Unilateral Ureteral Obstruction as a Model of Kidney Fibrosis and Increasing of Systolic Blood Pressure in Mice

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Abstract

Background: Obstructive nephropathy can lead to progressive and permanent loss of kidney function characterized by interstitial inflammation and tubulointerstitial fibrosis. Tubulointerstitial fibrosis presents as the result of various kidney injuries in general and can cause chronic kidney disease (CKD), which can progress to end-stage kidney disease and the increase of systolic blood pressure in mice.

Objective: This study aimed to determine the effectiveness of unilateral ureteral obstruction (UUO) as a model of renal fibrosis and increasing of systolic blood pressure in mice.

Method: Sixteen male Rattus norvegicus mice (150-200 g) were divided into control groups and UUO by ureteral ligation, eight mice each. The systolic blood pressure (SBP) was measured every seven days. After 30 days the animals were dissected to analyze the changes in renal interstitial fibrosis. Statistical analysis was carried out by unpaired t-test or alternative tests.

Results: There was a significant increase in interstitial fibrosis in the UUO rat group [1% (0% - 5%) vs. 75% (20% - 90%), p <0.001] and SBP [85.38 ± 1.69 mmHg vs 144.75 ± 4.27 mmHg, p <0.001].

Conclusion: UUO can be used as a model of fibrosis and hypertension, which can be used as the basis for the development of anti-fibrotic and anti-hypertensive drugs.

Keyword: interstitial fibrosis, hypertension, unilateral ureteral obstruction

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Background

Chronic kidney disease (CKD) is a major public health problem throughout the world where its prevalence continues to increase annually as life expectancy increases. CKD is a general term for a heterogeneous disorder that affects kidney structure or its function with various clinical manifestations that ends with end-stage renal disease. CKD progression is defined as a decrease in glomerular filtration rate irrespective of the primary disease. Progression of CKD is characterized by loss of renal cells and deposition of extracellular matrix (ECM), regardless of the cause. One conse-
quence of CKD is tubulointerstitial fibrosis that occurs due to an imbalance between synthesis and reduction of ECM deposition.³

Chronic hypoxia is involved in various pathogenic conditions, including the development of CKD. Chronic renal hypoxia is induced by a number of pathogenic conditions, including renal ischemia, reduced peritubular capsules, and tubulointerstitial fibrosis.⁴ Blockage of the ureter both unilaterally and bilaterally will lead to progressive damage to kidney structure and cause chronic kidney dysfunction as the main cause of end-stage CKD in children in the United States.⁵ The unilateral ureteric obstruction (UUO) model in mice causes kidney ischemia and hypoxia which will lead to interstitial inflammation and tubulointerstitial fibrosis, resulting in progressive and irreversible loss of kidney function. The initial injury at the acute onset of kidney obstruction in UUO leads to changes in glomerular filtration rate, renal blood flow, and interstitial edema.⁶ Prolonged obstruction in UUO resulted in the development of hydronephrosis, renal atrophy, interstitial fibrosis, and eventually renal dysfunction.⁷

Obstructive nephropathy can cause progressive and permanent loss of kidney function characterized by interstitial inflammation and tubulointerstitial fibrosis. Tubulointerstitial fibrosis is the result of various kidney injuries in general and can cause CKD that can end up being end-stage kidney disease and hypertension.³ Hypertension can be a cause and consequence of CKD. Continuously high systemic blood pressure will eventually disrupt the kidney’s autoregulation system and cause the pressure to be passed on to the kidneys. This will injure the kidneys and over time will result in CKD.⁸ How hypertension causes damage to the kidneys is not yet fully known.⁹ Therefore animal models of kidney fibrosis and hypertension can be used as a basis for the development of anti-fibrotic and anti-hypertensive drugs for inhibiting the progression of CKD.

Materials and Methods

Male Norvegicus mice (150-300 g) are placed in clean plastic cages with controlled temperature and humidity, a constant 12 hour light / dark cycle, and free access to food and water. One week after adaptation, UUO was induced. After a 7-day quarantine period, 16 mice were divided randomly into two groups: mice in Group 1 (n=8) were controls and mice in group 2 (n=8) underwent unilateral ureteric ligation. The use of animals and experimental protocols were approved by the Ethics Committee under the Guidelines for the Care and Use of Animal Laboratories at the Research Council.

Briefly, after induction of general anesthesia with intramuscular ketamine injections (0.5 mg/kg body weight), the abdominal cavity was excised through a midline incision and the left ureter was ligated at two sites using silk thread 3-08. Systolic blood pressure (SBP) was measured using a tail-cuff method using a blood pressure analyzer for test animals. This method allows researchers to measure systolic blood pressure. The blood pressure of the test animals was measured every week from the beginning to the end of the experiment in mmHg. On the 30th day, the animals were given anesthesia with intramuscular ketamine injections and were executed. Kidney samples were collected for histopathological examination. Renal interstitial fibrosis will appear as type-I collagen buildup around fibroblast and proximal tubule in reddish colored with Verhoeff-Van Gieson staining. The thickness of renal interstitial fibrosis is assessed using an ocular micrometer at 400x magnification.

The results of the two groups are shown as the mean ± standard deviation. Statistical analysis of renal interstitial fibrosis and SBP used an unpaired T-test or alternative test, with a p-value of <0.05 accepted as a significant statistical value.

Results

When compared with the average level of tubulointerstitial fibrosis in the control group, the ureteric ligation
group tended to a higher level of tubulointerstitial fibrosis. The Mann-Whitney test results for renal tubulointerstitial fibrosis are shown in Table 1.

Tabel 1. Tubulointerstitial fibrosis between groups

| Group                      | n  | Median (minimum–maximum) | Sig. |
|----------------------------|----|--------------------------|------|
| Control                    | 8  | 1% (0% – 5%)             |      |
| Unilateral ureteral ligation | 8  | 75% (20% – 90%)         | 0.001** |

**significant if p <0,01

The average independent sample using the Mann-Whitney test above shows that the test of the variable level of tubulointerstitial fibrosis between control and UUO group was significant with p=0.001. It can be argued that in unilateral ureteric ligation groups the mean rate of tubulointerstitial fibrosis was higher (increased) significantly compared to the control group.

Clinical effects arising from unilateral ureteral ligation as CKD rat models, one of which is systolic blood pressure (SBP). SBP measurements in this study were carried out every week from the beginning of the study to the end of the study. An increase in SBP was seen at the beginning of the first week after ureteral ligation, with the results detailed in Figure 3.

**Figure 2. Interstitial fibrosis of each group at 400x magnification.**

The appearance of interstitial fibrosis looks reddish (yellow arrow). A: control group; B: Ureter Ligation group (UUO);

**Figure 3. Weekly systolic blood pressure measurement of each group**

Table 2. Mean systolic blood pressure (mmHg) of control and ureteral ligation group

| Group                     | n  | mean ± SD    | Sig. |
|---------------------------|----|--------------|------|
| Control                   | 8  | 85.38±1.69   |      |
| Ureteral ligation         | 8  | 144.75±4.27  | 0.001** |

**significant if p <0,01**

**Figure 1. Distribution of interstitial fibrosis in each group at 100x magnification.**

**Figure 2. Distribution of interstitial fibrosis in each group at 400x magnification.**
The results of the unpaired t-test analysis above show that the mean systolic blood pressure of the unilateral ureter ligation group was significantly higher than the control group (p=0.001).

Discussion

This study’s result showed unilateral ureteral ligation have significantly increased fibrosis and SBP in test animals. The increase of systolic blood pressure was also found significant since the first week after ligation. This can be explained based on the results of several existing studies showing that increased blood pressure in humans is associated with an increase in pro-inflammatory mediators such as TNF-α, IFN-γ, IL-6, IL-17 and reduced anti-inflammatory cytokines such as IL-10. The association between TNF-α and IL-6 polymorphisms with hypertension further support the findings. Experimental and clinical evidence confirms that the immune system plays an important role in hypertension. Increased sympathetic outflow as a consequence of CNS stimulation causes an increase in blood pressure, causing tissue injury and neoantigen and/or damage-associated molecular pattern (DAMP) formation. APC activation by DAMP and direct CNS stimulation leads to activation of CD4+ and CD8+ and differentiation of CD4+ T cells to Th1 / Th17 cells. Th1 / Th17 effector cells play an important role in the development of hypertension by producing pro-inflammatory mediators, including ROS, IFN-γ, TNF-α, and IL-17, to encourage low-grade inflammation. ROS is an important medium for the occurrence of hypertension caused by exposure to angiotensin II.11,12

The kidneys play an important role in the metabolism of advanced glycation end products (AGEPs) and modulate the production of ROS. When kidney function has decreased, retention of AGEPs and pro-oxidants will trigger oxidative damage which will contribute to mononuclear cell activation and stimulation of the inflammatory response. This study shows unilateral ureteral ligation can lead to kidney ischemia that causes kidney injury, which can lead to immune system activation and increased production of inflammatory cytokines.12,13 This proinflammatory role is important in the pathogenesis of kidney disease as a result of hemodynamic changes, tubular epithelial cell apoptosis kidney, kidney tissue fibrosis or inflammation.14

Histopathological examination results in this study showed that the UUO group had a significantly increased renal tubulointerstitial fibrosis compared to control group. This result is in accordance with Chevalier’s (2016) study, that the first week after induction of UUO, the inflammatory, vasoactive and apoptotic processes have resulted in a display of signs of tubular atrophy and features of tubulointerstitial fibrosis important at the beginning of UUO, because this event is the foundation for all subsequent developments. An increase in the number of macrophages occurred as early as four hours after UUO in mice. Leukocyte recruitment after UUO involves increased expression of chemokines, chemokine receptors, and adhesion molecules such as osteopontin, galectin-3, and selectin. These induced molecules support systemic recruitment and the local proliferation of macrophages.15 The mechanism of interstitial fibrosis in the development of CKD is related to proteinuria, ROS, vasoactive substances, tubular hypertrophy, hypermetabolism, and endothelial dysfunction.16

The pathophysiological mechanisms that contribute to hypertension are very complex and include inflammation, remodeling, stiffness, calcification, and atherosclerosis. Oxidative stress is generally caused by excess production of ROS, decreased levels of NO and reduced antioxidant ability in the cardiovascular, renal and central nervous system. In physiological conditions, ROS regulates cellular processes, such as differentiation, proliferation, apoptosis, cycles, and cell migration. ROS controls endothelial function and vascular tone, so increased production of ROS and / or weak antioxidant defense mechanisms contribute to endothelial
dysfunction and smooth muscle cells, which ultimately leads to progressive organ failure.\textsuperscript{12,16,17}

An increase in SBP showed severe damage to UUO mice. This could be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by UUO.\textsuperscript{12,18}

UUO can be used as a model of fibrosis and hypertension, which will then be used as a basis for the development of anti-fibrotic and anti-hypertensive drugs.

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