MICROCIRCULATORY DYSFUNCTION OF THE RAT URINARY BLADDER

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Abstract

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1. INTRODUCTION

1.1. The microcirculation of the urinary bladder

Until recently, technical limitations made it difficult to observe and measure microcirculatory variables. The advent of intravital video-microscopy (IVM) resulted in the accumulation of new data from various fields of animal research. Most of the characteristic details of the microcirculation of the urogenital tract, however, remained to be elucidated. Up to now, no microcirculatory data were available on the microcirculatory characteristics of the bladder during circulatory dysfunction, such as ischemia-reperfusion (I/R) injury. Thus, an experimental study with fluorescence IVM on this field could be of importance, as it could be considered as a "*gold standard*" for further investigations.

An increased neutrophil adhesiveness for microvascular endothelium appears to be a critical first step in the overall pathogenesis of I/R-induced injury. IVM techniques could monitor the adherence and emigration of leukocytes and visualize "*no-reflow*" and "*reflow paradox*" components of I/R. With this method high resolution images and precise quantification of the parameters of microcirculation could be obtained in thin tissues.

1.2. Ischemia-reperfusion-induced inflammation of the bladder

Inflammation of the urinary bladder is a frequent diagnosis in clinical practice and widely investigated. However, many aspects of the pathogenesis are not completely understood. I/R is used in experimental research as a trigger of inflammation. So far, it has not been used to induce cystitis.

I/R of the urinary bladder can result from various pathologic conditions. Although re-establishment of perfusion is mandatory for the

survival of ischemic organs, reperfusion is associated with local post-ischemic inflammation and thereby paradoxically promotes further tissue injury.

1.2.1. *The role of endothelin-1 in ischemia-reperfusion injury*

The vascular endothelial lining has multiple functions as a diffusion barrier between the intravascular and the extravascular spaces. It synthesizes, metabolizes and releases a number of humoral and hormonal substances and plays a role in maintaining the local homeostasis via locally produced vasoactive mediators. As a result, functional alterations of the endothelium are rapidly manifested in micro- and macrocirculatory changes influencing the function of the affected organ.

Evaluations of the function of the vascular endothelium generally focus on the endothelium-dependent constriction and dilation of blood vessels, neutrophil-endothelial cells interactions or permeability changes.

One of the important factors in the vasoregulation after I/R and leukocyte activation is the release of the potent vasoconstrictive peptide endothelin-1 (ET-1). Endothelins are a family of 21-amino acid polypeptides, that are synthesized from precursor molecules by proteolytic cleavage. Three active isoforms have been identified. ET-1 is released predominantly from endothelial cells. ET-A receptors are predominately found on smooth muscle cells as well as cardiomyocytes and binding of ET-1 to these receptors elicit exclusively vasoconstriction.

ET-1 was shown to be involved in many states of critical illnesses. Since plasma levels of ET-1 are increased during I/R, hypoxia, inflammation and sepsis, the peptide may also contribute to the characteristic microcirculatory perturbations under these conditions. ET-1 induces activation and adhesion of polymorphonuclear leukocytes at the site of inflammation.

Endothelin receptors can be found on the surface of the urinary tract cells and all tissue components of the bladder, including urothelial and muscular layers.

1. 3. Enterocystoplasty

Enterocystoplasty (ECP) is a method to enlarge the capacity and to decrease the intravesical pressure of urinary bladder using intestinal segments. Bladder augmentation is a management of the intractable, small capacity, poorly compliant high-pressure neurogenic bladder. The inclusion of this population as candidates for ECP has been accompanied by the complication of late spontaneous rupture of the augmented bowel. This potentially life-threatening complication mostly occurred at a site remote from the bladder-bowel anastomosis. Several theories have been devised to explain the rupture. One supposed etiology is the microvascular insufficiency with resultant ischemia in the wall of the intestine in response to the increased intravesical pressure.

1.4. Aims of the studies

We aimed to answer the following questions:

1) What are the microcirculatory characteristics of the urinary bladder in rats?
2) How do microcirculatory parameters of the urinary bladder change after I/R injury?
3) What is the contribution of ET-1 to the pathomechanism of I/R injury in the urinary bladder?
4) What are the microcirculatory consequences of elevated intravesical pressure after enterocystoplasty?

2. MATERIALS, METHODS AND EXPERIMENTAL PROTOCOLS

2.1. Animals

Male Spraque-Dawley and Wistar rats (250-300 g) were used. The experiments were performed in accordance with the Hungarian and German legislations on protection of animals.

2.2. Surgical procedures and measurements

2.2.1. *Surgical procedures for examination of the bladder in rats*

After premedication with atropine sulfate and sodium pentobarbital anesthesia, the animals were placed on a heating pad. Catheters were inserted into the carotid artery and jugular vein to measure the mean arterial pressure, heart rate and for the injection of fluorescent dyes for IVM. The animals were intubated through a tracheotomy.

After a midline laparotomy the bladder was exposed. The urethra was ligated. The two ureters were cut through. A catheter was inserted into the bladder at the dome. The bladder was emptied first and then was filled with 0.5 ml of 0,9% NaCl solution. The intravesical pressure was continuously measured. At the given time points, the bladder was exteriorized and was placed on a stage for intravital fluorescence video-microscopy. Afterwards the animals were killed with an overdose of sodium pentobarbital.

2.2.2. *Method for ischemia-reperfusion of the rat urinary bladder*

Ischemia of the bladder was performed by clamping both cystic artery branches. After 60 min the abdomen was reopened, the clips were removed, allowed the 30 min reperfusion period. After reperfusion the bladder was exteriorized and put under the intravital video-microscope.

2.2.3. *Procedure of enterocystoplasty and measurement of leak point pressure*

Following midline laparotomy, a 1.5 cm ileal segment with good blood supply was chosen and resected. The intestinal continuity was re-established. Then the bladder was exposed and cannulated on the dome. Isotonic saline solution was injected into the bladder, and the pressure value at which the external urethral sphincter opened and a drop of liquid appeared in the meatus (leak point) was measured. Then the bladder top was incised. The resected intestinal segment was opened along its antimesenteric border. This detubularized ileal segment was anastomosed to the bladder. The animals were given fluid and analgetics and allowed to recover following the operation.

The second operation was performed 90 days later. Following atropine premedication and intraperitoneal sodium pentobarbital anesthesia the animals were placed on a heated operating table. Tracheotomy, jugular vein and carotid artery cannulations then a midline laparotomy was performed.

2.2.3.1. *The consequences of elevated intraluminal intestinal pressure*

Following laparotomy a 1.5 cm ileal segment was chosen, resected and cannuled on both open ends. The pressure was increased within the intestine by injecting isotonic saline solution. The ileal segment was put under the intravital videomicroscope. Pictures were then taken at the given pressure values.

2.2.3.2. *The consequences of elevated intravesical pressure*

Following laparotomy the urethra was resected, then catheter was fixed. Another cannula was inserted into the bladder dome thus the pressure could be checked. Isotonic saline solution was injected through the lower

cannula and the intravesical pressure was increased. The bladder was put under the intravital videomicroscope.

2.2.3.3. *The consequences of elevated intraluminal pressure of the enterocystoplasty*

The augmented bladder was exposed using an abdominal incision. The urethra was resected, and a cannula passed in. Isotonic saline solution was injected to increase the pressure. Another cannula was also fixed into the bladder dome, where the pressure was recorded. The augmented bladder was placed on a stage for IVM measurements. At each pressure value and time point pictures of the intestinal and bladder parts of the ECP were taken.

2.3. Histology

The examined organ was resected, fixed in 4% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for light microscopy.

2.4. Fluorescence intravital video-microscopy

2.4.1. *Intravital video-microscopy in ischemia-reperfusion experiments*

Contrast enhancement was achieved by iv injection of fluorescein isothiocyanate (FITC)–labeled for the plasma, and rhodamine for the leukocyte labeling. The microcirculatory network was visualized by fluorescence intravital microscope. The microcirculation was analyzed by using an epi-illumination technique. The microscopic images were recorded by video camera attached to a video recorder for computer-assisted evaluation.

2.4.2. *Video analysis*

Arteriolar and venular diameter, functional capillary density (FCD), venular red blood cell velocity (RBCV) and macromolecular leakage were determined. Leukocyte-endothelial cell interactions were analyzed with respect to rolling and adherent leukocytes.

2.4.3. *Intravital video-microscopy of enterocystoplasty*

Fluorescein isothiocyanate (FITC)-labeled erythrocytes were injected intravenously to visualize the microcirculatory networks. Fluorescent intravital microscope was used.

2.4.4. *Video analysis*

Quantitative analysis of microhemodynamics was performed by frame-to-frame analysis of videotaped images. RBCV, FCD, and capillary perfusion rate were determined in the longitudinal muscular layer.

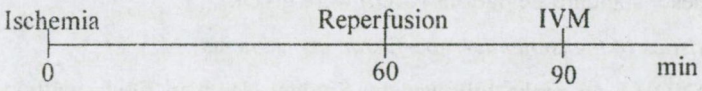
2.5. Experimental protocols

2.5.1. *Microcirculatory characteristics of the urinary bladder*

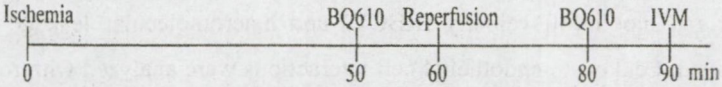
IVM measurements were made at the beginning, and 90, 120 and 180 min after the surgical exposure of the urinary bladder.

2.5.2. *Microcirculatory consequences of ischemia-reperfusion of the urinary bladder*

After 60 min ischemia and 30 min reperfusion fluorescence IVM measurements were performed. Sham-operated animals served as controls.



2.5.3. *The role of endothelin-1 in ischemia-reperfusion of the urinary bladder*



The animals were pretreated with the ET-A receptor antagonist BQ 610. The first dose was injected i.v. 10 min before the onset of ischemia, the second immediately at the beginning of the reperfusion. Microcirculatory changes were observed after 30 min reperfusion.

2.5.4. *The consequences of elevated intraluminal pressure of enterocystoplasty*

IVM pictures of the microcirculation of muscular layer of the examined organs were taken at intravesical pressure values of 10, 20, 25, 30 and 40 mmHg, at each pressure level in the 1^{st} , 5^{th} and 10^{th} min and in the 1^{st}, 10^{th} , 20^{th} , 30^{th} , 40^{th} , 50^{th} and 60^{th} min at intravesical pressure value of 20 mmHg in separate experiments.

The microcirculation of intact ileal segments and the intact bladder were also determined at the given time points and pressure levels as controls. Following the examinations tissue samples were taken for histology.

2.6. Statistics

$p < 0.05$ was considered significant.

2.6.1. *Statistics of the measurements for bladder microcirculation*

Friedman repeated measures for analysis of variance was applied. Mean values ± standard deviations (S.D.) were given.

2.6.2. *Statistics of the ischemia-reperfusion experiments*

ANOVA on ranks followed by Student-Newman-Keuls method was applied. Mean values ± S.D. were given.

2.6.3. *Statistics of the enterocystoplasty experiments*

Changes in variables within groups were analyzed by ANOVA tests followed by the Bonferroni's test. Differences between groups were evaluated by means of Student's unpaired *t* test. Data are expressed as means ± S.E.M.

3. RESULTS

3.1. Microcirculatory characteristics of the urinary bladder

After filling the bladder with 0.5 ml saline solution the intravesical pressure was 9±2 mmHg and did not chang significantly during the observation period. There were no significant changes in microcirculatory parameters during the 180-min period as compared

3.2. Ischemia-reperfusion injury of the urinary bladder

3.2.1. *Microcirculatory changes of the urinary bladder*

All investigated microcirculatory parameters were significantly changed after I/R as compared to the control group. The numbers of rolling and firmly adherent leukocytes were elevated by 828% and 4610%, respectively. The macromolecular leakage was also significantly increased by 33%. The RBCV and FCD decreased to 35% and 27% of the control values, respectively. The arteriolar and venular diameters were also reduced to 73% of the controls.

3.2.2. *Histological changes*

Increased lymphocyte and polymorphonuclear leukocyte migration into the submucosa, lamina propria and the epithelium and diffuse edema was observed.

3.2.3. *The microcirculatory effects of endothelin-A receptor antagonist*

BQ 610 did not influence the macrocirculatory parameters.

In the control group, only moderate leukocyte-endothelial cell interactions were observed. We found normal flow parameters and there were very few capillaries unperfused.

With the administration of BQ 610, the microcirculatory parameters were significantly different from the values of the non-pretreated I/R group, but did not reach the control values, except venular diameter and arteriolar diameter where the difference was not significant from the control. Compared to the I/R group, the numbers of rolling and firmly adherent leukocytes were decreased by 80% and 94%, respectively.

3.3. Microcirculatory disturbances of elevated intraluminal pressure of enterocystoplasty.

3.3.1. *Leak point pressure*

The urethral sphincter's closure pressure was 39±2 mmHg and was similar in the intact bladder to that measured after ECP.

3.3.2. *Microcirculatory changes of the intact ileum*

When intraluminar pressure was increased the microcirculation of the intact ileum showed the first significant disturbance at 25 mmHg. The microcirculation became even more compromised at higher pressures.

3.3.3. *Microcirculatory changes of the intact bladder*

In the intact bladder, the microcirculation was well preserved during the examination period.

3.3.4. *Microcirculatory changes of the enterocystoplasty*

In animals with ECP significant microcirculatory differences were not observed in the augmented intestine or bladder as compared to the the intact organs.

When increasing the intravesical pressure significant microcirculatory damage started already below the leak point pressure (at 25 mmHg) in the ileal part. This dysfunction became worse with further increase of the pressure. In the bladder part of the ECP no significant microcirculatory damage could be observed.

3.3.5. *Microcirculation of the enterocystoplasty at low intraluminal pressure level*

Microcirculation of the ileal part of the ECP did not show significant damage at 20 mmHg pressure even after 60 min. The microcirculation was well preserved also in the bladder part during the examined period.

4. DISCUSSION

4.1. The microcirculation of the urinary bladder

Our first aim was to develop a reproducible rat model to precisely characterize the microvascular perfusion parameters of the bladder. We described the physiological characteristics of the microcirculation of the bladder. We filled the bladder with a constant volume of saline and measured the intravesical pressure during a 180-min examination period. The bladder was placed only for the time of the microscopic observation on a specially designed stage for the best visualization of the microcirculatory network, and between the measurements it was returned to the original position. All relevant microcirculatory parameters were determined and no significant change occurred during the observation period.

4.2. Ischemia-reperfusion-induced inflammation of the urinary bladder

The inflammatory mechanisms of different types of cystitis have been widely investigated using several animal models. So far, I/R has not been reported as a model for cystitis, in spite of the fact that this pathological mechanism triggers inflammation in other organs.

In the present study, I/R of the bladder significantly impaired microvascular flow and endothelial integrity. Leukocyte-endothelial cell interaction as a key parameter for inflammation was enhanced. Similarly, the histological changes after I/R of the urinary bladder are very similar to those observed in other models of cystitis.

4.3. The role of endothelin-1 in ischemia-reperfusion injury of the bladder

To date no data were available for the role of ET-1 on I/R-induced processes of the urinary bladder. In the present study, I/R of the bladder significantly affected all observed microcirculatory parameters. With the administration of BQ 610, the microcirculatory disturbances were attenuated, but control values were not achieved with exception of venular and arteriolar diameter. In this context, the fact that vasoconstriction is mediated predominantly by ET-A receptors might explain the protective effects of the ET-A receptor antagonist, since postischemic blood flow velocity and capillary perfusion were also improved after administration of BQ-610.

4.4. Enterocystoplasty

Despite of experience with ECP, late spontaneous rupture only recently has been reported as a complication. Previous reports have suggested

that there has been chronic overdistension of the augmented bladder before rupture.

Our ECP study represents a new *in vivo* model for studying the microcirculatory consequences of the ECP.

We demonstrated the greater sensitivity of the microcirculation of the intestinal segment of ECP to clinically relevant intraluminal pressure increases in the rat. Hypoxia can be the explanation for the site of the rupture. Over all, the fact that the microcirculation of the ECP remains well preserved at lower pressure even after a longer period warrants for the importance of self-catheterization of the patients to maintain the low intravesical pressure.

5. SUMMARY AND CONCLUSION

1. We successfully developed a new experimental rat model for the examination of the microcirculation of the urinary bladder and determined the *in vivo* microcirculatory characteristics of the organ.
2. I/R injury of the urinary bladder causes significant microcirculatory disturbances and induces microcirculatory inflammatory reaction within the muscular layer of the bladder wall.
3. ET-1 can act as an important player in the process of I/R-induced cystitis and microcirculatory damage. The ET-A receptor antagonist therapy significantly decreases the microcirculatory injury.
4. In our model we demonstrated the greater sensitivity of the microcirculation of the intestinal segment of ECP to clinically relevant intraluminal pressure increases. We demonstrated that the microcirculation of the ECP remains well preserved at lower intravesical pressure even after a longer period.

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List of full papers related to the subject of the thesis

I. Bajory Z., Hutter J., Krombach F., Messmer K.:
New method: the intravital videomicroscopic characteristics of the microcirculation of the urinary bladder in rats.
Urol Res 2002; 30(3): 148-52.

II. Bajory Z., Hutter J., Krombach F., Messmer K.:
Microcirculation of the urinary bladder in a rat model of ischemia-reperfusion-induced cystitis.
Urology 2002; 60(6): 1136-40.

III. Bajory Z., Hutter J., Krombach F., Messmer K.:
The role of endothelin-1 in ischemia-reperfusion induced acute inflammation of the bladder in rats.
J Urol 2002; 168(3): 1222-5.

IV. Bajory Z., Szabó A., Pajor L., Tiszlavicz L., Boros M.:
Intravital microscopic assessment of pressure induced microcirculatory changes after enterocystoplasty in rats.
J Urol 2001; 165(4): 1279-82.

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