Effect of the Traditional Chinese Medicines Chan Su, Lu-Shen-Wan, Dan Shen, and Asian Ginseng on Serum Digoxin Measurement by Tina-quant (Roche) and Synchron LX System (Beckman) Digoxin Immunoassays

Leonard Chow,¹ Myrtle Johnson,² Alice Wells,¹ and Amitava Dasgupta^{1*}

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

²Laboratory Services, Memorial-Hermann Hospital, Houston, Texas

Chan Su, Lu-Shen-Wan, Dan Shen, and Asian ginseng are traditionally used to treat a number of conditions, including cardiovascular disease. All of these traditional Chinese medicines exhibit cardioactive properties. Digoxin is a cardioactive drug with a narrow therapeutic range (0.8–1.9 ng/mL). A patient taking digoxin may also take these Chinese medicines for their cardiotonic effects. Moreover, the active components of these medicines that are responsible for cardiotonic effects bear structural similarities to digoxin. Therefore, we studied the potential interference of these Chinese medicines with two digoxin immunoassays—the Tina-quant (Roche Diagnostics) and the Beckman (Synchron LX system)—and compared the values with the fluorescence polarization immunoassay (FPIA; Abbott Laboratories). When very small amounts (2–5 μ L) of aqueous extract of Chan Su or Lu-Shen-Wan were added to drug-free serum, we observed high digoxin-like immunoreactivity with the FPIA. In contrast, when ethyl acetate extract of Dan Shen or microliter amounts of ginseng extract were added to

drug-free serum, we observed modest digoxin-like immunoreactivity with the FPIA, but no apparent digoxin activity with the Roche and Beckman digoxin immunoassays. When aliquots of a digoxin pool prepared from patients receiving digoxin were supplemented with these Chinese medicines, we observed the most significant interference with the FPIA. The presence of endogenous digoxin-like immunoreactive substances can have additive effects with these Chinese medicines and falsely increase apparent digoxin levels by the FPIA. On the other hand, the Roche and Beckman assays were free from interference from DLIS but showed significant interference from Chan Su and Lu-Shen-Wan. We conclude that the FPIA showed the most significant interference from all four of the Chinese medicines we studied. However, the Roche and Beckman assays showed no interference from two (Dan Shen and Asian ginseng) of the four Chinese medicines we studied. *J. Clin. Lab. Anal.* 17:22–27, 2003. © 2003 Wiley-Liss, Inc.

Key words: Chan Su; Lu-Shen-Wan; Dan Shen; Asian ginseng; digoxin; immunoassay

Traditional Chinese medicines are readily available without prescription from herbal stores. These medicines have been used for thousands of years to treat a variety of conditions in Asia. Chinese medicines such as Chan Su, Lu-Shen-Wan, and Dan Shen are used in treating cardiovascular diseases. Asian ginseng is used as a tonic for health.

Chan Su is prepared from the dried white secretion of the auricular glands and the skin glands of Chinese toads (*Bufo melanostictus* Schneider and *Bufo bufo gargarzinas* Gantor). Chan Su is also a major component

of the traditional Chinese medicines Lu-Shen-Wan and Kyushin (1,2). In small doses, Chan Su also exhibits antiinflammatory effects, pain relief, and simulation of

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

Received 15 August 2002; Accepted 9 October 2002

DOI: 10.1002/jcla.10064

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

myocardial contraction (3). Major bufanodienolides, such as bufalin, cinobufagin, and resibufogenin, are responsible for the cardiotonic effects of Chan Su (4). Bufalin is known to block vasodilatation and increase vasoconstriction, vascular resistance, and blood pressure by inhibiting Na^+/K^+ -ATPase. Bufanodienolides have structural similarity with digoxin.

Lu-Shen-Wan means "six-miracle pill" in Chinese. It contains six different medicines: Niu Huang (*Calculus bovis*), Zhen Zhu (*Margarita*), Xiong Huang (*Realgar*), Bing Pian (*Borneol*), Chan Su (*Bufo melanostictus*), and She Xiang (*Secretio moschus*). It is used to treat sore throat, difficulty in swallowing, and abdominal pain and agitation. The same bufanodienolides present in Chan Su are responsible for the cardiotonic effects in Lu-Shen-Wan.

Dan Shen is prepared from the root of a Chinese medicinal plant (*Salvia miltiorrhiza*). It is used to treat cerebrovascular diseases, and cardiovascular diseases such as angina pectoris (5). Twenty diterpene quinones, known as tanshinones, have been isolated from the root (6).

Ginseng means "essence of man" in Chinese. In general, ginseng is used as a tonic to invigorate the user physically and mentally. It also has a calming effect and is capable of relieving stress. *Panax ginseng* (or Asian ginseng), which grows in Manchuria, is one of several different varieties. Ginseng contains ginsenosides, which have some structural similarity with digoxin (7).

Digoxin is a cardiac glycoside that can increase the adequacy of circulation in patients with congestive heart failure and can also slow the ventricular rate in the presence of atrial fibrillation and flutter. The chemical structure of digoxin contains an aglycone ring (genin) that is attached to one to four molecules of sugar. The basic structure of the aglycone is a cyclopentanoperhydrophenanthrene nucleus, which is attached to an unsaturated lactone ring at C17. All four of these Chinese medicines have active components that show some structural similarity with digoxin. We studied the potential interference of these four Chinese medicines in serum digoxin measurement using two commonly used assays: the Tina-quant (Roche Diagnostics, Indianapolis, IN) and the Beckman digoxin immunoassays (Beckman, Brea, CA). The fluorescence polarization immunoassay (FPIA) was used for comparison because this assay uses a polyclonal antibody and is known to have high interference from Chan Su.

MATERIALS AND METHODS

Chan Su, Dan Shen, Lu-Shen-Wan, and Asian ginseng were purchased from a Chinese herbal store in Houston, Texas. The Chan Su was sold by weight from

a package without the name of the manufacturer. The Lu-Shen-Wan was manufactured by Shanghai Chinese Medicine Works (Shanghai, China). Shanghai Chinese Botanical Works in Shanghai, China, prepared the Dan Shen we used in this study. The Asian ginseng was sold under the Song Shiu Pan brand. The FPIA for digoxin was obtained from Abbott Laboratories (North Chicago, IL), and the assay was run on an FLX/TDx analyzer. The Tina-quant digoxin assay (Roche Diagnostics) was run on the Hitachi 717 analyzer, also obtained from Roche Diagnostics. The Beckman digoxin assay was run on the Synchron LX20 analyzer. We followed the manufacturers' instructions for these assays.

We prepared three different serum pools. The first pool was made using specimens from patients who were not taking digoxin. This pool showed an absence of digoxin-like immunoreactive substances (DLIS). The second pool was made using specimens from patients taking digoxin. Another pool was made using specimens from uremic patients also receiving digoxin (suspected high DLIS). These specimens are routinely submitted to our clinical laboratory for therapeutic monitoring of digoxin. We used specimens left over after the tests ordered by physicians were performed and reported. These specimens would otherwise have been discarded after 1 week. Standard solutions of Chan Su and Lu-Shen-Wan (1 mg/mL) were prepared in distilled water. The standard solution of Dan Shen was prepared in ethyl acetate (25 mg/mL). The Asian ginseng extract was added directly to aliquots of the serum pools. To avoid any matrix effect from ethyl acetate, the extract was placed in a test tube and ethyl acetate was evaporated under air. The dried extract was then reconstituted with serum. The Asian ginseng we purchased was aqueous extract. Therefore, the addition of 10 μL extract to 1 mL of serum pool should not have altered the matrix.

The specimens for the Roche and Beckman assays did not require any sample pretreatment, but the FPIA assay did. For this purpose, 200 μL of serum was mixed with 200 μL of sulfosalicylic acid in methanol. After protein precipitation, the specimen was centrifuged at a high speed and the clear supernatant was analyzed for the digoxin concentration. The assay is linear to a serum digoxin concentration of 5.0 ng/mL, and the assay sensitivity is 0.2 ng/mL. The Tina-quant digoxin assay (Roche Diagnostics) is linear up to a digoxin concentration of 7.5 ng/mL, and the detection limit is 0.15 ng/mL. The Beckman digoxin assay is linear up to a digoxin concentration of 4.5 ng/mL, and the detection limit is 0.2 ng/mL.

Statistical analyses were performed using an independent *t*-test, two-tailed. We considered a difference

statistically significant only at 95% confidence or higher ($P < 0.05$).

RESULTS

In this study we added 2–5 μL of Chan Su or Lu-Shen-Wan extract to 1 mL of serum specimens. This represents 1:200–1:500 dilutions, which are very high dilutions. However, these dilutions represent the amount that is expected to be ingested under the recommended dosages.

When aliquots of the drug- and DLIS-free serum pool were supplemented with very small amounts of Chan Su and Lu-Shen-Wan aqueous extracts, we observed significant digoxin-like immunoreactivity with all three digoxin immunoassays. The observed immunoreactivity was highest with the FPIA. In contrast, only FPIA showed digoxin-like immunoreactivity when Dan Shen extract or Asian ginseng extract were added to aliquots of drug-free serum. We observed no immunoreactivity using the Roche and Beckman digoxin assays in the presence of Dan Shen extract or Asian ginseng extract (Table 1).

We observed significant increases in serum digoxin concentrations when very small amounts (2–5 μL) of aqueous extract of Chan Su were added to aliquots of serum digoxin pools and subsequently analyzed by all three assays. For example, when 2.0 μL and 5.0 μL of aqueous extract of Chan Su were added to 1 mL of digoxin pool, the observed digoxin concentration was 4.36 $\mu\text{g/mL}$ and 9.30 $\mu\text{g/mL}$, respectively, when measured by the FPIA. The original digoxin concentration was 0.71 $\mu\text{g/mL}$. The Roche and Beckman assays also showed significant interference from Chan Su and Lu-Shen-Wan, but the magnitude of interference was less compared to the FPIA. For example, with the Roche digoxin assay, the concentration of digoxin increased from 0.77 $\mu\text{g/mL}$ to 3.03 $\mu\text{g/mL}$ in the

presence of 2.0 μL of aqueous Chan Su extract added to per milliliter of digoxin pool. Similar interference was also observed with the Beckman digoxin assay (Table 2).

Significant increases in serum digoxin concentration were also observed when very small amounts (2–5 μL) of aqueous extract of Lu-Shen Wan were added to aliquots of serum digoxin pools and subsequently analyzed by all three assays. However, the magnitude of interference was less remarkable than that seen with Chan Su. For example, the apparent digoxin concentration increased from a control value of 0.71 ng/mL to 4.36 ng/mL in the presence of 2.0 μL extract of Chan Su. However, in the presence of 2.0 μL of Lu-Shen-Wan, the apparent digoxin concentration increased from 0.71 ng/mL to 2.67 ng/mL using the FPIA. Both the Beckman and Roche digoxin immunoassays demonstrated significant interference with Lu-Shen-Wan, but the magnitude of interference again was less compared to the FPIA (Table 2).

We observed modest positive interference when ethyl acetate extract of Dan Shen or Asian ginseng extract was added to aliquots of digoxin pool. For example, when 25 μL and 50 μL of ethyl acetate extract of Dan Shen were added to 1 mL aliquot of digoxin pool, the observed digoxin concentration was 0.99 $\mu\text{g/mL}$ and 1.22 $\mu\text{g/mL}$, respectively, when measured by the FPIA. When 10 μL and 20 μL of Asian ginseng extract were added to the samples, respective values of 1.03 and 1.28 $\mu\text{g/mL}$ were observed by the FPIA. However, we observed no interference from Dan Shen or Asian ginseng when the Roche and Beckman digoxin assays were used for the serum digoxin measurement (Table 2).

DLIS showed a synergistic effect from all four Chinese medicines we studied in falsely increasing the apparent digoxin concentrations of serum when the FPIA digoxin assay was used. FPIA demonstrated high cross reactivity with endogenous DLIS. Miller et al. (8) reported an apparent digoxin concentration of

TABLE 1. Apparent digoxin concentrations as measured by three digoxin immunoassays after supplementing drug free serum with Chinese medicines

Specimen	Apparent digoxin, ng/mL, Mean (SD), n = 3		
	FPIA (Abbott)	Tina-quant (Roche)	Synchron LX (Beckman)
Serum Pool 1	None detected	None detected	None detected
Extract/mL serum + 2.0 μL Chan Su	3.65 (0.09)	2.06 (0.15)	2.60 (0.10)
Extract/mL serum + 5.0 μL Chan Su	8.57 (0.38)	4.63 (0.06)	4.50 (0.10)
Extract/mL serum + 2.0 μL Lu-Shen-Wan	1.73 (0.05)	0.27 (0.06)	0.33 (0.06)
Extract/mL serum + 5.0 μL Lu-Shen-Wan	2.69 (0.01)	0.37 (0.06)	0.47 (0.06)
Extract/mL of serum + 25 μL Dan Shen	0.45 (0.07)	None detected	None detected
Extract/mL serum + 50 μL of Dan Shen	0.76 (0.12)	None detected	None detected
Extract/mL serum + 10 μL Ginseng	0.52 (0.11)	None detected	None detected
Extract/mL serum + 20 μL Ginseng	0.76 (0.01)	None detected	None detected

TABLE 2. Effect of Chinese medicines on serum digoxin measurement by three immunoassays

Specimen	Apparent digoxin, ng/ml, mean (SD), n = 3		
	FPIA (Abbott)	Tina-quant (Roche)	Synchron LX (Beckman)
Digoxin Pool 1	0.71 (0.04)	0.77 (0.06)	0.67 (0.06)
Extract/ml serum + 2.0 µl Chan Su	4.36 (0.08) ^a	3.03 (0.06) ^a	3.20 (0.10) ^a
Extract/ml serum + 5.0 µl Chan Su	9.30 (0.42) ^a	5.40 (0.10) ^a	7.77 (0.12) ^a
Extract/ml serum + 2.0 µl Lu-Shen-Wan	2.67 (0.02) ^a	1.26 (0.12) ^a	1.33 (0.06) ^a
Extract/ml serum + 5.0 µl Lu-Shen-Wan	3.71 (0.50) ^a	1.43 (0.06) ^a	1.66 (0.06) ^a
Extract/ml of serum + 25 µl Dan Shen	0.99 (0.06) ^a	0.87 (0.06)	0.63 (0.06)
Extract/ml serum + 50 µl of Dan Shen	1.22 (0.02) ^a	0.83 (0.06)	0.73 (0.06)
Extract/ml serum + 10 µl Ginseng	1.03 (0.13) ^a	0.80 (0.10)	0.63 (0.06)
Extract/ml serum + 20 µl Ginseng	1.28 (0.11)	0.83 (0.06)	0.70 (0.10)

^aSignificantly greater than the corresponding digoxin pool value by *t*-test, two tailed ($P < 0.05$).

TABLE 3. Effects of Chinese medicines in serum digoxin measurement by three immunoassays in digoxin pools also containing elevated concentrations of DLIS

Specimen	Apparent digoxin, ng/ml, mean (SD), n = 3		
	FPIA (Abbott)	Tina-quant (Roche)	Synchron LX (Beckman)
Digoxin Pool 1	1.30 (0.04)	0.83 (0.06)	0.87 (0.06)
Extract/ml serum + 2.0 µl Chan Su	4.62 (0.02) ^a	3.60 (0.10) ^a	3.33 (0.06) ^a
Extract/ml serum + 5.0 µl Chan Su	8.42 (0.14) ^a	5.30 (0.10) ^a	4.43 (0.10) ^a
Extract/ml serum + 2.0 µl Lu-Shen-Wan	2.82 (0.12) ^a	1.33 (0.06) ^a	1.40 (0.10) ^a
Extract/ml serum + 5.0 µl Lu-Shen-Wan	3.50 (0.02) ^a	1.66 (0.06) ^a	1.90 (0.06) ^a
Extract/ml of serum + 25 µl Dan Shen	1.67 (0.05) ^a	0.87 (0.06)	0.83 (0.06)
Extract/ml serum + 50 µl of Dan Shen	1.77 (0.03) ^a	0.83 (0.06)	1.00 (0.17)
Extract/ml serum + 10 µl Ginseng	1.66 (0.08) ^a	0.90 (0.10)	0.97 (0.12)
Extract/ml serum + 20 µl Ginseng	1.80 (0.06) ^a	0.87 (0.06)	0.80 (0.10)

^aSignificantly greater than the corresponding digoxin pool value by *t*-test, two tailed ($P < 0.05$).

0.88 ng/mL in a volume-expanded patient who had never received digoxin. On the other hand, both the Roche and Beckman digoxin assays have minimal cross reactivity with DLIS. The digoxin pool prepared from volume-expanded patients showed a digoxin concentration of 1.30 ng/mL by the FPIA. The observed digoxin concentrations were 0.83 ng/mL by the Roche, and 0.87 ng/mL by the Beckman. The apparent discrepancy between the FPIA digoxin value and the values observed by the Roche and Beckman assays is due to DLIS. We observed further falsely increased digoxin levels in the presence of Chan Su, Lu-Shen-Wan, Dan Shen, and Asian ginseng using the FPIA, indicating that the presence of DLIS may have an additive effect on the interference of Chinese medicines in the FPIA assay. On the other hand, we observed no statistically significant change in the serum digoxin concentrations using the Roche and Beckman assays in the presence of Dan Shen and Asian ginseng, indicating that these Chinese

medicines do not interfere with these immunoassays even in the presence of elevated DLIS levels (Table 3).

DISCUSSION

Digoxin has a narrow therapeutic range (0.8–1.9 ng/mL), and the therapeutic monitoring of digoxin is essential for proper patient management. Because of the widespread use of Chinese medicines by the general population, it is possible that some patients receiving digoxin also are taking Chan Su, Lu-Shen Wan, Dan Shen, or Asian ginseng. Panesar (9) reported an apparent digoxin concentration of 0.88 ng/mL in a healthy volunteer who ingested a Lu-Shen-Wan pill. An apparent digoxin concentration of 4.9 ng/mL (measured by the FPIA) was reported in a woman who died after ingesting Chinese herbal tea containing Chan Su (10). Previously we reported positive and negative interference of Chan Su in serum digoxin measurement by

FPIA and MEIA digoxin assays (11). We also reported negative interference of Dan Shen in the MEIA digoxin assay (12). However, the effects of Chan Su, Lu-Shen-Wan, Dan Shen, and Asian ginseng on the Roche and Beckman digoxin assays have never been reported before. Moreover, the potential interference of Asian ginseng on digoxin immunoassays had not been studied heretofore. We recently acquired the ability in our laboratory to use both the Roche and Beckman digoxin assays. Therefore, we studied the effect of these popular Chinese medicines on these assays, which are also widely used in clinical laboratories. The FPIA values are given for comparison purposes only.

The amounts of Chan Su and Lu-Shen-Wan added to the digoxin pools were the expected concentrations of these compounds in vivo as calculated from the recommended dosages. We also reported in vivo digoxin-like immunoreactivity in mice after they were given Dan Shen in doses comparable to those taken by humans (based on body weight) (13). Experiments on humans were not performed, because of the unknown toxicities of these Chinese medicines. Nevertheless, our data clearly show significant interference of Chan Su and Lu-Shen-Wan with both the Roche and Beckman assays for digoxin. Although the magnitude of interference is less than with the FPIA, the falsely elevated values may cause a clinically significant bias in interpretations of serum digoxin concentrations.

Although the FPIA showed interference from Dan Shen, the Roche and Beckman digoxin assays showed no interference from either Dan Shen or Asian ginseng. Asian ginseng showed modest interference with the FPIA, but no interference with the Roche and Beckman digoxin assays.

The FPIA digoxin assay was mostly affected by these traditional Chinese medicines because the polyclonal antibody used in this assay lacks specificity. It is also well established that the FPIA assay is mostly affected by endogenous DLIS (14). The less marked interference from Chan Su and Lu-Shen-Wan, and absence of interference from Dan Shen and Asian ginseng in the Roche assay may be attributable to the more specific mouse monoclonal antibody against digoxin used in this assay. Monoclonal mouse antibody specific to digoxin is also used in the Beckman digoxin immunoassay, which is also less affected by these Chinese traditional medicines compared to the FPIA digoxin assay. However, in vivo data from humans are necessary in order to clearly establish these interferences in the therapeutic monitoring of digoxin by immunoassay.

Bufalin, the active component of Chan Su, is structurally very similar to digoxin, except for the presence of sugar moiety. This structural similarity may explain why monoclonal antibody-based immu-

noassays are also affected by Chan Su and Lu-Shen-Wan. On the other hand, active components of both Dan Shen and Asian ginseng demonstrate some structural similarity with digoxin. Thus, polyclonal-based immunoassays (such as the FPIA) are affected, while the two monoclonal antibody-based immunoassays are free from such interference.

According to a recent survey, ginseng is the most widely used herbal medicine in the U.S. (15). The Chinese herbalists in two different stores in Houston informed us that Dan Shen is the most popular Chinese herb for improving heart conditions. Therefore, it is more likely that a patient taking digoxin is also using Dan Shen and/or Asian ginseng than Chan Su. The lack of interference from Dan Shen and Asian ginseng in the Roche and Beckman digoxin assays indicates that these assays may be more useful in a clinical laboratory than the FPIA to eliminate interferences from Chinese medicines with digoxin immunoassays.

REFERENCES

1. Hong Z, Chan K, Yeung HW. Simultaneous determination of bufadienolides in traditional Chinese medicine preparations, Liu-Shen-Wan by liquid chromatography. *J Pharm Pharmacol* 1992;44:1023-1026.
2. Chan WY, Ng TB, Yeung HW. Examination of toxicity of a Chinese drug, the toad glandular secretion product Chan Su in pregnant mice and embryos. *Biol Neonate* 1995;67:376-380.
3. Chen KK, Kovarikove A. Pharmacology and toxicology of toad venom. *J Pharm Sci* 1967;56:1535-1541.
4. Morishita S, Shoji M, Oguni Y, et al. Pharmacological action of "Kyushin" a drug containing toad venom: cardiotoxic and arrhythmogenic effects and excitatory effect on respiration. *Am J Chin Med* 1992;20:245-246.
5. Panmami MB, Chen S, Bryant HJ, et al. Effect of three sodium-potassium adenosine triphosphate inhibitors. *Hypertension* 1991;18:316-324.
6. Zhou W, Ruigrok TJC. Protective effect of Dan Shen during myocardial ischemia and reperfusion: an isolated rat heart study. *Am J Chin Med* 1990;18:19-24.
7. Lee AR, Wu WL, Chang W, Lin C, King ML. Isolation and bioactivity of new tanshinones. *J Nat Prod* 1987;50:157-160.
8. Miller JJ, Straub RW, Valdes R. Analytical performance of a monoclonal digoxin assay with increased specificity on ACS:180. *Ther Drug Monit* 1996;18:65-72.
9. Panesar NS. Bufalin and unidentified substances in traditional Chinese medicine cross react in commercial digoxin assay. *Clin Chem* 1992;38:2155-2156.
10. Ko R, Greenwald M, Loscutoff S, et al. Lethal ingestion of Chinese tea containing Chan Su. *West J Med* 1996;164:71-75.
11. Dasgupta A, Biddell 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 concentration. *Am J Clin Pathol* 2000;114:174-179.
12. Wahed A, Dasgupta A. Positive and negative in vitro interference of Chinese medicine Dan Shen in serum digoxin measurement: elimination of interference by monitoring free digoxin concentration. *Am J Clin Pathol* 2001;116:403-408.

13. Dasgupta A, Actor J, Olsen M, Wells A, Datta P. In vivo digoxin-like immunoreactivity in mice and interference of Chinese medicine Dan Shen in serum digoxin measurement: elimination of interference by using a chemiluminescent assay. *Clin Chim Acta* 2002;317:231–234.
14. Datta P, Hinz V, Klee G. Comparison of four digoxin immunoassays with respect to interference from digoxin-like immunoreactive factors. *Clin Biochem* 1996;29:541–547.
15. Harnack LJ, Rydell SA, Stang J. Prevalance of use of herbal products by adults in the Minneapolis/St. Paul, Minn., metropolitan area. *Mayo Clin Proc* 2002;76:688–694.