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Urine uromodulin estimation in partial bladder outlet obstruction and cyclophosphamide-induced haemorrhagic cystitis models in rats*

Uromodulina w eksperymentalnych modelach blokady podpęcherzowej oraz pocyklofosfamidowego krwotocznego zapalenia pęcherza moczowego u szczurów

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: Uromodulin (UMOD) is a glycoprotein excreted by the thick ascending limb of the Henle's loop and distal convoluted tubule cells, playing various, yet still unclear roles. An abnormal urinary UMOD excretion is observed in many pathophysiological conditions. The aim of our study was to assess urine UMOD excretion in experimental partial bladder outlet obstruction (PBOO), reflecting BPH in humans, and in cyclophosphamide-induced haemorrhagic cystitis (CP-HC).

Materials and methods: PBOO and CP-HC rats and two appropriate control groups were studied. The PBOO model was surgically induced by partial proximal urethral obstruction and CP-HC by four i.p. cyclophosphamide administrations (every two days). 24-hour urine collections were performed in both PBOO (on 3rd, 7th, 12th and 15th day after surgery) and CP-HC rats (on 1st, 3rd, 5th and 7th day). UMOD was determined with the ELISA method. Both 24-hour urinary UMOD excretion and urinary UMOD concentrations were determined.

Results: In the overall assessment, PBOO rats were characterized by decreased mean urinary UMOD concentration. However, as the urine volume, except for transient drop on 3rd day following PBOO operation, was steadily increasing, the daily urinary uromodulin excretion did not differ from the control one.
Contrary to PBOO, CP-HC rats demonstrated mean urinary concentration similar to that of the control rats, while their 24hr UMOD excretion in urine was almost doubled due to urine volume increase (from 1.6 up to almost 3 fold). The highest UMOD urinary output was observed after the 3rd and 4th doses of cyclophosphamide.

Discussion: A reduced urinary UMOD excretion in early PBOO phase may be considered as a marker of distal tubular cells damage due to incomplete bladder emptying and increased pressure retrograding to distal tubules. This effect disappears with structural, adaptive histological changes of the bladder wall leading to an improved voiding. In CP-HC animals, the elevated

*The consent of the 1st Local Bioethics Committee in Krakow (No. 126/2010 and 28/2013) was obtained before commencement of the study.

urinary UMOD level may be associated with complex inflammatory response due to the cytotoxic CP action. UMOD assessment in this model may reflect renal and urological toxicity as UMOD excretion rises with the cumulative cyclophosphamide dose.

Keywords: **uromodulin • overactive bladder (OAB) • bladder outlet obstruction • haemorrhagic cystitis • cyclophosphamide**

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Abbrevations: **AHL** - ascending limb of the Henle's loop, **BPH** – benign prostatic hyperplasia, **BWW** – bladder wet weight, **CP** – cyclophosphamide, **CP-HC** – cyclophosphamide-induced haemorrhagic cystitis, **DCT** – the distal convoluted tubule, **LUT** – lower urinary tract, **OAB** – overactive bladder, **PBOO** – partial bladder outlet obstruction, **UMOD** – uromodulin.

Introduction

Normal voiding is a complex phenomenon which may be disturbed and manifested as urgency, with ("wet" form) or without ("dry") urge incontinence, usually in the presence of frequency and nocturia. That syndrome is referred to as "overactive bladder" (OAB) and is mainly used for description of those disturbances in which no precise, local or systemic etiological cause, could be identified. The pathophysiology of this "primary" OAB remains not completely understood, however some hypotheses (with the most accepted neurologic and myogenic ones) are proposed. They are given in details in some reviews [2,6,14], also of our authorship [9].

Despite the common accepted OAB definition given above, this term is also currently applied to the description of secondary bladder overactivity symptoms, observed in various organic dysfunctions. One of them is benign prostatic hyperplasia (BPH) in men - an abnormality that leads to detrusor overactivity due to the bladder wall restructure with hyper-proliferated IC cells and fibroblasts, increased production of nerve (NGF), vascular (VEGF) and fibroblastic (FGF) growth factors, overproduction of reactive oxygen species due to hypoxia, neuronal enlargement and supersensitivity to locally released acetylcholine [2,3]. The increased infravesical pressure due to incomplete bladder emptying and urine retention is moved backwards, possibly as far as renal distal tubes.

Secondary OAB symptoms due to acute bladder inflammation are also observed in patients treated with alkylating, cytotoxic oxazaphosphorine agents (e.g. cyclophosphamide - CP), used in pharmacotherapy of some neoplastic disorders. CP urotoxicity is related to its metabolic pathway into aldophosphamide form, and subsequent release of acrolein that exacerbates both cellular and chemical inflammatory changes [34]. We have provided a detailed pathophysiological description of CP-induced bladder inflammation in one of our previously published papers [10]. Summing up, in both obstruction-associated and chemical bladder injury (with subsequent damage of distal tubules), some secondary OAB symptoms are observed.

The partially bladder outlet obstruction (PBOO) in rat is an experimental model reflecting pathological conditions observed in human BPH [25]. Haemorrhagic cystitis with kidney damage may be also produced in rats by cyclophosphamide administration (CP-HC model) [5,8]. Thus, using those two experimental models, a secondary, overactivity-associated morphological bladder damage can be observed. According to our knowledge, currently there is no biochemical marker of bladder overactivity in those two conditions. Because kidneys are also involved in the pathophysiological process of both PBOO and CP-HC, it is hypothetically possible to use as a marker any constituent, that is excreted in an excessive amount by impaired kidneys. One of the potential candidates is a protein excreted into urine - uromodulin (UMOD).

Little is known about UMOD excretion secondary to either cytotoxic cyclophosphamide urinary tract damage or urine outflow obstruction, in the course of BPH. Thus, the aim of our study was to assess urine uromodulin excretion in two experimental OAB models: partial bladder outlet

obstruction (reflecting BPH in humans) and in cyclophosphamide-induced haemorrhagic cystitis.

Material and methods

ANIMALS: Forty 6-weeks-old Wistar rats obtained from the central animal house of the Pharmaceutical Faculty of the Jagiellonian University Collegium Medicum in Krakow were used for experiments. For acclimatisation to the new living conditions, in the first week animals were placed in five collective cages with unlimited access to a standard laboratory feed (Labofeed, Kcynia) and water. Constant temperature of 22°C was maintained in the room. At the beginning of the experiment rats were randomised into study groups, 10 animals each. During the experiment, animals in the particular group lived together in the same cage and had unlimited access to water and feed.

STUDIED GROUPS: We performed our study using two experimental secondary OAB models – obstructive, with partial bladder outlet obstruction (PBOO) and chemical, cyclophosphamide-induced (CP-HC), with two appropriate control groups (control (1) and (2), respectively). Animals in the control (1) group underwent a sham operation (PBOO surgery but without proximal partial urethral ligation). Control (2) animals were sham treated - they received normal (0.9%) saline solution instead of CP, in volume corresponding to the volume of CP applied in CP-HC group.

In the PBOO group, one rat did not survive the anaesthetic agent dose, and another one died in an early post-surgical period. The third PBOO subject was excluded as PBOO criteria failure because of a loosened urethral ligation revealed in final laparotomy. Finally, there were 7 animals in the PBOO group. In the appropriate control (1) group, two animals also did not survive the sham procedure, thus 8 control individuals completed the study.

Out of 10 rats included into the CP-HC group, only 6 completed the study and 4 died before reception of the last CP dose. A cumulative nephro- and urotoxicity of the applied CP dose was a possible reason of that high mortality rate in that group. The remaining animals were generally in poor condition, deteriorating with each CP dose. In control (2) group, all 10 animals completed the study and exhibited the best condition of all studied groups.

THE PBOO SURGERY: Ten animals had the proximal section of the urethra partially surgically ligated, in order to create the bladder outlet obstruction. According to the literature, a condition clinically corresponding to BPH with bladder overactivity develops within two weeks post-surgically [7,25]. The procedure was performed under pentobarbital anaesthesia (Morbital, Puławy, 45mg/kg b.w. administered i.p.). Following the anaesthesia, a medial incision was made in the projection over the bladder. The exposed bladder was carefully separated from the surrounding fatty tissue, and the urethra was catheterised (polyethylene catheter, diameter 0.58mm), ligating the proximal section of the urethra around the catheter. After the catheter was removed, the abdominal integument and skin were sutured in layers with standard surgical sutures (Medilen 4/0 USP; cutting needle DS2, 3/8). The surgical wound was sprayed with Neomycin and the surrounding skin was sprayed with Oxycort to minimise the risk of post-surgical infection. Two first days after the surgery were treated as a convalescence period. Data collection was started on the 3rd day.

CP-INDUCED HAEMORRHAGIC CYSTITIS: In another ten animals an experimental model of haemorrhagic cystitis with bladder overactivity was created by four (every two days – on days one, three, five and seven) intraperitoneal administrations of cyclophosphamide - CP (Sigma Aldrich) – at the dose of 75mg/kg b.w. The CP solution was prepared each time *ex tempore* before administration. According to the literature that dosage scheme leads to haemorrhagic cystitis following the fourth dose [5,8]. Control (2) animals received injections of normal saline. Data acquisition was started with administration of the 1st CP dose.

BODY WEIGHT MEASUREMENT AND DAILY URINE COLLECTION: Body weight was measured in conscious rats using standard laboratory electronic scale, and the daily urine output was taken using standard metabolic cages. During the 24-hour urine collection, each of the study individuals was placed in a separate cage with unlimited access to water and feed. In PBOO animals, body weight and daily urine output were measured on the 3rd, 7th, 12th and 15th day after the surgery. Control (1) rats were studied once at the beginning of the experiment on the 3rd day. The measurement was accepted as a reference value. In CP-HC individuals, body weight and daily urine output was measured on the 1st, 3rd, 5th and 7th day, consistently with the administration of CP doses. Body weight was taken before each CP administration and then the studied rats were placed in metabolic cages. Measurements of control (2) rats were taken on the day 1 only, and results were accepted as a reference point for CP-HC animals.

We did not study control rats in parallel to PBOO/CP-HC ones because we had made an assumption that in normal animals urine production and uromodulin excretion are stable over the time (15 or 7 days for PBOO and CP-HC, respectively). Therefore, we had decided to perform a single measurement in the control (1) group on the 3rd day after the sham surgery (those rats exhibited no signs of postoperative abnormalities at that time) and in the control (2) group on the 1st day of the experiment (those animals were totally intact except a single saline administration).

UROMODULIN ASSESSEMENT: Rat uromodulin level was measured in urine samples using a commercially available ELISA kit (WUHAN EIAAB SCIENCE CO.,LTD), strictly according to the manufacturer's instructions. Uromodulin concentrations (in [ng/ml]) in appropriate groups and time points (along with the total estimation)

were obtained. We calculated also the amount of daily excreted uromodulin in reference to the 24-hour urine measurement in all studied cases.

BLADDERS WET WEIGHT MEASUREMENT AND HISTOPATHOLOGICAL ASSESSEMENT: Following the last body weight and daily urine output measurement animals were sacrificed with a lethal dose of pentobarbital (Morbital, Puławy, Poland; 100 mg/kg b.w.). The bladder was collected from every study animal, following a previous separation from the surrounding fatty tissue and voiding. According to the literature data, the bladder wet weight (BWW) may be treated as an indirect evidence of the bladder reconstruction induced by inflammation (in the CP-HC model) or by outlet obstruction (in the PBOO model), associated with its overactivity [23,32,42]. Directly after collection, bladders were weighed on an analytical scale and then placed in 4% formalin solution with PBS for histopathological evaluation.

STATISTICAL ANALYSIS: The results were presented as means ± SD. Paired results were analysed for groups: PBOO-control (1) and CP-HC-control (2) using the Fischer Snedecor test. In uromodulin estimation, we juxtaposed total results as well as those assessed on a particular day of the experiment (in PBOO participants) or estimated after a respective CP dose (in CP-HC animals).

Results

Results of body weight and daily urine volume measurements

In PBOO animals, we observed a progressive body weight increase during the experiment. On the contrary, CP-HC rats exhibited a progressing body weight loss with administration of subsequent CP doses. Observed changes are given in Tables 1 and 2.

Along with the body weight increase, a slight progressive rise in 24-hour urine output was also noted in PBOO. However, on the 3rd day after PBOO induction, the rats excreted even less urine than the control. It may be a consequence of the surgery and reduced bladder contraction, inappropriate to existing partial proximal urethral obstruction. From the day 7 onwards a trend of increased urine volumes was observed in that group as compared to the control counterpart. CP-HC rats, on all studied days, were characterised by higher diurnal urine volumes excretion compared to the control ones.

The detailed results of observed 24-hour urine output in all studied groups are given in Tables 1 and 2.

Table 1. Partial bladder outlet obstruction (PBOO) rats characterization

	Control (1)	PBOO			
		D3 3rd day	D7 7th day	D12 12th day	D15 15th day
body weight [g]	177.13±8.41	174.60±6.80	190.60±8.17 *	198.60±6.88 ***	203.20±8.59 ***
24-hour urine volume [ml]	3.59±1.50	2.72±0.72	4.37±2.29	5.90±2.04 *	6.90±1.02 ***
bladder wet weight [g]	0.14±0.06	n/e	n/e	n/e	0.26±0.16

Statistical significance: n/e – not estimated, * - p<0.05; ** - p<0.01; *** - p<0.001

Table 2. Cyclophosphamide-induced haemorrhagic cystitis (CP-HC) rats characterization

	Control (2)	CP-HC			
		D1 (1st dose)	D2 (2nd dose)	D3 (3rd dose)	D4 (4th dose)
body weight [g]	185.88±9.96	188.00±11.83	184.50±9.09	183.67±9.37	181.67±10.61
24-hour urine volume [ml]	5.90±1.52	9.50±4.37	13.42±10.41	17.42±9.36 *	14.83±6.43 **
bladder wet weight [g]	0.17±0.06	n/e	n/e	n/e	0.13±0.03

Statistical significance: n/e – not estimated, * - p<0.05; ** - p<0.01; *** - p<0.001

EVALUATION OF BLADDER WET WEIGHT: According to the literature [23,32,42], the BWW measurement was intended as an indirect evidence of bladder dysfunction in studied models. In the PBOO group (group 1), on the day 15 of the experiment, the bladder wet weight was almost double compared to the control. It might be due to the suspected bladder wall reconstruction associated with the bladder attempts to overcome the partial urethral obstruction. On the other hand, CP-HC, CP-HC animals had lower BWW compared to the control. However, the above mentioned differences in both PBOO and CP-HC groups were not statistically significant. The detailed results of measured BWW are given in Tables 1 and 2.

HISTOLOGICAL ANALYSIS OF COLLECTED BLADDERS. According to the pathomorphological evaluation, bladders collected from PBOO animals demonstrated signs of oedema and congestion of the bladder wall, with a minimal hyperplasia of the muscular layer. In the CP-HC model, a clear oedema and signs of congestion (mostly of the cystic mucosa) were found, along with signs of focal proliferation of fibroblasts in the mucosal lamina propria, mostly around some fine, submucosal blood extravasations. Fine lymphocytic inflammatory infiltrations were visible in the vicinity of vessels of the mucosal lamina propria. Epithelium of the bladder lining demonstrated focal ulceration with signs of clear proliferation of cells and of *anisonuleosis focalis et papillosis*.

Uromodulin analysis – urine uromodulin concentration

THE PBOO MODEL: The total urine UMOD concentrations were significantly (p<0.01) lower in PBOO animals (4.497 ± 0.646 ng/ml, comparing to control (1) ones (5.784 ± 0.775 ng/ml). The differences were also revealed on individual days, and they were strongly statistically significant. A marked decreasing trend of that parameter was observed on subsequent days.

THE CP-HC MODEL: Contrary to PBOO findings, an increasing trend of urine UMOD concentrations was observed with subsequent CP doses. However, both the total result (5.410 ± 1.105 ng/ml) and values of UMOD concentrations calculated for three successive doses (1-3) were not statistically significant compared to the control (5.238 ± 0.960 ng/ml). Only the concentration value calculated following the 4th CP dose was significantly (p<0.05) higher in CP-HC than in the control.

Details of the results described above are given in Figures 1 and 2 which present UMOD concentration changes during the course of experiment.

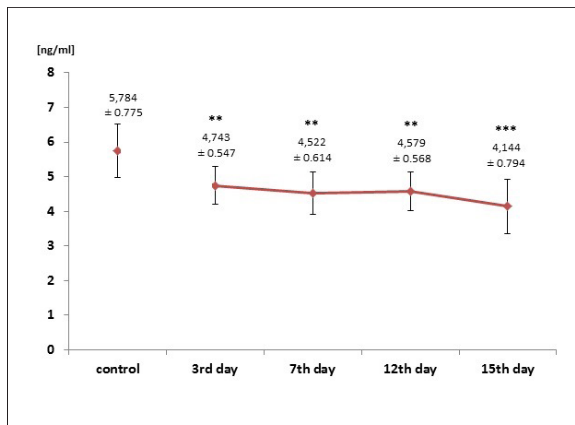

Fig. 1. Uromodulin urine concentration changes in PBOO rats

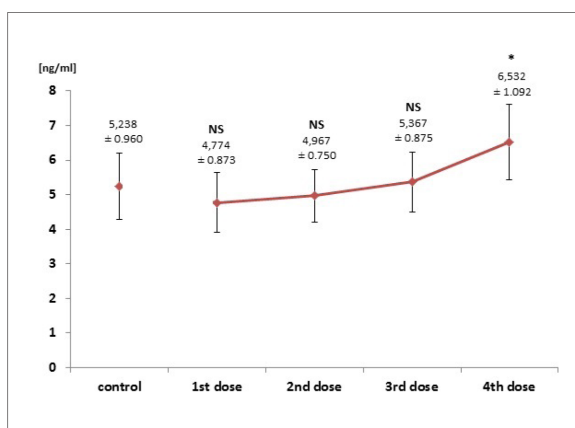

Fig. 2. Uromodulin urine concentration changes in CP-HC rats

Uromodulin analysis – 24-hour urine uromodulin excretion

THE PBOO MODEL: Total excreted UMOD amount was almost identical in PBOO (22.791 ± 8.827 ng/day) and control (1) individuals (22.819 ± 8.209 ng/day). However, considering 24-hour excreted UMOD changes, an increasing trend was revealed in PBOO rats, similar to the urine volume changes observed in that group. On the 3rd day a statistically significant reduction of the UMOD amount was observed, and on the 15th day the UMOD excretion was the highest, compared to the control.

THE CP-HC MODEL: Contrary to the findings in PBOO animals, the total 24-hour UMOD excretion was almost doubled in CP-HC rats (59.192 ± 26.710 ng/day) comparing to control (2) ones (34.159 ± 6.153 ng/day) and that difference was statistically significant (p<0,0001). The group demonstrated also an increasing trend of 24-hour excreted UMOD changes, except for the value calculated after the second CP dose. The highest excreted UMOD values were noted after the 3rd and the 4th CP dose and they were statistically significant comparing to control.

Details of the results described above are given in Figures 3 and 4. They illustrate changes of the UMOD excretion in time, also showing the hypothetical course of UMOD changes also on remaining days of the experiment, that we didn't make any calculations for.

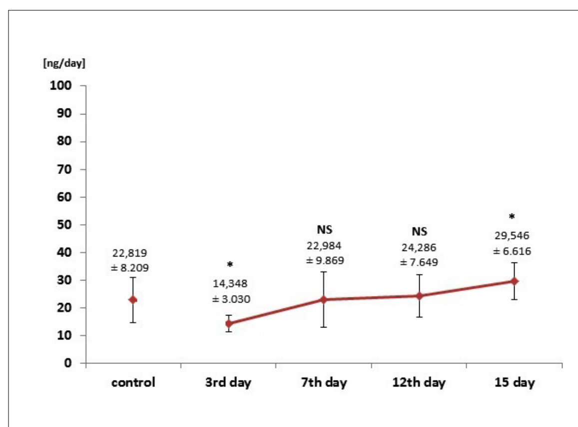

Fig. 3. 24-hour uromodulin excretion in PBOO rats

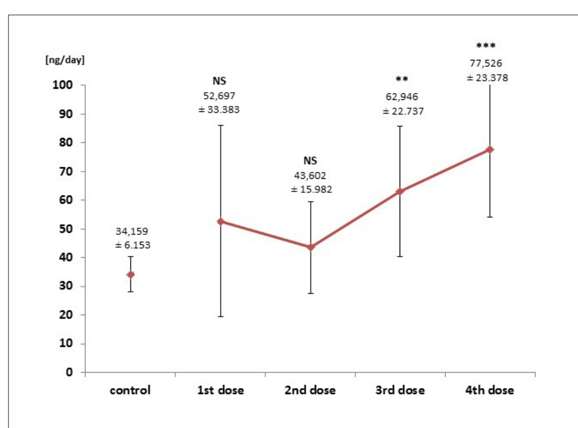

Fig. 4. 24-hour uromodulin excretion in CP-HC rats

Discussion

The main findings of our study are:

1. In the total assessment, PBOO rats were characterized by unchanged daily urinary uromodulin excretion compared to the control. However, due to the increased voiding, the UMOD mean urinary concentration in those animals was decreased. Contrary to PBOO findings, CP-HC rats demonstrated almost doubled total 24hr UMOD excretion with mean urinary concentration being unchanged compared to the control.

2. Considering time-dependent changes of the urinary UMOD we have found, that PBOO rats exhibited an increasing trend, with significantly decreased (on the 3 day after the BOO surgery) and increased (on the day 15, respectively) urinary UMOD amount. UMOD concentrations were significantly lower on all days of the experiment compared to the control. CP-HC individuals also demonstrated a generally increased trend in 24-hour UMOD urinary excretion. However, the more cumulative exposure to CP was applied (after 3rd and 4th doses), the higher UMOD amount excreted with urine was observed. The urinary UMOD concentrations were similar to those observed in the control, except for the calculated concentration following the reception of the fourth CP dose, that showed a significantly higher value compared to the control.

Uromodulin is the most abundant urinary protein in healthy individuals, excreted into urine by the thick ascending limb of the Henle's loop (AHL) and the distal convoluted tubule (DCT), constituting physiological hyaline casts [40]. UMOD is also known as the Tamm-Horsfall protein (THP), because it was discovered by Igor Tamm and Frank Horsfall in 1950 during a precipitation procedure of urine collected from healthy individuals, aimed at isolation of an inhibitor of hemagglutination of viruses (the authors attempted to obtain a compound that would prevent viruses from binding to susceptible cells) [37]. The same protein was re-discovered in 1985 by Muchmore and Decker who isolated it from urine of pregnant women and described as a glycoprotein of immunosuppressive activity [24]. Thereafter, in 1987, Pennica [26] and Hession [15] studied the structure of THP and UMOD and revealed that they are in the same protein. Thus, the terms "uromodulin" and "Tamm-Horsfall protein" may be used interchangeably; however, we have chosen to use the term uromodulin (UMOD) in our paper.

Despite the fact, that uromodulin has been discovered in the mid-20th century, the protein is still an object of interest of many researchers and its numerous physiological functions still remain rather elusive. UMOD was demonstrated to exhibit a tendency to gelation/aggregation in normal urine, that provides the water barrier on the luminal plasma membrane of AHL/DCT that serves as a physical barrier controlling water permeability and transcellular ionic transport. UMOD was also revealed to be a "scavenger" receptor for many ligands, including numerous cytokines, thus it may play a modulatory role in signal transduction in kidneys and the urinary tract (especially during an inflammatory process) [33,40].

One of the most important physiological UMOD feature seems to be an ability of binding to uropathogenic strains of *Escherichia coli*. That suggests the role of UMOD in the defense against urinary tract infections. During colonization of the urinary system, bacteria bind their lectin-like adhesin-containing fimbriae to carbohydrate structures and glycolipids (e.g. uroplakin Ia and Ib) present at the luminal urothelial cell surfaces, that act as cell receptors for fimbriated *E coli*. Binding to bacterial adhesins UMOD competes with uroplakins, therefore offering a protective effect decreasing uropathogenity of *E coli* [33,40].

Abnormalities in UMOD secretion (an excessive or reduced UMOD urine output) is observed in many renal conditions, such as acute renal failure [18], chronic nephropathies [27,31,39], interstitial cystitis [1,4], lithiasis [13,19,35,41], kidney transplantation [16,22,38], urinary tract infections [20,28,29] and others. UMOD is produced in kidneys only (however, there are also reports suggesting a possible extrarenal UMOD production, e.g. in salivary glands, je-

junum and glial cells of human brain, but UMOD mRNA has not been found in these tissues so far) [17].

Little is known about UMOD release in other stress conditions, such as BPH, associated with partial urethral obstruction or cyclophosphamide treatment, related to chemical bladder damage. Therefore we have quantified UMOD in urine samples obtained from rats with experimental models of BPH (PBOO model) and cyclophosphamide-induced bladder cystitis (CP-HC one). As it was mentioned above, in PBOO animals we have found a decline in urinary UMOD amount in the early phase after PBOO surgery. After two weeks, UMOD excretion was even elevated compared to the control. A reduced urinary UMOD excretion is considered to be a marker of distal tubular cells damage [33]. Thus, consistently, our findings suggest that PBOO induction and consequential intrabladder hydrostatic pressure rise, may cause subsequent DCTs damage. That effect is transitory: along with the structural bladder wall reconstruction in attempt to overcome the urinary outflow blockade, pressure conditions become improved allowing normalization of DCTs' activity. The increased UMOD level two weeks after PBOO surgery may be treated as a compensatory, temporary effect and longer period of observation would be necessary to determine if the increased UMOD excretion trend was stable. Our findings support the study reported by Storch et al. [36] who used different experimental model of the urine outflow blockade but with similar pathophysiological consequences for AHL and DCT cells. The authors revealed reduced UMOD level in both bilateral and unilateral ureteral obstruction in rats, as soon as 24 hours after the surgery. Upon release of the blockade, UMOD urinary level returned slowly to normal [36]. Thus, both our and Storch et al. results support the hypothesis that urinary tract obstruction leads to the retrograde pressure overloading and result in UMOD decrease. However, there is also the study by Fasth et al. [12], who demonstrated that unilateral ureteral obstruction for 24 hour in mice led to the increased formation of UMOD at the base of DCT cells, which was also detected in renal interstitium for as long as 3 weeks after the release of the obstruction [12].

Similarly, there are also controversies concerning the UMOD secretion following oxazaphosphorine, alkylating cytotoxic agents (cyclophosphamide, ifosfamide) administration. We have found, that CP in the experimental CP-HC model caused the essential total increase of UMOD secretion, particularly following subsequent CP doses. In our opinion, the elevated urinary UMOD level may be associated with a complex inflammatory response due to the cytotoxic CP action, particularly affecting the bladder in course of CP elimination. UMOD is reported to exert an anti-inflammatory effect binding to renal cytokines and lymphokines (interleukin 1, tumor necrosis factor α), thereby inhibiting inflammation during a kidney injury [15]. Taking those findings into consideration, our results would support the thesis that generalized inflammatory burst evoked by CP in urinary bladder affects also at least DCT/AHL cells contributing to UMOD over-release, in order to counteract proinflammatory mediators. On the other hand, however, there are also reports suggesting a pro-inflammatory role of UMOD. That protein was demonstrated to activate myeloid dendritic cells and macrophages *in vitro* via the Toll-like receptor 4 [30]. Those two contradictory UMOD properties may be reconciled if, two different UMOD forms – apically- and basolaterally-released are supposedly present, as suggested by El-Achkar and Wu [11]. The first of them may be responsible for the anti-inflammatory effect, reducing activity epithelial cells and serving as a signal for tissue repair processes. The second one may be characterized by an altered, different from the apical, form of glycosylation, that limits the interaction of the protein with specific receptors present on epithelial cells and mediating the anti-inflammatory effects. In our study, we were unable to differentiate individual UMOD forms, therefore it is impossible to determine clearly any pro- or anti-inflammatory role of the elevated UMOD level observed in the experimental CP-HC model. We have found no other experimental study related to UMOD estimation in experimental CP-HC cystitis, and therefore our results cannot be juxtaposed with any others. There is one corresponding study by MacLean et al. [21] who also assessed changes in urine protein excretion in patients suffering from chronic nephrotoxicity due to oxazaphosphorine agent treatment. However, we cannot refer to their findings because of major differences – comparison of results of experimental and clinical studies is impossible. Moreover, MacLean et al. [21] studied the effect of I.V. -administered ifosfamamide (not I.P. cyclophophosphamide) on complex protein excretion, assessed using the method of gel electrophoresis. They demonstrated that ifosfamide treatment resulted in the loss of the – so called – electrophoretic band A, which appears to be a normal urine constituent with molecular weight very close to that of UMOD. They concluded that extensive ifosfamide-induced tubular damage might lead to reduced UMOD excretion. Their finding would support the hypothesis that decreased urinary UMOD excretion is considered to be a marker of distal tubular cell damage. However, as we mentioned above, despite the fact that our results are conflicting, we cannot discuss the differences with MacLean et al [21] study due to too gross methodological differences.

To sum up, we have revealed an overall highly increased urinary UMOD level in the CP-HC experimental model and – depending on the total/detailed point of estimation – unchanged or only slightly elevated UMOD excretion in 24-hour urine collections in experimental PBOO. The pathophysiological interpretation of those findings seems to be difficult due to ambiguous UMOD properties. It seems that qualitative and quantitative UMOD excretion analysis in BPH and cystitis, as well as in other renal and urological abnormalities requires further studies to determine UMOD changes in various pathophysiological conditions. Moreover, it is possible that UMOD assessment in numerous renal and urological disorders could become one of the medical analytic tools used in diagnostic procedures to predict future clinical course of particular abnormalities.

References

[1] Bade J.J., Marrink J., Karrenbeld A., van der Weele L., Mensink H.J.: Increased urinary levels of Tamm_horsfall glycoprotein suggest a systemic etiology of interstitial cystitis. J. Urol., 1996; 156: 943-946

[2] Banakhar M.A., Al-Shaiji T.F., Hassouna M.M.: Pathophysiology of overactive bladder. Int. Urogynecol. J., 2012; 23: 975-982

[3] Briganti A., Capitanio U., Suardi N., Gallina A., Salonia A., Bianchi M., Tutolo M., Di Girolamo V., Guazzoni G., Rigatti P., Montors F.: Benign prostatic hyperplasia and its aetiologies. Eur. Urol. Suppl., 2009; 8: 865-871

[4] Canter M.P., Graham C.A., Heit M.H., Blackwell L.S., Wilkey D.W., Klein J.B., Merchant M.L.: Proteomic techniques identify urine proteins that differentiate patients with interstitial cystitis from asymptomatic control subjects. Am. J. Obstet. Gynecol., 2008; 198: 553.e1-553.e6

[5] Chopra B., Barrick S.R., Meyers S., Beckel J.M., Zeidel M.L., Ford A.P., de Groat W.C., Birder L.A.: Expression and function of bradykinin B1 and B2 receptors in normal and inflamed rat urinary bladder urothelium. J. Physiol., 2005; 562: 859-871

[6] Chu F.M., Dmochowski R.: Pathophysiology of overactive bladder. Am. J. Med., 2006; 119 (Suppl. 1): 3-8

[7] Das A.K., Leggett R.E., Whitbeck C., Eagen G., Levin R.M.: Effect of doxazosin on rat urinary bladder function after partial outlet obstruction. Neurourol. Urodyn., 2002; 21: 160-166

[8] Dinis P., Charrua A., Avelino A., Yaqoob M., Bevan S., Nagy I., Cruz F.: Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. J. Neurosci., 2004; 24: 11253-11263

[9] Dobrek Ł., Juszczak K., Wyczółkowski M., Thor P.J.: Overactive bladder - current definition and basic pathophysiology concepts. Adv. Clin. Exp. Med., 2011; 20: 119-129

[10] Dobrek Ł., Thor P.J.: Bladder urotoxicity pathophysiology induced by the oxazaphosphorine alkylating agents and its chemoprevention. Postepy Hig. Med. Dosw. (online), 2012; 66: 592-602

[11] El-Achkar T.M., Wu X.R.: Uromodulin in kidney injury: an instigator, bystander, or protector? Am. J. Kidney Dis., 2012; 59: 452-461

[12] Fasth A.L., Hoyer J.R., Seiler M.W.: Extratubular Tamm-Horsfall protein deposits induced by ureteral obstruction in mice. Clin. Immunol. Immunopathol., 1988; 47: 47-61

[13] Glauser A., Hochreiter W., Jaeger P., Hess B.: Determinants of urinary excretion of Tamm-Horsfall protein in non-selected kidney stone formers and healthy subjects. Nephrol. Dial. Transplant., 2000; 15: 1580-1587

[14] Hashim H., Abrams P.: Overactive bladder: an update. Curr. Opin. Urol., 2007; 17: 231-236

[15] Hession C., Decker J.M., Sherblom A.P., Kumar S., Yue C.C., Mattaliano R.J., Tizard R., Kawashima E., Schmeissner U., Heletky S. et al.: Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. Science, 1987; 237: 1479-1484

[16] Kaden J., Groth J., May G., Liedvogel B.: Urinary Tamm-Horsfall protein as a marker of renal transplant function. Urol. Res., 1994; 22: 131-136

[17] Kokot F., Duława J.: Tamm-Horsfall protein updated. Nephron, 2000; 85: 97-102

[18] Kokot M., Duława J., Nowicki M., Kokot F., Machowska J.: Urinary excretion of Tamm-Horsfall protein by patients with acute renal failure. Pol. Arch. Med. Wewn., 1992; 88: 225-229

[19] Lau W.H., Leong W.S., Ismail Z., Gam L.H.: Qualification and application of an ELISA for the determination of Tamm-Horsfall protein (THP) in human urine and its use for screening of kidney stone disease. Int. J. Biol. Sci., 2008; 4: 215-222

[20] Lose G., Sorensen K., Frandsen B., Nathan E.: Excretion of urinary Tamm-Horsfall glycoprotein in girls with recurrent urinary tract infections. Urol. Res., 1987; 15: 249-250

[21] MacLean F.R., Skinner R., Hall A.G., English M., Pearson A.D.: Acute changes in urine protein excretion may predict chronic ifosfamide nephrotoxicity: a preliminary observation. Cancer Chemother. Pharmacol., 1998; 41: 413-416

[22] McLaughlin P.J., Aikawa A., Davies H.M., Ward R.G., Bakran A., Sells R.A., Johnson P.M.: Uromodulin levels are decreased in urine during acute tubular necrosis but not during immune rejection after renal transplantation. Clin. Sci., 1993; 84: 243-246

[23] Morais M.M., Belarmino-Filho J.N., Brito G.A., Ribeiro R.A.: Pharmacological and histopathological study of cyclophosphamide-induced hemorrhagic cystitis - comparison of the effects of dexamethasone and Mesna. Braz. J. Med. Biol. Res., 1999; 32: 1211-1215

[24] Muchmore A.V., Decker J.M.: Uromodulin: a unique 85-kilodalton immunosuppressive glycoprotein isolated from urine of pregnant women. Science, 1985; 229: 479-481

[25] Parsons B.A., Drake M.J.: Animal models in overactive bladder research. In: Handbook of Experimental Pharmacology, ed: Andersson K.E., Michel M.C., Springer-Verlag Berlin Heidelberg 2011, 202: 15-43

[26] Pennica D., Kohr W.J., Kuang W.J., Glaister D., Aggarwal B.B., Chen E.Y., Goeddel D.V.: Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein. Science, 1987; 236: 83-88

[27] Prajczer S., Heidenreich U., Pfaller W., Kotanko P., Lhotta K., Jennings P.: Evidence for a role of uromodulin in chronic kidney disease progression. Nephrol. Dial. Transplant., 2010; 25: 1896-1903

[28] Reinhart H.H., Obedeanu N., Hooton T., Stamm W., Sobel J.: Urinary excretion of Tamm-Horsfall protein in women with reccurent urinary tract infections. J. Urol., 1990; 144: 1185-1187

[29] Reinhart H.H., Spencer J.R., Zaki N.F., Sobel J.D.: Quantitation of urinary Tamm-Horsfall protein in children with urinary tract infections. Eur. Urol., 1992; 22: 194-199

[30] Säemann M.D., Weichhart T., Zeyda M., Staffler G., Schunn M., Stuhlmeier K.M., Sobanov Y., Stulnig T.M., Akira S., von Gabain A., von Ahsen U., Hörl W.H., Zlabinger G.J.: Tamm-Horsfall glycoprotein links innate immune cell activation with adaptative immunity via a Toll-like receptor-4-dependent mechanism. J. Clin. Invest., 2005; 115: 468-475

[31] Samuell C.T.: Uromucoid excretion in normal subjects, calcium stone formers and in patients with chronic renal failure. Urol. Res., 1979; 7: 5-12

[32] Schröder A., Newgreen D., Andersson K.E.: Detrusor responses to prostaglandin E2 and bladder outlet obstruction in wild-type and Ep1 receptor knockout mice. J. Urol., 2004; 172: 1166-1170

[33] Serafini-Cessi F., Malagolini N., Cavallone D.: Tamm-Horsfall glycoprotein: biology and clinical relevance. Am. J. Kidney Dis., 2003; 42: 658-676

[34] Sinanoglu O., Yener A.N., Ekici S., Midi A., Aksungar F.B.: The protective effects of spirulina in cyclophosphamide induced nephrotoxicity and urotoxicity in rats. Urology, 2012; 80: 1392.e1-1392.e6

[35] Singh P.P, Pendse A.K., Rajkiran, Bhupesh Partani: Diurnal variations and 24 hr urinary excretion of mucoprotein and Tamm-Horsfall protein in renal stone patients. Indian J. Clin. Biochem., 1993; 8: 54-58

[36] Storch S., Saggi S., Megyesi J., Price P.M., Safirstein R.: Ureteral obstruction decreases renal prepro-epidermal growth factor and Tamm-Horsfall expression. Kidney Int., 1992; 42: 89-94

[37] Tamm I., Horsfall F.L.Jr.: Characterization and separation of an inhibitor of viral hemagglutination present in urine. Proc. Soc. Exp. Biol. Med., 1950; 74: 106-108

[38] Torffvit O., Kamper A.L., Strandgaard S.: Tamm-Horsfall protein in urine after uninephrectomy/transplantation in kidney donors and their recipients. Scand. J. Urol. Nephrol., 1997; 31: 555-559

[39] Torffvit O., Jorgensen P.E., Kamper A.L., Holstein-Rathlou N.H., Leyssac P.P., Poulsen S.S., Strandgaard S.: Urinary excretion of Tamm-Horsfall protein and epidermal growth factor in chronic nephropathy. Nephron, 1998; 79: 167-172

[40] Vyletal P., Bleyer A.J., Kmoch S.: Uromodulin biology and pathophysiology – an update. Kidney Blood Press. Res., 2010; 33: 456-475

[41] Wai-Hoe L., Wing-Seng L., Ismail Z., Lay-Harn G.: Proteomics and detection of uromodulin in first-time renal calculi patients and reccurent renal calculi patients. Appl. Biochem. Biotechnol., 2009; 159: 221-232

[42] Zeng J., Pan C., Jiang C., Lindström S.: Cause of residual urine in bladder outlet obstruction: an experimental study in the rat. J. Urol., 2012; 188: 1027-1032

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