Renovascular Morphological Changes in a Rabbit Model of Hydronephrosis

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Summary: Obstructive nephropathy ultimately leads to end-stage renal failure. Renovascular lesions are involved in various nephropathies, and most renal diseases have an ischemic component that underlies the resulting renal fibrosis. The aim of this study was to investigate whether morphological changes occur in the renal vasculature in hydronephrosis and the possible mechanisms involved. A model of complete unilateral ureteral obstruction (CUUO) was used. Experimental animals were divided into five groups: a normal control group (N) and groups of animals at 1st week (O1), 2nd week (O2), 4th week (O4) and 8th week (O8) after CUUO. Blood pressure was measured, renal arterial trees and glomeruli were assessed quantitatively, and renovascular three-dimensional reconstruction was performed on all groups. Glomerular ultrastructural changes were examined by transmission electron microscopy. The results showed that the systolic blood pressure was significantly increased in the obstructed groups (O1, O2, O4 and O8). Three-dimensional reconstruction showed sparse arterial trees in the O8 group, and a tortuous and sometimes ruptured glomerular basement membrane was found in the O4 and O8 groups. Furthermore, epithelial media thickness and media/lumen ratio were increased, lumen diameters were decreased, and the cross-sectional area of the media was unaltered in the segmental renal artery, interlobar artery and afferent arterioles, respectively. In conclusion, renal arterial trees and glomeruli were dramatically altered following CUUO and the changes may be partially ascribed to vascular remodeling. Elucidation of the molecular mechanisms of renovascular morphological alterations will enable the development of potential therapeutic approaches for hydronephrosis.

Key words: renal artery; hydronephrosis; ureteral obstruction; vascular cast; rabbit

Obstructive nephropathy is a common disease that is characterized by gradual increases over time in the severity of hydronephrosis, ultimately leading to end-stage renal failure [1]. Many renal diseases have an ischemic component accompanied by renal fibrosis, and ischemia is believed to be a critical underlying element of the pathological process leading to renal failure [2]. Numerous studies have demonstrated that changes in the renal microvasculature are involved in the process of various nephropathies. For example, epithelial cell foot process fusion and basement membrane thinning can be found in aminonucleoside nephrosis [3]. A series of changes, including glomerulosclerosis and reduced podocyte and basement membrane thickening, have been observed in IgA nephropathy [4].

At present, few publications exist concerning changes in renal microvasculature in obstructive nephropathy [5, 6]. It is also likely that during the course of analyzing the underlying pathology, some information about structural changes in renal arterial trees has been missed or ignored. Therefore, the aim of this study was to investigate whether morphological alterations in the renal vasculature play a role in hydronephrosis. Vascular casting of arterial trees and glomeruli and renovascular quantitative assessment were performed.

1 MATERIALS AND METHODS

1.1 Experimental Design

A total of 75 adult male New Zealand rabbits, weighing 2 to 2.5 kg, were randomly divided into five groups (n=15 per group): normal control group (N) and groups at 1st week (O1), 2nd week (O2), 4th week (O4) and 8th week (O8) after CUUO. The procedures for experiments and animal care were approved by the Animal Care and Use Committee of Wuhan University and conformed to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH Publication No. 80-23).

1.2 Establishment of Animal Model

The CUUO animal model was produced with New Zealand rabbits [7, 8]. Animals were anesthetized using 10% chloral hydrate (2.5 mL/kg) by injection into the ear vein; the left ureter was then exposed through a longitudinal 5-cm mid-abdominal incision and ligated with a 3–0 silk suture. After the incision was closed, animals received intramuscular injections of buprenorphine (0.03 mg/kg) to relieve pain and were treated by ear vein injection with penicillin G (20 000 U/kg) to prevent infection during the recovery period. Rabbits were kept in a warm
environment and given standard food and water ad libitum.

1.3 Blood Pressure Measurement

Rabbits were anaesthetized with 10% chloral hydrate. A floating polyethylene catheter was inserted into the right femoral artery for blood pressure measurement. The catheter was exteriorized through the inguinal skin. After a two-day recovery period, animals were placed in individual cages containing water and food for blood pressure recording. The catheter was connected to a transducer through a rotating swivel that allowed the animal to move freely in the cage. The blood pressure signal was digitized by a microcomputer after approximately 14 h of habituation[9].

1.4 Arterial Tree and Collecting System Casts

Arterial tree casts in all groups were prepared according to the following procedures. Under chloral hydrate anesthesia, the thoracic aorta was exposed and opened and a glass tube with internal diameter of 2 mm and length of 7 cm was inserted antegradely into the thoracic aorta. The left renal vein and hydronephrotic ureter were cut by a small incision for outflow and the renal vasculature was fixed using 2.5% glutaraldehyde. After 5 min, 20 mL perchloroethylene resin (20% concentration) was slowly infused with a hand-held syringe via the glass tube for a left renovascular cast. Meanwhile, the infusion pressure was adjusted to be equal to the mean artery pressure. The lower abdominal aorta was ligated at a distance of 5 cm from the renal artery and additional infusion of 5 mL perchloroethylene was performed. The perchloroethylene was coagulated in the vascular system after 48 h at room temperature. The left ureter was exposed and opened and a glass tube with internal diameter of 1 mm and length of 7 cm was inserted retrogradely into the ureter. A dental-based acrylic resin was then infused using a syringe via the ureter for a renal collecting system cast. The priming volume was 1.5 to 2 mL for the normal collecting system and 4 to 5 mL for the dilated collecting system. Cast specimens were preserved at room temperature for 48 h so that the fill material could be sufficiently coagulated. The renal tissue was digested and removed using a 20% hydrochloric acid solution at room temperature after 7 days. Finally, the cast specimens were rinsed with tap water and air-dried.

1.5 Glomerular Vasculature Casts

Procedures of thoracic aorta opening and intubation were identical with those described above for preparation of arterial tree casts. The lower abdominal aorta was ligated at a distance of 5 cm from the renal artery, and the superior mesenteric and right renal arteries were also ligated. The left renal vein and hydronephrotic ureter were opened by a small incision to allow the escape of blood, perfusate and urine. Immediately afterwards, the left kidney was transfused with heparin saline (5000 U/mL) to wash out the blood and the renal vasculature was fixed by treatment with 2.5% glutaraldehyde for 5 min. Methyl methacrylate (20 mL) was then infused to remove renovascular water and polymethyl methacrylate was transfused to prepare the glomerulus cast. After the cast fill material was sufficiently coagulated, renal tissue was digested and removed using 20% hydrochloric acid at room temperature. Finally, cast specimens were rinsed in tap water and air-dried. Glomeruli that accompanied interlobar arteries, afferent arterioles and efferent arterioles were removed from different regions using forceps and the samples were then pasted at an objective table and subsequently coated with gold palladium and examined with a scanning electron microscope (SEM, S-570; Hitachi, Japan) at an accelerating voltage of 20 kV and working distance of 10 mm.

1.6 Arterial Tree Three-dimensional (3D) Reconstruction

Perchloroethylene mixed with lead oxide at a concentration of 25% was applied as a fill material. The operating procedures for preparation of arterial tree casts were similar to the steps for preparation of arterial tree casts using perchloroethylene resin as a fill material. After the fill material was sufficiently coagulated, all cast specimens were scanned using 64 multidetector row computed tomography (CT, General Electric Co., USA) with a tube voltage of 120 kV and current of 250 mA. CT images were from 0.625 mm sections, 512 × 512 matrix and display field of view (DFOV) of 38.5 mm × 38.5 mm. The images were imported into a GE Adw 4.3 workstation to perform arterial tree 3D reconstruction.

1.7 Transmission Electron Microscopy

Renal tissues were pre-fixed with 2.5% glutaraldehyde for 2 h, rinsed three times in 0.1 mol/L phosphate-buffered saline (PBS), fixed using 1% osmium tetroxide for 2 h, rinsed three times in 0.1 mol/L PBS, and subsequently dehydrated in a graded series of ethanol, 15 min each time. The tissues were dehydrated again two times with pure acetone, 15 min each time, drenched 30 min in a solution of epoxy resin (Epon 812) and acetone (1:1), and subsequently embedded in absolute Epon 812 for 1 h. The tissues were solidified in absolute Epon 812 at 37°C for 24 h and at 60°C for 48 h. Finally, 70-nm ultra-thin slices were double-stained with uranyl acetate and lead citrate. The specimens were observed with a transmission electron microscope (H-600; Hitachi, Japan).

1.8 Quantitative Assessment of Arterial Trees and Glomeruli

Diameters of the main stem renal artery, the first-order branches of the renal artery, segmental renal artery, and interlobar artery were measured respectively on the cast specimens at three points with a sliding caliper (accuracy rating=0.02 mm) and averaged. In both afferent and efferent arterioles, diameters were measured on the SEM pictures using image analysis software (Image-Pro Plus) at three points (30, 40, and 50 μm from the glomerular vascular pole) and averaged. The renovascular media thickness, lumen diameter, media/lumen ratio, cross-sectional area of media and glomerular diameters were measured by light microscopy (OLYMPUS, CKX41, Japan) and image analysis software (Image-Pro Plus) in semi-thin sections (Hematoxylin-Eosin staining, tissue section thickness: 4 μm).

1.9 Statistical Analysis

The data were expressed as \( \bar{x} \pm s \). Statistical analysis was performed by one-way analysis of variance (ANOVA) and Student-Newman-Keuls (SNK)-\( q \) test. A value of \( P<0.05 \) was considered significant. We performed assessments of both inter- and intra-observer variation to assess reproducibility and variability of the measurements. The inter- and intra-observer variation was around 5% and 2% respectively.