STRAIN DIFFERENCE IN PLACENTAL DYSFUNCTION AND FETAL DEATH IN RATS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN

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Introduction

Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) during pregnancy causes intrauterine fetal death in many animal species. In an early study, we investigated the relationship between fetal death and alteration of placental functions after exposure to TCDD. Briefly, exposure of pregnant Holtzman (HLZ) rats to 1.6 μg TCDD/kg body weight resulted in a fetal death rate of approximately 13% on gestational day (GD) 20[1]. A large area of the placentas of TCDD-exposed surviving fetuses was found to contain glycogen cells and cysts filled with eosinophilic material (GC-EM), a greater amount of glycogen, and an increased expression level of glucose transporter 3 (GLUT3) mRNA[1]. Since alteration of glucose kinetics in the placenta is thought to be associated with reduced uterine blood flow in models of other diseases such as diabetic animals, which also exhibit intrauterine growth abnormalities[2,3], we hypothesized that the increased fetal death after TCDD-exposure is caused by the placental hypoxia observed by two-dimensional gel analysis[4]. This hypothesis is consistent with the finding that vascular maturation in the labyrinth zone is delayed in TCDD-exposed placenta (Ishimura *et al.*, this conference). This evidence of placental dysfunction, such as alteration of glucose kinetics and inhibition of vascular maturation in TCDD-exposed HLZ rats, provides new clues to the mechanism of fetal death in other animal species or strains.

A unique feature of TCDD toxicity is the differences in susceptibility among animal species or strains, including man. For example, the median lethal dose of TCDD (LD_{50}) in the hamster is 5000 fold higher than in the guinea pig[5]. Among mouse strains, C57BL/6 mice have been found to be much more susceptible than DBA/2 mice in terms of various endpoints, including CYP1A1 induction, cleft palate, thymic atrophy, and porphyria[6]. Since the difference in binding affinity of AhR for TCDD between these strains is almost exactly proportional to the differences in effective dose of TCDD for several endpoints, including CYP1A1 induction and thymic atrophy[5,7], in these strains, the aryl hydrocarbon receptor (AhR) is thought to play an important role in determining sensitivity to TCDD toxicity. On the other hand, the AhR does not always explain differences in TCDD toxicity, e.g., another gene besides AhR has been reported to be a determinant of sensitivity to the LD_{50} of TCDD in the rat[8]. Nevertheless, the extent to which AhR, and/or other genes, is responsible for TCDD-caused fetal death among different rat strains is worth investigating.

In the present study, we investigated differences in responsiveness between two strains of rats, the HLZ strain and Sprague-Dawley (SD) strain, in terms of induction of placental dysfunction and fetal death by TCDD, and we hypothesize novel underlying mechanisms that may determine sensitivity measured by using these endpoints.

Materials and Methods

Animals and sample collection: Rats were handled with care according to the guidelines on animal experiments at NIES. The protocol for TCDD administration was essentially the same as described previously[1]. Briefly, Holtzman rats were given a single oral dose of 1.6 μg TCDD/kg body weight or an equivalent volume of vehicle (control) on GD15. Similarly, SD rats were given 0, 2, 5, and 10 μg TCDD/kg on GD15. In the lower TCDD-dose experiments, both HLZ and SD rats were given 0, 0.0125, 0.05, 0.2, 0.8, 1.6 μg TCDD/kg on GD15, and all the rats were subjected to assessment of fetal survival or placenta collection on GD20.

Histological observations: The placental tissues were fixed in HistoChoice (Amresco, Solon, OH, USA) and embedded in paraffin. Both horizontal and transverse sections (5-μm thick) were prepared on silane-coated slides and stained with hematoxylin and eosin (HE). The horizontal sections were also stained for endothelium with BS-1 lectin, which identifies fetal capillaries, as described previously[9]. Images of maternal blood sinusoids (MBS) and fetal capillaries (FC) were constructed based on the BS-1 lectin stained sections.

Semi-quantitative RT-PCR and real-time PCR: Expression of AhR and CYP1A1 mRNAs was analyzed by semi-quantitative RT-PCR and/or real-time RT-PCR methods as described previously[1].

Sequence analysis: Total RNA was isolated from the liver, and cDNA was synthesized. Four sets of primers were designed to cover the whole AhR coding region. The products amplified by PCR were purified with a QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA, USA), and their sequence was determined by the direct sequence method with ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)

Results and Discussion

We administered TCDD to HLZ and SD rats on GD15 and evaluated these strains for differences in occurrence of placental dysfunction and fetal death on GD20. A fetal death rate of approximately 14% was observed at 1.6 μg TCDD/kg in HLZ rats, which is consistent with earlier results[1], whereas exposure to much higher doses of TCDD (max. 10 μg/kg) did not result in the fetal death in SD rats. These results suggest the presence of a large strain difference in fetal death caused by TCDD.

We histologically examined the labyrinth zone of the placenta on GD20 in horizontal sections. Both the MBS and FC in the placenta of TCDD-exposed HLZ rats were smaller than those in control placenta, but no reduction in size of either MBS or FC was observed in TCDD-exposed SD rats.

Next, we histologically examined the placenta for GC-EM on GD20. In HLZ rats, the presence of GC-EM was confirmed at 1.6 μg TCDD/kg, as shown previously[1], but no such effect was observed in SD rats, even at the highest TCDD dose. There also appears to be a large strain difference in placental dysfunction induced by TCDD, including abnormal blood vessel formation and alteration of glucose kinetics, and a close link between placental dysfunction and fetal death.

Based on the above-described difference in perinatal susceptibility to TCDD toxicity, we devised a working hypothesis that a difference in the primary sequence of AhR protein explains the difference in susceptibility, but determination of the nucleotide sequence of AhR cDNAs from HLZ rats and SD rats revealed that their primary structure was identical. We then investigated whether the AhR of HLZ and SD rats had the same activity *in vivo* by measuring cytochrome P4501A1 (CYP1A1) mRNA induction rate in placenta. Both HLZ and SD rats were exposed to 0, 0.0025, 0.05, 0.2, 0.8, and 1.6 μg TCDD/kg on GD15, and their placentas were analyzed on GD20. CYP1A1 mRNA was found to be induced in a dose-dependent manner in this dose-range, but no significant difference in CYP1A1 mRNA induction level was observed between these strains. These results showed no difference between HLZ and SD rats *in vivo* activity of AhR. Although our working hypothesis that the primary structure of AhR protein determines the susceptibility of HLZ and SD rats to TCDD was rejected, HLZ and SD strain rats appear to be unique experimental models for evaluating strain differences in TCDD toxicity.

The results of this study suggest that genetic factors other than AhR play an important role in determining susceptibility to induction of placental dysfunction and fetal death by TCDD. There is a study that has demonstrated the presence of a factor other than AhR in determining sensitivity to TCDD toxicity. In that study, Tuomisto *et al.* (1999) created a new rat line by mating a TCDD-sensitive Long-Evans (L-E) strain and a TCDD-resistant Han/Wistar (H/W) strain, and showed that the LD_{50} value in the new line differed from that of the parents despite having the same AhR allele, suggesting the existence of a gene independent from AhR[9]. Since the AhR of HLZ and SD strains has been shown to have the same structure, these two strains may be useful for detecting determinant genes other than AhR for endpoints other than placental dysfunction and fetal death.

There are experimental data showing that the primary structure of AhR protein is not responsible for the strain difference in susceptibility to TCDD-caused fetal death. For example, the strain difference in acute lethality between L-E and H/W rats depends mainly upon the primary structure of AhR[10], whereas the strain difference in fetal death does not[11]. It has been reported that susceptibility to fetal death after exposure to a TCDD congener cannot be deduced from the primary structure of AhR in the mouse. More specifically, the occurrence of cleft palate and hydronephrosis in C57BL/6 and DBA/2 mice exposed to 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) was positively correlated with the AhR activity of each strain, similar to the results of TCDD-exposure, but no difference in the incidence of fetal death was observed between these strains[12]. Although, the reason for the incidence of fetal death not being correlated with AhR activity in these studies was not discussed, we speculate that a gene other than AhR that determines susceptibility to induction of placental dysfunction and fetal death by TCDD is present in these strains. The manner in which genes other than AhR contribute to TCDD-induced effects in

the placenta and fetuses warrants further study.

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