A Study on Digoxin®-like Immunoreactivity and Its Nature in Patients with Chronic Renal Failure and Neonates

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= 국문초록 =

만성 신부전환자 및 신생아 혈청에서 Digoxin®과 교차 면역반응을 보이는 물질에 관한 연구

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이 명 식 · 이 중 기

Digoxin®을 복용하고 있지 않는 각 27명의 만성 신부전 환자 및 신생아의 혈청에서 Gammacoat Digoxin® kit와 Amerlex Digoxin RIA® kit를 이용하여 digoxin과 교차 면역반응을 보이는 내인성 물질(이하 DLI: Digoxin-like Immunoreactivity)을 측정하여 다음의 결과를 얻었다.

1) Gammacoat Digoxin® kit로 측정시 27명의 만성 신부전 환자중 12명에서 신생아의 경우는 27명 전원에서 측정 하한치 이상의 DLI가 검출된 반면, Amerlex Digoxin RIA® kit로는 1명을 제외한 양 군의 전원에서 DLI가 관찰되지 않았다. 만성 신부전 환자에서 Gammacoat Digoxin® kit로 측정된 DLI와 혈청 BUN/Creatinine 값 사이에는 유의한 상관 관계가 없었다.

2) 만성 신부전 환자 및 신생아의 pooled sera에 가한 digoxin의 수거율은 각각 49%, 40%였다.

3) 만선 신부전 환자 및 신생아의 pooled sera에서 측정되는 DLI의 희석 curve는 정상 희석 curve와 다르다고 할 수 없었다.

4) 만성 신부전 환자의 pooled sera에서 측정되는 DLI의 16%, 그리고 신생아의 pooled sera에서 측정되는 DLI의 20%가 trypsin에 sensitive하였다.

5) 만성 신부전 환자의 pooled sera에서 측정되는 DLI의 56%가, 그리고 신생아의 pooled sera에서 측정되는 DLI의 경우는 전체의 20%미만이 methylene chloride로 추출되었다.

이상에서 만성 신부전 환자 및 신생아의 혈청에서는 방사면역 kit의 종류에 따라 DLI가 검출되며 그 실체를 알기 위해서는 더욱 연구가 필요하다고 할 수 있었다.

Intrduction

Measurement of serum digoxin level with radioimmunoassay is being used prevalently since the initial development by Smith et al[1]. because of low toxic: therapeutic ratio and poor relationship between dose and serum concentration etc[2]. Moreover, measurement of serum digoxin level in cases with chronic renal failure seems to have even greater clinical significance considering frequent coexistence of cardiac diseases & renal failure and changes of pharmacodynamics due to renal failure. But it was found that there are significant differences in

the apparent serum digoxin concentrations measured with radioimmunoassay according to the types & kinds of assay kits[3,4]. It is considered to be caused by some endogenous substances having digoxin-like immunoreactivity (DLI), and DLI was also found in the sera of third trimester pregnant women[5], neonates[6,7] & amniotic fluid[6], subsequently.

Studies on DLI may be worthwhile not only in heightening of reliability & specificity of digoxin radioimmunoassay but also in investigation of Na-K ATPase inhibitor in consideration of the suspicion that the substance(s) having DLI may be related to the Na-K ATPase inhibitor found in the circulation of volume expanded experimental animals[8]. In the present study, we measured the levels of endogenous DLI in sera of patients with chronic renal failure & neonates and analysed various natures of DLI's.

Materials and Methods

Plasmata from 27 patients with end stage renal disease of serum creatinine level over 6 and 27 neonates below 39 days of age were obtained and stored at 20℃. All subjects were not receiving digoxin therapy and the samples were thawed & analysed within 60 days of collection. 10 healthy male volunteers comprised normal control group.

GammaCoat Digoxin kit (Clinical Assays) using coated tube as solid phase and Amerlex Digoxin RIA kit (Amersham) employing centrifugal separation method were used for assay of DLI. Normal pooled sera were used for dilution of respective pooled sera. Pooled sera of patients with chronic renal failure & neonates were treated with the 0.1% trypsin (Sigma, type I) for 45 min at 37℃ followed by addition of Soybean trypsin inhibitor (Sigma, type I-S) for measurement of trypsin sensitivity of DLI. Organic solvent extraction was done by addition of equal volume of methylene chloride (Sigma) to respective pooled sera, and organic phases were dried and reconstituted with same volume of normal pooled sera before radioimmunoassay. Digoxin recoveries were calculated as differences between amounts of the apparent immunoreactive digoxin before & after addition of appropriate amount of true digoxin to respective pooled sera divided by amount of digoxin added.

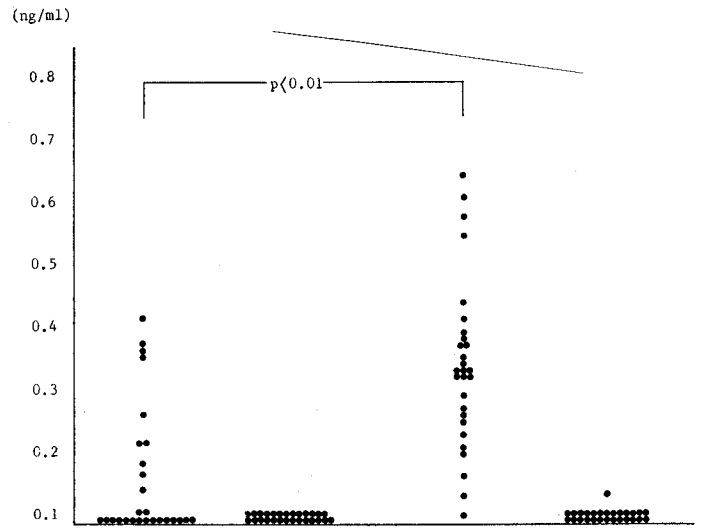

Fig. 1. Levels of endogenous digoxin-like substances in the sera of chronic renal failure & neonates.

Student's t-test, linear regression and test of parallelism[9] were used for statistical analyses and p-value below 0.05 was considered arbitrarily to be significant.

Results

1) DLI's in the Sera of Patients with Chronic Renal Failure & Neonates

DLI above lower limit of detection (0.1 ng/ml) was observed in sera from 12 (44.4%) of 27 patients with chronic renal failure using gammaCoat Digoxin kit (range: 0.11—0.41 ng/ml), whereas all sera had DLI values below lower limit of detection with Amerlex Digoxin RIA kit. In case of neonatal sera, all 27 samples gave positive DLI results (range: 0.11—0.64 ng/ml), and mean value of DLI's of 27 neonatal samples (0.36±0.13 ng/ml; mean±S.D.) was significantly higher than that of 27 samples from patients with chronic renal failure (0.11±0.14 ng/ml), using GammaCoat Digoxin kit. ($p<0.001$) with Amerlex Digoxin RIA kit, DLI above the lower limit of detection was not found in all neonatal sera except one sample (0.15 ng/ml) (Fig. 1).

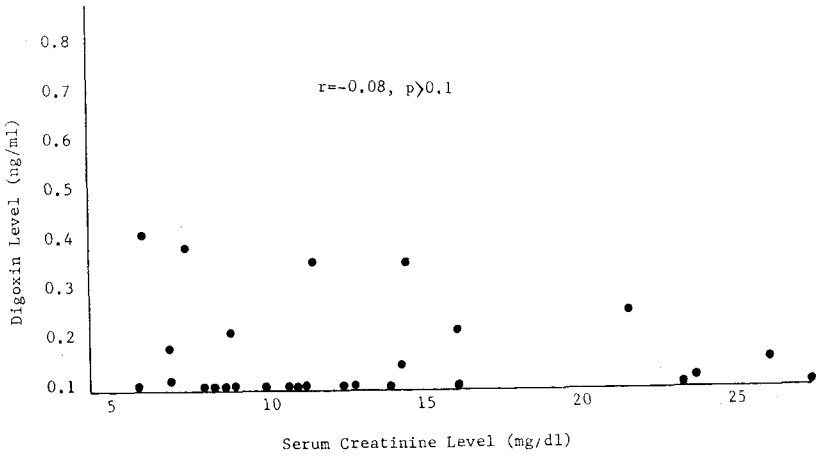

Fig. 2. Levels of endogenous digoxin-like substances versus serum creatinine levels.

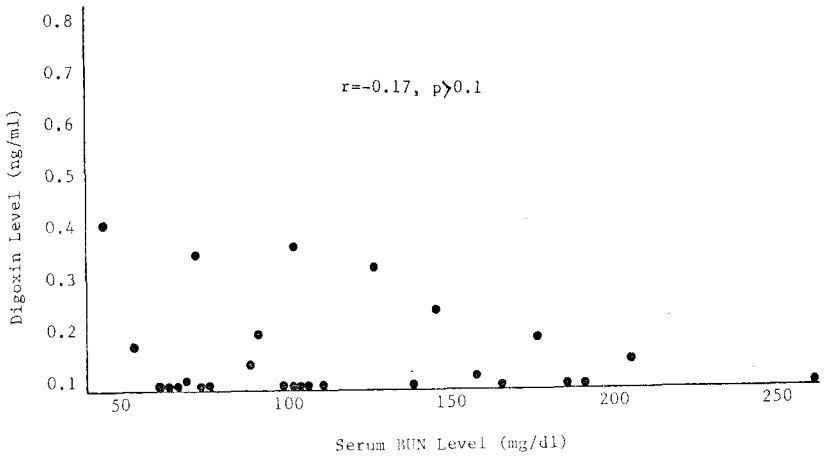

Fig. 3. Levels of endogenous digoxin-like substances versus blood urea nitrogen levels.

All of normal control sera gave negative DLI results with GammaCoat Digoxin kit except one (0.17 ng/ml).

There was no significant correlation between the DLI's of the patients with chronic renal failure measured with GammaCoat Digoxin kit and their serum blood urea nitrogen or creatinine levels($p>0.1$, $p>0.01$) (Fig. 2, Fig. 3).

2) Percent Recoveries of Digoxin

DLI's of pooled sera from patients with chronic renal failure & neonates with GammaCoat Digoxin kit were 0.24 & 0.40 ng/ml, respectively, and became 0.46 & 0.58 ng/ml after addition of 0.45 ng of true digoxin, yielding 49% & 40% recovories of added digoxin in the former & latter, respectively(Table 1).

3) Dilution Curves of DLI's

2 pooled sera from patients with chronic renal failure gave DLI values of 0.21 & 0.50 ng/ml using Gamma Coat Digoxin kit, and the DLI's became 0.11 & 0.23 ng/ml after 2-fold dilution with normal pooled sera, respectively. Neonatal pooled sera having DLI of 0.39 ng/ml gave DLI values of 0.27 & 0.18 ng/ml after 2-fole & 4-fold dilution, respectively, with GammaCoat Digoxin kit. The dilution curves of both groups of pooled sera were not significantly different from normal dilution curve when analysed with test of parallelism ($p>0.1$, $p>0.05$) (Fig. 4).

4) Trypsin Sensitivity of DLI's

DLI of pooled sera from patients with chronic renal failure was 0.5 ng/ml, and became lessened to 0.42 ng/ml after trypsin treatment with trypsin sensitivity of 16%, using GammaCoat Digoxin kit. DLI of pooled sera from neonates before & after trypsin treatment with GammaCoat Digoxin kit were 0.46 & 0.37 ng/ml respectively, to yield 20% trpsin sensitivity (Table 2).

5) Organic Solvent Extraction of DLI's

Of the total DLI of 0.50 ng/ml in pooled sera from patients with chronic renal failure, 0.28 ng/ml (56%) was extracted into organic phase with methylene chloride, whereas, no detectable DLI (below 0.01 ng/ml, below 21%) was extracted into organic phase of the total DLI, 0.46 ng/ml in neonatal pooled sera

Table 1. Per Cent Digoxin Recovery (Digoxin Recovered/Added)

	Original digoxin level (ng/ml)	Digoxin added (ng)	Final digoxin level (ng/ml)	Digoxin recovery (%)
CRF serum pooled	0.24	0.45	0.46	49
Neonatal serum pooled	0.40	0.45	0.58	40

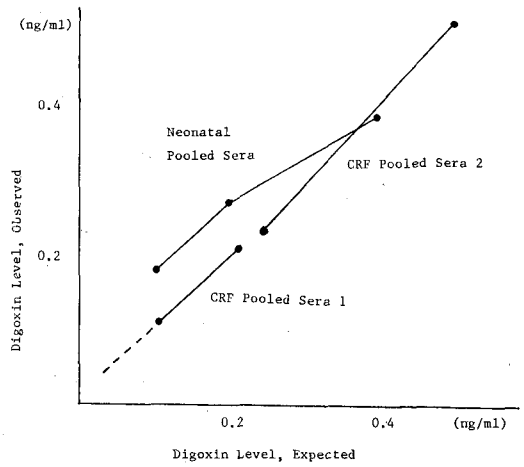

Fig. 4. Tests of parallelism in pooled sera.

Table 2. Trypsin Sensitivity of DLI's in Pooled Sera

	DLI before trypsinization	DLI before trypsinization	Trypsin sensitivity
CRF serum	0.50	0.42	16%
Neonatal serum	0.46	0.37	20%

(ng/dl)

Table 3. Extraction with Methylene Chloride

	Total DLI	Extracted DLI	% Extraction
CRF serum	0.50	0.28	56%
Neonatal serum	0.46	below 0.10	below 21%

(ng/dl)

using GammaCoat Digoxin kit (Table 3).

Discussion

This study documents the presence of variable levels of DLI according to radioimmunoassay kits in the sera of patients with chorinc renal failure & neonates, which might be clinically significant. Similar results were also reported by many investigators[3,4,6,10] but the true natures & identities of these DLI's are far from clear. Tracer degradation, nonspecific binding, variation in ionic strength or other factors which are independent of antigen-antibody reaction do not seem to be plausible causes of those results[4]. Dilution curves of pooled sera in this study also are in contradiction to the theory that DLI is due to nonspecific reaction, and suggest that some substance(s) displace radioactive tracer in a competitive manners. The apparent difference between the calculated slope of dilution curve and 1 in case of neonatal pooled sera as well as the apparently low recoveries of added digoxin might be attributable to the fact that these results were obtained in the lower extreme range of log-logit curve with possible higher error.

And the documented negligible cross immunoreactivity of commercial radioimmunoassay kits with digoxin analogues[12,14] and low against the possibility that DLI is caused by structural analogues of digoxin or their degradation product.

The suggestion by Graves et al[4]. that DLI detected in sera of patients with chronic renal failure might be related to "middle molecule"[15] in based on the findings that Na-K ATPase inhibitor simulating digitalis action was detected in the serum[16], urine[17] of patients with chronic renal failure, volume expanded human & experiemental animals[8,18]. Gruber et al[8]. and Klingmueller at al[18]. reported detection of Na-K ATPase inhibitor & DLI in the same post-salt peak using gel filtration chromatography. On the other hand, poor correlation between DLI's & serum blood urea nitrogen/creatinine levels and low trypsin sensitivity of DLI as documented in this study suggest that the substance(s) having DLI might be different from "natriuretic hormone" advocated by Gruber et al[8],. ACTH fragments with some natriuretic activity[19,20] or various atrial natriuretic peptides[21,23], but the possibility that "natriuretic hormone" is very small peptide and not susceptible to trypsin action must also be borne in mind.

Also controversial are the origin & identity of DLI found in the sera of premature or full-term neonate. DLI was also detected in the placental homogenate[24], third trimester pregnant women[5] & amniotic fluid[6] indicating that DLI is produced at the gestational unit. But the hypothesis that the steroids related to pregnancy engender DLI seems unlikely in consideration of the low cross immunoreactivity of the radioimmunoassay kits with structural analogues and low extractability into organic solvent[5]. This type of DLI is reportedly not related to the weight or Apgar score of the neonate[7] and seems to disappear after 60 days after birth[7,25], suggesting possible role of fetal adrenal in genesis of DLI[7]. But report of Pudek et al[26]. that level of DLI & dehydroepiandrostenedione sulfate had poor correlation is against the role of fetal adrenal in this regard.

In conclusion, DLI's are detected in the sera of patients with radioimmunoassay kits, but the origins & chemical identities remain to be established despite of intensive efforts by investigators. And apparent differences of the various digoxin radioimmunoassay kits in the measurement of digoxin or DLI must be kept in mind in the selection of assay kit and in the interpretation of results in patients with renal failure, neonates & pregnant women etc.

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REFERENCES

1) Smith TW, Butler VP Jr, Haber E: *Determination of Therapeutic and Toxic Serum Digoxin Concentrations by Radioimmunoassay. N Engl J Med 281:1, 212, 1969*
2) Aronson JK: *Plasma Digoxin Concentrations, Indications for Measurement. Med Progress May 65, 1985*
3) Craver JL, Valdes R Jr: *Anomalous Serum Digoxin Concentrations in Uremia. Ann Intern Med 98 No. 4:483, Apr 1983*
4) Graves SW, Brown B, Valder R Jr: *An Endogenous Digoxin-Like Substances in Patients with Renal Impairment. Ann Intern Med 99 Np. 5:604, Nov 1983*
5) Graves SW, Valdes R Jr, Brown BA, Knight AB, Craug HR: *Endogenous Digoxin-Immunoreactive Substance in Human Pregnancies. J Clin Endocrinol Metab 58:748, 1984*
6) Valdes R Jr, Graves SW, Brown BA, Landt M: *Endogenous Substance in Newborn Infants Causing False Positive Digoxin Measurements. J Pediatr 102: 947, 1983*
7) Pudek MR, Seccombe DW, Whitfield MF: *Digoxin-like Immunoreactivity in Premature and Full-term Infants not Receiving Digoxin Therapy. N Engl J Med 308:904, 1983*
8) Gruber KA, Whitaker JM, Buckalew VM Jr: *Endogenous Digitalis Like Substance in Plasma of Volume-expanded Dogs. Nature 297:743, Oct 1980*
9) Rodbard D: *Statistical Quality Control and Routine Date Processing for Radioimmunoassay and Immunoradiometric Assays. Clin Chem 20 No. 10:1, 255, 1974*
10) Graves S, Brown A, Valdes R Jr: *Digoxin-like Substance in Uremic Patients: Falsepositive Effects on Digoxin Assay. Clin Chem 29 No 6:1,166, 1983*
11) Thorell JI, Larson SM: *Radioimmunoassay and Related Techinques. p. 83, Saint Louis, The C.V. Mosby Company 1978*
12) Manual of GammaCoat Digoxin Radioimmunoassay Kit. *Clinical Assays*
13) Manual of Amerlex Digoxin RIA kit for immuno assay of Digoxin in Human Serum or Plasma. *Amersham*
14) Valdes R Jr, Brown BA, Graves SW: *Variable Cross-reactivity of Digoxin Metabolites in Digoxin Immunoassays. Brief Scientific Reports 82 No 2:210, 1983*
15) Contreras P, Navarro LJ, Touraine JL, Freyria AM, Traeger J: *Molecules in the Milddle Molecular Weight Range. Nephron 32:193, 1982*
16) Bourgognie JJ, Hwang KH, Espinel C, Klahr S, Bricker NS: *A Natriuretic Factor in the Serum of Patients with Chronic Uremia. J Clin Invest 51:1, 514, 1972*
17) Bourgoignie JJ, Hwang KH, Ipakchi E, Bricker NS: *The Presence of a Natriuretic Factor in the Urine of Patients with Chronic Uremia. J Clin Invest 53:1, 559, 1974*
18) Klingmueller D, Weiler E, Kramer HJ: *Digoxin-like Natriuretic Activity in the Urine of Salt Loaded Healthy Subjects. Klin Wochenschr 60:1,249, 1982*
19) Gruber KA, Klein MC, Hutchins PM, Buckalew VM Jr, Lymangrower JR: *Natriuretic and Hypertensive Activities Reside in a Fragment of ACTH. Hypertension 6:468, 1984*
20) Orias R, McCann SM: *Natriuretic Effect of alpha-MSH in the Water-Loaded Rat. Proc Soc Exp Biol Med 133:469, 1970*
21) De Bold AJ: *Atrial Natriuretic Factor of the Rat Heart. Studies on Isolation and Properties. Proc Soc Exp Biol Med 170:133, 1982*
22) Trippodo NC, MacPhee AA, Cole FE: *Partially Purified Human and Rat Atrial Natriuretic Factor. Hypertension 5 (supp I):I-81, 1983*
23) Yandle TG, Espiner EA, Nicholls MG, Duff H: *Radioimmunoassay and Characterization of Atrial Natriuretic Peptide in Human Plasma. J Clin Endocrinol Metab 63:72. 1986*
24) Besch HR Jr, Hufferd S, Lake M, Hurwita R, Watanabe AM: *False Elevation of Apparent Digoxin Levels in Plasma of Premature Infants. Clin Chem 22:1,168, 1976*
25) Hicks JM, Brett EM: *Falsely Positive Digoxin Results in Serum Specimens from Acute Care Infants. Clin Chem 29 No. 6:1,249, 983*
26) Pudek MR, Seccombe DW, Jacobson BE, Whitfield MF: *Seven Different Digoxin Immunoassay Kits Compared with Respect to Interference by a Digoxin-Like Immunoreactive Substance in Serum from Premature and Full-Term Infants. Clin Chem 29 No. 11:1972, 1983*