Effects of 2,3,7,8-TCDD and 2,2',4,4',5,5'-HxCB treatment on vitamin K-dependent blood coagulation and hepatic deposition in neonatal rats

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Introduction

The susceptibility of newborn infants to vitamin K deficiency may lead to disturbed blood coagulation and subsequent hemorrhages[1]. A major risk factor is the use of antiepileptics (e.g. phenobarbital) by the mother during pregnancy. Enzyme induction in the liver of the newborn is the suggested mechanism[2]. The increase in the late bleeding type since 1967 is hypothesised to be related to the presence of cytochrome P450 inducing PCBs and PCDDs in human milk[3].

In preliminary studies with adolescent rats both 2,2',4,4',5,5'-hexachlorobiphenyl (HxCB) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) affected vitamin K-dependent coagulation factors and vitamin K cycle enzymes via P450 induction. HxCB exerted stronger effects compared to TCDD with sex-dependent differences[4,5].

The present study was performed to examine the effects of TCDD and HxCB alone or in combination on vitamin K-dependent blood coagulation in neonatal rats. In addition, the possible occurrence of interactive effects on hepatic deposition of these compounds after mixed dosage was studied[6].

Methods

Pregnant female WAG/Rij-rats were given a single oral dose of either 164 mg 2,2',4,4',5,5'-HxCB/kg or 1.4 µg 2,3,7,8-TCDD/kg, alone or in combination, on day 16 of gestation (n=5). The control group received vehicle only (peanut oil, 1.67 ml/kg). Nests were normalized at 3 males and 3 females. During the nursing period the dams were kept on a vitamin K_3-deficient casein diet. At day 10 after delivery neonates and dams were sacrificed. Prothrombin levels in the blood were measured in a chromogenic assay and were determined relative to a pooled reference sample. Ethoxy- (EROD), pentoxyresorufin O-dealkylating (PROD) and vitamin K-epoxide reductase activities (VKOR) were measured in hepatic

microsomes. The hepatic concentrations of both compounds were measured by GC/MS analysis. Different dose groups were compared by a nested Analysis of Variance (ANOVA).

Results and Discussion

Plasma prothrombin levels of the neonatal rats were reduced significantly in all treatment groups relative to the controls (fig.1). VKOR activity in the offspring was significantly induced only after mixed exposure and surprisingly not by HxCB treatment alone (fig.1), as observed before[5]. Prothrombin levels were not related to VKOR activity. However, prothrombin levels did correlate significantly to both PROD activity (fig.2) and hepatic concentration of HxCB (fig.3). The absence of a relationship between prothrombin levels and VKOR activity suggests that the increase of this vitamin K cycle enzyme activity is not due to a feedback mechanism, resulting from a shortage in prothrombin. From the significant relationship between VKOR activity and the concentration of HxCB in the livers of the offspring, HxCB seems to induce this enzyme activity directly (fig.4). Prothrombin levels or VKOR activity did not correlate with hepatic concentrations of TCDD or EROD activity.

CYP1A and 2B, measured as EROD and PROD activity respectively, were significantly induced in all treatment groups (table). Coadministration of HxCB with TCDD resulted in a higher EROD induction rate. Although HxCB itself is unable to induce CYP1A in adult rats, EROD activity in the offspring is induced significantly by this PCB. This induction of CYP1A related enzyme activity by phenobarbital administration has been observed before in neonatal rats[7]. Exposure to the mixture resulted in increased PROD induction rates compared to exposure to HxCB.

Table: Hepatic 7-ethoxy and 7-pentoxyresorufin O-dealkylating activity and relative concentration of TCDD and HxCB in neonatal rats

	EROD (nmol/min.mgp)	PROD (nmol/min.mgp)	TCDD or HxCB (% dose/g liver)
Control	0.005 ± 0.002	0.004 ± 0.001	-
TCDD	2.737 ± 0.702***	0.029 ± 0.006***	1.45 ± 0.55
HxCB	0.027 ± 0.012	0.035 ± 0.014***	0.068 ± 0.014
TCDD/ HxCB	4.239 ± 1.154***	0.076 ± 0.017***	1.14 ± 0.20 0.158 ± 0.031*

The hepatic concentration of HxCB was doubled by cotreatment with TCDD (table). For TCDD no increased liver deposition could be observed in the mixed group, compared to the TCDD group. The absence of interactive effects on the hepatic TCDD deposition is likely explained by a maximum induction of its binding site

Fig.1: Plasma prothrombin (*100%) and VKOR activity

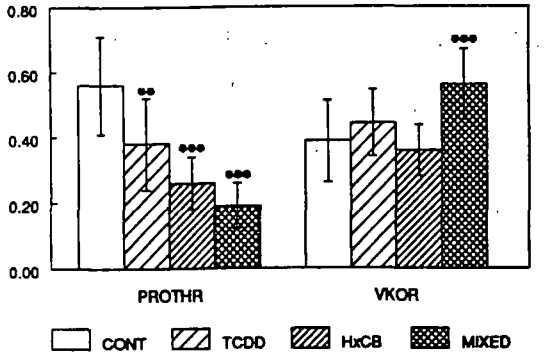


Fig.2: Correlation between prothrombin and PROD activity

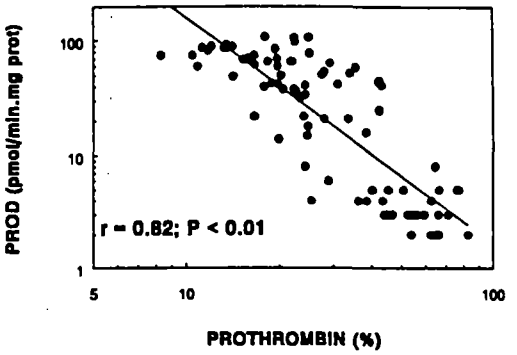


Fig.3: Correlation between hepatic HxCB and plasma prothrombin

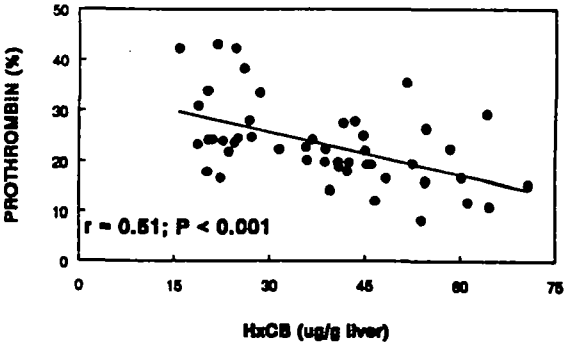
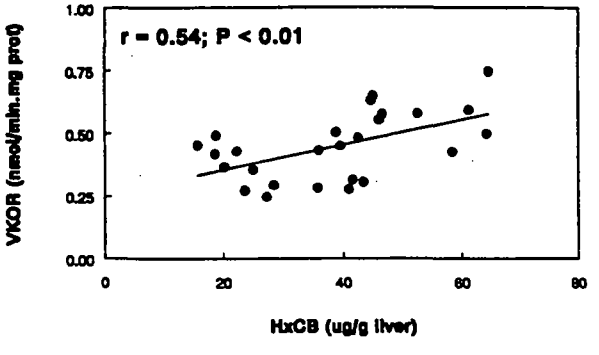


Fig.4: Correlation between hepatic HxCB and VKOR activity



CYP1A2[8]. The effect on hepatic HxCB deposition in the mixed dose group most likely resulted from a modulation by TCDD of the hepatic binding sites for HxCB. In contrast with TCDD, the HxCB binding site is not unequivocally identified as CYP1A2. Lipid droplets in the liver induced by TCDD may also function as a HxCB binding site.

No sex-dependent differences after TCDD and/or HxCB exposure on vitamin K-dependent coagulation, P450 induction or hepatic deposition were observed.

Conclusions

Both TCDD and HxCB decreased prothrombin levels in neonatal rats, which was additive after mixed dosage. VKOR activity was only induced after mixed exposure, and did not correlate to prothrombin. Although, both compounds exerted effect on vitamin K-dependent coagulation, only HxCB concentration in the neonatal liver and CYP2B induction were correlated to prothrombin and/or VKOR activity.

Mixed dosage of HxCB and TCDD resulted in a doubling of hepatic deposition of HxCB, but no effects were observed on TCDD.

No sex-dependent differences of TCDD and/or HxCB on biochemical parameters or hepatic deposition were found.

Acknowledgements

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