# INVOLVEMENT OF AH RECEPTOR ON DEVELOPMENTAL TOXICITY OF DIOXIN IN MOUSE FETUSES : SENSITIVITY IN AHR-MUTANT HETEROZYGOTES

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## Introduction

The aryl hydrocarbon receptor (AhR) has been considered to be one of the key molecules to mediate toxicity of dioxins. Dioxin induces cleft palate and hydronephrosis (dilated renal pelvis) in mouse fetuses. In orderto see whether this toxicity is mediated by AhR, we used mice whose *Ahr* gene were knocked out by homologous recombination. Homozygous *Ahr*-deficient (*Ahr*-/-) fetuses were resistant to the induction of cleft palate and dilated renal pelvis caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at a dose of 40μg/kg at gestation day 12.5 (GD, vaginal plug = day 0), whereas, in almost all wild type fetuses, cleft palate and dilated renal pelvis were induced[1]. Similar results were reported by Peters et al.[2]. In our experiment, as for fetuses having the heterozygous*Ahr*-mutant allele, all of them exhibited dilated renal pelvis upon treatment with TCDD. In contrast, in palatogenesis, the heterozygotes exhibited a lower incidence (24%) of teratogenic response to TCDD[1].

Two possibilities are held to explain for the discrepancy of the incidence of cleft palate and dilated renal pelvis. (1) The*Ahr* allele is inherited as a dominant trait in inducing dilated renal pelvis caused by TCDD, but not in inducing cleft palate. (2) Both of the teratogenic response (cleft palate and dilated renal pelvis) to TCDD are induced in a dose–response manner; as the dose of TCDD increases, the incidence increases.

The present study aimed at evaluating the effects of TCDD as to whether the teratogenic response was seen in a dose–response manner in heterozygous *Ahr*-mutant fetuses.

## Materials and Methods

*Animals.* Sexually mature C57BL/6J male and female mice from CLEA Japan, Inc. (Tokyo) were used. Homozygous *Ahr*-deficient males were maintained in Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine.

*Dioxin.* 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, product No. ED-901) was purchased from Cambridge Isotope Laboratories Japan (Osaka, Japan) and solved in corn oil.

*Sensitivity in C57BL/6J or heterozygous Ahr-mutant fetuses to TCDD. Ahr+/+* fetuses were produced by mating a C57BL/6J male and a C57BL/6J female. They were mated overnight

in a cage. The day when the vaginal plug was positive was designated as GD 0. Heterozygous *Ahr*-mutant fetuses were produced by mating homozygous*Ahr*-deficient males with C57BL/6J females.

*Dosing.* Pregnant mice were given TCDD at 0–80μg/kg at GD 12.5 by gavage. The doses were 0 (control), 0.3125, 0.625, 1.25, 2.5, 5, 10, 20, 40, and 80μg/kg. TCDD was diluted so that the volume of dosing was always 5,000μl/kg. The fetuses were harvested at GD 18.5 and examined for cleft palate and dilated renal pelvis.

**Table 1.** Experimental design and results concerning number of implantation sites and incidence of fetal death caused by TCDD in*Ahr*+/+ (WT), or heterozygous *Ahr*-mutant fetuses.

A) Result in *Ahr*+/+ fetuses

| Genotype of *Ahr* in fetuses (a) | Dose of TCDD (b) | No. of dams | Number of implantation | Number of fetal death | | Number of live fetuses |
|---|---|---|---|---|---|---|
| | | | | Early (c) | Late (d) | |
| *Ahr*+/+ | 0 (e) | 5 | 6.6 | 3 | 0 | 30 |
| | 0.3125 | 5 | 7 | 0 | 0 | 35 |
| | 0.625 | 5 | 8 | 0 | 0 | 40 |
| | 1.25 | 5 | 8.2 | 0 | 0 | 41 |
| | 2.5 | 5 | 8.4 | 4 | 3 | 35 |
| | 5 | 5 | 8.2 | 3 | 0 | 38 |
| | 10 | 6 | 8.2 | 1 | 2 | 46 |
| | 20 | 5 | 8.2 | 6 | 2 | 33 |
| | 40 | 6 | 7.2 | 5 | 0 | 38 |
| | 80 | N.D. (f) | | | | |

B) Result in *Ahr*+/- fetuses

| Genotype of *Ahr* in fetuses (a) | Dose of TCDD (b) | No. of dams | Number of implantation | Number of fetal death | | Number of live fetuses |
|---|---|---|---|---|---|---|
| | | | | Early (c) | Late (d) | |
| *Ahr*+/- | 0 (e) | 6 | 7.5 | 1 | 0 | 44 |
| | 0.3125 | N.D. (f) | | | | |
| | 0.625 | 5 | 9.2 | 1 | 0 | 45 |
| | 1.25 | 5 | 6.4 | 0 | 0 | 32 |
| | 2.5 | 5 | 6.4 | 0 | 0 | 32 |
| | 5 | 5 | 6.4 | 0 | 0 | 32 |
| | 10 | 5 | 7.6 | 0 | 1 | 37 |
| | 20 | 5 | 7.4 | 1 | 0 | 36 |
| | 40 | 5 | 7 | 0 | 0 | 35 |
| | 80 | 6 | 6.8 | 1 | 1 | 39 |

(a) *Ahr*+/+ fetuses were obtained from mating between C57BL/6J males and females; *Ahr*+/- between *Ahr*-/- males and C57BL/6J females.

(b) TCDD was given to pregnant mice by gavage at GD12.5 at dose levels of 0–80 μg/kg maternal body weight and fetuses were harvested at GD18.5.

(c) Death before completion of placenta. (d) Death after completion of placenta.

(e) Control. (f) Not done.

**Results and Discussion**

Table 1 shows the experimental design to see effects of TCDD on *Ahr*+/+ and *Ahr*+/- fetuses. Incidence of fetal death caused by TCDD (death after administration of TCDD) did not increase in *Ahr*+/+ and *Ahr*+/- fetuses as the dose increased. As the genotype of dams was *Ahr*+/+ in both experiments (effects on *Ahr*+/+ and *Ahr*+/- fetuses), the effect of maternal background is neglected.

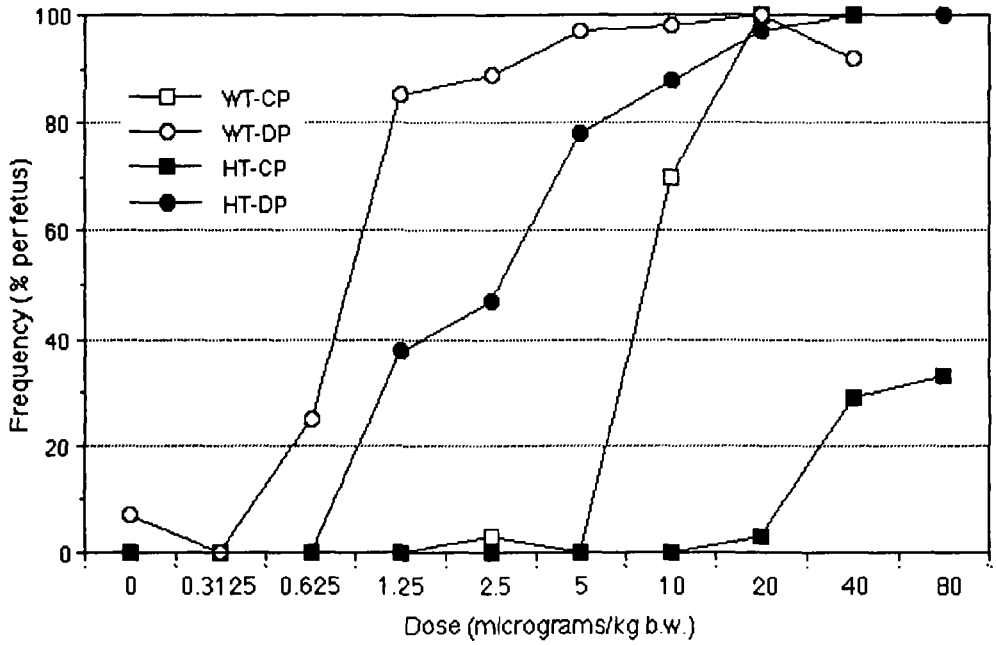


**Figure 1.** Dose–response relationship between dose of TCDD and induction of cleft palate (CP) and dilated renal pelvis (DP) in *Ahr*+/+ (WT) or heterozygous A*hr*-mutant (HT, *Ahr*+/-) fetuses. Dams were given TCDD by gavage once at GD 12.5, and killed at GD 18.5.

Figure 1 shows the dose–response relationship between *Ahr*+/+ and *Ahr*+/- fetuses. As for cleft palate, the incidence decreased as the dose decreased in heterozygous Ahr-mutant fetuses as well as in wild type (*Ahr*+/+) fetuses. It seems that the incidence of induction of cleft palate increases as the dose increases in heterozygous *Ahr*-mutant fetuses. In the present experiment, however, the maximal dose was 80 μg/kg in the experiment using heterozygotes, and it was not clarified whether incidence of cleft palate increases in higher doses than 80 μg/kg. Dilated renal pelvis was shown to be a more sensitive indicator of teratogenesis caused by TCDD compared to cleft palate. In wild type fetuses a clear dose–response was shown for dilated renal pelvis at dose levels between 0.10 μg/kg. In heterozygotes, a dose–response curve could be drawn and was shifted to the right, indicating that induction of dilated renal pelvis in heterozygotes was less sensitive than that in wild type fetuses.

The molecular mechanism of inducing cleft palate and dilated renal pelvis by dioxins is still unclear, although AhR is shown to be involved. We showed that TCDD inhibits proliferation of mesenchymal cells underlying the medial edge epithelium of the secondary palate at GD

13.5 and that cleft palate by TCDD results from poor development of palatal shelves[3]. We are investigating what molecules are regulated during the transcriptional processes by AhR.

In summary, heterozygous *Ahr*-mutant fetuses show haploinsufficiency for induction of cleft palate and dilated renal pelvis. Dose–response relationship was clearly shown also in induction of both teratogenesis in heterozygotes as well as in *Ahr*+/+ (WT) fetuses.

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