although mechanism of falsely elevated digoxin values due to cross-reactivity of spironolactone, potassium canrenoate, or canrenone is due to binding of such compounds with digoxin antibodies, mechanism of negative interference is not so straight forward. Valdes and Jortani speculated that negative interference is directly related to the physical design of the immunoassay and is fundamentally based on the fact that for binding of small molecules to antibodies, the rate of association is comparable for primary analyte and cross-reactants. It is the dissociation that is different and accounts for lower binding affinity of the cross-reactant. For example, in the MEIA assay which demonstrated negative interference with spironolactone, potassium canrenoate, and canrenone, during the wash step the dissociation rate for cross-reactant bound to antibody is greater than for digoxin bound to antibody, thus allowing more unoccupied sites to bind with the tracer, leading to reduced recovery of digoxin hence values could be falsely lowered (17).

Steimer et al. reported negative interference of spironolactone and related compounds in Dimension digoxin assays (11). However, in our study we did not observe any negative interference of spironolactone, potassium canrenoate, or their common metabolite canrenone in the Dimension Vista digoxin assay. We observed no significant interference in serum digoxin measurement *y* spironolactone concentration up to 500 ng/ml and canrenone concentration up to 100 ng/ml. In the presence of 500 ng/ml of canrenone, the apparent digoxin value was increased to 1.4 ng/ml (control value 1.3 ng/ml), which was not statistically significant. Therefore, based on these in vitro data we speculate that it is unlikely that spironolactone therapy is going to cause any significant problem in interpreting digoxin result in a patient receiving both digoxin and spironolactone and if therapeutic drug monitoring of digoxin is performed by Dimension Vista digoxin assay. Statistically significant interference was observed only when aliquots of digoxin pool were supplemented with 1,000 and 2,000 ng/ml of canrenone respectively. Such high amounts of canrenone are unlikely after oral administration of spironolactone and only can be achieved after intravenous administration of potassium canrenoate.

Although spironolactone, potassium canrenoate, and their common metabolite canrenone demonstrated modest cross-reactivity with Dimension Vista digoxin assay, our structurally related steroids such as cortisol, cortisone, 17-estradiol, estriol, prednisone, and progesterone did not show any cross-reactivity with this assay at up to 3 μg/ml concentration (package insert, Siemens Dimension Vista Flex reagent cartridge). Because potassium canrenoate is not used in the United States, we conclude that route therapy with spironolactone in patients receiving digoxin should not interfere with routine monitoring of digoxin using Dimension Vista digoxin assay.

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