IMMATURE ANGIOGENESIS IN THE RAT PLACENTAL LABYRINTH AFTER EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN

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Introduction

Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) during pregnancy causes fetal death in many animal species. The placenta, mainly composed of fetal endothelial cells and trophoblast cells which directly attach to maternal blood, plays an important role to supply nutrients and oxygen to developing fetuses. Normal blood vessel formation in the placenta, therefore, is one of the important aspects for fetal survival in the uterus.

In our earlier works we found that TCDD exposure at an oral dose of 1.6 μg TCDD/kg to pregnant Holtzman rats caused hypoxic state in their placenta[1] and an alteration of placental glucose kinetics including abnormal retardation of glycogen cells, increased expression of glucose transporter 3 (GLUT3) mRNA level, and increased glycogen level[2]. Similar to the TCDD-exposed rats, alteration of glucose kinetics and hypoxia in placentas have been reported in other disease models including maternal diabetes[3-5] and alcoholism[6] during pregnancy, all of which are known to increase the risk of fetal death or growth retardation. Thus, it is likely that there are common underlying mechanisms which eventually lead to placental hypoxia and alteration glucose kinetics. Although a decrease in blood flow in the placenta may account for etiology of fetal death or growth retardation in diabetic animals[5], there is little information about histological observations or genes associated with reduced blood flow in the placenta.

Blood vessels are constructed by two processes: the first process is vasculogenesis, whereby a primitive vascular network is established during embryogenesis, and the second one is vessel maturation/angiogenesis, which includes sprouting of preexisting vessels, morphogenic differentiation, and remodeling of endothelial cells[7,8]. Although many studies using knockout animals have shown that abnormal vasculogenesis of placenta was a primary cause for intrauterine death[9], little information is available about the maturation process of placental blood vessels.

In the present study, we observed the histology of the labyrinth zone of TCDD-exposed placenta by preparing a horizontal section and comparing them with vehicle-treated normal placentas. In addition, the expression of genes involved in vasculogenesis and angiogenesis were also compared. The present results, for the first time, showed that inhibition of vascular maturation occurred in the placentas of TCDD-exposed rats.

Methods and Materials

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 gestational days (GD) 15. The placentas were collected on GD20 (n=4 pregnant rats each for control and TCDD groups). Some placentas were immediately frozen in liquid nitrogen, and kept at –80°C until analyzed. The rest of the placentas were fixed in HistoChoice (Amresco, Solon, OH, USA) and embedded in paraffin.

Histological observations: Horizontal sections of placenta were prepared for on silane-coated slides to perform two kinds of staining. One preparation was applied for hematoxylin and eosin (H&E) staining, and the other for endothelial staining by using BS-1 lectin that identifies the fetal capillaries as described previously[10]. Briefly, sections (5-μm in thickness) were incubated with 0.3% H_2O_2 for 10 min to remove endogenous peroxidases, followed by treatment with 0.1% tyrpsin in 1 mg/ml $CaCl_2$, 0.1 M Tris-HCl pH 7.8 for 10 min. Non-specific binding was blocked with 1% BSA in PBS for 10 min, and avidin and biotin each for 15 min. Sections were incubated with 100 mg/ml biotin-labeled BS-1 lectin (Sigma, St Louis, MO, USA) for 1 h followed by incubation with streptavidin (1:400 dilution) conjugated with horseradish peroxidase for 1 h. Specific binding was detected using diaminobenzidine (DAB) solution.

Image analysis of maternal blood sinusoids (MBS) and fetal capillaries (FC): Images of MBS and FC were constructed by scanning a cross-section with an Olympus microscope connected to a digital camera (Tokyo, Japan), and manually processed on a computer. Morphometric measurement was performed by Scion Image (Scion Corporation, Frederick, MD, USA).

Semiquantitative RT-PCR: Expression of genes associated with blood vessel formation was analyzed by the semiquantitative RT-PCR method as described previously[1].

Results and Discussion

The histology of placental labyrinth zone from TCDD-exposed and vehicle-treated control rats was examined on horizontal sections. It was observed that TCDD exposure resulted in the enlargement of the cytoplasm of trophoblast giant cells (TGC), and made the trophoblastic layers of the interhemal membrane thicker than the vehicle treatment. As MBS and FC are tangled to each other in the labyrinth zone, immunohistochemical staining by using BS-1 lectin, that makes the identification of the FC more easily, was perfomed. TCDD-exposed placental specimens were found to show reductions in size and subdivision of MBS as well as undeveloped structure of FC compared to the control placenta. Morphometric measurement revealed that the average cross-sectional area of MBS and FC was reduced in TCDD-exposed placenta compared to control placenta. These results indicated that the TCDD-exposed placenta was reduced in size of both MBS and FC due to the expanded cell mass of TGC. This is the first histological evidence to support the hypoxic state of placenta and fetus.

Development of MBS and FC in the vehicle-treated control placenta from GD16 to GD20 was examined histochemically. On GD16, FC was not expanded but increased gradually in size until

GD20. The MBS occupied very limited area in the placenta on GD16, but it gradually expanded in size and was the majority area of placenta, which was shown by horizontal-sectional staining. The cell size of TGC including trophoblastic layer of the interhemal membrane was large on GD16, but reduced and attenuated as pregnancy proceeded. These results revealed that the maturation of both MBS and FC occurred in the labyrinth zone of placenta by expanding their size, which was concomitant with the reducing the cell size of TGC from GD16 to GD20. Taken together, histological study revealed that maturation of both MBS and FC in placenta was inhibited by exposure to TCDD.

We next examined genes associated with blood vessel formation in placentas by using semiquantitative RT-PCR. Two main systems, the vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) system and angiopoietin (ANG)/Tie2 system, are known to exist in embryonic vascular development[7,8]. The VEGF/VEGFR system and angiopoietin/Tie2 system are associated with vasculogenesis and vascular maturation, respectively. The expression of VEGF, FLK1, and FLT1 mRNAs which are involved in VEGF/VEGFR system was not different between control and TCDD-exposed placentas. In contrast, the expression of Tie2 mRNA which is involved in ANG/Tie2 system was decreased in TCDD-exposed placenta compared to control placenta. The expression of Tie2 mRNA in the vehicle-treated control placenta was increased as pregnancy proceeded from GD16 to GD20. Thus, these results suggested the possibility that TCDD inhibited the vascular maturation, which resulted in the decreased expression of Tie2 mRNA.

In the present study, vascular maturation of both MBS and FC were observed to be suppressed at histological level, the finding of which was further supported by a decreased expression of Tie2 mRNA. Taken together, the both results on histology and gene expression may allow us to present a novel hypothesis that TCDD disrupts vascular maturation rather than vasculogenesis in the placenta, which might increase the risk of fetal death.

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