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Differential susceptibilities of Holtzman and Sprague–Dawley rats to fetal death and placental dysfunction induced by 2,3,7,8-teterachlorodibenzo-*p*-dioxin (TCDD) despite the identical primary structure of the aryl hydrocarbon receptor

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Abstract

A single oral dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioin (TCDD) administered to pregnant Holtzman (HLZ) rats on gestational days 15 (GD15) caused placental dysfunction, resulting in fetal death (Ishimura, R., Ohsako, S., Miyabara, Y., Sakaue, M., Kawakami, T., Aoki, Y., Yonemoto, J., Tohyama, C., 2002a. Increased glycogen content and glucose transporter 3 mRNA level in the placenta of Holtzman rats after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 178, 161–171; Ishimura, R., Ohsako, S., Kawakami, T., Sakaue, M., Aoki, Y., Tohyama, C., 2002b. Altered protein profile and possible hypoxia in the placenta of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-exposed rats. Toxicol. Appl. Pharmacol. 185, 197–206). In order to investigate the mechanism underlying the TCDD-induced fetal death, we compared two outbred strains of rats, namely, the HLZ and the Sprague–Dawley International Genetic Standard rats (SD-IGS), a strain with characteristics resembling those of the HLZ rats. Pregnant HLZ and SD-IGS rats were administered TCDD as a single dose by gavage on GD15, as described within the parentheses (HLZ, 0, 1.6 μg TCDD/kg; SD-IGS, 0, 2, 5, 10 μg TCDD/kg). Whereas a high incidence (14%) of fetal death was observed on GD20 in the HLZ rats, no fetal deaths occurred in the SD-IGS rats, even at the highest dose of TCDD. A histological marker of cellular abnormality at the placental junctional zone, i.e., delay in the disappearance of the glycogen cells and cysts filled with an eosinophilic material (GC-EM), which normally disappear by GD20, was observed in the HLZ rats after exposure to the lowest dose of TCDD (1.6 μg TCDD/kg), but not in the SD-IGS rats even after exposure to the highest dose of TCDD. Furthermore, maternal blood sinusoids in the labyrinth zone were constricted following exposure to TCDD in the HLZ, but not SD-IGS rats. These observations indicate that HLZ rats are more susceptible to the adverse effects of TCDD on fetal growth and placental function, than SD-IGS rats. Direct sequencing analysis of the aryl hydrocarbon receptor (AhR) gene revealed no difference in the primary structure of the receptor between the HLZ and SD-IGS rats. In addition, no significant differences were observed between the two strains of rats in the levels of induction of placental cytochrome P450 1A1, 1B1, AhR, and AhRR mRNAs following administration of serially increasing doses of TCDD (0.0125, 0.05, 0.2, 0.8, and 1.6 μg TCDD/kg), indicating that the activity of TCDD-AhR complex in the placenta is similar between the HLZ and SD-IGS rats. Taken together, the above-described findings indicate that the higher susceptibility of HLZ rats to TCDD-induced placental dysfunction and fetal death may be modulated by other factor(s) in the genetic background of HLZ rats than the AhR.

Keywords: Fetal death; Placenta; TCDD; Susceptibility

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Introduction

Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes a wide variety of adverse effects in laboratory animals (Pohjanvirta and Tuomisto, 1994; Birnbaum, 1995; Tohyama, 2002), and it has been established that the majority of these adverse effects are mediated by an aryl hydrocarbon receptor (AhR)-dependent mechanism. Furthermore, it is well-known that the susceptibility to TCDD differs considerably among animal species: for example, the LD50 of TCDD in hamsters is approximately 8000-fold higher than that in guinea pigs (Schwetz et al., 1973; Henck et al., 1981). Even in the same animal species, the C57BL/6J strain of mice and Long–Evans (L–E, *TurkuA/B*) rats show an approximately 10-fold and more than 1000-fold higher susceptibility to TCDD-induced death than the DBA/2 strain (Chapman and Schiller, 1985) and Han/Wistar (H/W, *Kuopio*) rats (Pohjanvirta et al., 1988, 1993), respectively. Differences in the susceptibility to the adverse effects of TCDD among species or even strains of the same species have been ascribed to the distinct primary structures of the AhR in these animals, since the AhR structure determines its ligand-binding affinity and transactivation function. For instance, the transactivation domain in the C-terminal of the AhR protein is partially deleted in the C57BL/6J mice as compared to that in the DBA/2 mice, and replacement of single amino acid residue (A375V) in the ligand-binding domain causes a difference in the dissociation constant (K_d) (Ema et al., 1994; Poland et al., 1994). Mutation in the transactivation domain in the AhR of H/W rats was demonstrated in one study (Pohjanvirta et al., 1998).

In contrast, it was clearly demonstrated in a series of studies conducted using two experimental animal models (Tuomisto et al., 1999; Smith et al., 1998; Robinson et al., 2002) that susceptibilities to the adverse effects of TCDD are not necessarily always attributable to polymorphism of the *AhR* gene. Tuomisto et al. (1999) cross-bred and generated sublines of H/W and L–E rats, designated as Lines B and C. While both the rat lines had an identical structure of AhR, the LD50 value of TCDD in Line B was significantly higher, approximately 20-fold, than that in Line C, in which the LD50 value of the TCDD was found to be equivalent to that in the L–E rats. These results suggest the possibility of an as-yet unidentified gene (gene B^{hw}) of H/W origin being involved in the differential susceptibility between Lines B and C. By conducting a quantitative trait locus analysis of an F2 intercross between the susceptible C57BL/6 strain and resistant DBA/2 strain, Smith and his associates (Smith et al., 1998; Robinson et al., 2002) reported that the differential susceptibility of the animals to hepatic porphyria caused by the administration of iron compounds prior to that of TCDD was independent of the *AhR* gene loci. These observations suggest the possible existence of so-called modifier genes that determine the toxicity phenotype by modulating AhR gene expression.

Gestational exposure of many species of laboratory animals to TCDD has been established to result in fetal death (Couture et al., 1990; Olson and McGarrigle, 1992; Guo et al., 1999). Administration of a relatively low dose of TCDD (1.6 μg TCDD/kg) to pregnant Holtzman (HLZ) rats on gestation days 15 (GD15) was followed by a significantly elevated incidence (13%), the last stage of pregnancy, in the animals (Ishimura et al., 2002a). A novel finding in this study was the considerable delay in the disappearance of the glycogen cells and cysts filled with eosinophilic material (GC-EM) at the junctional zone of placenta caused by exposure to TCDD. Under normal physiological conditions, glycogen cells appear at around GD13, peak in number around GD16, and disappear by the time of delivery (Davies and Glasser, 1968). These findings suggested that TCDD retarded the normal degradation process of GC-EM and also disrupted the normal glucose kinetics in the placenta.

In our later study, TCDD administration during the gestation period was found to cause constriction of the maternal blood sinusoids in the labyrinth zone of placenta (Ishimura et al., 2003). The junctional zone, located on the uterus side, has been reported to play a role in glycogen storage and hormone secretion (Davies and Glasser, 1968), while the labyrinth zone in which fetal capillaries and maternal blood sinusoids intermingle, plays a role in the transport of oxygen and nutrients from the dam to the fetus though the trophoblastic cell layers. Our earlier observation of the appearance of protein markers of hypoxia suggested the possible involvement of placental hypoxia in the late stage of pregnancy in the toxic effects of TCDD on the fetus (Ishimura et al., 2002b).

In the present study, we analyzed the differential effects of TCDD on the fetus and placenta of HLZ rats imported from the United States and Sprague–Dawley International Genetic Standard (SD-IGS) rats introduced to Japan from the United States in 1994. While the SD-IGS rats exhibit close resemblance in characteristics to the HLZ rats, it was found that the SD-IGS rats were much less susceptible to the adverse effects of TCDD than the HLZ rats. We also demonstrated that the two rat strains had an identical AhR structure. These findings suggest that some modulating factors beside AhR may reside in the genetic make-up of the two outbred rats, which accounts for the strain difference in the susceptibility to the adverse effects of TCDD.

Materials and methods

Reagents. 2,3,7,8-TCDD (purity>99.5%) was obtained from Cambridge Isotope Laboratory (Andover, MA). Biotinylated BS-1 lectin, streptavidin-conjugated with horseradish peroxidase, n-nonane, and corn oil were purchased from Sigma (St. Louis, MO). HistoChoice was procured from Amresco (Solon, OH). Trizol reagent, SuperScript II RNase H-Reverse transcriptase, oligo (dT) 12–18 primer, and Platinum *Pfx* DNA Polymerase were obtained from Invitrogen (Carlsbad, CA). TaKaRa Ex Taq polymerase was purchased from Takara Bio (Otsu, Japan). QuantiTect SYBR® Green PCR Master Mix, RNeasy Mini kit, and QIAquick Gel Extraction kit were purchased from QIAGEN (Hilden, Germany). The BigDye terminator cycle sequencing kit was purchased from PE-Biosystems (Foster City, CA). The plasmid pGEM-T Easy vector was obtained from Promega Corp. (Madison, WI). Unless otherwise specified, all the other chemicals and reagents were purchased from Wako Chemical Industries (Osaka, Japan).

Experimental animals. Male and female HLZ rats were purchased from Harlan Co. (Indianapolis, IN). Male and female Sprague–Dawley International Genetic Standard (SD-IGS, Crj; CD(SD)IGS) rats (Mitsumori et al., 2001) were