Reinvestigation of Mechanism for Induction of Cleft Palate in Mice by Tetrachlorodibenzo-*p*-dioxin (TCDD)

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1. Introduction

Since the report by Pratt et al.[1] the mechanism for induction of cleft palate in mice by TCDD has seemed to be established. It has been believed that TCDD does not interfere with growth, elevation, or initial contact of the palatal shelves, but does interfere with firm adhesion and/or degeneration of the epithelial cells covering the medial edge of the palatal shelf. Furthermore, all-*trans*-retinoic acid (RA) coadministered with TCDD on gestational day 12 was reported to affect synergistically mouse palatal development[2]. However, we found that both TCDD and RA induced variant patterns of the palatal rugae, and that exencephalic mouse fetuses had fused palate even after transplacental exposure to high doses of TCDD or RA. These findings can hardly be explained by the effect of these chemicals on the medial epithelial cells. The present study aimed at reexamining the mechanism for induction of cleft palate by TCDD.

2. Materials and Methods

Colony-bred Jcl:ICR mice from CLEA Japan, Inc. (Tokyo) were used. Mature females were mated with males overnight. Copulation was ascertained by the presence of a vaginal plug on the following morning, and the day was designated as gestational day (GD) 0. For induction of exencephaly, the dams were treated with cadmium chloride (Cd) intraperitoneally at a dose level of 6 mg/kg at GD 7.5. For induction of cleft palate, the dams were treated with an oral dose of TCDD at dose levels of 10-80 μg/kg or RA at 0.6-160 mg/kg at GD 12.5. The dams were killed by cervical dislocation between GD 14.0 and GD 15.0 for examination of fetal palatogenesis. The fetuses were harvested, fixed in 2.5% glutaraldehyde in phosphate buffered saline or in Bouin's solution. Specimens fixed in glutaraldehyde were processed for observation by scanning electron microscopy. Those fixed in Bouin's solution were subjected for light microscopy. Incidences of cleft palate were calculated among fetuses observed at GD 18.5. Fetal palatal rugae were examined at GD 18.5 under a dissecting microscope after fixation in Bouin's solution. Anomalous ruga patterns were diagnosed according to Yasuda et al.[3]

3. Results

A. Observation of near term fetuses pretreated with Cd and treated with TCDD or RA

Table 1 summarizes the effects of coadministration of Cd and TCDD or RA. The Cd-pretreatment increased the fetal mortality rate and induced exencephaly in more than half of the surviving fetuses. In the groups not pretreated with Cd, more than 80% of the surviving fetuses had cleft palate. It should be noted, however, that none of the exencephalic fetuses had cleft palate, whereas more than 50% of fetuses without exencephaly had cleft palate.

Table 1. Effects of exencephaly on cleft palate induction by TCDD or RA

Groups[a)]	No. of dams	Total Implants	Fetuses				
			Dead	Live	EX[b)]	CP/EX[b)]	CP/Non-EX[b)]
Cd+TCDD	5	73	20	53	31	0/31	11/22
No+TCDD	5	74	1	73	1	0/1	67/72
Cd+RA	5	71	37	34	20	0/20	14/14
No+RA	5	68	9	59	0	-	45/59

a) Cd: 6 mg/kg at GD7.5, TCDD: 40 μg/kg at GD12.5, RA: 160 mg/kg at GD12.5
b) EX: Exencephalic fetuses, CP/EX: Fetuses with cleft palate among exencephalic fetuses
CP/Non-EX: Fetuses with cleft palate among fetuses without exencephaly

Table 2. Cleft palate and anomalous palatal rugae induced by TCDD or RA

Groups[a)]	No. of dams	Total Implants	Fetuses					
			Dead	Live	CP(+)[b)]	CP(-)[b)]	Anom. Ruga (%)[b)]	
Control	5	81	9	72	0	72	2	(3)
TCDD 10	5	74	3	71	10	61	8	(13)
TCDD 20	4	51	5	46	12	34	18	(53)
TCDD 40	4	59	0	59	54	5	5	(100)
RA 0.6	4	59	2	57	0	57	9	(16)
RA 2.5	5	75	3	72	1	71	18	(25)
RA 10	5	74	4	70	0	70	33	(47)
RA 40	6	78	4	74	21	53	35	(66)
RA 80	6	87	7	80	41	39	27	(69)

a) Numbers indicate doses in μg/kg for TCDD and mg/kg for RA.
b) CP(+): Fetuses with cleft palate, CP(-): Fetuses without cleft palate, Anom. Ruga: Fetuses with anomalous ruga patterns among fetuses without cleft palate

B. Palatogenesis in fetuses pretreated with Cd and treated with TCDD

In normal palatogenesis in Jcl:ICR mice, the palatal shelves attain a horizontal position between GD 14.0 and 14.5, and fusion of the medial edge begins in the region slightly anterior to the midpoint of the shelves, from which fusion spread both posteriorly and anteriorly. In exencephalic fetuses treated with TCDD, however, fusion of the medial edge began near the posterior end of the palatal shelves. At the time of initial fusion, the opposing medial edges of the anterior portion of the shelves were separated with a wide gap, hence the medial edges as a whole formed a "V" shape. Histological examination revealed signs of cell death in the medial edge epithelium at the fusion site.

C. Palatal rugae in fetuses treated with TCDD or RA

The results of observation of palatal rugae are summarized in Table 2. The incidence rates of cleft palate showed a clear dose response relationship in the groups treated with TCDD at and above 10 μg/kg or with RA at and above 20 mg/kg. The incidence rates of anomalous ruga patterns among fetuses without cleft palate also increased in a dose related manner. The dose response curve for cleft palate was almost similar to that for anomalous ruga patterns in the TCDD experiment, the two curves are rather different in the RA experiment. By RA anomalous ruga patterns were induced at dose levels much lower than the threshold level for induction of cleft palate. The incidences of anomalous ruga patterns classified by types are shown in Table 3. Short was the most prevalent type among the fetuses treated with TCDD, whereas supernumerary posterior to ruga 5 was the most common among the fetuses treated with RA.

Table 3. Anomalous patterns of palatal rugae induced by TCDD or RA

Groups[a)]	% Distribution of anomalous patterns[b)]					
	SN[c)]	SH[c)]	MF[c)]	LF[c)]	CR[c)]	Others
TCDD	6	74	3	26	6	0
RA	88	26	4	1	0	1

a) The groups treated with TCDD or RA at various doses are summed up into one group.
b) % of fetuses with anomalous ruga patterns
c) SN: supernumerary posterior to ruga 5, SH: short, MF: medial fusion, LF: lateral fusion, CR: cross

4. Discussion

The primary object of our study was to examine whether suppression by TCDD of the programmed cell death of the epithelial cells covering the medial edge of the palatal shelf is the actual mechanism for induction of cleft palate or not. The data given in Table 1 definitely show that the presence of exencephaly completely blocked induction of cleft palate by TCDD or RA. This block was apparently not due to pharmacokinetic alterations in exencephalic fetuses, because hydronephrosis, another manifestation of developmental toxicity of TCDD, developed among exencephalic fetuses as well as non-

exencephalic fetuses (data not shown). The discovery of this phenomenon gave us an opportunity to reinvestigate the mechanism for induction of cleft palate by TCDD.

Sato[4] reported that the palatal shelves of exencephalic fetuses were elevated earlier than non-exencephalic fetuses and that exencephalic fetuses showed two modes of palatal fusion different from the normal mode[4]. He suggested that these alterations of palatogenesis in exencephalic embryos are related to inhibitive mechanism(s) against cleft palate induction. Probably a narrow skull base in exencephalic fetuses promotes contact and fusion of the posterior part of palatal shelves. The "V" shaped medial edges in exencephalic fetuses at the beginning of palatal fusion is considered as a result of the narrow skull base. Our results showed that the posterior part of palatal shelves could contact and fuse even in the presence of TCDD or RA. The presence of cell death at the site of fusion in exencephalic fetuses suggests that TCDD could not suppress cell death after close contact of the medial edges of the palatal shelves. The suppression of cell death and active proliferation of medial edge epithelial cells after exposure to TCDD reported by Abbott and Birnbaum[5] may not be the causative mechanism for induction of cleft palate.

Palatal rugae in mice begin to develop around GD 13[6]. The increase of the anomalous patterns of palatal rugae in the fetuses treated with TCDD or RA indicate that TCDD and RA affect development of the secondary palate in a period earlier than the time of initial contact. The differences in the type of ruga anomalies between TCDD and RA groups suggest that these agents affect development of the palate in different ways. During the observation of palatogenesis of non-exencephalic fetuses exposed to TCDD, we got an impression that the elevation of the palatal shelves was delayed (data not shown). These findings indicate that TCDD interferes development of palatal shelves before initial contact, at least in Jcl:ICR mice.

This study was supported by Grants in Aid for Scientific Research on Priority Areas 02202128, 03202133, 04202132 from the Ministry of Education, Science and Culture of Japan, and a grant from the Ministry of Health and Welfare of Japan.

5. References

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