Epithelial Cell Death in TCDD-Induced Cleft Palate in Mice: Reexamination of the Mechanism for Clefting

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1. Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is highly teratogenic in mice, inducing cleft palate. It has been suggested that TCDD does not interfere with growth, elevation, or initial contact of the palatal shelves, but does interfere with the subsequent adhesion and/or degeneration of the epithelial cells covering the medial edges of both palatal shelves[1) 2)]. We found, however, that exencephalic mouse fetuses had fused palate even after transplacental exposure to high doses of TCDD, regardless of the cause of exencephaly, spontaneous, or cadmium- or hyperthermia-induced[3)]. Histological examination of these exencephalics exposed to TCDD revealed that the epithelial cells met at the midline underwent degeneration and disrupted in the process of fusion, arguing against the suggested mechanism of TCDD-induction of cleft palate. The present study aimed at reexamining whether TCDD exposure blocks the medial edge epithelial cell death *in vivo* or not. For this purpose, we employed supravital staining with acridine orange[4)] and *in situ* DNA nick end labeling (TUNEL) method[5)] to detect cell death.

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. At GD 12.5 the dams were treated with oral dose of TCDD at a dose of 40 μg/kg for induction of cleft palate. The dams were killed by cervical dislocation between GD 14.0 and GD 15.5 for examination of fetal palatogenesis. The fetuses were harvested in phosphate buffered saline (PBS), and some were fixed in Bouin's solution for conventional histological examination. For supravital staining with the fluorescent vital dye, acridine orange (AO), live fetuses were decapitated, and the heads were soaked in AO solution (5 μg/ml in PBS) after the mandible and tongue removed. After 15 min incubation at 37°C, they were washed in cold PBS, mounted on glass slide with their oral side down, and observed with an inverted fluorescent microscope. For the TUNEL staining of cell death, some fetuses were fixed in buffered formalin, and serial paraffin sections were processed after Gavrieli *et al.*[5)] with slight modification. Some dams were pretreated at GD 7.5 with cadmium chloride intraperitoneally at a dose level of 6 mg/kg for induction of exencephaly.

3. Results

Palatogenesis in control and TCDD-exposed fetuses:
In controls, the palatal shelves attained a horizontal position between GD 14.0 and 14.5, became closely apposed in the middle region of the shelves (opposite to the second and fourth pairs of palatal rugae), and began to fuse in this short period. At GD 14.5, fusion spread both posteriorly and anteriorly. In most of the TCDD-exposed fetuses, however, either one side or both of the palatal shelves were still in a vertical position at GD 14.5, giving us an impression that the elevation of the palatal shelves was somehow disturbed. By GD 15.0 the palatal shelves had elevated, but we never found that two shelves were in close contact. In exencephalic fetuses treated with TCDD, fusion of the medial edge did occur. It began near the posterior end of the palatal shelves toward GD 14.0, with the anterior portion still in vertical.

Supravital staining with AO:
Removal of the mandible and tongue from live fetuses interestingly resulted in the anterior portion of vertical shelves elevated horizontally in a flip-up fashion, suggesting that the tongue was a major obstacle against elevation of the shelves. Figure 1 illustrates cell deaths stained with AO. Developing palatal rugae were AO-positive in all the stages observed. In controls, at GD 14.25 cell death in the medial edge epithelium was not conspicuous before contact. During fusion, AO-labeled cells were observed at the midline position. On the contrary, in TCDD-exposed fetuses, a remarkable number of AO-labeled cells were found at GD 14.25 at the medial edge epithelium in the position between second and fourth pairs of palatal rugae. Cell death attained its peak around GD 14.75, and there remained some at GD 15.5.

TUNEL staining of cell death:
In situ DNA nick end labeling study confirmed and supplemented the findings of supravital staining. Some number of the apparently normal and also sloughing peridermal cells of the medial edge epithelium were TUNEL-positive even before contact, in controls. In exencephalic fetuses treated with TCDD, TUNEL-positive cells were found at the initial contact site in the posterior portion of shelves, and in the process of fusion, we found labeled cells in the disrupting midline epithelial seam.

4. Discussion

The objective of this study was to examine whether TCDD suppresses the programmed cell death of the medial edge epithelium of the palatal shelf *in vivo* for induction of cleft palate or not. We definitely showed that the epithelial cells underwent cell death in the presence of TCDD.

It is reported that, in organ culture systems, epithelial cell responses to TCDD (suppression of cell death and active proliferation) occurred over a narrow range of concentrations and TCDD exerted generalized cytotoxicity at a higher concentration[6) 7)]. The epithelial cell death shown here, however, is not the result of the cytotoxic effect of TCDD. First, at dose levels of 10 – 80 μg/kg cleft palate was induced in a dose response manner, and at the dose of 40 μg/kg that we applied in this study the incidence rate of cleft palate was about 90% with very low embryolethality (data not shown), suggesting that the results obtained here reflect well the cleft palate inducing effect of TCDD.

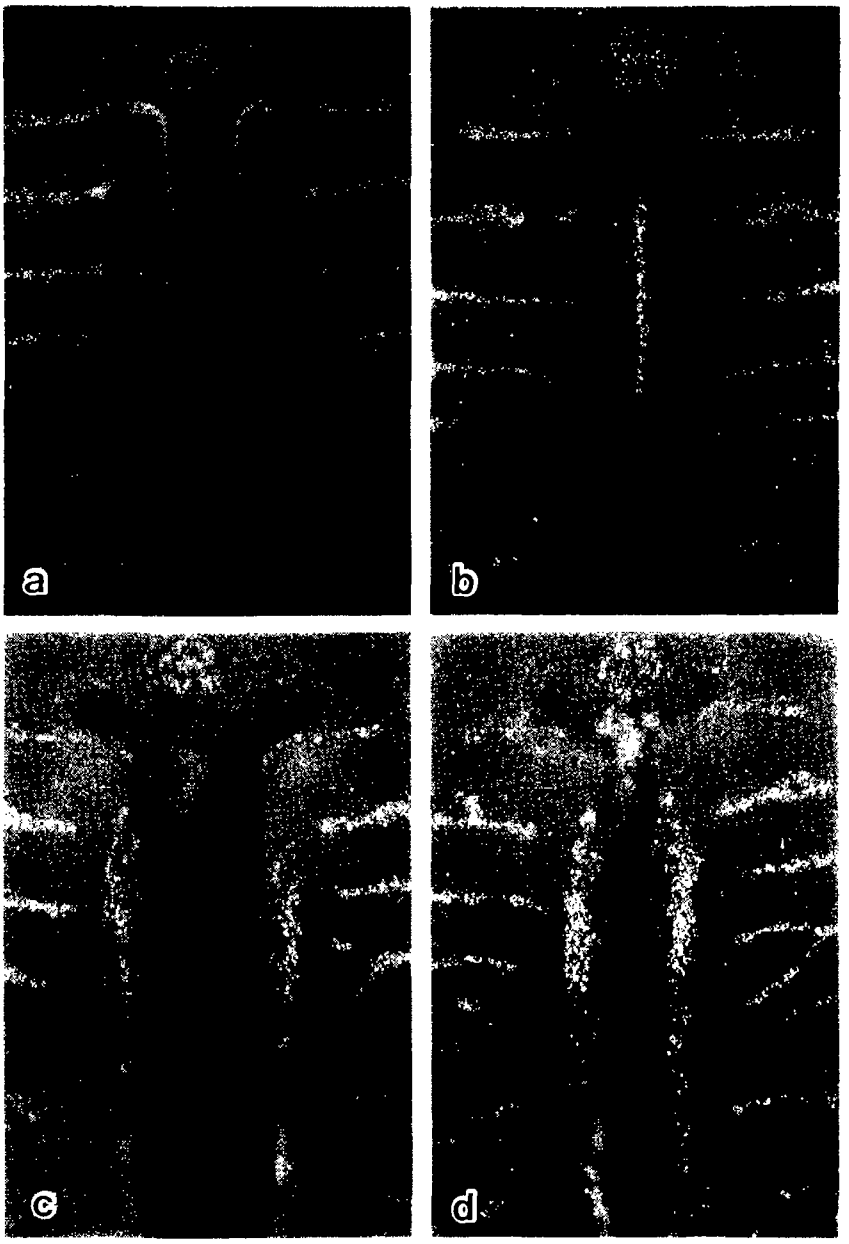

Fig. 1. Fluorescent micrographs illustrating AO-labeled cell deaths.
a and **b** Controls. GD 14.25 – before contact (a) and during fusion (b).
c and **d**. TCDD-exposed – GD 14.25 (c) and GD 14.75 (d).

Second, acridine orange is reported to stain selectively programmed or apoptotic forms of cell death and not to label cells undergoing necrotic death[4].

Observation of exencephalic fetuses treated with TCDD further supports the notion that the epithelial cell death is not suppressed by TCDD. In exencephalics the palatal shelves were elevated earlier than non-exencephalic fetuses and the posterior portions of palatal shelves were forced to make contact with each other, probably due to the narrowing of skull base[8]. The presence of cell death at the site of initial contact and the following fusion area in the exencephalic fetuses treated with TCDD suggested that TCDD could not suppress cell death after close contact of the medial edges of the palatal shelves.

In conclusion, the suppression of cell death and active proliferation of medial edge epithelial cells after exposure to TCDD reported by Abbott and Birnbaum[2] may not be the causative mechanism for induction of cleft palate, at least in Jcl:ICR mice. We propose that TCDD interferes development of palatal shelves before initial contact.

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5. References

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