ENHANCED EXPESSION OF COX2 AND CYP1A1 IN EARLY ACTIVE RENAL LESIONS DEVELOPED IN OFFSPRING OF RHESUS MONKEYS WITH EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) DURING GESTATION AND LACTATION

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Introduction

Prenatal exposure to a high dose of TCDD is known to induce hydronephrosis in the mouse[1]. Considering the pronounced difference between species observed in some previous studies, we investigated the effects of low dose of TCDD on development of the kidney in non-human primate after subcutaneous administration of TCDD into rhesus monkeys during pregnancy and lactation. In the previous report, we have for the first time described developmental abnormality with renal dysplasia develop in offspring of rhesus monkeys exposed during prenatal and lactational period to TCDD[2]. The renal lesions developed exclusively in offspring of dams exposed to relatively high dose (300ng/kg) of TCDD. However, the pathogenesis of the renal lesion is not clear. In the present study, we evaluated abnormalities of rennin-angiotensin system and cox2-prostaglandin system in the renal lesions of rhesus monkey offspring.

Materials and Methods

TCDD was purchased from Wellington Laboratories Inc., Guelph, Ontario, Canada) and was dissolved in a mixture of toluene/dimethyl sulfoxide (DMSO;1:2, v/v) at Kanto Kagaku Co., Ltd. (Tokyo, Japan). Final concentrations were confirmed by gas chromatography. Colony bred adult female rhesus monkeys (age, 3-10 years; weight, 4-7kg) were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). TCDD (0, 30, 300ng/kg of body weight) was subcutaneously administrated to pregnant female monkeys on gestation day 20 (GD20), followed by injection with 5% of the initial dose every 30 days during pregnancy and lactation until GD90. Offspring that died at or after birth and weaning or survived until 7 year old were examined pathologically. Immunohistochemical staining was performed on paraffin-embedded renal tissues using MAX-PO kit (Nichirei, Tokyo) or ENVISION kit (Dako Cytomation, Glostrup, Denmark) and

anti-vimentin (Dako), anti-alpha-smooth muscle actin (SMA)(Dako), anti-lymphocyte common antigen (LCA)(Dako), anti-renin (R&D Systems, Inc.), anti-CD10 (Novocastra), anti-cox2(Assaybio Tech), anti-cyp1A1(Santa Cruz), and anti-HSP70(Santa Cruz) antibodies. In addition, global gene expression was also conducted by using total RNA and gene chips (Human Expression Chip, Takara Bio).

Results and Discussion

Numbers of dams in each group (0, 30, 300ng/kg) were 23, 20, and 20, respectively. Numbers of abortions and stillbirths of offspring in each group were 5, 5, and , respectively; live births, 18, 15, and 16; postnatal deaths, 8, 6, and 10. Numbers of pathologically examined offspring in each group were 16, 13, and 17, respectively, including stillbirth and postnatal death cases (6, 4, 11, respectively), which made possible to examine early active stage of renal abnormalities, and 7 year old cases ((10, 9, 6, respectively), which made possible to evaluate the renal lesion after long-term follow-up. Renal lesions were found exclusively in 11 (65%) (6, early; 5, follow-up) of 17 offspring of dams exposed to relatively high dose of TCDD (300ng/kg). No remarkable histological abnormalities were detected in the kidneys of 16 and 13 offspring of controls and dams exposed to relatively low dose (30ng/kg) of TCDD.

Renal lesions in early postnatal death cases included tubulo-interstitial type with fibrosis (diffuse and severe form). Severe type of the renal lesions showed renal interstitial and peripelvic fibrosis with or without atrophic papilla. Tubular and glomerular dysgenesis was also indicated. An immunohistochemical study revealed predominant proliferation of vimentin-positive fibroblasts, not SMA-positive myofiboblast, in these lesions. LCA-posive lymphocyte infiltration was minimal. Renal lesions in 7 years old follow-up case showed mild and localized form of glomerulo-tubular dysplasia with glomerulosclerosis in the subcapsular region indicating predominant decrease of proximal renal tubules and increase of immature glomeruli with ectopic rennin-positive cells and SMA-positive cells. Ectopic renin-positive cells were localized only in affected glomeruli and arterioles of renal lesions and it suggested a secondary change following renal abnormal development.

Enhanced expression of cox2 and cyp1A1 proteins was evident in the juxtaglomerular apparatus and endothelial cells of arteries and veins in the kidneys of early postnatal death cases with renal lesions while no expression of both proteins was found in the renal vascular endothelial cells of all 7 year old cases with or without renal lesions as well as early death cases without renal lesions.

These results suggested that cox2 protein might play a role in pathogenesis of renal lesions in this primate model as indicated in rat model[3,4]. However, severe renal fibrosis found in offspring of rhesus monkey in the present study might be a secondary change following abnormal renal differentiation with dysgenesis or loss of nephrons because it is known that TCDD induces hydronephrosis without severe fibrosis in offspring of mice and, in addition, both renal dysgenesis and hydronephrosis without severe fibrosis was evident in kidneys of transgenic mouse models with developmental anomaly[5-7].

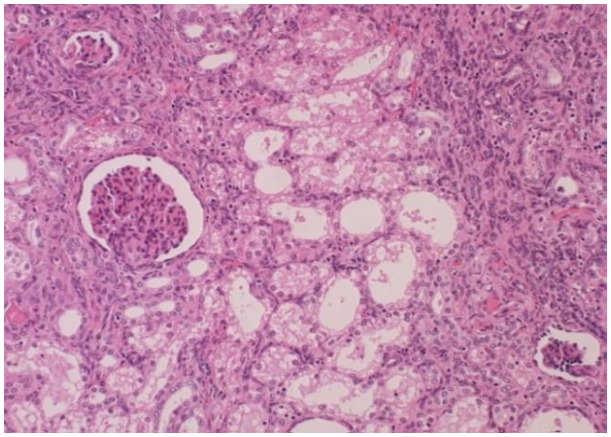

Figure 1. Glomerular dysgenesis and interstitial fibrosis with destruction or loss of renal tubules in the kidney of rhesus monkey offspring. HE stain.

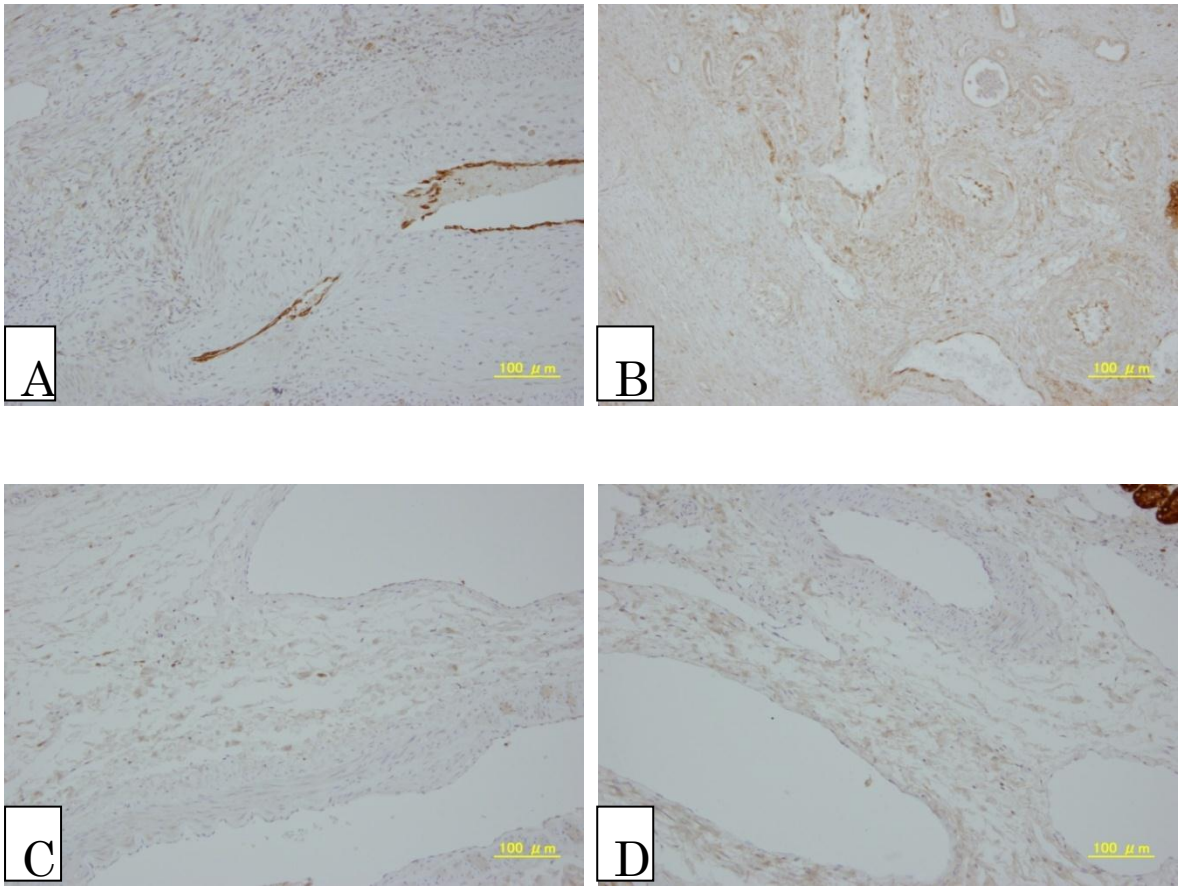

Figure 2. Enhanced expression of cox2 and cyp1a1 protein in vascular endothelial cells of the kidney with renal dysplasia developed in offspring died during postnatal period. Immunohistochemically, arteial endothelial cells in the kidney with active severe lesion are positive for cyp1A1 (A) and cox2 (B) while vascular endothelial cells in the kidney of 7 years old offspring are negative for both cyp1A1 (C) and cox2 (D).

Gene profiling analysis for renal lesions of 7 years old offspring revealed that 273 genes including osteopontin and uroplakin were up-regulated and 377 genes including kallikrein 1, podocin, Wilms tumor 1 were down-regulated, indicating renal tubular damages in renal lesions. However, significant upregulation or down-regulation of associated genes with cox2-angiotensin2 system was not indicated in this group.

In conclusions, these results indicate enhanced expression of cox2 protein in renal lesions with dysplasa developed in rhesus monkey offspring exposed during prenatal and lactational period to TCDD, suggesting the etiological association between sox2-angiotensin system and renal lesion. The renal lesions developed exclusively in offspring of dams exposed to relatively high dose (300ng/kg) of TCDD.

Acknowledgements

This study is supported by Health Science Research Grants for Research on Environmental Health from the Ministry of Health, Welfare and Labor of Japan.

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