MICROCIRCULATORY DYSFUNCTION OF THE RAT URINARY BLADDER

Ph.D. Thesis

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Summary

The main goals of our studies were **1.** to develop a standardized experimental model for the observation of the microcirculatory characteristics of the urinary bladder and **2.** to investigate the mechanism of microcirculatory dysfunction of the bladder under acute and chronic pathological conditions. In the first part, the microcirculatory consequences of ischemia-reperfusion (I/R) and the roles of the endogenous endothelin-1 (ET-1) were evaluated using selective endothelin receptor antagonist treatment and intravital video-microscopy (IVM) techniques. Secondly, the effects of elevated intravesical pressure were observed in a chronic rat model of enterocystoplasty (ECP) using a de-tubularised ileal segment for the augmentation of the urinary bladder.

In the acute studies, the consequences of 60 min ischemia followed by 30 min reperfusion were studied in a rat model. I/R of the bladder was elicited by clamping and releasing of the cystic arteries. The role of the ET-1 in this pathology was investigated with the administration of the selective endothelin-A receptor antagonist BQ 610 and the relevant microcirculatory parameters were evaluated using fluorescence IVM. Arteriolar and venular diameters, functional capillary density, venular red blood cell velocity, arteriolar and venular macromolecular leakage and leukocyte-endothelial cell interactions (rolling and adherent leukocytes) were assessed by a computer-assisted analysis system.

After I/R a severe inflammatory reaction evolved and significant microcirculatory failure were observed in the urinary bladder. Pretreatment with BQ 610 effective by attenuated the effects of I/R-induced inflammation, and partially prevented the microcirculatory disturbances.

In the chronic experiments with ECP the bladder was filled with a constant volume of saline solution to maintain the physiological intravesical pressure, or alternatively, the ECP also was cannulated and the intravesical pressure was elevated stepwise by 10 mmHg. The bladder or the ECP was positioned on a stage and fluorescence IVM measurements were performed during constant intravesical pressure or after each increment of pressure of the ECP.

Filling the bladder with a constant volume of saline solution resulted in a physiological intravesical pressure and microcirculatory parameters or intravesical pressure did not change significantly during the 180-min observation period. Stepwise increases of the intravesical pressure did not significantly affect microcirculatory flow and functional capillary

density of the bladder or the bladder part of ECP until the pressure reached 70 mmHg, and then significant microcirculatory disturbances were observed at higher pressure values. Microcirculation, however, was significantly diminished both in the intact bowel wall and in the intestinal graft part of the ECP at 25 mmHg intraluminal pressure. On the other hand, below this level (20 mmHg) no significant damage occurred in the microcirculation in both part of ECP even after a longer period.

These results demonstrate the sensitivity of the microcirculation of the intestinal part of the ECP to clinically relevant pressure increases. This model can serve as a basis for further investigations to better understand the basic pathophysiology of the urinary bladder at the microcirculatory level.

CONTENTS

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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1. Bajory Z., Szabó A., Pajor L., Boros M.
   Pressure-flow relationship after enterocystoplasty in rats. An intravital microscopy study.
   Seventh Vienna Shock Forum, Vienna 13-16 Nov. 1999
   Shock 1999; 12: 200.

2. Bajory Z., Szabó A., Pajor L., Boros M.
   Intravital microscopic examinations of the microcirculatory consequences of enterocystoplasty in the rat.
   Joint Meeting of The Physiological Society with the Hungarian Physiological Society, Budapest 27-29 May 2000
   The Journal of Physiology 2000; 526: 150.

3. Bajory Z., Hutter J., Krombach F., Messmer K.
   Microcirculatory changes in ischemia-reperfusion-induced cystitis in rats
   Congress of European Society of Surgical Research, Szeged, 13-16 May 2002
   European Surgical Research 2002; 34: 53.

4. Pákáski M., Bajory Z., Varga R., Szabó A., Pajor L., Boros M.
   Endothelin-A receptor antagonism reduces torsion-induced aspermatogenesis and microcirculatory failure in the rat.
   Congress of European Society of Surgical Research, Szeged, 13-16 May 2002
   European Surgical Research 2002; 34: 41.

1. INTRODUCTION

1.1. The microcirculation of the urinary bladder

The microcirculation consists of a network of blood vessels with an outside diameter of less than 300 μm. The primary function of this part of the circulatory system is a convective transport of substrates to the cells and delivery of metabolic products from the tissues. Local adaptation of these transport microvessels to an ever changing metabolic and functional demand is a prerequisite for a well functioning system.

Until recently, technical limitations made it difficult to observe and measure microcirculatory variables, such as the vessel diameter changes, red blood cell velocity, microvascular permeability or the state of cellular (leukocyte, endothelial cell, etc.) activation. However, in the last decade the advent of intravital video-microscopy (IVM) resulted in the accumulation of new data from various fields of animal research.[1.,2.] Microcirculation of many organs was observed under physiological and pathophysiological *in vivo* conditions,[1.-5] new perspectives of tumor and tissue transplantation research were opened,[2.] and microcirculatory observations of awake animals became also possible.[2.,6.] Most of the characteristic details of the microcirculation of the urogenital tract and the microcirculatory response to different pathophysiological stimuli, however, remained to be elucidated. In particular, up to now no microcirculatory data were available on the microcirculatory characteristics of the urinary 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.

In general, the pathophysiological consequences of hypoperfusion depend upon the previous state of an organ and the duration and degree of the ischemic insult. No single process can be identified as the critical event in ischemia-induced tissue injury as at least three possible mechanisms have been identified by which the tissues may become hypoxic. Firstly, the decreased blood flow will decrease oxygen delivery. Secondly, a decreased oxygen delivery may be related to an increased short-circuiting of oxygen as in the case of the countercurrent exchanger mechanism of the intestinal villi.[7.] Thirdly, hypoxic and cytotoxic cellular damage could be seen in sepsis - nevertheless, in septic conditions the above mechanisms might work in concert.

When a tissue is subjected to ischemia, a sequence of chemical reactions is initiated which may ultimately lead to cellular dysfunction and necrosis. It is evident that re-establishing blood flow is necessary for the rescue of ischemic tissues since this allows for the regeneration of cellular energy and the washout of toxic metabolites. However, reperfusion of ischemic tissues also leads to a sequence of events that paradoxically injure tissues.

The small intestine is one of the most widely investigated organ under ischemic conditions. The extreme susceptibility of the intestine to hypoxic injury is marked by the fact that 20 min total ischemia or 60-120 min severe partial ischemia regularly induces injury detectable by light microscope. Even before morphologic injury hypoxia causes increased mucosal permeability.[7] The main consequences of the increased permeability and the destruction of morphological barrier between the lumen and intestinal tissue are the increased translocation of bacteria from the lumen, absorption of bacteria to the portal venous system or to the lymphatics, and release of non-bacterial toxic substances from the gut.[8] Marshall and co-workers have proposed that the intestine might be the source of continuous release of bacteria and endotoxin, creating a background for the chronic septic complications often seen in patients in this condition.[9]

There is a large body of evidence which indicates that reactive oxygen metabolites play an important role in mediating the vascular and tissue-related cellular injury during reperfusion of ischemic intestine.[10] The oxygen radical hypothesis of reperfusion injury is based on the assumption that tissue injury is dependent on the reintroduction of oxygen followed by the production of reactive oxygen metabolites. Reperfusion greatly exacerbates the ischemia-induced leukocyte adherence and extravasation, and a potential source of reactive oxygen metabolites in postischemic tissues is the polymorphonuclear leukocyte (PMN). Neutrophils contain NADPH oxidase that reduces molecular oxygen to superoxide anion. Activated PMN secrete the enzyme myeloperoxidase (MPO), which catalyzes the formation of hydrochlorous acid from hydrogen peroxid and chloride ions. These cells can also release a variety of proteolytic enzymes that can injure the microvasculature. Thus, PMNs have the full potential to be a main source for the injurious effects of reperfusion injury.

An increased neutrophil adhesiveness for microvascular endothelium appears to be a critical first step in the overall pathogenesis of I/R-induced injury to the microvasculature. A number of factors influence the adhesive interactions between PMNs and endothelial cells. The adherence of leukocytes to venular endothelium largely depends on the expression of

adhesion molecules on the surface of activated PMNs and/or endothelial cells, hydrodynamic dispersal forces (e.g., blood flow velocity) that tend to sweep leukocytes away from the vascular wall, and products of PMN activation (e.g., elastase).

IVM techniques could monitor the adherence and emigration of leukocytes in postcapillary venules and visualize "*no-reflow*" and "*reflow paradox*" components of I/R injury. 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 in clinical and experimental research. However, many aspects of the pathogenesis are not completely understood. Several experimental animal models have been established to induce inflammation of the urinary bladder in various ways, in order to mimic the pathogenesis of human disease.[11.-14.] I/R is used in experimental research as a trigger of inflammation in various organs.[15.,16.] So far, it has not been used to induce cystitis, in spite of the fact that I/R also can be an ethiological factor of cystitis in patients.

I/R of the urinary bladder can result from various pathologic conditions, for example overdistension caused by lower urinary tract obstruction, thrombo-embolisation and recanalisation of bladder vessels, infrarenal aortic aneurysm, temporary ligation of cystic arteries, complications of pregnancy or from hemorrhagic or septic shock. 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.[15.-18.] In the bladder, the consequences of I/R-injury can range from increased urothelial permeability, recurrent infections, inflammations to necrosis or gangrene in the worst scenario.

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

The vascular endothelial lining is a widely-dispersed organ with a mass of approximately 1.5 kg. It has multiple functions as a diffusion barrier between the intravascular and the extravascular spaces of the blood and lymphatic vessels, whereby regulating permeability (fluid, metabolite and catabolic exchange). Additionally, 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.[19.-21.] The modulation of microvascular permeability, the regulation of leukocyte-endothelial interactions and angiogenesis are also dependent upon the endothelial integrity.[22.,23.] Pathological stimuli such as I/R, sepsis or hemorrhage modify the endothelium-dependent effector molecule expression.[24.,25.] 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 *(in vivo* experiments and *ex vivo* organ bath studies), neutrophil-endothelial cells interactions or permeability changes (microvascular permeability measurements).

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.[26.,27.] Three active isoforms (ET-1, ET-2 and ET-3) have been identified and cloned. ET-1 is released predominantly from endothelial cells, whereas ET-2 and ET-3 are expressed in the intestinal epithelium, renal tubular cells and in the brain. They exert their autocrine and paracrine action via two specific receptors, endothelin-A (ET-A) and endothelin-B (ET-B) receptors, respectively.[28.] After binding with ET-1, the ET-B receptors located on the endothelium (endothelin-B_1) mainly mediate vasorelaxation, while those located on vascular smooth muscle cells (endothelin-B_2) induce vasoconstriction. 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. A four-times higher affinity for ET-A as compared to ET-B renders ET-1 a potent endogenous vasoconstrictor.

ET-1 was shown to be involved in many states of critical illnesses, including cardiac dysfunction, pulmonary hypertension, hepatic failure, renal dysfunction as well as endocrine and metabolic imbalance. Since plasma levels of ET-1 are increased during I/R, hypoxia, inflammation and sepsis[29.], the peptide may also contribute to the characteristic microcirculatory perturbations under these conditions. However, in addition to its vasoconstrictive properties ET-1 induces activation and adhesion of polymorphonuclear leukocytes at the site of inflammation. Furthermore, it plays a role in platelet activation, probably via an increase of cytosolic free calcium followed by the expression of P-selectin.

These properties raise the possibility that an up-regulated endothelin release may be associated with the attachment of activated leukocytes to the vascular intima during pathophysiological states, such as I/R injuries. These actions probably are related to ET-A receptor activation, since binding of ET-1 to ET-A receptors was found to inhibit leukocyte-endothelium interaction and endothelial apoptosis.

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. The ratio of the ET-A receptors to ET-B is 4:1 in the bladder dome.[30] ET-1 can cause concentration-dependent contractions on the pig detrusor and vesical arterial smooth muscle.[31] Khan *et al.* studied the role of ET-1 in rabbit bladder hypertrophy and hyperplasia. With the administration of specific endothelin receptor antagonists the bladder neck smooth muscle proliferation was significantly inhibited.[32]

1. 3. Enterocystoplasty

Enterocystoplasty (ECP) as a method to enlarge the capacity and to decrease the intravesical pressure of urinary bladder using intestinal segments was first performed by Mikulicz in 1899.[33] For more than sixty years the procedure was limited to patients with shrunken bladder.[34] Bladder augmentation (using detubularized intestinal segment) as a management of the intractable, small capacity, poorly compliant high-pressure neurogenic bladder has become increasingly popular since 1967 when Servadio reported its use in a patient with neuropathic incontinence secondary to partial sacral agenesis.[35] Earlier concerns regarding subsequent retention of urine in patients with neuropathic bladders and secondary outflow obstruction have been largely eliminated by the widespread acceptance of intermittent clean catheterization in this patient population. Advances in urodynamics have greatly improved our understanding of the neuropathic bladder and led to refinement of our indications for augmentation ECP in these patients. Use of the artificial urinary sphincter and more recently, the urethral lengthening and reimplantation procedure have further extended these indications. The inclusion of this population as candidates for ECP has been accompanied by the complication of late spontaneous rupture of the augmented bowel with resultant peritonitis, sepsis and a few fatalities.[36-39] This potentially life-threatening complication occurred at a site remote from the bladder-bowel anastomosis in up to 5% of patients. Several theories have been devised to explain the rupture and these catastrophic

events after ECP including trauma from intermittent catheterization, chronic infection, chronic inflammation, avulsion of adhesions between the bowel patch and peritoneum. Another supposed etiology of this complication is the microvascular insufficiency with resultant ischemia in the wall of the intestine in response to the increased intravesical pressure.[40.]

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 (average weight 250-300 g) were housed in environmentally controlled rooms with a 12 hour day-night cycle and fed with standard laboratory food (18000 IU/kg vitamin A, 1280 IU/kg vitamin D_3, 120 mg/kg vitamin E) and water *ad libitum*. The circumstances of the housing met the requirements of NIH Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, NIH Publication No. 86-23, revised 1984). 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 urinary bladder in rats*

After premedication with atropine sulfate (0.1 mg/kg, subcutaneously; Braun, Melsungen, Germany) and sodium pentobarbital anesthesia (Narcoren®; Merial GmbH, Hallbergmoos, Germany, 45 mg/kg, intraperitoneally) the animals were placed in a supine position on a heating pad for maintenance of the normal body temperature during the experiment. All of the surgical procedures were made under operation microscope (Leica M651; Bensheim, Germany). Polyethylene catheters (ID 0.28 mm, OD 0.61 mm; SIMS Portex Ltd., Hythe, UK) were inserted into the left carotid artery and jugular vein to measure the mean arterial pressure (MAP), heart rate (Plugsys; Sachs Elektronik, March, Germany) and for the injection of fluorescent dyes for IVM. The animals were intubated (Abbocath®-T; 13G; ABBOTT, Sligo, Ireland) through a tracheotomy.

After a midline laparotomy the bladder was exposed and the median umbilical ligament was cut through near the bladder. The urethra was ligated with a 4-0 Perma-Hand® Silk (Johnson&Johnson Intl., Brussels, Belgium). The two ureters were cut through at their middle part to avoid the bladder from the overfilling during the experiment. A polyethylene catheter (ID 0.28 mm, OD 0.61 mm; SIMS Portex) was inserted into the bladder at the dome for filling the bladder and to measure the intravesical pressure. The bladder was emptied first and then was filled with 0.5 ml of bodywarm 0,9% NaCl solution. The intravesical pressure

was continuously measured with a pressure monitor (Plugsys). At the given time points, the bladder was exteriorized drawing gently by the stump of the umbilical ligament and was placed on a specially designed stage for intravital fluorescence video-microscopy. (Figure 1.) After the IVM measurements the bladder was replaced into the abdomen and the abdominal wall was closed by clamps until the next intravital observation. 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 using small metal clips (Figure 2.). Following the interventions the abdominal wall was closed by clipping. After 60 min the abdomen was reopened, the clips were removed, allowed the 30 min reperfusion period. After reperfusion the bladder was exteriorized again drawing gently by the stump of the umbilical ligament and put under the intravital video-microscope. Finally, the animals were euthanized with an overdose of sodium pentobarbital.

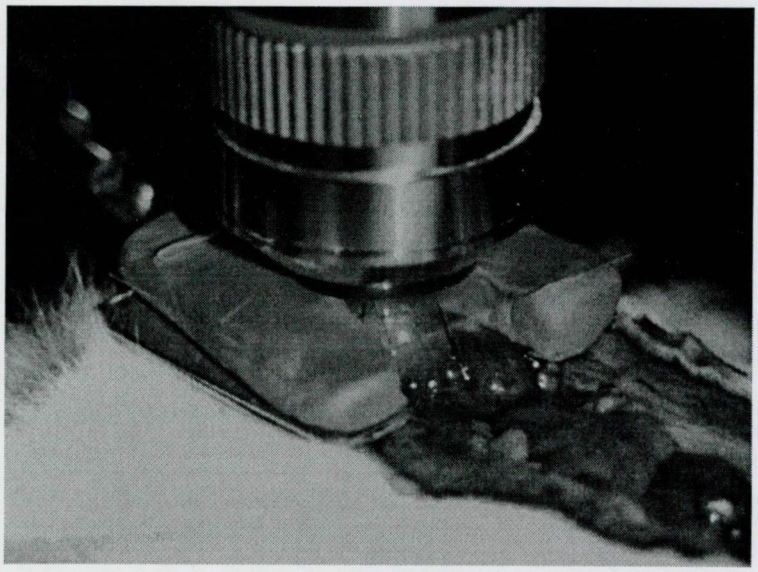


Figure 1. *The urinary bladder of the rat was placed on a specially designed stage for IVM.*

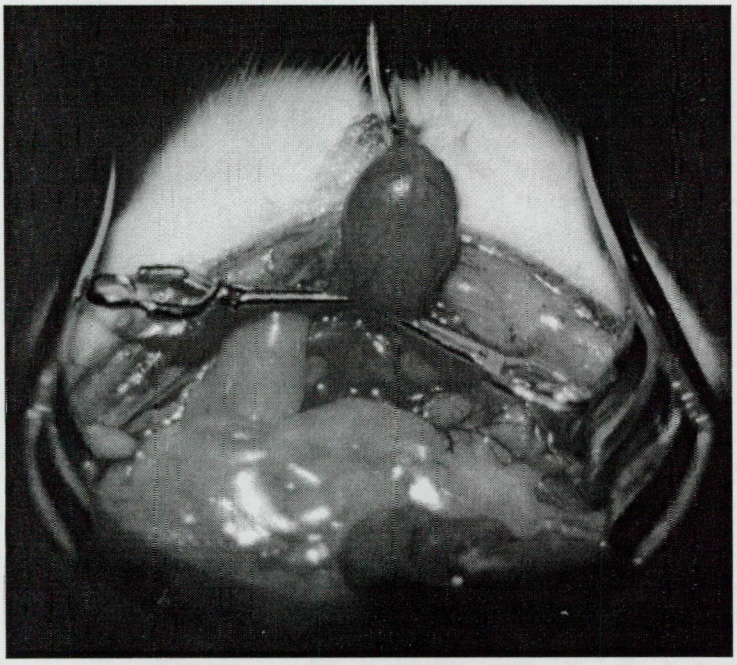


Figure 2. *The technique of urinary bladder ischemia in rat. Two metal clips were use to clamp both cystic artery branches of the rat.*

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

ECP was performed similarly to the method described by Guan et al.[41] Briefly, following midline abdominal laparotomy an approximately 1.5 cm ileal segment with good blood supply was chosen and resected. The intestinal continuity was re-established by end-to-end anastomosis with 6-0 Vicryl interrupted suture under operating microscope. Then the bladder was exposed and cannulated through a tiny gap that made on the dome using a 22-gauge polyethylene catheter. 36.5 °C isotonic saline solution was injected into the bladder through the cannula, 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 with microcauter in the middle line. The resected intestinal segment was opened along its antimesenteric border. This detubularized ileal segment was anastomosed to the bladder with 6-0 Vicryl suture run across all the four sides using operating microscope (Zeiss, 8 X) (Figure 3.). All the manipulations were performed carefully to avoid any injury to the vessels supplying the implanted segment. The operation was completed with wound closure in two layers using 4-0 Vicryl running suture. The animals were given fluid and analgetics and allowed to recover following the operation.

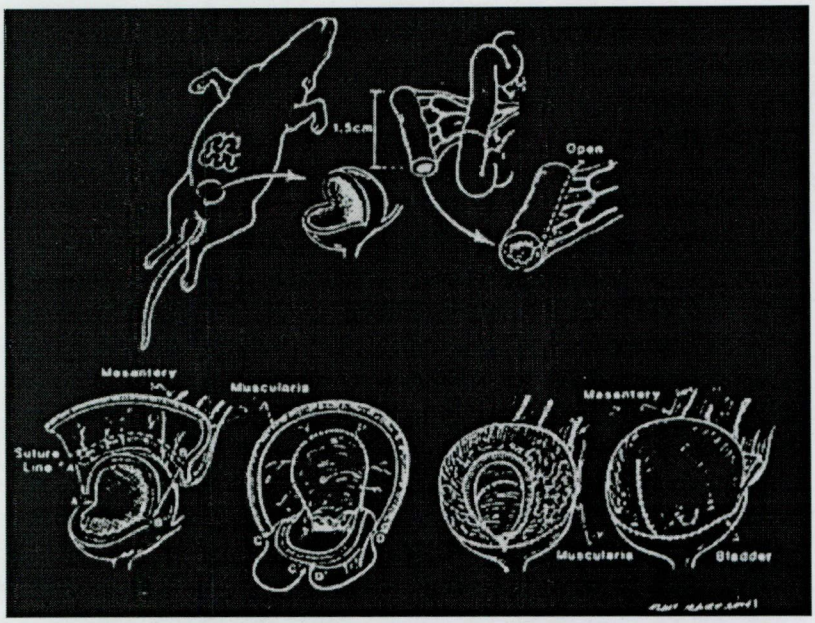


Figure 3. *The procedure of the enterocystoplasty in rat. The bladder was opened, a 1.5 cm-long ileal segment was resected, detubularized and anastomosed to the bladder. The continuity of the ileum was re-established by end-to-end anastomosis.*

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, left jugular vein and carotid artery cannulations then a midline laparotomy was performed in all animals.

2.2.3.1. *The consequences of elevated intraluminal intestinal pressure*

Following laparotomy an approximately 1.5 cm long ileal segment with good blood supply was chosen, resected and cannuled on both open ends. The pressure was increased within the intestine by injecting 36.5 °C isotonic saline solution through one cannula and on the other cannula the changes were recorded. The ileal segment was placed on a specially designed stage and 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 prepared and resected, then a 22-gauge polyethylene catheter was passed and fixed. Another cannula was inserted and fixed into the bladder dome thus the pressure could be checked. Isotonic saline solution (36.5 °C) was injected into the bladder through the lower cannula and the intravesical pressure was gradually increased. The bladder, placed on a specially designed stage, 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 a midline abdominal incision. The urethra was prepared and resected, and a 22-gauge polyethylene cannula passed in, through which 36.5 °C isotonic saline solution was injected to increase the pressure. A blood vessel-free area on the dome of the bladder was chosen, another cannula was also fixed into a tiny gap, where the pressure was recorded. The augmented bladder was placed on a specially designed stage for IVM measurements. At each pressure value and time point pictures of the intestinal and bladder parts of the ECP were taken.

Finally the animals were euthanized with an overdose of sodium pentobarbital in all groups.

2.3. Histology

The examined organ was resected, fixed in 4% formalin, embedded in paraffin, sectioned (5 μm) 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 albumin (MW 70000, 0.2 ml.; Sigma Chemicals, St. Louis, Missouri, USA) for the plasma, and rhodamine 6G (MW 479, 0.2%, 0.1 ml.; Sigma) for the leukocyte labeling

10 min before the fluorescence IVM evaluations. The microcirculatory network of the examined organ was visualized by using a high-resolution, modified Zeiss-Orthoplan fluorescence intravital microscope attached to a Ploemo-Pak illuminator with an $I_{2/3}$ blue (exitation filter (Ex): 495 nm., emission filter (Em): 515 nm.) and N_2 green (Ex: 525 nm., Em: 555 nm.) filter block (Leitz, Wetzlar, Germany). The microcirculation was analyzed by using an epi-illumination technique. With 25X water immersion objective (W 25X/0.6; Leitz) the magnification was 540X on the video screen (Sony, Tokyo, Japan). The microscopic images were recorded by a charge-coupled device video camera (FK 6990; Pieper GmbH, Schwerte, Germany) attached to an S-VHS video recorder (BR-S920E; JVC, Tokyo, Japan) for computer-assisted evaluation.[2.,42.]

2.4.2. *Video analysis*

Quantitative assessment of microcirculatory parameters was performed *off-line* by analysis of the videotaped images using a computer-assisted analysis system (CAMAS, Dr. H. Zeintl, Heidelberg, Germany). Arteriolar and venular diameter (μm) (Figure 4.), functional capillary density (FCD, length of erythrocyte perfused nutritive capillaries per observation area (1/cm)) (Figure 5.), venular red blood cell velocity (RBCV, mm/s) and macromolecular leakage (ratio of extravascular fluorescent intensity and intravascular fluorescent intensity) were determined in five observation fields of the muscular layer.[42.] Leukocyte-endothelial cell interactions[1.] were analyzed within five venules (n=5) per animal with respect to rolling and adherent leukocytes (Figure 6.). Rolling leukocytes were defined as cells moving significantly slower than the red blood cells moving in the centerline of the vessels and are given as number of rolling leukocytes per vessel diameter (mm) per second. Adherent leukocytes (stickers) were identified in each vessel segments as cells that did not detach from the endothelial lining within 30 seconds and are given as number of cells per square millimeter of endothelial surface, calculated from the diameter and length of the venule segment, assuming cylindrical geometry.[2.,43.]

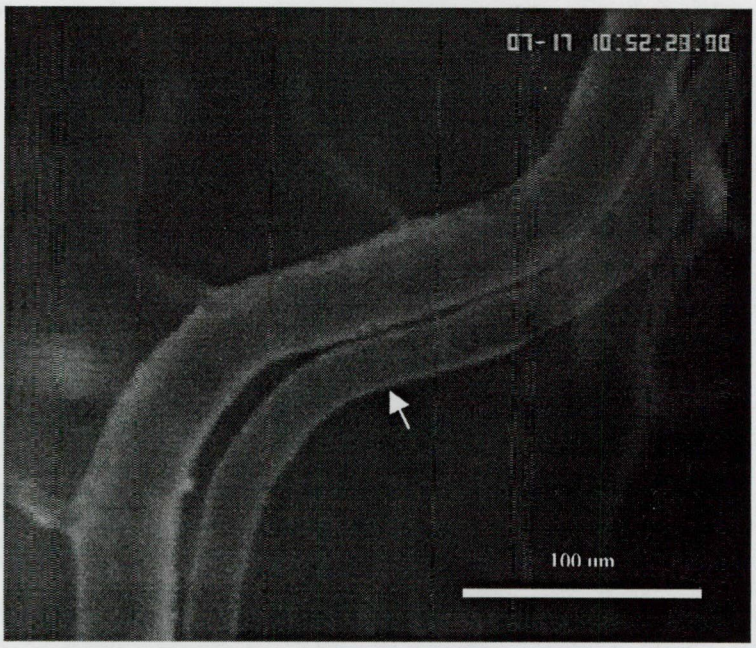


Figure 4. *An arteriole (arrow) and a venule of the muscular layer of the bladder. FITC-albumin plasma labeling.*

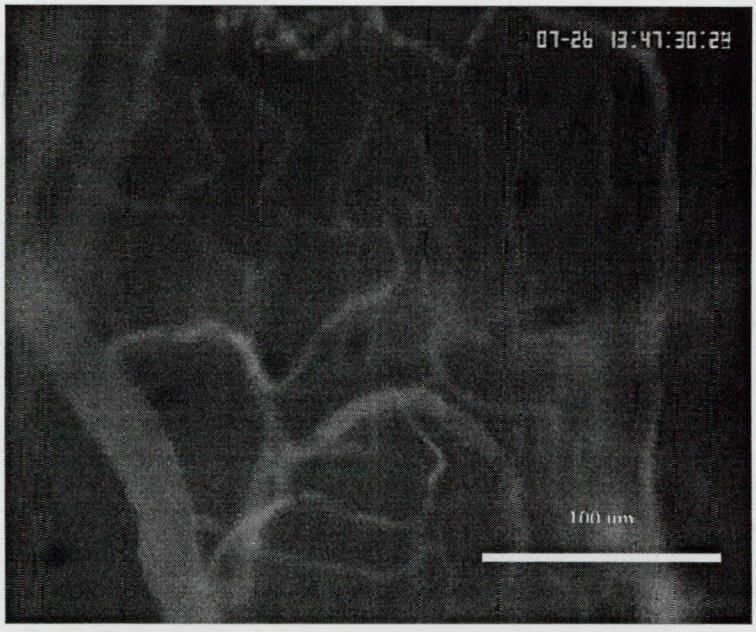


Figure 5. *The capillary network in the muscular layer of the bladder wall. FITC-albumin plasma labeling.*

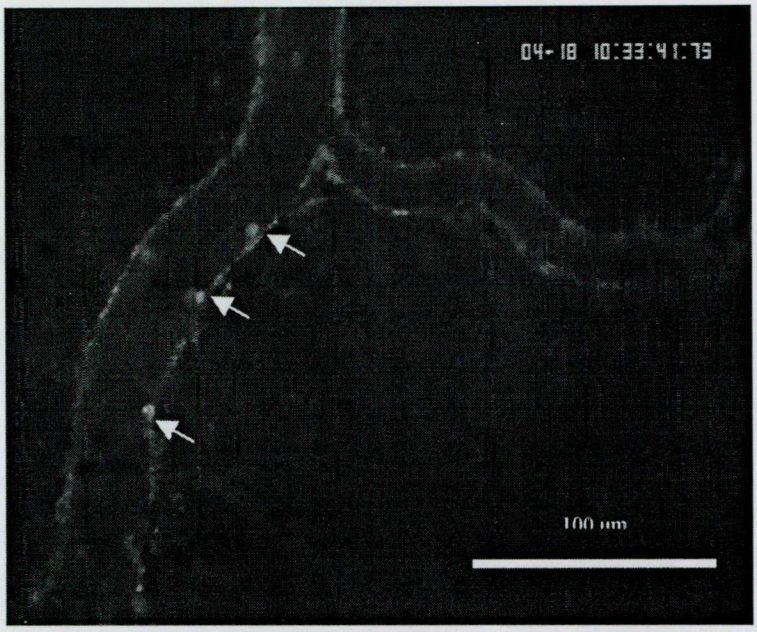


Figure 6. *Venules of the muscular layer of the urinary bladder. Rhodamine-6G labeled leukocytes (arrows) are rolling on the wall of the venule.*

2.4.3. *Intravital video-microscopy of enterocystoplasty*

The observed organs with intact neurovascular supply were placed on a specially designed stage. During intravital microscopy the tissue was continuously superfused with 36,5 °C Ringer's lactate solution to avoid drying, exposure to ambient air and maintaining the continuous immersion medium on the spherical surfaces. Fluorescein isothiocyanate (FITC)-labeled (Ex: 495nm., Em: 515nm.) erythrocytes were injected intravenously to visualize the microcirculatory networks.[44.]

The microcirculation was analyzed using an epi-illumination technique. A high resolution Zeiss Axiotech Vario 100HD fluorescent intravital microscope with a 100 W HBO mercury lamp, attached to an illuminator with blue filter block was used. With a 25 X (water immersion) objective (Acroplan, 20 X), the magnification on the video screen (Sony Multiscan 17se) was 450 X. The microscopic images were recorded by a charge-coupled device (CCD) video camera (AVT HORN-BC 12) and transferred to a video system (S-VHS Panasonic Video Cassette Recorder AG-MD 830) for off-line evaluation.[42.]

2.4.4. *Video analysis*

Quantitative analysis of microhemodynamics was performed by frame-to-frame analysis of videotaped images and with use of a computer-assisted digital image analysis system (Pictron®, Hungary).

Red blood cell velocity (RBCV, mm/s), functional capillary density (FCD, length of red blood cell-perfused nutritive capillaries per area) and capillary perfusion rate (PR, number of perfused capillaries per number of all capillaries) were determined in the longitudinal muscular layer.

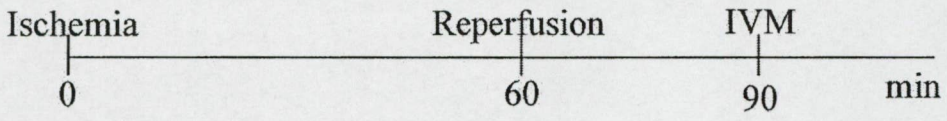
2.5. Experimental protocols

2.5.1. *Microcirculatory characteristics of the urinary bladder*

Fluorescence IVM measurements were made at the beginning, and 90, 120 and 180 min after the surgical exposure of the urinary bladder. (N=10, N:number of animals)

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

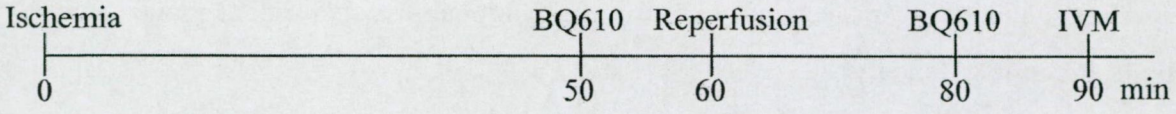
After 60 min ischemia and 30 min reperfusion fluorescence IVM measurements were performed. (N=10) Sham-operated animals served as controls, in this group (N=10) fluorescence IVM measurements were made after 90 min.



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

The animals (N=8) were pretreated with the ET-A receptor antagonist BQ 610 (i.v., 200 nmol/kg b.w.; homopiperidinyl-carbonyl-Leu-D-Trp-(CHO)-D-Trp-OH; Alexis Corp., Läufelfingen, Switzerland). BQ 610 was administered two times, 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*

The animals were fasted before the operations. The leak point pressure was measured in all animals. During the 90 days reconvalescence the animals were kept in the same place and were given regularly standard food. During the second operation and *in vivo* microscopy continuous infusion was administered and the mean arterial blood pressure was monitored. Animals with blood pressure under 90 mmHg were excluded from the experiment. During the operation threads of the same size, quality and label were used.

Fluorescence IVM pictures of the microcirculation of muscular layer of the same areas 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 (N=9) 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 (N=6).

The microcirculation of intact ileal segments (N=11) and the intact bladder (N=12) 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

2.6.1. *Statistics of the measurements for bladder microcirculation*

The data displayed a non-Gaussian distribution. For this reason, the necessary power of the statistical tests requires the use of minimum eight successful experiments. The analysis was performed with a statistical software package (SigmaStat 2.0 for Windows, Jandel Scientific, Germany). Friedman repeated measures for analysis of variance was applied for multiple comparisons between the different time points. Mean values ± standard deviations (S.D.) were given. $P < 0.05$ was considered significant.

2.6.2. *Statistics of the ischemia-reperfusion experiments*

The analysis was performed with a statistical software (SigmaStat 2.0). Due to the low number of animals, a non-normal distribution of the data was assumed. ANOVA on ranks followed by Student-Newman-Keuls method was applied for multiple comparisons between the different groups. Mean values ± standard deviations (S.D.) were given; $p < 0.05$ was considered significant.

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; p values < 0.05 were considered significant. 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 to the basal values (Table 1.).

	Baseline	90 min	120 min	180 min
Rolling leukocytes/ mm/s n=5	6.0±1.8	7.5±1.3	8.8±2.8	8.5±3.9
Adherent leukocytes (1/mm^2) n=5	12.7±17.3	20.0±9.5	28.8±17.8	26.1±13.8
Venular macromolecular leakage (%) n=5	0.74±0.10	0.76±0.12	0.78±0.11	0.79±0.10
Arteriolar macromolecular leakage (%) n=5	0.77±0.08	0.79±0.08	0.81±0.08	0.80±0.06
RBCV (mm/s) n=5	1.2±0.1	1.3±0.1	1.2±0.1	1.1±0.1
FCD (1/cm) m=5	141.6±12.2	138.3±9.8	132.2±6.0	136.2±9.2
Venular diameter (μm) n=5	41.2±22.1	39.2±14.2	37.2±15.3	40.2±3.4
Arteriolar diameter (μm) n=5	40.7±11.1	37.7±8.8	44.5±18.2	39.5±11.0

Table 1. *Microcirculatory characteristics of the urinary bladder in rats. Mean values ± S. D. (n: number of observed vessels, m: number of fields of interest). No statistically significant differences were found between the measurements.*

3.2. Ischemia-reperfusion injury of the urinary bladder

3.2.1. *Microcirculatory changes of the urinary bladder*

After filling the bladder with 0.5 ml saline solution, the intravesical pressure was 9±2 mmHg in both groups and did not change significantly during the experiments. The macrohemodynamic parameters were in the physiological range and did not differ between the groups (data not shown).

At the microcirculatory level of the bladder wall, all investigated parameters were significantly changed after I/R as compared to the control group. The numbers of rolling and firmly adherent leukocytes in postcapillary venules were elevated by 828% and 4610%, respectively (Figure 7.). The macromolecular leakage in arterioles and venules was also significantly increased by 33%. The venular red blood cell velocity and functional capillary density decreased to 35% and 27% of the control values, respectively. The arteriolar and venular diameters were also reduced to 73% of the controls (Table 2).

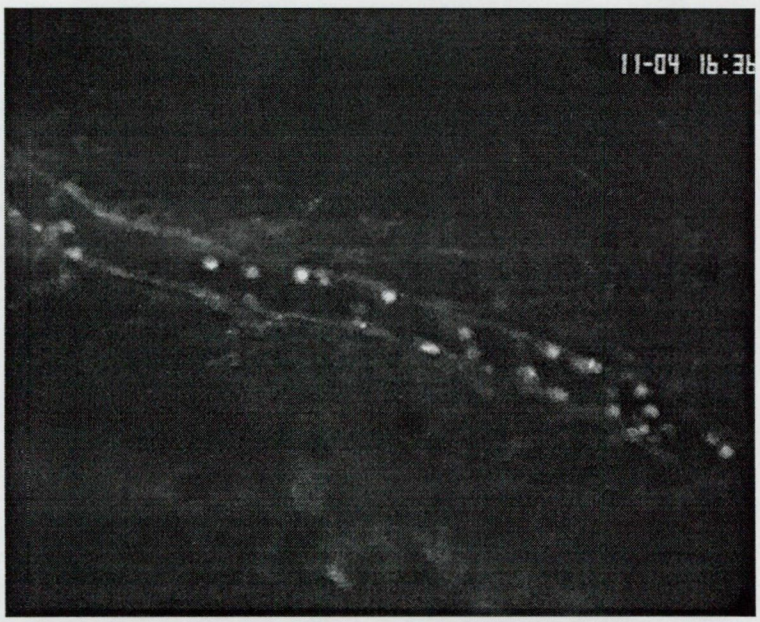


Figure 7. *A postcapillary venule in the muscular layer of the urinary bladder. Rhodamine-6G-labeled leukocytes rolling on and sticking to the endothelium during reperfusion.*

3.2.2. *Histological changes*

The histological examination of hematoxylin and eosin-stained sections revealed increased lymphocyte and polymorphonuclear leukocyte migration into the submucosa, lamina propria and the epithelium. Diffuse edema was observed in all layers of the bladder wall.

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

The administration of BQ 610 did not significantly influence the macrocirculatory parameters (data not shown). The intravesical pressure was 9±2 mmHg during the experiments and did not differ significantly between the groups.

In the control group, only moderate leukocyte-endothelial cell interactions were observed. We found normal flow parameters and there were very few capillaries unperfused by red blood cells. The macromolecular leakage was relatively high as compared to other organs, probably due to the relatively higher background fluorescence intensity of the urine.

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. (Table 2.).

	Sham	I/R	I/R+BQ-610
Rolling leukocytes/mm/s n=5	**7±2**	**58±11 ***	**12±4 ***
Adherent leukocytes ($1/mm^2$) n=5	**30±24**	**1383±543 ***	**87±66 ***
Venular macromolecular leakage n=5	**0.71±0.04**	**0.97±0.01 ***	**0.78±0.08 ***
Arteriolar macromolecular leakage n=5	**0.72±0.06**	**0.95±0.03 ***	**0.78±0.07 ***
RBCV n=5 (mm/s)	**1.19±0.07**	**0.42±0.09 ***	**1.08±0.1 ***
FCD m=5 (1/cm)	**149±20**	**41±7 ***	**113±7 ***
Venular diameter n=5 (μm)	**41±7**	**31±7 ***	**40±10**
Arteriolar diameter n=5 (μm)	**39±8**	**30±3 ***	**39±6**

Table 2. *Microcirculatory changes and the effects of ET-A receptor antagonism in I/R injury of the urinary bladder of the rat. Mean values ± S. D. Asterisks indicate significant ($p<0.05$) differences. (n: number of observed vessels, m: number of fields of interest)*

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 in all animals 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 gradually increased the microcirculation of the intact ileum showed the first significant disturbance at 25 mmHg. The microcirculation became even more compromised at higher pressures in terms of both red blood cell velocity, functional capillary density and perfusion rate (Figure 8., 9.).

3.3.3. *Microcirculatory changes of the intact bladder*

In the intact bladder, then microcirculation was well preserved during the examination period (Figure 8., 9.).

3.3.4. *Microcirculatory changes of the enterocystoplasty*

In animals with ECP significant differences were not observed in the augmented intestine or bladder in erythrocyte velocity, functional capillary density and perfusion rate parameters as compared to the microcirculation in the intact organs of the sham-operated rats.

When increasing the intravesical pressure significant microcirculatory damage started already below the leak point pressure (at 25 mmHg) in the ileal part of the ECP - similarly to the intact ileum. This dysfunction was maintained or became worse with further increase of the pressure. In the bladder part of the ECP no significant red blood cell velocity, functional capillary density or perfusion rate damage could be observed at the examined pressure values (Figure 8., 9.).

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 in terms of both red blood cell velocity, functional capillary density and perfusion rate. The microcirculation was well preserved in the bladder part during the examined period (Figure 10.).

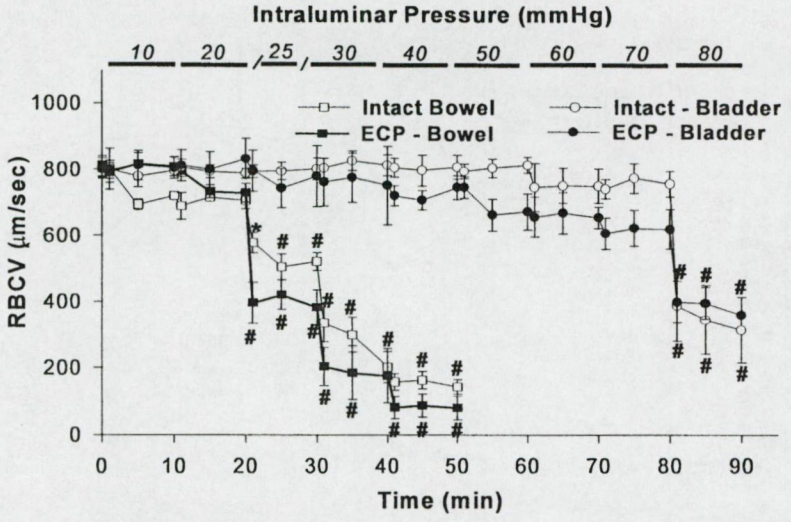


Figure 8. *Effects of various intraluminal pressures at 10 mmHg increments on red cell velocity (RBCV) of intact intestine and bladder, and the intestinal and bladder parts of enterocystoplasty (ECP). Data on ECP components were derived from simultaneous measurements of each anatomical neobladder site. Asterisk indicates $p<0.05$ versus baseline. Mean values ± SEM. Pound sign indicates $p<0.01$ vs baseline.*

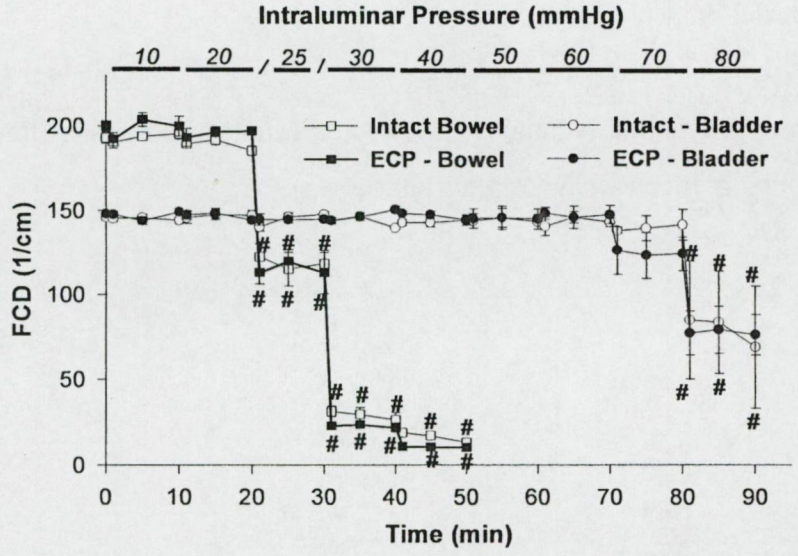


Figure 9. *Effects of increased intraluminal pressure at 10 mmHg increments on functional capillary density (FCD) of intact intestine and bladder, and intestinal and bladder part of enterocystoplasty (ECP). Data on ECP components were derived from simultaneous measurements of each anatomical neobladder site. Mean values ± SEM. Pound sign indicates $p<0.01$ vs baseline.*

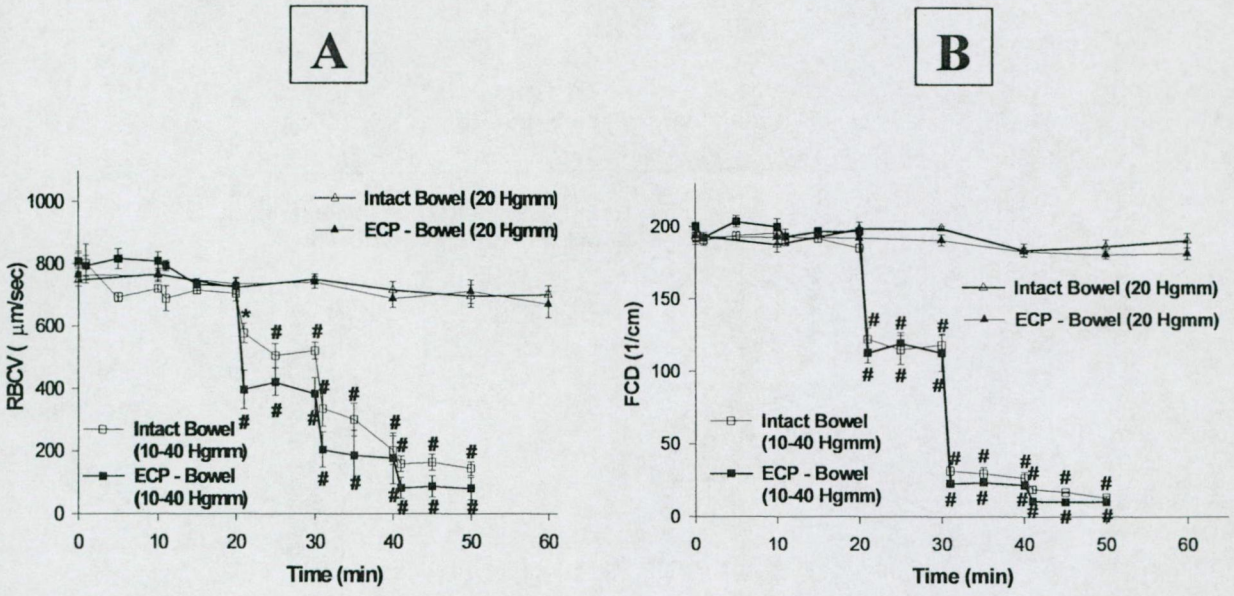


Figure 10. *Effects of increasing intraluminal pressure between 10-40 mmHg. A: Red blood cell velocity (RBCV) of intact bowel and intestinal site of enterocystoplasty (ECP) versus continuously elevated intraluminal pressure of 20 mmHg. Asterisk indicates $p<0.05$ vs baseline. Pound sign indicates $p<0.01$ vs baseline. B: Functional capillary density (FCD) of the intact bowel and intestinal site of ECP versus continuously elevated intraluminal pressure of 20 mmHg. Mean values ± SEM.*

3.4. Macrohaemodynamic parameters

The macrohemodynamic parameters were within the physiological limits during the experiments without significant changes (data not shown).

4. DISCUSSION

4.1. The microcirculation of the urinary bladder

Although the use of the fluorescence IVM became increasingly popular for studying the organ microcirculation[3.,5.,6.,45.-50.] very few reports deal with the intravital evaluation of the urinary bladder microcirculation. Our first aim was to develop a reproducible rat model to precisely characterize the microvascular perfusion parameters of the bladder. The rat is one of the most commonly used animals in microcirculatory research. The urinary bladder of the rat is very similar to that of the humans, both anatomically and functionally.[4.,51.] The main branches of the cystic arteries and large veins form an adventitial-serosal plexus which supplies the capillary network of the muscular layer and sends long perpendicular vessels to the mucosal plexus. Apart from few vascular connections between these layers, the submucosa is generally avascular. The muscular layer contains tortuous arterial and venous branches mostly derived from the adventitial-serosal plexus. In comparison to the extremely dense subepithelial capillary network, the muscularis is poor in capillaries.[51.] The microcirculatory network of the rat bladder can be visualized within the maximal depth of 300 μm of the muscular layer with fluorescence IVM, and can be distinguished from the vessels of other layers by their specific architecture.

The urinary bladder of the rat was chosen for our study because its thin tissue allows for high resolution images and precise quantification of the microcirculatory parameters. To study the microcirculation of the bladder with fluorescence IVM in an animal smaller than the rat would be disproportionately more difficult and the scatter resulting from technical problems would cause the need for a much larger experimental groups.

Reed et al. described some microcirculatory changes of the urinary bladder after photodynamic therapy using a fluorescence microscopic technique. The microcirculation was observed by transmitted light. In their study the organ was placed into a plexiglass tissue bath, fixed it by a suture on the bladder surface. The bladder was almost empty during the examination and the intravesical pressure and volume were not determined. Only the diameters of the red blood cell column and the presence or absence of platelet thrombi were observed as microcirculatory parameters.[52.]

In another study, *Young et al.* used an *in vivo* preparation to evaluate the effects of histamine, norepinephrine and prostaglandins on the arterioles and venules of the rat urinary

bladder. In their model the bladder was illuminated by light transmitted along a borosilicate glass without the use of fluorescein dye.[53] Consequently, data comprising all relevant microcirculatory parameters of the urinary bladder under physiologic conditions have not been available so far.

A large number of data exists about the microcirculation of other organs in various species.[3.,5.,6.,45.-50.] Among these, the microcirculatory parameters of the intestine and the mesentery are the most suitable for a comparison to our results.

Massberg et al. described the microcirculatory characteristics of the small intestine in the mouse. In their preparation, the functional capillary density of the muscular layer was 226±21 1/cm under normal conditions. The leukocyte-endothelial cell interaction was also analyzed. Under physiological conditions only a few white blood cells were found rolling along or firmly adherent to the endothelium (1±0.2 cells/mm/s and 18±7 cells/mm^2, respectively).[47]

Boros et al. also investigated the microcirculation of the small intestine in rats. In that model, the red blood cell velocity was 0.88±0.17 mm/s and the functional capillary density was 213±25 1/ cm, similar to the respective values of the mouse. In the longitudinal muscle layer, the respective values were 164±15 1/cm (FCD) and 0.78±0.09 mm/s (RBCV). The number of firmly adherent leukocytes (cells/mm^2) in the collecting venules was 8-10 under normal conditions.[2]

The microcirculation of the rat mesentery also was studied by fluorescence IVM. In contrast to the intestine, which is very similar to the bladder, the microcirculatory characteristic of the mesentery differs from the bladder in several aspects. Under control conditions, Kurose *et al.* reported 3.12±0.18 mm/s red blood cell velocity and leukocyte adherence of 5±2 cells/100μm vessel length. The higher flow can be explained by the larger vessel diameters, however the values of leukocyte adherence were similar to those found in the bladder.[45]

In our standardized rat model we described the physiological characteristics of the microcirculation of the bladder. We filled the urinary 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. There was a slight tendency for leukocyte-endothelial cell interactions and the functional capillary density slightly deviated from baseline values at the subsequent measurements. The anesthetic regimen could offer an explanation. Atropine is widely used in small animals for premedication. So far, there were no controlled studies on the influence of this drug on the microcirculation, but given that baseline measurements were obtained about 1 hr after atropine administration and a half-live of i.m. atropine is 3-4 hrs, the slight differences can not be explained by the use of this drug. Although the macrohemodynamic parameters remained unchanged throughout the experiments, the i.p.-administered pentobarbital could probably affect the microcirculation.

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. After administration of cyclophosphamide the development of cystitis was reported in rats[11.,54.] In this type of cystitis, plasma protein extravasation is significantly increased.[11.] Cystitis also can be induced by intravesical instillation of formalin (due to chemical irritation), or lipopolysaccharide. A foreign body in the bladder could lead to cystitis by mechanical stimulation, infection, or immunological reactions. Intramural edema, recruitment of inflammatory cells and mastocytosis were found in all forms of inflammation.[13.] Interstitial cystitis was induced by administration of bladder homogenate in complete Freund's adjuvant based on an autoimmune reaction.[55.] 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.[15.,16.]

I/R-induced failure of the microcirculation is characterized by decreased capillary perfusion leading to "*no-reflow*", and decreased functional capillary density. Reflow-associated events, termed "*reflow paradox*", include leukocyte-endothelial cell interactions and increased microvascular permeability.[56.] No single process can be identified as the critical event in ischemia-induced tissue injury but hypoxia and reoxygenation of a tissue is associated with a wide range of inflammatory reactions.[57.] *No-reflow* in nutritive microvessels prolongs local tissue hypoxia at the time of reperfusion, resulting in impairment of energy metabolism and cell death. Additionally, reperfusion of ischemic tissues also leads to a sequence of paradoxical tissue-injuring processes.[15.-18.]

To date only few animal models are available for the investigation of the characteristics of I/R of the urinary bladder. Some investigators studied the role of ischemia in the urinary bladder in terms of smooth muscle function. *Vanarsdalen et al.* investigated the consequences of bladder ischemia in a rabbit model. After 1 hr of ischemia a significant reduction was observed in the smooth muscle function of the bladder.[58] *Gill et al.* recorded a reduction in compliance and capacity of the urinary bladder after various ischemic periods.[59] The increase of intracellular Ca^{2+} after I/R may play an important role in these processes.[60] Others reported that mRNA levels of early response genes and protein synthesis were elevated after an ischemic episode of the urinary bladder.[61] *Saito et al.* studied the contractile function of the bladder after I/R and the role of the nitric oxide. 60 min ischemia followed by 30 min reperfusion caused a significant decrease in the maximum contractile response of the detrusor muscle. In the same study, histological examinations revealed that I/R causes infiltration of leukocytes in the submucosal region and in the smooth muscle. The authors concluded that nitric oxide may play an important role in the I/R-induced functional failure of the bladder, since the use of a nitric oxide synthase inhibitor significantly attenuated these pathologic alterations.[62]

In the present study, I/R of the urinary bladder significantly impaired the 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. IVM is an excellent technique for the detailed investigation of microcirculatory effects of drugs and compounds already used in research or in clinical practice. Therefore, this novel cystitis model, in addition to answering the particular question addressed in the present study, provides a basis for future investigations.

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

Ischemia of the urinary bladder may result in a wide spectrum of detrimental effects. While the etiology may vary, the consequences are always related to impaired arterial inflow, impaired venous outflow, or obstructed capillary circulation.

Elevated intravesical pressure caused by lower urinary tract obstructions may result in subtle changes in the microcirculation followed by the defect of urothelial antiadhesive effect with increased susceptibility to infections, functional disturbances, or inflammation.

Distention of the bladder results in decreased flow to all layers with a relative greater decrease in the mucosa than in the muscularis. Microcirculatory disturbance can also be an explanation for ulceration seen in interstitial cystitis.

More threatening diseases, even severe necrosis or gangrene of the bladder was observed after thrombophlebitis of pelvic veins as a complication of pregnancy and after ligation or embolization of hypogastric arteries. It is suspected that an interference between local infection, decreased blood supply, and systemic metabolic changes is necessary to develop these severe states.[63.]

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 microcirculatory parameters. With the administration of BQ 610, the microcirculatory disturbances were attenuated, but control values were not achieved with exception of venular diameter 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.

Several investigations showed that ET-1 plays a major role in ischemic organ injury and other inflammatory reactions.[26.] Under conditions of I/R, elevated amounts of ET-1 are released into the regional circulation. ET-1 is secreted predominantly in the abluminal direction and small changes in plasma levels might correlate well with significant effects of this peptide on local vascular reactivity. ET-1 has the ability to induce both primary (leukocyte rolling) and secondary (leukocyte sticking) microcirculatory inflammatory reactions by enhancing these leukocyte-endothelial cell interactions on several levels. Endothelial cell derived ET-1 release is sensitive to hemodynamic stimuli and thus microcirculatory perfusion failure would lead to leukocyte activation mediated by ET-A receptors.[2.] The other possibility is an interaction of ET-1 with endothelial cell membranes in the systemic circulation and the release of secondary chemoattractant mediators linked to ET-A and ET-B receptor activation. Once in the systemic circulation, ET-1 might induce phospholipase A_2 activation, or indirectly activate leukocytes through platelet-activating factor and leukotriene B_4 release from macrophages or endothelial cells. Furthermore, the effects of endothelins may interfere with the antiadhesive actions of endothelial cell-derived antiaggregatory mediators. The number of ET-A binding sites is increased in response to reperfusion, and the responsiveness to the vasoconstrictor effects of ET-1 is enhanced. It

could be hypothesized that an increased local endothelin level and a relative lack of antiaggregatory mediators play a concerted role in the process of neutrophil adherence and accumulation in the microcirculation. Another possibility is a direct activation of endothelial cells and neutrophils through up-regulation of adhesion molecules on the surface of the cell membranes. The *in vivo* effects of ET-1 on neutrophil functions are far from clarified, because most of the data on endothelin-induced changes are from studies using isolated cell lines or high ET-1 doses, and the evidence is largely circumstantial. ET-1 also plays an important role in the elevation of microvascular permeability in I/R-induced inflammation. ET-1 may increase microvascular albumin leakage[64] and in the present study administration of BQ-610 reduced the macromolecular leakage. In concert with an increased precapillary resistance, an enhanced microvascular permeability may lead to perivascular edema and hemoconcentration, and the rise in capillary viscosity might promote impairment of microvascular perfusion. These mechanisms might be related to both ET-1-induced perfusion failure and tissue injury.

In our study, 60 min ischemia and 30 min reperfusion was used since with this intervention histological signs of inflammation have already been shown.[4] Similarly, the dose of BQ-610 was previously shown in our laboratory to have no effect on hemodynamic and microvascular parameters when infused into the same rat strain.[2]

Our results show that I/R-induced cystitis causes significant microcirculatory damage and ET-1 can act as an important player in this process. However, our results also show that the role of the ET-1 is not exclusive in these pathologies and although the use of an ET-A receptor blocker can significantly inhibit the microcirculatory injury, further investigations are needed to find final solution to prevent these complex disturbances.

4.4. Enterocystoplasty

Despite several decades of experience with ECP, late spontaneous rupture only recently has been reported as a complication. Most of the reported ruptures occurred in patients after augmentation for a neurogenic bladder secondary to myelomeningocele. The perforation typically occurs in the intestinal segment away from suture lines. Cystograms have a high false-negative rate. Therefore, the high index of suspicion must be maintained whenever the clinical findings suggest peritonitis despite non-diagnostic radiographic findings.

Previous reports have suggested that there has been chronic overdistension of the augmented bladder before rupture, presumably the results of poor compliance with an intermittent catheterization program, mucous obstruction of the catheter or positional problems with inadequate catheter drainage. Human urodynamic studies after ECP have shown baseline resting pressures of 10-20 water cm. The intestinal segment may exhibit mass contractions with maximal pressures reaching 100 water cm. Lower pressures and fewer contractions prevailed when detubularized bowel segments were used.[65.-67.] Studies of bowel obstruction have shown that intraluminal pressures in acute obstruction may reach 52 cm water in humans.[68.] According to Wangensteen and Rea, sustained intraluminal pressures of more than 22 water cm for a longer period can cause alterations in bowel permeability, a change they attributed to decreased viability.[69.]

The physical relationship expressed by Laplace's law among intraluminal pressure, radius and wall tension can be applied to explain bowel wall failure in cases of ECP rupture as well as cecal perforation in cases of large bowel obstruction. Wall tension is the limiting factor for blood flow and its magnitude is directly proportional to intraluminal pressure and radius. Thus, increasing either the radius or the intraluminal pressure will have the same effect, i.e. increased wall tension and therefore, decreased intramural blood flow. In an irregularly configured vessel, such as an augmented bladder, wall tension will fluctuate as the radius varies. In such a vessel maximal wall tension at a given intraluminal pressure will be obtained where the radius is greatest.[70.] Since the intestinal segment represents the site of maximum radius in most chronically distended augmented bladders, it would also be expected to exhibit the greatest degree of wall tension and subsequent vascular compromise. The findings of chronic transmural ischemia and perforation through the bowel segment of these augmented bladders in patients with diminished sensation is consistent with this proposed pathophysiological mechanism.

Epi-illumination with fluorescence IVM of an ECP model has not been previously used. Our ECP study represents a new *in vivo* model for studying the microcirculatory consequences of the ECP and it provides an opportunity for the better understanding of the microcirculatory effects of any methods already used in the clinical practice. Similarly, there has been only minimal use of the urinary bladder as a microcirculatory model. In a different model described by *Young et al.*, the bladder was illuminated by light transmitted along a borosilicate glass rod. These authors studied the effects of topical administration of vasoactive drugs on vascular diameters of both arterioles and venules by dripping the

respective drugs on the bladder surface.[53] In another study *Schuschke et al.* used a urinary bladder model for the microcirculatory consequences of vasoconstriction, vasodilatation and macromolecular leakage. In their model the microcirculation was examined by transmitted light and the bladder was positioned in a tissue bath.[71] A model for studying the microcirculation of the bladder using fluorescence *in vivo* microscopy was also described by *Reed et al.* They observed the rat urinary bladder microcirculation in response to the photodynamic therapy.[52]

Only a few reports are available on the characteristic changes during ECP. In one case the authors examined the ischemic necrosis hypothesis of the rupture by quantifying the arterial hypoperfusion using fluorometric assessment in dogs.[72] There was a significant decrease in fluorescein uptake at high intravesical pressures. Other studies basically focussed on the effects of various surgical techniques[73], on the transport processes[74], on the capacity[75] and contractility[62] of the bladder and the augmented bladder.

There are some investigations on the macrohemodynamic consequences of distension of the bowel in animal models[76,77], but none of them provided information at the microcirculatory level. In a canine model, *Boley et al.* examined the perfusion in isolated bowel segments in increasing intraluminal pressure circumstances. They found no changes in blood flow below 30 mmHg, over this a stepwise diminution in flow was observed.[77]

Our experiments demonstrate the greater sensitivity of the microcirculation of the intestinal segment of ECP to clinically relevant intraluminal pressure increases in the rat. It also seems that the microcirculation of the ileum is diminished even below the previously described intraluminal pressure, which causes hypoperfusion in canine intestine.[77] Secondary 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

New experimental animal models help to answer scientific questions and provide bases for future investigations. We are aware that understanding a particular mechanism does not provide overall solution for a clinical problem, and often addresses new questions. We hope that our studies on microcirculatory disorders of the urinary tract will increase our knowledge in this field and strengthen the theoretical basis of future rational therapies.

In 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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8. ANNEX