Tubular changes in obstructed kidney of adult mice evaluated using immunohistochemistry for segment-specific marker

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Summary. The main focus of the present investigation is to examine obstructed kidneys due to unilateral ureteral obstruction (UUO) model in adult mice using segment-specific tubular marker and to confirm the detailed morphological evaluation of UUO that is a typical model for the tubulointerstitial fibrosis which is an endpoint outcome of chronic renal diseases. Adult mice were subjected to UUO, and kidneys were harvested 1, 3, 7 days after surgical operation. Expansion of interstitial space both in the cortex and the medulla was confirmed 3 days after UUO by HE- and azan-staining. Interstitial fibrosis developed especially around dilated tubules. Immunohistochemistry for segment-specific antibodies revealed that the proximal tubules and the descending limb of Henle's loop did not dilate until 7 days after UUO, whereas initial dilation of the ascending limb of Henle's loop appeared to occur one day after surgery. The segment from the distal tubules to the collecting ducts began dilating one day after surgery and afterward significantly dilated. The downstream segment of nephron was involved in dilating earlier than the upstream of nephron in obstructed kidney examined in the present study. Moreover, the tubules accompanying apoptosis of tubular epithelial cells significantly dilated compared with those without apoptotic tubular epithelia. From the above-mentioned findings, we conclude that tubular dilatation of distal segment (from the ascending limb of Henle's loop to the collecting ducts) of nephron develops tubular epithelial apoptosis caused by accumulated urine, which would link to tubular disappearance and its replacement with fibrous tissue in UUO kidney of adult mice.

Key words: Unilateral ureteral obstruction, Tubular dilatation, Apoptosis, Fibrosis, Segment-specific tubular marker

Introduction

Tubulointerstitial fibrosis is often recognized as an endpoint outcome of a wide range of chronic renal diseases, regardless of the underlying pathogenesis. A number of studies have shown that deterioration of renal function is largely determined by the extent of tubulointerstitial alterations in many forms of renal diseases both in the experimental animal models and patients. There are several experimental models of tubulointerstitial fibrosis, for example, serum- (Steblay and Rudofsky, 1973), drug- (Shihab et al., 1996), proteinuria-induced nephritis (Eddy, 1989), and so on. Unilateral ureteral obstruction (UUO) had been used as one of such experimental animal models developing renal tubulointerstitial fibrosis (Klahr, 1998). To elucidate tubular dilatation or tubular atrophy in various tubular segments at varying time intervals following ureteral obstruction, we used UUO model in the present investigation. Regional tubular changes have been observed in the UUO model within 1 week of the onset of ureteral ligation (Klahr and Morrissey, 2002). In the UUO model, the obstructed kidney induces increased production of transforming growth factor-beta (TGF-beta) which leads to interstitial fibrosis. TGF-beta transduces signals downstream via novel cytoplasmic transcription factors called Smad proteins. Both TGF-beta and Smad seem to be a main signaling pathway in the pathogenesis of renal fibrosis caused by UUO (Klahr and Morrissey, 2002). The phenomena occurring in the UUO model are similar to those observed in patients with obstructed nephropathy which abolishes renal function rapidly. At a light-microscopic level, similar morphological changes appear to occur both in the obstructed nephropathy and the kidney treated by urinary tract obstruction. Although various aspects of pathophysiological and molecular events related to renal fibrosis were elicited by the ureteral obstruction, detailed morphological evaluation of the obstructed kidney had
not yet been performed in adult mice. Moreover, there are few reports discussing how morphological changes of UUO models cause renal fibrosis. In the present investigation we elucidate tubular dilatation and/or tubular atrophy in various tubular segments at varying time intervals following the ureteral obstruction in UUO model using immunohistochemistry for segment specific marker and compare obstructed kidneys with controls in sham-operated mice.

**Materials and methods**

**Animal model**

Male C57BL/6 mice weighing 20 to 25 g at 8 weeks of age, received water and standard mouse chow. The handling of animals conformed to the guidelines for care and use of experimental animals established by the Ethical Committee of Animal Experiments of Tsurumi University School of Dental Medicine. The unilateral ureteral obstruction (UUO) was performed using an established procedure (Chevalier et al., 1999). Briefly, under general anesthesia by diethyl ether, complete ureteral obstruction was performed by double-ligating the left ureter using 3-0 silk after a left lateral abdominal incision. Mice were sacrificed 1 day, 3 days, and 7 days (n = 6 mice per group) after the obstruction of a left ureter, respectively. UUO day 1, UUO day 3, and UUO day 7 kidneys were defined as the kidney harvested 1 day, 3 days, and 7 days after the ureteral ligation, respectively. Mice were transcardially perfused with 0.1M phosphate-buffered saline (PBS) followed by the fixative solution containing 4% paraformaldehyde and 15% saturated picric acid for hematoxylin and eosin (HE) staining, azan-staining, and immunohistochemistry for segment-specific tubular markers. The left kidney (UUO kidney) was harvested, cut, and immersed in the same fixative solution for 4-6 h at 4°C. For paraffin embedding, one part of the kidney was dehydrated and embedded in paraffin. Serial paraffin sections were cut in 5 micrometer-thickness and maintained at room temperature on slides coated with gelatin until submitting to various types of staining. For cryostat sections, another part of kidney was immersed with 15% sucrose in 0.1M phosphate buffer (PB: pH 7.4) for 6 h and then 30% sucrose in PB for 6 h, respectively, at 4°C after fixation. Finally specimens were frozen in O.C.T. compound (Sakura Finetechnical, Tokyo). Serial cryostat sections were cut in 6 micrometer-thickness and maintained at -20°C on slides coated with gelatin until using for immunohistochemistry. We also performed sham-operations in mice by left lateral abdominal incision and exposing the left ureter without ligation. As a control of UUO day 1 kidney, we used kidneys from mice 1 day after the sham-operation (Sham day 1 kidney). Similarly, we used Sham day 3, Sham day 7 kidney as the control of UUO day 3, UUO day 7 kidney, respectively.

**Immunohistochemistry**

The primary antibodies included (1) anti-aquaporin-1 (AQP1) antibody (Chemicon, Temecula, CA), which is a specific marker for the proximal tubules and the thin descending limb of Henle’s loop (Nielsen et al., 2002), (2) anti-Na/K/2Cl cotransporter (NKCC2) antibody (Alpha Diagnostic, San Antonio, TX), which is a specific marker for the thick ascending limb of Henle’s loop (Delpire and Mount, 2002), (3) anti-Na/Cl cotransporter (NCC) antibody (Chemicon), which is a specific marker for the distal tubules (Delpire and Mount, 2002), (4) anti-AQP2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), which is a specific marker for both the connecting tubules and the collecting ducts (Nielsen et al., 2002), all of which had been generated in rabbits except the anti-AQP2 antibody which was a goat polyclonal antibody. Immunolabeling was performed by the streptavidin–biotin (SAB) method, as follows. (1) The sections were incubated with 0.3% hydrogen peroxide in 100% methanol for 20 min in order to block endogenous peroxidase activity. (2) The sections were incubated with normal goat serum (Dako, Copenhagen, Denmark) diluted 1:5 in PBS with 0.03% Triton-X-100 (Wako, Tokyo, Japan) for 30 min. (3) The sections were incubated with diluted primary antibodies in PBS with 1% bovine serum albumin (BSA, Sigma, St. Louis, MO) and 0.03% Triton-X-100 overnight. (4) The sections were incubated with biotinylated goat anti-rabbit immunoglobulins (Dako) diluted 1:600 in PBS with BSA and Triton-X-100 for 30 min. (5) Finally, the sections were incubated with horse radish peroxidase-conjugated streptavidin (Dako) diluted 1:300 in PBS with BSA and Triton-X-100 for 30 min. But, for anti-AQP-2 antibody, instead of diluted goat serum and biotinylated goat anti-rabbit immunoglobulins, 10% BSA and biotinylated bovine anti-goat immunoglobulins diluted 1:600 for 30 min were used. Immunoreaction was visualized by using 0.025% 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.065% sodium azide (Wako) in 0.05M Tris-HCl buffer (pH 7.4) containing 0.01% hydrogen peroxide for 10 min. All steps were performed at room temperature. The sections were counterstained with hematoxylin, dehydrated, and then mounted in Permount.

**Assessment of fibrous area in UUO kidney**

A fibrous tissue is stained in blue color with azan-staining. After azan-staining, we measured the blue-colored area in each section of the cortex and medulla of kidneys of sham-operated mice or UUO treated mice. We selected four high power fields (HPF) randomly in each mouse and measured the fibrous area by use of an Image Processor and Analyser (TRI/2 D-MES; RATO, Tokyo, Japan) under high magnification (x400) (n=6 mice per group). For each group, we represented the measured fibrous area as the relative value, when the
mean value of fibrous area in Sham day 1 kidney group was one. For determination of the degree of interstitial fibrosis, a grid was used and the number of intersecting points was counted by use of an Image Processor and Analyser (TRI/2 D-MES; RATOC) under high magnification (×400). Results are expressed as the mean number of grid points developing fibrosis (Cachat et al., 2003). The area of fibrosis surrounding individual tubule was determined in randomly selected 40 tubules in UUO day 7 kidney. To evaluate the relationship between tubular dilatation and the degree of interstitial fibrosis, randomly selected 40 tubules were analyzed (tubular luminal area vs. fibrosis area around the tubules) in UUO day 7 kidney (n=6 mice). The cortex was defined as the region extending from the capsule to the deepest glomerulus, whereas the medullary region was defined as that zone extending from below the deepest glomerulus down to the renal pelvis.

Assessment of luminal area in uriniferous tubules

From representative immunolabeling sections, the tubular luminal area of each segment was measured in the cortex and the medulla by use of an Image Processor and Analyser (TRI/2 D-MES; RATOC). Forty tubules of each segment were randomly selected (n=6 mice per group).

Analysis of apoptosis in tubular epithelial cells

In order to examine sensitive quantification of apoptotic tubular epithelium, we used the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick end-labeling (TUNEL) method, which labels the characteristic DNA double-strand breaks. The assay was carried out as previously described (Sasaki et al., 2001). We defined that TUNEL positive tubules were the tubules containing at least one TUNEL positive tubular epithelial cell. To evaluate the relationship between tubular dilatation and tubular epithelial apoptosis, we selected randomly 40 TUNEL positive tubules in each UUO kidney group, and measured the luminal areas of such tubules (n=6 mice per group) by use of an Image Processor and Analyser (TRI/2 D-MES; RATOC). In sham-operated kidney, we measured only five tubular luminal areas of TUNEL positive tubules, because we seldom found TUNEL positive tubules in sham-operated kidneys. Moreover, to correlate apoptosis and tubular dilatation, 40 TUNEL positive tubules were analyzed (tubular luminal area vs. number of apoptotic cells/tubule) in UUO day 7 kidney (n = 6 mice).

Statistical Analysis

The analysis was performed with a commercially available statistical package (SPSS Ver 7.0). The Dunnett’s t test was used to compare tubular luminal areas in sham-operated kidneys with those in corresponding UUO kidneys. All measurements of tubular luminal areas are expressed as the mean ± SD. We performed the statistical analysis by using linear regression for correlation (tubular dilatation vs. the degree of interstitial fibrosis around individual tubule, the tubular luminal area vs. the number of apoptotic cells/tubule). A P value of <0.05 was considered to be statistically significant. Any significant differences in the measurements were not recognized among three sham-operated kidney groups for all items in our present study.

Results

Segment-specific tubules from the ascending limb of Henle’s loop to the collecting tubule showed higher luminal enlargement than other segments of tubules and the intertubular area tended to be gradually increased in the experimental time course after the ureteral ligation. In addition, the lumen-expanded tubule was significantly prone to develop tubular epithelial apoptosis, suggesting that dilated tubules would disappear and be replaced with fibrous tissues.

HE- and azan-staining

A number of uriniferous tubules appeared to dilate both in the cortex and the medulla of UUO day 1 kidney, whereas such a morphological change was not observed in Sham day 1 kidney (Fig. 1A,C). In UUO day 1 kidney, some tubular epithelial cells were decreased in height and such tubules represented the luminal expansion (data not shown). The level of tubular dilation became higher in UUO day 3 kidney than those of UUO day1 kidney (data not shown). Such tubular dilation progressed further in UUO day 7 kidney (Fig. 1B,D). Moreover, tubulointerstitial fibrosis was apparently recognized both in the cortex and the medulla in UUO day 3 kidney (data not shown), and this fibrous area was remarkably increased in UUO day 7 kidney (Fig. 1B,D). We found many fibroblast-like cells were densely distributed in the tubulointerstitial tissue of the obstructed kidney of UUO day 7 (Fig. 1B,D). Regardless of the interval following the sham-operation, similar images were obtained among three sham-operated kidney groups.

We used azan-staining to examine whether the spaces of tubulointerstitial fibrous tissues increase in UUO kidneys of adult mice. In UUO day 1 kidney, the fibrous area did not significantly increase compared with Sham day 1 kidney (Fig. 2A,B). Azan-staining revealed a significant increase of the fibrous area both in the cortex and the medulla 3 days after the ureteral ligature compared with Sham day 3 kidney. The fibrous area in the cortex of UUO day 3 kidney increased 9.1 times and in the medulla 6.5 times as large as that of Sham day 3 kidney.
Fig. 1. Hematoxylin-eosin (HE) staining of the cortex in Sham day 1 (A), UUO day 7 (B) kidneys. HE staining of the medulla in Sham day 1 (C), and UUO day 7 (D) kidneys. Azan staining of the cortex in Sham day 1 (E), UUO day 7 (F and I) kidneys. Azan staining of the medulla in Sham day 1 (G), UUO day 7 (H) kidneys. Note the tubular dilatation and the expansion of interstitial fibrous space (blue colored area in F and H) in UUO day 7 kidneys. The degree of interstitial fibrosis around dilated tubules (arrowheads) was higher than that around non-dilated tubules (arrows) shown in Fig. 1I. J. In UUO day 7 kidney, there is strong correlation between tubular dilatation and the degree of fibrosis surrounding individual tubule with line of linear regression ($r=0.925$, $P<0.001$). G indicates a glomerulus. A, B, E, F, x 400; C, D, G-I, x 200.
kidney, respectively (Fig. 2A,B). Furthermore, fibrous tissue area remarkably increased both in the cortex and the medulla in UUO day 7 kidney. The fibrous area in the cortex of UUO day 7 kidney increased 20.2 times and in the medulla 21.8 times as large as UUO day 7 kidney (Fig. 1F,H, 2A,B). The degree of interstitial fibrosis around dilated tubules seemed to be higher than that around non-dilated tubules (Fig. 1I). In UUO day 7 kidney, there was strong correlation between tubular dilation and fibrosis surrounding individual tubules (r=0.935, P<0.001) (Fig. 1J).

**Immunolabeling of AQP1 in the proximal tubules and the descending thin limb of Henle's loop**

After any day of the ureteral obstruction, no dilated tubules were immunoreactive for AQP1: The proximal tubules did not appear to dilate on histologic sections at any stage after UUO. As time passed after UUO, AQP1 positive proximal tubules decreased in number, and appeared to be replaced by AQP1 immunonegative dilated tubules and fibrous tissues in the cortex after 7 days of UUO (Fig. 3A-D).

The descending thin limb of Henle’s loop, which was immunopositive for AQP1, did not seem to morphologically change in the medulla at any stage after the ureteral obstruction (Fig. 4A-D).

**Immunostaining of the thick ascending limb of Henle’s loop with anti-NKCC2 antibody**

NKCC2-immunoreactive products were localized exclusively on the luminal surface of the epithelial cell in the thick ascending limb of Henle’s loop of Sham day 1 kidney (Fig. 5A, 6A). NKCC2-positive tubules were found to dilate as early as one day after the ureteral ligation (Fig. 5B). The thick ascending limb of Henle’s loop appeared to gradually dilate both in the cortex and the medulla in the experimental time course after the ureteral obstruction (Fig. 5A-D). Many epithelial cells of the dilated tubules in the cortex were immunolabeled with NKCC2, suggesting that the thick ascending limb of Henle’s loop accounted for a large population of such dilated tubules in the cortex. In the medulla, most of the dilated tubules were immunopositive for NKCC2 after ureteral obstruction (Fig. 6A-D).

**NCC-Immunolabeling of the distal tubules**

NCC-immunopositive products were expressed exclusively on the apical surface of the convoluted distal tubular cells in Sham day 1 kidney (Fig. 7A; arrows). NCC-labeled dilated tubules were observed one day after ureteral ligation (Fig. 7B; arrows). In the cortex, some dilated tubules were immunopositive for NCC and others immunonegative (Fig. 7B-D). Therefore, a part of the dilated tubules were regarded as the distal tubules in obstructed kidneys.

**Immunolabeling of the connecting tubules and the collecting ducts with anti-AQP2 antibody**

The specific expression of AQP2 was identified both in the connecting tubules and the collecting ducts of Sham day 1 kidney (Fig. 8A, 9A). All of AQP2-immunostained tubules appeared to dilate both in the cortex and the medulla as early as 1 day after UUO (Fig. 8B, 9B). As time passed after UUO, the luminal area of AQP2 labeled tubules continued to increase until 7 days after UUO (Fig. 8C,D,9C,D). Thus, some dilated tubules seemed to belong to the connecting tubules and/or the collecting ducts both in the cortex and the medulla.

**Tubular luminal area of each tubular segment**

The luminal areas of AQP1-labeled tubules did not show significant enlargement either in the cortex or the medulla of obstructed kidneys until 7 days after the

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**Fig. 2.** Measurements of the fibrous area in the cortex (A) and the medulla (B) of Sham and UUO kidneys. The value of area in each group is presented as fold increases greater than that of area in Sham day 1 group and the results are represented as the mean value ± SD of 4 high power fields randomly selected from representative sections (n=6 mice per group). *: P<0.05 versus corresponding Sham kidney group, **: P<0.001 versus corresponding Sham kidney group. S and U indicate Sham and UOO kidney group, respectively.
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Fig. 3. Immunolabeling of aquaporin-1 (AQP1) in the cortex of Sham and UUO kidneys. Sham day 1 (A), UUO day 1 (B), UUO day 3 (C), and UUO day 7 (D). Note that dilated tubules (asterisk) are AQP1-negative in Fig. 3. Immunolabeling of AQP1 observed in the glomerulus (G) belongs to erythrocytes. x 400

Fig. 4. Immunolabeling of aquaporin-1 (AQP1) in the medulla of Sham and UUO kidneys. Sham day 1 (A), UUO day 1 (B), UUO day 3 (C), and UUO day 7 (D). x 200
Fig. 5. Immunolabeling of Na-K-2Cl cotransporter (NKCC2) in the cortex of Sham and UUO kidneys. Sham day 1 (A), UUO day 1 (B), UUO day 3 (C), and UUO day 7 (D). Note that NKCC2-positive tubules dilate in UUO kidneys. x 400

Fig. 6. Immunolabeling of Na-K-2Cl cotransporter type 2 (NKCC2) in the medulla of Sham and UUO kidneys. Sham day 1 (A), UUO day 1 (B), UUO day 3 (C), and UUO day 7 (D). Note that NKCC2-positive tubules dilate in UUO kidneys. x 200
Fig. 7. Immunolabeling of Na-Cl cotransporter (NCC) in Sham day 1 (A), UUO day 1 (B), UUO day 3 (C), and UUO day 7 (D) kidneys. Note that immunolabeling of NCC is localized on the apical surface of the distal tubules (arrows) in Sham day 1 kidney and that a part of dilated tubules (arrows) are NCC-positive in UUO kidneys. x400

Fig. 8. Immunolabeling of aquaporin-2 (AQP2) in the cortex of Sham and UUO kidneys. Sham day 1 (A), UUO day 1 (B), UUO day 3 (C), and UUO day 7 (D). Note that a part of dilated tubules (arrows) are AQP2-positive. x 400
ureteral ligation (Fig. 10A-1, A-2). In contrast, the luminal areas of NKCC2-immunoreactive tubules were significantly increased as soon as one day after ureteral ligation both in the cortex and the medulla. NKCC2-labeled tubules continued to dilate for 7 days after the ureteral obstruction. In the cortex, the luminal area of NKCC2-immunostained tubules in Sham day 7 kidney was significantly different from that of UUO day 7 kidney (234.1±117.3 microsquaremeters in UUO day 7 kidney compared with 7.1±0.8 microsquaremeters in Sham day 7 kidney, P<0.001) (Fig. 10B-1). In the medulla, almost similar results were obtained (192.9±103.7 microsquaremeters in UUO day 7 kidney compared with 3.0±1.0 microsquaremeters in Sham day 7 kidney, P<0.001) (Fig. 10B-2). The speed of luminal expansion was almost similar in the distal nephron segment from the thick ascending limb of Henle's loop to the collecting ducts. The luminal area of each segment was significantly different between Sham day 7 and UUO day 7 kidney (the distal tubules: 119.1±53.3 microsquaremeters in UUO day 7 kidney compared with 8.4±4.8 microsquaremeters in Sham day 7 kidney, P<0.001, AQP2-positive tubules in the cortex: 186.5±182.7 microsquaremeters in UUO day 7 kidney compared with 3.6±1.0 microsquaremeters in Sham day 7 kidney, P<0.001, AQP2-positive tubules in the medulla: 93.4±71.7 microsquaremeters in UUO day 7 kidney compared with 2.9±1.3 microsquaremeters in Sham day 7 kidney, P<0.001) (Fig. 10C,D).

**Relationship between tubuloluminal expansion and tubuloepithelial apoptosis**

Next, we examined whether the apoptosis of tubular epithelial cells directly correlated with the degree of tubular dilatation. The luminal area of TUNEL positive

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**Fig. 9.** Immunolabelling of aquaporin-2 (AQP2) in the medulla of Sham and UUO kidneys. Sham day 1 (A), UUO day 1 (B), UUO day 3 (C), and UUO day 7 (D). Note that a part of dilated tubules are AQP2-positive. x200
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Fig. 10. Measurements of the luminal area of tubules in each segment. A-1. The proximal tubule. A-2. The descending limb of Henle's loop. B-1. The ascending limb of Henle's loop in the cortex. B-2. The ascending limb of Henle's loop in the medulla. C. The distal tubule. D-1. The connecting tubule and collecting duct in the cortex. D-2. The connecting tubule and collecting duct in the medulla of Sham and UUO kidneys. Results are presented as the mean values ± SD of 40 tubules randomly selected from sections immunolabeled with each segment specific antibody (n=6 per group). NS: not significant versus corresponding Sham kidney group. *: P<0.05 versus corresponding Sham kidney group, **: P<0.001 versus corresponding Sham kidney group. S and U indicate Sham and UUO kidney group, respectively.
tubules significantly enlarged 1 day after ureteral ligature compared with Sham day 1 kidney (27.1±18.9 microsquaremeters in UUO day 1 kidney compared with 3.7±0.5 microsquaremeters in Sham day 1 kidney, P < 0.036) (Fig. 11B, E). In UUO day 7 kidney, the luminal area of TUNEL positive tubules further enlarged (124.9±60.3 microsquaremeters in UUO day 7 kidney compared with 5.9±0.5 microsquaremeters in Sham day 7 kidney, P<0.001) (Fig. 11D,E). After 7 days of obstruction, there was strong correlation between tubular dilatation and tubular epithelial apoptosis (r=0.785, P<0.001) (Fig. 11F).

Discussion

In the present investigation, we revealed regional tubular changes in the obstructed kidney of adult mice using segment-specific tubular marker. Moreover, we also showed that morphological changes, i.e., dilated tubules were more prone to develop the apoptosis of tubular epithelia in UUO model of adult mice. An immunohistochemical analysis demonstrated that the specific tubular segment from the thick ascending limb of Henle's loop to the collecting duct dilated rapidly, whereas the proximal tubules and the descending limb of Henle's loop did not dilate in treated kidneys. These findings were confirmed by measuring the luminal area of each tubular segment in obstructed kidneys. To our knowledge, the present paper is the first report suggesting that tubular dilatation happened in renal medulla in obstructed kidneys by use of tubular specific antibodies. Cachat and colleagues (2003) showed that tubular dilatation in the obstructed kidney was most severe in the collecting ducts and least severe in the proximal tubules, although their results were obtained by using neonatal mice and they analyzed three tubular segments including the proximal tubules, the distal tubules, and the collecting ducts. Although we thoroughly analyzed five tubular segments of obstructed kidneys, results obtained by Cachat and coworkers did not conflict with our present results in adult mice.

It is generally thought that several factors might be involved in the tubular dilatation occurring in obstructed kidneys. Especially, three important factors seem to strongly affect the tubular dilatation by the ureteral ligation. The first factor is intraluminal-pressure by urine accumulation in tubules, the second one is the stiffness of the renal tubular wall against intraluminal-pressure; and the third one is the interstitial pressure counteracting intraluminal pressure. In mice with obstructed kidney,
there is no report revealing both actual intraluminal-pressure in each segment and pressure in interstitium around each segment. Cortell and colleagues (1973) investigated the response of renal tubule diameter (D) to changes in tubular pressure (P) induced by partially obstructing single tubules, and they defined that response as the compliance of the tubule. According to their report, the compliance characteristics of the proximal and distal tubules were found to be markedly different; the proximal tubular pressure-diameter relationship was linear, delta D/delta P=0.45mmHg, whereas the distal tubular pressure-diameter relationship was curvilinear delta D/delta P=-0.1xP+2.2. However, these pressure-diameter relationships formed when the interstitial pressure was zero (Cortell et al., 1973). Since the interstitial pressure in the obstructed kidney would change in the course of urine accumulation by the ureteral ligation, the relationship between pressure and diameter could be more complex than we expected. Moreover, Cortell et al. (1973) isolated single proximal or distal tubules, and obstructed the lumen of isolated tubules partially not by ligation but by caster oil for only 4-5 hours. Therefore, although the in vitro results led by Cortell and coworkers could not be applied directly to our in vivo results, the distal tubules tended to dilate more easily than the proximal tubules in clinical obstructive nephropathy (Moller et al., 1984). However, their investigation could be one suggestion that the distal tubules dilate more rapidly than the proximal tubules observed in our present investigation when intraluminal-pressure is identical. We have no evidence showing that the intraluminal-pressure is identical through whole nephron segment. Why did the proximal tubules dilate more slowly than the distal tubules and the collecting ducts? One of the reasons we speculated is that the intraluminal-pressure would increase more rapidly in the downstream segment of nephron than the upstream segment, i. e., the proximal tubule and descending limb of Henle's loop, because urine accumulation by ureteral ligation firstly happens at the downstream segment of nephron.

Tubules with TUNEL-labeled epithelia were significantly increased in tubular luminal area compared with those without TUNEL-positive epithelia in UUO kidney of adult mice. In addition, there was a strong positive linear relationship between tubular dilatation and tubular epithelial apoptosis, suggesting that increased intraluminal-pressure would induce apoptosis of tubular epithlia in dilated tubules. Our experimental results do not conflict with the results in neonatal mice kidneys reported by Cachat and coworkers (2003). Tubular epithelial cell apoptosis probably accounts for renal tissue loss in obstructed nephropathy because a direct correlation between its degree and the decline in dry kidney weight is well-documented (Truong et al., 1998). Ohashi et al. (2002) reported that some of the peritubular capillary lumina were enlarged or misshapen and the expression level of vascular endothelial growth factor (VEGF) became locally intense one week after UUO. Intense VEGF expression in tubules of UUO kidneys showed ischemic state of tubules by decrease of microcirculating blood flow, which could lead to apoptosis of tubular epithelia by hypoxia (Gobe and Axelsen, 1987). We supposed that dilated tubules would compress intratubular tissues and crush peritubular capillaries. In our present study, we had the impression that the degree of interstitial fibrosis around dilated tubules was high compared with that around non-dilated tubules shown in Fig. II. We proved precisely the strong relationship between tubular dilatation and the degree of fibrosis around the dilated tubules by using linear regression, supporting our supposition.

In conclusion, the tubular segment from the ascending limb of Henle's loop to the collecting duct tended to dilate more rapidly than the other segment of tubules in a time-dependent manner after the ureteral obstruction of adult mice, whereas the proximal tubules and the descending limb of Henle's loop did not dilate during 7 days after the ureteral ligation. Dilated tubules tended to induce apoptosis of their epithelia, suggesting that epithelia in dilated tubules would disappear by the epithelial apoptosis and be finally replaced with fibrous tissue, which might lead to loss of renal function.

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