# EFFECTS OF 2,3,7,8-TCDD OR 2,2',4,4',5,5'-HxCB ON VITAMIN K DEPENDENT BLOODCOAGULATION-FACTORS IN MALE AND FEMALE GERMFREE WAG/RIJ-RATS.

Carolien A. Bouwman*, Willem Seinen[1], Janna G. Koppe[2] and Martin van den Berg[1].

[1]) Research Institute of Toxicology, Section Environmental Toxicology, University of Utrecht, P.O. Box 80.176, 3508 TC Utrecht, The Netherlands.

[2]) Department of Neonatology, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

Abstract

In young female rats 2,2',4,4',5,5'-HxCB induces a vitamin K deficiency, whereas in male rats this compound reduces the development of this deficiency. 2,3,7,8-TCDD has the same, but less pronounced effect in male rats, but no effect in females. Distinct differences in the development of a vitamin K deficiency between males and females were observed for both compounds, which might be attributed to hormonal differences.

## Introduction

At the end of the nineteenth century "hemorrhagic disease of the newborn" during the first two weeks of life has been noticed for the first time. The discovery that vitamin K deficiency possibly plays a vital role in the onset of these bleedings lasted till the 1930's (Dam, 1929).

Vitamin K is required for post-translational carboxylation of glutamate residues to gamma-carboxyglutamate residues in proteins, which include coagulation factors II (prothrombin), VII, IX and X. This carboxylation is necessary for the conversion of the pro-enzyme form to the active enzyme, that is capable of binding $Ca^{2+}$-ions. Eventually thrombin will activate fibrinogen, which is the actual clotting process. Administration of vitamin K diminishes or prevents the hemorrhagic disease in the neonate (Widdershoven et al., 1986).

Lane and Hathaway (1985) reported an increase in the late form of hemorrhages since 1975. This bleeding pattern was often found in exclusively breast-fed infants (age 1-3 months) without any underlying cause. In two Japanese epidemiologic studies the annual incidence rate was estimated to be 1 case in 5000 births (Nagao and Nakayama, 1984; Hanawa et al., 1988). The introduction of prophylactic administration of vitamin K to all newborn infants has decreased the incidence of this bleedings. Also in Western Europe an increase in this disease has been observed (McNinch et al., 1983).

Another type of hemorrhage due to vitamin K deficiency is the so called early form, occurring within 24 hours after birth. This form is known to be caused by the maternal use of certain medicines, e.g. anticonvulsants such as phenobarbital and phenantoin. These chemicals are

*)correspondent

able to cause induction of cytochrome P-450II iso-enzymes in the liver, in which the synthesis of coagulation factors also takes place.

During the last decade several pollutants with strong cytochrome P-450 inducing properties have been spread in the environment. From these enzyme inducing compounds the PCBs, PCDDs and PCDFs are most noticeable for their accumulation in the human food-chain. These three classes of chemicals represent cytochrome P-450II (e.g. PCBs), as well as cytochrome P-450I type inducers (e.g. lateral substituted PCDDs and PCDFs). All these chemicals have been observed in human milk and cause general concern for the breastfed infant.

The observations mentioned above formed the basis of a hypothesis, which suggests that the presence of these compounds in breastmilk induces a vitamin K deficiency in the newborn infant, possibly leading to late hemorrhagic disease (Koppe et al., 1989).

In view of this postulated hypothesis our aim is to study the possible interaction between the vitamin K cycle and these compounds in laboratory animals.

Experimental

To study this possible relationship two experiments were done with male and female, germfree WAG/Rij-rats, which received either 2,3,7,8-TCDD or 2,2',4,4',5,5'-HxCB as toxic agent. The rats were aged 4 weeks, and divided in four groups of five animals. The animals were housed in an isolator with free access to water and a standard laboratory diet. After an acclimatization period of one week the control values of the coagulation time were obtained by using thrombotests (which detect deficiencies in factors II, VII and/or X) on 0.5 ml bloodsamples. Next, the animals received a single dose of 1 μg 2,3,7,8-TCDD/kg bw or 30-35 mg 2,2',4,4',5,5'-HxCB/kg bw by oral gavage. Control animals were dosed with the vehicle. All groups were placed on a vitamin K deficient diet (Hope Farms, Woerden) and observed for three weeks. Thrombotests were carried out weekly in order to ensue a developing vitamin K deficiency. After three weeks the rats were sacrificed and the livers removed for enzyme assays.

Results and discussion

Comparison of the coagulation-times between female (table 1) and male (table 2) control rats shows a remarkable difference between the sexes. In the female control group the vitamin K deficient diet caused only a slight prolongation of the coagulation-time. Whereas the same diet led to a life threatening situation in the male control group.

Administration of a single dose of 35 mg 2,2',4,4',5,5'-HxCB/kg to female rats (table 1) sped up the coagulation-time approximately two times, but in contrast dosage with 1 μg 2,3,7,8-TCDD/kg had no significant effect.

Treatment of male rats with TCDD as well as HxCB caused a significant delay in the development of the coagulation problems, which were observed in the control group (table 2). It appears from the thrombotest-values one week after dosage, that HxCB had a stronger delaying effect compared to TCDD. Unfortunately, there were no comparable data for week 3.

because of some serious experimental problems in the HxCB-dosed group during the last week.

Table 1: Coagulation-time (sec) as measured by thrombotest of female WAG/Rij-rats after a single oral dose of 1 μg 2,3,7,8-TCDD/kg or 35 mg 2,2',4,4',5,5'-HxCB/kg.

| week | control | TCDD | HxCB |
|---|---|---|---|
| 0 | 22 ± 2 (n = 12) | | |
| 1 | 22 ± 1 (n = 3) | n.a. | 21 (n = 2) |
| 2 | 30 (n = 2) | n.a. | 41 ± 3 (n = 5) |
| 3 | 35 ± 4 (n = 5) | 40 ± 8 (n = 4) | 87 ± 24 (n = 4) |

n.a. = not analyzed

Table 2: Coagulation-time (sec) as measured by thrombotest of male WAG/Rij-rats after a single oral dose of 1 μg 2,3,7,8-TCDD/kg or 30 mg 2,2',4,4',5,5'-HxCB/kg.

| week | control | TCDD | HxCB |
|---|---|---|---|
| 0 | 25 ± 1 (n = 17) | | |
| 1 | 52 ± 15 (n = 9) | 38 ± 7 (n = 3) | 24 (n = 2) |
| 2 | n.a. | n.a. | n.a. |
| 3 | 277 ± 57 (n = 6) | 104 (n = 2) | n.a. |

n.a. = not analyzed

So, in females dosage with HxCB accelerated the development of a vitamin K deficiency, whereas in male rats this compound, and also TCDD, caused a delay.

These opposite results in male and female rats suggest a possible influence of sexual hormones on the bloodcoagulation. Jolly et al. (1977) showed already that the intestinal absorbtion of vitamin $K_1$ was increased significantly after estrogen treatment of castrated male and female rats. Also, Uchida et al. (1985) demonstrated that estrogens protected the rat from an increased prothrombin time induced by n-methyltetrazolethiol. Romkes and Safe (1988) concluded that in the female rat uterus 2,3,7,8-TCDD exhibited an antiestrogenic effect.

Besides the data of the thrombotest the results of two enzyme assays, i.e. EROD and PROD, of all groups will be presented. Ethoxyresorufin- and penthoxyresorufin-o-deethylation are indicative of cytochrome P-450I and P-450II induction. Correlations between the various enzyme assays and the results of the coagulation-test will be presented.

Conclusion

Administration of a single dose of 1 μg 2,3,7,8-TCDD/kg bw or 30-35 mg 2,2',4,4',5,5'-HxCB/kg bw to female, germfree WAG/Rij-rats resulted in a prolongation of the bloodcoagulation-time after three weeks. In male rats these compounds caused after one week a

delay in coagulation-time compared to the control group. Male rats were more susceptible to the development of a vitamin K deficiency than female rats. HxCB caused more pronounced effects on bloodcoagulation than TCDD.

Acknowledgement: this study was supported by the Praeventiefonds, proj. no. 28-1691. With special thanks to the biotechnicians of the Gemeenschappelijk Dieren Instituut Amsterdam (GDIA) for their expert technical assistance.

References

Dam H. Cholesterinstoffwechsel in Hühnereiern und Hühnchen. Biochem. Zeitschr. 215 (1929): 475.

Hanawa Y., Maki M., Murata B., Matsuyama E., Yamamoto Y., Nagao T., Yamada K., Ikeda I., Terao T., Mikami S., Shiraki K., Komazawa M., Shirahata A., Tsuji Y., Motohara K., Tsukimoto I., Sawada K. The second nation-wide survey in Japan of vitamin K deficiency in infancy. Eur. J. Pediatr. 147 (1988): 472.

Jolly D.W., Craig C., Nelson T.E. Estrogen and prothrombin synthesis: effect of estrogen on absorption of vitamin $K_1$. Amer. J. Physiol. 232 (1977): H12.

Koppe J.G., Pluim E., Olie K. Breastmilk, PCBs, dioxins and vitamin K deficiency: discussion paper. J. Roy Soc. Med. 82 (1989): 416.

Lane P.A., Hathaway W.E. Vitamin K in infancy. J. Pediatr. 106 (1985): 351.

McNinch A.W., Orme R L., Tripp J.H. Hemorrhagic disease of the newborn returns. Lancet (1983): 1089.

Nagao, T., Nakayama K. Vitamin K deficiency in infancy in Japan [Letter to the editor]. Pediatr. 74 (1984): 315.

Romkes M., Safe S. Comparative activities of 2,3,7,8-tetrachlorodibenzo-p-dioxin and progesterone as antiestrogens in the female rat uterus. Toxicol. Appl. Pharmacol. 92 (1988): 368.

Uchida K., Shike T., Kakushi H., Takase H., Nomura Y., Harauchi T., Yoshizaki T. Effect of sex hormones on hypoprothrombonemia induced by n-mehyltetrazolethiol in rats. Thromb. Res. 39 (1985): 741.

Widdershoven J.A.M., Kollée L.A.A., Oostrom C.G. van, Munster P. van, Monnens L.A.H. Vitamine K-deficiëntie bij zuigelingen. Ned. Tijdschr. Geneeskd. 130 (1986): 473.