Atorvastatin Ameliorates Tubulointerstitial Fibrosis and Protects Renal Function in Chronic Partial Ureteral Obstruction Cases

John P. Fitzgerald,* Shyan-Yih Chou, Israel Franco, Unni M. Mooppan, Hong Kim, Rajiv Saini and Frederick A. Gulmi

From the Department of Urology, Brookdale University Hospital and Medical Center, Brooklyn and Division of Pediatric Urology, New York Medical College, Valhalla, New York

Abbreviations and Acronyms

α-SMA = α-smooth muscle actin
BW = body weight
GFR = glomerular filtration rate
PE = polyethylene
PUO = partial ureteral obstruction
TBS = tris-buffered saline
TGF-β = transforming growth factor-β
TIF = tubulointerstitial fibrosis
UUO = unilateral ureteral obstruction

Study received institutional animal care and use committee approval.

* Correspondence: Department of Urology, Brookdale University Hospital, 1 Brookdale Pl., Brooklyn, New York 11212 (telephone: 718-240-5324; FAX: 718-240-6155; e-mail: jfitzgerald14@gmail.com).

Purpose: Tubulointerstitial fibrosis, the histological feature of chronic obstructive nephropathy, is delineated in complete unilateral ureteral obstruction models. Histological changes during chronic partial ureteral obstruction are not well studied. We describe changes in a rat model of partial ureteral obstruction. We examined the effects of atorvastatin on histological alterations, fibrosis and function in this model.

Materials and Methods: All rats underwent right nephrectomy. To create partial ureteral obstruction the left ureter was incorporated into the psoas muscle, which was split and reapproximated. Excretory urogram, histology, Western blot of α-smooth muscle actin and renal clearance were examined in rats with sham, 14-day or 30-day partial ureteral obstruction. Obstructed rats received a regular or a diet supplemented with 50 mg/kg body weight atorvastatin per day.

Results: At 14 days of partial ureteral obstruction pyelogram showed hydronephrosis, which was more pronounced on obstruction day 30. Histological studies on obstruction days 14 and 30 revealed tubulointerstitial fibrosis in the medulla and cortex. Atorvastatin significantly decreased tubulointerstitial fibrosis seen in α-smooth muscle actin expression. On obstruction day 14 or 30 the glomerular filtration rate in rats on a regular diet was significantly lower than in sham PUO rats or rats on atorvastatin.

Conclusions: This model of partial ureteral obstruction enables chronic studies of morphological and histological changes of the obstructed kidney. It showed progressive fibrosis and decreased filtration function. Atorvastatin ameliorated fibrosis and helped preserve kidney filtration function.

Key Words: kidney, ureteral obstruction, nephrectomy, atorvastatin, fibrosis

CHRONIC urinary tract obstruction is an important cause of chronic kidney disease and renal failure in all age groups. The decrease in renal function caused by chronic obstruction is associated with kidney structural derangements, characterized by TIF.[1] Initially renal function changes may be reversible. However, with the sustained obstruction and increased pelvic pressure seen in cases of ureteropelvic junction obstruction, ureterovesical obstruction, bladder outlet obstruction or posterior urethral valves permanent renal function loss is accompanied by histological alterations, including tubular dilatation, tubular cell apoptosis and progressive interstitial compartment fibrosis.[1,2] Interstitial fibrosis is a complex process involving inflammatory cell infiltration, fibroblast proliferation, excessive extracellular matrix accu-

0022-5347/09/1824-1860/0
THE JOURNAL OF UROLOGY®

Vol. 182, 1860-1868, October 2009
Printed in U.S.A.
DOI:10.1016/j.juro.2009.04.086

mulation and decreased matrix degradation. Shortly after obstruction inflammatory cells appear in the interstitial space, releasing cytokines and growth factors to stimulate the fibrotic process.[2,3] Many studies show that inhibiting the renin-angiotensin system or TGF-β ameliorates obstruction induced TIF, suggesting that angiotensin II and TGF-β are involved in fibrosis development.[2–6]

Recent studies show that statins provided renal protection in experimental models of urinary tract obstruction.[7,8] Statins have anti-inflammatory and antioxidant actions, and are capable of attenuating fibrogenesis.[9] Agents with antioxidant action provide renal protection in partial obstruction cases.[10,11] These properties of statins are independent of their cholesterol lowering actions and are termed pleiotropic effects.[12,13] In rats with complete UUO for 14 days atorvastatin decreased tubular damage and interstitial fibrosis in the obstructed kidney.[14] In mice with UUO for 10 days fluvastatin ameliorated oxidative stress and fibrosis in the obstructed kidney interstitium.[15] In rats with 3 or 12-day UUO atorvastatin decreased microalbuminuria and improved filtration function after UUO release.[16] All of these studies evaluated the pleiotropic effects of statins on the kidney with complete obstruction. However, to our knowledge it remains unknown whether statins protect the kidney with chronic partial obstruction, as in pediatric conditions such as ureteropelvic obstruction and posterior urethral valves. We investigated the effects of atorvastatin on TIF and renal function in a rat model of chronic PUO.

MATERIALS AND METHODS

Experiments were performed in male Sprague-Dawley rats (Hilltop Lab Animals, Scottdale, Pennsylvania) weighing 275 to 350 gm that were maintained on regular rat chow (PMI Nutrition International, Brentwood, Missouri) or on the same diet supplemented with 50 mg/kg BW atorvastatin per day. Experiments were done in accordance with National Institutes of Health guidelines for the care and use of laboratory animals, and we received institutional animal care and use committee approval.

To create unilateral PUO the rat was anesthetized with 50 mg/kg sodium pentobarbital intraperitoneally. A low midline abdominal incision was made and right nephrectomy was performed. The right kidney was mobilized with minimal dissection. Two small surgical clips were applied to the hilar vessels and the kidney was removed. After the bladder was identified the left ureter was traced to its insertion in the bladder, mobilized, isolated with minimal dissection and retracted off the field with vessel loops. The psoas muscle was identified and split by blunt dissection. A space was created in the muscle to accommodate two-thirds of the ureteral length. The left ureter was moved into the interstice. The muscle was reapproximated with 4, 5-zero silk sutures in interrupted fashion. The abdominal wound was closed with sutures. For sham operation the rat underwent the same surgical procedures as for unilateral PUO, including removal of the right kidney (right nephrectomy), but the left ureter was not obstructed. After recovery from anesthesia the rats were housed individually in metal cages and given 0.02 mg/kg BW buprenorphine hydrochloride intramuscularly to relieve postoperative discomfort. Before surgery and clearance experiments the rats fasted but had free access to water.

Clearance Experiments

Renal clearance experiments were performed in sham operated rats with PUO. Rats were anesthetized with 50 mg/kg sodium pentobarbital intraperitoneally and placed on a heating pad to maintain body temperature at 37C. Tracheostomy was performed and the jugular vein was cannulated with a PE-50 catheter to infuse solutions and additional sodium pentobarbital doses. A carotid artery was catheterized with a PE-50 catheter to continuously monitor arterial blood pressure and sample blood. Rats were infused with 0.9% saline at 30 μl per minute during surgery to maintain euvolemia. In sham operated rats and rats with PUO a low midline incision was made. To collect urine the bladder was cannulated with a PE-240 catheter secured in place with a 3-zero silk suture. The abdomen was closed to minimize heat and water loss. Inulin (10%) was added to the infusion solution to measure GFR. Sodium pentobarbital was administered intravenously as needed to maintain proper anesthesia.

Postoperatively the rats were equilibrated for 60 minutes before clearance measurements. During 3 consecutive 30-minute clearance periods urine was collected at the mid point of each period. A 2 ml arterial blood sample was obtained at the first and last 30-minute period. Hematocrit was measured in collected blood. At the end of clearance experiments the rats were sacrificed with a lethal dose of sodium pentobarbital administered intravenously.

Clearance experiments were performed in 6 groups of 6 rats each, including group 1—rats with 14-day sham PUO fed a regular diet studied 14 days after right nephrectomy, group 2—rats with 14-day PUO plus a regular diet studied 14 days after right nephrectomy and PUO, group 3—rats with 14-day PUO plus atorvastatin maintained on the same diet as group 1 but supplemented with atorvastatin and studied 14 days after right nephrectomy and PUO, group 4—rats with 30-day sham PUO fed a regular diet studied 30 days after right nephrectomy, group 5—rats with 30-day PUO plus the same diet as group 4 studied 30 days after right nephrectomy and PUO, and group 6—rats with 30-day PUO plus atorvastatin maintained on the same diet as group 4 but supplemented with atorvastatin and studied 30 days after right nephrectomy and PUO (fig. 1).

Urine volume was measured gravimetrically and blood cells were separated from plasma by centrifugation. Hematocrit was measured by centrifugation and visual measurement on standard scale. Plasma and urine inulin was measured by standard spectrophotometry. GFR was calculated by standard inulin clearance technique.