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BRIEF REPORT

WILEY

Convallatoxin, the active cardiac glycoside of lily of the valley, minimally affects the ADVIA Centaur digoxin assay

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Objective: Lily of the valley is a poisonous plant due to the presence of the cardiac glycoside convallatoxin which is known to interfere with serum digoxin measurement using the LOCI digoxin assay and other digoxin assays. We evaluated potential interference of convallatoxin as well as extract of lily of the valley with the ADVIA Centaur digoxin assay by comparing results obtained using the LOCI digoxin assay.

Materials and Methods: Aliquots of a drug-free serum pool and a digoxin serum pool were supplemented with nanograms to 1 μg quantities of convallatoxin or 1.0 and 2.5 μL of lily of the valley extract per milliliter of serum followed by measurement of digoxin concentrations using the LOCI and ADVIA Centaur digoxin assays.

Results: Apparent digoxin concentrations were minimal using the ADVIA Centaur digoxin assay when aliquots of drug-free serum were supplemented with convallatoxin or extract of lily of the valley but apparent digoxin levels were very high using the LOCI digoxin assay. Moreover, minimal interference in serum digoxin measurement using the ADVIA Centaur digoxin assay was observed when aliquots of serum digoxin pool were further supplemented with lily of the valley extract. As expected, the LOCI digoxin assay showed significant interference of convallatoxin in serum digoxin measurement.

Conclusions: Significant interference of convallatoxin in serum digoxin measurement using the LOCI digoxin assay could be minimized using the ADVIA Centaur digoxin assay.

KEYWORDS

ADVIA Centaur digoxin assay, convallatoxin, lily of the valley, LOCI digoxin assay

1 | INTRODUCTION

The lily family is composed of 280-300 genera made up of 4000-4600 different species but only 90 genera representing approximately 525 species are found in North America. Lilies are popular decorative plants and are also found in floral arrangements. The *Convallaria genus*, commonly called "lily of the valley," is used for decoration in the United States.[1] Lily of the valley usually grows in the cooler climate of North America and also in Europe and certain parts of Asia. The entire plant is toxic, containing cardiac glycosides causing digitalis-like toxicity. The principle toxic cardiac glycoside found in lily of the valley is convallatoxin. There are several reports of lily of the valley poisoning.[2] Symptoms of digitalis-like toxicity in a family after accidental ingestion of lily of the valley plant has also been reported.[3] Lethal poisoning of lily of the valley ingestion in a dog has also been reported.[4] Lily of the valley extract is used in herbal medicine despite known toxicity of the plant since the 16th century and is still used as a component in many herbal extracts as a tonic for heart.[5] Convallatoxin, the major cardiac glycoside present in lily of the valley, has digitalis-like properties by inhibition of the Na, K-ATPase thereby causing a positive inotropic effect.[6]

Convallatoxin has structural similarity with digoxin, and we reported earlier rapid detection of convallatoxin in human serum using luminescent oxygen channeling technology-based digoxin

assay (LOCI Digoxin assay).[7] More recently, we reported that the iDigoxin assay on the Architect i1000 analyzer marketed by Abbott Laboratories is more sensitive than the LOCI digoxin assay in detecting the presence of convallatoxin.[8] Fink et al[9] also compared 5 digoxin assays for rapid detection of convallatoxin. However, any digoxin immunoassay capable of rapid detection of convallatoxin is also unsuitable for therapeutic drug monitoring of digoxin in patients who are taking lily of the valley herbal supplements.

Recently, in our laboratory, ADVIA Centaur digoxin assay is available. We compared interference of convallatoxin with the ADVIA Centaur digoxin assay by comparing results obtained using the LOCI digoxin assay. The potential interference of convallatoxin in the ADVIA Centaur digoxin assay has never been reported before.

2 | MATERIALS AND METHODS

Convallatoxin was purchased from the Sigma Chemical Company (St. Louis, MO). Lily of the valley herbal extract was obtained from a local herbal store in Houston, TX. The LOCI digoxin assay kits were purchased from Siemens Healthcare (Deerfield, IL) and assays were run on a Vista 1500 auto analyzer (Siemens Healthcare) following the manufacturer's protocol. The LOCI Digoxin assay utilizes a specific mouse monoclonal antibody against digoxin and the analytical measurement range of this assay is from 0.06 to 5.0 ng mL^{-1} of serum digoxin concentration. The ADVIA Centaur digoxin assay was also obtained from Siemens Healthcare and digoxin assays were run on the ADVIA Centaur analyzer. The ADVIA Centaur digoxin assay is a competitive immunoassay using direct chemiluminescent technology. The assay utilizes mouse monoclonal anti-digoxin antibody. The analytical measurement range of this assay is .1-5.0 ng mL^{-1}. Standard solutions of convallatoxin were made in absolute ethanol, followed by further dilution with ethanol to prepare working standards.

Aliquots of digoxin-free serum pools were supplemented with either convallatoxin (10-1000 ng mL^{-1}), or liquid extract of lily of the valley (1.0 μL mL^{-1} or 2.5 μL mL^{-1}). Microliter quantities of convallatoxin ethanol working solution or lily of the valley extract were added to dry test tubes followed by evaporation of the solvent under a gentle stream of nitrogen. Then dry residue was reconstitution with an aliquot of drug-free serum. The apparent digoxin concentrations were then measured using the LOCI digoxin assay and the ADVIA Centaur digoxin assay. Each measurement was performed in triplicate and results were expressed as the mean and 1 SD.

One digoxin serum pool was prepared by combining de-identified serum specimens from patients receiving digoxin that were submitted for therapeutic drug monitoring to our clinical laboratory. The left-over specimens were used after performing and reporting all results to the ordering clinicians, and after holding these specimens for 1 week as required by our laboratory protocol and according to guidelines of the University of Texas-McGovern Medical School at Houston Institutional Review Board. Aliquots of the digoxin pool were also supplemented with various amounts of convallatoxin or lily of the valley extract, with measurement of digoxin concentrations before and after supplementation using both the LOCI and the ADVIA Centaur digoxin assay.

Statistical analyses were performed using the two-tailed student *t* test. A statistically significant difference was considered at 95% confidence interval or higher ($P < .05$).

3 | RESULTS

Significant apparent digoxin concentrations were observed when aliquots of drug-free serum pool were supplemented with either convallatoxin or liquid extract of lily of the valley and the LOCI digoxin assay was used for measurement. In contrast, observed apparent digoxin concentrations were relatively low using the ADVIA Centaur digoxin assay. For example, no apparent digoxin concentration was observed when aliquots of drug-free serum pool were supplemented with up to 100 ng of convallatoxin per milliliter of serum. In contrast, the observed apparent digoxin concentration was 1.08 ng mL^{-1} using the LOCI digoxin assay. Although measurable apparent digoxin levels (>.1 ng mL^{-1}) were observed when aliquots of the drug-free serum pool were supplemented with higher concentration of convallatoxin or lily of the valley extract, observed apparent digoxin levels were relatively low using the ADVIA Centaur digoxin assay. For example, highest apparent digoxin concentration of .24 ng mL^{-1} was observed using the ADVIA Centaur digoxin assay when an aliquot of the drug-free serum pool was supplemented with lily of the valley extract to achieve a final concentration of 2.5 μL of extract per milliliter of serum, the corresponding apparent digoxin value was 5.85 ng mL^{-1} using the LOCI digoxin assay (Table 1).

Because cross-reactivity of a substance should be tested in the presence of the primary analyte,[10] the effect of adding

TABLE 1 Effect of supplementing aliquots of drug-free serum pool with convallatoxin or lily of the valley extract on the LOCI digoxin and ADVIA Centaur digoxin assay

	Apparent Digoxin, ng mL^{-1}, Mean (SD), n = 3	
Specimen	LOCI Digoxin	ADVIA Centaur Digoxin
Drug-free serum	None detected	None detected
+10 ng mL^{-1} convallatoxin	0.26 (0.01)	None detected
+50 ng mL^{-1} convallatoxin	0.68 (0.01)	None detected
+100 ng mL^{-1} convallatoxin	1.08 (0.02)	None detected
+250 ng mL^{-1} convallatoxin	1.98 (0.03)	0.12 (0.04)
+500 ng mL^{-1} convallatoxin	3.04 (0.03)	0.12 (0.03)
+1000 ng mL^{-1} convallatoxin	4.48 (0.03)	0.21 (0.04)
+1.0 μL mL^{-1} Lily of the valley herb extract	3.42 (0.007)	0.18 (0.02)
+ 2.5 μL mL^{-1} Lily of the valley herb extract	5.85 (0.15)	0.24 (0.02)

convallatoxin or lily of the valley extract to aliquots of a digoxin pool to test cross reactivity of convallatoxin in the presence of the primary analyte digoxin was also investigated. Bi-directional (negative interference with smaller amounts of convallatoxin but positive interference with higher amounts of convallatoxin) interference of convallatoxin with serum digoxin measurement using the LOCI digoxin assay was observed. This observation is consistent with our previous report.[7] However, the ADVIA Centaur digoxin showed very little interference in serum digoxin measurement in the presence of convallatoxin or lily of the valley extract. We observed no statistically significant change in digoxin values when aliquots of digoxin pool were supplemented with convallatoxin. For example, in the presence of 1000 ng mL^{-1} of convallatoxin, the serum digoxin concentration was 0.99 ng mL^{-1} which was not statistically different from the baseline digoxin value of 1.05 ng mL^{-1}. In contrast, when the digoxin concentration was measured using the LOCI digoxin assay, the value was 4.37 ng mL^{-1} (baseline value was 1.01 ng mL^{-1}). However, in the presence of 2.5 μL of lily of the valley extract per milliliter of digoxin pool, the digoxin value was increased to 1.17 ng mL^{-1}, a value significantly higher than baseline value of 1.05 ng mL^{-1} by independent *t* test two tailed, but the increase in digoxin value was only 11.4%. In contrast, the observed digoxin concentration was 6.05 ng mL^{-1} using the LOCI digoxin assay, a fivefold increase from the baseline value (Table 2).

In general, bias over 20% (positive or negative) is considered clinically significant according to the criterion defined by the College of American Pathologists. Therefore, interference observed in the digoxin assay using the ADVIA Centaur assay may not be clinically significant.

TABLE 2 Effect of supplementing aliquots of digoxin pool with convallatoxin or lily of the valley extract on serum digoxin measurement using the LOCI digoxin assay and the ADVIA Centaur digoxin assay

	Apparent Digoxin, ng mL^{-1}, Mean (SD), n = 3	
Specimen	LOCI digoxin	ADVIA Centaur digoxin
Digoxin pool	1.01 (0.03)	1.05 (0.04)
+10 ng mL^{-1} convallatoxin	0.87 (0.01)*	1.04 (0.04)
+50 ng mL^{-1} convallatoxin	1.12 (0.02)**	1.08 (0.03)
+100 ng mL^{-1} convallatoxin	1.40 (0.02)**	1.05 (0.03)
+250 ng mL^{-1} convallatoxin	2.08 (0.01)**	1.08 (0.06)
+500 ng mL^{-1} convallatoxin	3.10 (0.03)**	1.02 (0.03)
+1000 ng mL^{-1} convallatoxin	4.37 (0.04)**	0.99 (0.06)
+1.0 μL mL^{-1} Lily of the valley herb extract	3.94 (0.02)**	1.02 (0.03)
+2.5 μL mL^{-1} Lily of the valley herb extract	6.05 (0.03)**	1.17 (0.04)**

*Significantly less than digoxin value of the digoxin pool by independent *t* test, two tailed ($P < .05$).

**Significantly greater than the value of the digoxin pool by independent *t* test, two tailed ($P < .05$).

4 | DISCUSSION

Accidental poisoning by lily of the valley in both animals and humans has been reported. In addition, it is possible that a person taking digoxin may also take lily of the valley herbal supplement due to increased and widespread use of herbal remedies. Dietary supplement is approximately $30 billion industry in the United States and based on recent surveys, 52% adults reported use of at least 1 supplement and 10% reported using at least 4 products.[11] Lily of the valley extract is available in health food store without prescription. Convallatoxin can only be analyzed using chromatographic methods. Therefore, indirect determination of lily of the valley poisoning using a digoxin immunoassay may have clinical application.

Exact concentration of convallatoxin after toxic ingestion of lily of the valley plant or herbal extract is not known. Carlier et al[12] developed an ultra-high performance liquid chromatography combined with tandem mass spectrometry for analysis of 34 toxic compounds from plants including convallatoxin and commented that analytical measurement range of 5-1000 ng mL^{-1} is adequate for analysis of these toxins including forensic application. We selected 10-1000 ng mL^{-1} concentration of convallatoxin for our study. Alexandre et al[2] reported case of an 87-year-old woman who arrived at the hospital 2 hours after accidental poisoning with lily of the valley. She showed symptoms of digitalis toxicity but apparent digoxin level was within therapeutic range. The patient was discharged 24 hours after admission with no apparent toxicity. The authors did not mention analytical method used for digoxin measurement but studies have shown that convallatoxin concentrations between 50 and 100 ng mL^{-1} are associated with apparent digoxin concentrations within therapeutic range using digoxin immunoassays.[7-9] Therefore, convallatoxin concentration range we studied would include expected in vivo convallatoxin concentrations mimicking both accidental exposure and sever poisoning. Moreover, the package insert of lily of the valley herbal supplement we used for the study recommends taking 1-2 mL of extract. Assuming normal blood volume of 5 L, if 2 mL of extract is absorbed 100%, then expected concentration of lily of the valley extract should be .4 μL mL^{-1} in blood. We used 1.0 and 2.5 μL of lily of the valley extract to 1 mL of serum to mimic expected in vivo concentration to reflect potential toxic ingestion. Our goal was to compare effectiveness of LOCI digoxin assay and ADVIA Centaur digoxin in rapidly detecting poisoning with lily of the valley indirectly using digoxin immunoassay. Our results clearly indicate that LOCI digoxin assay is effective in detecting convallatoxin in serum but the ADVIA Centaur digoxin assay is not suitable for that purpose.

However, the bigger problem is when a person taking digoxin may also take lily of the valley extract because this herbal supplement is marketed as a heart tonic. Digoxin is a cardiac glycoside with a narrow therapeutic range. Therefore, it is important to

accurately measure serum digoxin level in patients taking digoxin. Immunoassays are widely used for digoxin measurement in clinical laboratories but digoxin assays also suffer from various interferences.[13,14] Our results indicate that the LOCI digoxin assay is not suitable for therapeutic drug monitoring of digoxin in patients taking lily of the valley extract. However, due to minimal interference of convallatoxin in the ADVIA Centaur assay, it may be used for therapeutic drug monitoring of digoxin in patients receiving lily of the valley extract.

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