Clinically Insignificant Negative Interferences of Spironolactone, Potassium Canrenoate, and Their Common Metabolite Canrenone in New Dimension Vista LOCI Digoxin Immunoassay

Amitava Dasgupta,^{1*} Myrtle J. Johnson,² and Tamal K. Sengupta³

¹Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School, Houston, TX

²Laboratory Services, Memorial-Hermann Hospital at Texas Medical Center, Houston, TX

³Department of Surgery, N.R.S Medical College and Hospital, Kolkata, India

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, which is also metabolized to canrenone. Due to reported both positive and negative interference of spironolactone, potassium canrenoate, and their common metabolite canrenone with digoxin immunoassays, we investigated potential interference of these compounds with the new homogeneous sequential chemiluminescent assay for digoxin based on the luminescent oxygen channeling technology (LOCI digoxin) for application on the Dimension and Vista platform. When 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 LOCI digoxin assay, we observed no detected value except when

aliquots were supplemented with very high amounts of potassium canrenoate or canrenone. However, we observed that apparent digoxin concentrations were very low. When aliquots of a serum digoxin pool (prepared by pooling specimens from patients receiving digoxin), were further supplemented with various amounts of spironolactone, potassium canrenoate, or canrenone and serum digoxin concentrations were remeasured using the LOCI digoxin assay, only statistically significant falsely lower digoxin values (negative interference) were observed in specimens containing very high amounts of canrenone or potassium canrenoate. However, such small bias may not have any clinical significance. We conclude that new Dimension Vista LOCI digoxin assay is virtually free from interferences of spironolactone, potassium canrenoate, and their common metabolite canrenone. *J. Clin. Lab. Anal.* 26:143–147, 2012. © 2012 Wiley Periodicals, Inc.

Key words: spironolactone; canrenone; potassium canrenoate; LOCI digoxin

Digoxin is a cardiac glycoside used for increasing 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. Although it is traditionally assumed that digoxin has a therapeutic range of 0.8–2.0 ng/mL, lower therapeutic range has also been suggested (1). Due to the narrow therapeutic range, interference from endogenous and exogenous factors is troublesome in accurately measuring serum digoxin concentration (2).

Spironolactone, a competitive aldosterone antagonist, has been used clinically for treating hypertension and congestive heart failure and may be used concurrently in a patient receiving digoxin. Spironolactone is rapidly and

*Correspondence to: Amitava Dasgupta, Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston, 6431 Fannin, MSB 2.292, Houston, TX 77030.
E-mail: Amitava.Dasgupta@uth.tmc.edu

Received 19 October 2011; Accepted 4 February 2012

DOI 10.1002/jcla.21501

Published online in Wiley Online Library (wileyonlinelibrary.com).

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

extensively metabolized to canrenone, an active metabolite. Although not used in the United States due to potential carcinogenic property, potassium canrenoate is used in Europe and in other countries throughout the world (3). Potassium canrenoate is also metabolized to canrenone (4). Potassium canrenoate, spironolactone, and canrenone all have structural similarity with digoxin (Fig. 1 and may interfere with digoxin immunoassays. Significant interferences of these compounds with the fluorescence polariza-

tion immunoassay (FPIA) for digoxin for application on the TDx analyzer (Abbott Laboratories, Abbott Park, IL) have been reported in the literature (5–7). However, Abbott Diagnostics discontinued the FPIA digoxin assay in 2010. Okazaki et al. also reported that OPUS digoxin assay showed minimal cross-reactivity with spironolactone and related compounds (8). Steimer et al. first described negative interference of canrenone in digoxin measurements using microparticle enzyme immunoassay (MEIA: Digoxin II assay for application on the AxSYM analyzer, Abbott Laboratories). Misleading subtherapeutic concentrations of digoxin as measured on several occasions led to erroneous digoxin-guided dosing that lead to serious digoxin toxicity in patients (9, 10). Recently, Siemens Diagnostics has marketed a new digoxin immunoassay based on the luminescent oxygen channeling (LOCI) technology for application on the Dimension Vista analyzer but effect of spironolactone and related compounds on serum digoxin measurement using this immunoassay has not been reported before. Here, we report our findings on the effect of spironolactone, potassium canrenoate, and their common metabolite canrenone on serum digoxin measurement using LOCI digoxin immunoassay.

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 the catalyst (11). New LOCI digoxin immunoassay for application on the Dimension Vista analyzer was obtained from Siemens Diagnostics (Deerfield, IL) and all assays were run on the Dimension Vista 1500 analyzer following manufacturer's recommended protocol. The LOCI Digoxin assay is a homogenous sequential chemiluminescent assay based on the LOCI technology and utilizes a specific mouse monoclonal antibody, again digoxin, and requires no sample pretreatment. The analytical measurement range of this assay is from 0.06 to 5.0 ng/mL while the calibration range is 0–5.0 ng/mL. The limit of detection is 0.06 ng/mL. Therefore, any apparent digoxin value less than 0.06 ng/mL was considered as “none detected” digoxin value. We also evaluated between run precision of the LOCI digoxin assay during the one-month period of this study (September 1–September 30, 2011) by calculating mean, standard deviation, and coefficient variation (CV) based on control values run on each day of operation as a part of the quality control protocol.

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 DLIS (digoxin-like immunoreactive substances) free, we

further treated this serum pool 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. 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.

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 absolute alcohol. Then working solutions for all these compounds (0.01 and 0.1 mg/mL) were made by diluting stock solutions with absolute alcohol (ethanol). In the first set of experiments, aliquots of charcoal-treated drug and DLIS-free serum pool were supplemented with various amounts of spironolactone, potassium canrenoate, or 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 ethanol was evaporated under nitrogen at room temperature to obtain almost dry residue, which was reconstituted with drug-free serum pool. Then apparent 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 LOCI 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 ethanol under nitrogen prior to reconstitution of almost 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 ($P < 0.05$).

RESULTS

The between-run precision of the digoxin assay was calculated based on values of low and high control during

TABLE 1. Apparent Digoxin Concentration Measured by the New Dimension Vista LOCI Digoxin Assay After Aliquots of Drug-/DLIS-Free Serum Pool Were Supplemented With Various Concentrations of Spironolactone, Potassium Canrenoate, and Canrenone

Specimen	Digoxin concentrations (ng/mL), mean (SD), <i>n</i> = 3
Drug-/DLIS-free serum pool	None detected ^a
+ spironolactone	
100 ng/mL	None detected
250 ng/mL	None detected
500 ng/mL	None detected
1,000 ng/mL	None detected
+ 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	0.09 (0.01)
+ Canrenone	
100 ng/mL	None detected
250 ng/mL	None detected
500 ng/mL	None detected
1,000 ng/mL	0.10 (0.02)
2,000 ng/mL	0.12 (0.00)

None detected: Apparent digoxin concentration < 0.06 ng/mL, the detection limit of the assay.

the period of the study (September 1-September 30, 2011). The CV of the low control was 1.6% (Mean: 0.96 ng/mL, SD: 0.015, *n* = 33), while the CV of the high control was also 1.6% (Mean: 3.29, SD: 0.052, *n* = 33).

When aliquots of drug-free serum pools were supplemented with various amounts of spironolactone, potassium canrenoate, or their common metabolite canrenone, we observed no apparent digoxin levels in aliquots supplemented with various amounts of spironolactone including very high concentrations. However, when aliquots were supplemented with 2,000 ng/mL of potassium canrenoate as well as 1,000 and 2,000 ng/mL of canrenone, very small apparent digoxin concentrations were observed using the LOCI digoxin assay (Table 1).

Rigorous characterization of immunoassay interference due to a cross reactant should be performed in the presence of the primary analyte (12). 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 no statically significant difference in serum digoxin values even when aliquots of the digoxin pool were supplemented with very high amounts of spironolactone. However, in the presence of 1,000 and 2,000 ng/mL of potassium canrenoate and canrenone, a statistically

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

Specimen	Digoxin concentrations (ng/mL), Mean (SD), <i>n</i> = 3
Drug-/DLIS-free serum pool	1.42 (0.03)
+ Spironolactone	
100 ng/mL	1.41 (0.01)
250 ng/mL	1.42 (0.01)
500 ng/mL	1.37 (0.03)
1,000 ng/mL	1.38 (0.04)
+ Potassium canrenoate	
100 ng/mL	1.40 (0.02)
250 ng/mL	1.42 (0.03)
500 ng/mL	1.39 (0.03)
1,000 ng/mL	1.33 (0.01)*
2,000 ng/mL	1.31 (0.03)*
+ Canrenone	
100 ng/mL	1.42 (0.00)
250 ng/mL	1.40 (0.01)
500 ng/mL	1.36 (0.04)
1,000 ng/mL	1.33 (0.01)*
2,000 ng/mL	1.26 (0.01)*

*Significantly less than the digoxin value in the original digoxin pool by the independent *t*-test, two tailed (*P* < 0.05).

significant negative bias was observed in serum digoxin measurement using the LOCI digoxin assay. 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 decreased from 1.42 ng/mL (digoxin concentration of the original pool) to 1.26 ng/mL, a statistically significant 11.3% decrease (Table 2). This was the highest negative bias observed in serum digoxin measurement. Because of very low impression of the new LOCI digoxin assay, the observed digoxin values in the presence of high amounts of potassium canrenoate and canrenone showed statistically significant difference but such small negative bias may not have any clinical significance.

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 (13). After intravenous administration of potassium canrenoate, the peak plasma canrenone concentration usually reaches 2,066 ng/mL. However, the peak canrenone concentration can also be as low as 1,117 ng/mL (14). Sadde et al. used an oral dose of 400 mg of spironolactone or an intravenous dose of 380 mg of potassium canrenoate and observed that the mean plasma concentration of canrenone

was 1,400 ng/mL (15). 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 the present study.

The MEIA digoxin assay showed both clinically and statistically significant interference with spironolactone, potassium canrenoate, and their common metabolite canrenone. Steimer et al. observed up to 42% decline in serum digoxin value in the presence of canrenoate using MEIA assay (10). However, the highest negative bias observed in the presence of extremely high amount of canrenone in the LOCI digoxin assay was only 11.3%. Therefore such difference may not be clinically significant. Interestingly, new digoxin assays for application on ARCHITECT clinical chemistry platforms (*cDig*, particle-enhanced turbidimetric inhibition immunoassay) and ARCHITECT immunoassay platforms (*iDig*, chemiluminescent microparticle immunoassay), also manufactured by Abbott Diagnostics are also virtually free from interferences of spironolactone and related compounds (16).

We described earlier that we observed falsely elevated serum digoxin concentrations in the Dimension Vista digoxin assay using Flex reagent cartridge in the presence of high amounts of spironolactone and canrenone (17). The new LOCI digoxin assay for application on the Dimension Vista analyzer is free interference of spironolactone even in supraphysiological concentration. Therefore, this assay is an improvement over the old digoxin assay. Moreover, the statistically significant difference in serum digoxin measurement in the presence of very high amount of potassium canrenoate is unlikely to be clinically significant. We conclude that the new and improved LOCI digoxin assay is virtually free from interferences from spironolactone, potassium canrenoate, and their common metabolite canrenone and can be used in clinical laboratories for routine therapeutic drug monitoring of digoxin.

REFERENCES

1. Sameri RM, Soberman JE, Finch CK, Self TH. Lower serum digoxin concentrations in heart failure and reassessment of laboratory report forms. *Am J Med Sci* 2002;324:10–13.
2. Dasgupta A. Therapeutic drug monitoring of digoxin: Impact of endogenous and exogenous digoxin-like immunoreactive substances. *Toxicol Rev* 2006;25:273–281.
3. Cook CS, Hauswald CL, Schoenhard GL, et al. Difference in metabolic profile of potassium canrenoate and spironolactone in rat: Mutagenic metabolites unique to potassium canrenoate. *Arch Toxicol* 1988;61:201–212.
4. Kojima K, Yamamoto K, Fujioka H, Kaneko H. Pharmacokinetics of spironolactone and potassium canrenoate in humans. *J Pharmacobiodyn* 1985;8:161–166.
5. Morris RG, Frewin DB, Taylor WB, Glistak ML, Lehmann DR. The effect of renal and hepatic impairment and of spironolactone on serum digoxin assay. *Eur J Clin Pharmacol* 1988;34:233–239.

6. Pleasants RA, Williams DM, Porter RS, Gadsden RH. Reassessment of cross-reactivity of spironolactone metabolites with four digoxin assays. *Ther Drug Monit* 1989;11:200–204.
7. Stone JA, Soldin SJ. An update on digoxin. *Clin Chem* 1989;35:1326–1331.
8. Okazaki M, Tanigawara Y, Kita T, Komada F, Okumura K. Cross-reactivity of TDX and OPUS immunoassay system for serum digoxin determination. *Ther Drug Monit* 1997;19:657–662.
9. Steimer W, Muller C, Eber B, Emmanuilidis K. Intoxication due to negative canrenone interference in digoxin monitoring. *Lancet* 1999;354:1176–1177.
10. Steimer W, Muller C, Eber B. Digoxin assays: Frequent, substantial, and potentially dangerous interference by spironolactone, canrenone and other steroids. *Clin Chem* 2002;48:507–516.
11. Tal DM. 6,7-dihydroxy-6,7-dihydrocanrenone isomers: Improved synthesis and proton NMR study. *Steroids* 1989;54:113–122.
12. Miller JJ, Valdes R. Methods for calculating cross reactivity in immunoassays. *J Clin Immunoassay* 1992;15:97–100.
13. Gardiner P, Schrode K, Quinlan D, et al. Spironolactone metabolism: Steady state serum levels of the sulfur containing metabolites. *J Clin Pharmacol* 1989;29:342–347.
14. Karras K, Seifert W. Pharmacokinetics of canrenone after oral administration of spironolactone and intravenous injection of canrenoate-K in healthy man. *Eur J Clin Pharmacol* 1983;25:449–453.
15. Sadee W, Dagcioglu M, Schroder R. Pharmacokinetics of spironolactone, canrenone and canrenoate-K in humans. *J Pharmacol Exp Ther* 1973;185:686–695.
16. DeFrance A, Armbruster D, Petty D, Kelley C, Cooper KC, Dasgupta A. Abbott ARCHITECT clinical chemistry and immunoassay systems – Digoxin assays are free of interferences from spironolactone, potassium canrenoate and their common metabolite canrenone. *Ther Drug Monit* 2011;33:128–131.
17. Dasgupta A, Johnson MJ. Effect of spironolactone, potassium canrenoate and their common metabolite canrenone on Dimension Vista digoxin assay. *J Clin Lab Anal* 2010;24:413–417.