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Elevated tubular proteinuria, albuminuria and decreased urinary N-acetyl-β-D-glucosaminidase activity following unilateral total ureteral obstruction in rats

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Abstract Urinary tubular proteinuria and N-acetyl-β-D-glucosaminidase (NAG) activity has not yet been studied after unilateral total ureteral obstruction (UTO). The aim of the study was (1) to evaluate in a longitudinal study (7 weeks) the behaviour and the potential clinical value of tubular proteinuria and urinary NAG activity after UTO; (2) to study the physiopathology of the non-obstructed contralateral kidney by using these two different markers of tubular damage. Methods: in 28 female, adult Wistar rats (UTO: $n = 16$, sham: $n = 12$), tubular proteinuria and urinary NAG activity were measured before and 1 and 5 weeks after surgery. Results: a significant ($P < 0.01$) increase in tubular proteinuria/creatinine ratio and urinary creatinine and a decrease in urinary NAG activity was found 1 week after UTO. All parameters normalized after 6 weeks. Albuminuria increased progressively ($P < 0.01$) during the study. Conclusion: tubular proteinuria increases during the first week following UTO in rats. The initial increase of low molecular weight proteins following UTO is not due to tubular damage as no parallel increase of urinary NAG was found. We suggest an initial tubular overperfusion with primary urine, due to an increased single nephron glomerular filtration and overruling the reabsorption capacity of the proximal tubules.

Key words Ureteral obstruction · Proteinuria · Hydronephrosis · Glucosaminidase · Tubular proteins

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Introduction

All ureteral obstruction rapidly provokes renal tubular damage [5, 9, 12, 13, 20] and in general increases tubular proteinuria and urinary enzymes. However, no increased tubular proteinuria or urinary N-acetyl-β-D-glucosaminidase (NAG) excretion are expected after unilateral total ureteral obstruction. As far as we know, no studies have yet evaluated tubular proteinuria or renal enzymes after a prolonged total unilateral ureteral obstruction.

It has been suggested that tubular proteinuria or renal enzymuria allows the evaluation of proximal tubular damage [14]. Tubular proteins are proteins with a low molecular mass (< 68 kDa) that are as such filtered across the glomerulus and are very efficiently reabsorbed by the proximal tubule. Only extremely low concentrations of these micromolecular proteins appear in urine in physiological situations. Malfunction of this reabsorption mechanism results in leakage of these small proteins in the urine (Fig. 1). This so-called tubular proteinuria has been observed after partial ureteral obstruction, intoxication with heavy metals, diabetes and pyelonephritis. Several small proteins have been described: β-2-microglobulin, retinol-binding protein and the α-1-microglobulin [7, 13, 14, 16, 17]. Renal enzymes such as γ-glutamyl transferase (a brush-border enzyme), lysozyme and NAG (both lysozomal enzymes of the tubular cells) appear in the urine after injury to the tubular cell membranes [7, 14, 16]. NAG is also found in serum but is not filtered. It appears in the urine after damage of the cell membranes of the proximal tubules (Fig. 1). Huland et al. [10] evaluated urinary NAG activity as a parameter of the destructive phase after unilateral partial ureteral obstruction in rats. In the

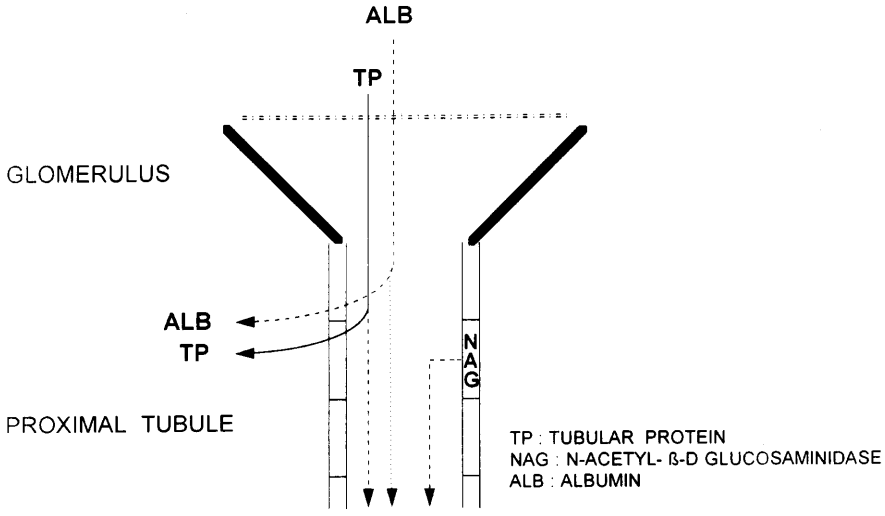

Fig. 1 Evaluation of glomerular and tubular function with albuminuria (ALB), tubular proteinuria (TP) and urinary N-acetyl-β-D-glucosaminidase (NAG) activity

unipapillary kidney of the rat, this destructive phase lasts for 2 weeks followed by stable atrophy [5]. During the destructive phase, the urinary NAG activity was significantly elevated [10].

The aim of the study was first to evaluate longitudinally (7 weeks) the behaviour and the potential clinical value of tubular proteinuria and urinary NAG activity after unilateral total ureteral obstruction and second, to study the pathophysiology of the non-obstructed contralateral kidney by using these two different markers of tubular damage.

Materials and methods

Animals

Experiments on 28 females Wistar rats weighing 200–300 g were performed. Unilateral total ureteral obstruction (UTO) was applied to 16 animals (2 died, 14 survived) while a sham procedure was done in 12 animals (1 died, 11 survived). Principles of laboratory animal care were followed, as well as specific national laws. The study was approved by the ethics committee for animal research of the hospital (April 1997).

Methods

All animals were followed for 7 weeks. At the end of week 1, a laparotomy was performed under general anaesthesia using intraperitoneal pentobarbital (Nembutal, 40–60 mg/kg). Unilateral total ureteral obstruction was created with three non-absorbable 2-0 sutures and the ureter was transected between the two distal sutures. No antibiotics or other drugs were administered. Animals were kept warm until they woke up and were then put back in their cages.

Morning urine samples were collected three times a week [11] in weeks 1, 2 and 6 and were pooled per rat and per week. Urine was obtained by putting the animal on a glass or transparent plastic plate and awaiting micturition. The urine was collected by aspiration and stored at −20°C. At week 7, the animals underwent a second laparotomy and urine was taken, by bladder puncture, for culture. A blood sample was taken for determination of serum creatinine.

All urine samples were centrifuged at 5000 rpm for 5 min. Serum and urine creatinine were assayed following the method of Jaffé, using commercial reagent (Boehringer Mannheim, Germany). For the determination of the urinary NAG activity, a colorimetric assay was used (Boehringer Mannheim). The NAG activity (units/litre) was measured photometrically at 580 nm. Urinary NAG excretion was expressed per gram urinary creatinine [8]. Tubular proteinuria and albuminuria were measured with gel permeation chromotography. An advanced protein purification system (Waters 650, Millipore, Milford, Mass.) was used together with a spectrophotometer at 280 lambda. A protein Pak Glass 300 5 W (Waters) column was used. The elution buffer was a phosphate-buffered saline (pH = 7.3; 0.1 M). The injection volume was 25 ml and the run time was 40 min at 0.8 ml/min. Tubular proteinuria and albuminuria are also expressed per gram urinary creatinine [8].

Statistical Analysis

Statistical analysis was done with Statistica 5-0 (Statsoft, Tulsa, USA). Results are given as median and quartile range [median (range)] or as mean ± standard deviation (SD) when appropriate [1]. Comparison between groups was performed using non-parametric tests (Wilcoxon, Kruskal-Wallis).

Results

Tubular proteinuria, urinary NAG activity, albuminuria before and after UTO

Results of urine samples are listed in Table 1. No tubular proteinuria was detected in the pre-experimental period. One week (week 2) after operation a significant increase ($P < 0.01$) in tubular proteinuria/creatinine ratio was found (Fig. 2). At week 6, tubular proteinuria/creatinine ratio decreased significantly ($P < 0.01$). No significant difference was found in tubular proteinuria between weeks 1 and 6. A significant decrease in urinary NAG activity/creatinine ratio (Fig. 3) was observed in the first week after UTO but at week 6 the urinary NAG activity significantly increased ($P < 0.01$). No significant difference was found in urinary NAG activity/creatinine ratio between weeks 1 and 6 (Table 1). A significant difference in albuminuria/creatinine ratio was found between weeks 1 and 2. No significant difference was found between

Table 1 Tubular proteinuria (TP), urinary NAG activity (NAG), albuminuria (ALB) and urinary creatinine after unilateral total ureteral obstruction (UTO) and in the sham population at weeks 1, 2 and 6

	Week 1, prior to surgery	Week 2	Week 6
UTO (n = 14)			
TP/creatinine	0 mg/g (0–0)	393.7 mg/g (101.8–728.9)**	126 mg/g (8.5–232.4)**
NAG/creatinine	45.6 U/g (25.6–63.3)	24.3 U/g (21.7–32.2)**	51.7 U/g (32.1–58.9)**
ALB/creatinine	35 mg/g (0–170)	143.5 mg/g (65.3–193.7)*	261 mg/g (171.2–570)°°
Urinary creatinine	89.3 mg/dl (74.5–95.1)	138.9 mg/dl (100.5–145.2)**	99 mg/dl (67.5–113.9)*
SHAM (n = 11)			
TP/creatinine	0 mg/g (0–0)	0 mg/g (0–0)	0 mg/g (0–0)
NAG/creatinine	36.2 U/g (16.1–43.0)	23.4 U/g (22.1–61.9)	36.7 U/g (26.0–57.1)
ALB/creatinine	0 mg/g (0–0)	0 mg/g (0–0)	22.9 mg/g (0–51.2)
Urinary creatinine	91.5 mg/g (37.9–114.3)	127.5 mg/g (52.4–196.9)	93.8 mg/g (81.7–11.8)

Data given as median (quartiles); *$P < 0.05$ versus previous test result, **$P < 0.01$ versus previous test result, °°$P < 0.01$ versus week 1

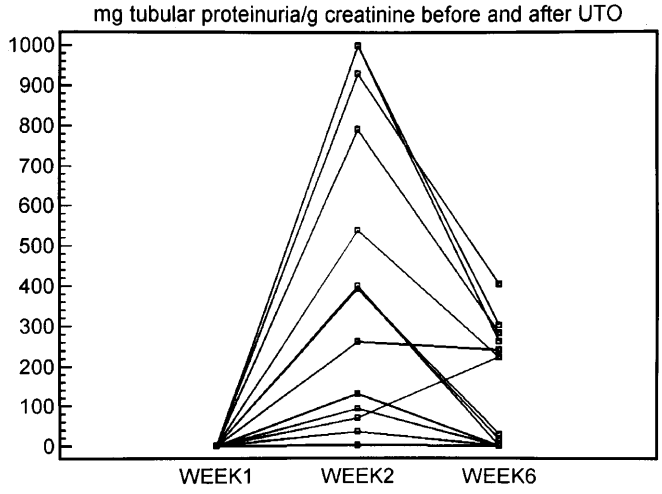

Fig. 2 Following (end of week 1) unilateral total ureteral obstruction (UTO), tubular proteinuria increases significantly (week 2) and normalizes at week 6

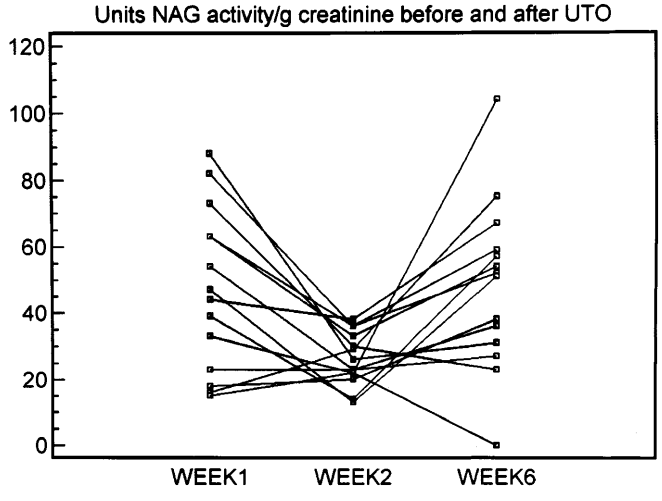

Fig. 3 Following (end of week 1) UTO, urinary NAG activity decreases significantly (week 2) and normalizes at week 6

weeks 2 and 6 ($P < 0.05$). A significant increase ($P < 0.01$) in albuminuria/creatinine concentration was found between weeks 1 and 6 (Table 1, Figure 4). The

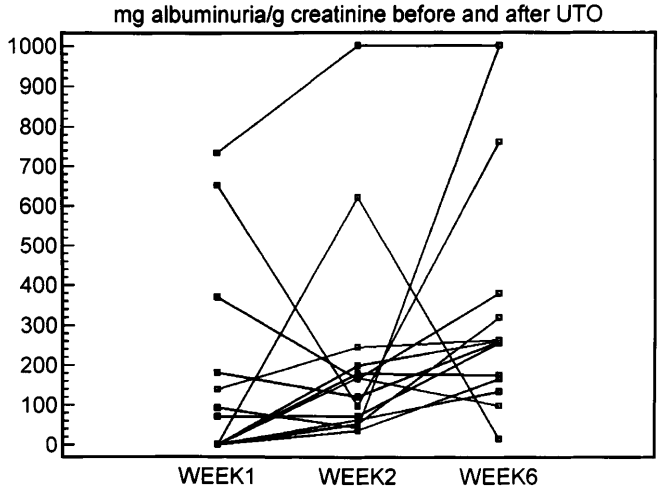

Fig. 4 Following (end of week 1) UTO, albuminuria increases progressively during the longitudinal study

urinary creatinine concentration increased significantly ($P < 0.01$) in the first postoperative week and normalized ($P < 0.01$) at week 6 (Table 1).

The sham-operated animals

In the sham group, no significant (Kruskal-Wallis) difference was found in urinary NAG activity/creatinine ratio, tubular proteinuria/creatinine ratio, albuminuria/creatinine ratio or urinary creatinine concentration between weeks 1, 2 and 6 (Table 1).

Serum creatinine concentration and urine culture at week 7

Serum creatinine was higher following UTO compared with the sham animals but this difference was not statistically significant (mean ± standard deviation) [UTO n = 9:0.83 mg/dl ± 0.13 mg/dl, sham group n = 11: 0.72 mg/dl ± 0.11 mg/dl]. All surviving animals (n = 25) had sterile urine or non-significant bacterial growth.

Discussion

In humans, UTO is seen in acute situations with symptoms of a renal colic. In certain patient populations (i.e. children, spinal cord injury patients, brain-injured patients, mentally retarded patients, postoperative pain syndromes) the differential diagnosis of renal colic and other causes of abdominal pain can be difficult. In these patients, an easily accessible screening tool for UTO is needed. Renal colic is also seen after acute partial ureteral obstruction and in these conditions, an increased urinary excretion of both tubular proteins and NAG activity is found [8, 10, 12]. Conversely, following UTO an increased tubular proteinuria is seen in combination with a decreased urinary NAG activity. Therefore, the combined determination of tubular proteinuria and urinary NAG activity can differentiate unilateral partial from total ureteral obstruction. However, this statement needs to be confirmed in a prospective study and as humans have multipapillary kidneys and rats have unipapillary kidneys, direct extrapolation from rat experiments cannot be made and further studies are needed.

Tubular proteinuria rises after UTO but this cannot be considered as a marker of tubular damage. When tubular damage provokes tubular proteinuria in a situation with normal glomerular filtration rate (GFR) and impaired tubular reabsorption, an increase in urinary NAG activity (leakage from the proximal tubules) in parallel with tubular proteinuria is expected. However, urinary NAG activity did not increase after UTO. Therefore, tubular proteinuria must be explained by an increased total renal and single nephron GFR or an elevated serum concentration [2,4,8]. After UTO, the GFR drops by 50%. A progressive elevation of the single nephron GFR in the contralateral kidney is seen until the GFR normalized. The single nephron GFR increases due to anatomic growth of glomeruli and an increase of the blood flow per glomerulus [4]. During this initial phase, the tubules are overperfused with filtrate containing serum low molecular weight proteins. As compensatory tubular hypertrophy is not yet established and high quantities of these proteins pass into the proximal tubules, the reabsorption threshold is exceeded. Consequently these small proteins leak into the urine. This phenomenon was also described by Bianchi et al. [2] in uninephrectomized rats. A second explanation for the elevated tubular proteinuria following UTO is that after the initial decrease in total GFR, the serum concentrations of low molecular weight proteins increase. These small proteins are easily filtered through the glomerulus and the reabsorption threshold in the proximal tubule is exceeded. Other examples of these "overflow proteinuria" are Bence Jones proteinuria [8], caused by elevated serum concentrations of light chains in myeloproliferative disease and severe renal failure (GFR decreased $>70\%$), where low molecular weight proteins accumulate in the serum [8].

Albuminuria increases significantly 1 week after UTO. This can also be explained by the initial elevation of the single nephron GFR and overperfusion of the proximal tubules with small proteins. Once compensatory tubular hypertrophy is established, a normalization of albuminuria is expected. Contrarywise, a progressive increase of albuminuria was found at week 6. Several authors [3,6,15] found hypertension in most of the animals following UTO. An increase of systemic blood pressure, an ipsilateral intrarenal vasoconstriction and a contralateral intrarenal vasodilatation were the most relevant changes associated with UTO [3,6,15]. The predominant mechanisms for these observations were stimulation of the renin-angiotensin system as vasoconstrictor (systemic and in the ipsilateral kidney) and of eicosanoids (prostaglandin, thromboxane) and nitric oxide as vasodilators (increase the single nephron GFR) [3, 6, 15, 19]. As hypertension is a common complication following (weeks) UTO in rats and a well-known cause of albuminuria, an increased blood pressure could explain the progressive increase in albuminuria throughout our study.

The urinary NAG activity decreases after UTO and normalizes at week 6. This suggests the presence of inhibitors for the enzymatic NAG determination, a decrease in the NAG pool or an increase in the fractional excretion of water. In a pilot study we performed a dialysis experiment on several rat urine samples with different creatinine concentrations and found no evidence for inhibitors of the enzymatic reaction used for the estimation of the NAG activity. More extensive experiments on this subject were done by Noto et al. [18] who found no evidence that urinary inhibitors interfere with the method that was used for the estimation of the NAG activity. In the present study, the fractional excretion of water was not increased as the urinary creatinine concentration was elevated. Finally, the initial NAG pool was reduced by 50% and the single nephron glomerular filtration rate increased progressively [4] in the first weeks following UTO. This can account for the observed decrease in urinary NAG excretion until compensatory tubular hypertrophy is established.

The urinary creatinine concentration increased initially following UTO, but not after the sham operation. Although no objective parameters are available on this subject, we noticed that the sham-operated animals voided easier, larger volumes of macroscopically diluted urine then did the animals after UTO. Animals that undergo UTO are likely to have more pain as they suffer from renal colic. In humans, due to stimulation of the vagal nerve, nausea and vomiting is seen during renal colic, resulting in concentrated urine and decreased production of urine (decreasing intrapyelic pressures and pain).

Conclusion

Tubular proteinuria is a sensitive marker for the diagnosis of UTO in rats. The initial increase of low mo-

lecular weight proteins following UTO is not due to tubular damage as no parallel increase of urinary NAG was found. We suggest that the tubules are initially overperfused with primary urine due to an increased single nephron glomerular filtration, overruling the reabsorption capacity of the proximal tubules. In a later phase normalization occurs probably by compensatory tubular hypertrophy.

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