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Histopathological characterization of renal tubular and interstitial changes in 5/6 nephrectomized marmoset monkeys (*Callithrix jacchus*)

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ABSTRACT

Common marmosets (*Callithrix jacchus*) have become a useful animal model, particularly for development of biopharmaceuticals. While various renal failure models have been established in rodents, there is currently no acceptable model in marmosets. We analyzed the damaged renal tubules and tubulointerstitial changes (inflammation and fibrosis) of 5/6 nephrectomized (Nx) common marmosets by histopathological/immunohistochemical methods, and compared these findings to those in 5/6 Nx SD rats. In Nx marmosets and rats sacrificed at 5 and 13 weeks after Nx, variously dilated and atrophied renal tubules were seen in the cortex in common; however, the epithelial proliferating activity was much less in Nx marmosets. Furthermore, the degrees of inflammation and fibrosis seen in the affected cortex were more severe and massive in Nx marmosets with time-dependent increase. Interestingly, inflammation in Nx marmosets, of which degree was less in Nx rats, consisted of a large number of CD3-positive T cells and CD20-positive B cells (occasionally forming follicles), and a few CD68-positive macrophages. Based on these findings, lymphocytes might contribute to the progressive renal lesions in Nx marmosets. Fibrotic areas in Nx marmosets comprised myofibroblasts expressing vimentin and α-smooth muscle actin (α-SMA), whereas along with vimentin and α-SMA expressions, desmin was expressed in myofibroblasts in Nx rats. This study shows that there are some differences in renal lesions induced by Nx between marmosets and rats, which would provide useful, base-line information for pharmacology and toxicology studies using Nx marmosets.

1. Introduction

Chronic kidney disease (CKD) is emerging as a worldwide public health problem. Because the pathophysiology of CKD is very complicated, animal models suitable for CKD are needed, particularly for the sake of newly developing pharmaceuticals.

The development of biotechnology-derived pharmaceuticals (biopharmaceuticals) such as antibody-based and gene therapies could revolutionize the prevention and treatment of incurable diseases (Brennan et al., 2004). For pharmacological and safety studies on such biopharmaceuticals, non-human primates may become a useful animal model (Brennan et al., 2004); the primary reason is that in many cases, target proteins of antibody-based products are shared both by humans and primates. Therefore, pre-clinical studies using primates may be carried out with the great predictive value of pathophysiological roles of target proteins, leading to the significant extrapolation on the efficacy and toxicity of such products (Haley, 2003). With the increase of biopharmaceuticals, demand for disease models of non-human primates has increased (Thomas et al., 1987; Yoo et al., 1988).

A small New World monkey, the common marmoset (*Callithrix jacchus*), has recently been focused on as a pre-clinical animal model. Because of the small size (200–500 g), availability and relatively rapid generational turnover, the marmoset is considered to be suitable for biomedical researches (Zühlke and Weinbauer, 2003).

Abbreviations: α-SMA, α-smooth muscle actin; CKD, chronic kidney disease; EMT, epithelial to mesenchymal transition; H&E, hematoxylin and eosin; Nx, nephrectomized; PCNA, proliferation cell nuclear antigen; TGF-β1, transforming growth factor-β1; VA cells, cells co-expressing of vimentin and α-SMA; VAD cells, cells co-expressing of vimentin, desmin and α-SMA; VD cells, cells co-expressing of vimentin and desmin.

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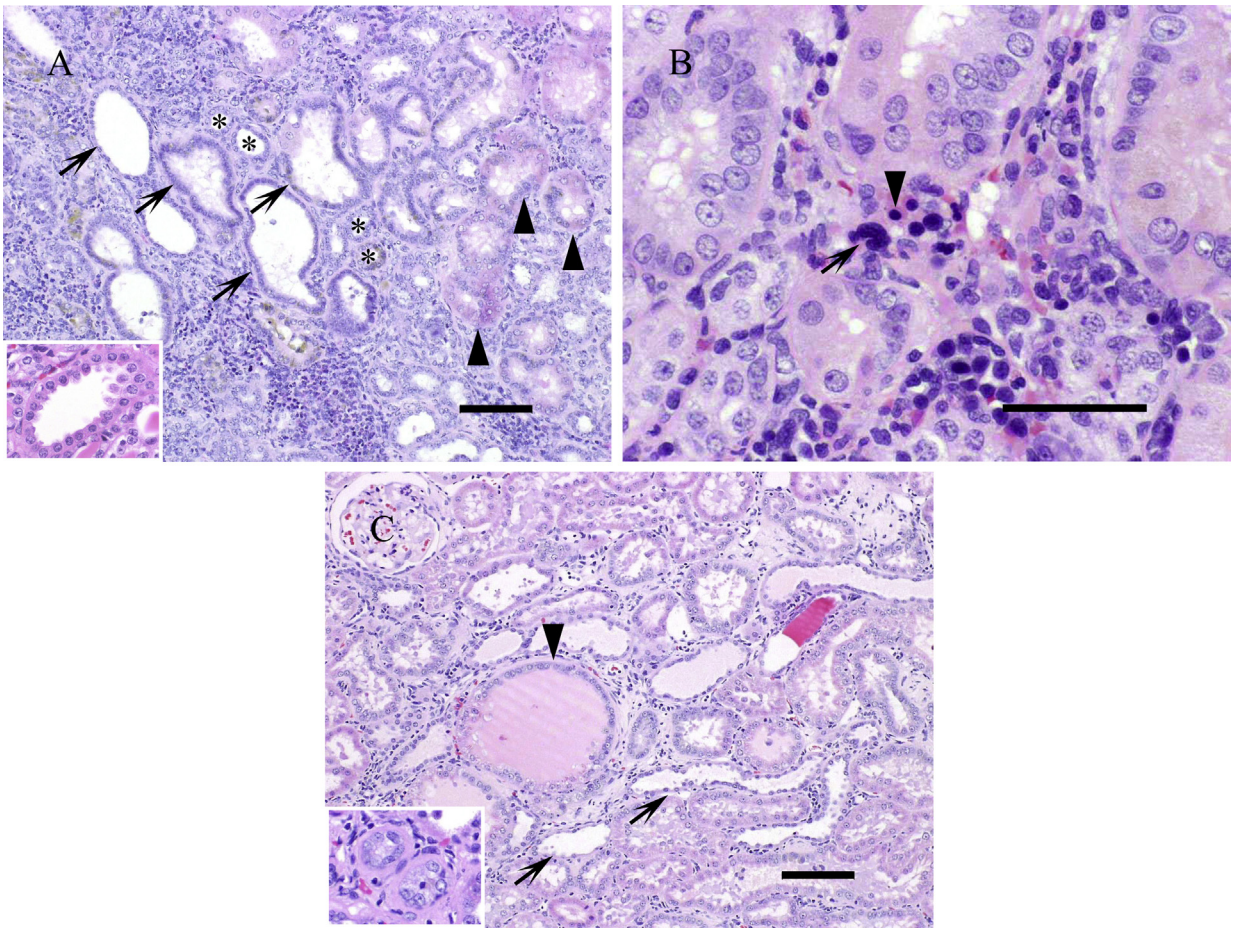

Fig. 1. Histopathological findings. (A) A marmoset, 13 weeks after Nx. Various kinds of renal tubular changes are seen in the area with inflammatory reaction; atrophic (asterisks), dilated tubules with basophilic epithelia (arrows), and tubules with single or multilayered, eosinophilic, tall cuboidal to columnar epithelia (arrow heads and insert). H&E. Bar = 100 μm. (B) A marmoset, 13 weeks after Nx. Extramedullary hematopoiesis consisting of myelocytes (arrow) and erythroblasts (arrow head) is observed. H&E. Bar = 50 μm. (C) A rat, 13 weeks after Nx. The renal tubular damages are seen; dilated tubules with flattened to cuboidal epithelia (arrows) and atrophic tubules (insert). Some of affected tubules contain hyaline casts in the lumen (arrow head). H&E. Bar = 100 μm.

We have attempted to develop a CKD model using common marmosets. In order to establish the CKD model, in the present study, we employed 5/6 nephrectomized (Nx) model, because this Nx model has been widely used for studying CKD by using various experimental animals, particularly rats (Kliem et al., 1996). Although Nx CKD models can induce the dysfunction of renal tissues, it is reported that there are differences in levels of renal dysfunction between animal strains or species; it may imply the different resistance to renal mass reduction (Bourgiognie and Ghraoui, 1992; Gross et al., 2004; Yamate et al., 2005; Fleck et al., 2006).

By using Nx marmosets, previously, we reported glomerular lesions, in comparison with those in Nx rats (Suzuki et al., 2013); interestingly, the glomerular lesions were weaker in Nx marmosets than in Nx rats; that was partly because the glomerular basement of the marmosets was thicker than that of the rats, presumably resulting in greater resistance to blood pressure in the glomeruli of the marmosets. Although proteinuria in the Nx marmosets was weaker than in the Nx rats reflecting less glomerular injury in the Nx marmosets, the Nx marmosets showed significant increases in several serum biochemical and urine parameters relating to renal failure in the same manner as seen in the Nx rats (Suzuki et al., 2013; Yamaguchi et al., 2014). Renal tubulointerstitial injury is the hallmark of human CKD patients (Risdon et al., 1968), and also important in Nx animals. Therefore, the present study is to analyze renal tubular and interstitial injury in the Nx marmosets in detail by the means of histopathological and immunohistochemical methods. By comparing those in the Nx rats, in addition, possible pathogenesis of renal tubulointerstitial injury in the Nx marmosets was investigated. This is the first study providing the base-line data for renal tubular damage and tubulointerstitial injury of the Nx marmosets.

2. Materials and methods

2.1. Animals and husbandry

Female marmosets and female Crl:CD(SD) rats were obtained from CLEA Japan, Inc. (Tokyo, Japan) and Charles River Co. (Shiga, Japan), respectively. Marmosets (aged 42–65 months) and rats (9 weeks old) were used. Each animal underwent a 5/6 surgical Nx in two steps or sham operation (rats only). Control marmosets did not receive sham operation, because of animal welfare; we have confirmed that sham-operation for Nx (only laparotomy) did not have any influences on serum biochemical and urine analyses, as well as renal histopathology; in fact, sham-operated rats in this study showed findings in serum biochemical, urinary and pathological analyses that are within background data (Suzuki et al., 2013). For Nx operation, briefly, the area around the proposed incision site was shaved and cleaned with an antiseptic solution (iodine and 70% alcohol solution). Under inhalational anesthesia