2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) AFFECTS GLUCOSE KINETICS IN RAT PLACENTA

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Introduction

Exposure to relatively high doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes the wasting syndrome, which is characterized by loss of body weight and depletion of energy stores followed by hypoglycemia due to wide variety of alterations in metabolism (1). Although the intrinsic mechanisms were still unclear, it is obvious that exposure to TCDD is associated with the alteration of carbohydrate metabolism. TCDD derived from maternal compartments pass through the placenta before reaching the fetus. As the placenta has a variety of functions including nutrients transport in order to support normal fetal growth, it is conceivable that disruption of placental functions precedes the abnormal fetal growth. However, it remained unclear what kinds of biological alterations occurred in placenta. The rodent placenta is organized into two morphologically distinct zones: the junctional zone (JZ) and labyrinth zone (2). The JZ is located adjacent to the uterine decidua and contains three morphologically distinct trophoblastic cells: trophoblast giant cell, spongiotrophoblast cell, and glycogen cell. The glycogen cell contains substantial amounts of glycogen in the cytoplasm, therefore it is speculated that they function as energy resources for fetus or placenta. In the rat, glycogen cells appear around gestational day (GD) 13 with a maximum numbers of the cell population around GD16, after which the cell numbers decrease followed by the disappearance of the glycogen cells by the end of pregnancy (2). There is a chemically-induced diabetic animal model which shows fetal growth abnormality accompanied with histological changes of glycogen cells in the placenta. When rats were administered streptozotocin, they showed intrauterine fetal growth retardation. In these rats, the placental glycogen level was increased, and the numbers of cysts in which glycogen cells were degenerated and filled with eosinophilic materials were increased in the placenta, particularly in late pregnancy (3). This model provides the evidence that the glycogen cells are closely associated with the fetal growth.

In the present study, pregnant rats were exposed to a relatively low dose of TCDD and the histochemical observation of placentas showed an increase in numbers of large cysts having degenerated glycogen cells with eosinophilic materials. We also observed alteration of glycogen content and glucose transporter expression in the TCDD-exposed placenta. We propose that the placenta would be an appropriate model to study the effect of TCDD on carbohydrate metabolism.

Materials and Methods

Animals and sample collection: Animal experiments were performed according to the guideline on animal welfare at NIES. The protocol for TCDD administration was essentially the same as described previously (4). Briefly, Holtzman rats were given a single oral dose of 800 or

1600 ng TCDD/kg body weight or an equivalent volume of vehicle (control) on GD15. The placentas and fetuses were collected on GD16 and GD20 (n=6,3, and 6 pregnant rats for control group, 800 ng/kg group, and 1600 ng/kg group, respectively). The fetus and placenta were weighed, and placentas were frozen by liquid nitrogen and then kept in –80 °C until analysis. Some placentas were fixed for histological analysis.

Histopathology: Placental tissue was fixed in Bouin's fluid and embedded in paraffin. Transverse sections, 5-μm thick, were prepared on silane-coated slides and stained with hematoxylin and eosin (H&E).

Glycogen assay: The placentas were digested in a boiling bath for 30 min with 30% KOH saturated with Na_2SO_4. The glycogen was precipitated by adding 95% ethanol to make the final concentration 43%. The glycogen precipitates were dissolved in water and analyzed by the phenolsulfuric acid colorimetric method.

Semiquantitative RT-PCR: Expression of glucose transporter 1 (GLUT1) and GLUT3 mRNA was analysed by the semiquantitative RT-PCR method performed as described previously (4). Briefly, the purified RNA samples (4 μg) were reverse-transcribed by the standard protocol of the supplier. For the mRNA amplification, the volume of the reaction mixture was 25 μl which contained 0.5 μl of the reverse transcriptase reaction products, 1 unit of TaKaRa Ex Taq polymerase, 1 x Ex Taq buffer, 0.2 mM of each dNTP mixture, and 0.2 μM of each primer. The cyclophilin primers were used to control for variation in RT-PCR efficiency. PCR was initially performed by several cycles ranged, and the data in the logarithmic phases were adopted. The relative amounts of RT-PCR products for GLUT1 and GLUT3 were then quantified by standardizing with PCR product of CP using Scion Images software (Scion Corporation, USA).

Statistical analysis: All data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test as a post hoc test for comparison of means. Significance was set at $p < 0.05$.

Results and Discussion

The weights of the fetuses and the placentas were measured on GD16 and GD20. No significant differences were observed in fetal or placental weight between control and TCDD-exposed groups both on GD16 and GD20. In contrast, histological changes were observed in placenta. On GD16 the glycogen cells occupied the majority of the JZ in the placentas, and the area occupied by the glycogen cells was reduced by GD20 in the control rats. On GD20 in both 800 ng and 1600 ng TCDD/kg groups, a larger area occupied by the glycogen cells and cysts filled with eosinophilic materials surrounded by glycogen cells (GC-EM) were seen compared with the control group, suggesting the alteration of glycogen metabolism in TCDD-exposed placenta. The glycogen assay revealed that average glycogen concentration of the placentas on GD20 from the control rats, 800 ng/kg rats, and 1600 ng/kg rats was 196 ± 26 μg/g tissue, 274 ± 27 μg/g tissue, and 307 ± 30 μg/g tissue, respectively.

The glycogen content in placenta was partially dependent on the amount of glucose transported from the maternal circulation. In order to examine the implication of glucose transporter with glycogen content in placenta, the expression of glucose transporters in the placentas was analyzed by semiquantitative RT-PCR. The GLUT1 and GLUT3 proteins are predominantly expressed in rat placenta, and the both proteins are known to be key enzymes in placental glucose transport (5). The GLUT1 mRNA levels of placentas on GD16 were not changed between control and TCDD-exposed groups, and on GD20 marginally increase of GLUT1 mRNA level was observed only in 1600 ng/kg group. The GLUT3 mRNA level was not changed on

GD16 either whereas the expression of GLUT3 mRNA was increased approximately 2-fold in both 800 and 1600 ng/kg groups compared to the control group on GD20.

The GLUT3 has been suggested to play an important role in transporting the glucose from maternal circulation into trophectoderm cells (6). Although the function of glycogen cell is still unclear, it exists presumably to supply the glucose to fetus in order to sustain remarkable growth of the fetus in late pregnancy. In the present study, GLUT3 gene expression was up-regulated by TCDD, which might increase the glucose uptake into placenta from maternal circulation. This probably caused an oversupply of glucose to fetus despite the necessity of glucose supply to fetus from placental glycogen, which then resulted in the increase of glycogen content in the placenta.

The present study showed histological changes of glycogen cells, increase of glycogen content, and up-regulation of GLUT3 mRNA level in the placenta after exposure to a relatively low dose of TCDD. To our knowledge, this is the first report indicating the alteration of placental glucose kinetics by TCDD exposure.

References

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