Modulation of vitamin K-dependent blood coagulation in rats by dioxins and PCBs

Carollen Bouwman, Willem Seinen and Martin van den Berg
Research Institute of Toxicology, University of Utrecht, P.O. Box 80176, NL-3508 TD Utrecht, The Netherlands

1. Introduction

Newborn infants are at risk of hemorrhages due to a vitamin K deficiency ('hemorrhagic disease of the newborn' or HDN)[1]. The early type of HDN, occurring within 24 hours after birth, may be related to induction of mixed function oxidase activity in the fetal liver after usage of anticonvulsants such as phenobarbital or phenytoin by the mother during pregnancy[2]. During the last several decades the Late Hemorrhagic Disease (LHD), which mainly occurs at the age of 6 weeks in breast fed infants, has been increasing[3,4] . The absence of a causal agent in the late bleeding syndrome led to the hypothesis that PCDDs, PCDFs and PCBs present in human milk might be involved in the development of a vitamin K deficiency[5], possibly as a result of hepatic cytochrome P450 induction. This hypothesis is supported by the observation of hemorrhages in (fetal) rats and rhesus monkeys following exposure to chlorinated or brominated dioxins[6,7].

2. Material and Methods

Several studies on the effects of 2,3,7,8-TCDD and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) on vitamin K-dependent blood coagulation were performed in four week old female and male rats, and in neonatal rats after exposure of the dams on gestation day 16. TCDD and HxCB were chosen as model compounds representing CYP1A1/2 and CYP2B1/2 inducers respectively. In all studies a single oral dose ranging from 0.003-30 nmol TCDD or 0.75-500 μmol HxCB per kg bodyweight was administered by gavage. During the experiments the rats were kept on a synthetic vitamin K_3-deficient diet.
The following parameters of vitamin K-dependent blood coagulation were determined: plasma levels of coagulation factors VII and prothrombin, enzyme activities of the hepatic vitamin K cycle and hepatic concentrations of vitamin K_1 and menaquinone-4 (a type of vitamin K_2). In addition, P450 activities were determined by EROD (CYP1A1/2), PROD (CYP2B1) and testosterone hydroxylation (CYP2B1, 2A1 and 3A1/2) assays.

3. Results

Both TCDD and HxCB caused the following dose-dependent effects on all vitamin K-dependent coagulation parameters in the rats: 1) reduced factor VII and prothrombin *levels; maximally 88% in male* and 44% in female rats; 2) 15-30% reduction in vitamin K_1 and menaquinone-4 concentrations in liver; 3) induction of the activities of the vitamin K

cycle enzymes γ-glutamyl carboxylase and vitamin K 2,3-epoxide reductase (fig). However, the effects depended strongly on sex and age. In general, male pubescent rats were more susceptible to disturbance of vitamin K-dependent blood coagulation than female or neonatal rats. HxCB was the most potent compound in both neonatal and male rats. In neonatal rats no effects on the vitamin K cycle were observed.

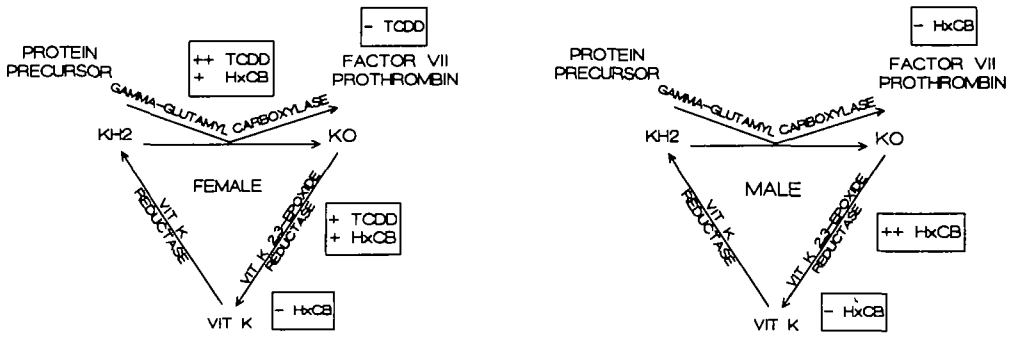

Fig.: Schematic representation of sex-dependent effects on vitamin K-dependent blood coagulation factors, vitamin K cycle enzyme activities and vitamin K_1 concentrations in male and female rat liver after administration of a single dose of 2,2',4,4',5,5'-HxCB or 2,3,7,8-TCDD; - indicates reduction; + induction

In male rats the Lowest Observed Adverse Effect Level (LOAEL) of HxCB on factor VII reduction was 36 mg/kg bw; no LOAEL of TCDD could be determined. In contrast, the LOAEL of TCDD on female factor VII was 96 ng/kg bw, whereas no LOAEL of HxCB was found. No sex-dependent differences were observed in the neonatal rats.

4. Discussion and conclusions

The effects of HxCB on male vitamin K-dependent blood coagulation were dose-dependently related to induction of CYP2B1/2 and 3A1/2 activities. In female rats, TCDD-induced CYP1A1/2 activity was related to decreased prothrombin levels. Although TCDD administration caused a slight induction of CYP2B1/2 and 3A1/2 activities, these responses did not correlate with vitamin K-dependent parameters.
The numerous correlations between induced P450 activities by HxCB and vitamin K-dependent coagulation suggest involvement of P450 in the onset of a vitamin K deficiency. However, whether the interaction is direct or indirect could not be determined in these experiments. It is possible that vitamin K might be a suitable substrate of P450 isoenzymes due to the naphthoquinone structure. Our results indicated that vitamin K may be metabolized directly by P450. On the other hand, vitamin K-dependent blood coagulation could be modulated by changes in steroid metabolism resulting from induced P450 activity. Generally, estrogens protect against a vitamin K deficiency, whereas androgens exert a counteracting effect[8]. Antiestrogenic properties of TCDD might be responsible for the observed effects in our female rats. Steroid metabolism can be influenced by induction of P450 subfamilies such as CYP1A2 or 2B, but also by induction of male-specific CYP3A2 or female-predominant CYP2A1. The observed sex-dependent effects in pubescent rats support this possible explanation. In our experiments administration of HxCB resulted in CYP2A1 and 3A1/2 induction, whereas TCDD only induced the latter P450 subfamily. Correlations with vitamin K-dependent coagulation were only found following induction by HxCB. In summary, the induction of P450 isoenzymes by

HxCB administration is related to vitamin K deficiency in rats. However, whether the vitamin K deficiency is a result of direct metabolic conversion of vitamin K or occurs indirectly through the modulation of steroid hormone metabolism remains unclear.
Apart from the possible role of P450 induction as the mechanism of action of HxCB and TCDD on vitamin K-dependent coagulation, several other possibilities could be rejected: 1) No blockage of the vitamin K cycle was observed with either of these compounds. On the contrary, the associated γ-glutamyl carboxylase and vitamin K 2,3-epoxide reductase activities were increased in rats of both sexes. This could suggest enhancement of the vitamin K cycle in response to decreased vitamin K concentrations and/or coagulation factor levels; 2) Coagulation factor synthesis in the liver cells was not inhibited by HxCB or TCDD; 3) Induction of DT-diaphorase, which might also reduce vitamin K, is considered to be unimportant when the vitamin K cycle is functional[9].
Remarkably, HxCB administration resulted in slightly larger decreases of hepatic vitamin K1 and menaquinone-4 in female rats, although male rats were much more sensitive to a vitamin K deficiency. Our experiments indicated that female rats not only maintained higher hepatic levels of vitamin K than male rats, but were probably able to use menaquinone-4 as a cofactor in the carboxylation process of the coagulation factors. Previously, female rats were observed to restore decreased levels of vitamin K-dependent coagulation factors[10]. This might have involved the switch over to bacterially synthesized menaquinones by practicing coprophagy.
Recent data on TCDD and HxCB concentrations in Dutch human milk samples[11] indicate that the total dose to which newborn infants are exposed after eight months of breast feeding would be approximately 2.6-10.5 ng TCDD/kg and 0.124-0.316 mg HxCB/kg bodyweight. Consequently, the LOAEL for TCDD on factor VII in the female rats was 10-35 fold higher than the estimated cumulative dose of the newborn infant, while for HxCB, the LOAEL in the male rats was 100-300 fold higher. On the other hand, TCDD and HxCB represent less than 10% of the total TEQ of human milk[11]. In addition, other potent P450 inducers like polycyclic aromatic hydrocarbons might contribute to induction of P450 and associated effects on vitamin K-dependent coagulation in newborn infants.
Breast fed newborn infants are very susceptible to a vitamin K deficiency for several reasons: first, low plasma vitamin K_1 levels reflect an intake which is approximately 20% of the recommended daily intake[12]. Moreover, total hepatic storage of vitamin K_1 is less than 1 µg which results in a state of potential vitamin K deficiency[13]. Consequently, increased elimination of vitamin K_1 from the liver of the newborn can pose a serious risk in developing intracranial hemorrhages.
Although the mechanism of action of vitamin K deficiency was not elucidated the results obtained in these studies in rats support the tentative conclusion that PCDDs, PCDFs and PCBs may be involved in LHD in newborn infants. However, the role of environmental chemicals in LHD may not be unequivocally determined, since vitamin K_1 prophylaxis for all newborn infants has been introduced in most industrialized countries. Nevertheless, Japanese epidemiologic studies on this subject reveal some interesting trends since the introduction of vitamin K prophylaxis in 1984[4,14]. Although the total incidence of LHD has decreased, the percentage of breast fed infants as well as the number of intracranial hemorrhages were increased within the LHD-affected group. Secondly, the number of LHD-affected and breast fed infants who did receive vitamin K prophylaxis after birth raised from 2.6% (1981-1985) to 12.7% (1985-1988). Recently, the extension of vitamin K_1 prophylaxis from a single dose directly after birth to the daily administration of 25 µg during the first three months after birth, practiced in The Netherlands, resulted in complete protection from vitamin K deficiency[15].
In view of the results described in this study, which support the possible involvement of

dioxins and PCBs in the etiology of LHD, the extension of vitamin K_1 prophylaxis from a single to multiple administration to all breast fed newborn infants in industrialized countries is strongly recommended.

5. References

1) Lane P.A., and W.E. Hathaway (1985): Vitamin K in infancy. J.Pediatr. 106, 351-9.
2) Keith D.A., and P.M. Gallop (1979): Phenytoin, hemorrhage, skeletal defects and vitamin K in the newborn. Med.Hypotheses 5, 1347-51.
3) McNinch A.W., R.L'E. Orme, and J.H. Tripp (1983): Haemorrhagic disease of the newborn returns. The Lancet 1, 1089-90.
4) Hanawa Y., M. Maki, B. Murata, E. Matsuyama, Y. Yamamoto, *et al.* (1988): The second nation-wide survey in Japan of vitamin K deficiency in infancy. Eur.J.Pediatr. 147, 472-7
5) Koppe J.G., E. Pluim, and K. Olie (1989): Breastmilk, PCBs, dioxins and vitamin K deficiency: discussion paper. J.Roy.Soc.Med. 82, 416-9.
6) McConnell E.E., J.A. Moore, and D.W. Dalgard (1978): Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Rhesus monkeys (Macaca mulatta) following a single oral dose. Toxicol.Appl.Pharmacol. 43, 175-87.
7) Olson J.R., B.P. McGarrigle, D.A. Tonucci, A. Schecter, and H. Eichelberger (1990): Developmental toxicity of 2,3,7,8-TCDD in the rat and hamster. Chemosphere 20, 1117-23.
8) Uchida K., T. Shike, H. Kakushi, H. Takase, Y. Nomura, *et al.* (1985): Effect of sex hormones on hypoprothrombinemia induced by n-methyltetrazolethiol in rats. Thromb.Res. 39, 741-50.
9) Wallin R., and L.F. Martin (1985): Vitamin K-dependent carboxylation and vitamin K metabolism in liver. Effects of warfarin. J.Clin.Invest. 76, 1879-84.
10) Lans M.C., A. Brouwer, J.G. Koppe, and M. VandenBerg (1989): Enzyme induction and alterations in thyroid hormone, vitamin A and K levels by TCDD in neonatal and maternal rats. Chemosphere 20, 1129-34.
11) Koopman-Esseboom C., M. Huisman, N. Weisglas-Kuperus, C.G. VanderPaauw, L.G.M.Th. Tuinstra, *et al.* (1994): PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere, submitted.
12) Canfield L.M., J.M. Hopkinson, A.F. Lima, B. Silva, and C. Garza (1991): Vitamin K in colostrum and mature human milk over the lactation period - a cross-sectional study. Am.J.Clin.Nutr. 53, 730-5.
13) Guillaumont M., L. Sann, M. Leclercq, B. Vignal, and A. Frederich (1993): Changes in hepatic vitamin K_1 levels after prophylactic administration to the newborn. J.Pediatr.Gastroenterol.Nutr. 16, 10-4.
14) Hanawa Y., M. Make, E. Matsuyama, H. Tada, T. Urayama, *et al.* (1990): The third nation-wide survey in Japan of vitamin K deficiency in infancy. Acta Pediatr.Jpn. 32, 51-9.
15) Cornelissen E.A.M., L.A.A. Kollée, T.G.P.J. VanLith, K. Motohara, and L.A.H. Monnens (1993): Evaluation of a daily dose of 25 µg vitamin K_1 to prevent vitamin K deficiency in breast-fed infants. J.Pediatr.Gastroenterol.Nutr. 16, 301-6.