EFFECTS OF TCDD AND POLYCHLORINATED TERPHENYLS (PCTS) ON THE DEVELOPMENT OF CLEFT PALATE IN MOUSE EMBRYOS

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Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a teratogenic in mice, inducing cleft palate and hydronephrosis [1]. C57BL/6 mice are well known to be sensitive on the TCDD toxicity. On the other hand, it has been reported that fetuses of ddY mice fed PCTs (polychlorinated terphenyls), which have similar uses as polychlorinated biphenyls and have been identified as environmental contaminants, showed a cleft palate and the incidence of cleft palate was higher than C57BL/6 mice [2]. Therefore, the present studies was undertaken to determine if the incidence of TCDD-induced cleft palate was higher in ddY mice than C57BL/6 mice and involvement of AhR in PCTs-induced cleft palate was examined by using AhR-deficient mice. A molecular mechanism for the effects of TCDD on the embryogenesis was also examined by embryoid body and c-DNA miroarray analysis.

Methods and Materials

1) Female C57BL/6 and ddY mice were obtained from SLC Co. (Hamamatsu, Japan) at 8-10 weeks of age and were housed overnight with male C57BL/6 or ddY mice, respectively. Female mice were checked for vaginal plugs the next morning, which was designated as GD 0.5. The mice were given rodent chow (CRF-1, Oriental Co.) and distilled water ad libtum and housed under controlled conditions of temperature and light (12-h light; 12-h dark cycle). On GD 18.5, fetus were removed and analyzed. TCDD was dissolved in corn oil and singly administered to the pregnant mice from GD 8.5 to GD 14.5. The administration routes were p.o. or s.c. and the doses were 10 to 80 microgram/kg body weight. 2) To clarify the involvement of AhR in PCTs induced cleft palate, Ahr-deficient mice, which were kindly provided by Prof. A. Fujii (Tsukuba Univ. Japan) were fed the PCTs at dose of 4000 ppm in the diet from GD0.5 to GD 18.5 and the incidence of cleft palate in the fetus were examined. 3) To clarify the molecular mechanisms of TCDD-teratogenesis including cleft palate, mouse embryoid body (EB) which mimics the embryo were treated wih TCDD and c-DNA microarray analysis was conducted. Total RNA (20 micro gram, 4-days-culture) was prepared with RNeasy (QIAGEN K.K. Japan) and was fluorescently labeled with Atlas Glass Fluorescent Labeling Kit (CLONTECH Laboratories, Inc.). The probes were hybridized to glass microarray (Atlas Glass Mouse 1.0 Microarray, CLONTECH Laboratories, Inc.). The fluorescent signals were detected with Gene Pix 4000 microarray scanner (Axon Instruments, Inc.USA).

Results and Discussion

The dose-dependent effect of TCDD on the cleft palate in C57BL/6 mice was shown in Fig.1. After single s.c. administration of TCDD, cleft plate was induced in dose-dependent manner at administration of GD 12.5. In the other days, cleft palate was only observed at 80 microgram/kg bw group. In contrast, there were little induction of cleft palate by TCDD treatment in ddY mice (Fig.2).

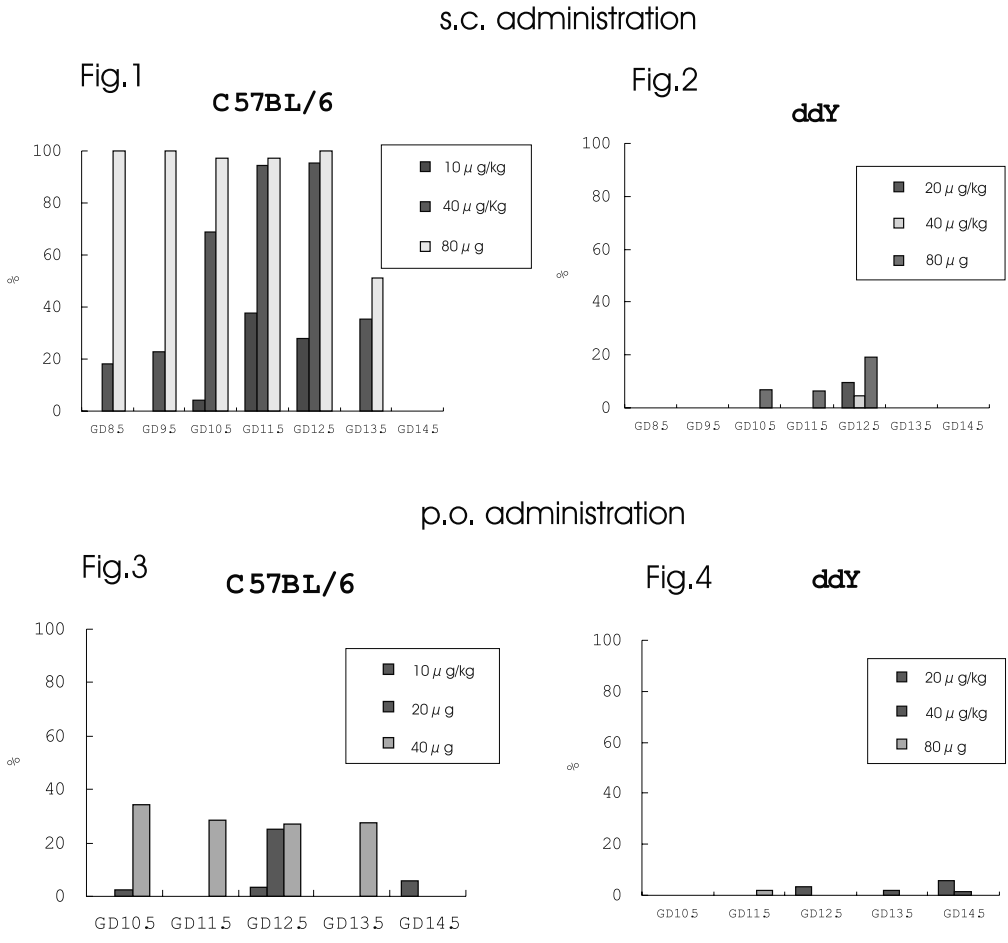

Table 1. Cleft palate incidence of AhR-deficient mice fed PCTs

Wild	8/29	(27.6 %)
Hetero	11/50	(22.0 %)
Homo	14/37	(37.8 %)

The difference was more evident in the p.o. administrated-groups. The dose-response effect of TCDD on the cleft palate in C57BL/6 mice was shown in Fig.3. After single p.o.. administration of TCDD, cleft plate was induced in dose-dependent manner at administraion of GD 10.5-12.5. In contrast, slight induction of cleft palate by TCDD in ddY mice were only observed at GD10.5-12.5 (Fig.4). The results

indicate that ddY mice were less sensitive than C57Bl/6 mice in the cleft palate induction-ability of TCDD. To demonstrate the PCTs-induced cleft palate was AhR independent, PCTs was administered to AhR deficient mice. As the results, there were no significant differences of cleft palate-incidence among the AhR-wild, -hetero and -homo mice (Table 1). The results clearly demonstrate that the PCTs-induced cleft palate was AhR independent. The mechanisms of PCTs-induced cleft palate was not clear at this time and further studies will be required. In the c-DNA microarrays analysis, nearly 50 % of the genes are expressed in EBs. We find that exposure to 10 nM TCDD for 4 days showed that several genes which are required for epithelial-mesenchymal interaction was changed. The results may help to assess the molecular mechanisms of TCDD-induced teratogenesis. In conclusion, we have demonstrated that cleft palate-induction pathway in TCDD and PCTs were different. And Ahr-deficient mice is useful system for its analysis.

References

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