Effects of 2,3,7,8-TCDD or 2,2',4,4',5,5'-HxCB on the Activity of the Vitamin K-Cycle Enzymes in Rats: a Novel Mechanism of Action

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INTRODUCTION

In newborns vitamin K-dependent bleeding disorders, so called Haemorrhagic Disease of the Newborn (HDN) occur and can be caused by maternal medication with e.g. phenobarbital or phenytoin[1]. Induction of fetal liver enzymes was suggested to lead to this vitamin K-deficiency[2]. The increase in the late type of HDN in exclusively breast-fed infants during the last decades was hypothesised to be caused by enzyme inducing contaminants like PCDDs and PCBs[3].

In previous studies on WAG/Rij-rats dose and sex-dependent effects of 2,3,7,8-TCDD as well as 2,2',4,4',5,5'-HxCB on vitamin K-dependent blood coagulation were observed[4].

In order to obtain insight in the effects of TCDD and HxCB on the vitamin K-dependent blood coagulation the enzymes involved in the vitamin K-cycle were analysed in liver microsomes of some groups from our dose-response studies. Vitamin K-dependent carboxylase carboxylates the glutamic acid residues of blood coagulation factors II, VII, IX and X, while cofactor vitamin K-hydroquinone (KH_2) is converted to vitamin K 2,3-epoxide (KO). KO is reduced to KH_2 again in two steps, involving vitamin KO reductase and vitamin K reductase. In addition the non-carboxylated coagulation factors were determined as endogenous substrate in the liver microsomes.

METHODS

Male and female WAG/Rij-rats (SPF) of four weeks old were exposed to a single oral dose of 30 nmol 2,3,7,8-TCDD/kg bodyweight (females), 0.3 nmol TCDD/kg bw (males) or 500 µmol 2,2',4,4',5,5'-HxCB/kg bodyweight (both females and males). Control animals received vehicle only. Treatment groups and one set of control groups were placed on a synthetic vitamin K_3-deficient casein diet (Hope Farms B.V., Woerden, The Netherlands), while another control group received identical diet with vitamin K_3.

After 10 days (males) or 21 days (females) the animals were sacrificed. Blood was collected in vials containing EDTA and centrifuged to obtain plasma. In the plasma samples factor VII content was measured using the chromagenic substrate S-2765 in a spectrofotometric assay (Chromogenix, Amsterdam, The Netherlands).

Livers were removed for microsomal preparation. Enzyme activity and protein content were measured as described previously [4]. Vitamin KO reductase activity, was determined by HPLC-analysis[5]. Carboxylase, vitamin K reductase activity and endogenous substrate were assayed by means of $^{14}CO_2$ incorporation using a descarboxyprothrombin substitute[6]. All measurements were done in duplicate and analysed by means of ANOVA and LSD-tests.

RESULTS

TCDD significantly increased carboxylase activity and the endogenous substrate content in female rats compared to the control group kept on the vitamin K-deficient diet (fig. 1a). The deficient diet alone also increases both parameters, but the extent is significantly less than with coadminstration of TCDD. HxCB only elevated carboxylase activity, but to a lesser extent than TCDD. The vitamin K reductase/carboxylase activity was increased in all female groups comparable to the elevation in carboxylase activity. Total cytochrome P450 content was induced by both compounds (table). Factor VII in plasma showed a decrease in case of TCDD administration.

Table : Factor VII in plasma and total cytochrome P450 content in liver of female and male WAG/Rij-rats

	Cyt P450 (nmol/mg protein)		Factor VII (relative to +K group)	
	avg	sd	avg	sd
Female/+K	0.397	0.116	1.00	0.36
-K	0.438	0.074	0.80	0.14
TCDD	1.029[a]	0.036	0.51	0.17
HxCB	0.640[b]	0.049	0.91	0.24
Male/+K	0.639	0.135	1.00[b]	0.02
-K	0.560	0.063	0.36	0.32
TCDD	0.804[c]	0.023	0.52	0.17
HxCB	1.350[a]	0.098	0.06	0.02

[a] $P<0.001$; [b] $P<0.01$; [c] $P<0.05$ compared to -K

In male rats a vitamin K-deficient diet alone led to a significant increase in vitamin K reductase/carboxylase activity and endogenous substrate content (fig. 1b). When HxCB was administered vitamin KO reductase activity was significantly elevated compared to the controls. Administration of TCDD did not significantly change any enzymatic activity involved in the male vitamin K-cycle. TCDD as well as HxCB increased total P450 content significantly (table). Only HxCB showed, in contrast with TCDD, a strong decrease in factor VII plasma content concurring with elevated levels of vitamin KO reductase.

The data from this study were used to determine any statistical relationship between carboxylase or vitamin KO reductase against cytochrome P450 activities or factor VII in both sexes. In females linear relationships were observed between carboxylase activity and total P450 content or factor VII (fig. 2,3). In male rats factor VII was correlated with carboxylase or vitamin KO reductase activity (fig. 3,4). In addition a relationship between total

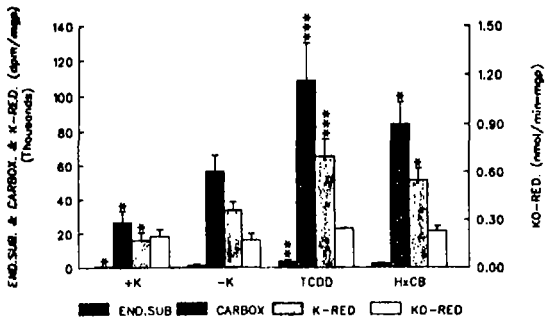
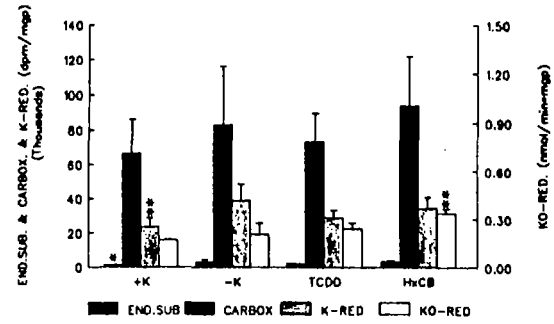


Fig. 1a: Vitamin K-cycle enzyme activity and endogenous substrate in female rats



* P<0.05; ** P<0.01; *** P<0.001 compared to -K

Fig. 1b: Vitamin K-cycle enzyme activity and endogenous substrate in male rats

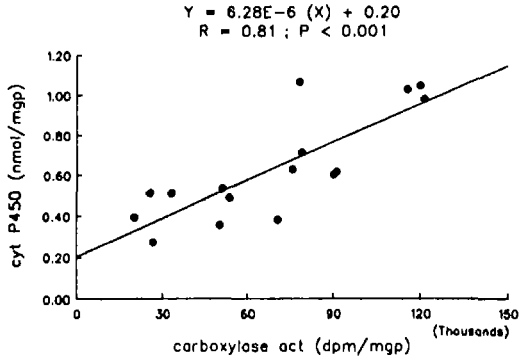


Fig. 2: Relation between carboxylase activity and cytochrome P450 content in female rats

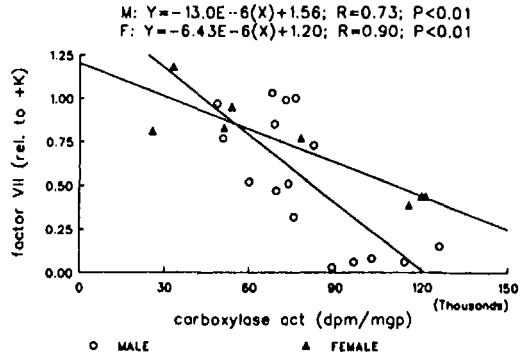


Fig. 3: Relation between carboxylase activity and factor VII

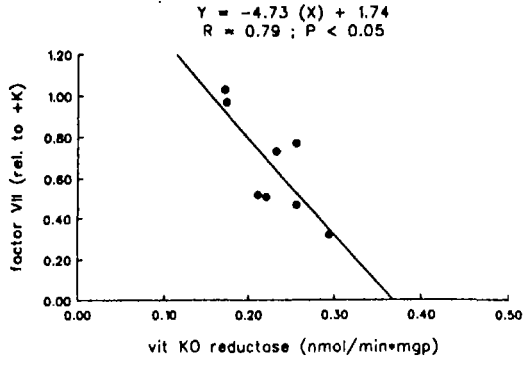


Fig. 4: Relation between vitamin KO reductase activity and factor VII in male rats

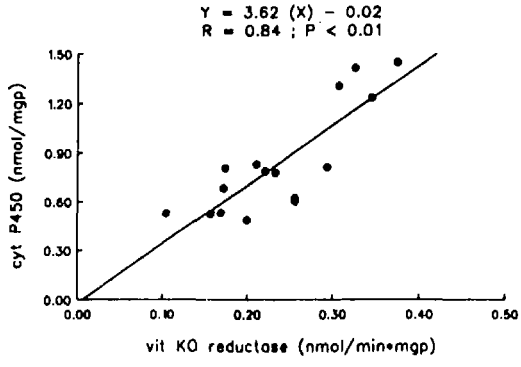


Fig. 5: Relation between cytochrome P450 content and vitamin KO reductase activity in male rats

cytochrome P450 and vitamin KO reductase was found in males, but not in the females (fig. 5).

DISCUSSION AND CONCLUSIONS

In rats with a low vitamin K status TCDD and HxCB cause a decrease in factor VII content in plasma, while they increase several enzyme activities in the vitamin K-cycle. Our results indicate that modulation of the vitamin K-cycle is different for male and female rats. TCDD caused the strongest effect on factor VII and carboxylase activity in females. Whereas HxCB increased vitamin KO reductase and diminished factor VII in males. For TCDD the difference in dose between the sexes might have played some role in these results. Sex-dependent differences in acquiring a vitamin K-deficiency and effects of TCDD and HxCB on vitamin K-dependent blood coagulation have been observed before, with male rats being most sensitive[4].

Though various effects of TCDD or HxCB on factor VII and enzymes of the vitamin K-cycle were observed, the mechanistic pathway causing the vitamin K-deficiency is still obscure. A vitamin K-deficiency induced by HxCB or TCDD is apparently not caused by blocking one of the enzymes of the vitamin K-cycle like e.g. warfarin does[5]. The observed correlations between carboxylase or vitamin KO reductase activity and total cytochrome P450 content are noticeable. As TCDD and HxCB are enzyme inducers of cytochrome P450 1A1/2 and 2B1/2, respectively, these iso-enzymes are apparently not involved as modulators of the vitamin K-cycle. The relationship observed with total cytochrome P450 might indicate the involvement of (an)other P450 iso-enzyme(s), inducible by TCDD as well as HxCB.

The results of this study support the hypothesis that some PCDDs and PCBs decrease the vitamin K-dependent blood coagulation. It is recommended to administer vitamin K to infants in the industrialised world during the whole breast feeding period.

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