Endogenous and Exogenous Digoxin-like Immunoreactive Substances

Impact on Therapeutic Drug Monitoring of Digoxin

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Key Words: Digoxin; Digoxin-like immunoreactive substances; DLIS; Chinese medicines; Spironolactone; Free digoxin

Abstract

Endogenous digoxin-like immunoreactive substance (DLIS) was first reported in volume-expanded dogs. Its presence has been confirmed in blood, urine, and other body fluids. Elevated DLIS concentrations are encountered in patients with volume-expanded conditions such as uremia, essential hypertension, liver disease, and preeclampsia. DLISs cross-react with antidigoxin antibodies and falsely elevate serum digoxin concentrations, interfering in interpretation of results for therapeutic digoxin monitoring. Falsely lower digoxin values due to the presence of DLISs have been reported. The association of DLISs with volume expansion led to speculation that they could be natriuretic hormones. Several structures have been proposed for DLISs, including nonesterified fatty acid, phospholipid, lysophospholipid, bile acid, bile salt, and steroid. Exogenous DLISs can be found in serum after ingestion of various Chinese medicines and therapy with spironolactone, canrenone, or potassium canrenoate. Like endogenous DLISs, exogenous DLISs interfere with serum digoxin assays, complicating therapeutic digoxin monitoring. However, most reported endogenous and exogenous DLISs are strongly protein-bound while digoxin is weakly protein-bound. Therefore, interference of both endogenous and exogenous DLISs in serum digoxin measurement can be eliminated by monitoring digoxin concentrations in the protein-free ultrafiltrates.

Discovery of Endogenous Digoxin-like Immunoreactive Substances

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 by blocking the atrioventricular node. After the discovery of endorphins, the endogenous equivalent of opiates, there was a hypothesis for the presence of the endogenous equivalent of cardiac glycosides. It was further hypothesized that antidigoxin antibody may be able to detect the presence of digoxin-like immunoreactive substances (DLISs) in body fluids. Gruber et al1 first demonstrated the presence of endogenous DLISs in 1980, in volume-expanded dogs. Then Craver and Valdes2 reported an unexpected increase in the serum digoxin concentration in a patient with renal failure and already receiving digoxin. The apparent serum digoxin level was still present after discontinuation of digoxin therapy.2 Balzan et al3 also confirmed the presence of DLISs in human plasma and urine. DLISs were found in various human body fluids and tissues including cord blood, placenta, amniotic fluid, bile meconium, cerebrospinal fluid (CSF), and saliva.4,5 DLISs cross-react with antidigoxin antibodies and inhibit Na+, K+-adenosine triphosphatase (ATPase).

Detection of DLISs in Body Fluids

DLISs can be detected in serum and other body fluids by using commercial immunoassays for digoxin, taking advantage of the cross-reactivity of DLISs with antidigoxin antibody.
Apparent digoxin concentrations as detected by radioimmunoassay (RIA) digoxin assays had been reported by several investigators in patients not receiving digoxin. Some of those RIA digoxin assays later were discontinued owing to high interference from DLISs. Early reports also indicated cross-reactivity of DLISs with the fluorescence polarization immunoassay (FPIA) marketed by Abbott Laboratories, Abbott Park, IL. Although many investigators used commercially available digoxin assays for detecting DLISs in body fluid, other approaches also have been documented. Panesar used bufalin as an antigen and developed polyclonal antisera for detecting DLISs, and they also developed a Fab of the antidigoxin antibody–based enzyme immunoassay for detecting DLISs, and they also developed a Fab of the antidigoxin antibody–based enzyme immunoassay for this purpose. They concluded that a polyclonal antibody–based ouabain assay was more efficient for detecting DLISs in human blood. More specific high-performance liquid chromatographic techniques using reverse phase columns also had been used for detecting DLISs in biologic fluid. However, these techniques are time consuming and technically more difficult than automated immunoassays.

**Criteria for DLISs**

DLISs can be divided into 2 groups. One class of DLISs interferes only with digoxin immunoassays owing to their cross-reactivity with antidigoxin antibody. The other class of compounds inhibits or binds with Na+, K+-ATPase. These compounds also may cross-react with antidigoxin antibody. Because of the ability of DLISs to inhibit Na+, K+-ATPase, it was hypothesized that DLISs are natriuretic hormones. Several studies had been reported in which investigators took advantage of the digoxin-like immunoreactivity and Na+, K+-ATPase binding ability of DLISs to purify these compounds from intact cells or isolated receptors. These investigators also studied the biochemical and or physicochemical parameter of isolated DLISs.

**DLIS Concentrations in Serum Samples of Healthy People and in Disease**

**Healthy People**

DLIS concentrations in serum samples of subjects not taking any digoxin depend on the immunoassay used. The FPIA marketed by the Abbott Laboratories has substantial cross-reactivity with DLISs. However, even with the FPIA, the concentrations of DLISs in healthy people usually are below the detection limit of the instrument (0.2 ng/mL [0.3 nmol/L]). Many diseases can substantially increase the concentration of DLISs.

**Volume Expansion**

Volume expansion is a major cause of elevated DLIS concentrations in the blood. Elevated concentrations of DLISs have been reported in uremia, essential hypertension, hypertension of water volume expansion, liver disease, preeclampsia, liver and kidney transplantation, congestive heart failure, prematurity, and other conditions.

**Intensive Care Unit**

Howarth et al reported elevated DLIS concentrations in the plasma of patients in the intensive care unit. Although some patients showed hepatic or renal dysfunction, another 42 patients who showed elevated DLIS concentrations had neither hepatic nor renal dysfunction. The authors used an FPIA assay for measuring DLISs. The DLIS concentrations were 0.0 to 1.3 ng/mL (0.0-1.7 nmol/L) in 16 patients with coexisting hepatic and renal dysfunction, while 38 patients with hepatic dysfunction but normal renal function showed a range of DLIS concentrations of 0.0 to 0.6 ng/mL (0.0-0.8 nmol/L). Four patients with only renal dysfunction had DLIS concentrations between 0.0 and 0.2 ng/mL (0.0-0.3 nmol/L), and the remaining 42 patients had DLIS concentrations ranging from 0.0 to 0.3 ng/mL (0.0-0.4 nmol/L).

**DLIS Concentrations in Disease and the Therapeutic Index of Digoxin**

The concentrations of DLISs found in these patients were significant given the narrow therapeutic range of digoxin (0.8-1.9 ng/mL [1.0-2.4 nmol/L]). The therapeutic range of digoxin in patients with congestive heart failure is even lower (0.8-1.5 ng/mL [1.0-1.9 nmol/L]). This group of patients also demonstrated elevated DLIS concentrations in serum. Miller et al reported a DLIS concentration of 0.9 ng/mL (1.1 nmol/L) in a patient not receiving digoxin. A DLIS concentration of 1.2 ng/mL (1.5 nmol/L) in a patient with liver failure but who had received no digoxin also has been reported. Logoglu et al used an RIA (double-antibody RIA) for detecting DLISs in serum samples of patients with normal and preeclamptic pregnancies. The mean DLIS concentration in the normotensive group (n = 14) was 0.3 ng/mL (0.4 nmol/L), while the mean was 0.3 ng/mL (0.4 nmol/L) in the preeclamptic group (n = 17). The authors concluded that there was no statistical difference between DLIS concentrations in these 2 groups. Doolittle et al described a patient in whom the serum digoxin concentration increased from 2.5 ng/mL (3.2 nmol/L) to 9.3 ng/mL (11.9 nmol/L) 2 days after cardiac surgery; the patient also had experienced a cardiac arrest. The digoxin concentration...
apparent digoxin concentrations in 57% of the patients when using the RIA (range, 0.2-0.6 ng/mL [0.3-0.8 nmol/L]), in 55% of the patients when using the FPIA (range, 0.2-1.6 ng/mL [0.3-2.0 nmol/L]), and in only 28% of the patients when using the fluorometric assay (range, 0.2-0.4 ng/mL [0.3-0.5 nmol/L]) and concluded that FPIA and RIA digoxin assays were more susceptible to DLIS interference. The authors also showed discrepant digoxin values (>2 Sy/ Sx from the regression line). These patients had renal disease or hepatic disease. The discrepant digoxin values always were lower with the CLIA than with other digoxin assays. The authors concluded that the CLIA digoxin assay on the ACS:180 analyzer showed almost no interference from DLISs compared with an RIA (Magic, Ciba-Corning) assay.

Miller et al studied analytic performance of the CLIA digoxin assay on the ACS:180 analyzer (originally Ciba-Corning, currently marketed by Bayer Diagnostics) by comparing this assay with the FPIA (Digoxin II), Stratus II digoxin assay (Dade Behring), and an RIA digoxin assay (Magic). The authors detected no DLISs in serum samples using the CLIA, but measurable concentrations of DLIS were observed with the FPIA, Stratus II digoxin assay, and the RIA method. The authors also compared digoxin levels in 121 serum samples from 49 patients and observed comparable values with all digoxin assays. However, 11 patients showed discrepant digoxin values (>2 Sy/Sx from the regression line). These patients had renal disease or hepatic disease. The discrepant digoxin values always were lower with the CLIA than with other digoxin assays. The authors concluded that the CLIA digoxin assay on the ACS:180 analyzer had improved the specificity for digoxin measurement.

Way et al evaluated the Vitros digoxin assay (Johnson and Johnson, Rochester, NY) for interference from DLISs. The Vitros digoxin assay is an enzymatic heterogeneous competitive immunoassay that uses dry-slide technology. The authors compared this assay with the OnLine digoxin assay (Roche, Indianapolis, IN), which is a homogeneous microparticle immunoassay based on the aggregation of digoxin-coated microparticles in the presence of antidigoxin antibody. Digoxin in the specimen partly inhibits the aggregation, and, thus, the rate of aggregation (as measured by light scattering) is inversely proportional to the digoxin concentration. The authors also used a microparticle enzyme immunoassay (MEIA; Abbott Laboratories) that uses a digoxin–alkaline phosphatase conjugate and 4-methylumbelliferyl as a substrate. The authors compared 3 digoxin assays using 26 adult patients receiving digoxin and observed mean ± SD digoxin concentrations of 1.3 ± 0.7 ng/mL (1.7 ± 0.9 nmol/L) with the Roche assay, 1.3 ± 0.6 ng/mL (1.7 ± 0.8 nmol/L) with the Abbott assay, and 1.5 ± 0.7 ng/mL (1.9 ± 0.9 nmol/L) with the Vitros assay. The mean digoxin concentrations found by using the Vitros assay were significantly different by the Student t test. To study potential interference of DLISs (suspected causes of such discrepancy) in these assays, the authors added known amounts of digoxin in serum samples prepared from newborns (high DLIS concentrations) and adults (no DLISs). The samples from newborns showed a mean digoxin level of 0.4 ng/mL (0.5 nmol/L) by the Roche method and 0.7 ng/mL (0.9 nmol/L) by the Vitros assay. The specimens from adults showed a mean value of 0.7 ng/mL (0.9 nmol/L) by the Roche method and 0.8 ng/mL (1.0 nmol/L) by the Vitros assay. The authors concluded that the positive bias in the Vitros assay compared with the Roche OnLine assay was probably due to DLISs.

Bonagura et al reported high specificity of the Roche OnLine assay for digoxin, which had no cross-reactivity with DLISs and negligible cross-reactivity with the noncardioactive metabolites of digoxin. Marzullo et al reported that the EMIT 2000 (Dade Behring) digoxin immunoassay and the Roche OnLine digoxin immunoassay were least affected by DLISs compared with other digoxin assays. Sacco et al also confirmed improved specificity of the EMIT 2000 digoxin assay and very low cross-reactivity from DLISs compared with the FPIA digoxin assay and concluded that the EMIT 2000 had adequate specificity, sensitivity, precision, and accuracy for routine monitoring of digoxin concentrations in clinical laboratories.

Although most investigators reported positive interference of DLISs with serum digoxin measurement, negative interference (falsely lower digoxin values) of DLISs in the MEIA for digoxin has been reported. This could be a serious problem because a clinician may increase a digoxin dose based on a low digoxin concentration due to elevated DLISs, and digoxin toxicity may result.

**Elimination of DLIS Interference in Digoxin Immunoassay by Ultrafiltration**

Valdes and Graves first reported strong serum protein binding of DLISs. Therefore, DLISs usually are absent in the protein-free ultrafiltrate. Taking advantage of high protein binding of DLISs and poor protein binding of digoxin (25%), both positive and negative interference of DLISs in serum digoxin measurement can be eliminated completely by measuring digoxin concentrations in the protein-free ultrafiltrate. Protein-free ultrafiltrate of digoxin can be prepared easily by centrifuging the specimen at 1,500g to 2,000g in an ultrafiltration device (Centrifree Micropartition System, Amicon, Danvers, MA; molecular weight cutoff, 30,000) for 20 to 30 minutes. Digoxin is only 25% protein-bound, and approximately 75% of digoxin is found in the ultrafiltrate. Immunoassay kits used for monitoring total
increased rapidly to 4.0 ng/mL (5.1 nmol/L) during the next several hours and then slowly to 1.0 ng/mL (1.3 nmol/L) during the next 11 days despite no digoxin being administered. Analysis of serum samples by another digoxin assay confirmed the original values. The authors used an immuno-fluorometric digoxin assay for their study, an ACA analyzer (Dupont Diagnostic System, Dade Behring, Deerfield, IL) for their initial study, and a digoxin RIA method (Dupont-NEN, Dade Behring) for reconfirmation of the original digoxin values.20 Garbagnati27 measured DLIS concentrations in children (age range, 5-16 years) using an FPIA digoxin assay and observed measurable DLIS concentrations in 50% of the children (range, 0.0-0.4 ng/mL [0.0-0.5 nmol/L]).

Lusic et al28 reported comparable plasma and CSF levels of DLISs in 40 patients with aneurysmal subarachnoid hemorrhage. On the first day, DLIS concentrations were detected in the serum samples of 34 patients (range, 0.0-0.9 ng/mL [0.0-1.2 nmol/L]) and CSF samples of 32 patients (range, 0.0-1.0 ng/mL [0.0-1.3 nmol/L]). On the seventh posthemorrhage day, DLISs were present in plasma samples of 37 patients (range, 0.0-1.5 ng/mL [0.0-1.9 nmol/L]) and CSF samples of 38 patients (range, 0.0-1.7 ng/mL [0.0-2.2 nmol/L]). The authors used a fluorescence polarization immunoassay (Digoxin II, Abbott Laboratories) for their study.28 Elevated DLIS concentrations (mean, 0.5 ng/mL [0.6 nmol/L]) using the same FPIA assay also have been reported.29

**Decreased DLIS in Bipolar Disease**

Although most reports in the literature described increased DLIS concentration, Gridet al30 reported decreased DLIS concentrations in patients with manic bipolar disorder compared with healthy control subjects. The authors used an RIA for measuring DLIS concentrations. The mean DLIS concentration in the control group was 296.6 pg/mL (379.6 pmol/L), while the mean DLIS concentration in the bipolar disorder group was 143.6 pg/mL (183.8 pmol/L).30 Conditions that cause an abnormality or a change in DLIS concentrations are summarized in **Table 1**.6,7,20-22,25,28-41

**Positive and Negative Interference of DLISs in Serum Digoxin Measurement: Impact on Therapeutic Drug Monitoring of Digoxin**

Concentrations of DLIS found in various patient groups are significant in the light of the narrow therapeutic window of digoxin. Positive interference of DLISs in the FPIA digoxin assay (Abbott Laboratories) is very well documented in the literature. Many investigators used this assay to measure DLIS levels in a variety of patients not receiving digoxin. Avendano et al40 reported findings of 89% false-positive digoxin values in blood drawn from peripheral veins of newborns and a striking 100% false-positive digoxin levels in the corresponding cord blood when FPIA (Digoxin II) was used for measurement. The authors also observed 60% false-positive values in patients with severe hepatic disease and concluded that digoxin levels must be interpreted very carefully in these patients.40 Frisolone et al7 studied apparent serum digoxin levels in patients with liver disease using an FPIA (Digoxin II, TDx analyzer, Abbott Laboratories), an RIA (gamma goat iodine 125), and a fluorometric assay (Stratus, Dade Behring). These patients did not receive digoxin or spironolactone. The authors observed measurable

### Table 1

**Causes of an Abnormality or a Change in the Level of Digoxin-like Immunoreactive Substances**

<table>
<thead>
<tr>
<th>Condition or Factor</th>
<th>Effect</th>
<th>Measurement Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential hypertension</td>
<td>↑</td>
<td>RIA; ouabain binding</td>
<td>Ahmad et al21, Cloix et al37</td>
</tr>
<tr>
<td>Hypertension or volume expansion</td>
<td>↑</td>
<td>Rubidium uptake</td>
<td>Krep et al30</td>
</tr>
<tr>
<td>Uremic syndrome</td>
<td>↑</td>
<td>RIA; FPIA; ACA†</td>
<td>Schrader et al6, Ahmad et al21, Howarth et al22, Frisolone et al7, Howarth et al22, Lucena et al33, Tamura et al35, Avendano et al40</td>
</tr>
<tr>
<td>Liver disease, liver failure</td>
<td>↑</td>
<td>EMIT 2000†; FPIA</td>
<td>Schrader et al6, Tamura et al35, Avendano et al40</td>
</tr>
<tr>
<td>Transplantation</td>
<td>↑</td>
<td>FPIA; RIA; ACA†</td>
<td>Tamura et al35, Avendano et al40</td>
</tr>
<tr>
<td>Prematurity, newborn</td>
<td>↑</td>
<td>FPIA</td>
<td>Logoglu et al25, Gilson et al39, Avendano et al40</td>
</tr>
<tr>
<td>Pregnancy and preeclampsia</td>
<td>↑</td>
<td>RIA; FPIA; Na+, K+—ATPase inhibition</td>
<td>Shilo et al20, Dasgupta et al38</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>↑</td>
<td>FPIA</td>
<td>Hayashi et al32</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>↑</td>
<td>FPIA</td>
<td>Bagrov et al34,41</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>↑</td>
<td>FIA; mass spectrometry</td>
<td>Howarth et al22</td>
</tr>
<tr>
<td>Need for intensive care</td>
<td>↑</td>
<td>FPIA</td>
<td>Straub et al23, Tamura et al35</td>
</tr>
<tr>
<td>Diabetes</td>
<td>↑</td>
<td>FPIA</td>
<td>Lusic et al28</td>
</tr>
<tr>
<td>Mucocutaneous lymph node syndrome</td>
<td>↑</td>
<td>RIA</td>
<td>Bentur et al31</td>
</tr>
<tr>
<td>Aneurysmal subarachnoid hemorrhage</td>
<td>↑</td>
<td>FPIA</td>
<td>Grider et al30</td>
</tr>
<tr>
<td>Postmortem blood</td>
<td>↑</td>
<td>FPIA</td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>↓</td>
<td>RIA</td>
<td></td>
</tr>
</tbody>
</table>

ATPase, adenosine triphosphatase; FIA, fluorimunoassay; FPIA, fluorescence polarization immunoassay; RIA, radioimmunoassay; †, increased; ↓, decreased.

* References are representative only. Other investigators may have reported similar findings.
† ACA, an affinity-mediated immunoassay from Dupont Diagnostic System (Dade Behring, Deerfield, IL).
digoxin concentrations have adequate sensitivity to measure free digoxin concentrations in the protein-free ultrafiltrate. It also is possible to calculate the true total digoxin concentration and the extent of DLIS interference in digoxin measurement by measuring albumin and total and free digoxin concentrations and then using mathematical equations.51,52

Is DLIS a Natriuretic Hormone?

Serum DLIS concentrations are elevated in patients with conditions associated with volume expansion, and DLISs also can inhibit Na+, K+-ATPase. In addition, it has been associated with natriuresis, thus raising the possibility that DLISs have a role in water and sodium homeostasis.21 Garbagnati27 demonstrated that children with elevated concentrations of DLISs showed significantly lower natremia, higher urinary and fractional excretion of sodium, and increased systolic blood pressure compared with children with no measurable DLIS concentration. Elevated DLIS concentrations in serum and urine samples in children with nephrotic syndrome had been reported.53 The authors concluded that DLISs may be involved in natriuresis and may regulate active sodium transport in children with nephrotic syndrome.53 Acute volume expansion and high sodium intake increased plasma levels of DLISs in rats and in patients with essential hypertension.54 A significant positive correlation also was found between urinary DLISs and sodium excretion in hypertensive subjects. More interestingly, ultrafiltrate from patients with renal failure and high sodium excretion in hypertensive subjects. More interestingly, ultrafiltrate from patients with renal failure and high plasma DLIS concentrations caused natriuresis when infused in the renal artery of a dog.55 Goodline56 reported a case in which the blood pressure of a pregnant woman with preeclampsia was reduced significantly after intravenous treatment with the Fab of the antidigoxin antibody, probably owing to the binding of free DLIS with the Fab. However, there are other reports in the literature that dispute the link between elevated DLISs and natriuresis. Scott et al57 found no difference in the circulating levels of DLISs in normotensive and hypertensive rabbits despite marked alterations in dietary sodium intake. Trachtman et al58 concluded that an increase in the DLIS concentration does not lead to an increase in blood pressure.

Structure of DLISs

Instead of being a single compound, DLISs may be a class of compounds. Several investigators identified nonesterified fatty acids, phospholipids, and lysophospholipids as DLISs.59,60 When the first antidigoxin antibody was introduced, it was recognized that most steroids cross-react to some extent with these antibodies. Several investigators reported progesterone, 17-OH progesterone, cortisol, and glycodihydroxy and glycotrihydroxy bile salts as DLISs.51 Shaikh et al62 reported that DLISs have a molecular weight of 780 d, made up of one 390-d aglycon and several sugar moieties. De Angelis et al63 characterized DLISs as a single peak by high-performance liquid chromatography from human serum with a retention time similar to that of digoxin and concluded that the structure of DLISs was similar to that of digoxin. Qazzaz et al64 reported that subtle structural differences exist between DLISs and digoxin at or near the lactone ring as well as in the nature of sugar. Moreover, deglycosylated congeners of DLIS also exist in human serum. Bagrov et al41 characterized DLISs as marinobufagenin with a molecular weight of 400 d.

Exogenous DLIS

Interference of Spironolactone and Its Metabolites in Digoxin Assays

Spironolactone, a competitive aldosterone antagonist, has been used clinically in therapy for hypertension and congestive heart failure for a long time. Spironolactone is rapidly and extensively metabolized, and the metabolite canrenone also is 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 treatment of a patient, interference of spironolactone and canrenone in the therapeutic monitoring of digoxin is troublesome. Positive interference of spironolactone and its active metabolite canrenone in the RIA for digoxin was reported as early as 1974.65 Potassium canrenoate also showed positive interference with serum digoxin monitoring by both RIA and enzyme immunoassay.66,67 Morris et al68 first reported positive interference of spironolactone in digoxin measurement using the FPIA in 1988. Later, other authors verified the interference of spironolactone and canrenone in the FPIA and other commonly used immunoassays for digoxin.59,70 Okazaki et al71 also reported falsely elevated digoxin levels in patients receiving digoxin and potassium canrenoate. The authors reported 2 cases in which cross-reactivity of the assay system caused clinical problems, and they recommended use of the OPUS digoxin assay (OPUS Diagnostics, Fort Lee, NJ), which showed minimum cross-reactivity.71

Steimer et al72 described negative interference of canrenone in digoxin measurement. Canrenone and spironolactone caused falsely low digoxin values due to negative interference in the serum digoxin measurement when an
MEIA for digoxin was used. Misleading subtherapeutic concentrations of digoxin as measured on several occasions led to falsely guided digoxin dosing, which in turn led to serious digoxin toxicity in the patients.72 By taking advantage of strong protein binding of spironolactone and its metabolites and poor protein binding of digoxin, it might be possible to eliminate the interference of these compounds in serum digoxin measurement by monitoring free digoxin.

**Interference of Chinese Medicines in Digoxin Measurement**

**Chan Su**

Traditional Chinese medicines are readily available without prescription from herbal departments in local Chinese stores. One such Chinese medicine is Chan Su, which is prepared from the dried white secretion of the auricular glands and the skin glands of Chinese toads (Bufo melanostictus Schneider or Bufo bufo gargarizans Gantor). Chan Su also is a major component of traditional Chinese medicines Lu-Shen-Wan and kyushin.73,74 These medicines are used for the treatment of conditions such as tonsillitis, sore throat, and palpitations. Traditional use of Chan Su given in small doses also includes stimulation of myocardial contraction, anti-inflammatory effects, and pain relief.75 The cardiotonic effect of Chan Su is due to its major bufadienolides, such as bufalin, cinobufagin, and resibufogenin.76 Bufalin is known to block vasodilatation and increase vascular resistance, and blood pressure by inhibiting Na+, K+–ATPase.77

Fushimi and Amino78 reported a serum digoxin concentration of 0.4 ng/mL (0.5 nmol/L) in a healthy volunteer after ingestion of kyushin tablets containing Chan Su as the major component. Panesar79 reported an apparent digoxin concentration of 0.9 ng/mL (1.2 nmol/L) in a healthy volunteer who ingested Lu-Shen-Wan pills. The author used the FPIA and TDx analyzer for the study. Chan Su is the major component of Lu-Shen-Wan pills. An apparent digoxin concentration of 4.9 ng/mL (6.3 nmol/L; measured by the FPIA) was reported in 1 woman who died after ingestion of Chinese herbal tea containing Chan Su.80

Chan Su extract also falsely increased the serum digoxin concentration in vitro when the FPIA (Digoxin II, TDx analyzer) was used for serum digoxin measurement. In contrast, serum digoxin levels were falsely lowered (negative interference) when the MEIA, also marketed by Abbott Laboratories (AxSYM analyzer), was used. However, the components of Chan Su responsible for digoxin-like immunoreactivity are bound substantially to serum proteins (>80%). By taking advantage of the high protein binding of Chan Su and the 25% protein binding of digoxin, this interference can be eliminated by monitoring the free digoxin concentration.81

**Siberian Ginseng**

There is one case report of the interference of Siberian ginseng in serum digoxin measurement. A 74-year-old man had a steady serum digoxin level of 0.9 to 2.2 ng/mL (1.2-2.8 nmol/L) for 10 years. His serum digoxin level increased to 5.2 ng/mL (6.7 nmol/L) on one occasion after taking Siberian ginseng. Although the level was toxic, the patient did not experience any signs or symptoms of digoxin toxicity. The patient stopped taking Siberian ginseng, and his digoxin level returned to normal.82

**Dan Shen**

Dan Shen is another Chinese herb prepared from the root of the Chinese medicinal plant Salvia miltiorrhiza. This drug has been used in China for many years in the treatment of various cardiovascular diseases, including angina pectoris.83 This herbal product also is widely available in the United States. More than 20 diterpene quinones, known as tanshinones, have been isolated from Dan Shen.84 These compounds have structural similarity with digoxin. Feeding Dan Shen to mice caused digoxin-like immunoreactivity in serum samples.85 However, the extent of DLIS activity was less remarkable than that observed in mice after feeding with Chan Su. In vitro studies indicated that Dan Shen can falsely increase serum digoxin levels if the FPIA is used. In contrast, digoxin values were falsely lower with the MEIA. Again, the components of Dan Shen that cause the DLIS activity are strongly protein-bound, and monitoring free digoxin levels eliminates this interference.86

There are a few other reports of interference of Chinese medicines and other products in serum digoxin measurement. These alternative medicines are listed in Table 2.

**Conclusion**

Both endogenous and exogenous DLISs can cause significant interference in serum digoxin measurements. DLISs cause low to moderate false increases in serum digoxin values in most digoxin immunoassays. However, the FPIA (Digoxin II) showed significant interference from DLISs. Negative interference of DLISs in the MEIA digoxin assay also may be problematic because the clinician may increase the digoxin dose based on falsely low serum digoxin concentrations. However, both positive and negative interference in serum digoxin measurements can be eliminated completely by monitoring free digoxin concentrations. Therefore, monitoring free digoxin concentrations may be recommended if a clinician has questions about a digoxin level in a patient with a volume-expanded condition.

Interference in digoxin assays owing to ingestion of Chinese medicines can cause more confusion. Most patients
do not inform their physicians when they use alternative medicines. Present studies indicate that components of the Chinese medicines that cause DLIS activity are strongly protein-bound. Monitoring free digoxin levels may eliminate such interferences. Limited information is available in the literature on the interference of Chinese medicine in serum digoxin assays. Therefore, it is possible that some Chinese medicines may have components that cause DLIS activity but are not strongly protein-bound. Monitoring free digoxin levels cannot eliminate such interference in serum digoxin measurement by immunoassays.

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