INTERSPECIES SENSITIVITY TO TCDD EXAMINED WITH EMBRYONIC PALATAL ORGAN CULTURE

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ABSTRACT

Embryonic mouse, rat, and human palatal shelves respond to TCDD in vitro with alterations in proliferation and differentiation of medial epithelial cells which could contribute to induction of cleft palate. The palatal cells of the mouse are approximately 200 times more sensitive than the cells of rat or human palatal epithelium.

INTRODUCTION

Sensitivity to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is highly dependent on the species exposed and endpoint examined with effects ranging from mild to debilitating and lethal. TCDD is teratogenic in C57BL/6N mice, inducing cleft palate and hydronephrosis at doses which do not produce overt maternal toxicity or embryolethality. In contrast, pregnant F344 rats respond to TCDD exposure with maternal toxicity, and embryonic and fetal death are the main developmental responses rather than cleft palate and hydronephrosis. The potential for TCDD to cause developmental toxicity in humans has been evaluated through epidemiological studies and no increased risk of birth defects associated with TCDD exposure has been detected. However, these epidemiological studies are limited in their ability to detect increased risk by the small numbers of exposed pregnancies and the absence of accurate measures of the level of TCDD exposure.

In the present study the embryonic palatal shelf organ culture system is used as a model to determine the cellular responses to TCDD of mouse, rat and human palatal cells. Palatal

shelves of mouse, rat, and human embryos pass through similar developmental stages prior to forming the hard palate. The development of mouse palatal shelves in vivo has been compared to development in organ culture, and the cultured shelves develop on a comparable schedule and pass through the same critical stages as those in vivo. For each species, the model demonstrates the ability of TCDD to induce specific alterations in the regulation of proliferation and differentiation of palatal cells, the level of exposure required to produce the alterations, and the concentration of TCDD at which cytotoxicity is observed.

METHODS

Palatal shelves for organ culture were dissected from normal, untreated C57BL/6N mouse embryos on GD 12, or from F344 rat embryos on GD 14 or 15. Human embryonic craniofacial tissues were obtained from Dr. T. Shephard, University of Washington, Seattle, and human palatal shelves were dissected from tissues aged GD 52, 53, or 54. Palatal shelves from all three species were cultured in standard organ culture dishes on supported filters, over IMEM:F12 medium containing either 1 or 5% FBS. TCDD was disolved in DMSO and the final level of DMSO in the medium was 0.1%. TCDD concentrations in the medium ranged from 1×10^{-13} to 1×10^{-7} M, control medium contained only the DMSO. Standard procedures were followed for preparation of the tissues for scanning and transmission electron microscopy, immunohistochemical analysis, and autoradiography of ^{3}H-thymidine incorporation.

RESULTS AND DISCUSSION

Exposure of palatal shelves of all three species to TCDD in vitro inhibited the degeneration of the medial peridermal cells; the medial epithelial cells continued to proliferate, expressed EGF receptors, and differentiated into a stratified, squamous, oral-like epithelium. These effects are identical to the changes produced in embryonic mouse palatal shelves after exposure to TCDD in vivo. The main difference between these species is the level of TCDD required to elicit the responses and to induce cytotoxicity (i.e. degenerative alterations in ultrastructure of mesenchymal and epithelial cells). In the embryonic mouse, after in vivo exposure to a teratogenic dose of TCDD (30 μg/kg, GD 11), 1 pg total TCDD was found in the palatal shelf, a level comparable to the effective in vitro concentration, 5×10^{-11} M TCDD (16 pg/ml). Exposure in organ culture to 1×10^{-10} M TCDD resulted in cytotoxicity. Rat palatal shelves did not respond in vitro to 5×10^{-11} M TCDD, but all of the shelves exposed to 1×10^{-8} M had medial epithelial alterations identical to those occurring in the mouse. Toxicity in the rat shelves was observed at 1×10^{-7} M. It is likely that such concentrations cannot be reached in the rat palate in vivo due to maternal toxicity and embryolethality. The sensitivity of the human shelves is similar to that of the rat, as a response to TCDD occurred at 1×10^{-8} M, toxicity appeared at 1×10^{-7} M, and only one shelf responded at 5×10^{-11} M. Present data suggest that relatively high levels of human exposure to TCDD would have to occur before a cellular response was elicited in the human embryonic palatal shelf.

DISCLAIMER

This document has been reviewed in accordance with U. S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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