Journal of Clinical Laboratory Analysis 16:172–177 (2002)

Bidirectional (Positive/Negative) Interference of Spironolactone, Canrenone, and Potassium Canrenoate on Serum Digoxin Measurement: Elimination of Interference by Measuring Free Digoxin or Using a Chemiluminescent Assay for Digoxin

Amitava Dasgupta,[1]* Helene Saffer,[1] Alice Wells,[1] and Pradip Datta[2]

[1]*Department of Pathology and Laboratory Medicine, University of Texas–Houston Medical School, Houston, Texas*

[2]*Bayer Diagnostics, Tarrytown, New York*

Spironolactone and potassium canrenoate (aldosterone antagonist diuretics) are often used with digoxin in clinical practice. Spironolactone, potassium canrenoate, and their common metabolite canrenone cross-react with the fluorescence polarization immunoassay (FPIA) for digoxin, and can falsely elevate serum digoxin concentrations. Serum digoxin concentrations were falsely lowered when the microparticle enzyme immunoassay (MEIA) was used. Aliquots of drug-free serum were supplemented with therapeutic and above-therapeutic concentrations of spironolactone, canrenone, and potassium canrenoate, and apparent digoxin activities were measured. We observed digoxin-like activities in the FPIA, but observed no activity with the MEIA or the chemiluminescent assay (CLIA). However, when serum digoxin pools prepared from patients receiving digoxin were supplemented with these compounds, we observed suppression of total digoxin levels with the MEIA. In contrast, no interference was observed in the presence of these compounds when CLIA was used for digoxin measurement. These compounds are strongly protein-bound, and no apparent digoxin activity was observed in the protein-free ultrafiltrate when drug-free sera were spiked with high levels of these compounds. Taking advantage of strong protein binding of these compounds and weak protein binding of digoxin (25%), interference of spironolactone, canrenone, and potassium canrenoate in FPIA and MEIA digoxin assays can be mostly eliminated by monitoring free digoxin concentration. Another approach to avoid this interference is to use the CLIA digoxin assay. J. Clin. Lab. Anal. 16:172–177, 2002. © 2002 Wiley-Liss, Inc.

Key words: spironolactone; metabolite; interference; fluorescence polarization immunoassay; microparticle enzyme immunoassay; chemiluminescent assay; digoxin

INTRODUCTION

Digoxin is a cardiac glycoside used most frequently to increase the adequacy of circulation in patients with congestive heart failure, and to slow the ventricular rate in the presence of atrial fibrillation and flutter. Spironolactone, a competitive aldosterone antagonist has been used clinically in the therapy of hypertension and congestive heart failure for a long time. Spironolactone is rapidly and extensively metabolized, and the metabolite canrenone is also pharmacologically active. Spironolactone and canrenone are strongly bound to serum proteins (90%) and have structural similarity with digoxin.

Because spironolactone and digoxin may be used concurrently in the management of a patient, interference of spironolactone and canrenone in the therapeutic monitoring of digoxin is troublesome. Digoxin has a narrow therapeutic range (0.8–1.9 ng/mL). Moreover, toxicity may be encountered at a digoxin concentration as low as 2.5 ng/mL. Positive interference

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

Presented in part at the 36th Annual Meeting of the Academy of Clinical Laboratory Physicians and Scientists, Seattle, Washington, 2001.

Received 17 December 2001; Accepted 5 April 2002

DOI 10.1002/jcla.10039

Published online in Wiley InterScience (www.interscience.wiley.com).

of spironolactone and its active metabolite canrenone in the radioimmunoassay for digoxin was reported as early as 1974 (1). Potassium canrenoate also showed positive interference with serum digoxin monitoring by both radioimmunoassay and enzyme immunoassay (2,3). In 1988 Morris et al. (4) first reported positive interference of spironolactone in digoxin measurement using the fluorescence polarization immunoassay (FPIA). Subsequently, other authors verified the interference of spironolactone and canrenone in the FPIA and other commonly used immunoassays for digoxin (5,6). Okazaki et al. (7) also reported falsely elevated digoxin levels in patients receiving digoxin and potassium canrenoate. The authors reported two cases in which cross-reactivity of the assay system caused clinical problems, and they recommended the use of an OPUS digoxin assay, which showed minimum cross-reactivity.

Recently, Steimer et al. (8) described negative interference of canrenone in digoxin measurement. Canrenone and spironolactone caused falsely low digoxin values due to negative interference in serum digoxin measurement using a microparticle enzyme immunoassay (MEIA). Misleading subtherapeutic concentrations of digoxin as measured on several occasions led to falsely guided digoxin dosing, which led to serious digoxin toxicity in the patients.

We studied potential interference of spironolactone, potassium canrenoate, and canrenone in serum digoxin monitoring using a chemiluminescent assay (CLIA). CLIA uses a specific monoclonal antibody against digoxin, whereas both MEIA and FPIA use rabbit polyclonal antibody. We previously reported interference of digitoxin in digoxin assays and elimination of interference using the CLIA, and also described the importance of antibody specificity (9). Recently, we reported negative interference of Chan Su in the MEIA, and positive interference in the FPIA for digoxin. We also demonstrated in our report that this interference can be completely eliminated by using the CLIA, again due to specificity of the antibody (10). In this work we report our findings on elimination of interference from spironolactone, canrenone, and potassium canrenoate in digoxin measurement by using the CLIA digoxin assay. Moreover, both negative interference in the MEIA and positive interference in the FPIA can be mostly eliminated by monitoring free digoxin.

MATERIALS AND METHODS

Spironolactone and potassium canrenoate were purchased from Sigma Chemical Company (St. Louis, MO). We prepared canrenone, the active metabolite of both potassium canrenoate and spironolactone, by acid catalyzed lactonization of potassium canrenoate using p-toluenesulfonic acid as a catalyst. The FPIA and MEIA for digoxin were purchased from Abbott Laboratories (Abbott Park, IL). The FPIA was run on a TDx/FLX analyzer and the MEIA was run using an AxSYM analyzer (Abbott Laboratories, Abbott Park, IL). The CLIA digoxin assay was run on an ACS:180 analyzer (Bayer Diagnostics, Tarrytown, NY). Centrifree Micropartition System ultrafiltration devices were obtained from Amicon Corporation (Danvers, MA).

The FPIA for digoxin requires a serum pretreatment whereby 200 µl of serum is treated with 200 µl of 50% sulfosalicylic acid in methanol. After protein precipitation, specimen is centrifuged at a high speed and protein-free supernatant is used for digoxin measurement. To avoid a matrix problem, we also treated protein-free ultrafiltrate of serum (for measuring free digoxin) with 50% sulfosalicylic acid. As expected, we observed no protein precipitation. The assay is linear up to a serum digoxin concentration of 5.0 ng/mL, and the sensitivity of the assay is 0.20 ng/mL. The MEIA digoxin assay requires no sample pretreatment. The assay is linear up to a serum digoxin concentration of 4.0 ng/mL, and the detection limit is 0.30 ng/mL. The CLIA digoxin assay also does not require any sample pretreatment. The assay is linear up to a serum digoxin concentration of 5.0 ng/mL, and the sensitivity of the assay is 0.15 ng/mL of serum digoxin concentration.

Aliquots of drug and digoxin-like immunoreactive substance (DLIS) free serum were supplemented with various concentrations of spironolactone, canrenone, and potassium canrenoate, and apparent digoxin concentrations were measured by FPIA, MEIA, and CLIA assays for digoxin. We also measured apparent digoxin activity in the protein-free ultrafiltrate prepared by centrifuging specimens with the Centrifree Micropartition System.

We routinely receive serum for therapeutic monitoring of digoxin in our clinical laboratory. After performing and reporting all results to the ordering clinician, we discard these specimens after 1 week. For this study, we prepared several serum pools containing various amounts of digoxin using the leftover specimens. Then we supplemented different aliquots of these digoxin pools with various amounts of spironolactone, canrenone, and potassium canrenoate. Digoxin concentrations were measured again using FPIA, MEIA, and CLIA, and the values were compared with the values observed in the original pool.

In separate experiments, aliquots of digoxin pool were supplemented with various concentrations of spironolactone and canrenone, or potassium canrenoate and canrenone. Then total and free digoxin concentrations were measured using either MEIA or FPIA. The objective of this experiment was to study whether

TABLE 1. Cross reactivity of spironolactone, potassium canrenoate, and canrenone with FPIA, MEIA, and CLIA digoxin assay

	Digoxin concentrations, ng/ml mean (SD), n = 3					
	FPIA		MEIA		CLIA	
Specimen	Total	Free	Total	Free	Total	Free
Spironolactone						
25 ng/ml	ND	ND	ND	ND	ND	ND
50 ng/ml	ND	ND	ND	ND	ND	ND
100 ng/ml	ND	ND	ND	ND	ND	ND
250 ng/ml	ND	ND	ND	ND	ND	ND
500 ng/ml	ND	ND	ND	ND	ND	ND
1000 ng/ml	0.27 (0.01)	ND	ND	ND	ND	ND
Potassium canrenoate						
100 ng/ml	ND	ND	ND	ND	ND	ND
250 ng/ml	ND	ND	ND	ND	ND	ND
500 ng/ml	0.29 (0.01)	ND	ND	ND	ND	ND
1000 ng/ml	0.42 (0.02)	ND	ND	ND	ND	ND
2000 ng/ml	0.60 (0.02)	ND	ND	ND	ND	ND
Canrenone						
100 ng/ml	ND	ND	ND	ND	ND	ND
250 ng/ml	0.20 (0.01)	ND	ND	ND	ND	ND
500 ng/ml	0.34 (0.02)	ND	ND	ND	ND	ND
1000 ng/ml	0.65 (0.03)	ND	ND	ND	ND	ND
2000 ng/ml	0.89 (0.02)	ND	ND	ND	ND	ND

ND, none detected, below the sensitivity of the assay.

interference of spironolactone, canrenone, and potassium canrenoate in the FPIA and MEIA can be eliminated by monitoring free digoxin concentrations. Free digoxin concentrations were measured in a protein-free ultrafiltrate prepared by centrifuging serum in a Centrifree Micropartition System for 20 min at 1,500 *g*.

Statistical analysis was done using an independent, two-tailed *t*-test. We considered a difference significant only at a 95% confidence interval or higher.

RESULTS

We observed low interference of spironolactone with the FPIA, as evidenced by a low apparent digoxin concentration of 0.27 ng/mL when the concentration of spironolactone was 1,000 ng/mL, the highest concentration studied. We observed no apparent digoxin activity with MEIA and CLIA, even when an aliquot of serum was supplemented with 1,000 ng/mL of spironolactone. We observed higher cross-reactivity of potassium canrenoate and canrenone with the FPIA, while no apparent digoxin activity was observed with either MEIA or CLIA. In addition, we observed no apparent digoxin level in the protein-free ultrafiltrate with any assay, as expected from high protein binding of spironolactone, canrenone, and potassium canrenoate (Table 1). The concentration of spironolactone, canrenone, and potassium canrenoate chosen for this study was based on the expected concentration after ingestion of spironolactone or intravenous administration of potassium canrenoate. Okazaki et al. (7) used similar concentrations for their in vitro study demonstrating cross-reactivity of spironolactone and potassium canrenoate with the FPIA digoxin assay.

Rigorous characterization of interference in an immunoassay due to a cross-reactant should be performed in the presence of the primary analyte (11). However, in most studies, cross-reactants are added to ligand-free sera. Conceptually, a cross-reactant should lead to a positive interference in an immunoassay, thus falsely elevating the concentration of the primary ligand. We added various amounts of spironolactone, canrenone, and potassium canrenoate in two different serum pools containing digoxin. Then total digoxin concentrations were measured using FPIA, MEIA, and CLIA. We observed falsely elevated digoxin concentrations using FPIA with spironolactone, canrenone, and potassium canrenoate. Spironolactone showed less interference than canrenone and potassium canrenoate. In contrast, we observed falsely lowered digoxin values when MEIA was used for measuring digoxin concentrations. For example, the original digoxin concentration in digoxin pool 1 was 1.37 ng/mL. In the presence of 1,000 ng/mL of spironolactone, the digoxin value was falsely elevated to 1.58 ng/mL. In the presence of 1,000 ng/mL of canrenone, the observed digoxin value was 1.74 ng/mL

TABLE 2. Effect of spironolactone, potassium canrenoate, and canrenone on serum digoxin concentrations as measured by FPIA, MEIA, and CLIA

	Digoxin concentration, ng/ml mean (SD), n = 3		
Specimen	FPIA	MEIA	CLIA
Digoxin pool 1	1.37 (0.09)	1.32 (0.03)	1.31 (0.08)
+ 500 ng/ml; Spironolactone	1.44 (0.02)	1.15 (0.06)[b]	1.33 (0.03)
+ 1000 ng/ml; Spironolactone	1.58 (0.10)[a]	1.08 (0.03)[b]	1.30 (0.02)
+ 1000 ng/ml; K-Canrenoate	1.72 (0.11)[a]	1.06 (0.04)[b]	1.27 (0.04)
+ 2000 ng/ml; K-Canrenoate	1.84 (0.08)[a]	1.04 (0.03)[b]	1.28 (0.03)
+ 1000 ng/ml; Canrenone	1.74 (0.04)[a]	1.10 (0.03)[b]	1.26 (0.08)
+ 2000 ng/ml; Canrenone	2.02 (0.04)[a]	1.06 (0.06)[b]	1.25 (0.08)
Digoxin pool 2	0.40 (0.02)	0.41 (0.01)	0.36 (0.04)
+ 500 ng/ml; Spironolactone	0.47 (0.03)[a]	0.39 (0.02)	0.35 (0.03)
+ 1000 ng/ml; Spironolactone	0.62 (0.01)[a]	0.37 (0.01)[b]	0.33 (0.04)
+ 1000 ng/ml; K-Canrenoate	0.67 (0.07)[a]	0.34 (0.02)[b]	0.34 (0.02)
+ 2000 ng/ml; K-Canrenoate	0.88 (0.04)[a]	0.33 (0.03)[b]	0.35 (0.02)
+ 1000 ng/ml; Canrenone	0.67 (0.02)[a]	0.36 (0.02)[b]	0.35 (0.02)
+ 2000 ng/ml; Canrenone	1.02 (0.03)[a]	0.31 (0.02)[b]	0.34 90.02

[a]Significantly greater than the control value by independent t-test, two ailed ($P < 0.05$).
[b]Significantly less than the control value by independent t-test, two-tailed ($P < 0.05$).

using FPIA. In contrast, the observed digoxin values were 1.32 ng/mL (control), 1.15 ng/mL (1,000 ng/mL spironolactone), and 1.10 ng/mL (1,000 ng/mL canrenone) when measured by MEIA. Interestingly, the corresponding digoxin concentrations did not change when CLIA was used. The control value was 1.31 ng/mL. In the presence of 1,000 ng/mL of spironolactone, the observed digoxin concentration was 1.30 ng/mL. In the presence of 1,000 ng/mL of canrenone, the observed digoxin value was 1.26 ng/mL, which was not statistically different from the control value of 1.31 ng/mL (Table 2).

Spironolactone, canrenone, and potassium canrenoate are strongly bound to serum proteins. Therefore, we explored the possibility of eliminating such interference using an ultrafiltration technique. We prepared other serum pools from patients receiving digoxin and supplemented aliquots of the pool with various concentrations of these compounds. Then total and free digoxin concentrations were measured using the FPIA or MEIA digoxin assay. Because the CLIA is free from such interference, free digoxin monitoring is not required.

When a digoxin pool was supplemented with spironolactone and canrenone, we observed a modest change in total digoxin concentration in the presence of 100 ng/mL of spironolactone, and 250 ng/mL of canrenone using FPIA. However, free digoxin concentration did not change significantly, indicating that interference of spironolactone and canrenone in the FPIA can be eliminated by monitoring free digoxin concentration. With a very high concentration of spironolactone and canrenone, the free concentration also increased significantly (Table 3), but such high concentrations are unlikely in a clinical situation. Similarly, with the MEIA the concentration of total digoxin also dropped modestly in the presence of 100 ng/mL of spironolactone and 250 ng/mL of canrenone, but the free digoxin concentrations did not change from the control value. In the presence of a very high concentration of spironolactone (200 ng/mL) and canrenone (500 ng/mL), the total digoxin dropped more significantly than the free digoxin (Table 3). In the presence of a moderate amount of potassium canrenoate and canrenone, the total digoxin concentration increased significantly when measured by the FPIA. Fortunately, free digoxin concentration did not change significantly, indicating that monitoring free digoxin (Table 3) can eliminate such interference. However, in the presence of a high concentration of potassium canrenoate and canrenone, monitoring free digoxin concentration cannot eliminate this interference.

DISCUSSION

Oral administration of 100 mg spironolactone usually led to a peak serum spironolactone concentration of 83 ng/mL, and a peak canrenone concentration of 202 ng/mL (12). A previous study (13) reported a mean peak canrenone concentration of 177 ng/mL after oral administration of spironolactone. After intravenous administration of potassium canrenoate, the peak plasma canrenone concentration was 2,066 ng/mL. However, the peak canrenone concentration can be as low as 1,117 ng/mL (13). Sadee et al. (14) studied the pharmacokinetics of spironolactone, canrenone, and

TABLE 3. Total and free digoxin concentrations in digoxin serum pools prepared from patients receiving digoxin and further supplemented with spironolactone, canrenone, and potassium canrenoate

	Digoxin concentrations, mean (SD), n = 3			
Specimen[a]	Total (FPIA)	Total (MEIA)	Free (FPIA)	Free (MEIA)
Digoxin pool 3	1.48 (0.02)	1.45 (0.03)	1.17 (0.04)	1.12 (0.05)
+ 50 S and 100 C	1.56 (0.01)[b]	1.35 (0.02)[c]	1.19 (0.02)	1.10 (0.02)
+ 100 S and 250 C	1.59 (0.03)[b]	1.30 (0.03)[c]	1.23 (0.06)	1.08 (0.02)
+ 200 S and 500 C	1.66 (0.01)[b]	1.14 (0.04)[c]	1.34 (0.02)b	1.02 (0.03)[c]
Digoxin pool 4	2.10 (0.03)	NP	1.67 (0.02)	NP
+ 100 KC and 200 C	2.34 (0.02)[b]	NP	1.69 (0.03)	NP
+ 250 KC and 500 C	2.51 (0.04)[b]	NP	1.70 (0.03)	NP
+ 1000 KC and 2000 C	2.78 (0.06)[b]	NP	1.82 (0.04)[b]	NP
Digoxin pool 5	NP	1.80 (0.02)	NP	1.23 (0.04)
+ 100 KC + 200 C	NP	1.71 (0.01)[c]	NP	1.24 (0.02)
+ 250 KC + 500 C	NP	1.60 (0.02)[c]	NP	1.22 (0.02)
+ 1000 KC and 2000 C	NP	1.24 (0.06)[c]	NP	1.10 (0.01)[c]

[a]50 S and 100 C (spironolactone 50 ng/ml, canrenone 100 ng/ml); 100 S and 250 C (spironolactone, 100 ng/ml, canrenone 250 ng/ml); 200 S and 5000 C (spironolactone, 200 ng/ml, canrenone, 500 ng/ml); 100 KC and 200 C (potassium canrenoate 100 ng/ml, canrenone, 200 ng/ml); 250 KC and 500 C (potassium canrenoate, 250 ng/ml, canrenone, 500 ng/ml); 1000 KC and 2000 C (potassium canrenoate, 1000 ng/ml, canrenone, 2000 ng/ml).
[b]Significantly greater than the control value by independent t-test, two tailed ($P < 0.05$).
[c]Significantly less than the control value by independent t-test, two tailed ($P < 0.05$).
NP, not performed.

potassium canrenoate in humans. They used either an oral dose of 400 mg of spironolactone or an intravenous dose of 380 mg of potassium canrenoate. Although the 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 (14).

Our results demonstrated that oral administration of spironolactone in a standard dose of a 100-mg tablet should not have a very significant effect on serum digoxin measurement using FPIA or MEIA. Nevertheless, such interference can be completely eliminated by monitoring free digoxin or using a CLIA digoxin assay. On the other hand, intravenous injection of potassium canrenoate may have a very significant effect on serum digoxin measurement. However, this interference can be completely eliminated by using CLIA. This assay uses a specific monoclonal antibody and is more specific for digoxin measurement compared to FPIA and MEIA, which both use polyclonal antibody. Other investigators also reported that the CLIA is free from interference from endogenous digoxin-like immunoreactive substances, whereas FPIA may lead to falsely increased digoxin levels and MEIA may show falsely lower digoxin values (15–17).

The positive interference of spironolactone, canrenone, and potassium canrenoate in the FPIA digoxin assay, and negative interference in the MEIA are due to different assay designs. Jortani et al. (18) first demonstrated that, based on assay architecture (i.e., a wash step or its absence), the same antibody may provide a positive or negative cross-reactivity of the interfering substance in the presence of the primary analyte. While most cross-reactive substances in a competitive immunoassay cause a positive interference, negative interferences caused apparently by the specific architecture of an immunoassay have been reported (9,10). Negative interference has been found when the bound ligand is first separated from the free ligand by a wash step, followed by binding of the tracer (label) and then generation of the signal. The MEIA assays on the AxSYM analyzer follow this architecture. A plausible mechanism of this phenomenon could be that during the first incubation of the sample and capture antibody, both the primary analyte and the interfering substance bind with the capture antibody. During the subsequent wash step, followed by incubation with the tracer substance, the more loosely bound cross-reacting substance dissociates, allowing the generation of falsely elevated signal. Because signal is inversely proportional to the concentration, a falsely low value of the primary analyte can be observed. In assay architecture such as in the FPIA, where there is no wash step to separate bound and free ligand, the cross-reactants bound to antibody prevent binding of the tracer compound, resulting in decreased signal. Again, a decreased signal leads to a falsely increased value of primary analyte due to the inverse relationship between signal and observed concentration.

Taking advantage of the strong protein binding of canrenone and potassium canrenoate, we demonstrated

that interference of canrenone in a digoxin assay could mostly be eliminated by an ultrafiltration technique. We observed no apparent digoxin concentration in the protein-free ultrafiltrate, even when aliquots of drug and DLIS-free serum pool were supplemented with high concentrations of canrenone. Moreover, when we supplemented digoxin serum with potassium canrenoate and canrenone, the free digoxin concentrations were minimally affected, indicating that the interference from the expected steady-state concentration of potassium canrenoate and canrenone can be eliminated by monitoring free digoxin. However, if digoxin is monitored less than 4 hr after injection of potassium canrenoate, during which time the concentration of potassium canrenoate can be as high as 1,000 ng and the concentration of canrenone as high as 2,000 ng/mL, free digoxin measurement is also subject to interference. Therefore, it is recommended that CLIA be used to avoid any interference under such circumstances.

We conclude that interference of spironolactone, potassium canrenoate, and canrenone in serum digoxin monitoring can be easily eliminated by using the CLIA. Alternatively, monitoring free digoxin concentration can also mostly eliminate such interference.

REFERENCES

1. Huffman DH. The effects of spironolactone and canrenone in digoxin radioimmunoassay. Res Commun Chem Pathol Pharmacol 1974;9:787–790.
2. Lichey J, Rietrock N, Borner K. The influence of intravenous canrenoate on the determination of digoxin in serum by radio and enzyme immunoassay. Int J Clin Pharmacol Biopharmacol 1979;17:61–63.
3. Silber B, Sheiner LB, Powers JL, Winter ME, Sadee W. Spironolactone-associated digoxin radioimmunoassay interference. Clin Chem 1979;25:48–50.
4. 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.
5. 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.
6. Foukaridis GN. Influence of spironolactone and its metabolite canrenone on serum digoxin assays. Ther Drug Monit 1990; 12:82–84.
7. 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.
8. Steimer W, Muller C, Eber B, Emmanuillidis K. Intoxication due to negative canrenone interference in digoxin drug monitoring [letter]. Lancet 199;354;1176–1177.
9. Datta P, Dasgupta A. Bidirectional (positive/negative) interference in digoxin immunoassays: importance of antibody specificity. Ther Drug Monit 1998;20:352–357.
10. Dasgupta A, Biddle D, Wells A, Datta P. Positive and negative interference of Chinese medicine Chan Su in serum digoxin measurement: elimination of interference by using a monoclonal chemiluminescent digoxin assay or monitoring free digoxin. Am J Clin Pathol 2000;114:174–179.
11. Miller JJ, Valdes R. Methods for calculating cross reactivity in immunoassays. J Clin Immunoassay 1992;15:97–100.
12. Gardiner P, Schrode K, Quinlan D, Martin B, et al. Spironolactone metabolism: steady state serum levels of the sulfur containing metabolites. J Clin Pharmacol 1989;29:342–347.
13. 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.
14. Sadee W, Dagcioglu M, Schroder R. Pharmacokinetics of spironolactone, canrenone and canrenoate-K in humans. J Pharmacol Exp Ther 1973;185:686–695.
15. Miller JJ, Straub RW, Valdes R. Analytical performance of a monoclonal digoxin assay with increased specificity on the ACS:180. Ther Drug Monit 1996;18:65–72.
16. Dasgupta A, Schammel D, Limmany A, Datta P. Estimating concentration of total digoxin and digoxin-like immunoreactive substances in volume expanded patients being treated with digoxin. Ther Drug Monit 1996;18:34–39.
17. Dasgupta A, Trejo O. Suppression of total digoxin concentrations by digoxin-like immunoreactive substances in the MEIA digoxin assay: elimination of negative interference by monitoring free digoxin concentrations. Am J Clin Pathol 1999;111:406–410.
18. Jortani SA, Miller JJ, Helm RA, Johnson, Valdes R. Suppression of immunoassay results by cross reactivity. J Clin Ligand Assay 1997;20:177–179.