PATHOGENESIS OF DIOXIN-INDUCED CLEFT PALATE AND HYDRONEPHROSIS IN MICE

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ABSTRACT: Pathogenesis of cleft palate and hydronephrosis in Jcl:ICR mice with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was studied by electron microscopy and lectin histochemistry. Transmission electron microscopy revealed degenerative changes in the TCDD-treated hyperplastic ureteric epithelium. Lectin binding patterns suggested changes in sugar residue distribution in the TCDD-treated unfused palate.

INTRODUCTION: Among dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent teratogen in mice. It induces cleft palate and hydronephrosis in mouse fetuses transplacentally treated during the period of organogenesis. The pathogenesis of cleft palate is considered to be due to disturbed epithelial adhesion of the palatal shelves (Pratt et al., 1984). Hydronephrosis results from hyperplasia of the ureteric luminal epithelium (Abott et al., 1987). To reveal changes involved in TCDD-induced teratogenesis, we examined the palate and ureter in mouse fetuses transplacentally treated with TCDD by electron microscopy and lectin histochemistry.

MATERIALS AND METHODS: Jcl:ICR mice were used in this study. TCDD in nonane solution was purchased from Cambridge isotope laboratories, and diluted with corn oil. Pregnant mice (detection of vaginal plug = day 0) were orally given by gavage 80 μg/kg of TCDD at day 12.5 of gestation. This dose had been shown to produce neally 100% cleft palate and hydronephrosis in previous experiments. The dams were killed between days 14.5 and 18.5, and the embryos were freed from the uterus and extraembryonic membranes.

For electron microscopy, palatal shelves and ureters were removed from embryos in 2.5% glutaraldehyde solution in 0.1 M Millonig's phosphate buffer (pH 7.4) and cut into small pieces. The specimens were immersion-fixed in the fixative at 4°C for 2 hours and postfixed in 1% osmium tetroxide in 0.1 M Millonig's phosphate buffer at 4°C for 1 hour. Then the tissue pieces were rinsed in 10% sucrose solution 5 times and stained en bloc in 3% uranyl acetate solution. Tissue specimens were dehydrated in graded ethanol series and doubly stained in 3% uranyl acetate and Reynolds' lead citrate solution were observed in a JOEL 1200EX-type electron microscope.

For lectin histochemistry, heads and trunks from the mouse embryos were fixed in Carnoy's fixative at 4°C for 2 days, dehydrated in ethanol series, and embedded in paraffin. Sections for the palate were cut coronally and sections for the ureter were cut horizontally. They were then hydrated, pre-incubated in 0.01 M phosphate buffered saline (PBS) containing 1% bovine serum albumin for 10 minutes at 4°C before histochemical staining. Sections were incubated in a solution containing 20 μg/ml of biotinylated lectins (purchased from Vector Laboratories, Burlingame, California) in 0.01 M PBS at room temperature overnight. Lectins used were Con-A, PSA, LCA, DBA, SBA, SJA, RCA-I, BSL-I, WGA, s-WGA, PHA-E, and PHA-L. After rinsing 3 times in 0.01 M PBS, the sections were incubated in a solution containing 50 μg/ml

FITC-conjugated avidin (Zymed Laboratories, San Francisco) at room temperature for 1 hour. The sections mounted in glycerol were observed in a NIKON Optiphoto microscope equipped with a EFD2-type fluorescence generating system.

For controls, embryos from vehicle (corn oil) treated dams were similarly observed.

RESULTS AND DISCUSSION:

Palate: Temporal and spatial changes in lectin binding patterns were noted during palatal development. The majority of changes were observed similarly in control and TCDD-treated palatal shelves. However, staining patterns with PHA-E showed differences between control and TCDD-treated specimens around the time of palatal fusion at day 14.5 of embryonic age. In TCDD-treated palatal shelves, the medial epithelial cells were hyperplastic, and peridermal cells covering this area showed a strong affinity to PHA-E. The control palatal medial epithelium and the TCDD-treated palatal epithelium covering the oral and nasal side showed no affinity to PHA-E. Abott et al. (1989) reported that programmed cell death of the medial peridermal cells was prevented by the TCDD treatment. The above mentioned changes in the PHA-E binding pattern may be related to the alteration in the medial peridermal cells.

Ureter: No apparent differences in lectin binding patterns were noted between control and TCDD-treated ureteric epithelium and mesenchyme sorounding the ureter during the embryonic days 14-16, although ureteric epithelium treated with TCDD had become hyperplastic by day 16.5. Transmission electron microscopic observations of TCDD-treated ureters revealed protrusion of the apical cells and degenerative changes, as reported by Abott and Birnbaum (1990). Many vacuoles and occasional phagosomes were observed in TCDD-treated epithelial cells. Although apical tight junctions were fairly well preserved, intercellular spaces were widened in TCDD-treated ureteric epithelium. The basal lamina was kept intact. These findings support the view that TCDD-induced hydronephrosis is a consequence of occulusion of the ureter by epithelial cells (Abott and Birnbaum, 1990).

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