Ureter, the Guardian Angel of Nephron: The Impact of Pelvicalyceal System on Apoptotic Activity in an Experimental Obstructive Uropathy Model

Nefronun Koruyucu Meleği Üreter: Deneysel Obstrüktif Üropati Modelinde Pelvikalisiyel Sistemin Apoptotik Aktivite Üzerine Etkisi

Serhat Gürocak[1], Volkan Ergin[2], Ahmet Cumaoğlu[3], İyimser Üre[1], İpek Işık Gönül[4], Aysel Arıcıoğlu[3], İbrahim Bozkırlı[1] Adnan Menevşe[2]

[1]Gazi University, Faculty of Medicine, Department of Urology, Ankara, Turkey
[2]Gazi University, Faculty of Medicine, Department of Medical Biology and Genetics, Ankara, Turkey
[3]Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey
[4]Gazi University, Faculty of Medicine, Department of Pathology, Ankara, Turkey

ABSTRACT

Objective: We aimed to assess the impact of obstructive uropathy and its revision on renal apoptotic and inflammatory activity in an experimental model in rabbits.
Methods: Twenty-four rabbits were separated into four different groups. Ureteropelvic junction (UPJ) obstruction model was performed in Group 1 and 2. Ureterovesical junction (UVJ) obstruction model was performed in Group 3 and 4. Thirty days after the operations, bilateral nephrectomy was performed in Group 1 and 3. Thirty days after the initial surgery, the obstruction was revised in Group 2 and 4. Thirty days after the revision surgeries, bilateral nephrectomy was performed in Group 2 and 4. The apoptotic and inflammatory activity was measured at the protein level and nephrectomy specimens were examined histologically.
Results: Caspase 3 levels were significantly higher in operated left kidneys than the levels obtained in the right counterparts. The increase in procaspase 9 and caspase 3 activities in the UVJ obstruction group was less than those of the UPJ obstruction group ($p<0.05$, $p<0.01$, respectively). After the revision of obstruction, the decrease in procaspase 9 and caspase 3 activities was more significant in Group 4 than in Group 2 ($p<0.05$, $p<0.01$, respectively). Cyclooxygenase-2 expression decreased insignificantly in Group 2 and 4 when compared to Group 1 and 3 ($p>0.05$, $p>0.05$, respectively).
Conclusion: The rabbits with a UVJ obstruction had lower apoptotic indexes as compared to those with a UPJ obstruction. The apoptotic activity decreased to almost normal levels following an adequate revision of the obstruction in both groups, but this improvement was more significant in animals with a UVJ obstruction.

Key Words: Ureteral obstruction, inflammation, genetics

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ÖZET

Amaç: Bu çalışmamızda deneysel obstrüktif üropati modeli uyguladığımız tavşanlarda pelvikalisiyel sistemin renal apoptotik aktivite üzerine etkisini değerlendirmeyi amaçladık.
Yöntemler: Yirmi altı adet tavşan obstrüktif üropatinin seviyesine göre 4 gruba ayrıldı. Birinci ve ikinci gruba üreteropelvik (UP) darlık modeli uygulandıktan sonra 1.gruba postoperatif 30.günde bilateral nefrektomi uygulandı. İkinci gruba postoperatif 30.gün piyeloplasti uygulandıktan 30 gün sonra bilateral nefrektomi yapıldı. Üçüncü ve 4. gruba ise üreterovezikal (UV) darlık modeli uygulandıktan sonra 3.gruba postoperatif 30.günde bilateral nefrektomi uygulandı. Dördüncü gruba postoperatif 30.gün üreteroneosistostomi uygulandıktan 30 gün sonra bilateral nefrektomi yapıldı. Nefrektomi materyallerindeki apoptotik aktivitenin incelenmesi amacıyla western blot yöntemleriyle apoptotik genlerin protein düzeyinde ifadelenmeleri çalışıldı.
Bulgular: UP ve UV obstrüksiyon uygulandığında her iki grupta da apoptotik aktivite yükselmesine rağmen UV obstrüksiyondaki artış UP obstrüksiyona göre daha azdı ($p:0.023$, $p<0.001$). Obstrüksiyon revize edildiğinde prokaspaz 9 ve kaspaz 3 aktiviteleri anlamlı şekilde geriledi ($p<0.001$, $p<0.001$). Bu gerileme UV obstrüksiyonun revizyonunda daha fazlaydı ($p:0.011$, $p<0.001$).
Sonuç: UV darlığın nefron üzerindeki moleküler etkisi UP darlığa göre daha benin seyretmektedir. Bunun muhtemel sebebinin UP ile UV bileşke arasındaki üreterin obstrüksiyona sekonder oluşan basınç artışını anlamlı şekilde kompanse edebilme yeteneğine ve böylece nefrona bu basıncın daha az yansımasına bağlı olduğunu düşünmekteyiz.

Anahtar Sözcükler: Üreteral obstrüksiyon, inflamasyon, genetik

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Address for Correspondence / Yazışma Adresi: İyimser Üre, MD, Gazi University Faculty of Medicine, Department of Urology, Ankara, Turkey, Phone: +90 530 347 33 01, E-mail: iyimserure@yahoo.com

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INTRODUCTION

Physiological turnover of the cell including both cell proliferation and programmed cell death is essential for preserving the functional integrity of the kidney. Obstructive uropathy (OU), a major cause of chronic renal failure in childhood, is characterized by the decreased cell proliferation and increased apoptosis (1). The ureteropelvic junction (UPJ) and ureterovesical junction (UVJ) obstruction are among the most common known causes of the obstructive uropathy in children (2). Both the pelvicalyceal system and the ureter are dilated in the UVJ obstruction as compared to the UPJ obstruction.

The interstitial inflammation is an important factor contributing to the early damage of nephrons and the renal failure in OU. It constitutes an early response to the unilateral urinary obstruction (3) and this response is mainly mediated by the renal infiltration by macrophages that are attracted by a variety of cytokines and chemokines (4). The inflammation leads to the generation of reactive oxygen species (ROS) (5). The overproduction of ROS has been identified as a key component of apoptotic pathways involving the activation of endogenous endonucleases and the direct DNA fragmentation (6).

Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins. Caspase-9 is an important initiator caspase, and upon an apoptotic stimulation, caspase-9 further processes other caspase members including caspase-3 to initiate a cascade, which leads to apoptosis (7). Apoptosis has a significant role in the pathogenesis of the renal cellular injury derived from urinary tract obstruction, and the renal tubular cell damage caused by increased hydrostatic pressure is one of the factors regulating the renal apoptotic response. It can provide a powerful mechanical stimulus to apoptosis in the obstructed kidney (8,9). A probable pressure reducer can be the collecting duct itself. The expandable structure of the whole collecting system, including the pelvicalyceal system and the ureter, can balance this pressure overload on the tubular cells.

In this study, our aim was to investigate the differences of apoptotic and inflammatory activity in kidneys with a ureteropelvic or ureterovesical junction obstruction by creating two different obstructive uropathy models in rabbits.

MATERIAL AND METHOD

Twenty-four rabbits were separated into four groups. A UPJ obstruction model was created in the first and the second groups, and a UVJ obstruction model was created in the third and the fourth groups initially. Thirty days after the initial operations, bilateral nephrectomies were performed in the first and the third groups. In the second and the fourth groups, rabbits underwent revision and the obstruction was relieved by surgery at the 30th day. In the second and the fourth groups, bilateral nephrectomies were performed 30 days after the revision. Nephrectomy specimens were evaluated in order to measure the apoptotic activity at protein level, and the pathological specimens were examined by light microscopy following the routine tissue processing.

Surgical Method

UPJ obstruction model

A two-cm-long left flank incision was applied to the rabbits. After the medial deflection of the intestinal structures, we advanced to the retroperitoneal space. Kidney was identified and the dissection of the hilum was performed. The modification of the partial ureteral obstruction as defined by Shokeir et al. was used in our model (10). Renal pelvis and ureteral junction were found and suspended. A 0,035-mm guide wire was placed parallel and adjacent to the proximal ureter. A 2/0 silk suture was inserted around the wire and the ureteropelvic junction, and then the suture was tied. The loop point of the suture was lubricated with the lubricating jelly in order not to harm the junction point, and the guide was removed. By this way, ureteropelvic junction size was reduced to the diameter of the wire, and a partial obstruction model was formed (Figure 1a).

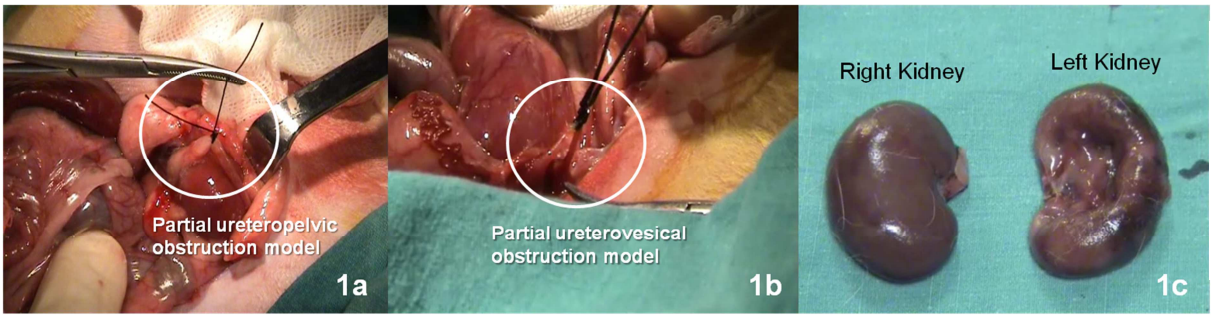

Figure 1: Macroscopic view of;
a) Partial ureteropelvic obstruction model
b) Partial ureterovesical obstruction model
c) The right and left kidneys after sacrifice.

UVJ obstruction model

A two-cm- long left inguinal incision was applied to the rabbits. Following the superior deflection of the intestinal structures, we advanced to bladder. The left ureter was found and the dissection of the distal portion of the left ureter was performed. The same partial obstruction model was applied to the UV junction. (Figure 1b).

Revision and obstruction release surgery

Rabbits underwent the same incisions according to their previous surgery, and the same procedures were applied to dissect the junction points. The silk sutures were found in the places which they were applied to 30 days ago. They were cut and removed. A ureteral re-implantation and pyeloplasty were performed by using 7/0 sutures.

Genetic Method

Quantitative Real-time PCR

The total RNA isolation from kidney tissue was performed by phenol-guanidine thiocyanate extraction using RNAzol RT reagent (Molecular Research Center, Inc. Cincinnati, OH) according to the manufacturer's instructions. Total RNA was reverse-transcribed to cDNA using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) in a 20 µL reaction mixture. Random hexamers were used to prime the cDNA synthesis. A real-time PCR was performed using a Light Cycler 480 II System (Roche Diagnostics, Mannheim, Germany). To quantify cDNA, we performed Real-time quantitative PCR using LightCycler 480 Probe Master mix and hydrolysis probes. Primer and probe used in Real-time PCR for the gene were as follows. Cyclooxygenase-2 (COX-2) forward: 5'-CGGAATTTCTGACCAGAATCA-3', reverse: 5'-AAGGATGTAGTGCACCGTGTC-3' (UPL probe #68); Real-time PCR was carried out in 20 µL of a reaction mixture containing 10 µL of 2X Master reaction mix (Roche Diagnostics GmbH, Mannheim, Germany), 0.25 pmol of primer (Alpha DNA, Montreal, QC, Canada), 0.1 µM of probe, and 1 µL of cDNA. Thermocycling conditions were: DNA denaturation at 95°C for 10 minutes, followed by 45 amplification cycles for 10 seconds at 95°C and 20 seconds at 60°C and finally a cooling step to 40°C. The mRNA level was normalized to GAPDH level.

Western Blot Analysis

Tissue samples were homogenized with lysis buffer. Lysates were sonicated and incubated on ice for 5 min before centrifugation at 13,000 *g* for 15 min at 4^0C. Supernatants were transferred to microcentrifuge tubes, protein contents were measured using a BCA protein assay kit (PIERCE, Rockford, IL, USA). For electrophoresis, 30 µg of each homogenate was prepared with 4X Laemmli sample buffer and incubated at 95^0C for 5 min. The samples were loaded into SDS-PAGE gels, and electrophoresis was performed, followed by transfer to PVDF membranes. Nonspecific binding sites were blocked by treating membranes in 5% non-fat dried milk in TBS-Tween 20 for 1 h at the room temperature. To study protein expression, membrane blots were incubated with polyclonal cleaved caspase-3, procaspase-3, procaspase 9, and GAPDH (Stressgen, San Diego, CA) antibody diluted in TBS-Tween 20 0.1% plus non-fat dried milk 5%. Thereafter, membranes were washed three times for 10 min and incubated for 1 h at RT with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Beverly, MA, USA). Immuno-detection was performed with SuperSignal West Pico Chemiluminescent Substrate system (PIERCE, Rockford, IL, USA), according to the manufacturer's instructions and then exposed to CL-Xposure film (PIERCE, Rockford, IL, USA). Western blots were quantified using the Image J 1.32 software after densitometric scanning of the films.

Pathological Examination

Nephrectomy specimens from each animal were fixed in 10% buffered formalin for 12 hours and processed for routine paraffin embedding. The 4 µm sections were prepared from paraffin blocks and stained with hematoxylin-eosin, trichrome and periodic acid-Schiff (PAS) stains. The tubulointerstitial injury was examined semi quantitatively in renal cortical parenchyma, with X20 objective and scored on a scale of 0 to 4, as follows: 0, no tubulointerstitial injury, 1, <25% of the tubulointerstitium injured; 2, 25-50% of the tubulointerstitium injured; 3, 51 to 75% of the tubulointerstitium injured; 4, >75% of the tubulointerstitium injured (11). Tubulointerstitial injury was defined as tubular dilation, tubular atrophy, sloughing of tubular epithelial cells and interstitial fibrosis with inflammation. Interstitial fibrosis was examined on Masson's trichrome stained sections. The presence or absence of focal segmental and global glomerulosclerosis in the renal cortical parenchyma was also recorded. Since neither of them was observed in the nephrectomy specimens, scoring was not applied.

Statistics

Expression of the target genes' mRNA relative to that of the housekeeping gene mRNA was calculated using the relative expression software tool (REST 2009, V2.0.13), and statistical significance was determined using the pair-wise fixed reallocation randomization test. *P*<0.05 was considered statistically significant. Statistical analysis was performed by the Student's t test.

Ethical Committee

This study was approved by Gazi University Ethical Committee with document number GÜET-08.054 and date 17/12/2008.

RESULTS

Normalization of procaspase-9 and procaspase-3 levels according to GAPDH was performed. Relative expression of procaspase-9 and procaspase-3 of the right kidneys were accepted as 1. Procaspase-3 levels were significantly higher in the left operated kidneys than the levels obtained in the right counterparts (Figure 2). When procaspase-9 expression levels were considered, there was no statistically significant difference between the right and the left kidneys. When we gave a statistical score of 1 to the procaspase-9 and cleaved caspase-3 levels in UPJ obstruction, we found that scores for procaspase-9 and cleaved caspase-3 levels were 0.84 and 0.82 respectively (Figure 3). Similarly, scores of procaspase-9 and cleaved caspase-3 levels were found to be 0.86 and 0.84 respectively in the UVJ obstruction. After the revision of UVJ obstruction, scores decreased to 0,67 and 0,6 respectively.

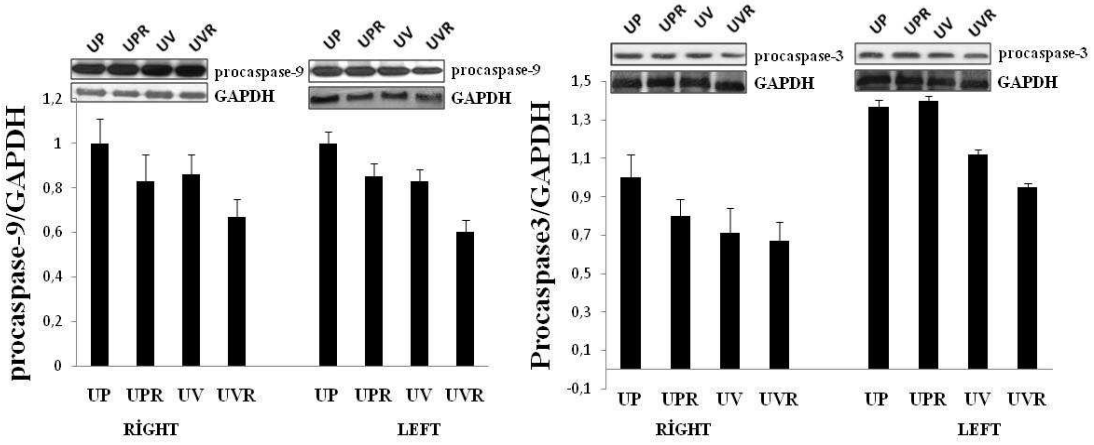

Figure 2: Procaspase-9 and active procaspase-3 expression level comparison between the right control and the left operated kidneys.
UP: Ureteropelvic obstruction; UPR: Ureteropelvic obstruction revision; UV: Ureterovesical obstruction; UVR: Ureterovesical obstruction revision.

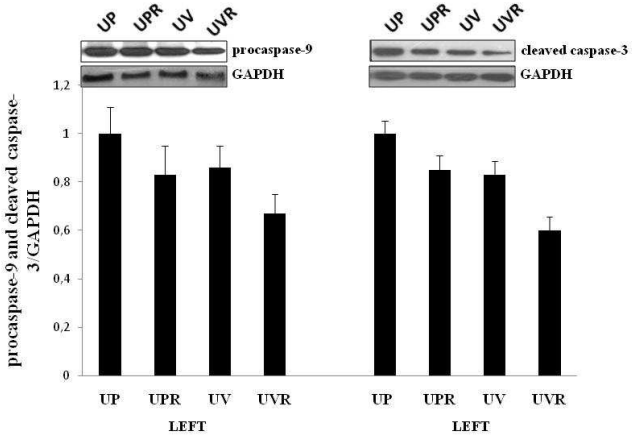

Figure 3: Procaspase-9 and cleaved caspase-3 expression levels in each group of the left operated kidneys. UP: Ureteropelvic obstruction; UPR: Ureteropelvic obstruction revision; UV: Ureterovesical obstruction; UVR: Ureterovesical obstruction revision.

To determine the role of inflammation in the pathogenesis of obstructive uropathy, normalization of COX-2 levels according to GAPDH was performed. When the relative expression of COX-2 levels in UPJ obstruction was accepted as 1, revision of obstruction led to the relative expression level of 0.79 for COX-2. In UVJ obstruction, the relative expression of COX-2 level was 0,74. After the revision of the UVJ obstruction, the relative expression of COX-2 level decreased to 0.38 (p<0.05) (Figure 4).

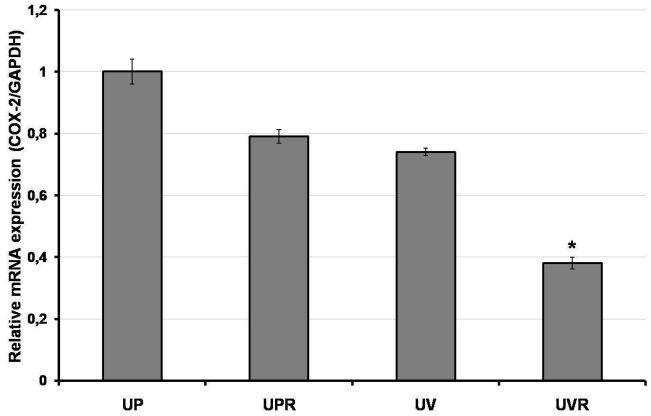

Figure 4: Relative mRNA expression levels of COX-2 gene in each group of the left operated kidneys. * p< 0.05 *vs.* UP group. UP: Ureteropelvic obstruction; UPR: Ureteropelvic obstruction revision; UV: Ureterovesical obstruction; UVR: Ureterovesical obstruction revision.

The increase in procaspase-9 and cleaved caspase-3 activities in the ureterovesical obstruction group was less than that of the ureteropelvic obstruction group (p<0.05, p<0.01, respectively).

After the revision of obstruction, the decrease in procaspase-9 and cleaved caspase-3 activities was more significant in Group 4 than in Group 2 ($p<0.05$, $p<0.01$, respectively). The decrease in COX-2 expression was insignificant in Group 2 and 4 when compared to Group 1 and 3 ($p>0.05$, $p>0.05$, respectively).

Histopathologic Findings

Although mean histopathological scores of interstitial fibrosis, interstitial inflammation and tubular atrophy were less severe in the revised group of UPJ obstruction as compared to the unrevised counterpart, only the score for interstitial fibrosis showed a statistically significant difference between the groups ($p<0.05$). The results for the UVJ obstruction group and its revised counterpart were similar for the pathologies examined and were not statistically significant (p value for interstitial fibrosis: >0.05, p value for interstitial inflammation: >0.05 and p value for tubular atrophy: >0.05).

Mean scores obtained from histopathological analysis were summarized in Table 1. Figure 4 represents the histopathological findings of renal parenchyma in different groups.

Table 1. Mean histopathologic scores of each groups.

	Interstitial Fibrosis	Interstitial Inflammation	Tubular Atrophy	Tubular Dilatation
Group 1	3.8	2.4	3	2.2
Group 2	2	2	2	3
p value	**p<0.05**	p>0.05	p>0.05	p>0.05
Group 3	2.5	2.33	3	2.5
Group 4	1.83	1.5	1.83	2.83
p value	**p<0.05**	p>0.05	p>0.05	p>0.05

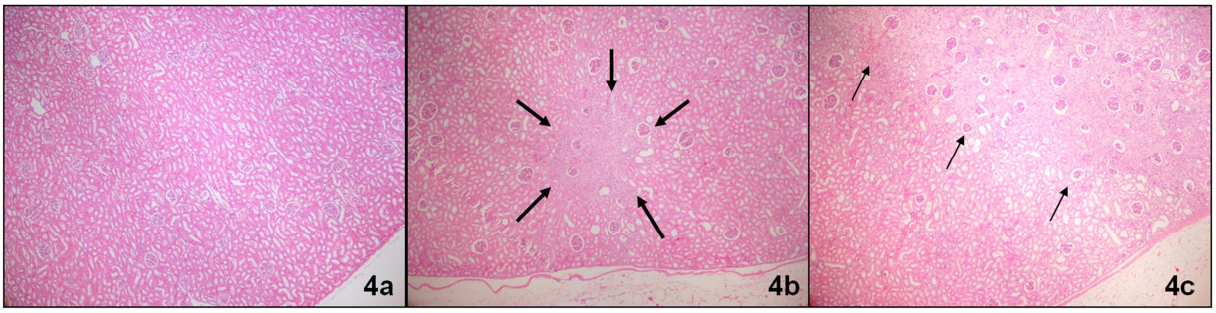

Figure 4: a. Normal cortical parenchyma of a right rabbit kidney, without any inflammation or fibrosis and tubular atrophy, H&E X40. **b**. Minute cortical scar characterized by focal fibrosis with atrophic tubules and accompanying mild inflammatory infiltrate, in the kidney of a rabbit with ureterovesical obstruction, group 3, H&E X 40. **c.** Moderately large segmental scarring area of the rabbit kidney, contrasting with the surrounding normal looking cortical parenchyma, group 1, H&E X40.

DISCUSSION

The urinary obstruction results in hydronephrosis which can cause renal parenchymal damage. The level, severity and duration of obstruction causes varying degrees of obstructive uropathy. The effects of obstructive nephropathy, including apoptosis and cell death, glomerulotubular injury, tubular dilatation, interstitial inflammation, and progressive interstitial fibrosis (12), can be considered as the result of consecutive cellular events. Progressive fibrosis is triggered by interstitial inflammatory cell infiltration (13), myofibroblastic activation and extracellular matrix deposition (14,15). Renal tubular apoptosis is activated by transforming growth factor-β1(TGF-β1) (16), tumor necrosis factor-α (TNF-α) (17), Fas (18), p53 (19) and caspases (17). The down regulation of some growth factors such as epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) facilitates the progression of apoptosis as well (20).

Increased activity of the renin-angiotensin system and other vasoconstrictor systems are the first hemodynamic changes observed in the obstructed kidneys (18). The amount of urine increases in the collecting system, and this leads to the increase in the interstitial pressure. Accordingly, the hydrostatic pressure reflecting on the tubules increases, and the tubular distention damages the tubular epithelial cells (21). An interstitial inflammatory response is initialized by a macrophage infiltration afterwards. COX-2 has an essential role in the synthesis of prostaglandins which are associated with the inflammatory process. Norreguard et al. investigated the contribution of COX-2 to ureteric motility and pelvic pressure after obstruction (22). They showed increased COX-2 labeling in surface epithelium and smooth muscle layers of both rat and human proximal dilated ureters as compared to control ureters. They concluded that COX-2 activity contributes to the increased pelvic pressure after obstruction. In our study, the expression level of COX-2 was highest in the UPJ obstruction model. After the revision procedures, COX-2 expression levels decreased insignificantly both in UPJ and UVJ obstruction models. These results show that the inflamatory burden reached in UPJ obstruction is much more as compared to the UVJ obstruction, and their revision provides a reasonable recovery.

There is also a massive myofibroblastic accumulation in the interstitium. These cells lead to the contraction of the kidney parenchyma and the interstitial fibrosis. In our study, interstitial fibrosis increased after partial obstruction at UPJ and UVJ. After revision, histological scores significantly returned to the normal levels. Moreover, reactive oxygen species are significantly increased in the obstructed kidneys, and this is a factor that reduces the threshold of apoptosis (23). One would expect that the results observed in the apoptotic activity would be paralelled by the histopatological data, but the difference was not significant except for the interstitial fibrosis in our study. Perhaps this discrepancy may be the result of a more sensitive response to the increased intraluminal hydrostatic pressure at the molecular level than at the tissue level.

The relief of obstruction carries upmost importance for the recovery of uropathy but the appropriate timing for the treatment is still controversial for different obstruction entities. In this manner, many studies with different obstruction durations have investigated the recovery of the kidney with improved renal functions (24,25). Recently, Soliman et al. (26) in a unilateral ureteral obstruction model for four weeks, showed the recovery of renal functions by ≈ 51% of baseline values at 8 weeks after the relief of ureteral obstruction. In addition, Zhou et al. demonstrated that apoptosis in the renal parenchyma was significantly increased in the the obstructed group as compared to sham group, especially at the 28th day in an experimental animal model (27). Since apoptotic index was scientifically proved to be increased at the 30th postoperative day for the revision of the obstruction in animal models, we have also chosen this day for the revision of obstruction in our study. We found significant difference in procaspase-9 and procaspase-3 levels between the control right kidneys and the operated left counterparts. The expression levels in the right kidneys were significantly lower than the left kidneys. We can draw a conclusion that our model of ureteral obstruction at different anatomical localizations was successful.

With regard to the idea of the harmful effect of increasing pressure in the collecting system, the volume of the affected collecting duct may determine the progression of tubuloepithelial damage. Mechanical forces from hydronephrotic distension are transmitted to the renal tubular cells (28), and the mechanical stretch related to the increase of intratubular pressure might in part mediate obstruction-induced apoptosis (29). The mechanism of the stretch-induced apoptosis has been studied in renal epithelial cells by Nguyen et al (30). In their model, certain cell lines of various components in nephron underwent cyclic stretch and apoptotic index of these cell lines was determined (30). Stretched cells displayed an increased activity of caspase-3 and -9, and apoptosis was modulated by the inhibition of caspase-3 and to a lesser extent by caspase-9. They concluded that the stretch induced apoptosis in renal epithelial cells is dependent on the activation of caspase-3 and 9. In our study, similar to Nguyen et al, we found that procaspase-3 expression levels increased but procaspase-9 levels were not altered after the obstruction of UPJ and UVJ in left kidneys when compared to the normal right counterparts.

In addition, this increased apoptotic activity was more prominent in the UPJ as compared to the UVJ. In this respect, ureterovesical obstruction may provide more volume in terms of decreasing the pressure reflecting on the tubular cells with the significant contribution of ureter volume to the collecting duct. Therefore, ureter may be acting like the guarding angel of the nephron in this case.

Because of its severe expandability, ureter can reach significant volumes. Due to the relieving effect, less pressure might be reflected to the renal parenchyma, and this might lead to the less stretched nephrons.

Moreover, Nguyen et al. found that a 5–25% change in the maximal radial stretch did result in an increase in apoptosis, but the frequency of stretch did not affect the outcome (30). This indicates that the stretch-induced apoptosis is dependent upon maximal radial change rather than the frequency of cyclic stretch. Our study results were consistent in that rabbit kidneys with a UVJ obstruction had rather more benign expression levels of apoptotic proteins than those with a UPJ obstruction. Unfortunately, our study was limited in terms of interpreting the volume changes in each group. It would be more illustrative, if data relative to the pyelocalyceal pressures and diameters could also be pre- and post-operatively evaluated and presented, endorsing our conclusions.

The results of our study showed that the relief of obstruction gives a hand for better improvement for renal function after obstructive uropathy. Apoptotic expression levels were recorded at the lowest level in the UVJ revision group. This is further logical in that the group of UVJ obstruction suffered less than the group of UPJ before the revision. And especially in low volume obstructions like UPJ obstruction due to the low compensation capacity of the renal pelvis, we could reach better molecular results in the UVJ group. However, we approach our results critically in that we did not perform a radioisotope renography following the revision surgeries to determine whether we have achieved a non-obstructive curve in our study. This could be a subject of further studies in this issue.

CONCLUSION

The rabbits with a UVJ obstruction had lower apoptotic indexes as compared with those with a UPJ obstruction. The apoptotic activity decreases to almost normal levels after adequate revision of obstruction in both groups, but this improvement is more significant in the UVJ obstruction.

Conflict of Interest

No conflict of interest was declared by the authors.

REFERENCES

1. Chevalier RL. Molecular and cellular pathophysiology of obstructive nephropathy. Pediatr Nephrol Berl Ger. 1999;13:612-9.

2. Chevalier RL. Perinatal obstructive nephropathy. Semin Perinatol. 2004;28:124-31.

3. Misseri R, Rink RC, Meldrum DR, Meldrum KK. Inflammatory mediators and growth factors in obstructive renal injury. J Surg Res. 2004;119:149-59.

4. Kluth DC, Erwig L-P, Rees AJ. Multiple facets of macrophages in renal injury. Kidney Int. 2004;66:542-57.

5. Gupta A, Gupta A, Nigam D, Shukla GS, Agarwal AK. Profile of reactive oxygen species generation and antioxidative mechanisms in the maturing rat kidney. J Appl Toxicol Jat. 1999;19:55-9.

6. Fernandez A, Kiefer J, Fosdick L, McConkey DJ. Oxygen radical production and thiol depletion are required for Ca(2+)-mediated endogenous endonuclease activation in apoptotic thymocytes. J Immunol Baltim Md 1950. 1995;155:5133-9.

7. Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, et al. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J Cell Biol. 1999;144:281-92.

8. Manucha W, Carrizo L, Ruete C, Vallés PG. Apoptosis induction is associated with decreased NHE1 expression in neonatal unilateral ureteric obstruction. Bju Int. 2007;100:191-8.

9.Nguyen HT, Bride SH, Badawy AB, Adam RM, Lin J, Orsola A, et al. Heparin-binding EGF-like growth factor is up-regulated in the obstructed kidney in a cell- and region-specific manner and acts to inhibit apoptosis. Am J Pathol. 2000;156:889-98.

10. Shokeir AA. Partial ureteral obstruction: a new variable and reversible canine experimental model. urology. 1995;45:953-7.

11. Sheerin NS, Sacks SH. Chronic interstitial damage in proteinuria. Does complement mediate tubulointerstitial injury? Kidney Blood Press Res. 1999;22:47-52.

12. Chevalier RL. Obstructive nephropathy: towards biomarker discovery and gene therapy. Nat Clin Pract Nephrol. 2006;2:157-68.

13. Schreiner GF, Harris KP, Purkerson ML, Klahr S. Immunological aspects of acute ureteral obstruction: immune cell infiltrate in the kidney. Kidney Int. 1988;34:487-93.

14. Sharma AK, Mauer SM, Kim Y, Michael AF. Interstitial fibrosis in obstructive nephropathy. Kidney Int.1993;44:774-88.

15. Vaughan ED Jr, Marion D, Poppas DP, Felsen D. Pathophysiology of unilateral ureteral obstruction: studies from Charlottesville to New York. J Urol. 2004;172(6 Pt 2):2563-9.

16. Miyajima A, Chen J, Lawrence C, Ledbetter S, Soslow RA, Stern J, et al. Antibody to transforming growth factor-beta ameliorates tubular apoptosis in unilateral ureteral obstruction. Kidney Int. 2000;58:2301-13.

17. Misseri R, Meldrum DR, Dinarello CA, Dagher P, Hile KL, Rink RC, et al. TNF-alpha mediates obstruction-induced renal tubular cell apoptosis and proapoptotic signaling. Am J Physiol Renal Physiol. 2005;288:F406-11.

18. Hughes J, Johnson RJ. Role of Fas (CD95) in tubulointerstitial disease induced by unilateral ureteric obstruction. Am J Physiol. 1999;277(1 Pt 2):F26-32.

19. Choi YJ, Mendoza L, Rha SJ, Sheikh-Hamad D, Baranowska-Daca E, Nguyen V, et al. Role of p53-dependent activation of caspases in chronic obstructive uropathy: evidence from p53 null mutant mice. J Am Soc Nephrol Jasn. 2001;12:983-92.

20. Chung KH, Chevalier RL. Arrested development of the neonatal kidney following chronic ureteral obstruction. J Urol. 1996;155:1139-44.

21. Grande MT, Pérez-Barriocanal F, López-Novoa JM. Role of inflammation in túbulo-interstitial damage associated to obstructive nephropathy. J Inflamm Lond Engl. 2010;7:19.

22. Nørregaard R, Jensen BL, Topcu SO, Nielsen SS, Walter S, Djurhuus JC, et al. Cyclooxygenase type 2 is increased in obstructed rat and human ureter and contributes to pelvic pressure increase after obstruction. Kidney Int. 2006;70:872-81.

23. Kayanoki Y, Fujii J, Islam KN, Suzuki K, Kawata S, Matsuzawa Y, et al. The protective role of glutathione peroxidase in apoptosis induced by reactive oxygen species. J Biochem (Tokyo). 1996;119:817-22.

24. Chevalier RL, Thornhill BA, Wolstenholme JT. Renal cellular response to ureteral obstruction: role of maturation and angiotensin II. Am J Physiol. 1999;277(1 Pt 2):F41-7.

25. Chevalier RL, Smith CD, Wolstenholme J, Krajewski S, Reed JC. Chronic ureteral obstruction in the rat suppresses renal tubular Bcl-2 and stimulates apoptosis. Exp Nephrol. 2000;8:115-22.

26. Soliman SA, Shokeir AA, Mosbah A, Abol-Enein H, Barakat N, Abou-Bieh E, et al. Recoverability of renal function after relief of chronic partial unilateral ureteral obstruction: study of the effect of angiotensin receptor blocker (losartan). urology. 2010;75:848-52.

27. Zhou T-B, Qin Y-H, Zhou C, Lei F-Y, Zhao Y-J, Chen J, et al. Less expression of prohibitin is associated with increased caspase-3 expression and cell apoptosis in renal interstitial fibrosis rats. Nephrol Carlton Vic. 2012;17:189-96.

28. Harris KP, Klahr S, Schreiner G. Obstructive nephropathy: from mechanical disturbance to immune activation? Exp Nephrol. 1993;1:198-204.

29. Cachat F, Lange-Sperandio B, Chang AY, Kiley SC, Thornhill BA, Forbes MS, et al. Ureteral obstruction in neonatal mice elicits segment-specific tubular cell responses leading to nephron loss. Kidney Int. 2003;63:564-75.

30. Nguyen HT, Hsieh MH, Gaborro A, Tinloy B, Phillips C, Adam RM. JNK/SAPK and p38 SAPK-2 mediate mechanical stretch-induced apoptosis via caspase-3 and -9 in NRK-52E renal epithelial cells. Nephron Exp Nephrol. 2006;102:e49-61.