RENAL FIBROSIS INDUCED BY IN UTERO AND LACTATIONAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN RHESUS MONKEYS

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) might induce the proliferation of the interstitial tissues. A recent study showed that low dose administration of TCDD induced myocardial fibrosis in marmosets by short range exposure[1]. In fibrotic myocardium, it was confirmed that proliferated components were not only collagen, but also fibronectin and laminin. Furthermore, TCDD induces endometriosis in the female monkey at a daily dose of 0.15 ng/kg/day for 4 years[2]. The effects of TCDD on proliferation of the extracellular matrix are possibly related to the increasing activation of growth factors such as TGF-β1and its receptor[1]. On the other hand, effects of TCDD on proliferation of the interstitial tissues of the offspring have not been well confirmed. It has been reported that TCDD alters extracellular matrix development in the kidney in the mouse fetus, when TCDD was administered to the pregnant mother[3]. In this report, however, decreasing of components of the extracellular matrix, such as fibronectin and collagen type IV, was described. It was concluded that the decreasing of the extracellular matrix results ultrastructural maldevelopments of the kidney. Recently, we observed renal dysgenesis in two postnatally dead young from mothers exposed to TCDD during the pregnancy and lactational period. In these two cases, renal corpuscles were mostly hypoplastic, and the interstitial tissues seem proliferated. These two cases were obtained by exposure to a high TCDD level, and these young died at the age of 406 days and 422 days. In the present study, we report that these two cases had renal fibrosis induced by TCDD exposure during the pregnancy and lactation, and show collagen proliferation in the kidney with picrosirius red staining.

Materials and Methods

Animals and treatment. Adult rhesus monkeys were mated, and females with confirmation of pregnancy by ultrasonography were given TCDD subcutaneously on day 20 of gestation at an initial dose level of 30 or 300 ng/kg. Controls received the vehicle. The lower dose level was set at about one third of the LOAEL body burden in rodents, and the higher one at about three times of the LOAEL. For maintenance of a certain body burden, 5% of the initial dose was given to dams every 30 days during pregnancy and lactation until 90 days after delivery. As of

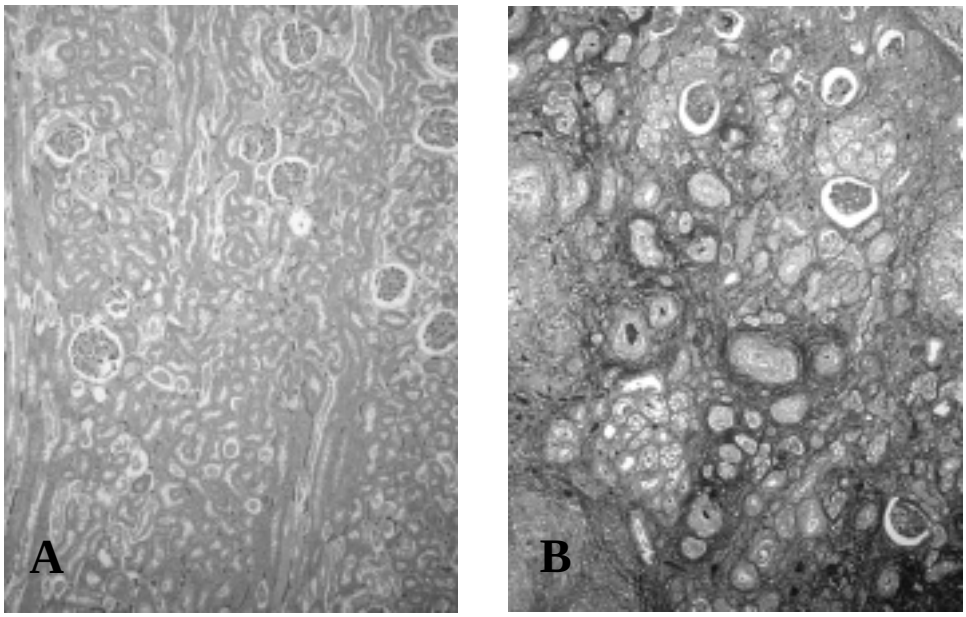

Figure 1. A: The kidney without dysgenesis. The tissue was not stained with picrosirius red. B: The kidney with dysgenesis. The renal corpuscle and tubules were very hypoplastic. The area of renal columns was fibrotic and intensely stained with picrosirius red.

March 2003, data on approximately 20 young in each group were obtained. Young that died postnatal were autopsied, and main organs were histopathologically examined.

Picrosirius red staining. The kidney was fixed in 10% buffered formalin, and embedded in paraffin. Thin sections were stained with 0.1% picrosirius red as a specific dye for collagen. Sections were photographed under a light microscope as digital images with a digital camera (Olympus C-4040).

Results and Discussion

Two cases of renal dysgenesis were detected in dead young from the 300 ng/kg group. These young died 406 days and 422 days after birth. Eight young died by 468 days after birth, thus, renal dysgenesis was seen in 25 % of dead young.

The kidney without dysgenesis showed almost no staining with picrosirius red (Figure 1, A). In contrast, the kidney with dysgenesis was fibrotic and then intensely stained with picrosirius red (Figure 1, B). In the dysgenetic kidney, the renal corpuscles and tubules developed hypoplastic. Collagen fibers surrounding vascular or the adventitia seemed proliferated, and invaded into the interstitial tissue. Lumen of some vascular was closured by proliferation of the intima (Figure 1,

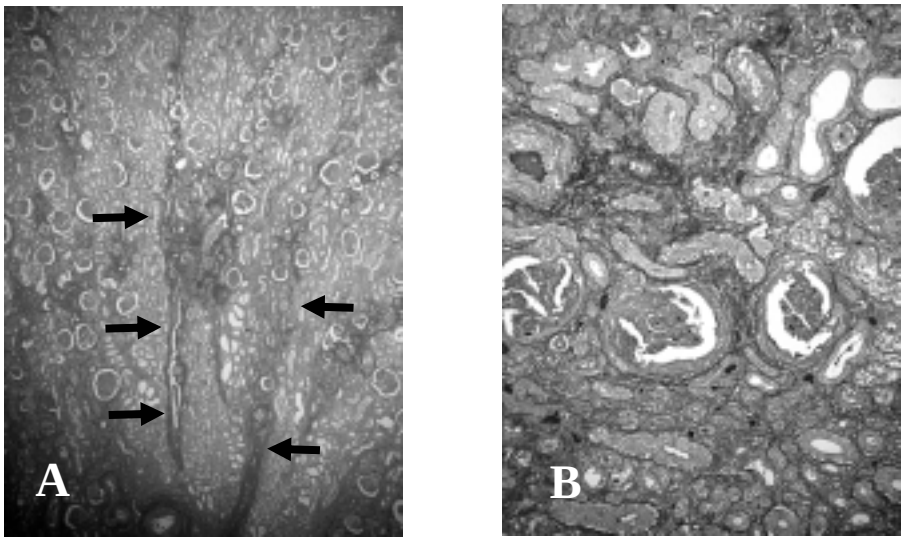

Figure 2. A: Low power microphotograph of fibrotic kidney. The renal columns (arroes) were staind with picrosirius red. Most of the renal corpuscles and tubules were not stained. B: High power microphotograph of fibrotic kidney. Some renal tubules was surrounded by collagen fibers. The wall of renal corpuscles was thickened.

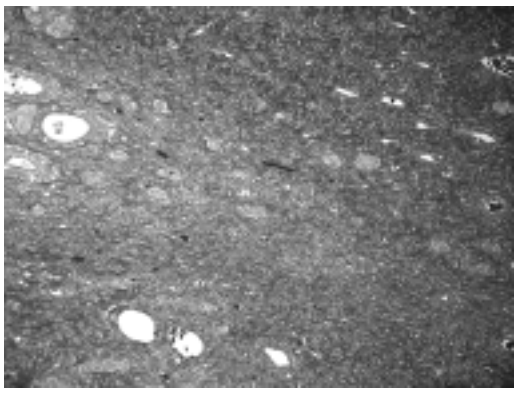

Figure 3. Sever fibrosis in the kidney. In the fibrotic area, most of renal corpuscles and tubules disappeared due to collagen fiber proliferation.

B).

At low power, the staining with picrosirius red was seen in the renal columns (Figure 2, A). Surrounding tissues of most of the renal corpuscles and tubules were not stained intensely. At

high power, however, it was recognized that collagen fibers surrounded some renal corpuscles and tubules (Figure 2, B). Remarkably, the wall of the renal corpuscles was thickened (Figure 2, B).

One kidney was highly fibrotic (Figure 3). In the fibrotic area, most of the renal corpuscles and tubules disappered, and collagen fibers markedly proliferated.

A previous immunohistochemical study[3] showed decreasing of collagen type IV in the fetal kidney from mouse dams exposed to TCDD. In the present study, however, proliferation of collagen in the kidney was clearly recognized. Furthermore, lumen of some vessels in the kidney was obliterated by proliferation of the intima. Further studies are needed whether TCDD induces atherosclerosis.

The effects of TCDD on collagen proliferation in the kidney are possibly linked to the effects of transforming growth factor-β1 (TGF-β1). This factor is known to induce fibrosis in various organs[4]. TGF-β1 has been recognized to induce marked fibrosis in the kidney[4]. Indeed, TGF-β1 was increased in marmosets with myocardial fibrosis by TCDD[1]. Clarification of relations between TCDD and increasing of growth factors is expected.

Acknowledgements

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