PALATAL RUGA ANOMALIES INDUCED BY DIOXINS IN MICE

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Introduction

The oral surface of the palate in the mouse or rat is characterized by eight or nine pairs of transverse ridges, or rugae. Various teratogens given to pregnant animals during the period of palatogenesis induce abnormal patterns of palatal rugae in fetuses[1,2]. We reported that dioxins such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3,3',4,4',5-pentachlorobiphenyl (PeCB) produced palatal ruga anomalies in mice[1,3]. The present study aimed at revealing pathogenesis of dioxin-induced anomalies of palatal rugae by examination of cell proliferation and cell death during palatal ruga formation.

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 0:00 am of the day was designated as the start of gestation day (GD) 0. At GD12.5, TCDD (Cambridge Isotope Laboratories, originally solved in nonane at a concentration of 50 μg/1.2 ml, and diluted with corn oil) or PeCB (Cambridge Isotope Laboratories, originally solved in isooctane at a concentration of 100 μg/ml, and diluted with corn oil) was orally given by gavage. The dose levels applied were 10, 20, and 40 μg/kg body weight for TCDD, and 50, 100, 200, and 400 μg/kg body weight for PeCB. Control mice received the vehicle. The dams were killed by cervical dislocation at GD18.5, and fetuses were removed by cesarean section. The live fetuses were examined for external abnormalities including cleft palate. After fixation in Bouin's solution, the mandible of fetuses was removed, and rugae on the oral surface of the secondary palate were examined in fetuses without cleft palate under a dissecting microscope. Abnormal patterns of the palatal rugae were recorded according to the definition by Yasuda, et al.[4].
Some of the control and TCDD-treated dams were sacrificed between GD13.5 and 14.5 for studies of cell kinetics. Cell proliferation was studied after intraperitoneal injection of BrdU at 300 mg/kg body weight to dams 2 hours before sacrifice. For examination of cell movement, dams were sacrificed at intervals after BrdU injection. For detection of BrdU labeling, Cell Proliferation Kit (Amersham) was used following the standard procedure[5]. Cell death was detected by the TUNEL method[6] with DIG Oligonucleotide Trailing Kit and DIG Nucleic Acid Detection Kit (Boehringer), or by vital fluorochrome staining with acrydine orange[7].

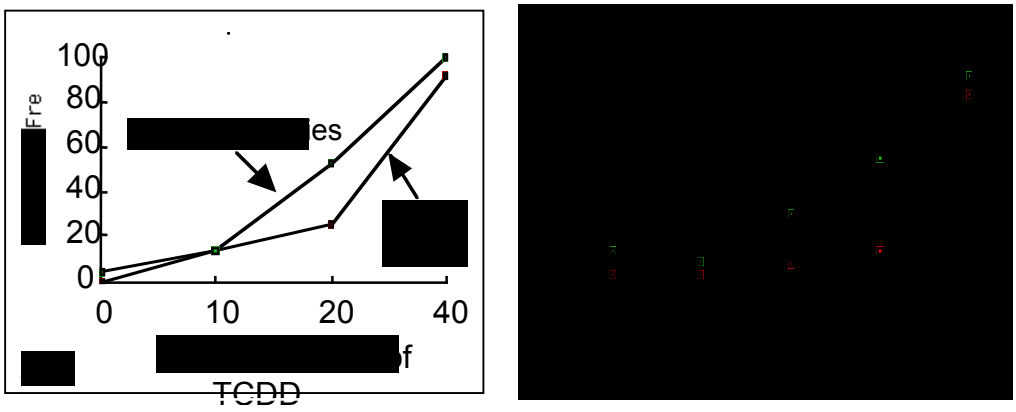

Fig. 1 Dose-response relationships of cleft palate and ruga anomalies. A: TCDD B: PeCB

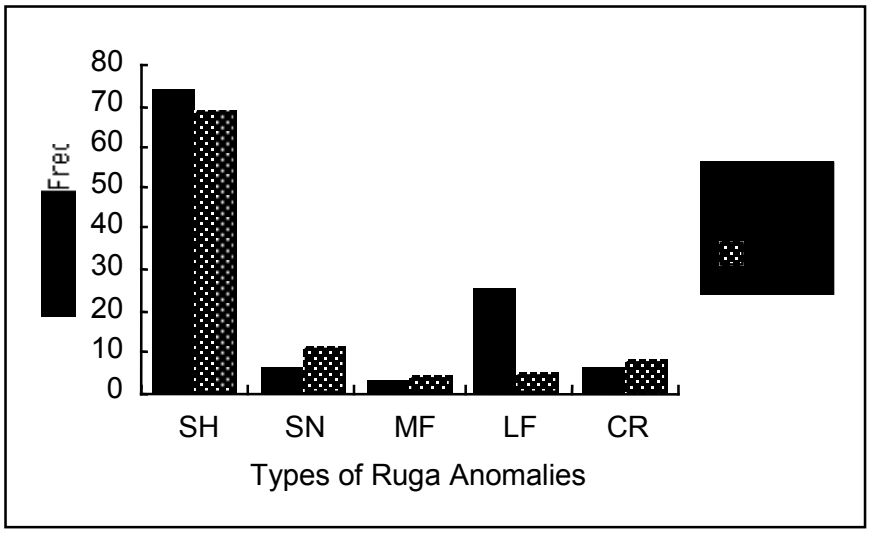

Fig. 2 Relative frequencies of types of ruga anomalies induced by TCDD and PeCB. SH: shortness, SN: supernumerary between rugae 4 and 8, MF: medial fusion, LF: lateral fusion, CR: cross. Frequencies were calculated as percentages of fetuses with any type of ruga anomalies. Some fetuses had more than one type of anomalies, hence the total of percentages exceeds 100%.

Results

Cleft palate was induced by TCDD and PeCB given at GD 12.5 with a clear dose-respons

relationship. Fetuses without cleft palate were also affected with palatal ruga anomalies in a dose related manner. The dose response curves for ruga anomalies were slightly higher than those for cleft palate. The potency of PeCB to induce these anomalies was about one-tenth of TCDD (Fig. 1). The most common abnormal pattern of palatal rugae was shortness among fetuses exposed to either TCDD or PeCB (Fig. 2). Shortness was usually observed rugae posterior to the ruga 4. Other types of ruga anomalies were rather infrequent.

In control fetuses around GD 13.5, cells labeled by BrdU were detected abundantly in the oral epithelium and palatal mesenchyme. However, distribution of the labeled cells was not uniform . Cells between prospective rugae were more frequently labeled. When specimens were taken 16 hours after the BrdU injection, labeled cells were more numerous around developing rugae. Cell proliferation in fetuses treated with higher doses of TCDD or PeCB seemed to be decreased when compared with controls.

Cell death was clearly demonstrated on the tip epithelium of ruga primordia in controls as well as dioxin-treated fetuses. Cell death in the palatal mesenchyme was not conspicuous in both control and treated fetuses.

Discussion

Rugae develop in the mouse in an anteroposterior sequence, beginning at GD12.5[8] or GD13[9]. Sakamoto et al.[10] described variant palatal rugae in corticosteroid-treated mouse fetuses. We reported that various cleft palate inducing agents such as all-*trans*-retinoic acid (RA), methoxyacetic acid, TCDD, and PeCB given to pregnant mice induced palatal ruga anomalies in fetuses escaped from palatal clefting. Apparently palatal ruga anomalies are a more sensitive indicator of toxicity to the developing palate. We noted some differences between TCDD- and RA-induced ruga anomalies[11], suggesting that these agents affect development of the palate in different ways. In the present study, TCDD and PeCB induced a similar pattern of ruga anomalies, shortness being the most frequent. This result indicates that PeCB, one of co-planar BCBs, affects palatal development by the similar mechanism mediated by the aryl hydrocarbon receptor (AhR), which was clearly showed to be involved in TCDD teratogenesis by our experiment with *Ahr*-null mice[12].

Mayura et al.[13] reported that administration of PeCB to female C57BL/6 mice at doses from 130.5 to 522 μg/kg body weight resulted in the dose-dependent formation of fetal cleft palate and hydronephrosis. Our results in ICR mice confirmed their teratological findings, although they did not observe palatal rugae.

The results of our cell kinetics study suggest that cells proliferate in the epithelium and subjacent mesenchyme between rugae, migrate to prospective rugae, and some cells die in rugae. Shortness, the most prevalent type of ruga anomalies, probably resulted from insufficient proliferation of cells, as indicated by the less numerous BrdU labeling in the treated fetuses. The preferential location of shortness in rugae posterior to the ruga 4 is easily explained by the fact that the most anterior three pairs of rugae have been formed at the time of injection of TCDD or PeCB.

Previously, cleft palate was considered to result from interference of final adhesion of well grown and elevated palatal shelves[14]. Our present results appear to negate the inhibited adhesion hypothesis, and to confirm our former conclusion that dioxins interfere with the development of palatal shelves during the period of ruga formation, before initial contact of bilateral palatal shelves[15].

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