

Digoxin Immunoassay That Avoids Cross-Reactivity from Chinese Medicines, Ryo Fushimi,¹ Hachiro Yamanishi, Miyuki Inoue, Shigeru Iyama, and Nobuyuki Amino (Central Lab. for Clin. Invest., Osaka Univ. Hosp., 2-15 Yamadaoka, Suita, Osaka, 565, Japan; ¹ author for correspondence: fax 81-6-879-6635)

We reported earlier (1, 2) that Chinese medicine containing Ch'an Su (dried venom of the Chinese toad) had immunoreactive digoxin-like activity detectable by fluorescence polarization immunoassay (TDx analyzer; Abbott Labs., N. Chicago, IL), affinity column-mediated immunoassay (*aca* V discrete analyzer; Dupont, Wilmington, DE), and competitive ELISA (Enzymun-Test Digoxin; Boehringer Mannheim, Mannheim, Germany). We attributed these results to the cross-reactivity of the antibodies in these methods with bufogenine, a component of Ch'an Su, along with bufalin, bufotalin, and cinobufagin (1, 2). Recently, a competitive chemiluminescence immunoassay for digoxin, the ACS:180 digoxin assay (Ciba Corning Diagnostics, Medfield, MA), developed with a monoclonal anti-digoxin antibody, was reported not to cross-react with digoxin-like factors and digoxin metabolites (3, 4).

Using the ACS:180 analyzer, we have studied the digoxin-like immunoreactivity in extracts (2) of Kyushin (traditional Chinese medicine in Japan) tablets and Ch'an Su (Kyushin Pharmaceutical Co., Tokyo, Japan) and compared the results with those obtained with the *aca* V and TDx analyzers. Digoxin-like immunoreactivity in Kyushin extracts of 1.7, 3.3, and 10.0 tablet equivalents per liter showed, respectively, 2.00, 2.94, and >5.00 $\mu\text{g/L}$ (off-range high) on the *aca* V analyzer, and 3.61, >5.00, and >5.00 $\mu\text{g/L}$ on the TDx analyzer. On the ACS:180, however, the digoxin-like immunoreactivity was <0.10 $\mu\text{g/L}$ (off-range low) at all three Kyushin concentrations. Likewise, with Ch'an Su at concentrations of 0.2, 10.0, and 20.0 mg/L, the respective immunoreactivities were <0.20, 3.49, and >5.00 $\mu\text{g/L}$ on the *aca* and <0.20, 3.88, and >5.00 $\mu\text{g/L}$ on the TDx. On the ACS:180, immunoreactivities were again all <0.10 $\mu\text{g/L}$.

We then examined healthy volunteers who took two tablets of Kyushin three times a day, a common dose of this drug; using all three methods, we determined any digoxin-like immunoreactivity in serum samples collected every hour (Fig. 1). Serum digoxin-like immunoreactivity appeared 1 h after drug intake and reached 0.2–0.5 $\mu\text{g/L}$ within 2 h by the *aca* V and TDx assays; however, the ACS:180 assay showed <0.10 $\mu\text{g/L}$ at all times.

Ch'an Su has weak cardioactivities and contains indoles and several amino acids. The main differences between digoxin and bufogenine are an α -pyrone ring and three digitoxoses at the C3 position on the steroid nucleus of digoxin. Usually anti-digoxin antisera are obtained after immunization with a combined carrier protein and digitoxose; these antisera cross-react with bufogenine. The ACS:180 digoxin assay is based on an acridinium ester-labeled mouse monoclonal antibody to digoxin as the tracer, and uses digitoxin conjugated to paramagnetic particles as the capturing solid phase (5). The anti-digoxin monoclonal antibody was obtained by immunization with

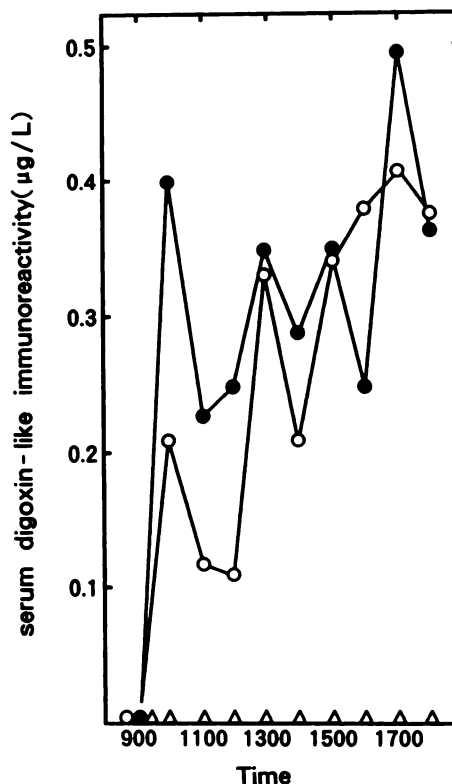


Fig. 1. Increase in serum digoxin-like immunoreactivity in healthy volunteers after taking Kyushin tablets.

Two tablets were administered at 900, 1200, and 1500 h. Immunoreactivities were measured with the TDx (●), *aca* V (○), and ACS:180 analyzers (△).

the combined carrier protein and steroid nucleus; therefore, this digoxin assay does not cross-react with Chinese medicines.

In Asian countries, Chinese medicines are easily obtainable at common pharmacies without a doctor's prescription, and some Chinese foods such as toad soup contain digitalis-like substances (6). In light of these facts, the ACS:180 analyzer should be used for assays of digoxin in areas where such medicines and foods are widely available.

References

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