Attenuation of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced cleft palate by dimethyl sulfoxide

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent experimental teratogen in mice. The most prominent effects induced by TCDD in fetus are cleft palate and hydronephrosis in mice. Recently, it has been reported that several compounds inhibited cleft palate in mice. For example, dimethylsulfoxide (DMSO) inhibited the secalonic acid-induced cleft palate in mice[1]. Methionine has been reported to inhibit all-trans retinoic acid (RA) or glucocorticoid (GC)-induced cleft palate in mice[2]. Therefore, in this experiment, the effects of these antiteratogenic compounds on the TCDD-induced cleft palate were examined. In addition, DMSO is known to be a hydroxyradical scavenger, the effect of dimethylthiourea (DMTU), a similar hydroxyradical scavenger[3], was examined. The effect of DMSO on RA-induced cleft palate was also examined.

Methods and Materials

2,3,7,8-TCDD was purchased from Radian International, Cambridge Isotope Laboratories, Inc. DMSO and L-methionine (Met) were purchased from Sigma Chemical Co. DMTU was purchased from Aldrich Co. RA was purchased from Wako Pure Chemicals Co. Female C57BL/6 mice were obtained from SLC Co. (Hamamatsu, Japan). TCDD was initially dissolved in a small volume of acetone and subsequently adjusted to a working concentration in corn oil. The mice were given rodent chow (CRF-1, Oriental Co.) and distilled water *ad libtum* and housed under controlled conditions of temperature and light (12-h light; 12-h dark cycle). On gestation day (GD) 12.5, the mice were given single oral administration of TCDD (10μg/kg bw). DMSO was administered by gavage at 5-10 ml/kg bw /day on GD 13.5 to 14.5 or 13.5 to 17.5. Met was dissolved in a vehicle of 0.9% sodium chloride and was administered by i.p. at 200mg/kg bw/day on GD 13.5 and 14.5. DMTU was dissolved in distilled water and administered by gavage at 200 mg/kg bw/day on GD 13.5 and 14.5. RA was suspended in corn oil and administered by gavage at 200mg/kg bw on GD 12.5. On GD18.5, the dams were killed and fetuses were examined to evaluate the incidence of cleft palate.

Results and Discussion

TCDD had induced cleft palate in 50-70% of the fetuses after administration of 10μg/kg bw on GD 12.5. The TCDD-induced cleft palate was partially attenuated by additional administration of DMSO at 10 ml/kg bw/day (Table 1) or 5ml/kg bw/day (Table 2) from GD 13.5. The critical period for this DMSO antiteratogenic effects seems to be around GD 13.5 and 14.5, since the pretreatment of DMSO at GD 11.5 and the treatment of DMSO on GD 11.5 and GD 13.5 did not show the antiteratogenic effect on the TCDD-induced cleft palate (data not shown), and the longer administration from GD13.5 to 17.5 did not decrease to cleft palate compared to the shorter administration on GD13.5 and 14.5 (Table 3). On the other hand, no protective effect of Met treatment on GD13.5 and 14.5 on the TCDD-induced cleft palate was observed (Table 4). Contrary to the effect of DMSO, a similar hydroxyradical scavenger DMTU treatment on GD13.5 and 14.5 increased the TCDD-induced cleft palate (Table 5). It is suggested from this data that the hydroxyradical scavenging property may not be involved in the antiteratogenic effect of DMSO. The mechanism of increase of cleft palate by DMTU is unknown. Contribution of the maternal toxicity of DMTU indicated by two dead cases in the TCDD+DMTU-treated group remains unclear, since same mortality was found in the TCDD+DMSO-treated group. To investigate the action of DMSO, we examined the effect of GD13.5-14.5 DMSO administration on the GD 12.5 RA-induced cleft palate. DMSO turned out to be non-protective in this study (Table 6). Additional to the Met, which counter acts against RA and GC, but not against the TCDD, DMSO would be the second chemical that can be used as a probe to dissect the multiple pathways of chemically-induced cleft palate. At this time, the mechanisms of antiteratogenic effects of DMSO against TCDD induced-cleft palate are unknown. Recently, Dhulipara VC et al.[4] reported that the relevance of protein kinase A pathway to the protective effect of

DMSO against the secalonic acid D-induced cleft palate. Therefore, there may be a possibility that the PKA pathway is involved in the TCDD-induced cleft palate, and DMSO is acting through the pathway. In conclusion, this is the first to demonstrate that DMSO attenuated the TCDD-induced cleft palate in mice.

Table 1

Effects of DMSO on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMSO	
No. of litters	6	6	
No. of dead mice	0	0	
No. of live fetuses	46	46	
No. of resorbed fetuses	0	0	
No. of dead fetuses	1	0	
No. of fetuses with cleft palate	25	11	
Cleft palate (%)	54.3	23.9	**

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMSO

was administered by gavage at 10 ml/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving

TCDD alone (*p<0.05, **P<0.01)

Table 2

Effects of DMSO on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMSO	
No. of litters	6	7	
No. of dead mice	0	0	
No. of live fetuses	46	61	
No. of resorbed fetuses	0	0	
No. of dead fetuses	0	0	
No. of fetuses with cleft palate	25	21	
Cleft palate (%)	54.3	34.4	*

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMSO

was administered by gavage at 5 ml/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving

TCDD alone (*p<0.05, **P<0.01)

Table 3

Effects of DMSO on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMSO	
No. of litters	8	6	
No. of dead mice	0	2	
No. of live fetuses	69	32	
No. of resorbed fetuses	1	1	
No. of dead fetuses	1	1	
No. of fetuses with cleft palate	42	10	
Cleft palate (%)	60.9	31.2	**

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMSO was administered by gavage at 10 ml/kg /day on days 13.5,14.5, 15.5, 16.5 and 17.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving TCDD alone (*p<0.05, **P<0.01)

Table 4

Effects of Methionine (Met) on TCDD induced-cleft palate in mice

	TCDD	TCDD+Met	
No. of litters	6	7	
No. of dead mice	0	0	
No. of live fetuses	51	59	
No. of resorbed fetuses	0	0	
No. of dead fetuses	1	0	
No. of fetuses with cleft palate	33	37	
Cleft palate (%)	64.7	62.7	

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and Met was administered by I.p. at 200mg/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving TCDD alone (*p<0.05, **P<0.01)

Table 5

Effects of dimethylthiourea (DMTU) on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMTU	
No. of litters	9	7	

No. of dead mice	0	2	
No. of live fetuses	74	43	
No. of resorbed fetuses	0	0	
No. of dead fetuses	3	2	
No. of fetuses with cleft palate	57	42	
Cleft palate (%)	77.0	97.7	**

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMTU

was administered by gavage at 200mg/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving

TCDD alone (*p<0.05, **P<0.01)

Table 6

Effects of DMSO on Retinoic acid (RA) induced-cleft palate in mice

	RA	RA+DMSO
No. of litters	5	5
No. of dead mice	0	0
No. of live fetuses	38	32
No. of resorbed fetuses	0	0
No. of dead fetuses	11	10
No. of fetuses with cleft palate	18	13
Cleft palate (%)	47.4	40.6

RA (200mg/kg) was administered by gavage on day 12.5 of gestation and DMSO

was administered by gavage at 5 ml/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving

RA alone (*p<0.05, **P<0.01)

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