Immunoreactive digitalis-like substance in pre-eclampsia

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Summary

An endogenous digitalis-like substance (DLS) may be involved in the pathogenesis of essential hypertension and pre-eclampsia. The digoxin levels in maternal and cord blood of 504 randomly selected patients were determined. Since none of the patients received digoxin, these levels indicated a cross-reacting substance (immunoreactive DLS). DLS levels were significantly higher in the cord blood of pre-eclamptic patients than in the cord blood of controls. DLS levels in cord blood increased with the severity of pre-eclampsia, and levels were higher in primigravidas than in multigravidas. The structure and biological activity of DLS must be determined before definite conclusions about its role in the pathogenesis of pre-eclampsia can be made.

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The existence of an endogenous digitalis-like substance (DLS) has recently received much attention.[1,2] There are interesting indications that DLS could be involved in the pathophysiology of essential hypertension,[3] and there seems to be an association between an immunoreactive digoxin-like substance and pre-eclampsia.[4] It is, however, still uncertain whether these reported digoxin-like substances are similar since they could possibly be different substances which cross-react with digoxin radioimmunoassays. Another important discovery was of high immunoreactive DLS values in the cord blood of newborn infants, which could falsely increase the desired levels should they require digitalis therapy.[5,6]

Endogenous DLS has also been reported in the plasma and urine of volume-expanded dogs,[7] in the serum of rats with experimental cardiac overload,[8] in extracts of mammalian brain[9] and in rat adrenal tissue.[10] A digoxin immunoreactive substance has also been found in the urine of salt-loaded healthy volunteers.[11]

The major mode of action of digitalis is to decrease sodium transport out of the cardiac cell by inhibiting Na^+K^+ATPase (the sodium pump). The accumulation of sodium results in an increase of intracellular calcium ions which is probably responsible for the positive inotropic effect of digitalis.

It is therefore possible that endogenous immunoreactive DLS could act on the sodium pump.[12,13] Furthermore, reduced activity of the sodium pump has been described in essential hypertension. This leads to increased intracellular sodium levels and therefore also calcium retention in the cell. The raised calcium levels then cause an increase in smooth-muscle contractility and therefore vasoconstriction. In pre-eclampsia leucocyte sodium levels are elevated and potassium levels are depressed, probably also as a result of decreased sodium-pump activity.[14] Erythrocytes of infants born to pre-eclamptic mothers have reduced Na^+K^+ATPase activity and it could be postulated that this could be due to a substance which suppresses Na^+K^+ATPase activity.[15] In a previous pilot study, higher levels of immunoreactive DLS were found in the blood of pre-eclamptic patients.[4] Since immunoreactive or endogenous DLS could play a role in the pathophysiology of essential hypertension and pre-eclampsia, it was decided to study this interesting problem prospectively.

Patients and methods

Over a period of 10 weeks 504 patients delivered at Tygerberg Hospital were chosen at random for this study. None of the patients had received digoxin or any cardiac glycoside. Blood samples were obtained from the umbilical cord and a peripheral maternal vein immediately after delivery, collected in glass tubes and refrigerated. Serum digoxin levels were determined within 72 hours after collection by using a commercially available radioimmunoassay kit from Clinical Assays, Cambridge. A Hewlett-Packard autogamma counter was used to determine radioactivity.

According to the antenatal records and findings during labour, patients were categorized into a control group and a group with pre-eclampsia. For the diagnosis of pre-eclampsia the blood pressure had to be 140/90 mmHg or more, on two occasions or more at least 6 hours apart, and accompanied by proteinuria and/or oedema. Pre-eclampsia was regarded as mild when the blood pressure was 160/100 mmHg or less and the proteinuria +++ or less. It was regarded as severe when these values were exceeded.

In twin pregnancies the cord blood value of the second twin was disregarded in order to maintain paired mother-infant samples. The laboratory technician responsible for the digoxin determinations had no insight into patient data. Control sera were used to prepare standard curves from which digoxin levels ranging from 0 to 4 ng/ml could be determined. Concentrations of DLS were obtained automatically from the standard curve produced by the microprocessor of the gamma counter. As none of the patients received digoxin, the apparent digoxin level must have been caused by the cross-reacting substance (immunoreactive DLS). The levels of immunoreactive DLS are reported in ng/ml in terms of digoxin, as the composition of DLS and the degree of cross-reactivity are still unknown.

Blood was taken from 20 healthy female volunteers to assess values for non-pregnant patients. Informed consent was obtained from all patients and permission for the study was obtained from the ethical committee of Tygerberg Hospital.

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TABLE I. CLINICAL MEASUREMENTS AND DLS LEVELS IN CONTROL AND PRE-ECLAMPSIA GROUPS (MEAN ± SD)

	N	Patients without pre-eclampsia	*N*	Patients with pre-eclampsia	*t*-test
Systolic blood pressure (mmHg)	**400**	**117 ± 13**	**101**	**152 ± 17**	***P* < 0,0001**
Diastolic blood pressure (mmHg)	**400**	**73 ± 10**	**101**	**101 ± 11**	***P* < 0,0001**
Maternal age (yrs)	**398**	**25,4 ± 6,1**	**99**	**22,7 ± 5,1**	***P* < 0,0001**
Gravidity	**396**	**2,5 ± 1,8**	**100**	**1,8 ± 1,6**	***P* < 0,001**
Parity	**396**	**1,4 ± 1,7**	**100**	**0,8 ± 1,4**	***P* < 0,001**
Mass of baby (g)	**401**	**3 021 ± 657**	**101**	**2 826 ± 645**	***P* < 0,01**
Placental mass (g)	**387**	**552 ± 129**	**96**	**541 ± 129**	**NS**
Gestational age (yrs)	**391**	**38,2 ± 2,7**	**101**	**38,0 ± 2,5**	**NS**
DLS of mother (ng/ml)	**401**	**0,57 ± 0,27**	**101**	**0,58 ± 0,25**	**NS**
DLS of cord blood (ng/ml)	**401**	**1,12 ± 0,25**	**101**	**1,23 ± 0,34**	***P* < 0,01**

NS = not significant ($P > 0,05$).

Results

There were 504 patients of whom 456 were coloured, 24 white, 22 black and 1 Asian. There were 384 vaginal deliveries, 57 caesarean sections, 38 forceps deliveries, 13 vacuum extractions and 10 breech deliveries. There were 3 sets of twins. Pre-eclampsia occurred in 101 patients, of whom 62 were primigravidas and 38 multigravidas (in 1 patient the gravidity was unknown). Of the 101 affected patients (of whom 41 were primigravidas) 70 had mild and 31 (21 primigravidas) severe pre-eclampsia. Diastolic blood pressure was 90 mmHg or above in 129 patients while 142 patients had proteinuria. Oedema was noted in 181 patients. Only 19 patients were known to be hypertensive before the latest pregnancy. There were 19 diabetics, 9 patients with an underlying renal disease, and 3 patients with rheumatic heart lesions.

When the group of patients without pre-eclampsia was compared with the pre-eclamptic group, it was noted that maternal age, gravidity and parity were significantly lower in the pre-eclamptic group (Table I). Babies born to pre-eclamptic mothers were also lighter, but the immunoreactive DLS values in the cord blood were higher than those from mothers without pre-eclampsia. Placental mass, gestational age and maternal immunoreactive DLS values did not differ significantly between the two groups (Table I).

Cord blood DLS values

Excellent correlation between maternal and cord blood values was found in patients without pre-eclampsia but poor correlation in patients with pre-eclampsia (Table II). Cord blood values tended to rise with the increasing severity of pre-eclampsia. In primigravidas cord DLS values also showed a tendency to rise with increasing severity of pre-eclampsia (Table III). It also appears that the variances increase with the severity of pre-eclampsia.

TABLE II. MEAN (± SD) MATERNAL AND CORD BLOOD DLS VALUES (ng/ml) AND THEIR CORRELATION WITH EACH OTHER

	DLS values	Significance of Spearman correlation coefficient
No pre-eclampsia		
Maternal value	**0,57 ± 0,27**	***P* < 0,0001**
Cord blood value	**1,12 ± 0,25**	
Mild pre-eclampsia		
Maternal value	**0,57 ± 0,22**	***P* < 0,05**
Cord blood value	**1,20 ± 0,29**	
Severe pre-eclampsia		
Maternal value	**0,60 ± 0,31**	***P* < 0,05**
Cord blood value	**1,29 ± 0,43**	

TABLE III. CORD BLOOD DLS IN CONTROL AND PRE-ECLAMPSIA GROUPS (MEAN ± SD)

	No pre-eclampsia	Mild pre-eclampsia	Severe pre-eclampsia
No. of patients	**401**	**70**	**31**
DLS (ng/ml)*	**1,12 ± 0,25**	**1,20 ± 0,29**	**1,29 ± 0,43**
No. of primigravidas	**136**	**41**	**21**
DLS (ng/ml)**	**1,17 ± 0,28**	**1,27 ± 0,28**	**1,34 ± 0,46**

* Levene's test for equal variances (df 2,449) *F* value 8,23; $P < 0,0005$.
** Levene's test for equal variances (df 5,490) *F* value 3,80; $P < 0,005$.

Lowest cord DLS values were seen in multigravidas without pre-eclampsia (mean value 1,09 ± 0,24 ng/ml) and highest values in primigravidas with pre-eclampsia (mean value 1,27 ± 0,29 ng/ml). The mean values for multigravidas with pre-eclampsia and primigravidas without pre-eclampsia were 1,12 ± 0,30 ng/ml and 1,17 ± 0,28 ng/ml respectively (Levene's test for equal variances (df 3,491) *F* value 3,08; $P > 0,05$).

Fetal sex also seemed to have influenced the cord blood DLS values since the mean value for boys was 1,17 ± 0,29 ng/ml and for girls 1,11 ± 0,25 ng/ml ($P < 0,01$).

Maternal DLS values

No difference in DLS values was found between patients with pre-eclampsia and the control group. When only patients without pre-eclampsia are considered, mean immunoreactive DLS values were 0,63 (± 0,27) ng/ml in the 136 primigravidas and 0,54 (± 0,26) ng/ml in the 260 multigravidas (pooled variance *t*-test, $P < 0,005$). Mean values for primigravidas with mild and severe pre-eclampsia were 0,60 (± 0,21) ng/ml and 0,63 (± 0,35) ng/ml respectively. Mean values for multigravidas with mild and severe pre-eclampsia were 0,52 (± 0,24) ng/ml and 0,56 (± 0,20) ng/ml respectively.

DLS values in non-pregnant control patients

In the control group of 20 non-pregnant patients the mean immunoreactive DLS value was 0,15 (± 0,15) ng/ml. The highest recorded value was 0,45 ng/ml. In 9 patients no immunoreactive DLS was found.

Discussion

Since the patients in this series were randomly selected the study and control groups differed in many ways. Patients in the affected study groups were younger and their parity and gravidity were lower than those without pre-eclampsia. This finding is not surprising since it is well known that pre-eclampsia is commoner in primigravidas. The finding of a reduced birthweight of infants born to pre-eclamptic patients is also well known.[15] There is less certainty about placental weight in pre-eclampsia; no specific effect on weight was demonstrated.

Immunoreactive DLS values were significantly increased in the cord blood of patients with pre-eclampsia, and the increase was related to the severity of the pre-eclampsia. Highest values were seen in primigravidas with severe pre-eclampsia and lowest values in the multigravidas without pre-eclampsia. The fact that immunoreactive levels were higher in boys than in girls is difficult to explain. As the molecular nature of DLS has not yet been established it would be premature to try to explain this difference at this stage. It is, however, interesting to note that eclampsia is commoner in patients with a male fetus.[16]

In the aetiology of essential hypertension, abnormalities in cell-membrane transport have recently received much attention.[3] It is possible that patients with essential hypertension have a diminished sodium excretory capacity and secondary to this a circulating substance which inhibits sodium transport in the kidney and elsewhere. This substance would enable the maintenance of sodium homeostasis in patients with decreased salt excretory capacity. The increase in intracellular sodium concentration may inhibit calcium efflux from the cells. Increased intracellular calcium concentration might in turn increase vascular reactivity and the tension in vascular smooth muscle and therefore cause hypertension.[12]

Since patients with essential hypertension have a higher incidence of pre-eclampsia, the aetiology of the two may be linked. The basic defect in pre-eclampsia may therefore also be reduced sodium excretory capacity. Because of the physiological salt retention in pregnancy a circulating substance may in turn increase in order to promote natriuresis, but one of the adverse effects of inhibition of sodium transport is intracellular calcium retention leading to hypertension. Since the possibility of an endogenous DLS exists[1] and also the possibility that the endogenous DLS and the circulating natriuretic factor have certain common effects, it may not be difficult to explain our finding of increased immunoreactive DLS levels in pre-eclampsia. It is, however, strange that cord levels are increased but not maternal levels. Before further progress can be made, the structure and biological activity of immunoreactive DLS needs to be established.

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