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Determination of free digoxin in sera of 8 patients with chronic cardiac insufficiency

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AIM: To establish a method for the determination of free digoxin in serum for clinical use and to study the relationship between the free and total digoxin concentrations in chronic cardiac insufficiency patients receiving digoxin with different renal function. **METHODS:** The ultrafiltration with fluorescence polarization immunoassay was used to determine the concentration of free digoxin. **RESULTS:** The concentrations of digoxin standards in serum were 0.96, 1.92, and 3.84 nmol·L^{-1}. The relative standard deviation was <7 % for intra-day and <6 % for inter-day determinations. The average recovery was 99.95 ± 2.18 %. The ratio of free/total digoxin in chronic cardiac insufficiency patients with renal dysfunction was lower than that in patients with normal kidneys (63.5 ± 4.7 % *vs* 75.1 ± 3.9 %, $P<0.01$). **CONCLUSION:** The present method is simple and reliable. In these patients there is an over-measurement for total digoxin concentration, suggesting the presence of elevated endogenous digoxin-like immunoreactive substances.

KEY WORDS digoxin; ultrafiltration; fluorescence polarization immunoassay; creatinine; blood urea nitrogen; congestive heart failure; kidney failure

Measurement of serum digoxin concentrations is recommended as a routine in patients undergoing digoxin therapy because its therapeutic range is narrow and the pharmacologic effect and toxicity well correlate to its concentrations in serum[1,2]. A highly significant, nonlinear relationship was found between serum digoxin concentration and clinical effect after digoxin dosing[3,4]. But the free drug is an important determinant of pharmacodynamic activity because only the free (unbound) drug is transported to its site of action where it binds to receptors to effect a response[5]. The purpose of this study was to establish a method for the determination of free digoxin in serum for clinical use and to study the relationship between the free and total digoxin concentrations in chronic cardiac insufficiency patients receiving digoxin with different renal function.

MATERIALS AND METHODS

Instruments and reagents TDX analyser, Digoxin II assay kit, digoxin calibrators and controls (Abbott Laboratories, USA); digoxin (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China).

Serum digoxin standards Digoxin was dissolved in 50 % ethanol to 300 μg·L^{-1}. Serum digoxin standards were prepared by adding appropriate amounts of digoxin stock solution to the mixed serum (pretreated with acetonitrile) from normal subjects to attain 3 concentrations: 0.96, 1.92, and 3.84 nmol·L^{-1}.

Ultrafiltration We modified the method of Dasgupta *et al*[6]. Serum digoxin standards 350 μl were pipetted into the sample reservoir of a filter (Ultrafree-MC, PLTK-membrane, molecular exclusion limit 30 kDa, Millipore, USA). It was spun at 25±3 (

Abbreviations: DLIS = digoxin-like immunoreactive substances; FPIA = fluorescence polarization immunoassay; MP = magnitude of polarization; RSD = relative standard deviations

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Tab 1. Eight inpatients with chronic cardiac insufficiency. The values of serum alanine aminotransferase for these patients were <667 nmol·s^{-1}·L^{-1}.

Man	Age /a	Body wt /kg	Creatinine /μmol·L^{-1}	Blood urea nitrogen /mmol·L^{-1}	Albumin /g·L^{-1}	Globulin /g·L^{-1}	Renal function
1	69	87	72.2	5.2	43.4	23.3	Normal
2	61	68	70.1	5.1	43.6	24.4	Normal
3	64	53	107.0	5.1	37.5	25.6	Normal
4	85	86	89.0	5.5	41.8	19.5	Normal
5	79	71	131.0	8.7	39.8	17.8	Abnormal
6	78	78	133.0	9.5	45.0	24.9	Abnormal
7	70	70	114.0	11.2	37.5	26.6	Abnormal
8	72	57	130.2	8.2	41.3	27.0	Abnormal

Tab 2. Precision of intra-day and inter-day for free digoxin by ultrafiltration with FPIA. $n=5$ repetitions. $\bar{x}\pm s$.

Digoxin added/nmol·L^{-1}	Digoxin measured/nmol·L^{-1}		RSD/%	
	Intra-day	Inter-day	Intra-day	Inter-day
0.96	0.98±0.06	0.97±0.04	6.12	4.12
1.92	1.95±0.12	1.87±0.11	6.15	5.88
3.84	3.77±0.10	3.80±0.03	2.65	0.79

using a 45° fixed angle-rotor TDX centrifuge (6000 × g for 15 min). An aliquot (200 μl) of the ultrafiltrate was diluted with 200 μl of dilution agent prepared by our laboratory, and then analyzed by fluorescence polarization immunoassay (FPIA).

FPIA Digoxin determinations were done in the Abbott TDX analyzer with FPIA reagents purchased from Abbott. The FPIA Digoxin II assay was performed according to the manufacturer's directions. Serum-based calibrators and controls were used throughout.

Analysis of serum digoxin The total and free digoxins in serum were determined in 8 hospitalized coronary disease patients with congestive heart failure (New York Heart Association classification, class II) (Tab 1). Renal dysfunction was defined as a serum creatinine greater than 130 μmol·L^{-1} and a blood urea nitrogen greater than 7.5 mmol·L^{-1}. They received prolonged digoxin treatment at daily doses of 62.5—125 μg *po*. Blood samples were collected just before the next dosing (serum valley concentration) at a steady state. The total digoxin was determined by FPIA, and free digoxin was determined by ultrafiltration with FPIA. The duration of centrifugal ultrafiltration for serum was 60 min.

Results were expressed as $\bar{x}\pm s$ and compared by t test.

RESULTS

Methodology The concentrations of digoxin calibrators were 0, 0.64, 1.28, 2.56, 3.84, and 6.40 nmol·L^{-1}. The relationship between digoxin concentration (C) and magnitude of polarization (MP) was found to be $MP=165.37-14.11\,C$, $r=-0.9966$. The lowest measurable level was 256 pmol·L^{-1}.

For each concentration tested, the relative standard deviations (RSD) for intra-day and inter-day (5 repetitions each) were <7 % and <6 %, respectively. The RSD for intra-day and inter-day were the smallest for the digoxin standards containing the highest concentration (3.84 nmol·L^{-1}, RSD 2.65 % and 0.79 %, respectively) (Tab 2).

The analytical recovery (9 repetitions) for

ultrafiltrates of serum digoxin standards showed that the higher concentration gave the less recovery (Tab 3).

Tab 3. Recovery for free digoxin by ultrafiltration with FPIA. $n=9$ repetitions.

Digoxin added /nmol·L^{-1}	Digoxin measured /nmol·L^{-1}	Recovery /%
0.96	0.98	102.43
1.92	1.90	99.07
3.84	3.78	98.35

Serum digoxin in chronic cardiac insufficiency patients Mean ratios of free to total digoxin in patients without or with renal dysfunction were 75.1 ± 3.9 % and 63.5 ± 4.7 %, respectively ($P<0.01$) (Tab 4).

Tab 4. Total digoxin and free digoxin in serum of patients with chronic cardiac insufficiency receiving digoxin. [a] $P<0.01$ vs the group with normal renal function.

№	Renal function	Total digoxin /nmol·L^{-1}	Free digoxin /nmol·L^{-1}	Free:Total /%	$\bar{x}\pm s$
1	Normal	0.72	0.58	80.6	75.1±3.9
2	Normal	2.64	1.89	71.6	
3	Normal	0.87	0.64	73.6	
4	Normal	1.18	0.88	74.6	
5	Abnormal	0.68	0.46	67.6	63.5±4.7[a]
6	Abnormal	1.28	0.86	67.2	
7	Abnormal	0.90	0.55	61.1	
8	Abnormal	1.57	0.91	58.0	

In a patient of digoxin intoxication with normal renal function, the fraction of free (4.16 nmol·L^{-1}) to total (5.77 nmol·L^{-1}) digoxin was 72.1 %, although his total digoxin concentration in serum attained a high level.

DISCUSSION

A method for measuring free digoxin in serum ought to be precisely accurate, easily performed in clinical laboratories, and to have a quick turnover time[7]. Our results showed that the measurements of free digoxin by FPIA in ultrafiltrate conformed to these advantages. The entire procedure for the measurement can be completed within 1.5 h. Accordingly, results would be available to the physicians within a clinically allowable time during therapeutic drug monitoring of digoxin.

The average protein binding of serum digoxin at room temperature in 4 cardiac insufficiency patients with normal renal function was 24.9 ± 3.9 %. The results were well consistent with the previously reported values of 27.3 % at 25 ℃[6] by ultrafiltration with FPIA, and of 23 %[8] and 25 %[9] at 37 ℃ by equilibrium dialysis with FPIA.

The ratio of free to total digoxin in cardiac insufficiency patients with renal dysfunction was significantly lower than that in patients with normal kidneys. Similar binding of digoxin to serum albumin was expected in the 2 groups because the concentrations of albumin in both groups are not statistically different[6]. The lower ratio of free/total digoxin in patients with renal dysfunction indicated an over-estimation for total digoxin concentrations, suggesting the presence of increased concentrations of endogenous digoxin-like immunoreactive substances (DLIS).

Elevated concentrations of DLIS have been reported in a number of pathophysiological conditions including hepatic diseases and renal insufficiency[10]. In fact, we found that the concentration of apparent digoxin in serum was 0.546 nmol·L^{-1} in a patient of kidney failure with uremia although he did not take any digoxin or receive any other cardiac glycosides therapy. These DLIS cross-reacted with digoxin antibodies when determinations of serum digoxin were performed with

immunoassay, resulting in falsely high measurements of serum digoxin[11]. But it was difficult to evaluate the extent of interference from DLIS in these patients receiving digoxin[11].

In a digoxin intoxication patient with normal kidneys the ratio of free to total digoxin was 72.1 %, higher than that from patients with renal insufficiency, suggesting the binding of protein for digoxin in serum does not depend on what level has attained in serum digoxin concentration.

In conclusion, the present method for determination of free digoxin by ultrafiltration with FPIA is reliable and useful. It is necessary to analyze the free digoxin, the pharmacologically active fraction, in chronic cardiac insufficiency patients receiving digoxin with renal dysfunction.

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八位慢性心功能不全患者血清游离地高辛检测

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目的：建立能为临床所用的检测游离地高辛的方法，并研究不同肾功能慢性心功能不全患者血清中游离和总地高辛浓度的关系。 **方法**：用超滤及荧光极化免疫测定法检测游离地高辛（Dig）。 **结果**：Dig 血清标准液浓度为0.96，1.92，3.84 nmol·L^{-1}。 日内、日间相对标准差分别小于7 % 和 6 %，平均回收率为99.95 ± 2.18 %。 接受 Dig 治疗的八位慢性心功能不全病人，肾功能不全与正常者的血清 Dig 游离/总浓度比率分别为63.5 ± 4.7 %、75.1 ± 3.9 %（$P < 0.01$）。 **结论**：肾功能不全病人血清 Dig 总浓度测定值偏高，提示其血清中存在过量的内源性 Dig 样免疫活性物质。

关键词 地高辛；超滤法；荧光极化免疫测定法；肌酸酐；血尿素氮；充血性心力衰竭；肾功能衰竭