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Digoxin-Like Immunoreactive Substance in Postmortem Blood of Infants and Children

REFERENCE: Spiehler, V. R., Fischer, W. R., and Richards, R. G., **"Digoxin-Like Immunoreactive Substance in Postmortem Blood of Infants and Children,"** *Journal of Forensic Sciences,* JFSCA, Vol. 30, No. 1, Jan. 1985, pp. 86-91.

ABSTRACT: A digoxin-like immunoreactive substance (DLIS) has been reported in the serum of infants not receiving digoxin. This study was undertaken to determine if DLIS is present in the postmortem blood and tissues of infants or children and whether the endogenous substance could interfere with forensic toxicological analysis in suspected overdose. Ninety blood specimens taken from the heart at autopsy of children or infants were screened for DLIS using commercial radioimmunoassay kits. The average age at death in these cases was 8.6 months, the median age was 2 months. DLIS equivalent to 0.25 to 2.0 ng/mL digoxin was found in one third of the cases. The incidence of positive findings was 5/6 stillborns, 10/45 Sudden Infant Death Syndrome (SIDS), 10/15 deaths as a result of infection, 4/7 homicides, 1/8 deaths caused by congenital defects, and 0/9 accidental deaths. The body distribution of DLIS was investigated and highest levels were found in the liver. Findings of DLIS in blood were correlated with renal failure, (elevated vitreous urea nitrogen), electrolyte imbalance, and liver trauma. Apparent concentrations were in the equivalent therapeutic range of digoxin and would not be confused with accidental or intentional overdose with digoxin.

KEYWORDS: toxicology, digoxin-like immunoreactive substance (DLIS), blood, radioimmunoassay

A digoxin-like immunoreactivity has been reported in the serum of neonates not receiving digoxin therapy [*1-4*]. An endogenous substance present in the circulation of premature and full-term infants that cross-reacts with antibodies to digoxin might give false positive results in radioimmunoassays for digoxin. The objectives of this study were to determine: (1) if the digoxin-like immunoreactive substance (DLIS) is present in the postmortem bloods of infants and children taken from the heart at autopsy and (2) whether the digoxin-like substance could be confused with accidental or intentional overdose with digoxin.

Methods

In 1983 there were 97 deaths of infants and children under 5 years of age in Orange County that were investigated by the Coroner. In four cases no or insufficient blood was obtained for testing. Three cases were omitted from the study because the child received digoxin or might have accidently taken digoxin belonging to a family member. Blood from the remaining 90 cases was screened for digoxin using radioimmunoassay. Since the study

Presented at the 36th Annual Meeting of the American Academy of Forensic Sciences, Anaheim, CA, 21-25 Feb. 1984. Received for publication 3 May 1984; accepted for publication 29 May 1984.

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was begun in June of the year, bloods from the first six months were analyzed in June. Some of these were as much as six months old. All bloods were stored at room temperature preserved with sodium flouride and potassium oxalate. DLIS appears to be stable in stored blood as is digoxin. No fewer or greater incidence of DLIS was noted in the six-month-old bloods.

The vitreous specimens were collected with a 10-ml syringe and an 18-gauge needle from the lateral canthus of both eyes, combined, and refrigerated until chemical analysis could be performed. The analysis was done on the Technicon Simultaneous Multiple Autoanalyzer Computer (SMAC), Technicon, which provides 20 chemical analyses and 4 calculations. The method for determination of urea nitrogen is a modification of the carbamido-diacetyl reaction. In a relatively weak acid solution, diacetyl monoxime is hydrolyzed to diacetyl, which, in turn, reacts directly with urea in the presence of acidic ferric ions. The presence of thiosemicarbazide intensifies the color of the reaction. The absorbance of the reaction mixture is measured at 520 nm in a 10-mm path flow cell.

Radioimmunoassay

The blood or tissue homogenate was analyzed directly with each of the radioimmunoassay (RIA) kits. These kits were Gamma Coat™ ^{125}I-Digoxin Radioimmunoassay Kit from Clinical Assays, Cambridge, MA 02139; Coat-A-Count® Digoxin Solid Phase Radioimmunoassay from Diagnostic Products Corp., double antibody; ^{125}I-Digoxin RIA kit from Diagnostic Products Corporation, Los Angeles, CA 90045; and the double antibody Digoxin RIAphase™ kit from Beckman Instruments Inc., Fullerton, CA 92634. All four kits used digoxin labelled with iodine-125 as the labelled antigen. ^{125}I-labelled antigen competes with unlabelled antigen from a standard or from a sample for available binding sites on an antibody (the primary antibody). The first two kits use a primary antibody which is immobilized onto the lower inner wall of a polypropylene tube so that no centrifugation is required in the performance of the assay. After incubation of the standards and samples with ^{125}I-labelled digoxin in antibody-coated tubes, the reaction mixture is decanted and the radioactivity bound to the tube via the antibody is counted. The Coat-A-Count assay also contains blocking agents which serve to free digoxin from its binding to circulating proteins.

The second two kits are double antibody assays using a second, precipitating antibody to complex with the anti-digoxin antibody with its bound labelled and unlabelled drug. In the RIAphase kit the second or precipitating antibody is chemically bonded to microcrystalline cellulose to aid in precipitating the complex. In the Diagnostic Products assay, polyethlyene glycol (PEG) is added to the second antibody to speed the rate of flocculation reaction and to produce a firm and easily visible pellet. The labelled and unlabelled digoxin that has reacted with the primary antibody will be found in the precipitate. The incubation mixture in these assays was centrifuged at 8000 $\times$ *g* in a Microfuge® centrifuge (Beckman Instruments, Inc., Fullerton, CA 92634) for 5 min to separate the bound and free fractions and, after decanting the supernatant, the precipitate was counted in a gamma counter.

Results and Discussion

In order of relative cross-reactivity with DLIS the kits were Clinical Assays > Diagnostic Products > Beckman Instruments. The extent of this cross-reactivity with DLIS is shown by the magnitude of the positive results expressed as equivalents digoxin in Table 1. Clinical Assays Gamma Coat gave the highest as well as the most frequent positives. The range of 0.25 to 2.0 ng/mL equivalents digoxin is the same as that reported for this kit by Pudek et al [*1,2*]. The two Diagnostic Products kits, the coated tube Coat-A-Count and the double antibody procedure, were less reactive. The Beckman Instruments Inc. RIAphase Digoxin double antibody assay less often registered a positive than the others, but when it did, the levels

TABLE 1—*Cross-reactivity of commercial kits to DLIS.*

	% Positive	Average, ng/mL	Range Equivalents Digoxin	*N*
Clinical Assays Gamma Coat	70	0.75	0.25-2.0	64
Diagnostic Products				
Coat-A-Count	58	0.43	0.25-1.0	51
Double Antibody	46	0.27	0.25-0.50	34
Beckman Instruments, Inc.	32	0.61	0.26-1.6	33
RIAPhase				

were similar to the values from the Clinical Assays Gamma Coat. A case was considered positive if all three kits indicated the presence of DLIS and the average value was taken as the level.

Incidence

Pudek et al [*1,2*] and Valdes et al [*3*] have shown that DLIS peaks at four to six days after birth in premature babies. Our findings in stillborns and neonates are similar. Of the six stillborns that became coroner's cases in Orange County in 1983, five were positive for DLIS and the level was generally equivalent to 0.5 ng/mL digoxin (Fig. 1). Both the neonates in the survey (one day old and one week old) were similarly positive for DLIS.

Pudek reported that DLIS is not found in infants older than two months. However DLIS was found in some children in this survey older than two months. On Orange County in 1983 there were 50 cases of sudden infant death syndrome or crib deaths. The average age of these cases was 2.5 months. DLIS was found in 10 of the 45 SIDS cases for which blood was obtained for the survey.

Of the nine accidental deaths none were positive for DLIS. Five of these were drownings for which the average age was 2.2 years old at the time of death. Of the eight deaths as a

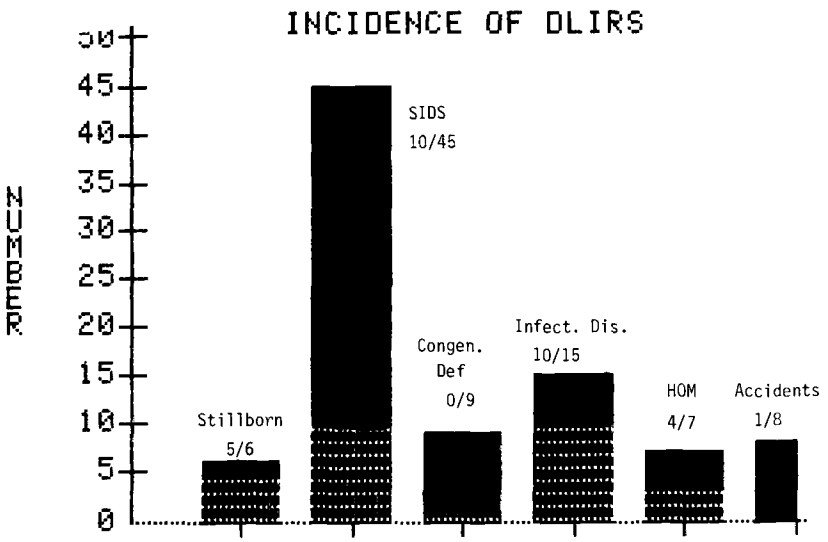

FIG. 1—*Incidence of positive findings of DLIS.*

result of congenital defects only one was positive for DLIS. Renal failure was complication in this case.

In four of the seven homicides of children, high levels of DLIS were found. The ages were 7 months, 8 months, 23 months, and 2 years. These four were battered child cases with abdominal trauma. DLIS was not present in the one case where death was caused by whiplash shaking or the two cases in which death was a result of gunshot wound.

The other category where high levels of DLIS were often encountered was deaths caused by infection particularly with diarrhea and consequent dehydration and electrolyte imbalance and different types of pneumonia. Ten out of fifteen in this category were positive for DLIS.

Digoxin-like immunoreactivity has been measured in the blood of hypertensive individuals [*5*], in monkeys with spontaneous hypertension [*6*], in rats with cardiac overload [*7*], and in volume-expanded dogs [*8*]. DLIS has been shown to be a ($Na^+ + K^+$) adenosine triphosphatase (ATPase) inhibitor [*9*]. It appears that endogenous inhibitors of the sodium pump share some structural characteristics with the cardiac glycosides. DLIS from the blood of hypertensives competitively inhibits ouabain binding to erythrocyte ($Na^+ + K^+$) ATPase [*5*]. The degree of inhibition (level of DLIS) was correlated with the degree of hypertension observed clinically and the measured urinary sodium output [*5*].

Blood levels of DLIS appear to be increased by sodium loading and volume expasion in animals and man. The injection of antidigoxin antibodies lowers the blood pressure of deoxycorticosterone acetate-salt hypertensive rats. [*10*]. The children with fatal infectious disease often had electrolyte imbalance which might have elicited a response from the endogenous sodium pump inhibitor or nariuretic hormone and they might have had cardiac stress as well.

Graves et al [*11*] and Valdes et al [*12*] reported that in 54 adult uremics not on digoxin, DLIS in the range 0.1 to 0.5 ng/mL digoxin were found. As a measure of renal failure vitreous urea nitrogen values obtained during SMAC analysis of the vitreous humor were compared to DLIS levels. Overall the correlation of DLIS and elevated vitreous urea nitrogen was poor ($r = 0.75$). However very high vitreous urea nitrogen values were often observed in older children with positive DLIS. The correlation for the SIDS or crib death cases between vitreous urea nitrogen and blood DLIS was $r = 0.85$.

Body Distribution

The liver, brain, stomach contents, and urine from five children not receiving digoxin were analyzed for DLIS (Table 2). Also shown are the tissue distribution values for a child who was receiving digoxin therapeutically and a child who received an overdose of digoxin, for comparison. The highest levels were found in the liver. DLIS was detected in tissue even though none was found in the blood. DLIS was detected in the brain, however the distribution was quite different from that found for exogenous digoxin in therapy or overdose [*13*]. DLIS was highest in the frontal cortex. It was not found in the choroid plexus which concentrates exogenous digoxin [*13,14*]. In this respect DLIS follows the distribution pattern of the brain digoxin receptors.[2] DLIS has been reported in human cerebrospinal fluid [*15*], in mammalian brain tissue, and has been shown to bind competitively to brain membrane digoxin receptors [*16*] as well as to ($Na^+ + K^+$) ATPase of erythrocytes and chicken embryo fibroblasts [*17*].

Conclusions

Digoxin-like immunoreactive substance is normally found in the postmortem blood and tissues of neonates, fetuses, and infants under two months of age. DLIS can be found in the

[2]F. L. La Bella, University of Manitoba, Manitoba, Canada, personal communication, 1983.

TABLE 2—*Body distribution of DLIS and digoxin, ng/mL or ng/g.*

Age	Sex	Weight, kg	Blood	Liver	Brain[a]	Urine	Stomach Contents
			DID NOT RECEIVE DIGOXIN				
0	F	1	0.6	0.8	0.25 CTX	ND	ND
0	M	2	0.45	0.63	ND	ND	ND
2.5 months	F	6	1.0	1.6	0.25 CTX ND CB ND BG	ND	0.3 ng/mL
3 months	M	5.5	0	0.8	0.45 CTX 0.60 CB	ND	0.8 ng/mL
10 months	F	8.2	0.5	1.2	ND CP 0.25 CB 0.3 CTX	ND	ND
			CONGENTIAL HEART DISEASE—RECEIVED DIGOXIN				
3 months	M	5.5	2.4	2.4	4.0	>6	150 μg
			ACCIDENTLY RECEIVED 4-MG DIGOXIN				
3 days	M	2.2	3.03	35.3	0.8	>40	...

[a] CTX = frontal cortex, CB = cerebellum, BG = basal ganglia, CP = choroid plexus of the ventricles, and ND = not detected.

blood of older infants with renal failure, electrolyte imbalance, or severe illness. DLIS is high in liver tissue and can leak into blood subsequent to liver damage and trauma. The cross-reactivity found with the commercial kits surveyed might result in DLIS concentrations similar to therapeutic levels of digoxin found in postmortem blood or tissue, these concentrations would not be confused with levels of digoxin found in accidental or intentional overdose with digoxin. These values range from 30 to 500 ng/mL [*18-20*]. In addition the pattern of regional brain distribution of the endogenous substance is distinct from that of exogenous digoxin [*13*].

DLIS can probably be separated from digoxin by extraction, high pressure liquid chromatographic separation, and radioimmunoassay of the resultant eluent fractions, [*20,21*]. DLIS may be not just an interference in digoxin immunoassay but a hormone or neuromodulator of interest in various physiological responses to stress and disease.

Acknowledgment

The authors would like to think Diagnostic Products Corp., Los Angeles, CA and Beckman Instruments, Inc., Fullerton, CA for supplying the kits used in this study.

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