Reduction of Vitamin K_1 and Menaquinone-4 Concentrations in Female and Male Rats after Exposure to 2,2',4,4',5,5'-Hexachlorobiphenyl

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1. Introduction

Vitamin K hydroquinone is an essential cofactor in the post-translational carboxylation of vitamin K-dependent coagulation factors II (prothrombin), VII, IX and X. A deficiency of this vitamin increases the risk of bleeding disorders, because the blood clotting process is disturbed by the absence of active coagulation factors. In the liver vitamin K is recycled constantly by several reductases [1]. As a result of this vitamin K cycle the daily requirement of vitamin K for healthy persons is low and provided by the vitamin K_1 (VK1) content of a normal diet [2,3]. The vegetable VK1 is considered to be the major vitamer in the carboxylation process. The relevance of bacterially synthesized menaquinones (or vitamin K_2) *in vivo* is still questionable [4].

Newborns often display a borderline vitamin K deficiency which incidently results in hemorrhages. Bleeding disorders as a result of a vitamin K deficiency are known as hemorrhagic disease of the newborn (HDN) [5]. A major risk factor to develop HDN are maternal anticonvulsant therapy. It is suggested that the elimination of vitamin K in the infant is accelerated by induction of hepatic mixed function oxygenases [6]. The incidence of late hemorrhagic disease (LHD) has been increased since the 1970s and is associated with breast-feeding [7-11]. Recently, the presence of PCBs, PCDDs and PCDFs in human milk was hypothesized to be involved in the increased risk of LHD [12]. PCBs, PCDDs and PCDFs are potent inducers of cytochrome P450 (CYP) isoenzymes, components of the mixed function oxygenases in the liver [13,14]. Di-ortho-substituted PCBs resemble the anticonvulsant phenobarbital (PB) in induction of CYP2B1/2 and CYP3A1/2 in rats [13,15]. 2,2,4,4'5,5-Hexachlorobiphenyl (HxCB) is the major representative of this class of PCBs in human milk [16].

Previous studies had revealed dose-dependent reductions in vitamin K-dependent factor VII and prothrombin levels in HxCB-exposed male and neonatal rats. In addition, the observed reductions in factor VII and prothrombin after HxCB administration were statistically related to hepatic CYP2B1, CYP3A1/2 and CYP2A1 induction in the rats. Female rats were not or only minimally affected by HxCB [17]. In the current study we

wished to examine whether HxCB might have affected vitamin K-dependent blood coagulation in rats by reducing VK1 and menaquinone-4 (MK-4) concentrations and whether this was associated with specific P450 activities.

2. Experimental design

Four week-old female and male WAG/Rij-rats (SPF) were divided in two groups. One group (n=5) of each sex received a single oral dose of 39.3 mg per kg bodyweight (110 µmol/kg) 2,2',4,4',5,5'-HxCB dissolved in peanut oil (1.5 ml/kg bw). The remaining group (n=4) of each sex received a single oral dose of the vehicle. Tap water and a standard laboratory diet were provided *ad libitum*. At day 10 after dosing the experiment was terminated. Blood was sampled by orbital puncture under a light ether anaesthesia and collected in heparinized tubes. Plasma was isolated by centrifugation (10 min at 3000 rpm) and stored at -70°C. Livers were isolated, frozen in liquid nitrogen and stored at -70°C until processing. Liver S9-fractions and microsomal fractions were prepared and stored at -70°C. VK1 and MK-4 concentrations were measured in blood plasma and hepatic S9-fractions (and homogenates) of each rat [18]. In the microsomal fraction of each liver 7-pentoxyresorufin O-dependentylation (PROD), hydroxylation of testosterone (OHT) and protein content were measured to determine the activities of CYP2B1, 3A1/2 and 2A1 isoenzymes [17]. Statistical analysis was performed by using two-way ANOVAs.

3. Results

VK1 and MK-4 levels. Ten days after dosing with 39 mg/kg bw HxCB VK1 plasma levels were reduced by 33% in female rats and by 30% in males, compared to the corresponding control groups. Yet, only in female rats the decrease in VK1 after HxCB administration was statistically significant. Plasma MK-4 levels were not affected by HxCB exposure in either sex. The average VK1 and MK-4 concentrations in plasma of the female control group resembled those of the male control group (table 1).

Table 1: VK1 and MK-4 concentrations in blood and liver of female and male rats 10 days after a single dose of 39 mg 2,2',4,4',5,5'-HxCB/kg bw

		Plasma		Liver (S9-fraction)	
		VK1 (pg/ml)	MK-4 (pg/ml)	VK1 (pg/mg prot.)	MK-4 (pg/mg prot.)
Female	Control (n=4)	4145 ± 516	672 ± 186	128 ± 11	116 ± 13
	HxCB (n=5)	2774 ± 537*	597 ± 164	87 ± 14***[a]	84 ± 11***
Male	Control (n=4)	3198 ± 1150	592 ± 117	96 ± 17**	44 ± 9***
	HxCB (n=5)	2246 ± 821	618 ± 193	82 ± 6[a]	41 ± 8***

* Significantly (P<0.05) different from control group; *** (P<0.001)
** Significantly (P<0.01) different from female group; *** (P<0.001)
[a] n = 4

Administration of HxCB to female rats also resulted in a 32% decrease of VK1 levels in the liver (S9-fraction). Also MK-4 concentrations were significantly reduced by 28% in the

female HxCB group (table 1). In male rats hepatic VK1 concentrations, measured in S9-fractions, were only slightly reduced (15%) compared to control rats (table 1). However, when measured in homogenized livers VK1 concentrations were significantly lower ($P<0.01$) in HxCB-exposed male rats than in control males, 8.39 ± 1.56 ng and 14.56 ± 2.03 ng/g liver respectively (42%). Exposure to HxCB had no effect on male MK-4 concentrations measured in hepatic S9 fractions (table 1). The average MK-4 concentrations in liver homogenates was reduced by 37% to 5.19 ± 1.88 ng/g liver in HxCB-exposed rats (control: 8.16 and 8.36 ng/g liver). This reduction was not statistically significant.

In male control rats VK1 as well as MK-4 concentrations (S9-fraction) were significantly lower than the concentrations measured in female rats; this also applied to MK-4 levels in male HxCB-exposed rats. A statistically significant ($P<0.01$) interaction was observed on the MK-4 concentration in the liver between HxCB exposure and sex, indicating a sex-dependent effect.

Cytochrome P450 activities. In both sexes CYP2B1-related PROD activity was significantly induced after administration of HxCB. However, the same dose of HxCB caused a 48-fold induction rate in male rats and only a 24-fold induction in females (table 2). A statistically significant ($P<0.01$) interaction between treatment and sex on PROD activity indicated a sex dependency. The constitutional levels of PROD activity were comparable between male and female rats (table 3).

The activity of CYP2A1 isoenzymes, measured as 7α-OHT activity, was significantly induced in both female (1.2-fold) and male rats (1.7-fold) after administration of HxCB (table 2). As expected, the activity of the female predominant CYP2A1 was significantly higher in female rats.

In addition 6β-OHT activity, reflecting male specific CYP3A1/2 activity, was significantly induced by HxCB in both sexes. In female rats 6β-OHT was raised 2.0-fold after HxCB administration; in male rats 1.6-fold (table 2). The absolute activities observed in male rats were significantly higher than those of females.

Table 2: CYP2B1 (PROD), CYP2A1 (7α-OHT) and CYP3A1/2 (6β-OHT) activities (pmol/min.mg protein) in liver of female and male rats 10 days after a single dose of 39 mg 2,2',4,4',5,5'-HxCB/kg bw

		PROD	7α-OHT	6β-OHT
Female	Control (n=4)	33 ± 18	423 ± 77[b]	84 ± 32[b]
	HxCB (n=5)	798 ± 73***[a]	526 ± 80*	171 ± 39**
Male	Control (n=4)	34 ± 16	147 ± 50'	176 ± 21''
	HxCB (n=5)	1638 ± 676***'''	249 ± 50*'''	282 ± 42***'''

* Significantly ($P<0.05$) different from control; ** ($P<0.01$); *** ($P<0.001$)
' Significantly ($P<0.05$) different from female group; '' ($P<0.01$); ''' ($P<0.001$)
[a] n = 4; [b] n = 3

4. Discussion and conclusions

A single dose of HxCB reduced VK1 and MK-4 concentrations in the liver of female and male rats. Although the largest reductions of VK1 and MK-4 concentrations were observed in female rats exposed to HxCB, absolute concentrations of VK1 as well as

MK-4 were higher in female rats than in males. Higher levels of VK1 and MK-4 implicate that female rats are less susceptible to depletion of vitamin K. This observation might explain the lack of effect of HxCB on female blood coagulation factors in previous studies [17]. Induction of CYP3A1/2 (6β-OHT) activity was correlated to the reduced hepatic VK1 concentrations in female and male rats. In male rats CYP3A1/2 activity was also related to the decreased MK-4 concentrations in the liver. The observed relationships suggest involvement of CYP3A1/2 in the elimination of VK1 and MK-4 from the liver of HxCB-exposed rats. No further support for involvement of CYP2B1 (PROD) was derived from the present study. Especially the role of CYP3A isoenzymes deserves further research in view of their predominance in human liver.

5. References

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