EFFECT OF A CHINESE MEDICINE "KYUSHIN" ON SERUM DIGOXIN CONCENTRATION MEASUREMENT IN DOGS

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The effect of the Chinese medicine kyushin administered orally on serum digoxin measurement was studied in an animal model. Administration of 6 pills of kyushin caused a significant increase in the serum concentration of a digoxin-like immunoreactive substance (DLIS). The DLIS concentration increased to a peak value of 1.14 ng/ml at 1½ hours and declined to 0.5 ng/ml after 24 hours. A one-week administration of 10 pills of kyushin a day produced a steady-state DLIS concentration ranging from 0.91 to 1.07 ng/ml. Among the 7 different kyushin ingredients, toad venom (ch'an-su in Chinese) was the only drug that produced DLIS in the dogs' serum. The similarity in structures of digoxin and toad venom most likely plays an important role in producing a cross-reactivity of DLIS with immunoassay antibody to digoxin.

Although these findings require confirmation in human beings, they suggest that this nonprescription drug may seriously compromise the accuracy and interpretation of digoxin concentration measurements.

MEASUREMENT of serum digoxin concentration is sometimes complicated by the presence of a digoxin-like immunoreactive substance (DLIS) that cross-reacts with immunoassay antibody to digoxin¹. This may result in false elevation of digoxin level.

Kyushin (Kyushin Seiyaku Co., Tokyo, Japan) is a compound Chinese medicine which is commonly used in Taiwan. It consists of 7 different ingredients: toad venom (Ch'an-Su in Chinese) 5 mg/6 pills, oriental bezoar (Niu-Huang in Chinese) 2.4 mg/6 pills, musk (She-Hsiang in Chinese) 1.2 mg/6 pills, ginseng 12 mg/6 pills, rhinoceros horn (Hsi-Chiao in Chinese) 6 mg/6 pills, pearl (Chen-Chu in Chinese) 7.5 mg/6 pills, borneol (Lung-Nau in Chinese) 2.4 mg/

6 pills. According to the kyushin usage instructions, the recommended dosage is 3 to 6 pills a day. The drug also has an effect on heart palpitation, dyspnea, dizziness, night sweating, and abdominal pain.

Recently, we measured the serum digoxin in a patient given kyushin but not receiving digoxin therapy. DLIS was found in her serum. In an attempt to confirm this finding, we performed an animal study to evaluate the serum DLIS production of kyushin in dogs.

MATERIALS AND METHODS

Six mongrel dogs weighing 9 to 10 kg were studied. Ten pills of kyushin per day were administered orally to 5 dogs for one week and the serum DLIS was measured every 24 hours after the treatment began until the DLIS became undetectable. Then the same method was repeated with the same dogs with 1 tablet (0.25 mg)

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Fig.1. Time course of mean ($\pm$ SD) serum concentration after a one-week consecutive administration of 10 pills/day of kyushin and 0.25 mg/day of digoxin in 5 dogs. The administration started at day zero and ended at day 6. n = number of dogs, open squares and open circles represent $\pm$ SD.

of digoxin (Sandoz Ltd., Switzerland) given each day.

A single dose of 0.25 mg of digoxin and 6 pills of kyushin were also orally administered consecutively to 3 dogs. The serum digoxin and DLIS concentrations were measured at $\frac{1}{2}$ - to 2-hour intervals for 8 hours and at 24 hours after each drug administration.

Traditional Chinese medicine containing 10 mg of toad venom, 4.8 mg of oriental bezoar, 2.4 mg of musk, 24 mg of ginseng, 12 mg of rhinoceros cornu, 15 mg of pearl and 4.8 mg of borneol were orally administered separately to 3 dogs. Serum DLIS was measured at one-hour intervals for 5 hours and at 24 hours after each drug administration. The control level of serum DLIS was zero before each administration.

The apparent DLIS and digoxin concentrations, using 200 μ L of serum, were determined by reactivity with antibody in the Fluorescence Polarization Immunoassay (FPIA) for digoxin (Digoxin II, Abbott Laboratories Inc., North Chicago, IL). Protein was precipitated from the sample with 200 μ L of 3% 5-sulfosalicylic acid and then centrifuged at $10,000 \times g$ for 90 sec. The supernatant was assayed immediately. The sensitivity of the FPIA assay is 0.2 ng/ml, and the within-run coefficient of variation is 7.65% at a concentration of 0.75 ng/ml. Samples with a DLIS concentration ≥ 0.2 ng/ml were considered positive; those with < 0.2 ng/ml were

considered negative and assigned a value of 0 ng/ml. The sera of each independent study were analyzed in a single run.

RESULTS

The concentration curve of apparent DLIS in the serum of digoxin-free dogs receiving 10 pills of kyushin per day, measured by the FPIA, is shown in Fig. 1. The DLIS increased from 0 to 0.95 ng/ml on the 4th day of kyushin administration and then maintained a steady-state range from 0.91 to 1.07 ng/ml. The DLIS disappeared 5 days after kyushin administration was stopped. Daily intake of 0.25 mg of digoxin by the same dogs resulted in a rapid serum digoxin concentration increase from 0 to 1.78 ng/ml on the 4th day and then reached a steady-state range from 1.55 to 1.78 ng/ml. The digoxin concentration subsided 4 days after the digoxin was stopped. At steady state the mean DLIS concentration of Kyushin (0.99 ng/ml) was about 59% of the digoxin concentration (1.69 ng/ml). Administration of kyushin and digoxin both presented a cumulative effect (Fig. 1), however, kyushin had a lesser cumulative effect than digoxin.

Figure 2 shows the serum digoxin and DLIS concentration curves of single-dose administration of 6 pills of kyushin and 0.25 mg of digoxin. In contrast to the peak serum concentration of digoxin (4.59 ng/ml) obtained at one

Fig. 2. Time course of mean serum concentration after consecutive administration of single dose of 6 pills of kyushin and 0.25 mg of digoxin in 3 dogs. The administration started at hour zero. n = number of dogs.

Fig. 3. Time course of serum DLIS concentration after administration of 10 mg toad venom at hour zero. n = number of dogs.

hour, kyushin had a much lower peak value of DLIS (1.14 ng/ml) obtained at 1½ hours. However, the digoxin concentration declined more rapidly than kyushin did. At the 24th hour the DLIS concentration of kyushin (0.5 ng/ml) was about 59% of the digoxin concentration (0.84 ng/ml).

The results of the administration of the separate ingredients of kyushin to the same dog revealed that serum DLIS was detected only after

toad venom administration. Fig. 3 shows the DLIS concentration curve after the administration of 10 mg of toad venom. The peak value of DLIS (1.27 ng/ml) occurred 3 hours after administration and the concentration declined to 0.64 ng/ml 24 hours later.

DISCUSSION

Previous studies have reported the presence of

Fig.4. Comparison of bufotalin and digoxin structures.

DLIS in the serum of adult patients with renal insufficiency,² pregnancies,³ and spironolactone administration.⁴⁻⁶ The present study indicates that kyushin administration can significantly increase serum DLIS in dogs.

The 24-hour DLIS concentration produced by a single dose of kyushin and the mean DLIS concentration at steady state of long term administration of kyushin are all about 59% of the concentration levels of 0.25 mg of digoxin produced in the corresponding period (Fig. 1 and 2). Thus, intake of kyushin may significantly interfere with the FPIA method of detecting digoxin concentration. The serum DLIS concentration curve of one week of kyushin administration presents a cumulative effect (Fig. 1). This cumulative effect may further influence serum digoxin measurement.

Although kyushin contains seven different kinds of Chinese medicine only the toad venom can produced DLIS in the serum of dogs (Fig. 3). Toad venom is prepared from the skin secretion of a toad called ch'an-su in Chinese. According to Chinese medical pharmacology, toad venom has a cardiotropic effect. Its action resembles that of digoxin.⁷ It also stimulates the central nervous system⁸ and has a local anesthetic effect.⁹ Toad venom has been found to have more than 20 different types of bufotoxin.¹⁰⁻¹² Their common basic chemical structure formula is a steroid nucleus and an alpha-pyrone ring attached at C-17 which is called bufadienolide. Fig. 4 shows the chemical structure of bufotalin, which is a main bufotoxin found in toad venom. Its chemical structure resembles that of digoxin. Thus, the similar chemical structures of digoxin and toad venom is believed to play an important role in producing a cross-reactivity of DLIS with immunoassay antibody to digoxin.

The chemical structure of digoxin is related

to toad venom. So when immunological techniques are used to measure its dosage, it is not surprising to record false positive results in individuals who have never received digoxin. Since the development of immunoassays for digoxin, many questions have been raised regarding the relationship of serum digoxin concentration to dosage and the therapeutic efficacy in the adult patient.¹³ For adults the goal of therapy is to maintain the serum or plasma digoxin concentration between 0.8 and 2.0 ng/ml.¹⁴ This narrow therapeutic range places vigorous demands on the accuracy and reproducibility of any assay for digoxin. Since kyushin produces a false elevation in digoxin concentration in dogs, the validity of serum digoxin concentration monitoring in patients given kyushin is being questioned. Kyushin is a commonly used nonprescription drug in Taiwan. Thirty-six percent of the patients visiting our clinic have taken this drug. Thus, the clinician must be aware of the potential of kyushin to falsely elevate apparent digoxin concentrations in a significant number of patients.

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