Original Article

Interference between eplerenone and digoxin in an enzyme multiplied immunoassay technique and chemiluminescent immunoassay

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Digitalis-like immunoreactive substances (DLISs) have been shown to cross-react with anti-digoxin antibodies. We previously reported that eplerenone, the structure of which is similar to that of digoxin, interfered with digoxin measurements in a fluorescence polarization immunoassay (FPIA), microparticle enzyme immunoassay (MEIA), and affinity column-mediated immunoassay (ACMIA), and also that the extent of interference was different in each assay.

Digoxin has a narrow therapeutic window; therefore, it is important to measure its serum concentrations without interference by clinically co-administered drugs. In this study, we performed two additional types of assays (enzyme multiplied immunoassay technique (EMIT) and chemiluminescent immunoassay (CLIA)) to clarify cross-reactivity between eplerenone and anti-digoxin antibodies. Furthermore, we used EMIT and CLIA to measure apparent digoxin concentrations in mixed solutions of eplerenone (1-100 μg/mL) and digoxin (1-3 ng/mL). Eplerenone was not detected as digoxin by EMIT and CLIA in cross-reaction tests. Furthermore, the apparent concentration of digoxin when co-administered with eplerenone was not significantly affected in EMIT and CLIA. These results suggest that EMIT and CLIA may be able to accurately measure serum digoxin concentrations in patients adjunctively receiving eplerenone.

Key words*: *DLIS, eplerenone, EMIT, CLIA, therapeutic drug monitoring

INTRODUCTION

Digoxin has been used for centuries as a therapeutic agent for congestive heart failure and is currently the only cardiac glycoside that is in widespread clinical use. Because of its narrow therapeutic range, therapeutic drug monitoring of digoxin is necessary and immunoassays are widely used (1). However, digitalis-like immunoreactive substances (DLISs) have frequently been reported to interfere with digoxin in immunoassays. Falsely high or low measurements of serum digoxin levels may cause side effects or poor therapeutic effects due to inappropriately administered dosages.

DLISs have been classified into three groups: 1. Compounds that are similar in structure to digoxin, of which aldosterone blockers, spironolactone, and can-

Received: December 7, 2012 Revised: June 21, 2013 Accepted: September 23, 2013
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renone are typical examples (2); 2. Endogenous digitalis-like factor (EDLF) (3, 4, 5, 6, 7, 8, 9, 10, and 11); and 3. Light scattering phenomena (12 and 13). We previously reported that eplerenone, which has a similar structure to and pharmacological effects as spironolactone, interfered with digoxin measurements in fluorescence polarization immunoassay (FPIA), microparticle enzyme immunoassay (MEIA), and affinity column-mediated immunoassay (ACMIA) (14), and that the extent of interference was different in each assay. Plasma digoxin concentrations are maintained at lower levels (0.5 - 0.8 ng/ml) (15) than the traditional therapeutic range (0.8 - 2.0 ng/ml) (1) and the extent of interference by DLISs is particularly high at lower concentrations; therefore, more accurate methods are increasingly required for the therapeutic drug monitoring of digoxin.

Enzyme multiplied immunoassay technique (EMIT) and chemiluminescent immunoassay (CLIA) are widely used for the therapeutic drug monitoring of digoxin in clinical settings. Previous studies showed that spironolactone and canrenone did not interfere with digoxin measurements in EMIT and CLIA (2, 16); therefore, EMIT and CLIA may be able to accurately measure digoxin in the presence of eplerenone. However, interference by eplerenone in EMIT and CLIA has not yet been examined. In the present study, we examined cross-reactivity between eplerenone and anti-digoxin antibodies in EMIT and CLIA to clarify interference between eplerenone and digoxin in commonly used methods in clinical settings.

MATERIALS and METHODS

Reagents and Assay Devices

Digoxin was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Eplerenone was provided by Pfizer Inc. (NY, USA). Horse serum was purchased from Invitrogen Corp. (CA, USA) as a solvent in which to dissolve digoxin. Methanol (99.7% <) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The digoxin assay regents used in this study were Emit 2000 (including an anti-digoxin rabbit polyclonal antibody, Siemens) for JCA-BM12 (JEOL Ltd, Tokyo, Japan) with a detection limit of 0.3 ng/mL for EMIT, and ARCHITECT *i* Digoxin (including an anti-digoxin mouse monoclonal antibody, Abbot) for ARCHITECT i 2000SR (Abbott, IL, USA) with a detection limit of 0.3 ng/mL for CLIA.

Assay procedure

Emit 2000: This assay was based on competition between a drug in the sample and a drug labeled with recombinant glucose-6-phosphatedehydrogenase for antibody binding sites. Because enzyme activity decreased upon binding to the antibody, the concentration of the drug in the sample could be measured in terms of enzyme activity. The active enzyme converted oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that was measured spectrophotometrically.

ARCHITECT *i* Digoxin: The sample, anti-digoxin-coated paramagnetic microparticles, assay diluent, and digoxigenin acridinium-labeled conjugate were combined to create a reaction mixture. The anti-digoxin-coated microparticles bound to digoxin present in the sample and to the digoxigenin acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions were added to the reaction mixture. The resulting chemiluminescent reaction was measured as relative light units (RLUs). An indirect relationship was observed between the amount of digoxin in the sample and RLUs detected by ARCHITECT i System optics.

All assays were performed according to the manufacturer's protocol.

Samples

Serum digoxin

Digoxin solution was prepared in methanol. This solution was further diluted with methanol to obtain 1, 2, and 3 ng/mL of digoxin. These solutions were evaporated and serum was added.

Serum eplerenone mixed with digoxin

An eplerenone solution was prepared in methanol. This solution was further diluted with methanol to obtain 1, 2.5, 10, and 100 μg/mL of eplerenone. These solutions were evaporated and each concentration of serum digoxin was added (0-3 ng/mL).

Sample preparations were performed in a similar manner to our previous study (14).

Precision of each assay for digoxin measurements

To test the veracity of the precision of digoxin measurements, each assay was repeated three times with

digoxin solutions of 0, 1, 2, and 3 ng/mL.

Cross-reactivity of eplerenone with digoxin in each assay

To assess the cross-reactivity of eplerenone with anti-digoxin antibodies, each assay was repeated three times with eplerenone solutions (with no digoxin present) of 0, 1, 2.5, 10, and 100 μg/mL.

Effects of eplerenone on each assay in the presence of digoxin

To assess the effects of eplerenone in the presence of digoxin, each assay was repeated three times with mixtures of known concentrations of digoxin (0, 1, 2, and 3 ng/mL) and eplerenone (0, 1, 2.5, 10, and 100 μg/mL) to test for a correlation between apparent and known digoxin concentrations at each eplerenone level. In addition, we compared the results of EMIT and CLIA with a previous study (the results of FPIA, MEIA, and ACMIA (14)) at a known digoxin concentration of 3 ng/mL.

Analysis

A simple regression analysis was used to evaluate the relationship between known and apparent digoxin concentrations measured by EMIT and CLIA. Significant differences were shown with $P < 0.05$. The coefficient of variation was calculated with the following formula:

Coefficient of variation (%)=100×Standard deviation / mean.

RESULTS

Precision of each assay for digoxin measurements

Digoxin concentrations measured in EMIT and CLIA were shown in Table 1. The coefficients of variation in EMIT ranged from 2.9 - 7.8 %, and in CLIA from 0.7 - 1.2 %.

In Table 1, the known concentrations of digoxin were consequently higher when using CLIA. However, these results were in the acceptable range according to the manufacturer's protocol.

Effects of eplerenone on each assay

No cross-reactivity was detected between eplerenone and anti-digoxin antibodies in both methods in the absence of digoxin (through 100 μg/mL of eplerenone).

The effects of eplerenone co-administered with digoxin were examined. The results of each assay were plotted by eplerenone levels, with the apparent digoxin concentration on the vertical axis and known digoxin concentration on the horizontal axis (Figures 1, 2). In EMIT, eplerenone alone did not cross-react with anti-digoxin antibodies; the y intercept of the regression line of 100 μg/mL with a known eplerenone concentration (0.52 ng/mL) was higher than those of the other regression lines (ranging from -0.03 to 0.24 ng/mL) (Figure 1). In CLIA, apparent digoxin concentrations were stable regardless of the presence of digoxin (Figure 2). A comparison between the present study (EMIT, CLIA) and previous study (FPIA, MEIA, and ACMIA) with a known digoxin concentration of 3 ng/mL revealed that apparent digoxin concentrations in EMIT and CLIA were more stable than those in FPIA, MEIA, and ACMIA (Figure 3).

Table 1 *Digoxin concentrations determined by EMIT and CLIA*

	Known digoxin concentration (ng/mL)			
	0	**1**	**2**	**3**
EMIT	< 0.3	0.97 ± 0.06	1.97 ± 0.06	2.97 ± 0.23
CLIA	< 0.3	1.10 ± 0.01	2.13 ± 0.02	3.12 ± 0.04

Solutions were measured three times for each sample by EMIT and CLIA.
Each coefficient of variation (%) was as follows: In EMIT, 1 ng/mL; 5.97, 2 ng/mL; 2.94, 3 ng/mL; 7.78. In CLIA, 1 ng/mL; 0.91, 2 ng/mL; 0.72, 3 ng/mL; 1.16.

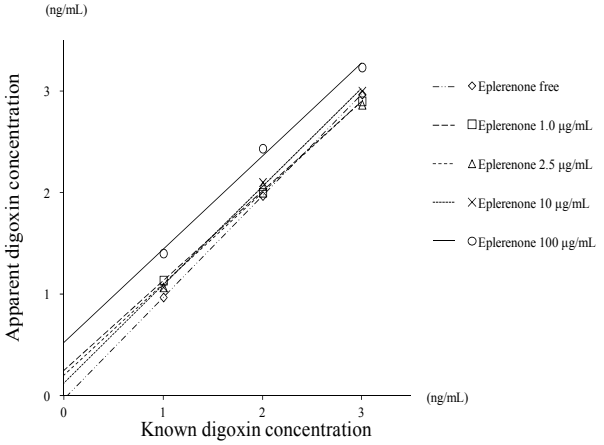

Fig.1 Correlation between the apparent digoxin concentration measured in EMIT and the known digoxin concentration in the eplerenone solution. A simple regression analysis was used to evaluate the relationship between known and apparent digoxin concentrations (0 μg/mL of eplerenone, r = 0.990, P < 0.001; 1 μg/mL of eplerenone, r = 0.995, P < 0.001; 2.5 μg/mL of eplerenone, r = 0.996, P < 0.001; 10 μg/mL of eplerenone, r = 0.999, P < 0.001; 100 μg/mL of eplerenone, r = 0.996, P < 0.001).

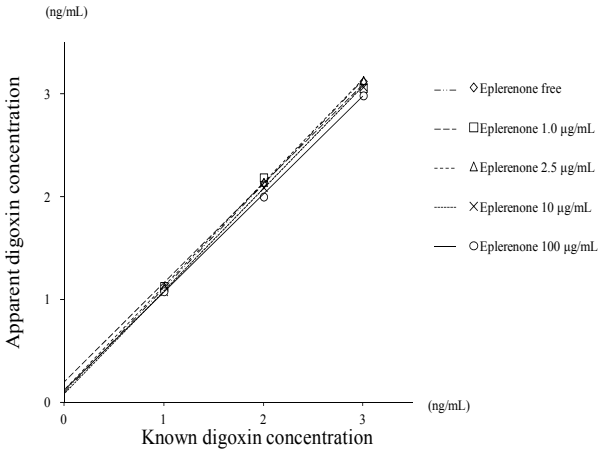

Fig. 2 Correlation between the apparent digoxin concentration measured in CLIA and the known digoxin concentration in the eplerenone solution. A simple regression analysis was used to evaluate the relationship between known and apparent digoxin concentrations (0 μg/mL of eplerenone, r = 0.999, P < 0.001; 1 μg/mL of eplerenone, r = 0.997, P < 0.001; 2.5 μg/mL of eplerenone, r = 0.999, P < 0.001; 10 μg/mL of eplerenone, r = 0.999, P < 0.001; 100 μg/mL of eplerenone, r = 0.999, P < 0.001).

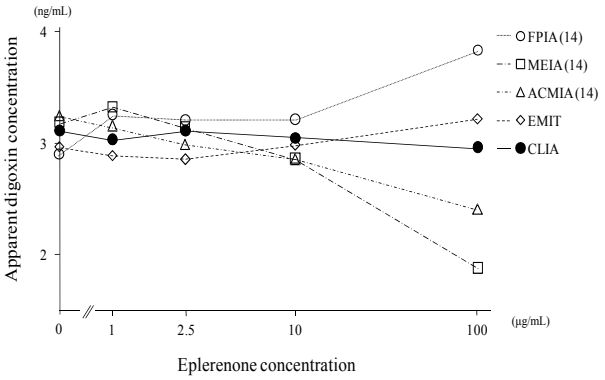

Fig. 3 Apparent digoxin concentrations in the presence of eplerenone. Eplerenone was prepared with drug-free serum. All samples were supplemented with 3 ng/mL digoxin.
Data for FPIA, MEIA and ACMIA were referred from reference 14.

DISCUSSION

Each known digoxin concentration (1, 2, and 3 ng/mL) correlated well in the results of EMIT and CLIA.

Falsely high or low measurements of serum digoxin levels may lead to side effects or poor therapeutic effects due to inappropriately administered dosages.

In the present study, no cross-reactivity was observed between eplerenone and anti-digoxin antibodies in EMIT. The measurement of apparent digoxin concentrations with 100 μg/mL eplerenone was higher than those with the other concentrations of eplerenone (Figure 1) in the same manner as FPIA (14). However, eplerenone concentrations cannot reach 100 μg/mL in clinical settings (17).

The results of the cross-reaction test showed that eplerenone was not detected as digoxin in EMIT because the detection limit of EMIT was 0.3 ng/mL. These results indicated that eplerenone (up to 100 μg/mL) could be detected as digoxin below 0.3 ng/mL in EMIT.

No cross-reactivity was observed between eplerenone and anti-digoxin antibodies in CLIA, regardless of the presence of digoxin (Figure 2).

The C_{max} of eplerenone was previously shown to be 1.87 ± 0.52 μg/mL following the administration of 100 mg/body (multiple doses) of eplerenone (17). A previous study demonstrated that eplerenone interfered with digoxin measurements in FPIA within its clinical dosage (14). Eplerenone was also shown to interfere with the measurement of apparent digoxin concentration in a digoxin and/or eplerenone concentration-dependent man-

ner (1-100 μg/mL) in MEIA and ACMIA (14). However, eplerenone was less likely to be detected in EMIT and CLIA than digoxin at its highest clinical dosage.

Our comparative analysis on EMIT, CLIA, FPIA, MEIA, and ACMIA revealed that the measurement of digoxin in CLIA was the least susceptible to interference from a wide range of eplerenone concentrations and that interference with digoxin measurements was less in EMIT than in FPIA, MEIA, and ACMIA.

In conclusion, EMIT and CLIA have an advantage over the other methods used in that they can accurately measure digoxin concentrations in a patient who has received not only a clinical dosage, but also an overdose of eplerenone.

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