EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON HEPATIC MONOOXYGENASES AND RESISTANCE TO TRICHINELLA SPIRALIS INFECTION IN RAT OFFSPRING AFTER PERINATAL EXPOSURE

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ABSTRACT

Wistar rats were treated subcutaneously on day 19 of gestation with 3 or 0.3 μg TCDD/kg body wt. After pretreatment with 3 μg TCDD/kg body wt TCDD liver concentrations and the activity of the ethoxyresorufin O-deethylase (EROD) in liver homogenate were determined in dams and offspring on day 22 prenatally and at different time points postnatally. 5 weeks postnatally 12 male offspring each of the control and of the two treatment groups were randomly selected and infected orally with 500 *Trichinella spiralis* larvae. Several times after infection antibody titers to *T. spiralis* (IgE, IgM, IgG) were measured. 6 weeks after infection the weight of relevant organs was determined and the tongue prepared for the counting of *T. spiralis* muscle larvae. Higher TCDD concentrations were found in the liver tissue of the offspring (34 ng TCDD/g liver) when compared with the peak concentrations in the dams (26.4 ng TCDD/g liver). EROD activity in dams was increased 46-fold (648 pmol resorufin/mg x min) on day 22 of pregnancy (i.e. 3 days after treatment with TCDD) in comparison to historical controls. In the offspring the highest EROD induction was observed on day 1 after birth (346 pmol resorufin/mg x min), and the activity decreased in the postnatal period. After infection with *T. spiralis* the mortality rate seemed to be increased and body weight was decreased significantly in the group of offspring exposed to 3 μg TCDD/kg body wt; relative weights of thymus and liver were also decreased after exposure to 0.3 μg TCDD/kg body wt. No significant differences were found in the antibody titers to *T. spiralis* (IgE, IgM, IgG) and the number of *T. spiralis* muscle larvae between the control- and the TCDD-treated groups of offspring.

KEYWORDS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin = TCDD; Wistar rats; TCDD liver concentrations; Ethoxyresorufin O-deethylase = EROD; *Trichinella spiralis*; Antibody titers to *T. spiralis*; *T. spiralis* muscle larvae; Offspring

ABBREVIATIONS

TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; EROD = Ethoxyresorufin O-deethylase; AHH = Arylhydrocarbon-hydroxylase; DMSO = Dimethylsulfoxide; PCDDs = Polychlorinated dibenzo-*p*-dioxins; PCDFs = Polychlorinated dibenzofurans; TBTO = Bis(tri-n-butyltin)oxide

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and other polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/PCDFs) have been shown to interfere with various immunological functions in several animal species (Vos and Moore, 1974; Vos and Luster, 1989). Besides gross-morphological alterations, such as thymus atrophy, alterations of immune functions were demonstrated in infectivity models (Vos and Luster, 1989). More recent studies indicate that changes in the percentage of specific lymphocyte subpopulations in peripheral blood may occur after application of single doses as low as 10 ng TCDD/kg body wt (Neubert et al., 1990).

The immunosuppressive potential of TCDD may be especially pronounced in young animals (Vos and Moore, 1974). TCDD is also detectable in human breast milk (e.g. Beck et al., 1987; Fürst et al., 1989) and the breastfed babies seem to represent a population at risk with respect to exposure to PCDDs and PCDFs. Theoretical considerations suggest that the concentrations achieved in the baby after 3 months of breast-feeding may be higher than the corresponding concentrations in maternal tissue (Neubert, 1988).

Here we report on the results of studies using a *Trichinella spiralis* infection model to evaluate effects of TCCD on the immune system of rat offspring exposed perinatally to TCDD. The decreased resistance to *Trichinella spiralis* has been shown to represent a thymus dependent reaction (Vos et al., 1983). Since the induction of hepatic monooxygenases is a sensitive biological effect which can be observed after perinatal exposure to TCDD (Korte et al., 1989), we concomitantly also determined the EROD activity. Because of different toxicokinetics of TCDD in different species it was also necessary to determine TCDD-liver concentrations which provided a better basis for an extrapolation of our findings to other species, including man.

MATERIAL AND METHODS

Animal maintenance, mating procedure and drug application
Female Wistar rats (Bor: Wisw/spf, TNO, Winkelmann, Borchen, FRG) weighing 200 to 230 g were kept in Macrolon^{R} cages under spf- (specific pathogen-free) conditions (room temperature: 21°C ± 1°C, relative humidity: 55% ± 5%, constant day/night cycle with light from 9:00 to 21:00 h). They received a standard pellet feed (Altromin^{R} 1324) and water *ad libitum*. The first 24 h period following the (2 hrs) mating procedure was called day 0 of pregnancy if sperm were detected in vaginal smears (Chahoud and Kwasigroch, 1977). Day 1 postnatally was the day on which the delivery was finished at 10 a.m.

^{14}C-TCDD supplied by Cambridge Isotope Laboratories (Woburn, USA) had a radiochemical purity of 97% and a specific activity of 122 mCi/mmol (according to the manufacturer). It was dissolved in a mixture of toluene/DMSO (1 + 2, v/v) and was administered with a Hamilton^{R}-microsyringe (Bonaduz, Switzerland) under the skin of the back (0.2 ml/kg body wt).

Experimental conditions
Pregnant Wistar rats were treated subcutaneously on day 19 of pregnancy with a single dose of 3 μg or 0.3 μg TCDD/kg body wt. Five weeks postnatally 12 male offspring each were randomly selected from group I (control group), II (0.3 μg TCDD/kg body wt) and III (3 μg TCDD/kg body wt) for infection with *Trichinella spiralis*. TCDD concentrations in liver tissue and the activity of the EROD in liver homogenate were determined at different time points in dams, fetuses and offspring (pooled livers of the fetuses and the offspring of one litter) of the high dose group. Corresponding data with 0.3 μg TCDD/kg body wt have been established recently in our laboratory (Korte et al., 1989).

Trichinella spiralis infection
The *Trichinella spiralis* strain used was originally isolated from an infected pig in Poland in 1960 and is maintained at the National Institute of Public Health and Environmental Protection (Bilthoven, Netherlands) in Wistar rats or Swiss mice. *T. spiralis* muscle larvae are obtained by digestion of carcasses of infected mice with hydrochloric acid and pepsin (Köhler and Ruitenberg, 1974). The randomly selected offspring were treated with 500 *Trichinella spiralis* larvae by oral intubation 5 weeks postnatally. Six weeks later the animals were sacrificed. Muscle (tongue) samples were prepared and fixed in formalin for microscopic counting of the larvae and for the evaluation of the inflammatory reaction around *T. spiralis* muscle larvae. The weights of thymus and other relevant organs were determined.

Antibody responses
Blood was taken from a tail vein 2 and 3 weeks after infection. A modification of a method originally described by Furuhama and Onodera (1983) as described in detail elsewhere (Stahlmann et al., 1988) was used for obtaining the blood. At the end of the experiment, six weeks after infection, blood was taken by decapitation of the animals. Plasma was separated and stored at -20°C. Antibody titers to *T. spiralis* (IgM, IgG, IgE) were determined using an ELISA-method which has been described before (Vos et al., 1983).

Determination of the TCDD content
All experiments were performed using ^{14}C-TCDD. Since TCDD is metabolically rather stable and polar metabolites are excreted rapidly, the measured radioactivity essentially represents the unchanged substance (Rose et al., 1976; Abraham et al., 1988).

Determination of the EROD activity and protein content
The activity of the EROD was measured spectrofluorometrically in liver homogenates using the method of Burke and coworkers (Burke et al., 1985). Protein contents in liver homogenates were determined using a biuret-method with an automated bichromatic analyser (ABA 100, Abbott, Wiesbaden, FRG) and Preciset^{R} protein standards (Boehringer Mannheim, FRG).

Statistical evaluations
For statistical evaluations we used a standard software program (Minitab, Pennsylvania State College, 1987).

RESULTS

Postnatal mortality and organ weight alterations of the offspring
After maternal treatment with 3 μg TCDD/kg body wt mortality of the offspring in the first 5 weeks postnatally was about 7% (6/83) which corresponds to our historical control value of untreated rats. After infection with *Trichinella spiralis* 3 of 12 animals from this group died during the following 6 weeks (25%). This result is noteworthy since among the non-infected offspring (n=65) of this group (being only TCDD-exposed) no animal died during the later postnatal period. After exposure to the lower dose (0.3 μg TCDD/kg body wt) plus *T. spiralis* infection no mortality was observed.

At the end of the experiment a statistically significant reduction of the body weight of the infected animals was observed after exposure to 3 μg TCDD/kg body wt (control: 201 g ± 34 g; 3 μg TCDD/kg body wt: 140 g ± 45 g). A significant decrease in the relative weight was seen in thymus and liver after exposure to 0.3 μg TCDD/kg body wt; in the high dose group the weight of these organs were also lower than in the controls (thymus: control: 462 mg ± 91 mg; low dose group: 335 mg ± 55 mg; high dose group: 310 mg ± 157 mg; liver: control: 8.9 g ± 1.4 g; low dose group: 7.3 g ± 4.2 g; high dose group: 6.0 g ± 1.9 g).

TCDD concentration and EROD activity in liver tissue
The maximum TCDD concentration in the liver tissue of the dams treated with 3 μg TCDD/kg body wt was measured on day 22 prenatally, 3 days after treatment (Table 1) and the level declined rapidly during the postnatal period. The elimination half-life can be estimated to be about 2 weeks. Peak concentrations in the liver tissue of the offspring were observed 1 week postnatally (34 ng TCDD/g liver). These concentrations were higher than the peak concentration measured in the dams (26.4 ng/g liver).
EROD activity was strongly enhanced in dams and offspring after exposure to 3 μg TCDD/kg body wt. On day 22 of pregnancy the activity in dams was increased about 46-fold in comparison to control values (14.3 ± 3.7; n=42) obtained in our laboratory under similar conditions (Abraham et al., 1988) for adult Wistar rats (Table 1). The maximum EROD induction in the liver homogenate of the offspring was observed between day 1 and 7 postnatally. The EROD activity measured was about half of the peak activity of the dams. The EROD activity in dams and offspring declined during the postnatal period. Eleven weeks postnatally the EROD activity in dams was induced 2-fold, values measured in the offspring were in the range of controls.

Table 1: **TCDD concentrations in liver tissue and EROD activity in the liver homogenates of dams and offspring after maternal treatment with 3 μg TCDD/kg body wt on day 19 of pregnancy.**

EROD activity in control rats: 14.3 ± 3.7 pmol/mg x min (n = 42)

	DAMS		FETUSES or OFFSPRING	
	TCDD (ng/g liver)	EROD (pmol/mg x min)	TCDD (ng/g liver)	EROD (pmol/mg x min)
Prenatally				
day 22	26.4	648	3.1	248
Postnatally				
day 1	22.9	602	5.7	346
1 week	16.7	137	34.0	343
2 weeks	10.1	159	26.8	223
4 weeks	0.9	30	5.5	127
6 weeks	1.1	48	1.6	111
7 weeks	0.5	31	0.4	47
11 weeks	0.4	33	0.1	17

Trichinella spiralis antibody titers, number of *T. spiralis* muscle larvae and inflammatory reaction around the muscle larvae

Blood was taken at different time points after infection to determine the *T. spiralis* antibody titers. For the IgM titers obtained 2 and 3 weeks after infection no clear differences were seen between controls and exposed groups (Table 2).

Six weeks after infection the offspring were sacrificed and blood was taken to detect the *T. spiralis* antibody titers IgE and IgG and the tongue was prepared for counting the *T. spiralis* muscle larvae. No differences in IgE and IgG responses to the parasite infection was recognizable between the control and the TCDD exposed animals (Table 2). Also, the numbers of *T. spiralis* muscle larvae/cm^2 were not significantly different between control animals and exposed offspring.

Table 2: Antibody titers to Trichinella spiralis and the number of T. spiralis muscle larvae

Several weeks after infection the animals were sacrificed and the tongue was prepared for counting the *T. spiralis* muscle larvae. *T. spiralis* antibody titers and the number of *T. spiralis* muscle larvae are shown as Mean ± SD and the range is given in parenthesis.

Dose (µg/kg body wt)	IgM (^{2}log titer)		IgE (^{2}log titer)	IgG (^{2}log titer)	larvae Nr./cm^2
	2 weeks after infec.	3 weeks after infec.	6 weeks after infec.	6 weeks after infec.	6 weeks after infec.
Vehicle	5.1 ± 1.6 (3 - 7)	6.7 ± 1.2 (5 - 7)	4.9 ± 2.3 (2-8)	12.2 ± 1.4 (9-14)	132 ± 139 (9-375)
0.3	5.4 ± 1.7 (3 - 8)	6.3 ± 1.9 (3 - 9)	3.8 ± 2.3 (2-8)	9.7 ± 2.6 (3-13)	113 ± 99 (8-364)
3	5.5 ± 1.7 (3 - 7)	5.7 ± 1.3 (4 - 7)	3.8 ± 2.2 (2-7)	11.9 ± 0.8 (11-13)	202 ± 174 (15-420)

DISCUSSION

The immunotoxic properties of TCDD have been shown in numerous studies (Vos and Moore, 1974; Vos and Luster, 1989). In almost all publications the typical effects, such as thymus atrophy are described after rather high doses of TCDD. An exception is the report from Clark et al. (1981; 1983) describing immunological alterations in mice after 4 ng TCDD/kg body wt. Since the induction of the hepatic monooxygenases is an especially sensitive biological effect which can be observed after exposure to very low doses of TCDD we have included this variable. An induction of AHH or EROD in adult female rats could be observed after a single treatment with 2 to 3 ng TCDD/kg body wt (Kitchin and Woods, 1979; Abraham, 1988).

TCDD tissue concentration and EROD activity

The peak concentrations measured in the liver tissue of the offspring after maternal treatment on day 19 of pregnancy with 3 µg TCDD/kg body wt were higher than corresponding values obtained in the dams. Maternal treatment with 0.3 or 0.03 µg TCDD/kg body wt leads to similar results as observed in studies recently performed in our laboratory (Korte et al., 1989).

Abraham et al. (1988) reported a 53-fold induction of EROD activity after application of 3 µg TCDD/kg body wt to adult Wistar rats. The EROD activity in the liver tissue was strongly induced in dams and offspring after perinatal exposure to 3 µg TCDD/kg body wt. Such an effect may already be observed after perinatal exposure to 0.3, 0.03 or even 0.003 µg TCDD/kg body wt (Korte et al., 1989). No other data are available up till now on the EROD activity of offspring after perinatal exposure. Lucier and McDaniel (1979) and Lucier et al. (1975) reported on an induction of the AHH in rat offspring after maternal treatment during pregnancy.

Trichinella spiralis infection

In infectivity models TCDD has been shown to decrease the resistance against infections (Vos and Luster, 1989). Up till now the effect of TCDD on the response of animals to a *Trichinella spiralis* infection has not been investigated. Since the *Trichinella spiralis* test, as practised in this investigation, was suitable to detect the thymotoxic potential of TBTO (Vos et al., 1984) we used this test to investigate a possible alteration of the immune function after perinatal TCDD exposure of rat offspring.

In the high-dose group only we observed a pronounced mortality (25%; 3 of 12 rats) after infection in five-week-old rats with *T. spiralis*. It might be discussed that this observation in only 3 animals is due to chance and not a result of the *T. spiralis* infection, but we would rather interpret this finding as a specific effect, since mortality neither occurred in the infected rats after the lower dose nor in uninfected rats after exposure to the high dose (3 µg/kg body wt) nor in untreated controls. Surprisingly, we could not observe any alterations in the other variables studied: no significant differences were found either in the number of *T. spiralis* muscle larvae, or in the *T. spiralis* antibody titers (IgM, IgG and IgE) between the controls and the exposed offspring. It may be agreed that the concentrations of TCDD in the tissue of the offspring had already considerably declined at the time of the infection with *T. spiralis* and thus a TCDD-induced immunosuppression may be demonstrable in this infectivity model with a shorter time period between exposure and infection. However, as assessed from hepatic concentrations, the TCDD levels at the time of infection with *T. spiralis* were still very high (i.e. > 2000 ppt) in comparison to the concentrations which are relevant for man.

ACKNOWLEDGEMENTS

These studies were supported by grant Nr. 07VDX 019 from the Bundesministerium für Forschung und Technologie.

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