EFFECTS OF TCDD IN UTERO ON REPRODUCTIVE DEVELOPMENT OF RHESUS MONKEY OFFSPRING

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Introduction

In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) results in wide variety of effects including developmental abnormality in the reproductive organs, immunological dysfunction, teratogenicity and carcinogenesis[1-7]. It was reported that a single oral dose of 200 ng/kg of TCDD to pregnant rats resulted in abnormalities of reproductive organs in the female offspring[3]. The maternal body burden at LOAEL was estimated as approximately 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. This value was divided by an uncertainty factor of 10, and the tolerable daily intake (TDI) of dioxin and dioxin related compounds was determined at 4 pg TEQ/kg/day in Japan in 1998. Considering the pronounced difference between species observed in some previous studies, it is necessary to obtain more reliable LOAEL. Therefore, we started the present study in 1999 to clarify effects of low dose TCDD on development of the reproductive organs, in non-human primate after subcutaneous administration of TCDD into rhesus monkeys during pregnancy and lactation.During the course of the present study, we found abnormal protein expression in liver and brain[8], and liver injury[9] in F0 monkeys exposed to TCDD, and abnormal development of teeth[10] in F1 monkeys exposed to TCDD. In this study, we examined effects of TCDD exposure *in utero* and lactational periods on development of the reproductive organas in F1 rhesus monkeys.

Materials and Methods

Adult rhesus monkeys were mated, and females with confirmation of pregnancy by

ultrasonography were given TCDD subcutaneously on day 20 of gestation at an initial

dose level of 30 ng/kg or 300 ng/kg group. The lower dose level was set at about one third of the LOAEL body burden in rodents, and the higher one at about three times of the LOAEL. For maintenance of a certain body burden, 5% of the initial dose was given to dams every 30 days during pregnancy and lactation until 90 days after delivery. Twelve control F1 monkeys, 11 F1 monkeys of 30 ng/kg group, and 8 F1 monkeys of 300 ng/kg group were studied concerning developmental abnormality in the reproductive organs based on body weights, anogenital distance, testicular descent, and size of testis and penis. CA125 concentrations in peripheral blood in F0 female monkeys were assayed using EIA kit to evaluate the status of endometriosis.

Results and Discussion

Menses was observed in 5 out of 8 control female monkeys, 3 out of 5 female monkeys of 30ng/kg TCDD group and 3 out of 3 female monkeys of 300ng/kg TCDD group. Body weights and anogenital distance were measured, and the data are summarizes in Table 1. There were no significant differences among control group, 30ng/kg TCDD group, and 300ng/kg TCDD group. As TCDD is known to cause endometriosis, serum CA125 concentrations in female monkeys were measured. The mean levels of CA 125 in control group, 30ng/kg group and 300ng/kg group were 17.3±12.3 U/ml (n=12), 12.3±2.8 U/ml (n=13) and 15.5±9.5 U/ml (n=14), respectively. There was no significant

difference among three groups.

As shown in Table 2, testicular descent was observed in 3 out of 4 male controls, 4 out of 6 male monkeys of 30ng/kg TCDD group, and 4 out of 5 male monkeys of 300ng/kg TCDD group. The size of testis and penis is summarized in Table 2. At this point there was no significant differences among controls, 30ng/kg TCDD group, and 300ng/kg TCDD group. Considering the size of testis and penis we speculated that it was difficult to evaluate the function of sperm in terms of the number and motility of the sperm. We continue to observe F1 monkeys and will evaluate the number and motility of the sperm in a year or two years.

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Table 1 Effects of TCDD *in utero* on body weight, menses and anogenital distance of

Female monkey offspring

Control group

Animal number	Body weights (kg)	Anogenital distance (mm) From anus To clitoris To vaginal orfice	Menses	Days after birth On January 12, 2005

1	4.1	42.4 19.9	+	1709
2	3.8	39.0 16.5	-	1700
6	4.4	40.8 17.7	+	1684
7	3.8	42.0 20.7	+	1711
11	3.9	42.2 19.5	-	1697
12	3.4	38.7 19.2	+	1687
46	2.6	29.9 15.5	-	1677
63	4.1	37.8 22.7	+	1651

30ng/kg TCDD group

Animal number	Body weights (kg)	Anogenital distance (mm) From anus To clitoris To vaginal orfice	Menses	Days after birth On January 12, 2005
18	3.2	32.3 17.6	-	1692
21	4.2	33.2 17.9	+	1696
30	3.9	35.6 22.7	+	1680
59	4.0	38.6 27.2	+	1669
65	3.9	43.2 25.0	-	1612

300ng/kg TCDD group

Animal number	Body weights (kg)	Anogenital distance (mm) From anus To clitoris To vaginal orfice	Menses	Days after birth On January 12, 2005
31	3.9	30.6 17.6	+	1701
35	4.1	35.3 17.3	+	1696
42	4.0	37.4 19.8	+	1686

Table 2 Effects of TCDD *in utero* on body weight, testicular descent and size of testis and penis of male monkey offspring

Control group

Animal number	BW (kg)	Size of testis and penis (mm) Testis(rt) Testis(lt) Penis	Testicular descent	Days after birth On January 12, 2005
4	3.5	8.8x13.9 7.4x12.1 24.4	-	1695
13	5.2	18.4x30.5 19.5x30.6 34.5	+	1697
62	3.8	12.3x20.2 11.4x20.6 25.6	+	1651
64	4.9	18.8x25.8 18.1x27.8 31.0	+	1655

30ng/kg TCDD group

Animal number	BW (kg)	Size of testis and penis (mm) Testis(rt) Testis(lt) Penis	Testicular descent	Days after birth On January 12, 2005
19	4.1	12.5x19.9 13.7x23.6 29.3	+	1705

22	3.9	10.1x16.1 9.5x14.9 23.2	-	1688
25	4.8	20.3x31.0 18.4x27.4 34.0	+	1693
27	4.7	26.1x37.6 26.3x39.0 38.9	+	1670
47	4.1	16.8x27.0 14.7x23.1 36.4	+	1677
53	4.1	8.8x14.6 8.4x15.1 17.9	-	1669

300ng/kg TCDD group

Animal number	BW (kg)	Size of testis and penis (mm) Testis(rt) Testis(lt) Penis	Testicular descent	Days after birth On January 12, 2005
33	3.9	9.3x17.5 10.3x20.9 17.8	+	1719
39	4.8	25.4x41.8 27.3x39.6 41.8	+	1681
44	4.4	21.1x40.8 22.5x35.0 31.3	+	1686
60	3.1	11.8x18.4 10.3x18.0 23.2	+	1661
66	3.6	9.0x16.7 8.9x17.6 29.6	-	1611