GESTATIONAL AND LACTATIONAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) INDUCES ABNORMAL DEVELOPMENT OF THE KIDNEY IN OFFSPRING OF RHESUS MONKEYS

Masato Watanabe[1], Shunichiro Kubota[2], Akihiro Arima[3], Atsunobu Muneoka[3], Hiroshi Sumida[4], Mineo Yasuda[4], Toshio Fukusato[1]

[1]Department of Pathology, Teikyo University School of Medicine, 2-11-1, kaga, Itabashi-ku, Tokyo, Japan, [2]Department of Life Sciences, Graduate school of Arts and Sciences, The University of Tokyo, Tokyo, Japan, [3]Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan, [4]Departments of Clinical Radiology, Faculty of Health Science, Hiroshima International University, Horoshima, Japan

Introduction

Renal involvement by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity has been reported in rodents, horses, and cats[1]. Prenatal exposure to a high dose of TCDD is known to induce hydronephrosis in the mouse[2]. 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 first described a peculiar form of renal interstitial and peripelvic fibrosis with nephron dysgenesis developed in offspring of rhesus monkeys exposed during prenatal and lactational period to TCDD[3]. The renal lesions developed exclusively in offspring of dams exposed to relatively high dose (300ng/kg) of TCDD. In the present study, we disclosed the spectrum and long-term persistence of abnormal development with tubular and glomerular dysgenesis in the kidney 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 kitt (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) antibodies. Electron microscopic observation and molecular analysis of renal tissues was also done. In addition, global gene expression was also conducted by using total RNA and gene chips (Human Expression Chip, Takara Bio). MicroRNA levels in the renal cortex were analyzed by quantitative RT-PCR (miScrip primer Assay, miScrip SYBR Green PCR Kit, QIAGEN).

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 4; 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. Renal lesions were found exclusively in 11 (65%) 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 included three subtypes: tubulo-interstitial type with fibrosis (diffuse and severe form), 4; glomerulo-tubular type with glomerulosclerosis (localized and mild form), 4; and mixed intermediate type, 3. Severe type of the renal lesions showed renal interstitial and peripelvic fibrosis with or without atrophic papilla (Figure 1). The renal lesion was associated with renal function failure which was the cause of death in two

offspring. Tubular and glomerular dysgenesis was also indicated. Electron microscopic observation showed interstitial collagen deposition and loss of renal tubules. An immunohistochemical study revealed predominant proliferation of vimentin-positive fibroblasts, not SMA-positive myofiboblast, in these lesions. LCA-posive lymphocyte infiltration was minimal. Mild type of renal lesions localized in the subcapsular region showed predominant decrease of proximal tubules and increase of immature glomeruli with ectopic rennin-positive cells and SMA-positive cells (Figure 2). This type of renal lesions was indicated in offspring that survived until 7 year old.

The spectrum of abnormal development shown in the present study of rhesus monkey offspring might be equivalent to abnormal renal differentiation including renal dysplasia and renal dysgenesis. However , the renal lesions appeared to be different from renal tubular dysgenesis because they were not always in the diffuse distribution but rather often seen as the localized form.

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[4-6].

Gene profiling analysis revealed that 273 genes including osteopontin and uroplakin were up-regulated and 377 genes including kallikrein 1, podocin, and Wilms tumor 1 were down-regulated, indicating renal tubular damages in renal lesions. MicroRNA analysis demonstrated increased levels of mir-141, 192, 200c in renal cortical tissues of the male offspring.

In conclusions, this is the first report that describes the spectrum and long term persistence of abnormal development with tubular and glomerular dysgenesis in the kidney of rhesus monkey offspring exposed during prenatal and lactational period to TCDD. The renal lesions developed exclusively in offspring of dams exposed to relatively high dose (300ng/kg) of TCDD.

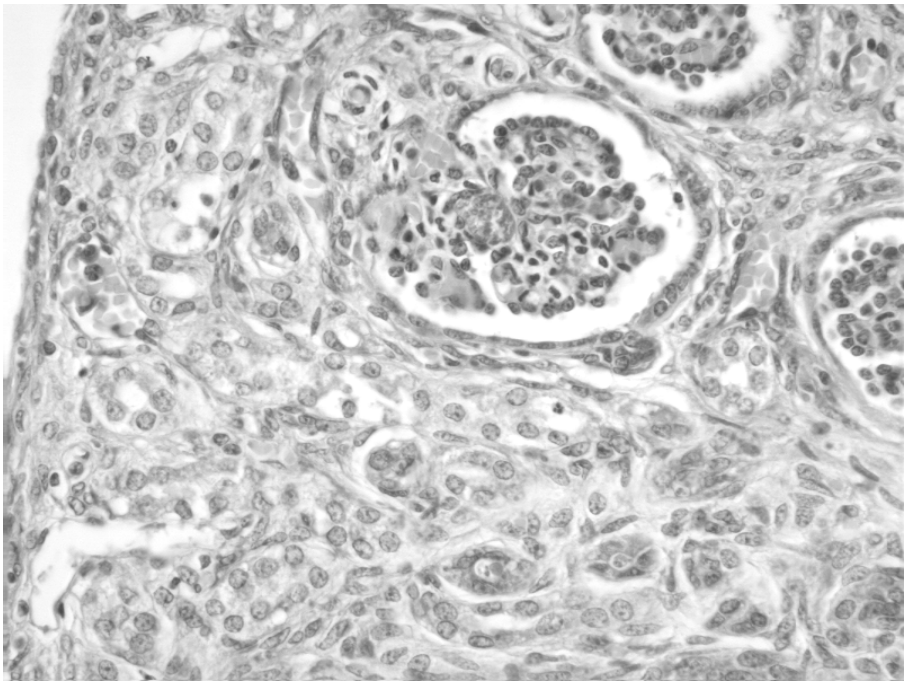

Figure 1. Interstitial fibrosis with destruction or loss of renal tubules in the kidney of rhesus monkey offspring involving the extensive or entire area of renal cortex (hematoxylin-eosin stain).

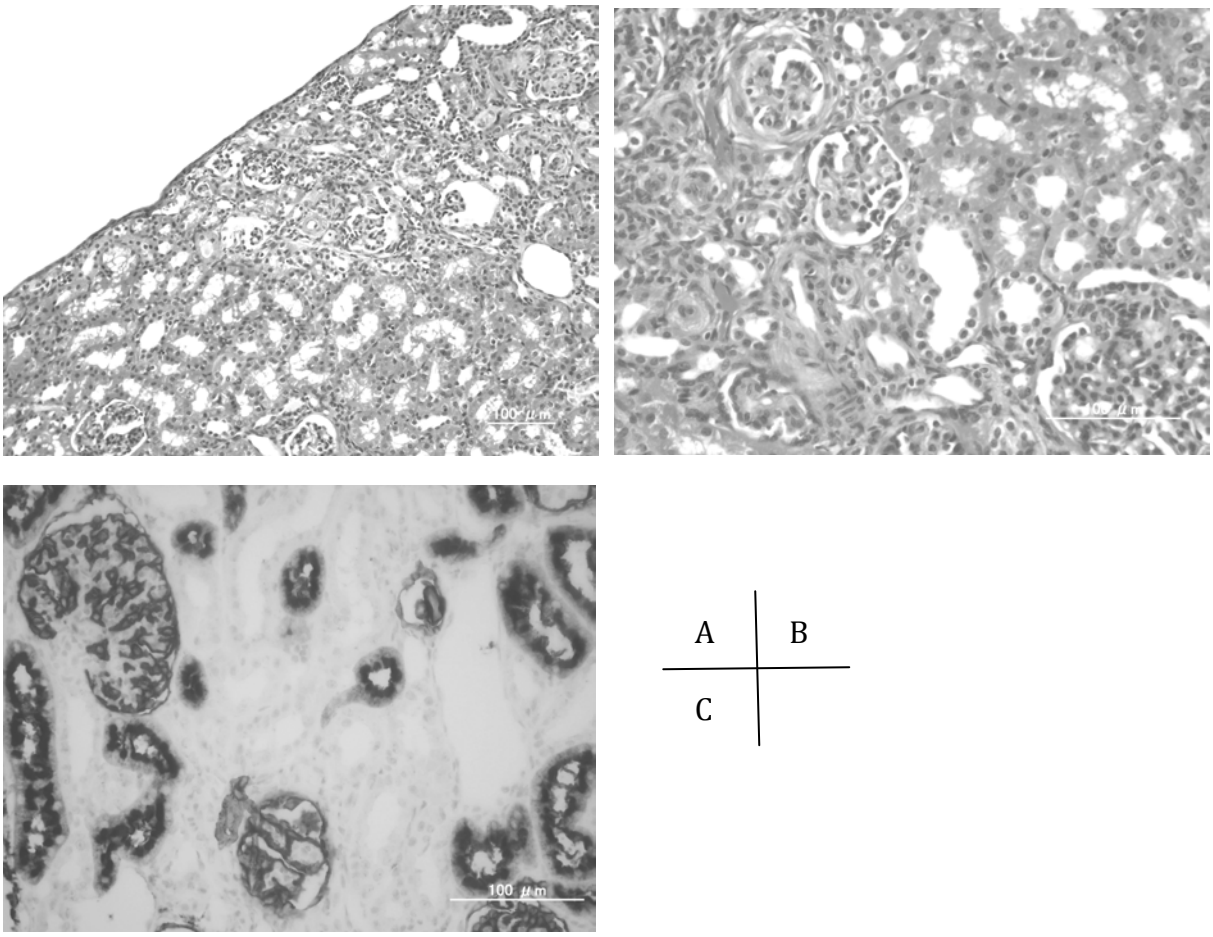

Figure 2. Localied lesion with proximal tubular loss and immature glomeruli. Renal cortical lesions with tubular and glomerular dysgenesis were localized in subcapsular region (A, B, hematoxylin-eosin stain). CD10 immunostain indicated decreased number of proximal tubules (C).

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